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Influence of biochar application on growth of *Oryza sativa* and its associated soil microbial ecology

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Abstract

In this study, biochar was produced from three biomass feedstocks such as fruits of *Cassia fistula* and *Caesalpinia* sp. and barks of *Eucalyptus globulus*. The samples of the obtained biochar were characterized for pH, physiochemical properties, surface morphology, and surface functional groups. The obtained biochar samples were further studied with/without the combination of urea for their plant growth enhancement properties including the germination studies and effect on shoot and root growth of rice plants. Biochar produced from *C. fistula* fruits at 1.5% concentration increased the plant shoot height 18% higher than the control plants. *Eucalyptus* sp. barks' biochar application at 0.5% concentration also increased the plant shoot height 12% longer than the control. However, the biochar produced from *Caesalpinia* sp. did not increase the shoot length. Yet, a generalized increase in root length was observed with the application of biochar. The combined application of nitrogen fertilizer (urea) and biochar together reverted the effect of biochar on the shoot length increase. Phospholipid-derived fatty acid (PLFA) characterization of soil revealed that soil biota shifts when soil was supplemented with the biochar. The bacterial community increased and a loss of fungal community was observed with the application of biochar.

Keywords Biochar · Agro residues · PLFA · Gasifier · Biomass feedstocks · Soil ecology

1 Introduction

Soil and crop management practices in agriculture such as fertilization, crop rotation, and land-use change exert a considerable influence on the soil chemical and biological properties over time. Continuous use of inorganic fertilizer increases nutrient availability to plants, but also at the same time, they can also affect soil microorganisms [1]. Soil microbial biomass is essential for nutrient cycling as well for soil formation, and thus, the soil microbes indirectly affect the soil quality. The use of the chemical fertilizers increases the soil efficiency but also affects the soil quality along with it [2].

Carbon sequestration is the carbon entrapment process, leading to the reduced carbon concentration in the atmosphere. One of the viable methods of the carbon sequestration is the biochar supplementation to the agricultural land [3]. The deposition of biochar into agricultural soil seems to confer several benefits on soil quality. Laboratory research [4] and historical findings [5] indicate that the incorporation of biochar into the soil pose demonstrable benefits in increasing soil fertility. In conventional agricultural practices, lime is applied to the soil to ameliorate the alkalinity [6]. However, alkaline nature of biochar could confer the soil alkalinity, thereby reducing the level of lime application. Lee et al. [7] have shown that the functional groups change the pyrolysis of biomass which can increase the alkalinity of the biochar.

Soil organic matter (SOM) can increase the crop productivity by improving the soil fertility and water holding capacity. However, SOM has a very less half-life and can be degraded fast [8, 9]. On the other hand, biochar has higher half-life than the SOM and can stay in the field for a much more extended period [10]. Furthermore, biochar application to the soil can increase the pH and cation exchange capacity (CEC)

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which are many preferred characteristics for plant growth enhancement [11].

In the tropical regions' agricultural practices, biochar produced from sugarcane filter cake enhanced the soil fertility [12]. Biochar produced from straw biomass has increased the available water holding capacity by 17–42% of soil and enhance the shoot and root growth by 40–165 and 50–57%, respectively, on Spring Barley (*Hordeum vulgare* L.) [13]. Hydrochar application has resulted in the better growth of soya bean plants and also showed to increase the plant growth-promoting bacteria *Rhizobacteria* in the soil rhizosphere [14]. A recent review by Rangabhashiyam and Balasubramanian [15] provides an insight on overall prospectives of various lignocellulosic biomass precursors for biochar production as well modification procedures to enhance the biochar properties are also highlighted.

Biochar may enhance the soil fertility and nutrient availability to plants but also affects the soil microbial biota [16]. Biochar has a wide range of physicochemical [17] as well as organochemical properties, which can affect the soil microbial ecology [18]. Phospholipid fatty acid (PLFA) analysis is a widely used method to identify various microbial biomass compositions in soil and other environmental samples. Using the PLFA pattern to study the communal structure of soil microorganisms has become one of the most commonly used methods [19]. The PLFA-based methods have several advantages, and some of them are rapid, inexpensive, sensitive, and reproducible [20].

The primary objective of this investigation is to expose the possibility of using biochar produced from three different biomass feedstocks on plant and shoot growth. For the production of biochar, three biochar biomass feedstocks, namely, (i) fruits of *Cassia fistula*. (ii) barks of *Eucalyptus globulus*, and (iii) fruits of *Caesalpinia* sp., were used in this investigation. The selected biomass samples were pyrolysed in top-lit up-draft (TLUD) gasifier. The obtained biochar was characterized and used to study its potential application for plant shoot and root growth enhancement. Furthermore, the soil microbial ecology was also carried out through PLFA characterization and the corresponding results are presented in this paper. The authors had earlier attempted to model the pyrolysis process using different biochemical and empirical approaches to evaluate the product yield along with its composition [21].

2 Materials and methods

2.1 Biochar production and characterization

The biomass was collected from the National Institute of Technology (NIT) Rourkela premises and segregated into three significant feedstocks, viz., (i) fruits of *C. fistula*, (ii) barks of *E. globulus*, and (iii) fruits of *Caesalpinia* sp. These

feedstocks were pyrolysed individually in the TLUD reactor system, and the biochars were collected separately. TLUD had been initially designed in SolidWorks 2015 platform and subsequently fabricated using the simple carpentry and welding equipment from the central workshop of the campus. The primary air outlet can be adjusted to provide control over the supply of primary air and availability of oxygen. The dry feedstocks were loaded onto the reactor, and with the help of gasoline, the feedstock is lit with the supply of air (oxygen) through the primary air inlet. Air exchange is adjusted through the primary and secondary air exchangers. Once the required heat is generated, both the airlets are shut to cut off the air exchanges for the biochar production. Before the biomass turns into ash, the fire is turned off to keep the char, and later, the produced biochars were collected from the top of the TLUD reactor system.

A well-crushed powder biochar (0.3 g) was suspended in 30 ml of deionized water and subjected to shaking for 30 min. It was then centrifuged at 5000 rpm for 5 min and the supernatant was taken to measure the parameters such as pH, electrical conductivity (EC), total dissolved solids (TDS), and salinity. The surface morphology of the biomass and biochar was analyzed using the Quanta FEG250 Environmental Scanning Electron Microscope (ESEM analysis). Fourier transform infra-red (FTIR) spectroscopy analysis was carried out to analyze the functional groups present on the biochar surface. The FTIR spectra were obtained using a PerkinElmer spectrum instrument. The surface area of the biochars was obtained using a Quantachrome BET surface area analyzer.

2.2 Plant growth study

The effect of biochar application on the preliminary vegetative plant growth was tested on a glasshouse experiment. The experimental setup consisted of growth pots with growth media filled up at various treatments or concentrations. The plant growth media was prepared in such a way to estimate the effect of biochar on the preliminary vegetative growth of plants. Crop species chosen to study the effect of biochar was *Oryza sativa* (rice plant). Various combinations of growth media were prepared to study the effect of fertilizer, biochar, and also the various combinations of both.

