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Impact of biochar application on different forms of organic carbon in acidic soil conditions

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Abstract
A field experiment was conducted to investigate the effects of biochar on soil organic carbon (SOC) content and its active fractions (DOC and SMBC). The experiment was laid out in randomized block design with three replication and seven treatments. The treatments were T_1-RDF, T_2- RDF + 1.0 ton ha^{-1} biochar at the time of initiation of the project, T_3- RDF + 1.0 ton ha^{-1} in every season, T_4- RDF + 2.0 ton ha^{-1} biochar at the time of initiation of the project, T_5- RDF + 2.0 ton ha^{-1} in every season, T_6- RDF + 3.0 ton ha^{-1} biochar at the time of initiation of the project, T_7- RDF + 3.0 ton ha^{-1} biochar in every season. Soil samples were air dried and analyzed for soil reaction (pH), electrical conductivity (EC), very labile carbon (VLC), labile carbon (LC), less labile carbon (LLC), recalcitrant carbon, dissolved organic carbon (DOC), oxidisable carbon, total organic carbon (TOC) and soil microbial biomass carbon (SMBC). The data pertaining to soil was statistically analyzed for their level of significance. pH (5.62-5.93), EC (0.15-0.28 dSm^{-1}), very labile carbon (1.83-2.36 g kg^{-1}), labile carbon (1.16-1.49 g kg^{-1}), less labile carbon (0.51-0.67 g kg^{-1}), recalcitrant carbon (2.25-2.89 g kg^{-1}), dissolved organic carbon (3.0-5.0 kg^{-1}), oxidisable carbon (4.35-5.60 g kg^{-1}), total organic carbon (5.74-7.39g kg^{-1}) soil microbial biomass carbon (249.60-328.30 mg kg^{-1}). The results revealed that different forms of carbon were highest in treatments T_7 (RDF + biochar @ 3 t/ha in every season) and lowest in T_1 (RDF). Increased status of carbon forms might be due to continuous addition of high quantities of biochar i.e.21 ton ha^{-1} from Rabi 2018 to Rabi 2021. Based on the present study, it may be inferred that continuous application of RDF + 3.0 ton ha^{-1} of biochar in every season would be viable option for improving the different forms of organic carbon in soil.

Keywords: Linseed, biochar, soil organic carbon, soil microbial biomass carbon

Introduction
Soil organic carbon (SOC) is an essential component of the global carbon cycle, involving the movement of carbon through soil, vegetation, oceans, and the atmosphere. The SOC pool stores a substantial 1500 PgC within the first meter of soil, surpassing the combined carbon content in the atmosphere (roughly 800 PgC) and terrestrial vegetation (500 PgC). The ratio of labile SOC to total SOC significantly impacts SOC sequestration and soil health. SOC is categorized into different pools based on its physical and chemical stability. These SOC pools are classified as very labile, labile, less labile, and non-labile, depending on their susceptibility to degradation through oxidation with sulfuric acid. The very labile and labile pools show a strong correlation with mineralizable nitrogen and water stable aggregate stability. In contrast, less labile and non-labile pools constitute about 35% of total SOC and 30-40% of soil organic carbon in the passive pool. Non-labile carbon exhibits chemical resistance due to the presence of alkyl C chains, aromatic structures, and phenolics, while labile carbon results from the breakdown of cellulose, hemi-cellulose, lignin, protein, lipid, and tannin from plant, animal, and microbial sources. SOC fractions are also distinguished by their mean residence times, with active fractions persisting for 1-5 years, slow fractions for 20-40 years, and passive fractions for 400-2000 years. The proportion of these carbon pools is influenced by factors such as the type of organic matter, soil type, and climate conditions.
Biochar has gained significant attention as a cost-effective amendment for carbon sequestration and soil rehabilitation (El-Naggar *et al*., 2018) [6].

It is a carbonaceous product obtained from biomass thermochemical conversion in an oxygen-limited environment. Utilizing biochar for waste disposal and enhancing soil quality has been widely studied (Zhang *et al*., 2018; Berek, 2014) [19, 1]. The main mechanisms of soil improvement involve biochar's ability to increase cation exchange capacity (CEC), pH, water holding capacity, and provide mineral nutrients directly (Chathurika *et al*., 2016; Hilioti *et al*., 2017; Cornelissen *et al*., 2018) [3, 8, 4]. Its alkaline composition, including ash and carbonates of Ca^{2+}, K^+, and Mg*2+*, and surface properties contribute to pH and CEC enhancement (Yuan *et al*., 2011; Jien and Wang 2013) [18, 9]. Additionally, biochar's resistance to degradation ensures its carbon remains in the soil for extended periods, ranging from 100 to 10,000 years. To preserve carbon and nutrients in a stable form, innovative strategies for soil retention are essential, particularly in preventing the oxidation of nutrients like phosphorus, magnesium, manganese, and carbon dioxide through pyrolysis processes.

