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Depth-dependent Influence of Biochar Application on the Abundance and Community Structure of Diazotrophic Under Sugarcane Growth

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Abstract

Despite progress in understanding diazotrophic distribution in surface soils, few studies have investigated the distribution of diazotrophic in dipper soil layers. Here, we leveraged high-throughput sequencing (HTS) of nifH genes obtained to assess the influence of biochar amended soil (BC) and control (CK), and soil depths (0-20, 20-40 and 40-60 cm) on diazotrophic abundance and community structures, and soil biochemical properties. Although soil physio-chemical properties, enzymes activities, diazotrophic genera and enriched OTUs were significantly influenced across the entire soil profiles, our results also revealed that BC amended soil significantly increased cane stalk height and weight, soil NO_3^-, NH_4^+, OM, TC and AK, and enhanced diazotrophic genera in soil depth 0-20 cm compared to CK treatment. Soil TC, TN, OM and NH_4^+ were the major impact factors shifting diazotrophic community structures in soil depth 0-20 cm. Moreover, multivariate ANOVA analysis revealed that changes in majority of the soil parameters measured were associated with soil depth rather than fertilization. More diazotrophic genera were to a greater extent significantly and positively associated with many soil environmental variables especially in the surface soil 0-20 cm than diazotrophic phyla. Overall, these results were more pronounced in 0-20 cm soil depth in BC than CK treatment.

Introduction

Sugarcane (*Saccharum officinarum* L.) is an economically vital crop planted in tropical and subtropical regions globally with an annual production of approximately 16 million tons. It contributes considerably to the sugar and biofuel-producing industries[1–2]. Fertilization is a crucial agricultural approach that not only improves plant nutrient storage but also simultaneously alters soil attributes and microbial communities[3–4]. In the past few decades, extensive fertilization, especially nitrogen fertilizer, has been used to raise sugarcane production to meet the growing sugar demand[5–6]. Although inorganic fertilization has a positive effect on sugarcane yield, on the other hand, it has unfavorable indirect effects on soil quality by causing soil acidification, enhancing soil pathogens, intensifying nitrification and leaching of nitrates[7–8]. In contrast, organic fertilization is an alternative approach to chemical fertilization to mitigate soil acidification and improve soil nutrient status, thus ensuring sugarcane productivity[2].

Biochar (BC) a soil supplement that is derived from pyrolysis of organic materials at a higher temperature under a limited oxygen environment is a stable organic material, dark in color, porous and carbonaceous[9–10]. BC as a soil amendment is a promising approach to improve soil fertility because it plays an important role in establishing reliable carbon storage, facilitating environmental functions, and mitigating greenhouse gas emissions[11,12]. In addition, adding BC to the soil can increase nutrient retention by absorbing carbon and reducing soil acidity[13]. Biochar induced changes may influence not only nutrients and organic materials cycling, but also plant growth and development [10]. In addition, studies have shown that BC amendment to the soil can cause changes in soil bacterial abundance and community composition[12–14]. However, our understanding of the BC amendment on soil fertility, soil bacterial community composition and their activities with a focus on soil depth as well as overall impact on sugarcane growth is limited.

Soil microbial community plays a pivotal role in nutrients recycling, by changing inorganic compounds and decaying organic materials[15–16], and maintaining quality underground water, thereby improving environmental functions[17]. Soil profiles serve as an ecological filter for many soil microorganisms[18]. Moreover, the existence of the microbial web in soil is very complex, as well as their compositions and diversity differ in soil layers[19]. Soil microbial are essential in N cycling, and they modulate soil available N that are required by plants[15].

Biological nitrogen fixation (BNF) is an important N source which provides nutrient for crops[16]. Diazotrophic (N2 fixers) are diverse group of soil bacteria that are capable of transforming atmospheric N2 to plant-available ammonium (NH_4^+)[20,21], by nitrogenase, a universal enzyme[20]. This process serves as a major source of N, accounting for about 97% into the environment[22,23]. Recently, a molecular marker of nifH gene family which encodes for the nitrogenase reductase subunit has been used to analyze diazotrophic community structures[21]. N2 fixer is sensitive to soil amendment practices. For instance, short-term application of biochar increased diazotrophic abundance and changed community compositions as well as increased the biological nitrogen fixation rate in an alkaline soil under soybean cultivation[12]. Similarly, Wang et al.[20] found that BC amended soil revealed the highest abundance of nifH gene. Diazotrophic abundance and community structures decrease with increasing soil depth. Wang et al.[24] reported that soil depth was the main indicator that altered diazotrophic structures rather than fertilization or sampling time. In addition, Reardon et al.[22] documented that soil depth was the influencing factor that altered nifH gene abundance, which was less in 10–20 cm as compared to 0–10 cm soil layer. However, our knowledge of the distribution pattern of soil microbe community structure and their related activities have been limited to topsoil 0–15 cm[18], where soil bacterial and organic matters are abundant[25]. Moreover, not much is known about diazotrophic relative abundance and community structure, as well as their relationship with soil environmental variables in dipper soil layer under biochar application during sugarcane growth, which is important for better understanding the role of N2-fixers distribution patterns in the soil environment. To address these knowledge gaps a field experiment was designed using biochar as soil amendment during sugarcane cultivation to assess the effect on diazotrophic, soil physio-chemical properties, soil enzyme activities, as well as sugarcane parameters. The study sought to: (a) investigate the variation in diazotrophic abundance and community structures in three soil profiles (0–20, 20–40 and 40–60 cm) under biochar application; (b) explore the relationships between the diazotrophic community structures and soil environmental variables using high-throughput sequencing (HTS).

Results

Response of Sugarcane Parameters to Inorganic Fertilizer and Biochar Amended Soil

BC amended soil significantly increased sugarcane stalk number, stalk weight compared with CK treatment ($P < 0.05$) (Fig. 1A,C). However, compared to CK treatment, BC amended soil had no impact on sugar content (Fig. 1B).

Soil Environmental Variables

Soil physio-chemical properties varied considerable with increasing soil depth, and soil admendmets practices (Fig. 2). Soil NO_3^-, NH_4^+, TC, AP, AK and TN significantly decreased with soil depth ($p < 0.05$) (Fig. 2A,B,D,E,G,H). Moreover, soil OM decreased with increasing soil depth in BC treatment, while, in CK OM fluctuated (Fig. 2C,). In BC treatment, TC/TN fluctuated across the entire soil depth, but revealed no change in entire soil profile in CK, while soil pH in both CK and BC were significantly higher ($p < 0.05$) in 20–60 cm than 0–20 cm soil depth (Fig. 2F,I). Regarding various treatments,, BC amended soil significantly improved ($p < 0.05$) soil nitrate (NO_3^-), NH_4^+, OM, TC, and AK in one soil depth (0–20 cm) (Fig. 2A,B,C,D,) compared to CK. Moreover, BC treatment significantly enhanced ($p < 0.05$) TC/TN and AK at soil depths (20–40, 40–60 cm, and 0–20 and 20–40 cm, respectively) compared with CK treatment (Fig. 2F,G). However, in soil depth (40–60 cm) soil TN significantly diminished ($p < 0.05$) under BC

amended soil compared with CK treatment (Fig. 2E). In the whole soil profile (0–60 cm), BC treatment did not change soil pH in comparison to that under CK treatment (Fig. 2I).