Soil material was obtained from an agricultural field nearby the NIT Rourkela campus with the coordinates 22° 15′ 24.9″ N and 84° 54′ 42.3″ E. The soil material was found to be clay nature. The pH of soil was found to be 7.416 with the electrical conductivity of 76.9 μS. The cation exchange capacity of the soil through ammonium displacement method was found to be around 48.9 meq/100 g of soil. This soil was mixed with various biochar and fertilizer treatments for the plant growth studies. Biochar produced from all the three types of feedstock were utilized for the study. Biochar applications (0.5, 1.0, 1.5, and 2.0% (*w/w*)) with and without the combination of nitrogen

fertilizer (urea) were supplemented to study the effect on the preliminary vegetative plant growth. Amount of urea to be supplemented was kept constant at 22 kg/ha and accordingly calculated for the study as 0.014 g/100 g of soil with the depth of 10 cm. Table 1 gives the experimental design undertaken to study the effects of biochar, fertilizer, and the combination of both. In this investigation, three trials were conducted for each combination.

Table 1 Experimental design for the plant growth study

Treatments	Description	Experiments
Control	The plants are grown only on soil and no additional supplements are given	Soil
Soil + fertilizer	The plants are grown in the soil supplemented with urea at the rate of 0.014 g/100 g of soil	Soil + 0.014 g urea
Soil + biochar	The plants are supplemented with three types of biochars separately at the concentrations of 0.5, 1, 1.5, and 2.0% supplementation	Soil + 0.5% *Cassia* biochar
		Soil + 0.5% *Eucalyptus* biochar
		Soil + 0.5% *Caesalpinia* biochar
		Soil + 1.0% *Cassia* biochar
		Soil + 1.0% *Eucalyptus* biochar
		Soil + 1.0% *Caesalpinia* biochar
		Soil + 1.5% *Cassia* biochar
		Soil + 1.5% *Eucalyptus* biochar
		Soil + 1.5% *Caesalpinia* biochar
		Soil + 2.0% *Cassia* biochar
		Soil + 2.0% *Eucalyptus* biochar
		Soil + 2.0% *Caesalpinia* biochar
Soil + fertilizer + biochar	The plants are grown in the soil supplemented with urea at the rate of 0.014 g/100 g of soil and biochars applied separately at the concentrations of 0.5, 1, 1.5, and 2.0% as a supplement to the plants	Soil + 0.014 g urea + 0.5% *Cassia* biochar
		Soil + 0.014 g urea + 0.5% *Eucalyptus* biochar
		Soil + 0.014 g urea + 0.5% *Caesalpinia* biochar
		Soil + 0.014 g urea + 1.0% *Cassia* biochar
		Soil + 0.014 g urea + 1.0% *Eucalyptus* biochar
		Soil + 0.014 g urea + 1.0% *Caesalpinia* biochar
		Soil + 0.014 g urea + 1.5% *Cassia* biochar
		Soil + 0.014 g urea + 1.5% *Eucalyptus* biochar
		Soil + 0.014 g urea + 1.5% *Caesalpinia* biochar
		Soil + 0.014 g urea + 2.0% *Cassia* biochar
		Soil + 0.014 g urea + 2.0% *Eucalyptus* biochar
		Soil + 0.014 g urea + 2.0% *Caesalpinia* biochar

All set of experiments were conducted in triplicates

The pots were sowed with the rice seeds and incubated in the controlled conditions for 4 weeks. Incubation conditions were 24 ± 2 °C temperature, 85% humidity, and 2000–4000 lx light intensity with 16/8 photoperiodism (16 h light and 8 h of darkness). Germination percentage was calculated on the fourth day after sowing. Shoot and root lengths were used as the plant growth indicators to study the effect of biochar and the biochar-fertilizer combination. The shoot and root lengths were measured after 4 weeks of incubation.

2.3 Soil microbial study

To understand the effect of biochar on soil microbial community, analysis of PLFAs was carried out. The soil microbial ecology was studied after 20 days of the incubation period for the control soil and biochar-amended soil in which the plants were grown. In this study, the procedure described by Buyer and Sasser [20] was followed in carrying out the soil microbial ecology study. The gas chromatography-mass spectroscopy (GCMS) analysis to quantify the PLFAs was done on the HP5MS column, and the gas used for mobile phase was the mixture of nitrogen-helium. Table 2 provides the fatty acid markers used in the study to categorize the identified PLFAs corresponding to different organisms. These fatty acid markers were used to understand the soil microbial community and the effect of biochar on the soil microbial community. Soil ecology characterization through PLFA pattern was carried out only on the best positive results obtained from the plant growth studies on each biochar combination.

3 Results and discussion

3.1 Physical properties of biochar

The physiochemical properties of the biochar are provided in Table 3. The pH, EC, TDS, and salinity of the biochar generally depend on the biomass feedstock and the pyrolysis temperature. The pH of all the three types of biochars was found

Table 2 PLFA markers used in the current study

S. no.	Fatty acid type	Biomarker group
1	Iso and anti-iso saturated branched fatty acids	Gram-positive bacteria
2	Monounsaturated fatty acids and cyclopropyl 17:0 and 19:0 fatty acids	Gram-negative bacteria
3	10-Methyl fatty acids	Actinobacteria
4	18:2 ω6 fatty acids	Fungi
5	20:3 and 20:4 fatty acids	Protozoa
6	15:0, 17:0 cyclo, 19:0 cyclo, 15:1 iso, 17:1 iso and anti-iso fatty acids	Eubacteria
7	Poly unsaturated fatty acids	Eukaryotes

to be alkaline as indicated in Table 3. It can also be inferred from Table 3, which the biochar produced from the fruits of *C. fistula* has the increased EC, salinity, and TDS than the biochar produced from the other two biomass feedstocks. The cation exchange capacity of the biochar from *Cassia* sp. fruits, *Eucalyptus* bark, and *Caesalpinia* sp. fruits was found to be 82.6 meq/100 g, 55.8 meq/100 g, and 7.80 meq/100 g of sample, respectively. Higher salinity and TDS and CEC could be directly correlated with the higher available mineral content. It shows that the biochar produced from *Cassia* sp. fruits had higher dissolved and exchangeable ions which could improve the plant growth.

3.2 Surface area analysis

The surface area and total pore volume of the biochars were studied using the Brunauer–Emmett–Teller (BET) analysis and the results are provided in Table 3. It can be observed from Table 3 that the biochar produced from the bark of *E. globulus* had a higher surface area (163m^2/g) and total pore volume (0.4576cm^2/g). This value is higher than the already reported value of 5–25 and 8–60 m^2/g by Mohammadi et al. [22] and Brewer et al. [23], respectively. However, the surface area of the biochars produced from the fruits of *C. fistula* and *Caesalpinia* sp. had the surface area around 70 and 78 m^2/g which is almost equal to the reported value of Brewer et al. [23] on biochar.

