Characterisation, stability, and microbial effects of four biochars produced from crop residues

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ABSTRACT
In recent years the importance of biochar application in soil has increased tremendously as pyrogenic carbon (C) may act as an important long-term C sink because its microbial decomposition and chemical transformation is very slow. Biochar was prepared from maize stover, pearl millet stalk, rice straw and wheat straw in a pyrolysis kiln at the temperature of 400 °C. The biochar was characterised for various physical, chemical and structural properties. The stability of biochar in soil was studied by CO2 efflux for one year. The effect of biochar on available N, P, K and microbial properties was also studied in a separate experiment continued for 67 days. The wheat and rice biochar exhibited higher cation exchange capacity (CEC) than the other biochar materials, while the pH values of maize and pearl millet biochar were higher over rice and wheat biochar. The maize biochar was richer in C, N and P contents. The energy dispersive X-ray spectrometry (EDS) analysis showed that wheat and rice biochar was richer in K and Si, respectively. Total C content was highest in maize biochar (66%) followed by pearl millet biochar (64%), wheat biochar (64%) and rice biochar (60%). The Fourier-transform infrared spectroscopy (FTIR) analysis showed the presence of various functional groups in biochar. The maize biochar exhibited stronger structural surface functional groups including aromatic C=O stretching. Among the four different biochars used for CO2 efflux study, the maize biochar was found to be the most stable showing reduced C mineralization to protect the native soil organic C. The reduced C mineralization was also observed in the case of pearl millet and wheat biochar. Contrarily, rice biochar exhibited higher C mineralization. The maize biochar being most stable in soil showed highest C enrichment in soil. The maize biochar enhanced the available N and P in soil, while wheat biochar increased the available K content in soil. The rice biochar being relatively labile in soil fuelled the proliferation of microbial biomass and thereby enhancing the physiological efficiency of microbes measured in terms of dehydrogenase activity. Maize biochar with higher nutrient values especially N and P and C stability could be advocated for enhancing soil fertility and long-term C sequestration. Rice biochar might be advocated for higher microbial activities in restoring biological fertility of degraded soils.

1. Introduction

Rice and wheat are grown sequentially over an area of 24 Mha throughout South-East Asia of which the Indo-Gangetic plains grow 32 and 42% of rice and wheat, respectively; representing the region’s share of one quarter to one-third of the total production (Ladha et al., 2003). Besides rice, maize or pearl millet is also grown in sequence with wheat as a wet season crop. The major problem which cropped up recently with this wheat based cropping system is how to dispose the large quantities of crop residues left over in the field due to the use of mechanised combined harvester. In order to clear the land ready for the next crop, the easiest option available to the farmers is to burn the residues in the field which cause losses of essential plant nutrients and environmental pollution by releasing suspended particulate matter, smoke and greenhouse gases. It is a matter of concern that in Indian state of Punjab alone, some 70 to 80 million tons of rice and wheat straw are burned annually releasing approximately 140 million tons of CO2 to the atmosphere, in addition to methane, nitrous oxide and air pollutants (Punia et al., 2008). In this scenario, biochar, a pyrolysed product of biomass offers a significant, multidimensional opportunity to transform large scale agricultural waste streams from a financial and environmental liability to valuable assets. Interestingly if these residues are converted into biochar, 50% of initial biomass C can be recovered as compared to only 3% during open burning and <10–20% after 5–10 years during biomass decomposition (Baldock and Smernik, 2002). Biochar, produced by the pyrolysis of biomass under limited oxygen, is highly stable and resistant to microbial decay. Thus there is considerable interest in the concept of applying biochar to soil as a long-term sink for carbon (C) thereby mitigating climate change (Prayogo et al., 2014). This concept has been further strengthened by the realisation that biochar application to soil can promote soil fertility and crop growth.
due to impacts on soil physicochemical properties (Glaser et al., 2002; Lehmann et al., 2006; Yamato et al., 2006). Typically, P appears more available in soils to which biochar has been applied (Edelstein and Tonjes, 2012), and P sorption rates to the surface of ferrhydrate were markedly decreased in the presence of biochar (Cui et al., 2011). Biochar application has received growing interest as a sustainable technology to improve highly weathered or degraded tropical soils (Lehmann et al., 2006). Zimmerman et al. (2011) found that at month to year timescales, biochar reduced mineralisation of soil organic matter (SOM), probably via physical protection of sorbed C within biochar pores or at the surface of biochar, as occurs with organic matter sorbed onto siliceous materials (Semple et al., 2013). Protection of soil C could also be the result of greater aggregation, protecting both biochar and SOM from degradation, changes in microbial enzyme activity as a result of enzyme sorption to biochar (Prayogo et al., 2014). Before the application of biochar as a soil amendment, it is essential to characterise the biochar for efficient management. The physical and chemical properties of biochars are influenced by the properties of the feedstock and by pyrolysis conditions such as highest treatment temperature and furnace residence time (Downie et al., 2009). Feedstocks differ in their composition concerning elemental composition; the presence of soil and dust particles; moisture content; and lignin, cellulose, and hemicellulose content, which, in turn, affect the properties of the respective biochars after pyrolysis (Ubbelohde and Lewis, 1960; Boehm, 1994; Alexis et al., 2007; Yip et al., 2007). Scanning electron microscopy (SEM) has been successfully used for the recognition for physical and morphological characterisation (Saleh et al., 2012). Furthermore, the combination of both SEM and chemical fingerprints using the Energy-dispersive X-ray spectrometry (EDS) allows for the determination of the chemical composition of particles, and thus their O/C ratio, at micrometre or even nanometre-scale resolution. FTIR is considered as a powerful tool to identify surface organic functional groups in biochar (Yao et al., 2011; Peng et al., 2011; Saleh et al., 2012). In spite of the substantial production of crop residues with special reference to wheat and rice annually, these materials are rarely tapped for making biochar and therefore linking characteristics of biochar with its stability in soil is extremely important for long-term C sequestration.

