# Priming Effect of Pigeon Pea and Wood Biochar on Carbon Mineralization of Native Soil Organic Carbon and Applied Municipal Solid Waste Compost

Vassanda Coumar Mounissamy,[a] Deeksha Parla Chandrashekar,[b] Tapan Adhikari,[a] Abhijit Sarkar,[a] Sangeeta Lenka,[a] Rajendiran Selladurai,[a,c] Dinesh Kumar Yadav,[a] Madhumonti Saha,[a] Bharat Prakash Meena,[a] Ajay,[a] and Jayant Kumar Saha [a]

A laboratory incubation experiment was conducted for 36 days to study the effect of pigeon pea biochar (PPB) and wood biochar (WB) on carbon mineralization of native soil organic carbon (SOC) and municipal solid waste compost (MSWC) applied to soil. The MSWC addition enhanced soil respiration by 2-fold (231 mg C $kg^{-1}$ soil) over the control (118 mg C $kg^{-1}$ soil). The PPB addition significantly ($P < 0.05$) increased cumulative loss of carbon as $CO_2$, whereas WB significantly decreased the cumulative loss of C over control. Addition of PPB at 5% and 10% levels increased SOC mineralization (positive priming) +22.9% and +31.2%, respectively; whereas reduction in SOC mineralization (negative priming) was noticed in WB (5% and 10%) treated soils by -3.1% and -21.7%, respectively. Similarly, WB induced strong negative priming effects (-21.9% and -29.5%), while PPB caused a weak positive priming effect (+3.0% and +11.6%) at 5% and 10% levels on mineralization of added labile carbon substrate (MSWC), respectively. Results indicate the hardwood (*Prosopis juliflora)* biochar exhibits refractory properties that inhibit mineralization of both native SOC and applied organic compost (MSWC), and thereby it can be used as an amendment to stabilize native and applied organic matter in soil, which may significantly contribute to soil carbon sequestration.

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*Contact information: a: ICAR-Indian Institute of Soil Science, Nabibagh, Berasia Road, Bhopal- 462 038, India; b: Rani Lakshmi Bai Central Agricultural University, Jhansi- 284 003, India; c: ICAR-IIHR-Central Horticultural Experiment Station, Chettalli-571 248, Kodagu District, Karnataka, India;*
** Corresponding author: vassanda.coumar@gmail.com*

## INTRODUCTION

The increase in atmospheric $CO_2$ concentration and global warming has resulted in an increased awareness of carbon sequestration in soil. Terrestrial carbon sequestration plays a major role in the global carbon cycle and it has been successfully utilized to offset anthropogenic $CO_2$ emissions. In most of the tropical countries, including India, soils are very low in organic matter content and because of intensive cultivation coupled with high temperature, most of the soil carbon is lost to the atmosphere as $CO_2$. Therefore, there is increased interest towards long term sequestration of terrestrial soil organic carbon primarily by protecting recalcitrant organic matter in soil physically through organo-mineral complexes and charcoal (biochar) formation. Recent studies have demonstrated

and created considerable interest on biochar to mitigate climate change by sequestering carbon in soil (Lehmann *et al.* 2021)

The fine-grained porous nature of biochar is comparable in its appearance to charcoal formed by natural burning. Biochar is produced through pyrolysis, a thermo-chemical process in which biomass is heated in the absence of oxygen at a controlled temperature. One of the most important characteristics of biochar, particularly regarding carbon sequestration, is its long-term stability in soil. Because of its highly condensed nature, biochar in general has been regarded as a chemically and biologically recalcitrant material (Skjemstad *et al.* 2002).

Several researchers have indicated that the addition of biochar influences the microbial community and its activity (Nan *et al.* 2016; Wu *et al.* 2017). According to Zhang *et al.* (2017), biochar can alter the soil microenvironment, leading to shifts in bacterial communities and biodiversity. However, these effects can vary depending on the soil type, the nature and amount of biochar used, and other factors. Recent investigations also showed that carbonized materials from the incomplete combustion of organic material (*i.e.*, black C, pyrogenic C, charcoal, *etc.*) are accountable for increased soil organic matter and plant available nutrients in soils of the Brazilian Amazon basin (Glaser *et al.* 2000; Blanco-Canqui 2021).

Even though biochar is relatively stable, it can still be partially mineralized by both abiotic (Liang *et al.* 2008) and biotic mechanisms (Hamer *et al.* 2004; Zheng *et al.* 2022). Kuzyakov *et al.* (2009) found that the decomposition of biochar was relatively rapid during the first three months following incorporation to soil and slow, partial decomposition occurred during the following 3.2 years of the experiment.

Biochar could also cause positive or negative priming effect on mineralization of native SOM as well as on labile amended carbon source following its application in soil. Positive (C mineralization stimulation) or negative (C mineralization suppression) priming effects and magnitude varied with soil and biochar type. This positive priming could occur if biochar provides a mineralizable C source, nitrogen, phosphorous, and micronutrients or even a habitat favoring increased microbial heterotrophic activity (Thies and Rillig 2009). In contrast, the porous nature of biochar and its greater affinity for natural organic matter may possibly stabilize SOM through organo-mineral complex or sorption mechanism, thereby creating a negative priming effect (reduced carbon mineralization) (Sollins *et al.* 1996). Therefore, an increased mineralization of biochar following the introduction of a substrate or a positive priming effect on soil organic C caused by biochar addition might clearly offset the role of biochar in soil carbon sequestration.