3.3 Surface morphology

The surface morphology of the selected feedstock and the obtained biochars are depicted in Figs. 1 and 2, respectively. This surface morphology was observed using the ESEM. The feedstocks showed superficial structures with the ordered arrangements of cells over the surface without any porous structures. However, the biochars exhibited porous structure irrespective of the biomass feedstock pyrolysed, which is exhibited in Fig. 2. However, the shape of the porous structure varied among the biochars depending upon the biomass. Biochar produced from the fruits of *C. fistula* had the oval-shaped porous structure, whereas the polygonal-shaped porous structure was observed in the biochar produced from the barks of *E. globulus*. Longitudinal porous structures were observed in the biochar produced from fruits of *Caesalpinia* sp. Furthermore, a lot of interconnecting microporous channels were observed in all the biochars irrespective of the feedstock type. Similar type of microporous structure was earlier reported by Hilioti [24] in the castor plant-derived biochar. This porous structure could have acted as the release route for the gases to vaporize during the pyrolysis process [25].

Table 3 Physicochemical analysis of the biochar produced from three types of biomass feedstocks

| Type of biomass used for the production of biochar	pH	EC (μS)	TDS (ppm)	Salinity (ppt)	BET surface area (m²/g)	Total pore volume (cm³/g)	CEC (meq/ 100 g)
Fruits of *Cassia fistula*	9.89 ± 0.11	1672 ± 5.86	840 ± 7.02	0.82 ± 0.06	70.22 ± 0.95	0.2597 ± 0.0756	82.60 ± 0.10
Barks of *Eucalyptus globulus*	8.34 ± 0.07	937 ± 7.64	469 ± 1.53	0.41 ± 0.12	163.77 ± 1.07	0.4576 ± 0.0667	55.83 ± 0.15
Fruits of *Caesalpinia* sp.	9.23 ± 0.08	484 ± 2.52	243 ± 5.03	0.17 ± 0.10	78.28 ± 0.57	0.7245 ± 0.0646	7.80 ± 0.06

3.4 Surface functional group analysis

The FTIR analysis of the selected biomass feedstocks in this study and the obtained biochar were carried out and presented in Fig. 3. The FTIR analysis was done to identify the surface functional groups present in the biomass feedstocks and the obtained biochar and to understand the functional group changes that took place during the pyrolysis process.

A comparative observation between biomass feedstock and the obtained biochar revealed that specific peaks diminished gradually in the obtained biochar, irrespective of the biomass feedstock. This trend of diminishing or decrease in the intensity of the peaks was also observed and reported by Li et al. [26]. In the biochar produced from the bark of *Eucalyptus* sp. and *Caesalpinia* sp. fruits, the peak corresponding to the hydroxyl group (-OH) (3400–3000 cm^{-1}) found to be decreased in intensity than the biomass samples after pyrolysis. However, in the biochar produced from the fruits of *Cassia* sp., the sharpness of peak corresponding to hydroxyl group got increased. The concentration symmetric nitro groups in the biochar produced from the fruits of *C. fistula* were reduced (1376 cm^{-1}), while the intensity of

the asymmetric nitro group (1557 cm^{-1}) increased when compared with the biomass samples. The peak corresponding to the carbonyl functional group (–CO) (1315 cm^{-1}) had marginally increased intensity in the biochar produced from *C. fistula* fruits than the biomass samples. Furthermore, aromatic ring structure was found on the biochar surface (742 cm^{-1}) which could have formed during the pyrolysis process.

In the biochar produced from the barks of *E. globulus*, the peak corresponding to the hydroxyl group was found to be widened than the biomass feedstock (3400–3000 cm^{-1}). In the eucalyptus bark feedstock, two distinct sharp peaks at 1622 and 1316 cm^{-1} corresponding to carbon-carbon double bond and the acyl/phenyl carbonyl bonds (–CO–), respectively, were observed. However, in the biochar, flattened and blunt peaks were observed between the wavenumbers 1620 and 1315 cm^{-1}. The shift in the peaks could be due to the loss of intensity in the functional groups. Furthermore, aromatic ring structure was identified through a very sharp peak at 780 cm^{-1} in the biochar produced from barks of *Eucalyptus* sp. which shows the increased aromaticity on the biochar surface.

Fig. 1 Surface morphologies of the biomass feedstocks of **a** fruits of *Cassia fistula*, **b** bark of *Eucalyptus globulus*, and **c** fruits of *Caesalpinia* sp.
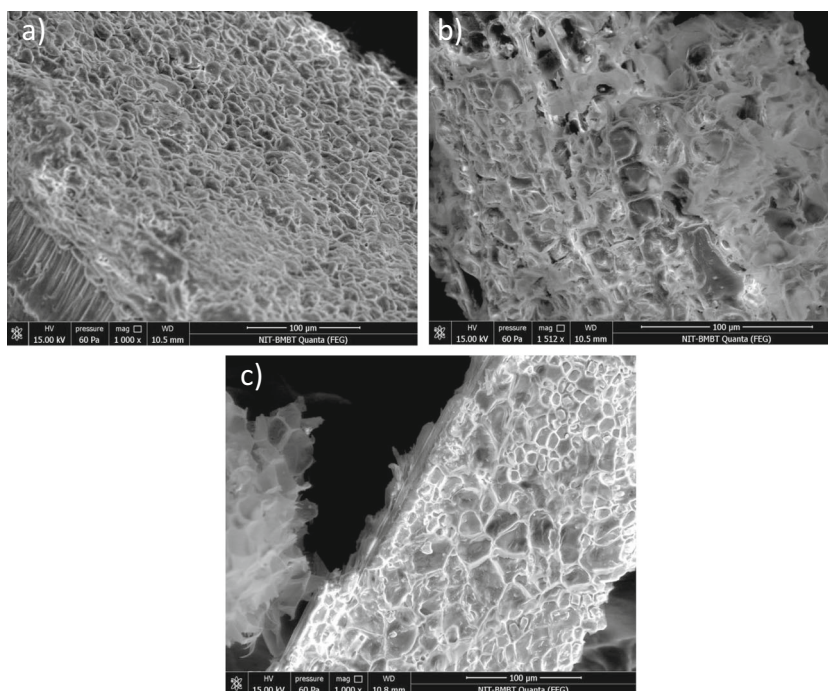
Fig. 2 Surface morphologies of the biochar produced from **a**, **b** fruits of *Cassia fistula*, **c**, **d** bark of *Eucalyptus globulus*, and **e**, **f** fruits of *Caesalpinia* sp.
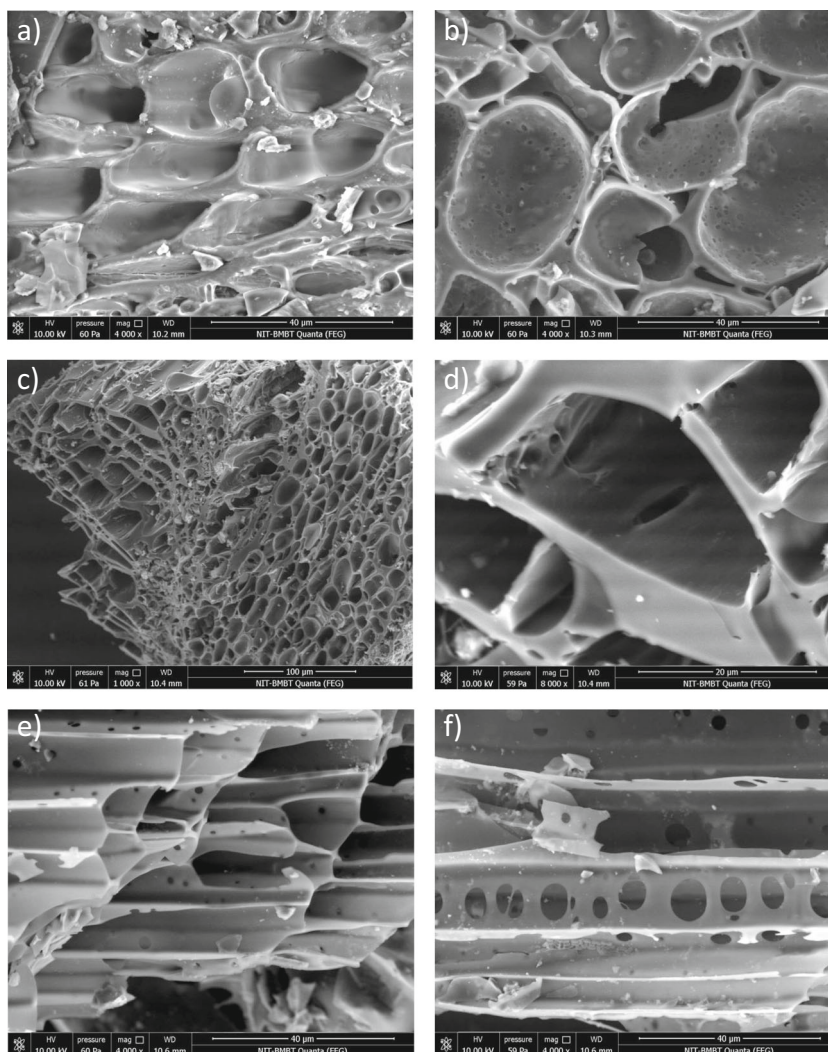
In the biochar produced from *Caesalpinia* fruits, mostly the functional groups were observed to be decreased or lost. The hydroxyl group concentration on the biochar surface was found to be very less than the biomass, and a general loss of nitrogen containing functional groups was also observed, namely, nitro group (1384 cm^{-1}) and carbon-nitrogen bond (1246 cm^{-1}). A decrease in the alkoxy group (1054 and 1125 cm^{-1}) intensity on the biochar surface.

