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ORIGINAL RESEARCH

Effect of invasive weed biochar amendment on soil enzymatic activity and respiration of coal mine spoil: a laboratory experiment study

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Abstract

Mining and excavation activities cause massive degradation of land, leading to complete loss of soil resources, vegetation, and biodiversity. Mine spoils support invasive weeds (predominantly *Lantana*) which can strive in these harsh conditions, causing allelopathy during plantation stage of reclamation. It is hypothesised that biochar produced from invasive weeds will enhance enzymatic activity, CO_2 flux and overall fertility of coal mine spoil. A 6-month incubation study was conducted on the effect of biochar amendment (2 and 3%, w/w) on mine spoil enzymatic activities (dehydrogenase, invertase, amylase and cellulase), respiration and coal mine spoil fertility. The study showed that biochar significantly improved dehydrogenase (83%) and cellulase activity (78%) at 3% amendment. Geometric mean of enzymatic activities increased from 1.87 in control to 4.51 at 2% and 3.25 at 3% biochar amendment. Mine spoil physio-chemical properties such as soil organic carbon (65%), cation exchange capacity (54%), bulk density (25%) and water holding capacity (19%), were improved significantly compared to the unamended mine spoil. Biochar amendment reduced mine spoil CO_2 flux at 2% (2.85 μmol CO_2 m^{-2} s^{-1}) and 3% (2.60 μmol CO_2 m^{-2} s^{-1}) compared to control (4.92 μmol CO_2 m^{-2} s^{-1}). The cost of biochar production and application (2%, w/w) in pit plantation during reclamation is estimated to be 844 USD t ha^{-1} (plantation density: 1600 trees ha^{-1}). On the basis of present study, biochar preparation from invasive weeds can be used for sustainable reclamation of coal mine spoil.

Keywords *Lantana* · Geometric mean of enzymatic activity · Economics · Reclamation · Pit plantation · Coal mining

1 Introduction

Coal is an important contributor to worldwide energy generation but its environmental impact is quite catastrophic (Ahirwal and Maiti 2017). Coal ranks highest amongst the fossil fuels reserves in India and is responsible for 55% of energy requirement of the nation (Maiti 2013). 729 million tonnes (MT) of coal was produced in 2019–2020 in India, out of which > 90% of coal extraction was by surface mining. This causes loss of soil and vegetation cover and biodiversity, air and water pollution, complete disturbance of drainage and permanently alters natural landforms (Ahirwal et al. 2017). Mine spoil is nutrient deficient and characterised by low cation exchange capacity, nutrient unavailability, impoverished organic content and poor physical–chemical and biological characteristics (Frouz et al. 2008; Ussiri and Lal 2008). 2021–2030 is declared as the decade for ecosystem restoration by the UN General Assembly. Its main objective is the restoration of degraded land which provides essential ecosystem services. Therefore, the restoration of mine affected land is essential to fulfil the goals of global land-use policies. Biochar, a carbonaceous product of pyrolytic reaction of biomass has been reported to reclaim degraded land (Ghosh et al. 2020; Ghosh and Maiti 2020a, b). It is suitable for improving plant growth and microbial biodiversity (Pandey et al. 2020).

Enzymatic assays help quantify the microbial activity in soils which are responsible for important operations such as mineralization and humification (Palansooriya et al. 2019). This in turn influences the biogeochemical cycles of elements such as carbon, nitrogen, phosphorous and sulphur (Futa et al. 2020; García-Ruiz et al. 2008; Novak et al. 2018). Activities of these enzymes affect soil physio-chemical

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characteristics and support the growth of vegetative cover (Gascó et al. 2016; Ghosh and Maiti 2020a, b; Paz-Ferreiro et al. 2012). Thus, enzyme activity is a sensitive indicator for the evaluation of effect of an amendment in mine spoil reclamation (Ahirwal and Maiti 2018b; Palansooriya et al. 2019). Biochar application has been reported to increase activities of various extracellular and intracellular enzymes related to C, N, P and S cycles (Masto et al. 2013; Paz-Ferreiro et al. 2012; Gascó et al. 2016) and also improve enzymes such as dehydrogenase and catalase which are involved in the life process of microbes (Khadem and Raiesi 2017; Gascó et al. 2016). Other enzymatic activities such as urease, alkaline phosphatase, β-glucosidase, arylsulphatase, oxidase, fluorescein diacetate hydrolase has also been reported to improve by biochar application (Munir et al. 2021; Masto et al. 2013). The effect of biochar on enzymatic activity of coal mine spoil depends on the soil characteristics, microbial activities, types of enzymes and their interaction with biochar (Mohan et al. 2018). Sorption of enzyme molecules to the functional groups present on biochar surface influences the enzymatic reaction (Raiesi et al. 2019; Sandhu et al. 2019).

Surface mining has been reported to significantly decrease the terrestrial carbon pool (Ahirwal and Maiti 2018a), therefore, development of remediation technology which effectively sequesters carbon in the reclaimed coal mine spoil is necessary to combat global warming (Ahirwal et al. 2020). Biochar as an amendment during mine spoil reclamation not only enriches C pool in the derelict soil but also supports plant growth (Masto et al. 2013; Mohan et al. 2018). In an unfertilised soil, an increased CO_2 flux after biochar application has been observed due to the soil microbial activity. Also, the substrate provides matrix for microbial decomposition resulting in the increase in the CO_2 released. However, in reclaimed coal mine spoil, the labile fraction of carbon is much less compared to the recalcitrant carbon (Ghosh et al. 2020). Thus, CO_2 flux in a barren soil has been reported to increase up to a certain period and then remain stable post biochar application. Carbon in biochars, originally removed from the atmosphere as CO_2 during plant growth, remains in soils for hundreds of years (Camps-Arbestain et al. 2015; Lehmann et al. 2015). However, both positive and negative mineralization and CO_2 emission have been reported. Biochar stability and its interaction with soil biota depend upon the biochar feedstock, the production method, the pyrolysis residence times and temperature (Ameloot et al. 2013). Biochar can act as an amendment to address problems related to remediation of degraded land (Ducey et al. 2021). However, a few studies have also reported the negative impact of biochar application on plant growth at high application rate (60 t ha^{-1}) possibly due to increased soil alkalinity resulting in decreased nutrient availability and potentially Na toxicity (Gonzaga et al. 2018). However, the coal mine spoil is so impoverished that there will be probably no negative impact of biochar application.

Invasive weeds like *Lantana camera* naturally colonise coal mine overburden dumps and often cause allelopathy during plantation stage of reclamation. *Lantana* has very high biomass content and lignocellulosic composition (Ghosh et al. 2020). Invasive weeds like *Lantana* can be easily uprooted, converted to biochar in the site itself and used in the pit plantation technique during restoration of coal mine spoils (Kaur et al. 2018; Radhaboy et al. 2019). Thus, we propose that abundantly growing weeds in abandoned overburden dumps can be harnessed during the dry tropic summer months and converted to biochar. The biochar produced can be very easily applied in the pit plantation method. Biochar can be mixed with mine spoil and commercially important trees can be planted in such pits.

Influence of biochar in coal mine spoil enzymatic activities has not been reported widely, and experiments are essential for designing practical applicability in field scale for mine spoil quality and fertility enhancement. Thus, the influence of biochar application on enzyme activity in coal mine spoil and its correlation with the soil organic matter and mine spoil CO_2 flux need attention. The study tested the hypothesis that biochar produced from invasive weeds will improve enzymatic activities, CO_2 flux and fertility of coal mine spoil. The objectives of this study were to assess the impact of *Lantana* biochar (1) on the mine spoil physiochemical properties after 6 months incubation in pilot scale application; (2) mine spoil enzymatic activity involved in C cycling and respiration, and (3) the economics involved for its application in a field scale.