In recent years, there has been considerable work on the investigation of mineralization of biochar following its application in soil; however, much less work has been done to investigate effect of different types of biochar application on mineralization rate of native SOC or amended labile carbon source, particularly municipal solid waste compost (Wardle *et al.* 2008; Sohi *et al.* 2010).

Keeping this in view, the current research work aimed to determine the priming effect of hardwood and pigeon pea biochar on short-term changes in the rate of mineralization of native soil organic carbon (SOC) and amended labile carbon source (municipal solid waste compost).

## EXPERIMENTAL

### Materials Used for Incubation Experiment

Biochar from pigeon pea (PPB) (*Cajanus cajan*) and *Prosopis* hardwood (WB) (*Prosopis juliflora*) was collected from Central Institute of Agricultural Engineering (CIAE), Bhopal, Madhya Pradesh. The pigeon pea stem and *Prosopis* hardwood were cut into pieces (10 to 20 cm), and after drying, the biomass was pyrolyzed at 300 °C for 2 h followed by quenching and subsequent drying in oven at 105 °C. The biochars were then crushed using a 24 blade Rotar Mill (Model. No. Pulversittee 14) and sieved to obtain a uniform 53 to 75 µm particle size. Bulk municipal solid waste compost (MSWC) collected from Adampur Municipal Dumpyard, Bhopal was sieved to obtain uniform particle size of 53 to 75 µm and the processed MSWC was used in the incubation experiment as a source of labile carbon substrate. The biochars and the MSWC were analyzed for total organic C (CHN- analyzer, Thermo Fisher Flash 2000 model) and oxidizable C content by dichromate oxidation method (Walkley and Black 1934) prior to incubation. The experimental soil for incubation study was collected from the research farm of Indian Institute of Soil Science, Bhopal, Madhya Pradesh. The soil sample was air-dried, sieved to 0.2 mm, and stored in the dark prior to chemical characterization and incubation. The soil was classified as Typic Haplustert (clayey texture) with pH 8.31 and organic carbon 0.47%. Physico-chemical properties of the experimental soil are presented in Table 1.

**Table 1.** Physico- Chemical Properties of Experimental Soil

| Parameters | Mean ± SD (n=3) | Method/Reference |
|---|---|---|
| pH (soil: water, 1:2) | 8.31 ± 0.47 | Jackson (1973) |
| EC (soil: water, 1:2) ($dSm^{-1}$) | 0.26 ± 0.03 | Jackson (1973) |
| Sand (%) | 35.8 ± 1.54 | International Pipette method (Piper 1966) |
| Silt (%) | 20.5 ± 1.01 | |
| Clay (%) | 43.7 ± 1.22 | |
| Textural class | Clayey Soil | |
| SOC (%) | 0.47 ± 0.05 | Walkley and Black (1934) |
| AN ($kgha^{-1}$) | 172 ± 11.2 | Subbiah and Asija (1956) |
| AP ($kg\ ha^{-1}$) | 10.1 ± 0.61 | Olsen *et al.* (1954) |
| AK ($kg\ ha^{-1}$) | 412 ± 12.7 | Black (1965) |
| AS($kgha^{-1}$) | 10.8 ± 0.88 | Chesnin and Yien (1950) |
| Total Heavy Metal (ppm) | | Lindsay and Norvell (1978) |
| Cu | 85.6 ± 2.22 *(1.59 ± 0.06) | |
| Cd | 00.10 ± 0.01 (0.05 ± 0.01) | |
| Pb | 32.6 ± 1.51 (0.49 ± 0.05) | |
| Cr | 27.7 ± 1.87 (0.07 ± 0.02) | |
| Ni | 75.1 ± 2.18(0.23 ± 0.02) | |
| Zn | 54.3 ± 1.33 (0.69 ± 0.04) | |

Note: EC: Electrical Conductivity; SOC: Soil Organic Carbon; AN: Available Nitrogen; AP: Available Phosphorus; AK: Available Potassium; AS: Available Sulphur; *DTPA extractable heavy metals content

### Scanning Electron Microscopy Techniques

Surface morphology of the biochar samples (PPB and WB) was assessed through scanning electron microscopy (SEM; model: JEOL JSM 5600). For observation of biochar samples, the same magnifications (1000X) were employed. Further, one spot was selected at a time and replicated observations were taken for each sample to study the morphological

features. Tungsten filament was used as the electron source, which was operated at 10 kV HV, and the focal length was 14.39 mm.