Soil Enzyme Activities

In both CK and BC treatments, cellulose decreased with soil depth (Fig. 3A). Phosphatase acid activity and β-glucosidase activity were significantly higher ($p < 0.05$) in topsoil (0–20 cm), than soil depth 20–60 cm in both CK and BC treatments (Fig. 3C,D). Additionally, urease activity was significantly higher ($p < 0.05$) in soil layer 20–40 cm than 0–20 and 40–60 cm soil profiles in BC treatment, whereas in 0–40 cm soil layer it urease activity was stable compared to 40–60 cm soil profile in CK (Fig. 3B). With respect the different treatments, cellulase and β-glucosidase activities in BC amended soil revealed no significant change in the entire soil layer (0–60 cm) compared to CK. In soil layer (20–40 cm), urease activity increased significantly under BC treatment compared with CK, while in one soil depth (0–20 cm) urease activity was significantly higher ($p < 0.05$) in CK treatment (Fig. 3B). In CK treatment, phosphatase acid activity was significantly higher ($p < 0.05$) in soil depth (0–20 cm) than BC amended soil and in 20–60 cm under both treatments (Fig. 3D).

Relative Abundance of Dominant Diazotrophic Phyla and Genera

The relative abundance of dominant diazotrophic phyla and genera levels were examined under BC and CK treatments at three different soil depths (0–20, 20–40 and 40–60 cm). Our result revealed that soil depth and various soil amendment practices did not significantly altered diazotrophic phyla across the entire soil profiles (Fig. 4A). However, *Proteobacteria* (80.2–92.0%) was the highly dominant diazotrophic phylum followed by *Cyanobacteria* (0.0-8.6%) and *Verrucomicrobia* (0.2–0.6%) in both CK and BC tretments. (Fig. 4A).

Diazotrophic genera significantly changed with soil depth. At genera level of diazotrophic, the relative abundance of *Geobacter* (88.6–93.6%) was highly dominant, followed by *Anaeromyxobacter* (4.5–10.8%), *Dechloromonas* (0.4–1.4%), *Burkholderia* (0.1–1.3%), *Desulfovibrio* (0.2-1.0%) *Methylomonas* (0.1–0.8%) and *Azotobacter* (0.0–1.0%) (Fig. 4B) in all the samples. However, *Anabaena* and *Burkholderia* in 0–20 cm soil layer were significantly influenced in both CK and BC treatments compared to 20–60 cm soil profile ($p < 0.05$) (Fig. 4B). Moreover, Azotobacter were also significantly altered in 0–20 cm soil profile compared to 20–60 cm soil depth in BC treatment ($p < 0.05$), while they were slightly altered in the entire soil layer in CK (Fig. 4B).

With regard to various treatments, BC amended soil improved genus *Anabaena, Stenotrophomonas* and *Burkholderia* relative abundance in one soil layer (0–20 cm) compared to CK. However, compared to CK, BC amendment significantly diminished *Anaeromyxobacter* and *Geobacter* relative abundance in one soil layer (0–20 cm) ($p < 0.05$) (Fig. 4B).

Bacterial beta diversity

A nonmetric multidimensional scaling (NMDS) analysis employed clearly revealed the separation of the different soil depths (0–20, 20–40 and 40–60 cm) (Fig. 5). Moreover, an analysis of similarities (ANOSIM) reinforced further the significant differences ($R^2 = 0.56$, $p < 0.001$) among the different soil depths. From the results obtained from the (NMDS) analysis, a redundancy analysis (RDA) was employed in different soil depths to assess the effect of soil environmental variables on diazotrophic community structure. The analysis revealed that soil TC ($R^2 = 1.1024$, $p < 0.001$) ($R^2 = 1.0984$, $p < 0.001$), OM ($R^2 = 1.0872$, $p < 0.001$) and NH_4^+ ($R^2 = 1.0792$, $p < 0.001$) were the minor impact

factors that caused a shift in diazotrophic communities structure in soil depth 0–20 cm (Fig. 6A). At soil depths 20–40 and 40–60 cm, soil AP (R^2 = 1.2679, p < 0.001), (R^2 = 1.2168, p < 0.001) was the main driver shifting diazotrophic community structure, respectively (Fig. 6B,C).

Diazotrophic distribution patters in different soil depths

To better understand diazotrophic community distribution patter in the different soil profiles, we identified OTUs that were specifically enriched in various soil depths. Due to the fact that MNDS analysis on diazotrophic community structure did not show significant difference in different soil depths between CK and BC treatments (Fig. 5), we performed analysis using combined samples from diazotrophic OTUs (Fig. 7). Vann diagram analysis shows the similarity and overlap of species among various soil profile. Our results revealed that 59 (31.9%), 55 (29.7%) and 52 (28.1%) enriched diazotrophic OTUs were identified in 0–20, 20–40 and 40–60 cm soil depths, respectively. Thus indicating that diazotrophic OTUs community in the topsoil (0–20 cm) were higher compared to 20–40 and 40–60 cm soil depths (Fig. 7A). Next, we characterized enriched diazotrophic OTUs across the three soil depths, ternary plot analysis was employed to identify enriched diazotrophic OTUs using the combined samples. Enriched diazotrophic OTUs were identified in different soil depths (Fig. 7B). Unsurprisingly, subsequent boxplot analysis further revealed that these identified diazotrophic OTUs significantly depleted with soil depth (p < 0.05) (Fig. 7C).

Table 1
Multivariate ANOVA for the effects of soil depth, and different treatments on nifH OTUs, diversity, species richness, coverage and soil enzyme activities

	OUT	Shannon	Chao1	Coverage	Urease	Cellulase	Glucosidase	Phosphatase
Treatment	NS	NS	NS	NS	NS	NS	NS	NS
Depth	***	**	***	***	***	**	***	***
T X D	***	**	**	***	***	**	***	***

Depth stands for soil depth with 0–20 cm, 20–40 cm and 40–60 cm soil layers, treatment stands for control (CK); and biochar (BC).

Table 2
Multivariate ANOVA for the effects of soil depth, and different treatments on soil biochemical properties

	pH	EC	TN	TC	TC/TN	AP	OM	AK	NO_3^-	NH_4^+
Treatment	NS	NS	NS	NS	**	NS	NS	NS	NS	NS
Depth	***	***	***	***	NS	***	NS	***	***	***
T X D	***	***	***	***	**	***	*	***	***	***

Depth stands for soil depth with 0–20 cm, 20–40 cm and 40–60 cm soil layers, treatment stands for control (CK); and biochar (BC).

Multivariate ANOVA for the effects of soil depth, and different treatments on nifH OTUs, diversity, species richness, coverage and soil enzyme activities

Multivariate ANOVA analysis furthered revealed that soil depth significantly (p < 0.05) impacted diazotrophic OUTs, Shannon, chao1, coverage, as well as soil enzyme activities namely urease, cellulase, glucosidase and phosphatase

compared to fertilization (Table 1). Furthermore, soil biochemical properties were significantly ($p < 0.05$) affected by soil depth than the different fertilizations used (Table 2).