2 Materials and methods

2.1 Biochar production and characterisation

Lantana camara growing in the coal mine overburden dumps was used as feedstock for biochar preparation. Feedstock was pyrolysed in a pre-heated muffle furnace at 450 ℃ for 60 min residence time. Biochar yield was calculated by the formula: yield (%) = weight of biochar (g)/weight of feedstock (g) × 100. pH and electrical conductivity of the filtrate (1:5; solid: deionised water; w/v) were measured by using multi-parameter probe (HI-2020, Hanna Instruments, India) (Singh et al. 2010). Organic carbon was determined by the potassium dichromate oxidation method (Walkley and Black 1934). The elemental analysis (C, H and N) was done using a CHNS-O Elemental Analyser-Euro vector EuroEA 3000, Italy. Bulk density and porosity of the biochar samples were estimated by using a column experiment (Ghosh et al. 2020). The surface morphology of the biochar was determined by field-emission scanning electron microscopy

(FE-SEM Supra55, Carl Zeiss, Germany) and an FTIR (Fourier-transform infrared spectrophotometer) spectrum was used to analyse surface functional groups present in the biochar samples.

2.2 Collection of coal mine spoil

Coal mine spoil sampling was done from Tetulmari opencast project (OCP) (23° 48′ 210″ N and 86° 20′ 27″) Dhanbad (Fig. 1). Spoil samples were sieved in the field itself (< 2 mm) to get rid of the large boulders and rocks present in the mining site. Bulk spoil samples were brought back to the institute campus to conduct a pilot scale study on the pit application of biochar.

2.3 Coal mine spoil characterisation

pH and EC of the mine spoil were determined with a multiparameter probe (HI-2020, Hanna Instruments, India) in a spoil and water slurry (spoil: water, 1:2.5, w/v). Organic carbon (OC) was calculated by chromic acid wet oxidation method (Walkley and Black 1934). Cation exchange capacity (CEC) was determined by the ammonium acetate method (Jackson 1973). A Kjeldahl distillation unit (KJELODIST-EAS VA, Pelican equipment's Inc. India) was used for available nitrogen (Av-N) content in the mine spoil. Total C, H and N were measured by CHNS-O Elemental Analyser (Eurovector EuroEA 3000, Italy) by flash combustion technique (980 °C). Water holding capacity (WHC) was determined by placing the spoil samples in a Keen box (5.6 × 1.6 cm) in a water bath for 24 h (Maiti 2013). Bulk density (BD) was measured by soil core method (Maiti, 2013). Available potassium and sodium were determined by ammonium acetate method. Available phosphorus was extracted by alkaline sodium bicarbonate (pH 8.5) and measured by a UV–VIS Spectrophotometer, Shimadzu Corporation, Japan (Ahirwal et al. 2017).

2.4 Experimental design of biochar amendment study

The experimental design was a completely randomised block design with mine spoil as control and biochar amendment at 2% and 3% (w/w) with five replicates for each dose (Fig. 2a). The mine spoil samples collected from the field were air dried at room temperature in laboratory condition (25–25 °C), and divided into three equal parts. One part was mixed with biochar at 2% (w/w) and another with 3% (w/w). The control and amended mine spoil samples were placed in PVC lined pits each of 45 cm × 45 cm × 30 cm dimension (Fig. 2b). A total of 15 (3 amendments × 5 replicates) such pits were prepared in the ESE department of Indian Institute of Technology (Indian School of Mines) Dhanbad campus. A PVC collar (11 cm in height and 21 cm in diameter) was placed up to a depth of 5 cm at each plot having overburden dump as shown in Fig. 2b, c. Initially, distilled water was added to obtain a field capacity of 50%. The coal mine spoil was left naturally to incubate for 6 months in mid-June. The spoil enzymatic activity was measured on the beginning in June, mid-way in August (3 months) and the end of

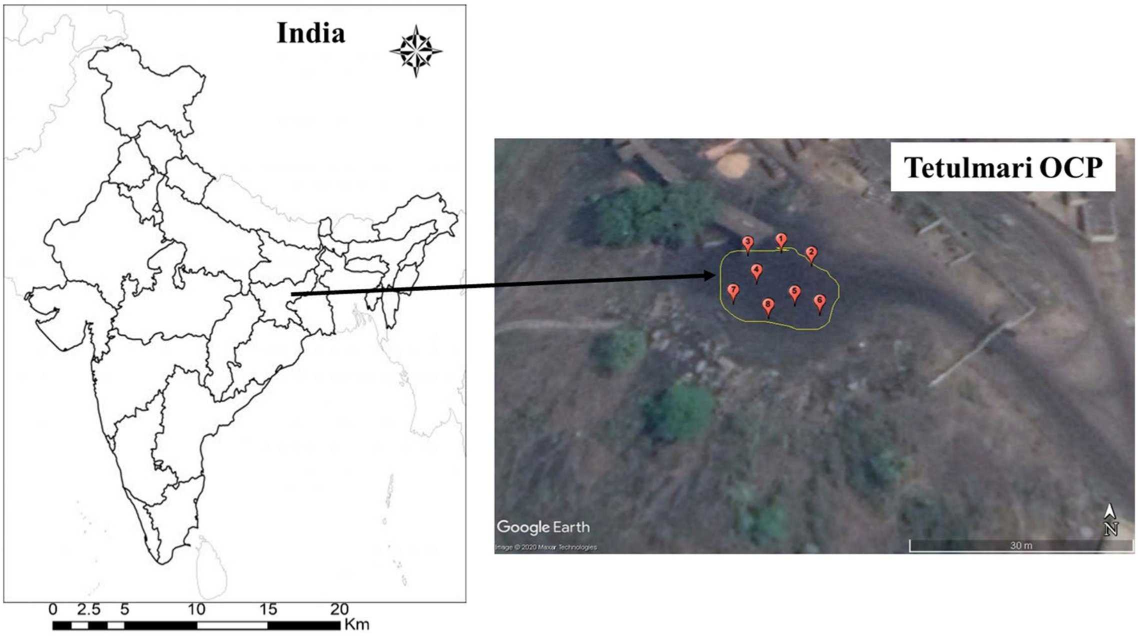


Fig. 1 Location of coal mine spoil collection site in Tetulmari OCP, Jharkhand, India