Materials and Methods

Site description and field experiment

The field experiment was conducted at Research Farm of Department of Soil Science, Birsa Agricultural University, Ranchi, Jharkhand during Rabi, 2021. This experiment was started during the Rabi season in 2018. Geographically, Ranchi is located at a longitude of 85°19' E, altitude of 23°17' N and an altitude of 625 m above mean sea level. This site has a tropical climate characterized by hot and wet summers and mild winters. The temperature during summers can rise up to 42 °C. The monsoon season lasts from July to September, and the state receives an average annual rainfall of 1450 mm. The texture of soil is sandy loam soil with pH of 5.4 and EC,the contents of organic carbon, Available nitrogen (AN), phosphorous (AP), potassium (AK) were 0.24dS/m, 0.43%,191.30 kg/ha, 14.56 kg/ha, 152.4 kg/ha respectively. In this experiment, seven treatments with different levels of biochar rates, such as 1 t/ha, 2 t/ha and 3 t/ha were applied to topsoil (0–15 cm depth) at the time of initiation of the project (2018) and in every season each with three replicate plots (7.5 × 4 m^2). The biochar was made from maize stone under 350 to 500 °C for four hours in the absence or limited supply of oxygen, with contents of organic carbon, nitrogen, phosphorous, potassium of 94.0, 0.32, 0.16 and 1.08% respectively, and had a pH of 7.06.

Soil sampling and analysis

With the aid of an auger, soil samples were taken from the experimental site after the linseed crop was harvested at a depth of (0 to 15 cm) for the seven selected treatments and three replications. The soil samples were mixed thoroughly and were passed through 2 mm sieve and then stored in cellophane bags in a clean, dry environment.

In order to understand different carbon pools in soils, we adopted modified Walkley and Black method. To evaluate the oxidizable carbon, a modified procedure was employed using either 5 or 10 ml of concentrated sulphuric acid, as opposed to the 20 ml recommended by Walkley and Black (1934) [16]. This adjustment resulted in three distinct ratios of acid to aqueous solution (0.5:1, 1:1, and 2:1), which corresponded to different concentrations of sulphuric acid (12 N, 18 N, and 24 N). By subjecting the extracted organic carbon to increasing oxidizing conditions, as described by Walkley in 1947, a comparative analysis of the results was made possible. There are four categories of total organic carbon, each representing a different level of labile behavior.

1. Very labile carbon, organic carbon that is oxidized with 12 N H_2SO_4.
2. Labile carbon, is an oxidizable organic carbon which is obtained by calculating the difference between 18 N and 12 N H_2SO_4.
3. Less labile carbon, reflects the difference in oxidizable organic carbon which is obtained by calculating the difference between 24 N and 18 N H_2SO_4. It is worth noting that the 24 N H H_2SO_4 is the equivalent of the standard Walkley-Black method.
4. Non-labile carbon/Recalcitrant carbon, also known as Fraction 4, which is the residual organic carbon remaining after reaction with 24 N H_2SO_4. This category is determined by comparing the total organic carbon (TOC) measured by the TOC analyzer with the organic carbon remaining after reaction with 24 N H_2SO_4 (TOC-24 N H_2SO_4)

Oxidisable carbon content of the soils was determined using the wet digestion method described by Walkley and Black (1934) [16]. Dissolved organic carbon was determined by taking soil water suspension in the ratio 1:2 as described by Mc Gill *et al*. (1986) [16]. It is followed by Walkley and Black (1934) [16] method. Microbial biomass carbon (MBC) was determined by the fumigation-extraction technique (Brookes *et al*.,1985) [2]. Ten grams of soil was fumigated with chloroform ($CHCl_3$) for 24 h at 25 °C, and samples were extracted with 50 ml 0.5 M dipotassium sulphate (K_2SO_4) for 30 min on a horizontal shaker at 200 rpm and filtered through paper (Whatman No. 42). Similarly, 10 g soil was extracted for non-fumigation at the same time (Brookes *et al*. 1985) [2]. Soil organic carbon (SOC) in the extracts was measured by the titration method.

Results and Discussions

Organic carbon

Different fractions of soil organic carbon were studied namely very labile carbon (VLC), Labile carbon (LC), less labile carbon (LLC), non-labile Carbon (NLC), dissolved organic carbon (DOC) and Oxidisable carbon as influenced by different levels of biochar is presented in Table 1. The active pool of carbon consists of the very labile pool and labile pool while the passive pool consists of the less labile and non-labile carbon fractions. It is found that the passive pool of carbon is present in a relatively higher proportion than the active pool.