A loss or decrease in the intensity of the peaks in the biochar, after the biomass pyrolysis process, was observed and already been reported by Yuan et al. [27]. Formation of aromatic rings on the surface of biochar was also reported by Weber and Quicker [28]. This aromatic ring structure formation could have resulted due to the decreased hydrogen/carbon and oxygen/carbon ratio in the biochar.

3.5 Effect of biochar on plant growth

Effect of the separate application of biochar and the biochar-fertilizer combination on the plant growth was studied in the glasshouse study over a period of 4 weeks. The effect on seed germination by the various treatments was measured after 4 days. The growth indicators, namely, the shoot height and the root length were measured after the 4 weeks.

3.6 Biochar effect on seed germination

Four days after sowing rice seeds, seedling growth was evaluated. During the study, no evidence was found that the biochar or the biochar-fertilizer combination was affecting the overall germination percentage of the rice seeds. Similar kind of result has already been reported that biochar does not affect the seed germination percentage of the tomato plant [29]. However, in some cases, the positive effect of biochar on the seed germination percentage and early germination was also reported [29]. Thus, the effect of biochar on the germination percentage and early germination of plant seeds needs to be studied and assessed individually.
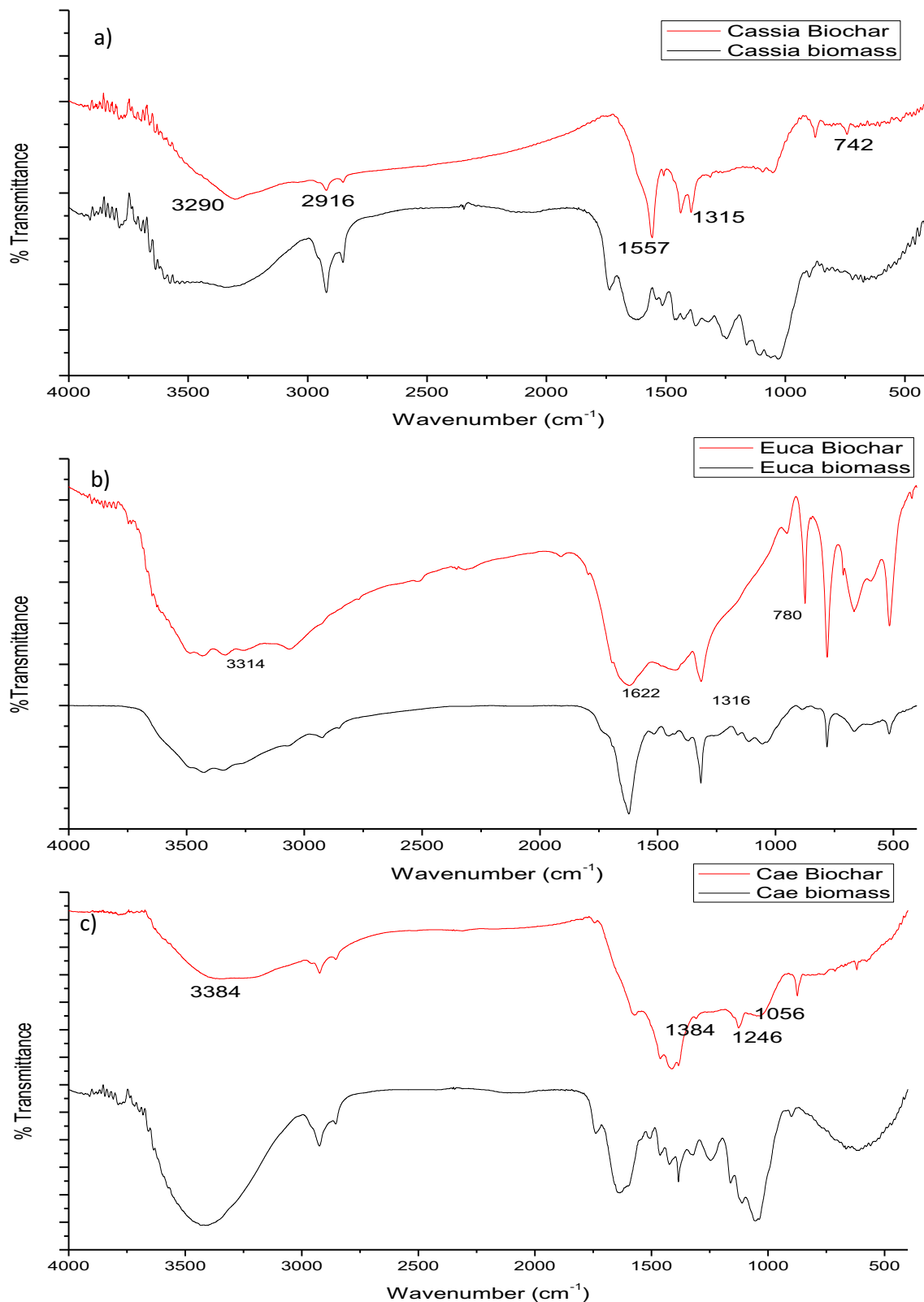
Fig. 3 FTIR spectra for various biomass feedstocks and biochars produced from the feedstocks **a** fruit of *Cassia fistula*, biochar produced from fruit of *Cassia fistula*; **b** bark of *Eucalyptus globulus*, biochar produced from bark of *Eucalyptus globulus*; and **c** fruit of *Caesalpinia* sp., biochar produced from fruit of *Caesalpinia* sp.