Correlation between bacterial composition and soil properties

Pearson's correlation coefficients were calculated to investigate the relationship between soil physio-chemical properties and the most abundant diazotrophic phyla and genera in three different soil depths. Our findings showed that diazotrophic phyla were to a lesser extent significantly and positively or negatively related to soil physiochemical properties compared diazotrophic genera across that entire soil depth gradient. Soil pH was significantly and positively associated with the relative abundance of *Chlorobi* at soil depth 0–20 cm. Furthermore, phyla *Firmicutes* and *Proteobacteria* showed a very strong and positive relationship with soil NH_4^+ and AP at soil depths 20–40 cm, respectively. Whereas soil TC and *Verrucomicrobia* were positively and significantly correlated in soil depth 20–40 cm. However, *Cyanobacteria* and *Chlorobi* were significantly and negatively correlated with soil AP and pH at soil depth 20–40 cm, respectively. At soil depth 40–60 cm, phylum *Firmicutes* revealed a strong and positive relationship with soil pH, however, NO_3^- was significantly and negatively related to phylum *Cyanobacteria* (Fig. 8A).

Meanwhile, our analysis revealed that more diazotrophic genera were to greater extent significantly and positively associated with many soil environmental variables especially in the surface soil 0–20 cm than diazotrophic phyla. *Anaeromyxobacter*, *Anabaena*, *Enterobacter* genera were significantly and positively associated with soil TC, NH_4^+ and AK, OM as well as TC and TN in soil surface soil 0–20 cm, respectively. Furthermore, in 20–40 cm soil layer, we observed that *Burkholderia* and *Dechloromonas* showed a strong and positive relationship with soil TC and NO_3^-, respectively. However, in 40–60 cm soil depth, *Anaeromyxobacter*, *Methylomonas* and *Dechloromonas* were significantly and negatively correlated with soil OM and AK, respectively (Fig. 8B).

Discussion

BC amended soil significantly increased the stalk weight and height of sugarcane. Similarly, Sarfraz et al.[26] and Ehsanullah et al.[27] mentioned that BC application significantly improved crop yields. Soil depth is an important environmental gradient, as well as soil amendmet practices affecting soil physio-chemical properties[28]. In the current study, soil OM, TC, NH_4^+ NO_3^-, and AK significantly decreased with soil depth. BC is a promising alternative to improving soil fertility owing to the pivotal role it plays in building up reliable carbon storage[9]. Studies have revealed BC amendment had profound effect on soil environmental variable, especially N and C cycles[29]. Similarly, our study revealed that biochar amended soil significantly increased soil OM, TC, NH_4^+ NO_3^-, and AK in one soil depth 0–20 cm compared to CK treatment, which we believed were resposible for the improved growth of sugarcane plant.

Soil enzyme activities are considered important indicators of fertile soil due to their important role they carry out in biochemical reaction and sustaining soil fertility[29]. In our previous study, enzymes associated with C and N cycles were enhanced in the topsoil than in subsoil[30]. In our study, numerous patterns in the activity of soil enzymes were observed. Soil cellulase activity decreased with soil depth, while β-Glucosidase activity and phosphatase activity were significantly enhanced in 0–20 cm than 20–60 cm soil profile in both CK and BC treatments. On the other hand, the application of BC may harm soil enzyme activities by impeding or enhancing soil organic matter contents[31]. In the present study, cellulase and β-glucosidase activity in BC in the entire soil profile revealed no

significant change compared to CK. BC treatment significantly reduced urease and phosphatase acid activity in soil layer (0–20, 40–60 cm) and (0–20, 20–40 and 40–60 cm), respectively than CK. The diminishing trend of these enzymes could be attributed to the application of biochar.

Chu et al.[32] revealed that soil microbial community structure is naturally sensitive to environmental fluctuations, and is an important indicators of fertile soil. Diazotrophic abundance decrease with soil depths, Reardon et al.[22] revealed that diazotrophic abundance reduced in soil depth subsoil (10–20 cm) than upper soil layers (5–10 and 0–5 cm). Diazotrophic relative abundance was assessed at the phyla and genera level to measure the impact of soil depth. We observed that biochar significantly influenced diazotrophic genera in 0–20 cm soil layer, but did not affect the diazotrophic phyla.

The utilization of biochar to amend soil can have a significant influence on the biotic and abiotic components of soil, thus significantly altering soil microbial abundance and community composition [30,33,34]. In the current study, BC amendment had no significant influence on diazotrophic abundance at the phyla level. However, compared to CK treatment, BC amended soil had profound impact on diazotrophic abundance at the genera level.

Geobacter was highly abundant in both BC and CK treatments. However, genera *Geobacter* was improved in one soil depth (20–40) compared to CK treatment. This finding is roughly incontinence with studies conducted by Liao et al.[35] and Liu et al.[12], in which it was reported that in biochar amended soil, *Geobacter* were among the dominant bacterial. Our previous study also revealed that *Geobacter* in the subsoil was enhanced compared to the topsoil[30]. This result further validates that *Geobacter* tends to grow in an anaerobic environment, which sequentially improves the lower soil with *Deltaproteobacteria* genera.

Information on free-living N-fixers in the bulk soil is hindered due to the diverse nature of soil microorganisms community and the challenges of linking N fixation activities to each genus of diazotrophic genera[36]. However, Agnihotri[37] mentioned that *Anabaena* genus is known as a nitrogen fixer of a filamentous cyanobacteria genera. Chen et al.[38] reported that biochar amended soil increased soil microbial relative abundance at soil depth 0–15 cm. Similarly, relative abundance *of Anabaena* was significantly high in one soil layer (0–20 cm) in BC amended soil compared to CK treatment.

Azotobacter is a nitrogen fixation bacteria that can stimulate soil rhizosphere microorganisms, protects plant against phytopathogen, as well as boosts nutrient absorption which eventually enhancing biological N fixation. In the current study, *Azotobacter* was significantly in soil profile 0–20 cm. Our result agreed with Kalaigandhi et al.[39] and Abd-el-Malek et al.[40] who reported that *Azotobacter* abundance is depth-dependent, and was notably high in the rhizosphere soil[41].

Burkholderia is Gram-negative bacteria mainly compose of various soil-dwelling bacteria that exhibit different environmental functions namely fixing nitrogen mutualists, pathogen and saprophyte[36]. Its distribution pattern in the soil is not clearly understood[42], however in three different agriculture management regimes namely crop rotation, maize monoculture, and grassland. It was established that the area under maize monoculture and grassland cultivation was dominated by *Burkholderia* strains. In the current study, *Burkholderia* relative abundance was improved in the topsoil (0–20 cm) in BC amended soil compared to CK treatment. This finding partially agreed with Lin et al.[33], in which it was reported that *Burkholderia* relative abundance was significantly increased under organic fertilizers.