It was hypothesized that biochar produced from maize stover, pearl millet stalk, rice straw and wheat straw might have macromolecular structures dominated by aromatic C to make these more resistant to microbial decomposition than un-charged organic residues in soil. In this study four different biochars were prepared from the above ground residues of four different crops at 400 °C in an oxygen-limited pyrolysis kiln. The objective of the study was to compare the physicochemical and spectral characteristics of biochar samples linking to long-term microbial stability and C enrichment in soil. The other objective was to study the short-term effect of biochar on microbiological properties and available nutrients in soil.

2. Material and methods

2.1. Biochar production

Biochar was prepared from maize stover, pearl millet stalk, rice and wheat straw in a closed drum fire brick enclosure. The empty space between the two walls of the fire brick was thermally insulated with perlite. The barrel was filled with feedstock (approximately 8 kg) and it was sealed, with only a small opening to allow the off-gases to escape. The temperature probes were inserted at the top, middle and bottom positions of the barrel through small 3-mm holes and the barrel is closed from the top by an iron plate with small opening (50 cm) at the center with 5.1 cm pipe fitting welded to it. This allows directing the off gases to escape. A small fire was started with wood chips under the barrel. Temperature was controlled relatively easily by limiting the size of the fire under the barrel.

Temperature inside the barrel was continuously monitored by digital temperature probes. Then temperature started rising up and went up to 400 °C, especially at the top measurement point. Since heat rises, the pyrolysis zone tended to move from the top to the bottom of the barrel. Towards the end of the process, temperature rises to 400 °C at the bottom measurement point and it was maintained for half an hour and external heating was stopped for maintaining uniform heating in the barrel. After 2 h, the lid of the barrel was opened and water was added from the top to extinguish the left-over heat inside the biochar. On the next day the biochar was removed from the barrel and kept in open for drying under the sun. The charred yield of biochar provided by a kiln is given by $Y_{char} = m_{char} / m_{bio}$ (Antal and Gronli, 2003), where $m_{char}$ is the dry mass of charcoal taken from the kiln and $m_{bio}$ is the dry mass of the feedstock loaded into the kiln (Antal and Gronli, 2003).

2.2. Biochar characterisation

Biochar was characterised for bulk density (BD) (Veihmeyer and Hendrickson, 1948), water holding capacity (WHC) (Keen and Raczkowski, 1921), pH (1:2::soil:water, Jackson, 1973), electrical conductivity (EC) (1:2::soil:water, Jackson, 1973), cation exchange capacity (CEC) (Sumner and Miller, 1996), and P, S contents (Jackson, 1973). Total C in biochar was determined by dry combustion method with a C analyser (Elementar, Vario TOC Cube). Nitrogen content in biochar was measured by digestion with concentrated H2SO4 followed by distillation in a Kjeltech distillation unit (Brenner and Mulvaney, 1982). Scanning electron microscope (SEM) imaging analysis was conducted using a Zeiss EVOMA10 Scanning Microscope. Surface element (C, O, Si, Mg, Ca, K, Fe) analysis was conducted simultaneously with the SEM at the same surface locations using energy dispersive X-ray spectroscopy (EDS). Biochar samples were mounted on Al stubs and coated with gold/palladium for EDS and SEM. The beam energy used was 20 kV (Downie et al., 2011). The EDS can provide rapid qualitative, or with adequate standards, semi-quantitative analysis of elemental composition with a sampling depth of 1–2 μm. Biochar quality was analysed with FTIR spectroscopy (Bruker, model Alpha) with the help of Opus Wizard software. The tablet of biochar was prepared by mixing 5 mg biochar (0.1 mm sieved) with 1 g analytical grade KBr powder by applying pressure in a hydraulic press. The spectra were recorded from 4400 to 400 cm$^{-1}$ by averaging 200 scans at 2 cm$^{-1}$ resolution.