However, after 4 days of germination, the effect of the biochar and the biochar-fertilizer combination was observed on the root length of the plants. Plants supplemented with only biochar produced from the fruits of *C. fistula*, and the

bark of *Eucalyptus* showed an increased root length than the control plants. Root length of the rice seedlings nearly increased thrice than the control plants, when the plants were supplemented with the *C. fistula* fruit biochar. When the *Eucalyptus* bark biochar was supplemented to the seedlings, five times increased root length than the control seedlings was observed. Vaughn et al. [30] also showed the increased root length with the application of biochar in bentgrass species. However, the effect of *Caesalpinia* fruits biochar on the root length was not as comparable to the control plants.

3.7 Biochar effect on the seedlings—above- and belowground growth

After the incubation period of 4 weeks, the shoot and the root lengths were measured to understand the effect of various biochar, nitrogen fertilizer, and the combinations employed. Figure 4 shows the effect of various treatments on the plant growth.

Application of biochar produced from the fruits of *C. fistula* significantly increased the shoot and root lengths
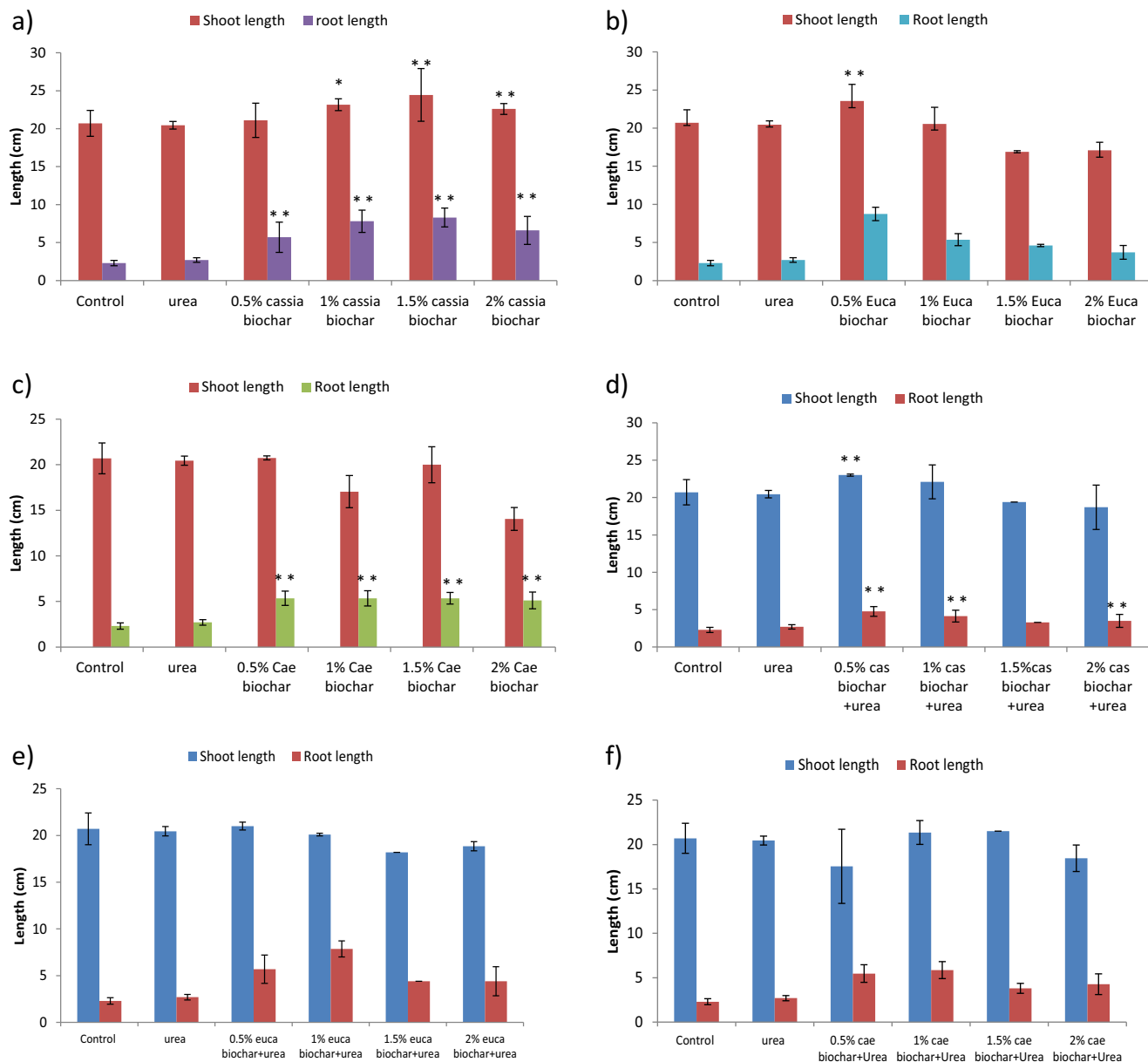
Fig. 4 Shoot and root lengths of various treatments employed. **a** Mean shoot and root lengths of rice plants supplemented with biochar produced from *Cassia* fruits. **b** Mean shoot and root lengths of rice plants supplemented with biochar produced from *Eucalyptus* bark. **c** Mean shoot and root lengths of rice plants supplemented with biochar produced from *Caesalpinia* fruits. **d** Mean shoot and root lengths of rice plants supplemented with biochar produced from *Cassia* fruits and fertilizer. **e** Mean shoot and root lengths of rice plants supplemented with biochar produced from *Eucalyptus* bark and fertilizer. **h** Mean shoot and root lengths of rice plants supplemented with biochar produced from *Caesalpinia* fruits and fertilizer. Single asterisk indicates significance difference at $p < 0.1$. Two asterisks indicate significance difference at $p < 0.05$

of the rice seedlings till the application of 1.5% biochar concentration. The plants supplemented with 1.5% *Cassia* fruits biochar increased the shoot length 18% more than the control plants and a significant fourfold increase in the root length which is shown in Fig. 4a. The plants supplemented 0.5% with the *Eucalyptus* bark biochar significantly increased the shoot length over 12% than the control plants. The root length of the plants was also increased fourfold at 0.5% biochar (*Eucalyptus* bark) application as depicted in Fig. 4b. Application of biochar produced from the *Caesalpinia* fruits did not affect the shoot length at a lower concentration. However, supplementing the plants with a higher concentration of biochar produced from the *Caesalpinia* fruits decreased the shoot height which is depicted in Fig. 4c. Only twofold increase in the root length was observed when the rice seedlings were supplemented with the *Caesalpinia* fruits biochar, which is not comparable to the other two biochar.