Stenotrophomonas genus has been classified as a disease suppression bacteria[43], as well as a plant growth promoter and a biocontrol agent[44]. A study found that *Stenotrophomonas* was significantly higher under no-mulching treatment at different soil depths. Similarly, our result revealed that *Stenotrophomona's* relative abundance was higher at one soil depth (20–40 cm) in BC amendments soil; and was evident in the current study, where sugarcane growth parameters were enhanced.

NMDS analysis was conducted to assess the impact of CK and BC treatments on diazotrophic community in different soil depths. The analysis revealed no significant difference in different soil depths between CK and BC treatments. However, using the combine samples, Venn clearly established that diazotrophic is depth-dependent, while ternary plot was able to distinctly identify enriched diazotrophic OTUs across the different soil profiles. Boxplot analysis further clearly exhibited significant difference in enriched OUTs in among the soil depths. Our finding roughly corroborated with the results of Zgadzaj et al.[45], in which it was reported that bacterial community was significantly enriched in the soil.

Furthermore, multivariate ANOVA analysis further confirmed that soil depth is one of the important environment gradients that greatly influence soil bacterial and soil physiochemical as well as soil enzyme activities[46]. We observed that soil depth significantly influenced diazotrophic OUTs, Shannon, chao1, coverage, nifH gene copies, as well as soil enzyme activities namely urease, cellulase, glucosidase and phosphatase and soil biochemical properties rather than fertilization. These results conformed with the findings of Fischer et al.[47], Lipson et al.[28] and Zhang et al.[30], in which they reported that soil bacterial, soil enzyme activities and soil physiochemical properties change were depth dependent.

Soil environmental variables play an important in influencing bacterial community structures [6,12]. Likewise, diazotrophic community structures are very responsive to soil environmental variables [49]. Zhang et al.[30] documented that soil pH and AK were the main factors affecting bacterial community compositions in the topsoil compared to AP and TC/TN, while in the subsoil, soil pH, AK and TC were the main factors in terms of changing bacterial community compositions. Similarly, in the current study, redundancy analysis (RDA) revealed that diazotrophic community structures were very sensitive to soil environmental namely TC, TN, OM, NH_4^+ and AP. Soil TC was the major impact factor, whereas soil TN, OM and NH_4^+ were the minor impact factor that caused a shift in diazotrophic community structures in soil depth 0–20 cm. While at soil depths 20–40 and 40–60 cm, soil AP was the main driver shifting diazotrophic community structures.

Pearson's correlation coefficients analysis revealed that more diazotrophic genera were to greater extent significantly and positively related to many soil physiochemical properties mainly in the surface soil 0–20 cm than diazotrophic phyla

Conclusions

This study explored the distribution patters of diazotrophic genera and phyla, soil enzyme activities and soil physio-chemical properties in different soil depths under control, (CK) and biochar, (BC) amendments. We also assessed the response of sugarcane to these different soil management practices. Our findings revealed that soil depth had profound impact on soil parameters measured. We also observed that diazotrophic relative abundance at the genera level, and enriched diazotrophic OTUs were significantly improved with soil depth. Diazotrophic genera were to greater extent considerably and positively related to many soil environmental variables mainly in the soil surface horizon (0–20 cm) than diazotrophic phyla. These results are likely to enhance our understanding of how

diazotrophic bacterial response different soil management practices, as well as and their relationship with soil physio-chemical properties in different soil profiles.

Materials And Methods

A field experiment was conducted in March 2017 at the Sugarcane Research Center of Fujian Agriculture and Forestry University in Fuzhou, Fujian Province (latitude 26°5'0" east longitude 119°13'47"). The local climate of the area is subtropical monsoon with an annual average temperature of 20°C and a rainfall of 1369 mm. The experiment was set in a randomized block design with two different treatments and each treatment was replicated three times; with each replicate covering an area of 25 m^2 (5 m x 5 m). The two treatments used were: control, (CK), biochar amended soil (BC). Biochar was applied at the rate of 20 t ha^{-1}. The biochar used in this study was bought from Nanjing Qinfeng Crop Straw Technology Company, China. The biochar was produced from sugarcane straw at the 550–650°C. The properties of the biochar were: TC = 3.08 g/kg; TN = 4.27 g/kg; content pH (H_2O) = 8.01; AP = 315.21 mg/kg and AK = 217.03 mg/kg. Whereas the basic properties for soil were: TC = 4.11 g/kg; TN = 5.07 g/kg; content pH (H_2O) = 6.99; AP = 205.91 mg/kg and AK = 45.02 mg/kg, NH_4^+ = 4.01 g/kg and NO_3^-. 3.90 = g/kg. The biochar was uniformly spread to the soil surface and instantly mixed into the plowed soil at the depth of 0–60 cm before cultivating the sugarcane. Sugarcane stems were cut at about 10–12 cm in length, maintaining two buds on each sett [50], and 10 setts were planted on each row with 0.3 m plant-to-plant spacing and 0.5 m row-to-row spacing.

Plant Cane Growth Parameters

Sugarcane height was measured in centimeter (cm) with the help of a meter rod from the soil surface to the top of the sugarcane. Mean plant height was calculated by taking the average of three replications. Twenty sugarcane stalks were randomly sampled from each row, milled and analyzed the juice for pol and Brox using the method of Legendre[51]. Cane fresh weights were used to determine individual stalk weight (kg $stalk^{-1}$).

Soil Sampling

Soils collection at different depths (0–20, 20–40 and 40–60 cm) were carried out in December 2019. For every plot, a sample was collected at five different spots, homogenized and mixed accordingly forming one sample. Sieving of soils was done using 2-mm mesh and plant residues and stones were removed. Portion of the soil was stored at -20°C for the extraction of DNA and enzyme activities, and the remaining soil samples were stored at 4°C for analyzing soil environmental variables.

Analysis of Soil Physio-chemical Properties

Air-dried soils were used to analyze soil pH, available phosphorus (AP), available potassium (AK), total carbon (TC), total nitrogen (TN) and organic matter (OM). Soil pH was determined using glass electrode pH meter. AP was estimated using the Molybdenum Blue method. The Flash Smart elemental analyzer (Thermo ScientificTM, Waltham, MA, USA) was used to measure the TN and TC. Soil AK was extracted by ammonium acetate and determined by atomic absorption spectrophotometry. Soil OM was measured using Nelson and Sommers methods[52]. A fresh soil sample was used for the extraction of NH_4^+ and NO_3^- with 2.0 M KCl and measured using the continuous flow analyzer (San++, Skalar, Holland).

Measurement of Soil Enzyme Activities

Soil enzyme activities were determined as described by Sun et al.[53]. Incubation of soil was carried out using buffer sodium carboxymethylcellulose solution, cellulose, (glucose, mg/g 24 h, 37°C) activity to measured colorimetrically by quantifying a decrease in 3,5-dinitrosalicylic acid from reducing sugar. Soil urea activity (NH_3-N, mg/g 24 h, 37°C) was measured using improved sodium phenolate and sodium hypochlorite colorimetry. While a nitrophenyl phosphate disodium substrate was used to determined acid phosphatase activity (phenol, ug/g, 1 h, 37°C). After buffering the soil with p-nitrophenyl- β-Glucosidase activity (p-nitrophenyl, ug/g, 1 h, 37°C) was determined using a colorimeteric p-nitrophenol assay.