## Incubation Experiment and $CO_2$ Measurements

An incubation experiment for a period of 36 days was conducted to investigate the effect of biochars (PPB and WB) addition on short term carbon mineralization of native SOC and applied municipal solid waste compost (MSWC). The incubation experiment was completed in 2-kg capacity poly-ethylene pots with an airtight lid, containing 1kg of 0.2 mm sieved soil. The experiment was comprised of ten treatments with three replications: ($T_1$) Control (soil alone), ($T_2$) MSWC, ($T_3$) MSWC + WB (5%), ($T_4$) MSWC + WB (10%), ($T_5$) WB (5%), ($T_6$) WB (10%), ($T_7$) MSWC + PPB (5%), ($T_8$) MSWC + PPB (10%), ($T_9$) PPB (5%), and ($T_{10}$) PPB (10%). The amount of MSWC added to soil was 10 t $ha^{-1}$ (on dry weight basis). The amount of biochars (PPB and WB) added was 5% and 10% of total weight of MSWC, *i.e.*, 0.5 and 1.0 t $ha^{-1}$, respectively. After 3 days of MSWC application, biochar was added to the soil as per the treatment details. After addition of MSWC and biochar, the soil was mixed thoroughly and moistened to maintain 70% of water holding capacity (WHC). Immediately after watering, a vial containing 20 mL of 1 *N* NaOH solution was placed inside the pot and the container was sealed with the lid. Three blanks consisting of pots with only NaOH as above were also included and incubated at 25 °C for 36 days. The NaOH vials were changed after 3 days for determination of $CO_2$ absorbed in the alkali. For this purpose, an excess amount of 3 *N* barium chloride solutions was added to the NaOH solution to precipitate the carbonate as in soluble $BaCO_3$. After adding a few drops of phenolphthalein indicator, the unreacted NaOH was back titrated with 1 *N* HCl solution. In a similar way, evolution of $CO_2$ was measured every three days up to the 36$^{th}$ day.

## Quantification of Priming Effect and Microbial Biomass Carbon

Based on Hamer *et al.* (2004) the priming effect (PE) of pigeon pea and *Prosopis* hardwood biochars on C mineralization from soil and applied MSWC was quantified. In the current experiment, as an assumption, the values of $CO_2$-C release in the control treatment (non-amended) were subtracted from all other treatments to obtain $CO_2$-C release values for biochar, MSWC and biochar + MSWC treatments.

The priming effect (PE) values of applied organic substrates (MSWC, pigeon pea, and *Prosopis* hardwood biochars) on short term mineralization of native SOC ($PE_{SOC}$) and applied MSWC ($PE_{MSWC}$) were calculated using Eqs. 1 and 2:

$$PE_{SOC} = \frac{Min.C_T - Min.C_C}{Min.C_c} X100 \quad (1)$$

where $PE_{SOC}$ is priming effect on native SOC (%), $Min.C_T$ is mineralization of C in the treated soils (MSWC/PPB/WB), and $Min.C_C$ is mineralization of C in the control soils. The carbon mineralization is assessed as $CO_2$ evolution over a period,

$$PE_M = \frac{Min.C_{B+M} - Min.C_M}{Min.C_M} X100 \quad (2)$$

where $PE_M$ is priming effect on applied MSWC (%), $Min.C_{B+M}$ is mineralization of C in the biochar plus MSWC-treated soils, and $Min.C_M$ is mineralization of C in the MSWC treated soils.

At the end of 36 days of incubation period the soil was sampled and the microbial biomass carbon was measured by Fumigation – Extraction method (Jenkinson and Powlson 1976).

### Statistical Analysis

Data obtained as treatment means for cumulative $CO_2$-C evolution and MBC were analyzed using one way analysis of variance (ANOVA) by the statistical package SPSS 9.0. Differences in mean values were considered significant at $P < 0.05$.

## RESULTS AND DISCUSSION

### Properties of Materials Used and Carbon Mineralization

The total organic C content in WB, PPB, and MSWC was 71.3, 53.2, and 25.4%, while dichromate oxidizable C (OC) contents were 16.55, 14.34, and 15.09%, respectively (Table 2).

**Table 2.** Chemical Composition of Biochars of Pigeon Pea and Prosopis Wood and MSWC

| Parameters | Mean± SD (n=3) | | |
|---|---|---|---|
| | PPB | WB | MSWC |
| pH (soil: water, 1:2) | 8.65 ± 0.11 | 8.78 ± 0.09 | 7.08 ± 0.23 |
| EC (soil: water, 1:2) ($dSm^{-1}$) | 0.47 ± 0.16 | 0.52 ± 0.11 | 1.93 ± 0.27 |
| TOC (%) | 53.2 ± 1.24 | 71.3 ± 1.85 | 25.4 ± 1.33 |
| TN ($kgha^{-1}$) | 0.96 ± 0.21 | 0.85 ± 0.15 | 1.31 ± 0.12 |
| C/N ratio | 55.4 ± 1.63 | 83.9 ± 2.68 | 19.4 ± 0.96 |
| TP ($kg\ ha^{-1}$) | 0.07 ± 0.01 | 0.06 ± 0.03 | 0.17 ± 0.04 |
| TK ($kg\ ha^{-1}$) | 0.54 ± 0.08 | 0.62 ± 0.06 | 0.14 ± 0.04 |
| TS ($kg\ ha^{-1}$) | 0.12 ± 0.01 | 0.10 ± 0.04 | 0.08 ± 0.02 |
| Total Heavy Metal Content (ppm) | | | |
| Cu | 22.9 ± 1.67 | 29.4 ± 2.36 | 197 ± 5.46 |
| Cd | 0.23 ± 0.05 | 0.18 ± 0.02 | 2.68 ± 0.24 |
| Pb | 0.67 ± 0.09 | 0.41 ± 0.02 | 113 ± 10.57 |
| Cr | 10.1 ± 1.33 | 8.29 ± 1.06 | 50.7 ± 2.07 |
| Ni | 2.11 ± 0.07 | 2.38 ± 0.09 | 42.3 ± 1.54 |
| Zn | 31.6 ± 1.64 | 35.9 ± 1.08 | 254.9 ± 9.66 |