2.3. Soil

The soil was collected from the farm of Indian Agricultural Research Institute, New Delhi. The soil is sandy loam in texture (Bouyoucos, 1962) and it belongs to the hyperthermic family of Typic Haplustept. The soil was air dried and passed through 2-mm sieve. It has pH 8.2 (1:2: w:v soil:water suspension, Jackson, 1973), electrical conductivity (EC) 0.28 ds m$^{-1}$ (1:2: w:v soil:water suspension, conductivity bridge, Jackson, 1973), total organic C 5.3 g kg$^{-1}$ (Elementar, Vario TOC Cube), available N 88.0 mg kg$^{-1}$, Olsen’s NaHCO3 extractable P 4.0 mg kg$^{-1}$ (Olsen and Sommers, 1982), and ammonium acetate (CH3COONH4) extractable K 54.0 mg kg$^{-1}$ (Knudsen et al., 1982).

2.4. Biochar effect on carbon dioxide evolution

The experimental design consisted of four treatments: control (soil without biochar), soil mixed with biochar prepared from maize, pearl millet, rice and wheat. Air dried soil weighing 70 g was mixed with 8.94 g (eqv. to application rate of 20 Mg ha$^{-1}$ to a soil depth of 15 cm) of biochar (0.1 mm sieved) and placed in a beaker. The moisture content of soil/soil with biochar in each beaker was maintained at 55% of soil porosity. The beakers were transferred in air tight jars (500 mL capacity) to capture evolved CO2 by NaOH trap kept inside. In decomposition study, soil respiration in-terms of amount of CO2 evolved was monitored periodically for one year for twice in a week for the first
two weeks and once in a week for the rest of the period. Absorbed CO₂ in NaOH was precipitated as BaCO₃ and the excess of NaOH was back titrated with standard HCl for estimation of C mineralization in soil (Zibilske, 1994a, 1994b). The alkali was replaced twice in the first 2 week period followed by once in a week for subsequent 50 weeks of incubation period. All the jars were kept inside a biochemical oxygen demand (BOD) incubator at 38 °C. At the end of one year of C mineralization, the available N (Subbiah and Asija, 1956), Olsen’s P (Olsen et al., 1954), ammonium acetate extractable K (Hanway and Heidel, 1952) and total soil C (Elementar, Vario TOC Cube) were estimated.

### 2.5. Biochar effect on microbial activities

In a separate set of incubation experiment in parallel to C mineralization, the soil biological activities in terms of dehydrogenase activity (DHA) (Casida et al., 1964), microbial biomass C (MBC) (Anderson and Domsch, 1978) and alkaline phosphomonoesterase activities (Eivazi and Tabatabai, 1977) were estimated after 21, 45 and 67 days of incubation.

### 3. Results

#### 3.1. Characterisation of biochar

Chemical, physical, morphological and spectral properties of biochar prepared from maize stover, pearl millet stalk, rice and wheat straw were assessed. The charring yield of biochar varied between 45 and 50%. The cation exchange capacity (CEC) was higher in maize stover biochar, while it was lower in pearl millet stalk biochar (Table 1). The pH values of all the biochar were alkaline and maize stover biochar showed the highest pH (10.7) followed by pearl millet biochar stalk biochar (10.6), wheat biochar (8.8) and rice biochar (8.6). The EC was recorded highest in wheat straw biochar (13.3 ds m⁻¹) followed by that in pearl millet stalk biochar (10.9 ds m⁻¹), rice straw biochar (7.68 ds m⁻¹) and maize biochar (3.84 ds m⁻¹). The bulk density of rice and wheat straw biochar was comparatively lower than that of maize stover and pearl millet stalk biochar. The water holding capacity was observed highest in wheat straw biochar (561%) followed by that in maize stover biochar (456%) and pearl millet stalk biochar (419%). Nitrogen content was highest in maize stover biochar (1.31%) followed by that in wheat straw biochar (1.23%), pearl millet stalk biochar (1.10%) and rice straw biochar (0.95%). Phosphorus content was highest in maize stover biochar (1.76%) followed by that in pearl millet stalk biochar (1.60%), rice straw biochar (1.52%) and wheat straw biochar (0.74%). Total C content was highest in maize stover biochar (66%) followed by that in wheat straw biochar (64%), pearl millet stalk biochar (64%) and rice straw biochar (60.0%) (Table 1). Oxygen content was highest in rice straw biochar (29.6%), while the other biochars were at par in oxygen content ranging from 25.4% to 25.9% (Fig. 1 a, b, c & d). The EDS analysis at points A and B indicated that pearl millet stalk biochar and rice straw biochar particles consisted of high calcium agglomerates (Fig. 1 e and f). Rice straw biochar was highest in Si content (5.54%) and in other biochar Si content ranged between 2.49 and 3.59%. Wheat straw biochar was richer in K content (3.15%) followed by pearl millet stalk biochar (2.52%), maize stalk biochar (1.96%) and rice stalk biochar (1.50%). Maize stover biochar and pearl millet stalk biochar were at par in Fe content (0.32 to 0.33%), while rice and wheat straw biochar had 0.18% and 0.12% Fe, respectively. Pearl millet stalk biochar was richer in Ca and Mg.