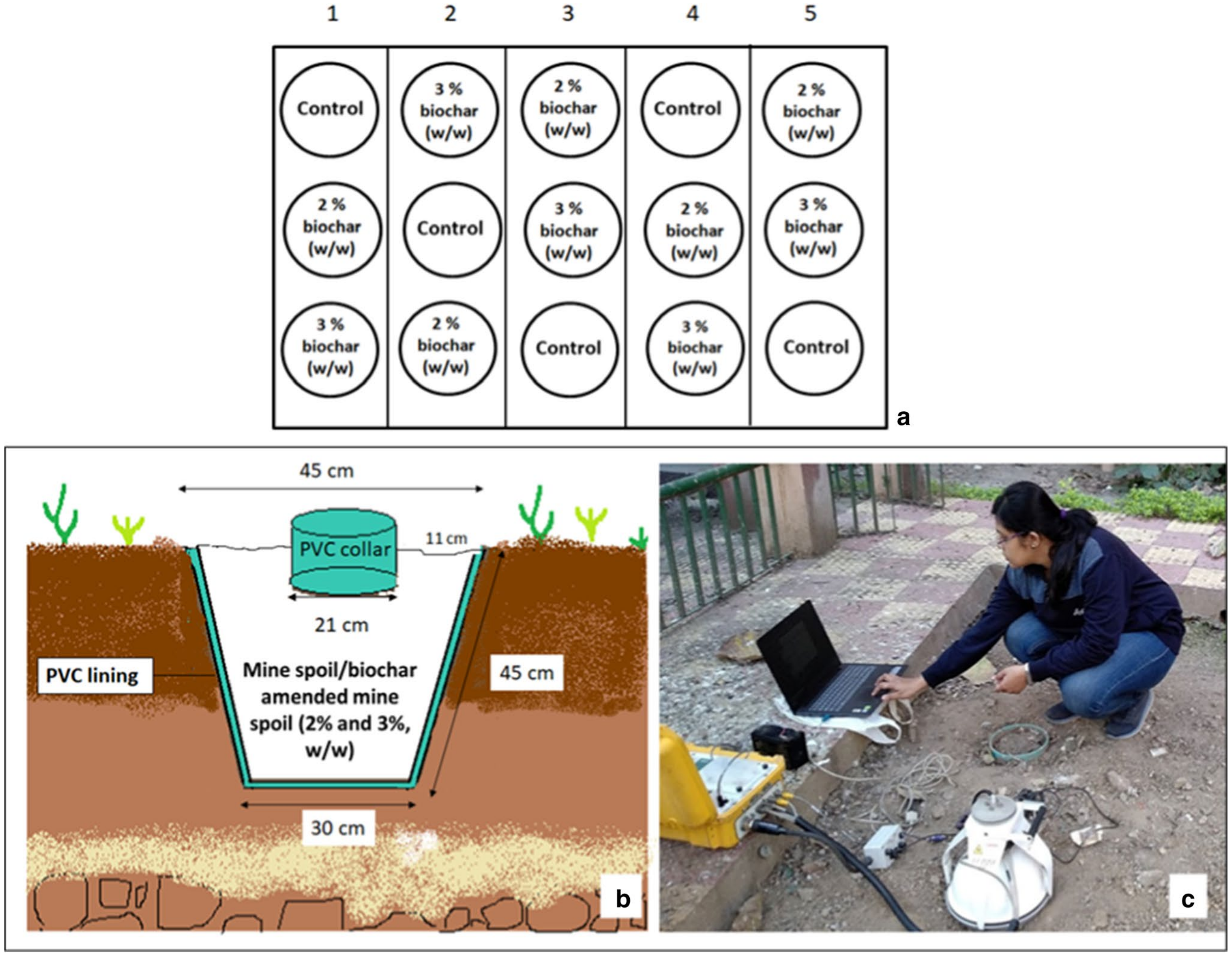


Fig. 2 **a** Randomised block design for biochar amendment study. **b** Diagrammatic representation showing the pilot scale set up for the biochar amendment study, **c** soil CO_2 flux measurement (LICOR LI -8100, LICOR Inc)

incubation in November (6 months). After the incubation period, mine spoil respiration was measured for 4 months (November–February).

2.5 Enzyme activities

The mine spoil samples were analysed for four enzymatic activities and the changes that occurred after 3 and 6 months of incubation were measured. The soil enzymatic activities such as dehydrogenase (DHA), invertase, amylase and cellulase were measured. Glasswares were previously treated by 2 M nitric acid for 24 h then further cleaned with distilled water. All chemical and reagents used were of analytical grade. DHA was determined by reducing triphenyl tetrazolium chloride (TCC) to triphenyl formazane (TPF) (Casida Jr 1977). The results were expressed as μg TPF g^{-1} soil 24 h^{-1}. For invertase (EC 3.2.1.26), amylase (EC 3.2.1.1) and cellulase (EC 3.2.1.4) activities, soil samples were incubated in sucrose, starch and carboxy methyl cellulose substrate, respectively, with Sorensen's buffer (pH 5.5, 0.06 M) for 24 h at 30 °C. The produced sugar was measured colometrically by 3, 5–dinitrosalicylic acid (Arey, 2010). The activities of invertase, amylase and cellulase were finally represented by μg glucose per g soil after 24 h.

2.6 Geometric mean of enzyme activities

The geometric mean of enzyme activities (GM_{ea}) can be used as an index to quantify the all enzymatic activity values in a single numerical value (García-Ruiz et al. 2008; Paz-Ferreiro et al. 2012). Literature review suggested that the reported GM_{ea} are of agricultural or garden soil, its value in coal mine degraded soil has not been reported yet (Zhang et al. 2015). Thus, GM_{ea} of the assayed enzymes activities of the biochar amended soil was calculated by Eq. 1:

$$\mathrm{GM}_{\mathrm{ea}} = \sqrt[4]{\mathrm{DHA} \times I \times A \times C}, \tag{1}$$

where, DHA, *I*, *A* and *C* are dehydrogenase, invertase, amylase and cellulase activities, respectively.

2.7 Measurement of CO_2 flux

CO_2 flux was monitored in control plot (only coal mine spoil) and biochar amended mine spoil (2–3%) which was incubated for a period of 6 months. Spoil CO_2 flux was monitored by LICOR LI-8100 (LICOR Inc. Lincoln, USA). A PVC collar (11 cm in height and 21 cm in diameter) was inserted up to a depth of 5 cm at each plot (Fig. 2b). The observations for CO_2 flux were taken for a period of four months (120 days) from November 2018 to February 2019. 12 h data from 8:00 to 20:00 (+5:30 GMT) was measured at 20 min interval, each lasting for 90 s. LI-8100 PC Client v3.0.1 windows interface software was used for the operation of the CO_2 flux system using Lenovo laptop.

Spoil temperature was recorded by inserting a temperature probe into the spoil (type E thermocouple, p/n 8100–201; 6.4 mm in diameter, 25 cm in length). Spoil moisture was recorded by placing a soil moisture probe up to a depth of 5 cm (ECHO Model EC-5, p/n 8100–202; 5 cm in length). Relative humidity was generated by the instrument itself. The moisture probe generated the output in mV (500–1300 mV) from which volumetric water content (VWC) was measured by Eq. 2:

$$\mathrm{VWC}\left(\mathrm{m^3 m^{-3}}\right) = -3.14 \times 10^{7} \times \mathrm{mV}^2 + 1.16 \times 10^{-3} \times \mathrm{mV} - 0.612. \tag{2}$$

Millivolts (mV) vs. moisture content (%, w/w) was in the range of 640 mV to 902 mV and corresponding moisture content ranged from 1.2% to 16.6%. The regression equation used is as follow (Eq. 3):

$$\mathrm{Moisture}\ \% = 0.032 \times \mathrm{mV} - 19.407. \tag{3}$$

2.8 Statistics

One-way ANOVA (analysis of variance) was used to compare the means of biochar and mine spoil characteristics. Differences between individual means were evaluated using Duncan's multiple range test at $p<0.05$. Pearson's correlation analysis with a significance level of $p<0.01$ and $p<0.05$ was performed to identify the correlation between variables. For all statistical analysis MS-Excel and IBM-SPSS 2019 software were used.

3 Results and discussions

3.1 Biochar properties

The general properties of biochar are given in Table 1. A 54.9% yield of biochar was obtained at this pyrolysis condition from *Lantana* feedstock. Biochar obtained had an alkaline pH (9.34) and EC of 4.76 mS cm^{-1}. The total elemental carbon, nitrogen, hydrogen and oxygen were 51.64%, 6.08%, 5.88% and 7.64%, respectively. The low H/C ratio (0.11) of *Lantana* biochar represents its high degree of aromatisation while the low O/C ratio (0.14) represents the polarity of the biochar. The C/N ratio of 9.18 indicates that the pyrolysis improves organic matter which enhances the labile carbon, thus making it available for microbial activity in the spoil. The ratios are indicative of the prolonged stability of carbon in the soil.

The FE-SEM of the biochar revealed a highly porous morphology (Fig. 3). This provided a large surface area and substrate for microbial activity and nutrient accumulation. The FTIR spectra showed five strong peaks representing various functional groups. The O–H bond at 3391 cm^{-1} was prominent due to the breaking of hydrogen bonded hydroxyl groups at higher temperatures (Fig. 4). Other bonds such as $–CH_3$ (2924 cm^{-1}), $–CH_2$ (2870 cm^{-1}), C=O (1600–1700 cm^{-1}) due to cellulose of the feedstock were also present.