PPB- Pigeon Pea Biochar; WB- Wood Biochar; MSWC- Municipal Solid Waste Compost; EC: Electrical Conductivity; TOC: Total Organic Carbon; TN: Total Nitrogen; C/N: Carbon/Nitrogen ratio; TP: Total Phosphorus; TK: Total Potassium; TS: Total Sulphur

The results further suggest that 23.2, 27.0, and 59.4% of the TOC in WB, PPB, and MSWC was oxidizable by the wet dichromate oxidation process, respectively. Surface morphology and structural pores of the biochars (PPB and WB) were witnessed through SEM and are presented in Fig. 1. The SEM images of biochar at 1000X magnification depicted that both the biochars are porous in nature with rough and undulated surface morphology. However, WB is more compact in nature and displayed longitudinal fibrous structures with more micropores than that of PPB that contained more macropores. This may be due to the presence of more recalcitrant structural C in the WB. This could be due to the presence of more lignin and lignocellulosic compounds in the hard woods used as the feedstock of WB. The surface morphology properties of the biochars are greatly

influenced by the feedstock types and the pyrolysis temperature. The presence of more recalcitrant C could negatively affect the native soil C mineralization and C mineralization from its structures.

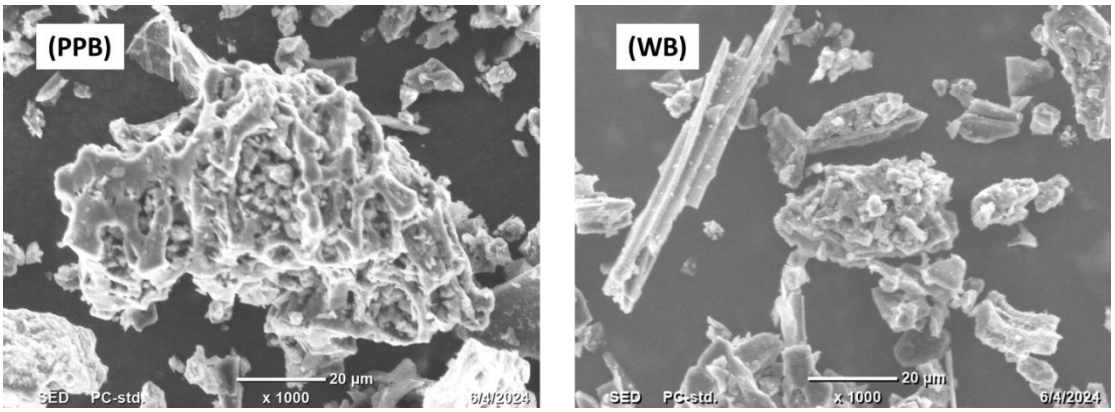


**Fig. 1.** The SEM of pigeon pea biochar (PPB) and wood biochar (WB) at 1000X magnification

The net loss of C due to microbial respiration under different treatments for 36 days is presented in Fig. 2. The results showed that the net loss of C from MSWC decreased significantly from 113 mg C $kg^{-1}$ soil to 61.9 and 44.8 mg C $kg^{-1}$ soil due to mixing of WB @ 5% and 10% of the weight of applied MSWC, respectively.

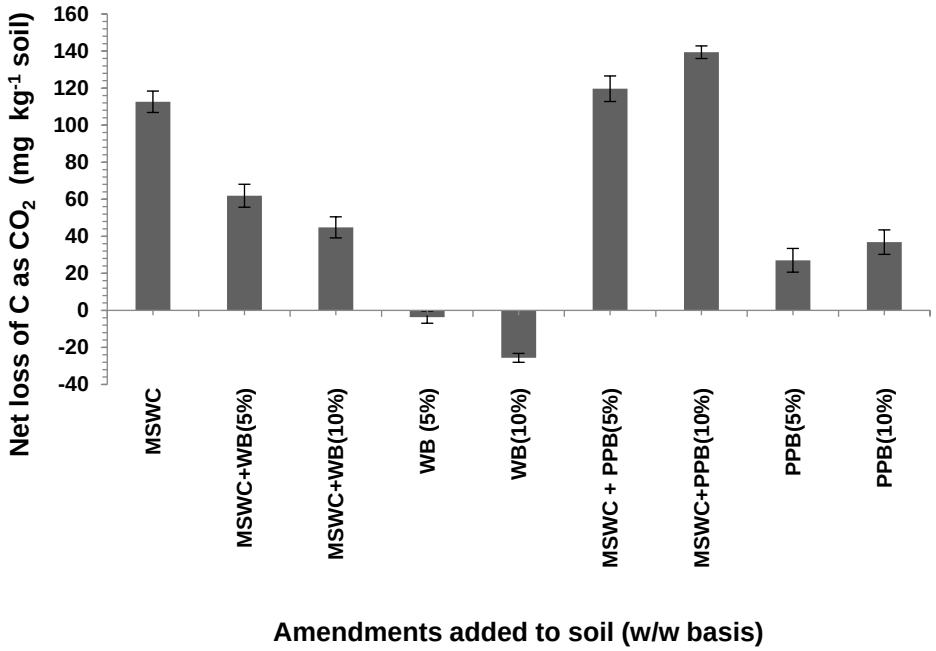


**Fig. 2.** Net loss of C from MSWC and in combination with WB and PPB after 36 days of incubation. Each bar depicts mean value of triplicates and the error bars depicted standard deviation