The FTIR spectra of all the biochar samples were in the range of 4000–400 cm⁻¹ (Fig. 2). It could be seen that the band between 3417 and 3434 cm⁻¹ was ascribed to the mixed stretching vibration absorption of amino and hydroxyl groups and maize stover biochar showed the maximum and wheat straw biochar showed the lowest absorption and the other two biochars were in between the above two in terms of energy absorption. The band in the range of 2854 to 2924 cm⁻¹ indicates the aliphatic C–H stretching vibrations (Sarmah et al., 2010; Tatzber et al., 2007; Schnitzer et al., 2007). The band in the range of 1596 to 1617 cm⁻¹ might be due to carbonyl group stretching vibration or assigned to C=O and C=C in plane aromatic vibrations (Smidt and Meissl, 2007). The peak at 1414 to 1448 cm⁻¹ was caused by the phenolic O–H bending. The peak at 1089 to 1101 cm⁻¹ is indicative of the OH in-plane bending cellulose. The small band at 802 and 617 cm⁻¹ represents aromatic C–H out of plane deformation, C–O out of plane deformation from carbonates and alkylic bend, respectively (Ibara et al., 1996; Tatzber et al., 2009, Smidt and Schwanninger, 2005).

#### 3.2. Decomposition of biochar in soil

The C mineralization from soil with rice straw biochar was higher than from the other biochar throughout the incubation period (Fig. 3). The C mineralization from the other biochar treatments was at par up to day 137 and thereafter the wheat straw biochar treatment showed decreased mineralization even below the control treatment up to day 365. At this point reduced C mineralization from the wheat straw biochar treatment was observed. This clearly demonstrated that wheat straw biochar contains labile C which could sustain positive priming up to day 137 and thereafter it showed reduced carbon mineralization. The C mineralization from pearl millet stalk and maize stover biochars were at par almost throughout the period. Pearl millet stalk and wheat straw biochars showed increased C mineralization up to day 270 and thereafter both the biochars showed reduced C mineralization on C mineralization up to 365 day. On the contrary, an increased C mineralization was observed throughout the incubation period in rice biochar indicating a good source of C that might have fuelled heterotrophic microbial activities.

One of the objectives of the experiments was to investigate whether the application of biochar could increase total soil C (TSC) at the end of one year of C mineralization in an incubation study. On an average the TSC increased in the range of 41 to 65% in biochar treated soil (Fig. 4). The TSC was highest in maize stover and wheat straw biochars treated soils, while it was observed lowest in the case of rice straw biochar treatment. Total C mineralization from maize stover biochar was marginally lower than that from wheat straw biochar and thus the two biochar showed TSC which were found to be non-significant. The low C content and high C mineralization from rice straw biochar were the prime reasons behind less enrichment of soil C in this treatment. The TSC in pearl millet stalk biochar treated soil was in between the above two biochar materials.

#### 3.3. Effect of biochar on available nutrients

The available soil nitrogen (N), phosphorus (P) and potassium (K) which were measured after 365 days of incubation varied