3.2 Changes in mine spoil physio–chemical properties

The change in the physio–chemical properties due to *Lantana* biochar amendment in a coal mine spoil has been shown in Table 2. Electrical conductivity showed a steep rise from 90.06 mS cm^{-1} in spoil to 721.66 mS cm^{-1} at 3%, w/w application rate ($p<0.05$). The increase in cation

Table 1 Physio-chemical properties of biochar ($n=5$)

Characteristics	Values
pH (1:5, w/v)	9.34 ± 1.22
EC (1:5, w/v; mS cm^{-1})	4.76 ± 2.11
OC (%)	61.64 ± 1.17
C (%)	55.819 ± 1.2
H (%)	5.883 ± 0.6
N (%)	6.08 ± 1.2
H/C	0.11 ± 0.01
C/N	9.18 ± 0.03
BD (g cm^{-3})	0.25 ± 0.03
Yield (%)	54.9 ± 0.76
Porosity (%)	88 ± 0.89

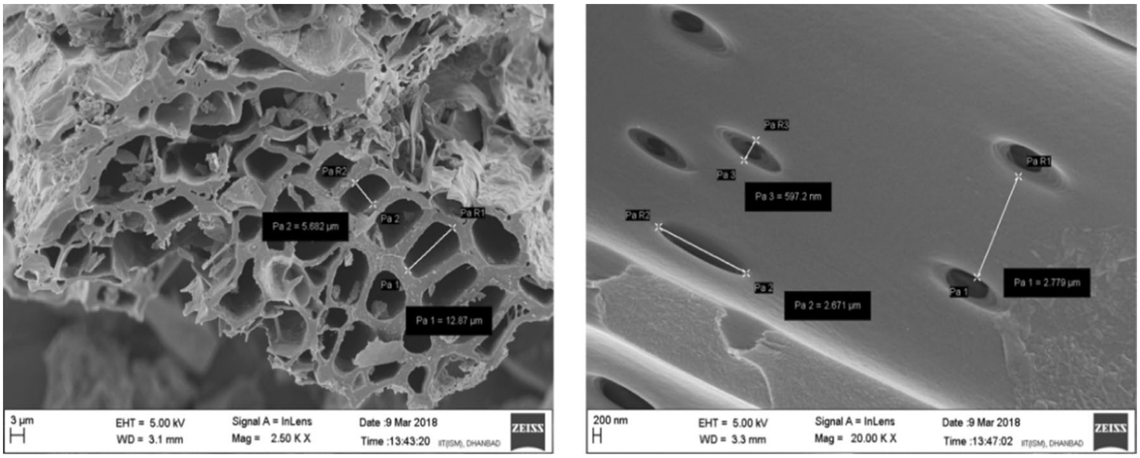


Fig. 3 The FE-SEM image of *Lantana* biochar showing the pores at 2.5 k × and 120 k ×

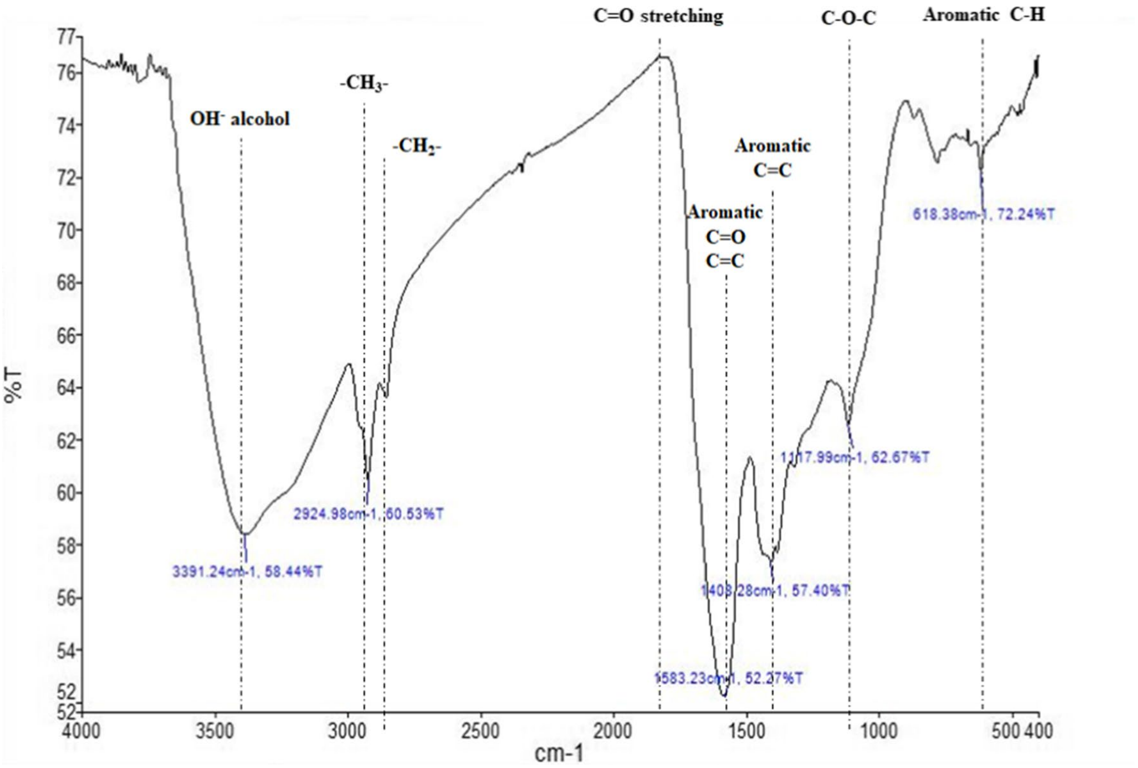


Fig. 4 FTIR spectra of *Lantana* biochar showing the surface functional groups

exchange capacity from control to 3% (w/w) amendment (7.24 vs. 15.85 Cmol kg^{-1}) might be responsible for the steep rise in electrical conductivity. Mohan et al. (2018) reported the similar results by rice husk and corn cover biochar application. The organic carbon increased with increasing biochar application rate in the mine spoil, and significant increase ($p < 0.05$) of 192% was observed at 3% (w/w) biochar amendment compared to the control (Table 2). Gonzaga et al. (2018) reported similar results by the application of coconut husks and pine wood chips biochar. Total carbon increased from 3% in mine spoil to 21% at 3% (w/w) biochar application rate. Total N and H did not show any significant changes ($p < 0.05$) by biochar application. Water holding capacity (27%) and bulk density (decreased by 13%) were also effected significantly by its application at 3% (w/w). Fellet et al. (2011) reported an increase in water-holding

Table 2 Physio-chemical properties of coal mine spoil and the amended mine spoil (2% and 3%, w/w) ($n = 5$, and values are given in mean ± standard deviation)

	Mine spoil (control)	Amended coal mine spoil (2%, w/w)	Amended coal mine spoil (3%, w/w)
pH (1:2.5, w/w)	6.49 ± 0.45	6.69 ± 0.57	7.41 ± 0.34
EC (mS cm^{-1}); (1:2.5, w/w)	90.06 ± 16.29	651.67 ± 25.65	721.66 ± 71.8
CEC (C mol kg^{-1})	7.24 ± 0.50	12.08 ± 0.79	15.85 ± 2.05
SOC (%)	0.74 ± 0.4	2.09 ± 0.075	2.16 ± 0.073
Available-N (mg kg^{-1})	17.0 ± 1.00	22.46 ± 0.5	44.53 ± 0.25
C (%)	3.00 ± 0.5	20.006 ± 1.2	21.072 ± 1.4
H (%)	0.359 ± 0.1	1.649 ± 0.3	0.649 ± 0.5
N (%)	0.693 ± 0.2	0.21 ± 0.2	0.424 ± 0.11
H/C	0.0847 ± 0.22	0.0819 ± 0.01	0.0307 ± 0.01
C/N	4.329 ± 0.53	24.69 ± 2.015	29.10 ± 1.7
WHC_{max} (%)	33.16 ± 0.03	37.92 ± 0.16	41.32 ± 0.19
BD (g cm^{-3})	1.2 ± 0.005	0.957 ± 0.05	0.95 ± 0.01
K (ppm)	91 ± 1	500 ± 55.67	470 ± 20
P (ppm)	0.163 ± 0.12	0.416 ± 0.02	0.4 ± 0.025

capacity by 5% at 10% biochar application in a mine spoil. In another mine spoil, Kelly et al. (2014) reported a decrease in bulk density decreased by 16.4% and 19.7% for soil sampled from different sites.