The biochar application rate had a significant effect on the SOC content based on the one-way ANOVA. The SOC content, DOC, and MBC changed over time. The data regarding different forms of organic carbon are presented in table 1 revealed that among the study of various treatments, very labile ranged from (1.83-2.36 g/kg), labile carbon ranged from 1.16-1.49 g/kg), less labile carbon ranged from 0.51-0.67 g/kg) and Recalcitrant carbon ranged from (2.25- 2.89 g/kg). Dissolved organic carbon ranged from (3-5 g/kg) and Oxidisable carbon ranged from (4.35-5.60 g/kg). The increase in the biochar application rate increased soil SOC storage because of the high carbon content in the biochar. Moreover, the functional groups such as phenolic and carbonyl C in biochar help to adsorb organic compounds (Zheng *et al*., 2022) [20]. The increased status of organic carbon with RDF + 3.0 ton ha^{-1} biochar in every season may be due to continuous addition of high quantities of biochar (21 ton ha^{-1}) from Rabi 2018 to Rabi 2021. Similar results were reported by Yang *et al*. (2018) [17] and Demisie *et al*. (2014) [5]. The study revealed that the order of carbon fractions in the acid soil was RC>VL>L>LL. Moreover, very labile carbon and recalcitrant carbon fractions contribute 70% of total organic carbon.

Soil microbes play a crucial role in soil organic carbon (SOC) processes, and it has been observed that microbial biomass contributes to more than 50% of the extractable soil organic matter fractions. Highest microbial biomass carbon was observed in the treatment T_7 when soil was treated with RDF and 3 t/ha biochar in every season. MBC varied from 249.60 mg/kg to 328.30 as presented in table 2. The results from this study established that SMBC increased with biochar application relative to control, which is an indication that biochar addition to soils can accelerate the growth of microbes (mostly bacteria and fungi). Same results have been reported by several authors (Lehmann *et al*., 2011) [13]. In the majority of studies, microbial biomass increases in response to biochar addition and microbial community composition is changed significantly (Jones *et al*., 2012; Lehmann *et al*., 2011) [11, 13]. These responses may be because biochar benefits soil microbial biomass and microbial communities by providing suitable habitat for microbial growth and protection from predators (Quilliam *et al*., 2013) [15], absorbing poisonous substances to prevent microbial poisoning (Gomez *et al*., 2014) [7], improvement of the soil's physicochemical characteristics (Jindo *et al*., 2012) [10], increased nutrient availability or the provision of a C substrate for decomposition (Khadem and Raiesi, 2017) [12].

Table 1: Effect of biochar application on different pools of soil organic carbon

Treatments	VL (g kg^{-1})	L (g kg^{-1})	LL (g kg^{-1})	RC (g kg^{-1})	DOC (g kg^{-1})	OC (g kg^{-1})	TOC (g kg^{-1})
T_1: RDF	1.83	1.16	0.51	2.25	3.0	4.35	5.74
T_2: RDF+1.0 ton/ha biochar (Once- At the time of starting the project	1.94	1.23	0.54	2.38	3.5	4.61	6.09
T_3:RDF+1.0 ton/ha biochar (Every season)	2.09	1.32	0.58	2.57	4.9	4.97	6.56
T_4: RDF+2.0 ton/ha biochar (Once- At the time of starting the project)	2.03	1.26	0.55	2.44	3.3	4.73	6.24
T_5:RDF+2.0 ton/ha biochar (Every season)	2.17	1.37	0.60	2.66	4.9	5.15	6.80
T_6: RDF+3.0 ton/ha biochar (Once- At the time of starting the project)	2.00	1.26	0.55	2.45	3.5	4.75	6.27
T_7: RDF+3.0 ton/ha biochar (Every season)	2.36	1.49	0.67	2.89	5.0	5.60	7.39
CD (P=0.05)	0.262	0.185	0.082	0.338	0.055	0.61	0.85
CV (%)	7.154	8.010	8.112	7.546	7.402	5.16	7.47

Table 2: Effect of biochar application on soil microbial biomass carbon

Treatments	SMBC (mg kg^{-1})
T_1: RDF	249.60
T_2: RDF+1.0 ton/ha biochar (Once- At the time of starting the project	250.20
T_3: RDF + 1.0 ton/ha biochar (Every season)	268.67
T_4: RDF+2.0 ton/ha biochar (Once- At the time of starting the project)	254.90
T_5: RDF + 2.0 ton/ha biochar (Every season)	287.10
T_6: RDF+3.0 ton/ha biochar (Once- At the time of starting the project)	259.76
T_7: RDF + 3.0 ton/ha biochar (Every season)	328.30
CD (P=0.05)	30.28
CV (%)	6.27

Conclusion

Our study revealed that a high biochar application rate significantly increased SOC content and soil organic carbon fractions (DOC and MBC). Based on one year of experimentation, it may be concluded that continuous application of RDF + 3.0 ton ha $^{-1}$ of biochar in every season would be viable option for improving the different forms of organic carbon in soil.

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