Throughout the incubation period, significantly lower amount of loss of C from MSWC was observed due to mixing of WB with MSWC (Fig. 3a). Even when the soil was treated with WB without MWSC, the loss of C from soil reduced marginally but was statistically significant at 10% WB level. In contrast, when MSWC was mixed with PPB

@ 5%, the loss of C from MSWC remained same but increased significantly due to mixing PPB @ 10% with MSWC (Fig. 3b). Even when the soil was treated with PPB @ 5% and 10% (without MSWC) the net loss of C of 27.0 and 36.8 mg C $kg^{-1}$ soil from PPB was observed, respectively over control treatment and such trend was evidenced throughout the incubation period. These results indicate that part of the C from PPB was used as substrate by the microbes for their metabolic activities. In these treatments, 32.1 mg C $kg^{-1}$ soil and 64.2 mg C $kg^{-1}$ soil were added to the soil as dichromate oxidizable C through PPB. If it is assumed that the dichromate oxidizable C of PPB was used by the soil microbes for their metabolic activities, then it could be said that 84.2% and 57.4% of the oxidizable C content of PPB was mineralized in 5% and 10% PPB applied treatments, respectively. In this experiment the trend of C loss in each treatment was depicted in Figs. 3 and 4. The rate of C loss was very high in initial 3 days period and then steadily declined throughout the incubation period. During the incubation period also, it was observed that in MSWC and PPB applied soils, the C loss was more than that of normal soil, whereas, in WB applied soil the C loss was less than that of normal soil.

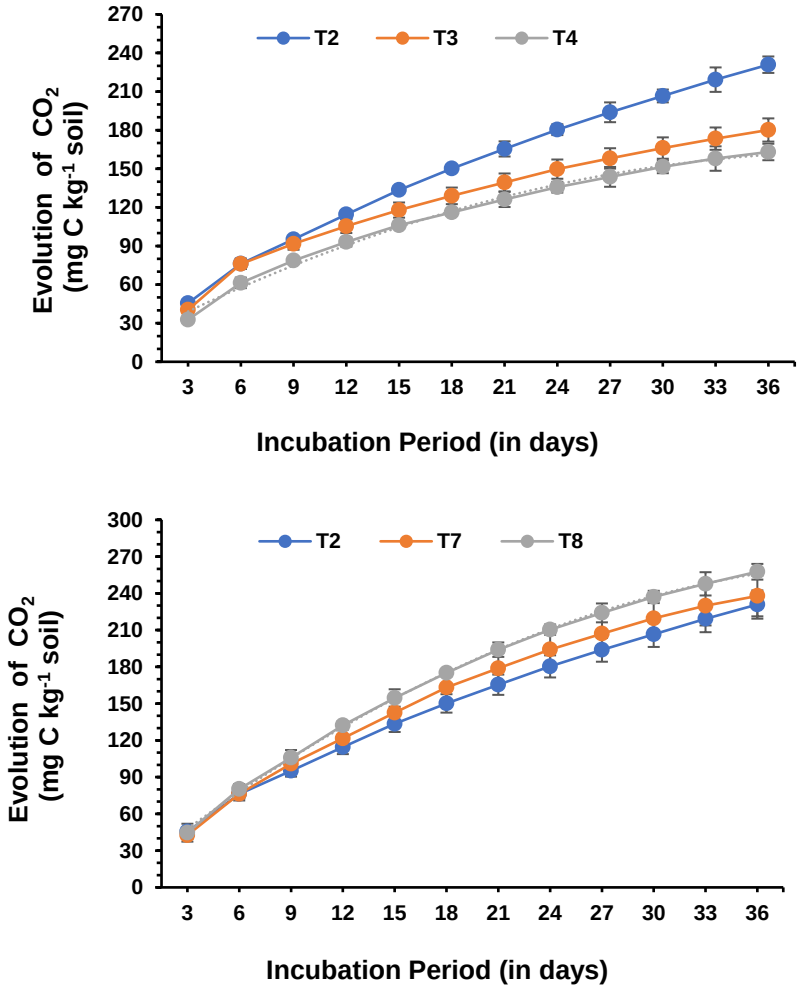


**Fig. 3.** (a) Cumulative evolution of $CO_2$ from MSWC ($T_2$) alone and in presence of 5% ($T_3$) and 10% ($T_4$) wood biochar (WB); (b) Cumulative evolution of $CO_2$ from MSWC ($T_2$) alone and in presence of 5% ($T_7$) and 10% ($T_8$) PPB. Each point depicts the mean value of triplicates and the error bars depicted standard deviation.

The cumulative loss of C as $CO_2$ from soil due to microbial respiration under different treatments is presented in Table 3. It was observed from the results that addition of organic substrate (MSWC and biochar) either alone or in combination significantly increased the cumulative loss of carbon over control except in the treatments where WB was added at 5% and 10%, respectively (Fig. 4a and 4b). In absolute control treatment where no organic input was added, the loss of C as $CO_2$ was 118 mg C $kg^{-1}$ in soil after 36 days of incubation.