Application of biochar has effects on the soil physiochemical characteristics [11]. The changes could be in the pH, electrical conductivity, salinity, and CEC of the soil which can aid in plant growth. Increased porosity and higher surface area-to-volume ratio in the biochar can support the plant growth by preventing the leaching of nutrients [31]. Also higher the nutrient availability by increased CEC can also support and increase the plant growth. This implies that the availability of nutrient for the plant growth with the application of biochar could be increased. The difference in the plant growth among three types of biochars was due to the difference in electrical conductivity and salinity which could affect the soil CEC and nutrient release from the soil. Increased shoot length in the rice seedlings was observed when the *C. fistula* fruit biochar was provided as a supplement. It can be due to the significant increase of CEC, EC, and salinity and the alkaline pH of the biochar. The presence of hydroxyl and the carbonyl functional groups in *C. fistula* fruits biochar could also favored the nutrient exchange between the soil and plants in a productive way. It can be observed from Table 3 that the CEC of the *Caesalpinia* sp. fruits biochar is very low. It can also be observed from Fig. 3 that the biochar produced from the *Caesalpinia* sp. fruits lost the hydroxyl group and the nitrogen containing functional groups. The loss of nitrogen group in the biochar could affect the C/N ratio and thereby the growth of the plants [32]. Further, the drastic loss of hydroxyl group also affected the nutrient exchange between soil and the plants.

The effect of combined application of biochar and nitrogen fertilizer (urea) on the shoot growth can be observed in Fig. 4. The plant's growth on 0.5 and 1% biochar (fruits of *Cassia* sp.) and fertilizer combination showed an increase in shoot length of 12 and 7%, respectively. A significant increase of shoot length was observed with the 0.5% *Cassia* biochar and urea combination. This increase was less significant than the 18% increase in plants' shoot length with biochar (from fruits of *Cassia* sp.) supplement alone. Also, the plants supplemented with the combination of urea and biochar produced from the barks of *Eucalyptus* did not show any significant difference in shoot length from control plants. Plants grown with a combination of urea and biochar from the *Caesalpinia* fruits did not yield any positive response in the shoot length than the control plants. However, root length was observed to increase with the combination of biochar and urea application than the control and urea-supplemented plants.

Soil nitrogen content is one of the key and significant nutrients that are necessary for the growth of plants. Nitrogen from the atmosphere should undergo ammonification (NH_4^+) followed by nitrification (NO_3^-) before being utilized by plants [33]. Thus, nitrogen cycle in soil plays a major role in plant growth, and it can be affected by the application of biochar [34]. It can be observed from Fig. 4 that the better results were achieved with the application of biochar alone rather than the combination of biochar and the nitrogen fertilizer. The enhanced effect of biochar alone on plants could be explained by different factors that were involved in the nitrogen cycle and alteration in the cycle. With the addition of biochar, extractable nitrogen as nitrate got decreased which in turn could affect the overall plant growth which can be attributed to the fact that biochar has a high carbon-to-nitrogen ratio [35, 36]. Brewer et al. [23] reported that the soil amended with both the urea and biochar had lower pH value than the soil amended with biochar alone which could be due to the nitrification of urea in the soil. It was also observed by Brewer (2011) that there was no significant variation in CEC and increased EC value in the soil amended with the biochar-urea combination than the biochar alone.

The use of freshly prepared biochar could also have implied adverse effects on the nitrogen metabolism. It was reported that the application of freshly prepared biochar immobilized the mineral nitrogen [37–40]. Asai et al. [41] reported that the application of nitrogen fertilizer after the biochar application in the rice field can limit the nitrogen availability to the plants. Further, biochar with low C/N ratio and low nitrogen and inorganic (ash) content could be responsible for the nitrogen adsorption and thereby limit the plant growth [32, 42]. These could be the reasons for the nullified effect of the biochar-urea combination.

Another attribute to a negative interaction between nitrogen fertilizer and biochar being increased nitrous oxide (N_2O) and ammonia (NH_3) emission from the field [43]. It was also reported that the higher rate of biochar application significantly increased the ammonia and nitrous oxide emissions from the field. It was also reported that higher nitrous oxide emission occurred during the period of initial basal application of the fertilizer to the field along with the biochar [44]. Ameloot et al. [45] put forth an argument that volatiles present in the biochar could act as an organic carbon source for the denitrifying organisms. Biochar application also increased the abundance of ammonia-oxidizing bacteria, thus increasing

the nitrate concentration in the soil [46]. Further, the addition of nitrogen fertilizer could increase the substrate (NO_3^-) for the denitrifiers present in the soil, thereby increasing the efflux of nitrogen containing gases and loss of nitrogen to the plants.

To minimize the efflux of N_2O and NH_3, it was proposed to adopt combined application of biochar and nitrification inhibitors along with the nitrogen fertilizer [44]. By the proposed way, the loss of nitrogen by efflux can be minimized.

3.8 Soil microbial community

The change in the communal structure of soil microbial biomass was studied through PLFA characterization. The PLFA study gives an insight into the soil microbial community and how the communal structure is affected by the change in soil physicochemical characteristics. PLFA characterization was done for control soil, urea-supplemented soil, soil supplemented with 1.5, 0.5, and 0.5% biochar produced from *C. fistula* fruits, *Eucalyptus* sp. barks, and *Caesalpinia* sp. fruits, respectively, and the soil supplemented with the combination of urea and 1, 0.5, and 1% biochar produced from the *C. fistula* fruits, *Eucalyptus* sp. barks, and *Caesalpinia* sp. fruits respectively.

Figure 5 depicts the overall PLFA profile for the different treatments. In control soil, the fungal concentration was found to be the highest at 37%, and it was followed by gram-negative bacteria at 24%. Eukaryotes and eubacteria occupy the next highest inhabitants. In urea-supplemented soil, the level of gram-negative bacteria was found to be highest at 38%, followed by gram-positive bacteria at 28%. Fungi
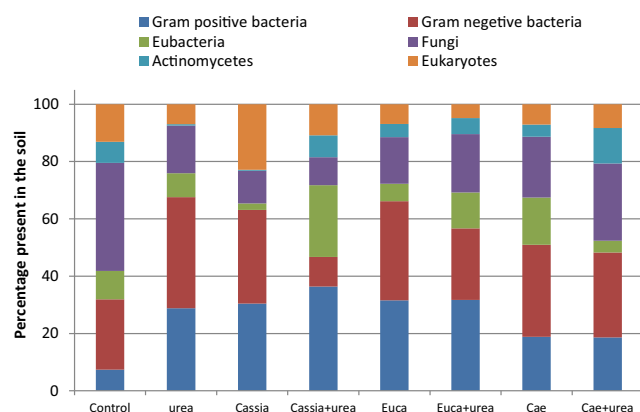
Fig. 5 PLFA characterization of various soil treatments: control soil, urea-supplemented soil, soil supplemented with only 1.5% biochar produced from *Cassia fistula* fruits, soil supplemented with the combination of urea and 1% biochar produced from *Cassia fistula* fruits, soil supplemented with only 0.5% biochar produced from *Eucalyptus* sp. barks, soil supplemented with the combination of urea and 0.5% biochar produced from *Eucalyptus* sp. barks, soil supplemented with only 0.5% biochar produced from *Caesalpinia* sp. fruits, soil supplemented with the combination of urea and 1% biochar produced from *Caesalpinia* fruits

occupied the third highest in the urea-supplemented soil, and remaining organisms constitute the rest. In the soil supplemented with the biochar produced from the *C. fistula* fruits, bacterial community occupies around 65%, and the eukaryotes occupy the next highest community. The fungal community got significantly reduced with the application of *C. fistula* fruit biochar. A similar type of community profile was obtained, when the soil was supplemented only with the application of other two biochar types.