3.3 Effect of biochar amendment on enzymatic activities of coal mine spoil

3.3.1 Dehydrogenase activity

Effect of biochar application on the spoil enzymatic activities at the end of 6-month incubation study has been shown in Fig. 5a. A significant increase ($p < 0.05$) of 83% was observed in dehydrogenase (DHA) value at 3% (w/w) compared to control. DHA values at 2 and 3% (w/w) application rates were significantly different during the middle of the study. DHA is considered to be the index of microbial metabolic activity and most importantly the indicator of respiration of the soil) (Maiti 2013; Gascó et al. 2016). Mukhopadhyay et al. (2020) reported that yard waste biochar addition at 10% (w/w) increased the coal mine spoil DHA activity from 34.02 μg TPF g^{-1} soil 24 h^{-1} in control to 59.71 μg TPF g^{-1} soil 24 h^{-1}. Wang et al. (2015a, b) reported similar findings in DHA activity by addition of biochar produced from maize at 300–600 °C. Liu et al. (2018) also reported a positive effect of coconut shell @5% (w/w) on the DHA activity in a soil incubation experiment.

3.3.2 Invertase activity

Invertase activity was also found to increase significantly ($p < 0.05$) up to 2% (w/w) biochar application while a decreased was observed at 3% biochar amendment (Fig. 5b). At 2% (w/w) invertase activity increased from 3 μg glucose g^{-1} soil 24 h^{-1} in the control mine spoil in the beginning of the experiment to 14 μg glucose g^{-1} soil 24 h^{-1} after 3 and 6 months of the experiment. However, at 3% (w/w) application rate, there were no significant changes in the invertase activity. Invertase enzyme in microorganisms catalyses the hydrolysis of sucrose to glucose and fructose. Thus, the changes in the invertase activity in the spoil act as an indicator of microbial activity. Zhang et al. (2015) reported an increase in invertase activity by using rice straw and peanut hull biochars in an acidic spoil. Similarly, Khadem and Raiesi, (2017) found a 1.3- to 5.8-fold increase in invertase activity by corn stalk biochar in calcareous soil. A decrease in enzymatic activity beyond 2% (w/w) biochar amendment can be due to its ability to adsorb organic and inorganic molecules. Nie et al. (2018) reported that sugarcane and bagasse biochar at different doses (1.5, 2.25 and 3.0 t ha^{-1}), increased the invertase activity 1.2, 1.5 and 1.7 times, respectively, compared to the control. At higher level of application, biochar particles might inhibit soil enzymes or their corresponding substrates by blocking their reaction sites (Bailey et al. 2011).

3.3.3 Amylase activity

Amylase activity increased significantly ($p < 0.05$) up to 2% (w/w) application rate; beyond this threshold a steep fall was observed at 3% biochar amendment (Fig. 5c). At 2% (w/w), the amylase activity increased form 11 μg glucose g^{-1} soil 24 h^{-1} in control to 38 μg glucose g^{-1} soil 24 h^{-1} after 3 months to 44 μg glucose g^{-1} soil 24 h^{-1} at the end of the incubation study. However, at 3% (w/w), a decrease in amylase activity was observed compared to the control. Awasthi

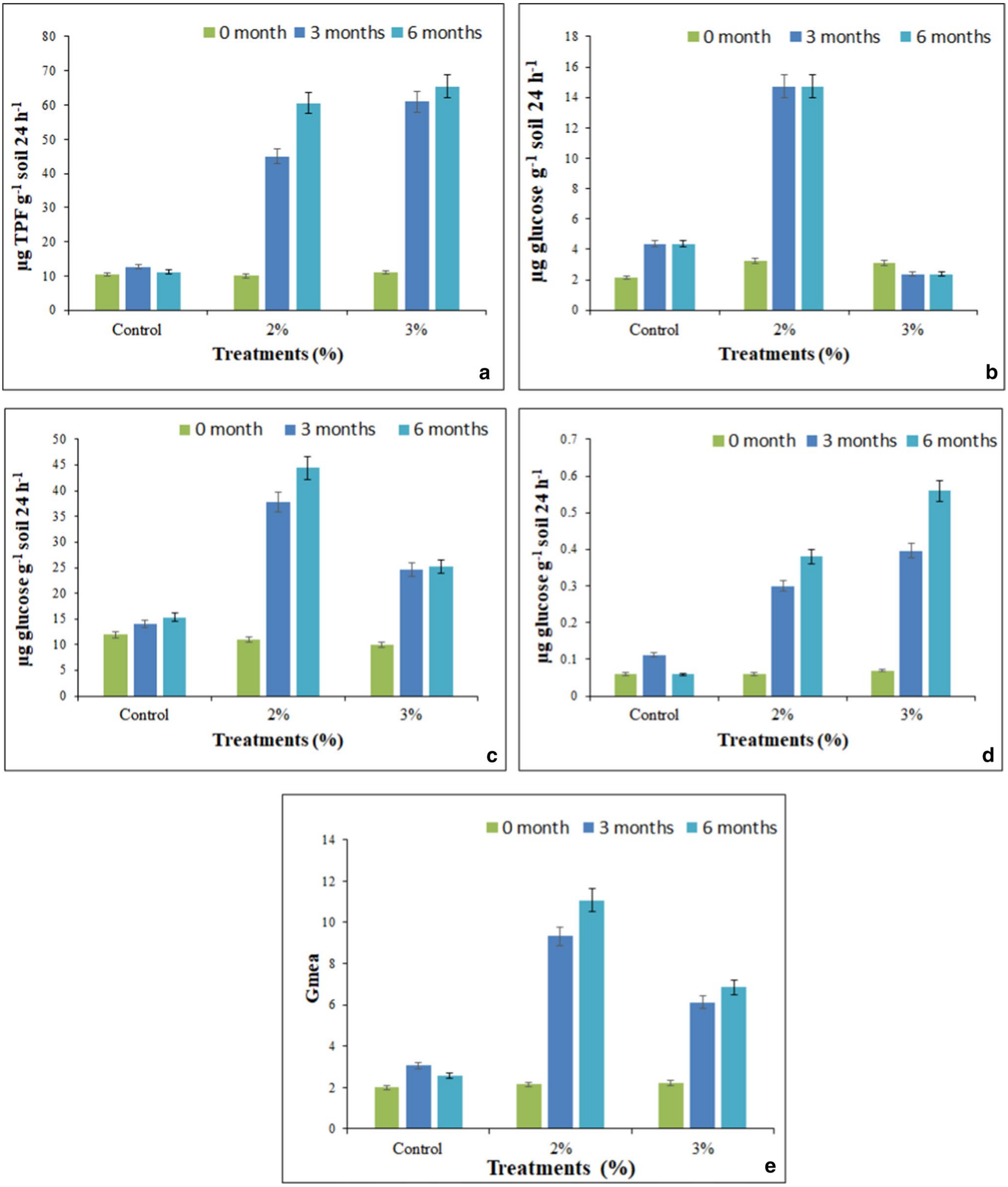


Fig. 5 **a** Dehydrogenase, **b** amylase, **c** invertase, **d** cellulase activities and **e** GM$_{ea}$ in control and 2% and 3% (w/w) *Lantana* biochar amendments at the beginning, middle and end of incubation

et al. (2016) reported that biochar produced from wheat straw significantly improved amylase activity in a substrate. Amylase plays an important role of catalysing the hydrolytic depolymerisation of polysaccharides such as starch in soil to simple glucose molecule. Its presence is significantly correlated with microbial populations and moisture content. A decrease in the amylase activity beyond 2% (w/w) indicates that an application rate beyond this negatively affected microbial population responsible for amylase secretion in the spoil. At higher application rate, enzymes may be inactivated in biochar amended soils, by blocking or absorption of the substrate (Bailey et al. 2011).