The addition of labile carbon source (MSWC) enhanced the soil respiration by 2-fold (231 mg C $kg^{-1}$ soil) at the end of 36 days period of incubation. This was in agreement with McLeod *et al*. (2011) who reported that organic material addition enhanced the soil respiration by 2- to 3-fold at the end of six months after incubation. Similarly, the cumulative loss was increased compared to the control (118 mg C $kg^{-1}$ soil) to 180, 163, 238, and 258 mg C $kg^{-1}$ soil when MSWC was applied with WB (5% and 10%) and PPB (5% and 10%), respectively. However, it was reported that the incorporation of organic substrates into soils also retards SOC mineralization instead of accelerating the process (Nottingham *et al.* 2009; Wang *et al.* 2016).

Results from the current experiment also showed that among the biochars, PPB (5% and 10%) addition increased the cumulative loss of carbon as $CO_2$, whereas WB resulted in reduced cumulative loss compared to control. Therefore, the increased carbon loss over control following the application of MSWC and PPB either alone or in combination reflects the mineralization of the added organic matter due to increased availability of mineralizable carbon and favourable C/N ratio as compared to WB.

Zimmerman *et al.* (2011) also reported that pyrolysis temperature and the nature of raw material used for biochar production plays a major role in SOM mineralization following its addition in soil. For example, biochar prepared from grasses increased the carbon mineralization whereas biochar from wood decreased the C mineralization in soils (Zimmerman *et al.* 2011).

**Table 3.** Cumulative Loss of C through Soil Respiration and Microbial Biomass Carbon (MBC) in Soil at the End of Incubation Period (36 days)

| Treatment | Cumulative Loss of C (mg C $kg^{-1}$ soil) | MBC (mg C $kg^{-1}$ soil) |
|---|---|---|
| Control | 118 ± 1.48f | 232 ± 1.25g |
| MSWC | 231 ± 2.05b | 333 ± 2.77c |
| MSWC+WB @5% | 180 ± 1.65c | 289 ± 2.13d |
| MSWC+WB @10% | 163 ± 1.78d | 281 ± 2.69de |
| WB @5% | 115 ± 2.33f | 226 ± 1.72g |
| WB @10% | 92.6 ± 1.12g | 208 ± 2.08h |
| MSWC+PPB @5% | 238 ± 2.22b | 345 ± 3.61b |
| MSWC+PPB @10% | 258 ± 23.5a | 368 ± 4.65a |
| PPB @5% | 145 ± 1.19e | 260 ± 2.17f |
| PPB @10% | 155 ± 1.07de | 273 ± 3.55e |

Data represents the mean value ± standard deviation from three replicates(n=3). Mean values followed by a different lowercase letter within a column are significantly different ($p < 0.05$)

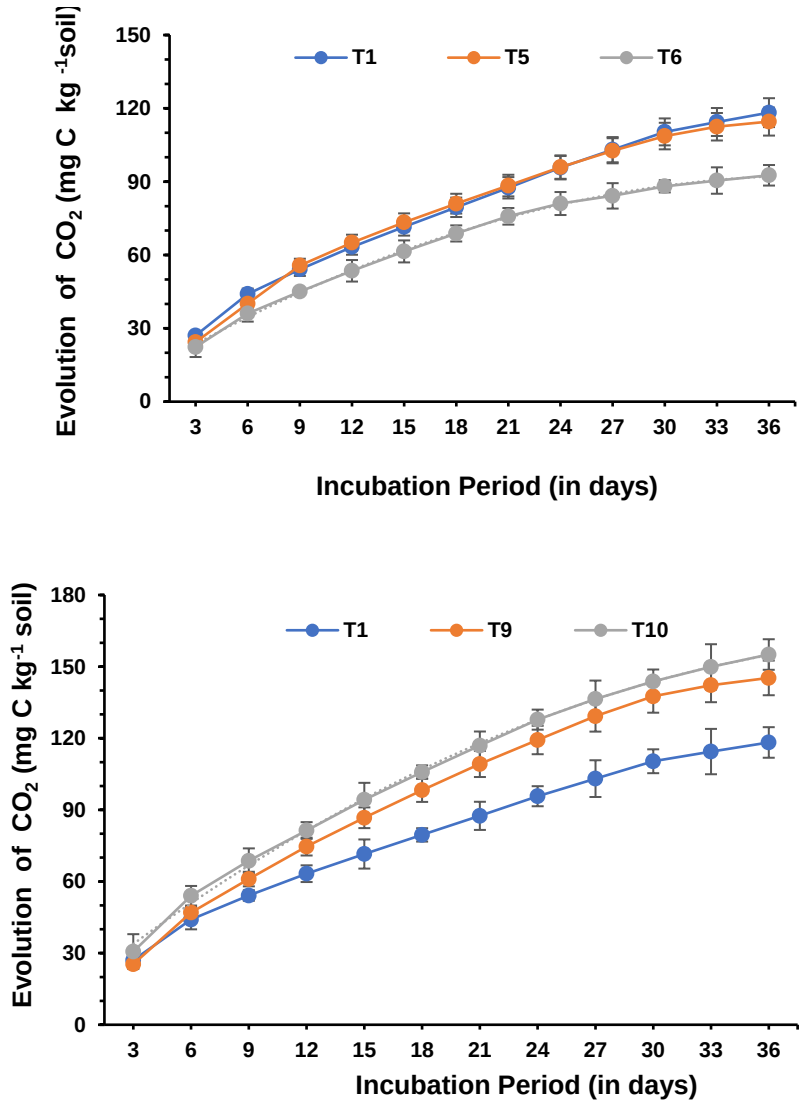


**Fig. 4.** (a) Cumulative evolution of $CO_2$ from soil ($T_1$) alone and in presence of 5% ($T_5$) and 10% ($T_6$) WB; (b) Cumulative evolution of $CO_2$ from soil ($T_1$) alone and in presence of 5% ($T_9$) and 10% ($T_{10}$) PP. Each point depicts the mean value of triplicates and the error bars depicted standard deviation