Soil supplemented with 1% biochar produced from the *C. fistula* fruits and urea bacterial community occupies 70% of the soil ecological community and the fungi species was at the lowest compared to the other treatments. However, in the other two biochar cases, the combined application with urea increased the fungal concentration. Combined biochar-urea supplement to the soil increased fungal community composition than the supplement of biochar alone to the soil.

Several factors play a role in affecting the soil biota including, biochar physical properties, organic and inorganic composition of biochar, physical and sorption properties of the biochar, and the response of soil biota to the biochar supplement. Lehmann et al. [16] listed out the numerous factors of biochar that could have impact on soil properties such as pH, surface area, a fraction of remained organic matter, recalcitrance, surface chemical properties, CEC, as well the amount of minerals present in it. Thus, different biochars have diverse effects on the soil microbial biota based on the characteristics of biochar.

Anders et al. [47] observed that the biochar application affects the soil microbial community structure rather than affecting the total microbial biomass present in the soil. The utilization of the biochar carbon by microbes takes specific timeframe since acclimatization of microbes to biochar-amended soils needs time [48]. The change in the pH, EC, and other physicochemical characteristics that also changes the available form of carbon present in the soil could change the structure of the microbial community based on the acclimatization pace of the various microbes. The present research also observed that the shift in community structure could be the indirect impact of biochar rather than the direct effect.

The results obtained from the experiments conducted were similar to the results of Gomez et al. [49] where the gram-negative bacteria dominate the soil microbial biota rather than the fungi and gram-positive bacteria. Ameloot et al. [45] observed that efflux of CO_2 and NO_2 gases from the soil after the addition of biochar to the soil could act as the readily available substrate for the soil microbial growth. This could be one of the reasons for the increased bacterial growth in the biochar-supplemented soil. Kim et al. [50] also reported that the bacterial diversity was 25% greater in biochar-amended soil than the biochar-un-amended soil. However, soil added with the combination of nitrogen fertilizer and the biochar showed different effect on the soil microbial biota which could be

explained by different needs of the plant to form a symbiotic relationship with the microbes under the changing nutrient conditions. With the addition of nitrogen fertilizer, the plants need not rely on the biological nitrogen fixation as much as under the nitrogen limitation [16].

4 Conclusion

Biochar was successfully produced from three different biomasses, namely, fruits of *C. fistula* and *Caesalpinia* sp. and barks of *E. globulus* using a TLUD gasifier system. Samples of biochars were characterized to be porous and alkaline. There were variations in the electrical conductivity and salinity properties of the biochar which were based on the feedstock biomasses used. A general loss or change of functional groups was observed between the biomass and biochar after pyrolysis. Germination studies revealed that the biochar application on soil had increased the root length of rice plant seedlings. Biochar produced from the *C. fistula* fruits at 1.5% concentration enhanced the plant shoot height by 18%. The *Eucalyptus* sp. barks' biochar application at 0.5% concentration also enhanced the plant shoot height by 12%. The combined application of urea (nitrogen fertilizer) and biochar reverted the effects of biochar on the plant shoot growth. The probable reasons could be adsorption of nitrogen on the biochar or increased efflux of N_2O that affect the nitrogen metabolism in plants. However, an increasing trend of root length was observed with the biochar application for the concentrations considered in this study. The PLFA characterization of soil revealed that soil biota shifts when the soil is supplemented with biochar. The trend of increased bacterial community and a decreasing fungal volume was observed with the application of biochar. However, in the biochar and nitrogen fertilizer combination, the increase in fungal volume as well decline in bacterial community in the soil might be due to the secondary effect of biochar on the physiochemical characteristics of soil.

References

1. Balasubramanian P & Karthickumar P (2017) Biofertilizers and biopesticides: a holistic approach for sustainable agriculture. In: Sustainable utilization of natural resources. pp 255–284. https://doi.org/10.1201/9781315153292
2. Savci S (2012) An agricultural pollutant: chemical fertilizer. Int J Environ Sci Dev 3(1):73
3. Matovic D (2011) Biochar as a viable carbon sequestration option: global and Canadian perspective. Energy 36(4):2011–2016
4. Lehmann J, Gaunt J, Rondon M (2006) Bio-char sequestration in terrestrial ecosystems—a review. Mitig Adapt Strateg Glob Chang 11(2):403–427
5. Glaser B (2000) Persistence of soil organic matter in archaeological soils (terra preta) of the Brazilian amazon region. pp. 190–194 (No. Reserva Biblioteca/631.417 S964s). En: Sustainable management of soil organic matter. CAB International, Oxon
6. Zhang J, Lü F, Zhang H, Shao L, Chen D, He P (2015) Multiscale visualization of the structural and characteristic changes of sewage sludge biochar oriented towards potential agronomic and environmental implication. Sci Rep 5:9406
7. Lee XJ, Lee LY, Gan S, Thangalazhy-Gopakumar S, Ng HK (2017) Biochar potential evaluation of palm oil wastes through slow pyrolysis: thermochemical characterization and pyrolytic kinetic studies. Bioresour Technol 236:155–163
8. Schulz H, Dunst G, Glaser B (2013) Positive effects of composted biochar on plant growth and soil fertility. Agron Sustain Dev 33(4):817–827
9. Gerzabek MH, Pichlmayer F, Kirchmann H, Haberhauer G (1997) The response of soil organic matter to manure amendments in a long-term experiment at Ultuna, Sweden. Eur J Soil Sci 48(2):273–282
10. Kuzyakov Y, Subbotina I, Chen H, Bogomolova I, Xu X (2009) Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. Soil Biol Biochem 41(2):210–219
11. Glaser B, Lehmann J, Zech W (2002) Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. Biol Fertil Soils 35(4):219–230
12. Eykelbosh AJ, Johnson MS, de Queiroz ES, Dalmagro HJ, Couto EG (2014) Biochar from sugarcane filtercake reduces soil CO2 emissions relative to raw residue and improves water retention and nutrient availability in a highly-weathered tropical soil. PLoS One 9(6):e98523
13. Hansen V, Hauggaard-Nielsen H, Petersen CT, Mikkelsen TN, Muller-Stover D (2016) Effects of gasification biochar on plant-available water capacity and plant growth in two contrasting soil types. Soil Tillage Res 161(1–9):1–9
14. Egamberdieva D, Wirth S, Behrendt U, Abd_Allah EF, Berg G (2016) Biochar treatment resulted in a combined effect on soybean growth and a shift in plant growth promoting rhizobacteria. Front Microbiol 7:209
15. Rangabhashiyam S, Balasubramanian P (2019) The potential of lignocellulosic biomass precursors for biochar production: performance, mechanism and wastewater application—a review. Ind Crop Prod 128:405–423
16. Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota—a review. Soil Biol Biochem 43(9):1812–1836
17. Downie A, Crosky A, Munroe P (2009) Physical properties of biochar. In: Lehmann J, Joseph S (eds) Biochar for environmental management: science and technology. Earthscan, London, pp 13–32
18. Czimczik CI, Preston CM, Schmidt MW, Werner RA, Schulze ED (2002) Effects of charring on mass, organic carbon, and stable

carbon isotope composition of wood. Org Geochem 33(11):1207–1223

19. Frostegard A, Tunlid A, Baath E (2011) Use and misuse of PLFA measurements in soils. Soil Biol Biochem 43(8):1621–1625