<table>
<thead>
<tr>
<th>Biochar</th>
<th>CEC (cmol kg⁻¹)</th>
<th>pH (soil:water::1:2)</th>
<th>EC (ds m⁻¹)</th>
<th>Bulk density (mg m⁻¹)</th>
<th>Water holding capacity (%)</th>
<th>C (%)</th>
<th>N (%)</th>
<th>P (%)</th>
<th>S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize biochar</td>
<td>63.5</td>
<td>10.7</td>
<td>3.84</td>
<td>0.52</td>
<td>456</td>
<td>66</td>
<td>1.31</td>
<td>1.76</td>
<td>0.20</td>
</tr>
<tr>
<td>Pearl millet</td>
<td>57.6</td>
<td>10.6</td>
<td>10.9</td>
<td>0.60</td>
<td>419</td>
<td>64</td>
<td>1.10</td>
<td>1.60</td>
<td>0.22</td>
</tr>
<tr>
<td>Rice biochar</td>
<td>97.3</td>
<td>8.60</td>
<td>7.68</td>
<td>0.46</td>
<td>543</td>
<td>60</td>
<td>0.05</td>
<td>1.22</td>
<td>0.05</td>
</tr>
<tr>
<td>Wheat biochar</td>
<td>10.9</td>
<td>8.76</td>
<td>13.3</td>
<td>0.48</td>
<td>561</td>
<td>64</td>
<td>1.23</td>
<td>0.74</td>
<td>0.29</td>
</tr>
</tbody>
</table>
significantly due to biochar amendment (Fig. 5). In general the available N, P and K content increased significantly due to biochar application. The extent of increase in available N, P and K in soil ranged between 25 and 40%, 21 and 61% and 62 and 83%, respectively. The available N in soil treated with wheat straw and maize stover biochars was significantly higher over the soil treated with rice straw and pearl millet stalk biochars. The available P in soil was observed highest in maize stover biochar followed by rice straw biochar. The available P in pearl millet stalk biochar amended soil was at par with that in wheat straw biochar treated soil.

3.4. Effect of biochar on microbial activities

Overall the soil microbial activities measured in terms of dehydrogenase activity (DHA), microbial biomass C (MBC) and alkaline phosphomonoesterase activity were enhanced due to biochar application. Dehydrogenase is considered as a respiratory enzyme, the activity of which measures the physiological efficiency of microbes in soil. The DHA increased from 21 days of incubation and reached its peak on day 45 and then it decreased on day 67 (Table 2). On day 21 all the biochar showed significantly higher DHA over the control treatment. Among the biochar, rice straw biochar showed highest DHA, while it was lowest in maize stover biochar treated soil. The DHA in soil amended with pearl millet stalk and wheat straw biochar were in between that obtained with rice and maize stover biochar. Rice straw biochar showed significantly higher DHA, while maize stover biochar exhibited lower DHA than others even on days 45 and 67. The MBC also followed the same trend as of DHA, rice straw biochar treated soil showed highest MBC and maintained it for up to day 67. Pearl millet stalk biochar comparatively showed higher MBC than wheat straw biochar. Wheat straw biochar consistently maintained higher alkaline phosphomonoesterase activity (APA) throughout the incubation period, while rice straw biochar showed lower APA especially on days 21 and 84. Maize stover biochar along with pearl millet stalk biochar and the control treatment showed comparable APA on days 21 and 45.

4. Discussion

4.1. Biochar characteristics

The charring yields of maize stover biochar, pearl millet stalk biochar, wheat straw and rice straw biochar on weight basis were 50%,
50%, 46% and 45%, respectively, which was inconsistent with that reported by Keluweit et al. (2010) and Zimmerman (2010). However, Kuzyakov et al. (2009) reported char yields of 33% and 56% on C and weight basis, respectively from $^{14}$C labelled shoot litter of Lolium perenne in a muffle furnace at a temperature of 400 °C. The biochar prepared from maize stover showed the highest CEC and pearl millet stalk biochar showed the lowest CEC. However, Yuan et al. (2011) reported higher CEC in maize biochar prepared under limited oxygen in a muffle furnace. Silber et al. (2010) reported a pH-dependent CEC for maize stover-derived biochar of between 179 and 888 mmol c kg$^{-1}$. The pH values of maize stover (10.7) and pearl millet stalk (10.6) biochars were higher than that in wheat (8.8) and rice straw (8.6) biochar, while EC of maize stover biochar was lowest. The pH was also reported more in maize stover biochar (10.8), soybean straw biochar (10.9) and peanut straw biochar (10.9) prepared at 500 °C (Yuan et al., 2011). Parvage et al. (2013) reported a comparable pH value of 8.9 of the biochar prepared from wheat residues. The pH of biochar, similar with the other properties, is influenced by the type of feedstock, production temperature, and production duration (Liu et al., 2012). Besides organic material, biochar is also composed of mineral compounds. Minerals that are likely to be found in biochars include SiO$_2$, CaCO$_3$, KCl, and CaSO$_4$ as well as nitrates, oxides, and hydroxides (Parr and Sullivan, 2005; Amonette and Joseph, 2009). Though maize stover biochar showed higher pH but it showed lowest EC. Probably maize stover biochar had higher quantities of carbonates, basic cations and organic anions which might have contributed high alkalinity (Chan and Xu, 2009; Yuan et al., 2011). Yuan et al. (2011) confirmed that the presence of carbonates in X-ray spectra is the major source of alkalinity. The Si content of rice biochar is higher which might be due to rice straw being naturally rich in Si. Biochar prepared from maize stover was rich in C, N, P and Fe. The wheat biochar was rich in K. It is clearly evident that the elemental composition of biochar is strongly influenced by the feedstock type. The feedstock which is inherently richer in a particular element should contain more of that element when biochar is prepared from the same feedstock. Carbon contents of the biochar used for our study are in conformity with that reported by others (Yuan et al., 2011). Sánchez et al. (2009) reported that the biochar from rape contains 0.76% N, 0.36% P$_2$O$_5$ and 4.40% K$_2$O and that for sunflower contains 1.19% N,
0.44% P₂O₅ and 7.26% K₂O. Parvage et al. (2013) reported that biochar prepared from the mixture of seed coat, chaff, and residues from winter wheat was rich in macronutrients consisting of 0.9% P, 2.9% N, 2.5% Ca, 1.8% K, 0.5% Mg, and 0.2% S.