Soil DNA Extraction, Amplification, Sequencing, and Data Processing

The extraction of soil metagenomics DNA from 0.5 g fresh soil was carried out using Fast DNATM Spin Kit (MP Biomedicals, LLC, Santa Ana, USA) following the manufacturer guidelines. Assessment of DNA quality and quantity were carried out by calculating their absorbance (A260 and 280nm) using a spectrophotometer, and extracts were then stored at -20°C awaiting sequencing.

High throughput sequencing was used to determine diazotrophic community structures using the Illumina Miseq platform, and nifH amplification was carried out using primer pair PolF and PolR [54] combined with Illumina adaptor sequences and barcode sequences[55]. Sample libraries were generated from the purified PCR products. The Miseq 300 cycle Kit was used for paired-end sequencing on a Miseq benchtop sequencer (Illumina, San Diego, CA, United States). The raw nifH gene sequences were separated using sample based on their barcodes and permitting up to one mismatch. Btrim[56] was used to do quality trimming. Forward and reverse reads were merged into full-length sequences using FLASH [57], and sequences with short or contained ambiguous bases were removed. Resampling was done with 10,000 sequences per sample randomly. UCLUST was used to classify the operational taxonomic units (OTUs) at the 97% similarity level, and singletons were removed. The frameshift caused by insertions and deletion in DNA sequences were checked and corrected by RDP FrameBox. Valid nifH gene sequences (300–320 pb) were translated in proteins sequences and taxonomic assignment was performed using RDP FrameBox tool[58], after the processing.

Statistical Analysis

A nonmetric multidimensional scaling (NMDS) analysis employed to assess the differences in diazotrophic community structures in different soil depths. Bray-Curtis dissimilarities were used to analyze similarities (ANOSIM) to estimate the dissimilarity in diazotrophic community structures in 0–20, 20–40 and 40–60 cm depths. Redundancy analysis (RDA) was further performed in the different soil profiles to determine the relationship between diazotrophic community structures and environmental variables. Vann diagrams were plotted to visualize unique and overlap diazotrophic genera in different soil depths (http://bioinfogp.cnb.csic.es/tools/venny/index.html). Ternary plot analysis was conducted using R language based package153 ggtern an extension of package ggplot2 to determine the enriched abundant OTUs of diazotrophic bacterial in different soil depths of the combined samples. Pearson's correlation coefficients were employed separately for the different soil depths to test the relationship between soil properties and diazotrophic community structures both at the phyla and genera levels, using R-software[59]. The test data were calculated by ANOVA using DPS software (version 7.05, www.dpssoftware.co.uk), and the differences between the mean values of each treatment were matched by Tukey's method at a 5% level.

Declarations

Author Contributions: All authors contributed to intellectual input and provided assistance for this study and for manuscript preparation. N.F., M.T, Z.Y., C.Z., A.Y.A., Z.P., H.Z. and Z.L. designed the research and conducted the experiments. N.F., C.Z and A.A. analyzed the data. N.F. wrote the manuscript. M.T, H.Z and C.Z. reviewed the manuscript. H.Z. supervised the work and approved the manuscript for publication.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Khalil, F. *et al.* Genome characterization of Sugarcane Yellow Leaf Virus with special reference to RNAi based molecular breeding. *Microbial pathogenesis.* **120**, 187–197 (2018).
2. Tayyab, M. *et al.* Short-term effects of different organic amendments on soil fungal composition. *Sustainability.* **11**, 198 (2019).
3. Pang, Z. *et al.* Liming positively modulates microbial community composition and function of sugarcane fields. *Agronomy.* **9**, 808 (2019).
4. Castro, S. & Franco, H. N-Fertilization Adjustment in Sugarcane Crop Cultivated in Intensive Mechanization. in (2019). doi:10.5772/intechopen.83445
5. Thorburn, P. J. *et al.* Prioritizing crop management to increase nitrogen use efficiency in Australian sugarcane crops. *Frontiers in Plant Science*. **8**, 1504 (2017).
6. Hussain, Z., Ramli, N., Mansor, N. & Man, Z. A comprehensive review on biodegradable polymers and their blends used in controlled-release fertilizer processes.*Reviews in Chemical Engineering***31**, (2015).
7. Liu, X. *et al.* Sustainable biochar effects for low carbon crop production: a 5-crop season field experiment on a low fertility soil from Central China. *Agric. Syst.* **129**, 22–29 (2014).
8. Lehmann, J., Gaunt, J. & Rondon, M. Bio-char sequestration in terrestrial ecosystems–a review. *Mitigation and adaptation strategies for global change.* **11**, 403–427 (2006).
9. Sohi, S. P. Carbon storage with benefits. *Science.* **338**, 1034–1035 (2012).
10. Spokas, K. A. & Reicosky, D. C. Impacts of sixteen different biochars on soil greenhouse gas production. *Annals of environmental science.* **3**, 179–193 (2009).
11. Liang, B. *et al.* Black carbon affects the cycling of non-black carbon in soil. *Org. Geochem.* **41**, 206–213 (2010).
12. Liu, X. *et al.* Impact of biochar amendment on the abundance and structure of diazotrophic community in an alkaline soil. *Science of The Total Environment.* **688**, 944–951 (2019).
13. Van Zwieten, L. *et al.* Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant and soil.* **327**, 235–246 (2010).
14. Zhang, M., Riaz, M., Zhang, L., Eldesouki, Z. & Jiang, C. Biochar Induces Changes to Basic Soil Properties and Bacterial Communities of Different Soils to Varying Degrees at 25 mm Rainfall: More Effective on Acidic

SoilsImage_1.TIFImage_2.TIFImage_3.TIFImage_4.TIFImage_5.TIFTable_1.docx.*Frontiers in Microbiology***10**, (2019).