3.3.4 Cellulase activity

In the current study, cellulase activity had a positive relation with biochar application (Fig. 5d). At 3% (w/w) of biochar amendment, the cellulase activity increased from 0.06 µg glucose g^{-1} soil 24 h^{-1} in control to 0.4 µg glucose g^{-1} soil 24 h^{-1} in the middle of the study period and 0.6 µg glucose g^{-1} soil 24 h^{-1} at the end of the incubation study. An average increase in 70% and 78% was observed at 2% and 3% (w/w) biochar application, respectively. A number of biochar-based studies reported that biochar application significantly stimulated the cellulase activity in the soil (Awasthi et al. 2016; Khadem and Raiesi 2017). Cellulase is another important enzyme involved in C-metabolism and produced by soil microbes that catalyse the decomposition of cellulose and some other related polysaccharides. The presence of cellulase indicates a nutrient-rich soil with enriched microbial activity.

3.4 Effect of biochar in geometric mean of enzyme activities

The geometric mean of enzyme activities (GM_{ea}) is an index to summarise the soil enzymes activities having various values and units (Paz-Ferreiro et al. 2012). GM_{ea} of the unamended mine spoil was 1.87 while it increased to 4.51 at 2% (w/w) and 3.25 at 3% (w/w) biochar amendments, clearly stating the fact that *Lantana* biochar at 2% application is best suited for improving the spoil biochemistry (Fig. 5e). The improvement in the GM_{ea} is related to soil organic carbon and microbial activity due to biochar application (Lehmann 2007) or by direct interaction between the enzymes and the biochar surfaces (Lehmann 2007).

3.5 Effect of biochar amendment on CO_2 flux

The effect of biochar amendments at 2% (w/w) and 3% (w/w) on CO_2 flux, surface CO_2 concentrations, moisture, temperature and relative humidity at 5 cm depth was recorded for a 4-month period (Table 3). The control had much higher average CO_2 flux value of 4.92 µ mol CO_2 m^{-2} s^{-1} compared to 2.85 and 2.60 µ mol CO_2 m^{-2} s^{-1} in 2% and 3% (w/w) biochar amendment. The CO_2 flux value showed a significant ($p < 0.05$) 50% decrease by *Lantana* biochar application. It was found to be highly correlated with organic carbon, GM_{ea}, cellulase and DHA activity (Table 2). Studies report that changes in CO_2 flux by biochar application is influenced by feedstock, pyrolysis temperature, substrate type and also the age of biochar (Gascó et al. 2019). Biochar amendment significantly increased the CO_2 flux due to the increase in the organic matter in the substrate. On the other hand, wood biochar has been reported to reduce the CO_2 emission by about 42.8%, and a mixture of saw dust and coffee husk biochar reduced the CO_2 emissions by about 50% (Awasthi et al. 2016). Biochar prepared at higher temperature are generally known to be highly aromatic with high recalcitrant carbon (Al-Wabel et al. 2013). Majumder et al. (2019) reported that biochar had dual effects on soil carbon mineralization, depending upon the source material and pyrolysis temperature. In the present study, the mine spoil was incubated in natural conditions for 6 months, during this period the labile fraction of the biochar was acted upon by microbial activity leaving behind the high recalcitrant carbon. Thus, the 6-months aged biochar on the mine spoil had a lower CO_2 flux than the unamended substrate.

Table 3 Effect of biochar amendment on CO_2 flux (µ mol CO_2 m^{-2} s^{-1}), volumetric water content (m^3 m^{-3}), relative humidity (%), spoil moisture (%) and temperature (°C) (12 h data from 8:00 to 20:00 (+5:30 GMT) were taken at every 20 min interval for 90 s duration for 4 months, considering each amendment was measured twice a week, $n = 3168$)

	Control	2% (w/w)	3% (w/w)
CO_2 flux (µ mol CO_2 m^{-2} s^{-1})	4.92 ± 0.67	2.85 ± 0.75	2.60 ± 0.72
Volumetric water content (m^3 m^{-3})	0.22 ± 0.01	0.27 ± 0.01	0.24 ± 0.003
Relative humidity (%)	45.45 ± 4.2	64.45 ± 10.2	69.12 ± 5.2
Spoil moisture (%)	1.18 ± 0.36	2.98 ± 0.43	3.17 ± 0.65
Temperature (°C)	22.25 ± 0.5	20 ± 1.00	19 ± 0.86

3.6 Volumetric water content, relative humidity, spoil moisture and temperature

Incubated spoil moisture content was highest at 3% (w/w) amendment and least in control (Table 3). Mean relative humidity was also found to be highest at 3% (w/w) amendment (69%) and lowest in the control (45%) (Table 3). Biochar application in spoil affects its water holding capacity by modifying soil texture and structural properties. In addition, internal porosity also stores plant available water which in turn affects soil water retention properties (Rasa et al. 2018). The daily mean spoil temperatures at a 5-cm depth were 22.5, 20 and 19 °C for control, 2%, and 3%, respectively, with clear diurnal differences. The possible changes in spoil temperature resulting from biochar addition might affect other biophysical–chemical properties of the spoil.

3.7 Monthly variation

Spoil respiration was recorded for the months of November to February. In the control mine spoil, CO_2 flux initially decreased from November to January, and started increasing after January. However, in the biochar amended mine spoil, a decrease was observed after November, which eventually remained stable up to the month of February (Fig. 6a). Similarly, the cumulative CO_2 flux in the control mine spoil increased over the period of time while the biochar amended spoil had a lower cumulative CO_2 flux value (Fig. 6b). A review conducted by Liu et al. (2016) reported that a positive response of soil CO_2 flux was observed in the soil without vegetation and the unfertilised soil treated with manure and crop residue biochars. An increase in CO_2 emission followed by stabilisation by poultry litter biochar was reported by Van Zwieten et al. (2013). The average surface CO_2 showed an opposite trend, and the highest value was observed at 3% (w/w) biochar amendment and the least in unamended (Fig. 7c). However, the control and 2% (w/w) values did not vary significantly. Spoil moisture content was highest in biochar amended mine spoil during the period of study (Fig. 4d). Luo and Gu (2016) reported that peanut hull biochar at 3% (w/w) application rate increased the cumulative CO_2 emission due to the enhanced organic