The spectral analysis of biochar clearly showed that the major peaks at 2854 to 2924 cm⁻¹ (Sarmah et al., 2010; Tatzber et al., 2007; Schnitzer et al., 2007), 1596 to 1617 cm⁻¹ (Smidt and Meissl, 2007) 1414 to 1448 cm⁻¹, 1089 to 1101 cm⁻¹, 802 and 617 cm⁻¹ (Ibbara et al., 1996; Tatzber et al., 2009; Smidt and Schwanninger, 2005) were common to all the biochar. The different energy absorption values indicate difference in aromaticity and amount of functional groups in biochar. The FTIR data clearly indicated that the absorption of IR in various wavenumber was highest in maize stover biochar which could explain the presence of various functional groups in higher quantities that contributed significantly to higher alkalinity (pH of maize stover biochar 10.7) and wheat straw biochar showed lowest energy absorption in all the wave numbers at which different stretching vibrations were observed. The pearl millet stalk and rice straw biochar showed vibration energy absorption in between that observed in wheat straw and maize stover biochar. The FTIR data further confirmed the highest CEC in maize stover biochar and lowest in wheat straw biochar. The functional groups in maize stover biochar contributed significantly to the cation exchange properties. Comparable FTIR results were obtained for chemically pyrolyzed peanut hull by Saleh et al. (2012) and Ozer et al. (2007). The decomposition of hemicellulose during pyrolysis can be followed by the disappearance of the bands at 1740 to 1730 cm⁻¹ and 1230 cm⁻¹, which indicates the removal of acetyl ester groups (Schwanninger et al., 2004; Stefke et al., 2008). A marked decrease was observed for the bands in the range between 1200 and 1000 cm⁻¹, which might mark the loss of polysaccharides during pyrolysis (Kloss et al., 2012). The decrease and disappearance of the above-mentioned moieties were accompanied by increases of bands associated with vibrations of aromatic structures, such as at 3417 to 3434 cm⁻¹, 1596 to 1617 cm⁻¹, 1414 to 1448 cm⁻¹, 1089 to 1101 cm⁻¹, 802 cm⁻¹ and 617 cm⁻¹. The band at 1400 cm⁻¹ and 800 cm⁻¹ may also be caused by carbonates.
4.2. Biochar decomposition in soil

It has been proven from many studies that the addition of biochar in soil caused an immediate flush of CO2 release (Luo et al., 2011; Zimmerman et al., 2011). After the short-term flush of C mineralization on application of biochar to soil, there is evidence that biochar is stable over periods of up to thousands of years (Kuzyakov et al., 2009). Aromatic C is the recalcitrant component of biochar that provides stability to biochar in soil. On the other hand, volatile matter, a mixture of aliphatic C, carboxyl and carbohydrate, may present a relatively labile component of biochar the presence of which might be responsible for enhanced C mineralization from rice biochar as observed from our study. Nevertheless, the positive priming effect of biochar could occur if biochar acts as a source of mineralizable C and nitrogen, phosphorus and micronutrients (Chan and Xu, 2009). Zimmerman et al. (2011) reported positive priming effect of biochar produced from grasses at low temperatures (250 and 400 °C) applied in soils of lower organic C content during early incubation stage (90 d) and negative priming effect of biochar produced from hard wood at high temperatures (525 and 650 °C) applied in soils of high organic C during later incubation stage (250 to 500 d). Recently, Prayogo et al. (2014) showed that 2% of biochar application rate which was equivalent to 60 t ha⁻¹, repressed mineralization of native organic matter by 10%, and added litter by 20%. Our study showed that the native soil C mineralization could decrease by 2%, 2% and 8% in soil treated with pearl millet stalk, wheat straw and maize stover biochar at 0.9%, respectively. In the case of rice straw biochar 5% of the biochar C was respired. Zavalloni et al. (2011) reported that only 2.8% of wheat straw biochar was respired over 84 day incubation experiment. Similarly a positive and negative priming effect was also reported by others (Spokas and Reicosky, 2009; Cross and Sohi, 2011). The maize stover biochar being stronger in structural surface functional groups including aromatic C=C stretching as obtained through FTIR analysis which added higher stability than other biochar with special reference to wheat straw biochar which showed weak stretching vibration energy. The stability of the biochar in soil is related not only to its chemical compositions but also to the C contents. Yang et al. (2014) reported