Because biochar provides higher surface areas for adsorption, its addition in soils might also retard or protect the other labile carbon sources from microbial decomposition or degradation (Cooney 1998). Andrews and Tien (1981) reported that the mineralizable portion of carbon in biochar acts as a carbon source for microorganisms, which facilitates rapid growth and in turn results in faster decomposition of other carbon sources. Results also showed that biochar addition significantly affected the mineralization of MSWC throughout the incubation period. When MSWC was applied to the soil, the cumulative loss of C was 231 mg C $kg^{-1}$ soil, but this loss of C was significantly reduced to 180 and 163 mg C $kg^{-1}$ soil when MSWC was mixed with WB @ 5% and 10%, respectively. However, when the applied MSWC was mixed with PPB @ 5% and 10%, the loss of C from soil was increased over MSWC treatment to 238 and 258 mg C $kg^{-1}$ soil, respectively; however, it was statistically significant only at 10% PPB level. From the results, WB decreased the loss of native soil carbon as well as externally applied carbon through

MSWC and of its own. Even though WB contains more carbon (total and oxidizable) as compared to PPB, the net carbon loss was more in PPB treatments than WB. It might be due to higher N (0.96%) and narrow C/N ratio in PPB (55.4) facilitates the fast and easy mineralization of applied and native organic matter.

## Priming Effect of Organic Substrate on C Mineralization

Addition of organic substrates to soil results in short term stimulation or suppression in the carbon mineralization, which are defined as positive or negative priming effects, respectively (Kuzyakov *et al.* 2000). At the end of 36 days period of incubation, both biochars and MSWC organic substrate caused significant priming effect on short term carbon mineralization of SOC (Fig. 5). However, the extent of priming effect depends on the nature of organic substrate added to the soil. Positive priming effects on SOC degradation were recorded from the current study in the presence of MSWC and PPB, whereas, negative priming effect in the WB treated soil. The MSWC treated soil with relatively more readily oxidizable organic substrate resulted in the highest positive priming effect to an extent of +95% of basal respiration. Kuzyakov *et al.* (1997) also observed that after the addition of shoot and root residues the soil increased basal respiration (positive priming effect) to +336%, indicating that native SOC mineralization can be greatly stimulated following the addition of easily available carbon substrate. Similarly, addition of PPB at 5% and 10% rate caused an increase in SOC mineralization +22.9% and +31.2%, respectively, and thus it resulted in positive priming effects. In contrast, reduction in SOC mineralization was noticed in WB-treated soil, resulting in negative priming effect of -3.10% and -21.7% of basal respiration at 5% and 10% rate, respectively. Among the biochar, the total and easily oxidizable C content was more in WB as compared to PPB; however, WB addition at both the levels (5 and 10%) still resulted in negative priming effect, which might be due to a higher C/N ratio in the WB (83.85) as compared to PPB (55.36). Hilscher *et al.* (2009) also noticed enhanced respiration with grass-derived biochar addition on a Swiss loam; whereas, no increase in respired $CO_2$ in pine wood-derived biochar treated soil. The results from this experiment clearly revealed that the direction and intensity of priming effects depends on the substrate type and substrate concentration.