15. Levy-Booth, D. J., Prescott, C. E. & Grayston, S. J. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. *Soil Biology and Biochemistry.* **75**, 11–25 (2014).
16. Cleveland, C. C. *et al.* Global patterns of terrestrial biological nitrogen (N2) fixation in natural ecosystems. *Global biogeochemical cycles.* **13**, 623–645 (1999).
17. Lourenço, K. S. *et al.* Resilience of the resident soil microbiome to organic and inorganic amendment disturbances and to temporary bacterial invasion. *Microbiome.* **6**, 142 (2018).
18. Eilers, K. G., Debenport, S., Anderson, S. & Fierer, N. Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. *Soil Biology and Biochemistry.* **50**, 58–65 (2012).
19. Canfora, L. *et al.* Vineyard microbiome variations during different fertilisation practices revealed by 16s rRNA gene sequencing. *Applied soil ecology.* **125**, 71–80 (2018).
20. Wang, C., Zheng, M. M., Hu, A. Y., Zhu, C. Q. & Shen, R. F. Diazotroph abundance and community composition in an acidic soil in response to aluminum-tolerant and aluminum-sensitive maize (Zea mays L.) cultivars under two nitrogen fertilizer forms. *Plant and soil.* **424**, 463–478 (2018).
21. Mohamed, N. M., Colman, A. S., Tal, Y. & Hill, R. T. Diversity and expression of nitrogen fixation genes in bacterial symbionts of marine sponges. *Environ. Microbiol.* **10**, 2910–2921 (2008).
22. Reardon, C. L., Gollany, H. T. & Wuest, S. B. Diazotroph community structure and abundance in wheat–fallow and wheat–pea crop rotations. *Soil Biology and Biochemistry.* **69**, 406–412 (2014).
23. Wang, J. *et al.* Temporal variation of diazotrophic community abundance and structure in surface and subsoil under four fertilization regimes during a wheat growing season. *Agric. Ecosyst. Environ.* **216**, 116–124 (2016).
24. Wang, J. *et al.* Temporal variation of diazotrophic community abundance and structure in surface and subsoil under four fertilization regimes during a wheat growing season. *Agriculture, Ecosystems and Environment.* **216**, 116–124 (2016).
25. Fierer, N. *et al.*,. . Variations in microbial community composition through two soil depth profiles. Soil Bio Biochem 35: 167–176. Soil Biology and Biochemistry 35, 167–176(2003).
26. Sarfraz, R. *et al.* Impact of integrated application of biochar and nitrogen fertilizers on maize growth and nitrogen recovery in alkaline calcareous soil. *Soil science and plant nutrition.* **63**, 488–498 (2017).
27. Ehsanullah, K. J., Asghar, G., Hussain, M. & Rafiq, M. Effect of nitrogen fertilization and seedling density on fine rice yield in Faisalabad, Pakistan. *Soil Environ.* **31**, 152–156 (2012).
28. Lipson, D. *et al.* Changes in microbial communities along redox gradients in polygonized Arctic wet tundra soils. (2016).
29. Nannipieri, P., Kandeler, E. & Ruggiero, P. Enzyme activities and microbiological and biochemical processes in soil. Enzymes in the environment. Marcel Dekker, New York 1–33(2002).
30. Zhang, C. *et al.* Bacteria with Di ff erent Assemblages in the Soil Profile Drive the Diverse Nutrient Cycles in the Sugarcane Straw Retention Ecosystem. **11**,194(2019).
31. Thies, J. E., Rillig, M. C. & Graber, E. R. Biochar effects on the abundance, activity and diversity of the soil biota. *Biochar for environmental management: science, technology and implementation.* **2**, 327–389 (2015).
32. Chu, H. *et al.* Soil Microbial Biomass, Dehydrogenase Activity, Bacterial Community Structure in Response to Long-term Fertilizer Management. *Soil Biology and Biochemistry.* **39**, 2971–2976 (2007).

33. Lin, W. *et al.* The effects of chemical and organic fertilizer usage on rhizosphere soil in tea orchards. *PloS one.* **14**, e0217018 (2019).
34. Zheng, J. *et al.* Biochar compound fertilizer increases nitrogen productivity and economic benefits but decreases carbon emission of maize production. *Agric. Ecosyst. Environ.* **241**, 70–78 (2017).
35. Liao, H., Yaying, L. & Yao, H. Biochar Amendment Stimulates Utilization of Plant-Derived Carbon by Soil Bacteria in an Intercropping SystemData_Sheet_1.docx.*Frontiers in Microbiology***10**, (2019).
36. Hall, C. M. *et al.* Diverse Burkholderia species isolated from soils in the southern United States with no evidence of B. pseudomallei.*PLoS One***10**, (2015).
37. Agnihotri, V. K. Anabaena flos-aquae. *Critical Reviews in Environmental Science and Technology.* **44**, 1995–2037 (2014).
38. Chen, J. *et al.* Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China. *Appl. Soil. Ecol.* **71**, 33–44 (2013).
39. Kalaigandhi, V., Kannapiran, E., Harimuraleedharan, M. A., Sivakumar, T. & Arasu, V. T. Azotobacter population in Rhizosphere and Non-Rhizosphere sediments of Tondi Coast. *International Journal of Biological Technology.* **1**, 63–65 (2010).
40. Abd-el-Malek, Y., Hosny, I. & Shawky, B. T. Nitrogen-fixing capacity of Azotobacter as affected by the type and depth of substrate. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Zweite Naturwissenschaftliche Abteilung: Mikrobiologie der Landwirtschaft, der Technologie und des Umweltschutzes.* **134**, 390–397 (1979).
41. Abdallah, S., Hassan, H. & Mansour, M. The effect of some plants on the dynamics of Azotobacter in the soil. *Zagazig Journal of Horticultural Science Vol.* **43**, 395–406 (2016).
42. Currie, B. J., Dance, D. A. B. & Cheng, A. C. The global distribution of Burkholderia pseudomallei and melioidosis: an update. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* **102**, S1–S4 (2008).
43. Raaijmakers, J. M. & Mazzola, M. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. *Annual review of phytopathology.* **50**, 403–424 (2012).
44. Berg, G. & Ballin, G. Bacterial antagonists to Verticillium dahliae Kleb. *Journal of Phytopathology.* **141**, 99–110 (1994).
45. Zgadzaj, R. *et al.* Root nodule symbiosis in Lotus japonicus drives the establishment of distinctive rhizosphere, root, and nodule bacterial communities. Proceedings of the National Academy of Sciences 113, E7996–E8005(2016).
46. Gu, Y. *et al.* Long-term fertilization structures bacterial and archaeal communities along soil depth gradient in a paddy soil. *Frontiers in microbiology.* **8**, 1516 (2017).
47. Fischer, C. *et al.* Horizon-Specific Bacterial Community Composition of German Grassland Soils, as Revealed by Pyrosequencing-Based Analysis of 16S rRNA Genes. *Applied and environmental microbiology.* **76**, 6751–6759 (2010).
48. Arafat, Y. *et al.* Spatial distribution patterns of root-associated bacterial communities mediated by root exudates in different aged ratooning tea monoculture systems. *International journal of molecular sciences.* **18**, 1727 (2017).
49. Jichen, W. *et al.* Temporal variation of diazotrophic community abundance and structure in surface and subsoil under four fertilization regimes during a wheat growing season. *Agriculture, Ecosystems and Environment.* **216**, 116–124 (2016).