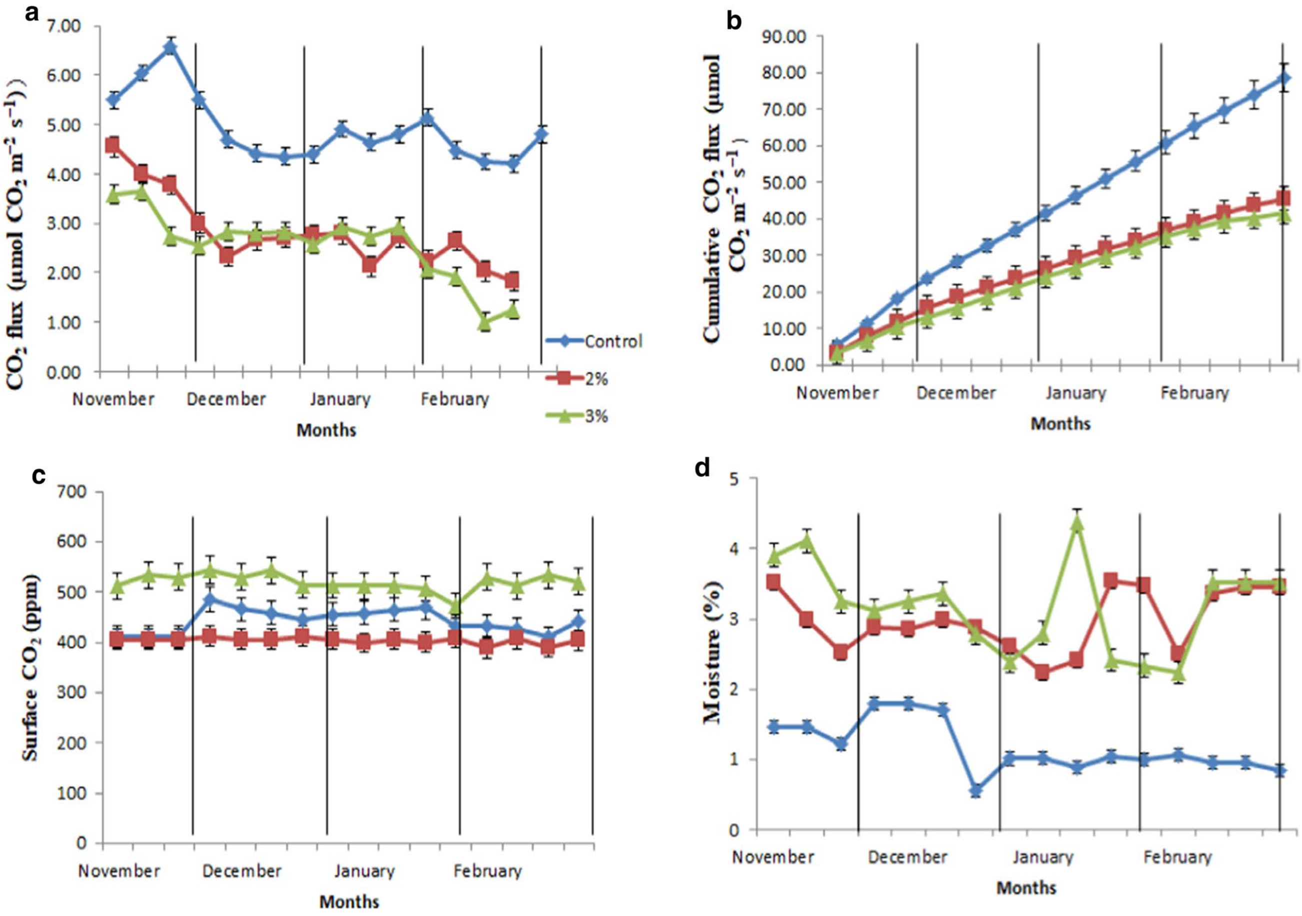
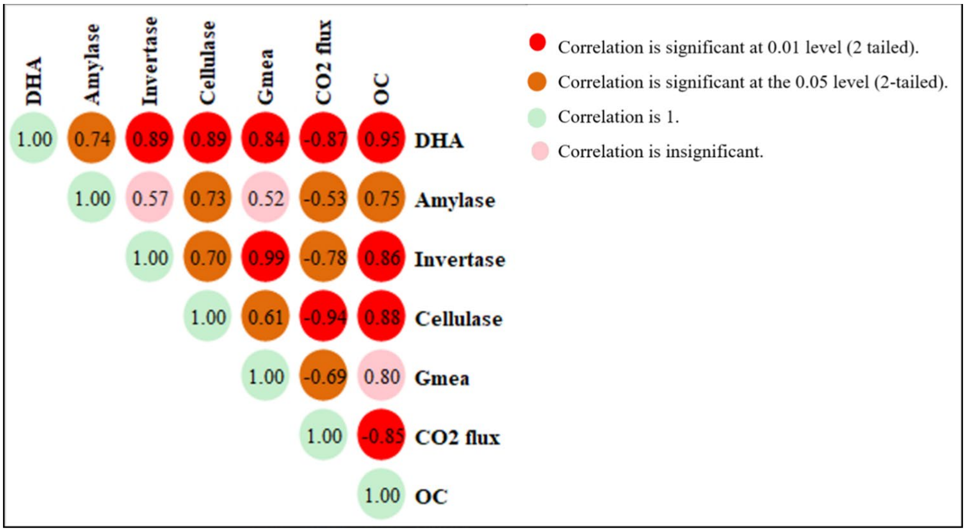


Fig. 6 Monthly variation in the control and biochar amended mine spoil (2% and 3%, w/w) for **a** CO_2 flux, **b** cumulative CO_2 flux, **c** surface CO_2 and **d** moisture content



	DHA	Amylase	Invertase	Cellulase	Gmea	CO2 flux	OC
DHA	1.00	0.74	0.89	0.89	0.84	-0.87	0.95
Amylase		1.00	0.57	0.73	0.52	-0.53	0.75
Invertase			1.00	0.70	0.99	-0.78	0.86
Cellulase				1.00	0.61	-0.94	0.88
Gmea					1.00	-0.69	0.80
CO2 flux						1.00	-0.85
OC							1.00

Fig. 7 Pearson correlation coefficients (*r*) between enzymatic activities of the soil and across biochar application rate ($n=5$)

carbon mineralisation caused by biochar addition. Similarly, Deng et al. (2020) reported that biochar addition increased carbon substrate for microbial activity which stimulated mineralisation and oxidation of biochar carbon and hence CO_2 emission.

4 Correlation of GM_{ea}, CO_2 flux and coal mine spoil properties

Pearson's correlation showed all the enzymatic activities and GM_{ea} were significantly correlated with organic carbon (Fig. 7). Many studies have reported a positive correlation between the soil enzymatic activities and organic matter contents (Khadem and Raiesi 2017). Soil enzymes catalyse a large number of important reactions such as decomposition of soil organic carbon. A positive correlation between extracellular enzymes such as DHA ($r=0.95$), amylase ($r=0.75$), invertase ($r=0.86$) and cellulase ($r=0.88$) and organic carbon was observed.

Correlation analysis showed that enzymatic activities were positively correlated with biochar application and mine spoil organic carbon (Fig. 8a). A similar trend was also observed for CO_2 flux (Fig. 8b). Biochar amendment and GM_{ea} and CO_2 flux were related with R^2 of 0.964 and 0.81, respectively ($p<0.05$). Similarly, GM_{ea} ($R^2=0.84$, $p<0.05$) and mine spoil organic carbon ($R^2=0.96$, $p<0.05$) also showed positive correlation by biochar amendment. The results indicate that biochar addition to mine spoil might generally increase the activities of enzymes associated with carbon mineralization.

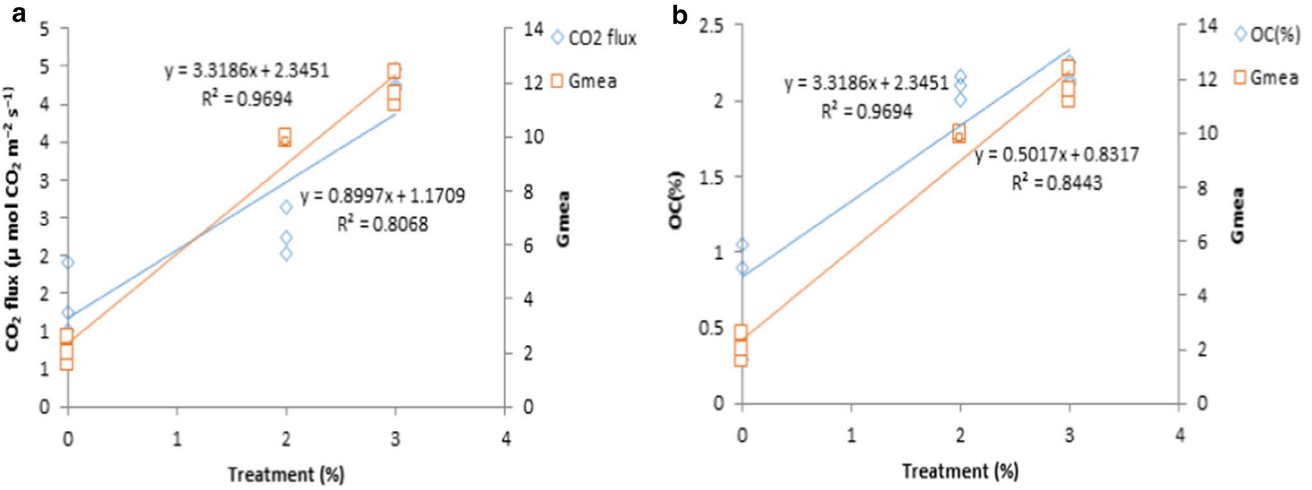


Fig. 8 Corelation of Gm_{ea} and **a** mine spoil CO_2 flux and **b** organic carbon in control, 2 and 3% (w/w) biochar amendment