![Fig. 3. Top: total carbon mineralization (CO2 efflux) in soil amended with various biochar (BC) materials, the bars with different lower case letters are significant according to Duncan’s multiple range test at P = 0.05. Bottom: changes in carbon mineralization (CO2 efflux) from soil with BC compared to the respective control treatments without BC addition. Error bars show standard errors (n = 4).](image-url)
that increased stability of corn and bamboo biochar in soil was observed with increase in C contents. Besides, the presence of higher alkalinity in maize biochar might have adversely affected the heterotrophic microbial population responsible for C mineralization process and thus showed reduced mineralization in the later stage of incubation. The other possibility speculated would be sorption of extracellular enzymes onto the biochar resulting in their removal from sites of organic matter turn over and release of soluble C from the biochar which acts as a preferential C source for the soil microbiota, thereby reducing utilisation of soil organic C (Jones et al., 2011). Thus total C mineralization over one year period was observed lowest in maize stover biochar followed by wheat straw biochar and pearl millet stalk biochar showed comparable C mineralization which was at par with the control treatment. The increased stability of biochar C is translated into enrichment of organic C contents in soil. Therefore, maize stover biochar being more stable in soil showed higherTSC. Wheat biochar was also at par with maize stover biochar in terms of C enrichment in soil. This might be due to higher C input from wheat straw biochar which was richer in C than maize stover biochar. Haeftelea et al. (2011) reported that the application of carbonized rice husks increased total organic C in rice growing soil of IRRI, Philippines. Prabha et al. (2013) reported that the application of rubber tree biochar at 16 t ha\(^{-1}\) increased the SOC concentration which was about 11.1% more than the control. Borchard et al. (2014) reported that historical charcoal production sites were enriched with BC and also exhibited increased stocks of natural SOC and total N possibly due to enhanced stabilization of natural SOC by the charcoal.

4.3. Biochar and microbial activities

The soil microbial biomass is the collective mass of all soil microorganisms, e.g. bacteria, fungi, protozoa (Jenkinson and Ladd, 1981). It has an important role in nutrient cycling and is therefore essential for plant growth (Brookes, 2001). Fumigation extraction (FE) is a well proven method to determine microbial biomass C (Vance et al., 1987b), nitrogen (N) (Brookes et al., 1985) and ninhydrin-N (nin-N) (Joergensen and Brookes, 1990), all of which are significantly correlated (Joergensen and Brookes, 1990). However, addition of activated charcoal to soil resulted in a significant decrease in K\(_2\)SO\(_4\) extractable C and ninhydrin-N in all three soils, whereas the addition of biochar generally did not (Durenkamp et al., 2010). Liang et al. (2010) suggested a correction factor 'E' to adjust the extraction efficiency factor 'k' when using soils with elevated amounts of biochar, to account for extra CHCl\(_3\)-released C sorbed by the biochar. In order to avoid the above limitation, substrate induced respiration put forward by Anderson and Domsch (1978) was used in our study. Our study clearly showed that the addition of biochar increased the microbial biomass C in soil. Kuzyakov et al. (2009) reported the incorporation of biochar into microorganisms after 624 days of incubation amounted to 2.6 and 1.5% of \(_{14}\)C input into soil and losses, respectively. Rice biochar being relatively more labile as ascertained in C mineralization study supported higher microbial biomass. Zavalloni et al. (2011) on the other hand reported that biochar prepared from hardwood pyrolysed at 500 °C did not influence microbial biomass or soluble organic N in soil. Schomberg et al. (2012) reported that the mineralizable C did not increase indicating that biochar addition did not stimulate microbial biomass. The labile fraction possibly supported the higher CO\(_2\) emission and higher microbial biomass in rice straw biochar. Bruun et al. (2012) reported the presence of unpyrolyzed carbohydrate fractions in wheat straw biochar which also fuelled the microbial population to increase. On the contrary, wheat straw and maize stover biochar being resistant as evident through the biochemical characterisation and biochar stability study maintained lesser MBC throughout the incubation period. As there was a spurt in the proliferation of microbes due to biochar amendment, the dehydrogenase activity increased significantly. Rice straw biochar which supported higher microbial biomass also showed enhanced dehydrogenase activity, while the maize stover biochar showed the reverse trend. However, a small pool of labile C can be present in fresh biochar depending on the pyrolysis technology applied (Bruun et al., 2012). Such labile biochar C may cause a short-lived increase in soil microbial metabolism immediately after biochar application (Kuzyakov et al., 2009; Smith et al., 2010; Farrell et al., 2013). However, alkaline phosphomonoesterase activities increased significantly due to the application of wheat straw biochar in soil. Rice straw biochar though supported higher dehydrogenase activity vis-à-vis higher microbial biomass but the alkaline phosphomonoesterate activity was low in this treatment. It is speculated that probably the p-nitrophenol released due to break down of p-nitrophenyl phosphate as a substrate was adsorbed more by the rice biochar than wheat straw biochar. From a 10-week laboratory study with biochar from sewage sludge, Paz-Ferreiro et al. (2012) likewise concluded that aroylsulfatase activity (ASA) increased from 26.4 μg NP g\(^{-1}\) h\(^{-1}\) in reference soils to 32.0 and 36.2 μg NP g\(^{-1}\) h\(^{-1}\) in soil with 4 and 8% biochar (w/w), respectively, though these increases were not statistically significant. However, Sun
et al. (2014) reported that the field plots with cumulative biochar rates of up to 100 Mg ha$^{-1}$, applied during two consecutive years, substantiated that biochar was not inhibitory to ASA as reference plots consistently showed lowest activities. The overall changes in biochemical properties could possibly be due to biochar induced different chemical changes such as increased pH, dissolved organic carbon and total carbon and nitrogen in soil and changed the microbial community structure (Muhammad et al., 2014).