20. Buyer JS, Sasser M (2012) High throughput phospholipid fatty acid analysis of soils. Appl Soil Ecol 61:127–130

21. Swagathnath G, Rangabhashiyam S, Parthsarathi K, Murugan S, Balasubramanian P (2019) Modeling biochar yield and syngas production during the pyrolysis of agro-residues. In: Green buildings and sustainable engineering. Springer, Singapore, pp 325–336

22. Mohammadi A, Cowie AL, Cacho O, Kristiansen P, Mai TLA, Joseph S (2017) Biochar addition in rice farming systems: economic and energy benefits. Energy 140:415–425

23. Brewer CE, Unger R, Schmidt-Rohr K, Brown RC (2011) Criteria to select biochars for field studies based on biochar chemical properties. Bioenergy Res 4(4):312–323

24. Hilioti Z, Michailof CM, Valasiadis D, Iliopoulou EF, Koidou V, Lappas AA (2017) Characterization of castor plant-derived biochars and their effects as soil amendments on seedlings. Biomass Bioenergy 105:96–106

25. Xin Y, Cao H, Yuan Q, Wang D (2017) Two-step gasification of cattle manure for hydrogen-rich gas production: effect of biochar preparation temperature and gasification temperature. Waste Manag 68:618–625

26. Li H, Dong X, da Silva EB, de Oliveira LM, Chen Y, Ma LQ (2017) Mechanisms of metal sorption by biochars: biochar characteristics and modifications. Chemosphere 178:466–478

27. Yuan JH, Xu RK, Zhang H (2011) The forms of alkalis in the biochar produced from crop residues at different temperatures. Bioresour Technol 102(3):3488–3497

28. Weber K, Quicker P (2018) Properties of biochar. Fuel 217:240–261

29. Solaiman ZM, Murphy DV, Abbott LK (2012) Biochars influence seed germination and early growth of seedlings. Plant Soil 353(1–2):273–287

30. Vaughn SF, Dinelli FD, Tisserat B, Joshee N, Vaughan MM, Peterson SC (2015) Creeping bentgrass growth in sand-based root zones with or without biochar. Sci Hortic 197:592–596

31. Laird D, Fleming P, Wang B, Horton R, Karlen D (2010) Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 158(3–4):436–442

32. Rajkovich S, Enders A, Hanley K, Hyland C, Zimmerman AR, Lehmann J (2012) Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. Biol Fertil Soils 48(3):271–284

33. Atkinson CJ, Fitzgerald JD, Hipps NA (2010) Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant Soil 337(1–2):1–18

34. Chan KY, Xu Z (2009) Biochar: nutrient properties and their enhancement. Biochar Environ Manag Sci Technol 1:67–84

35. Prasad M, Tzortzakis N, McDaniel N (2017) Chemical characterization of biochar and assessment of the nutrient dynamics by means of preliminary plant growth tests. J Environ Manag 216:89–95

36. Agegnehu G, Srivastava AK, Bird MI (2017) The role of biochar and biochar-compost in improving soil quality and crop performance: a review. Appl Soil Ecol 119:156–170

37. Ding Y, Liu YX, Wu WX, Shi DZ, Yang M, Zhong ZK (2010) Evaluation of biochar effects on nitrogen retention and leaching in multi-layered soil columns. Water Air Soil Pollut 213(1–4):47–55

38. Graber ER, Elad Y (2013) Biochar impact on plant resistance to disease. Biochar Soil Biota 278

39. Jin H (2010) Characterization of microbial life colonizing biochar and biochar-amended soils

40. Taghizadeh-Toosi A, Clough TJ, Sherlock RR, Condron LM (2012) Biochar adsorbed ammonia is bioavailable. Plant Soil 350(1–2):57–69

41. Asai H, Samson BK, Stephan HM, Songyikhangsuthor K, Homma K, Kiyono Y, Horie T (2009) Biochar amendment techniques for upland rice production in Northern Laos: 1. Soil physical properties, leaf SPAD and grain yield. Field Crop Res 111(1–2):81–84

42. Chan KY, Van Zwieten L, Meszaros I, Downie A, Joseph S (2008) Agronomic values of greenwaste biochar as a soil amendment. Soil Res 45(8):629–634

43. Sun H, Lu H, Chu L, Shao H, Shi W (2017) Biochar applied with appropriate rates can reduce N leaching, keep N retention and not increase NH3 volatilization in a coastal saline soil. Sci Total Environ 575:820–825

44. He T, Liu D, Yuan J, Luo J, Lindsey S, Bolan N, Ding W (2018) Effects of application of inhibitors and biochar to fertilizer on gaseous nitrogen emissions from an intensively managed wheat field. Sci Total Environ 628:121–130

45. Ameloot N, De Neve S, Jegajeevagan K, Yildiz G, Buchan D, Funkuin YN, Sleutel S (2013) Short-term CO2 and N2O emissions and microbial properties of biochar amended sandy loam soils. Soil Biol Biochem 57:401–410

46. Lin Y, Ding W, Liu D, He T, Yoo G, Yuan J, Fan J (2017) Wheat straw-derived biochar amendment stimulated N2O emissions from rice paddy soils by regulating the amoA genes of ammonia-oxidizing bacteria. Soil Biol Biochem 113:89–98

47. Anders E, Watzinger A, Rempt F, Kitzler B, Wimmer B, Zehetner F, Stahr K, Zechmeister-Boltenstern S, Soja G (2013) Biochar affects the structure rather than the total biomass of microbial communities in temperate soils. Agric Food Sci 22(4):404–423

48. Watzinger A, Feichtmair S, Kitzler B, Zehetner F, Kloss S, Wimmer B et al (2014) Soil microbial communities responded to biochar application in temperate soils and slowly metabolized 13C-labelled biochar as revealed by 13C PLFA analyses: results from a short-term incubation and pot experiment. Eur J Soil Sci 65(1):40–51

49. Gomez JD, Denef K, Stewart CE, Zheng J, Cotrufo MF (2014) Biochar addition rate influences soil microbial abundance and activity in temperate soils. Eur J Soil Sci 65(1):28–39

50. Kim JS, Sparovek G, Longo RM, De Melo WJ, Crowley D (2007) Bacterial diversity of terra preta and pristine forest soil from the Western Amazon. Soil Biol Biochem 39(2):684–690