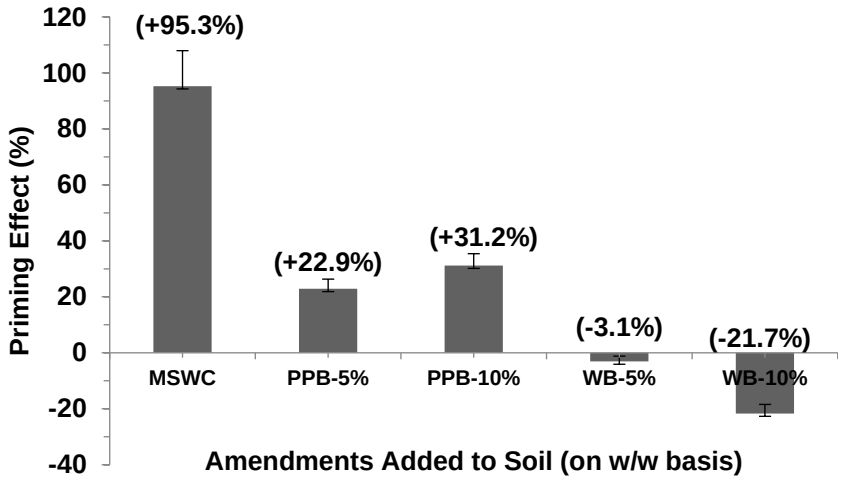


**Fig. 5.** Priming effect of MSWC, PPB, and WB on mineralization of SOC (The bars shown for the means are ± SE (n=3)

In recent years, several attempts were made to quantify the effect of organic substrate addition on short term mineralization rate of SOC (Fu *et al.* 2022; Yan *et al.* 2023) and biochar (Hamer *et al.* 2004; Kuzyakov *et al.* 2009); however, there has been much less work investigating the effect of biochar addition on degradation of more labile carbon substrate. Among the biochar, WB generally induced strong negative priming effects, while the addition of PPB caused only a weak positive priming effect on short term mineralization of more labile carbon substrate (MSWC). At 5% and 10% level of WB addition, MSWC mineralization was reduced by -21.9% and -29.5%, respectively. In contrast, PPB addition at 5 and 10% level increased the mineralization of MSWC +3.0% and +11.6%, respectively (Fig. 6). Few authors also reported that presence of biochar in soils either enhanced the degradation of more labile C substrates, such as ryegrass residue (Hilscher *et al.* 2009) and switchgrass residue (Novak *et al.* 2010), resulting in positive priming effects or even a negative priming influence of biochar on organic matter degradation (Hilscher *et al.* 2009). Possibly, the positive priming effect observed in the PPB treated soil might be due to increased microbial activity and the decomposition of labile carbon substrate (MSWC) rather than stabilizing them against degradation (Jenkinson 1971). In contrast, the negative priming effect on C mineralization following the addition of WB may be possibly due to decreased microbial activity and inhibition of enzymatic activity (Gianfreda *et al.* 1993; Fierer *et al.* 2001).

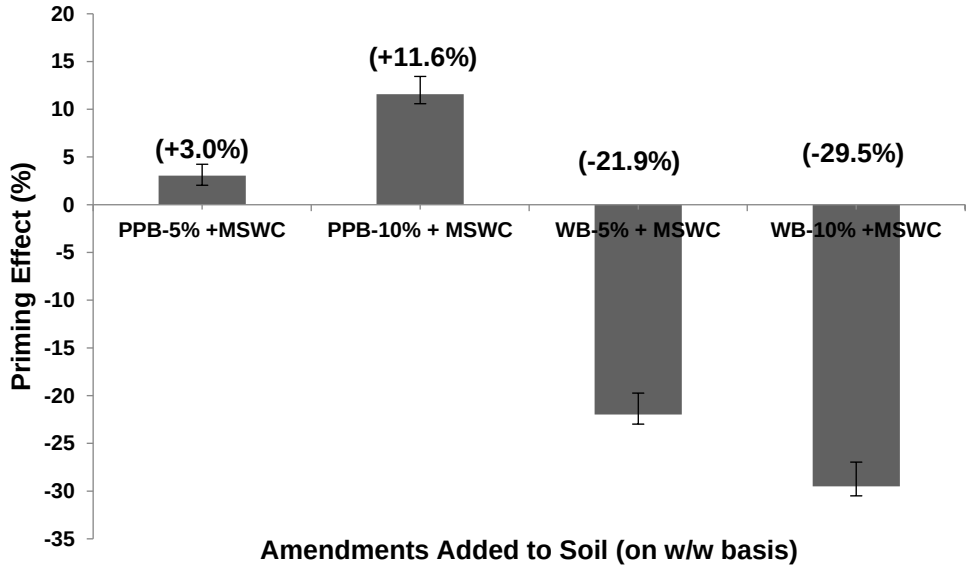


**Fig. 6.** Priming effect of PPB and WB on mineralization of MSWC (The bars shown for the means are ± SE (n=3)

## Microbial Biomass Carbon (MBC)

Soil MBC is a direct indicator of microbial activity, and it indirectly reflects the availability of organic material (Zeng *et al.* 2018; Zhu *et al.* 2020). The MBC in soil after 36 days of incubation was measured, and the results are presented in Table 3. The results showed that MBC in soil increased significantly from 232 to 333 mg C $kg^{-1}$ soil due to application of MSWC. However, when MSWC was mixed with WB @ 5% and 10%, the MBC in soil decreased significantly to 289 and 281 mg C $kg^{-1}$ soil, respectively. There was a significant decrease in MBC in soil due to addition of WB @ 10% while the MBC content

remained same in WB @ 5% added soil as compared to that observed in control treatment, indicating that WB has some refractory property to inhibit microbial activities in soil. In contrast to WB, the MBC in soil increased significantly when MSWC applied with PPB @ 5% and @ 10%, as compared to application MSWC alone. Even when PPB alone was applied to soil @ 5% and 10%, there was a significant increase of the MBC in soil as compared to control, suggesting that part of the C in PPB was used as substrate by the soil microbes.

## CONCLUSIONS

1. From this study, no noticeable carbon mineralization was observed in soil after 36 days of incubation from carbon-rich material of hardwood biochar (WB). Instead, there was marginal reduction of mineralization of native soil organic carbon due to incorporation of WB.
2. Combined application of municipal solid waste compost (MSWC) along with 5% and 10% WB also resulted in reduction of loss of C from applied MSWC of 21.9% and 29.5%, respectively. However, increased mineralization (positive priming effect) of native soil organic carbon (SOC) and labile carbon source (MSWC) was prominent in pigeon pea biochar (PPB) added soil.
3. The microbial biomass C (MBC) also showed that MSWC and PPB addition resulted in increased MBC over control, whereas, MBC was decreased in the WB treated soil.
4. The results clearly provide some evidence to indicate that hardwood biochar has some refractory property to inhibit the mineralization of both native soil organic C and applied organic matter (MSWC), and thereby can be used as an amendment to stabilize the native and applied organic materials in soil, which helps to sequester carbon for longer period in soil.
5. Similar studies on the long-term effects of applying soil amendments on carbon emissions and sequestration under various cropping systems in field conditions can be conducted for better understanding and enhance carbon storage in soil.

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