50. Nalawade, S. M., Mehta, A. K. & Sharma, A. K. Sugarcane planting techniques:A Review. (2018).
51. Legendre, B. L. The core/press method for predicting the sugar yield from cane for use in cane payment. *Sugar J.* **54**, 2–7 (1992).
52. Nelson, D. W. & Sommers, L. E. Total carbon, organic carbon, and organic matter. *Methods of soil analysis: Part 3 Chemical methods.* **5**, 961–1010 (1996).
53. Sun, J., Zhang, Q., Zhou, J. & Wei, Q. Pyrosequencing technology reveals the impact of different manure doses on the bacterial community in apple rhizosphere soil. *Applied soil ecology.* **78**, 28–36 (2014).
54. Poly, F., Monrozier, L. J. & Bally, R. Improvement in the RFLP procedure for studying the diversity of nifH genes in communities of nitrogen fixers in soil. *Research in microbiology.* **152**, 95–103 (2001).
55. Caporaso, J. G. *et al.* Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME journal.* **6**, 1621–1624 (2012).
56. Kong, Y. Btrim: a fast, lightweight adapter and quality trimming program for next-generation sequencing technologies. *Genomics.* **98**, 152–153 (2011).
57. Magoc, T. & Salzberg, S. F. L. A. S. H. Fast Length Adjustment of Short Reads to Improve Genome Assemblies. *Bioinformatics (Oxford, England).* **27**, 2957–2963 (2011).
58. Wang, Q. *et al.* Ecological patterns of nifH genes in four terrestrial climatic zones explored with targeted metagenomics using FrameBot, a new informatics tool. *MBio.* **4**, e00592–13 (2013).
59. Team, C. *Team RDC.R: A Language And Environment For Statistical Computing. R Foundation for Statistical Computing: Vienna, Austria. R: A Language and Environment for Statistical Computing* (2012).

Figures

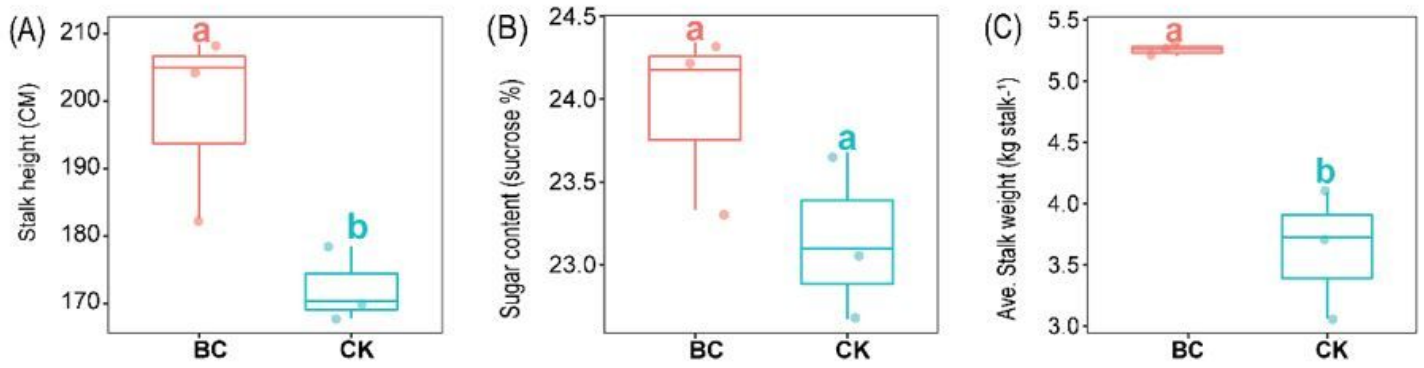

Figure 1

Impact of inorganic fertilizer and biochar amendment on sugarcane agronomic parameters. Boxplots with various lowercase letters indicate significant ($p < 0.05$) differences between treatments based on Tukey's HSD test. CK: control; BC: fertilization with biochar

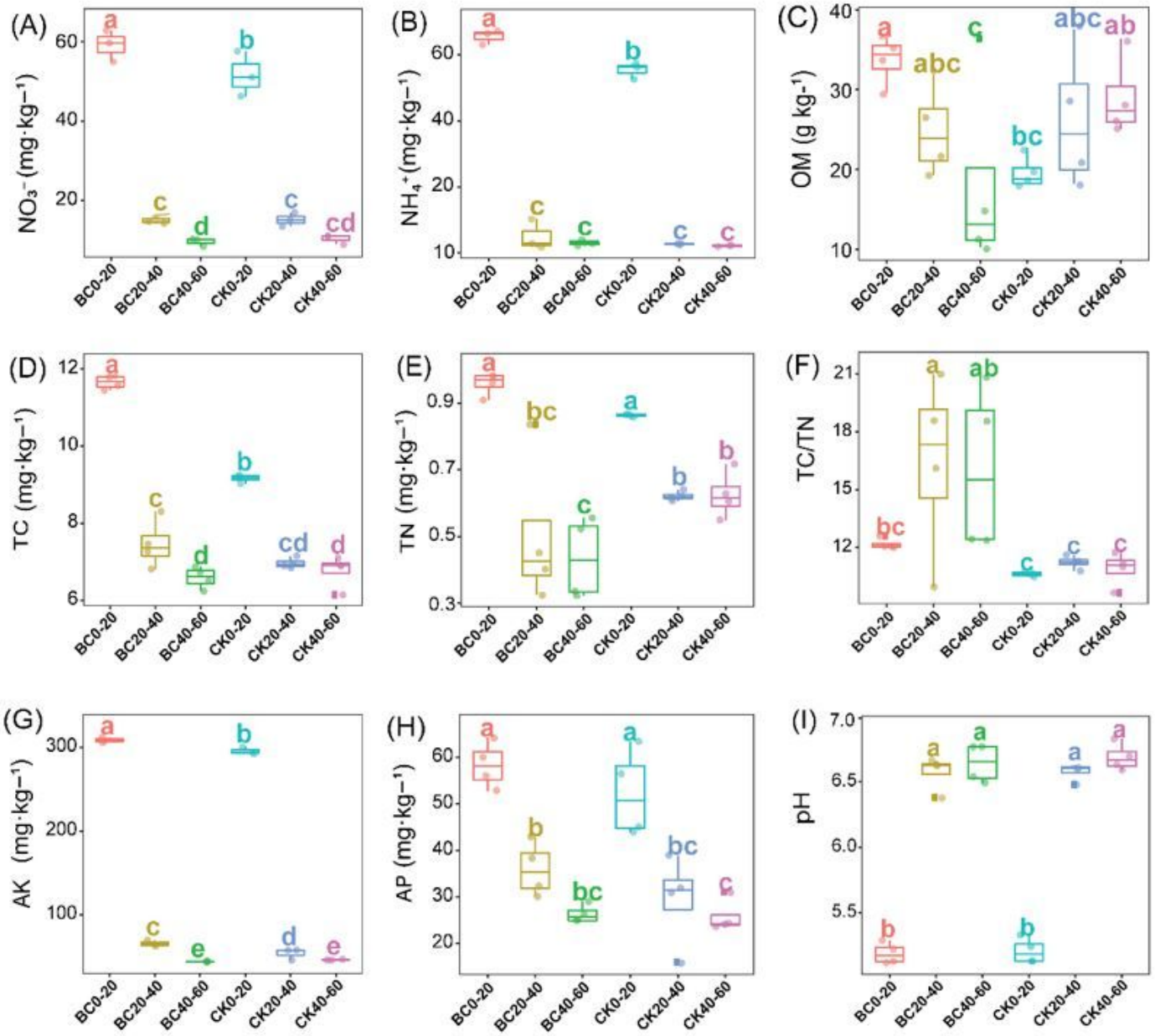

Figure 2

Soil environmental variables at three soil layers under biochar (BC) amended soil compared to control (CK). Boxplots with different lowercase letters depict significant differences between treatments (Tukey test, $p < 0.05$). (A) NH4+, ammonium; (B) NO3-, nitrate; (C) OM, soil organic matter; (D) TC, total soil carbon; (E) TN, total nitrogen; (F) TC/TN, carbon/nitrogen ratio; (G) AK, available potassium; (H) AP, available phosphorus; (I) pH potential hydrogen.