4.4. Biochar and available nutrients

Available N, P and K contents in soil increased significantly in biochar amendment. The wheat biochar showed higher increase in available N and K, while maize stover biochar showed higher increase in available P content in soil. Maize stover and wheat straw biochar being richer in N content might increased available N in soil. Similarly, wheat straw biochar being richer in K also increased the available K in soil. Among the nutrients (N, P, K), the maximum increase in available pool due to biochar application was observed in the case of K. This might be due to greater contribution of biochar towards nutrient element in water soluble pools. Potassium, being a non-structural element in plant biochar contributed the most in the above pool. In this regard, Parvage et al. (2013) reported a significant increase in water-soluble P in acidic silt loam, clay loam, and an intermediate loam soils treated with 1% wheat biochar. Haefelea et al. (2011) reported that the application of carbonized rice husks increased total N, available P and K in rice growing soil of IRRI, Philippines. Hao et al. (2011) reported that the amount and rate of P adsorption on the surface of ferrihydrite decreased with the presence of biochar and the desorbability of adsorbed P on ferrihydrite can be enhanced when combined with biochar. The application of poultry litter biochars at 2% (w/w) increased Mehlich 1 soil extractable P concentrations between 20- and 28-folds, while extractable Na increased between 99- and 145-folds (Novak et al., 2009). Similar property changes occurred to between 20- and 28-folds, while extractable Na increased between 99- and 145-folds (Novak et al., 2009). Similar property changes occurred to between 20- and 28-folds, while extractable Na increased between 99- and 145-folds (Novak et al., 2009). Similar property changes occurred to 145-folds (Novak et al., 2009). Similar property changes occurred to between 20- and 28-folds, while extractable Na increased between 99- and 145-folds (Novak et al., 2009). Similarly, wheat straw biochar being richer in K could be used in crops with higher requirement for K.

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References


Table 2

<table>
<thead>
<tr>
<th>Biomass</th>
<th>DHA (µg TPF g$^{-1}$ 24 h$^{-1}$)</th>
<th>MBC (mg kg$^{-1}$)</th>
<th>APA (µg pNP g$^{-1}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days 21</td>
<td>45</td>
<td>67</td>
</tr>
<tr>
<td>Maize</td>
<td>130 ± 0.42d</td>
<td>186 ± 0.40b</td>
<td>131 ± 0.25c</td>
</tr>
<tr>
<td>Pearl millet</td>
<td>134 ± 0.68c</td>
<td>209 ± 0.41a</td>
<td>131 ± 0.40c</td>
</tr>
<tr>
<td>Rice</td>
<td>155 ± 0.52a</td>
<td>208 ± 0.49a</td>
<td>155 ± 0.42a</td>
</tr>
<tr>
<td>Wheat</td>
<td>141 ± 0.50b</td>
<td>208 ± 0.55a</td>
<td>134 ± 0.34b</td>
</tr>
<tr>
<td>Control</td>
<td>125 ± 0.40e</td>
<td>173 ± 0.35c</td>
<td>118 ± 0.19c</td>
</tr>
</tbody>
</table>

Values (mean ± standard error) in each column followed by different lower case letters are significant according to Duncan’s Multiple Range Test at P = 0.05.