5 Implication of the study

The main objective of the current study was to test the feasibility of biochar application in a pit plantation method of mine reclamation. The pit size is generally 45 cm × 45 cm × 30 cm and filled with OB/topsoil along with tree sapling (Maiti 2013). Pit plantation with fruit bearing/timber trees saplings along with biochar will promote plant growth and immobilise potentially toxic metals (Ghosh and Maiti 2020a, b). Thus, from the current study it can be concluded that biochar at 2% (w/w) application rate in a pit can ameliorate mine spoil properties for supporting plant growth.

Thus, from the findings of the present pilot scale study, it can be estimated that in 1 ha of mine degraded land, if the pits are at distance of 2.5 m, there will be a total of 1600 pits (100/2.5 in each row × 100/2.5 in each column). The diagrammatic representation of a 1 ha mine spoil is shown in Fig. 9. Each pit has a volume of 47,006 cm^3 (45 cm × 45 cm × 30 cm) and the bulk density of the mine spoil is 1.2 g cm^{-3} (Table 1). Thus, 56 kg of mine spoil would be required in each pit and considering biochar application at 2% (w/w), 1.12 kg of biochar would be required per pit. Thus, the estimated amount of biochar required for 1 ha mine spoil remediation would be 1792 kg or 1.7 t ha^{-1}. This method of pit plantation would reduce biochar requirement many folds, as the conventional spreading method has been reported to use 5–60 t ha^{-1} biochar application for amendment purpose (Ghosh and Maiti, 2020a, b).

In a mining area, before the eco-restoration practices start, the extracted overburden materials are often left for 1–2 years. During this period, the invasive weeds like *Lantana* grow abundantly in these areas which cause problems due to allelopathy. These weeds can be uprooted during the dry tropical months before monsoon, sun-dried and converted to biochar by charcoal-pit production method which is labour extensive. The estimated unit cost (C_E, USD t^{-1}) for biochar production and application can be calculated by Eq. 2 (Dickinson et al. 2015; Ghosh and Maiti 2020a, b):

$$C_E = \left(U_A + U_B + U_C\right) \times C_i, \tag{4}$$

where, U_A = unit labour input for feedstock harvest and biochar production (d t^{-1}); U_B = unit labour input for biochar transport (d t^{-1}); U_C = unit labour input for biochar application to plantation pit (d t^{-1}); C_i = labour cost (USD d^{-1}).

Based on the above equation, the cost estimations have been shown in Table 4. The cost of biochar application is 120 USD t ha^{-1} by pit plantation method.

For upliftment of livelihood of stakeholders and employment generation, plantation of timber and fruit bearing trees having economical significance are often suggested. Also, plantation of native species will promote species richness and will promote better growth (Maiti and Ghosh 2020). Fruit bearing trees such as *Psidium guajava* (Guava), *Mangifera indica* (Mango), *Artocarpus heterophyllus* (Jackfruit), *Syzygium cumini* (Black plum) and *Citrus* spp. can provide economic benefits in the long run. Apart from those multipurpose trees such as *Albizia lebbeck* (Siris), *Tectona grandis* (Teak), *Dalbergia sissoo* (Shisham) and *Gmelina arborea*, Beechwood has high

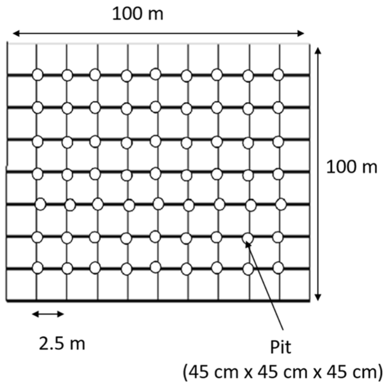


Fig. 9 A schematic diagram showing the pit plantation layout for 1 h land. The pits are at a distance of 2.5 m and each pit has a dimension of 45 cm × 45 cm × 30 cm, with volume of 47,006 cm^3 and biochar 1.7 t ha^{-1} (the diagram is not on scale and the number of pits is not exact in number)

Table 4 Cost of biochar production and application for 2% (w/w) biochar application, plantation pit preparation and plantation with fruit and timber bearing trees (area 1 ha; conversion 1 USD = Indian ₹ 75)

Sr No.	Expenses	INR	USD
1	Unit labour input for feedstock harvest and biochar production (d t^{-1}) (@ 6 h/d, each labour collects 50 kg feedstock) $U_A = 20$ d t^{-1}	9000	120
	Unit labour input for biochar transport (d t^{-1}) (Biochar produced in charcoal pits in the field itself) $U_B = 0$ d t^{-1}		
	Unit labour input for pit digging and application in a plantation pit (d t^{-1}) $U_C = 30$ d t^{-1}		
	labour cost (INR d^{-1}) $C_i = 180$		
	Sub- total cost of biochar production and application by Eq. 4 (@1 t ha^{-1}) C_i		
2	Timber saplings @₹35 and fruit bearing tree @₹25 for 1600 plantation pits (considering 50% timber and 50% fruit tree saplings)	48,000	640
	Total cost of sapling (ha^{-1})		
	Total cost t ha^{-1}	57,000	760
	Total cost at 2% (w/w) application (1.7 t ha^{-1})*	63,300	844#

*Total cost at 3% (w/w) amended by biochar will be 950 USD t ha^{-1}

#1 USD = Indian ₹ 75

economic significance. Thus, the cost of fruit bearing tree sapling 50% (@₹25) and timber saplings 50% (@₹35) will cost 640 USD ha^{-1} (Table 4). Therefore, the total cost of biochar production, preparation of plantation pit and plantation will cost 844 USD ha^{-1}.

6 Conclusions

Lantana biochar significantly improved GM_{ea} up to 2% (w/w) application rate. At 3% (w/w) mine spoil physio-chemical properties such as organic carbon (65%), WHC (19%), CEC (54%), BD (25%) were improved significantly compared to the unamended mine spoil. In general, DHA and invertase activity were increased up to 3% biochar amendment while amylase and cellulase activities increased up to 2% amendment and eventually decreased beyond this level. Decreases in mine spoil CO_2 flux by 42% and 47% by 2 and 3% biochar amendment respectively, compared to the control were observed. Thus, *Lantana* biochar effectively remediates the physio–chemical and biological aspects of degraded and can be easily applied during ecological restoration by pit plantation method. The application rate with this method will be 1.7 t ha^{-1}, which is lower than the reported rates of application. The cost of biochar preparation, application and plantation in this pit plantation technique at 2% is estimated to be 844 USD t ha^{-1}. If fruit orchards and timber bearing plants are used for plantation in mine spoil, it will provide livelihood to stakeholders.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dipita Ghosh. Prof. Subodh Kumar Maiti was the overall supervisor of the work.

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Availability of data and materials The datasets generated and/or analysed during the current study are not publicly available due as this is a part of an on-going Ph.D. thesis, but are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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