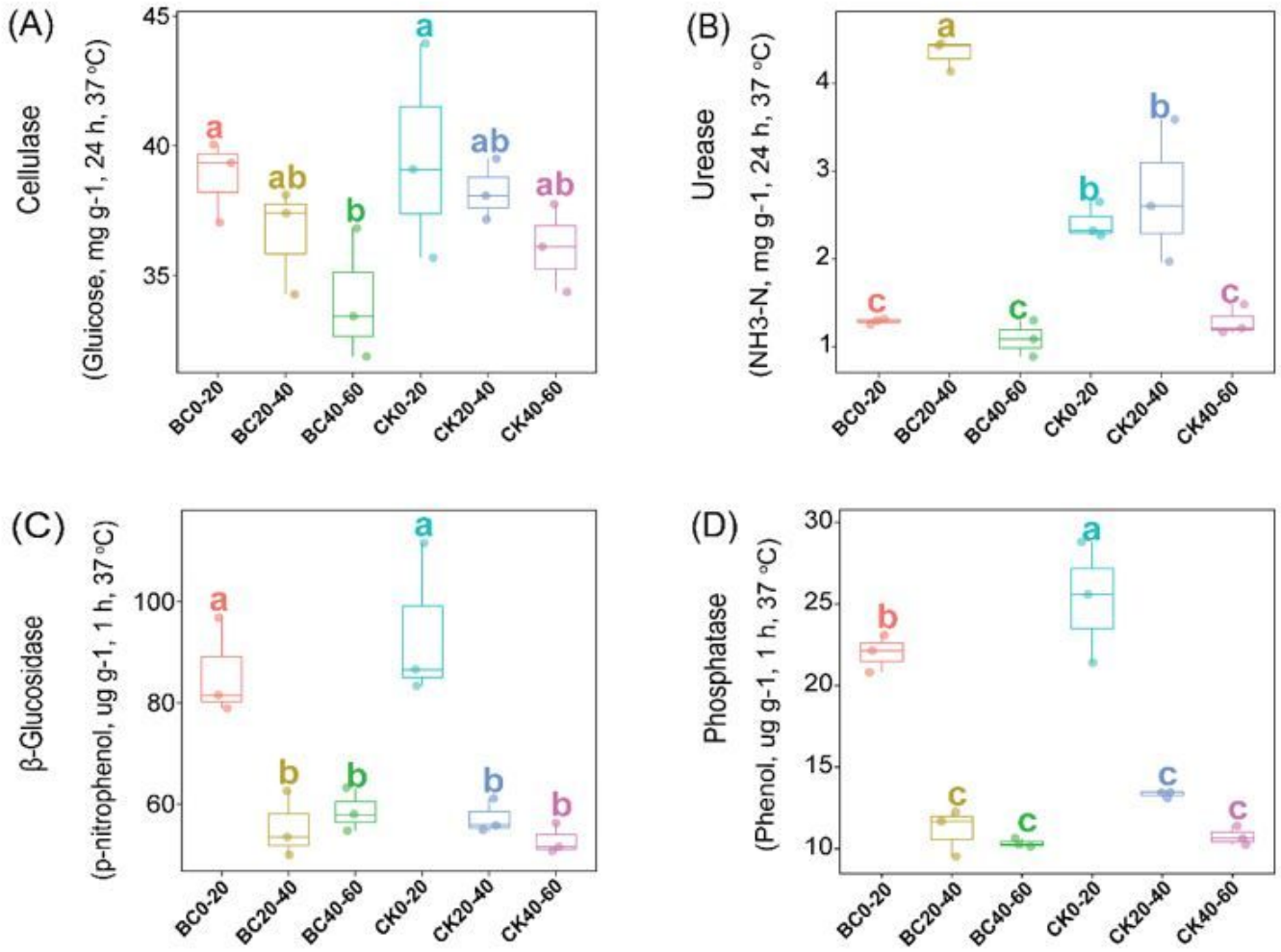

Figure 3

Soil enzymatic activities such as cellulase activity (A), urease activity (B), β-Glucosidase (C) and phosphatase activity (D), under BC, biochar amendment; compared to the CK, control at different soil depths. Boxplots with various lowercase letters indicate a significant difference between treatments based on Tukey's HSD test ($p < 0.05$).

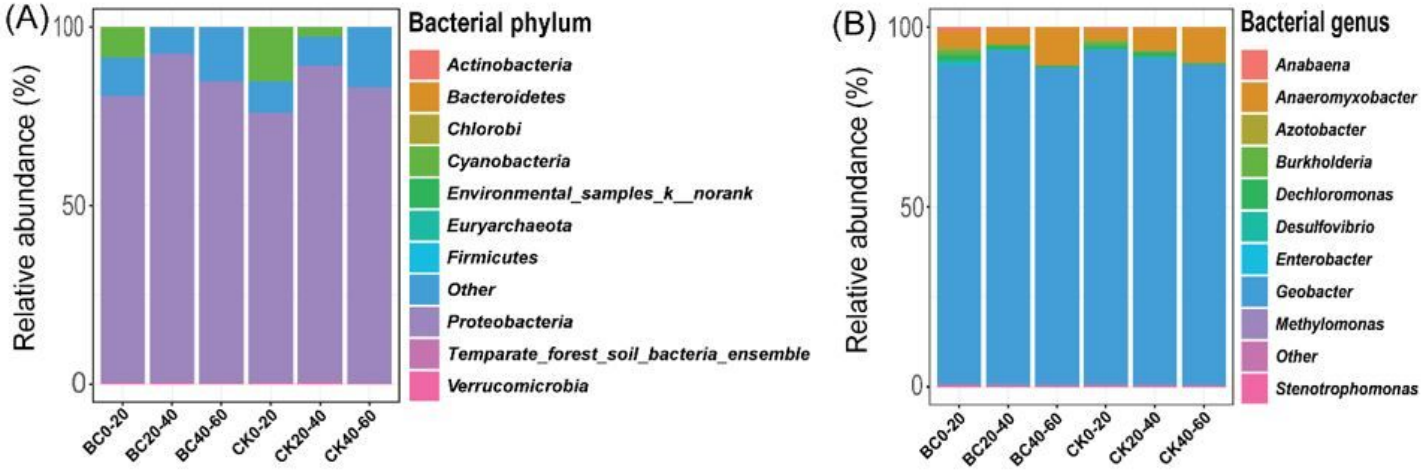

Figure 4

Distribution of diazotrophic phyla (A), diazotrophic genera (B) at different soil depths in BC amended soil (B) and control (CK). “Other” indicates those identified phyla and genera that were beyond the top 10 phyla and genera.

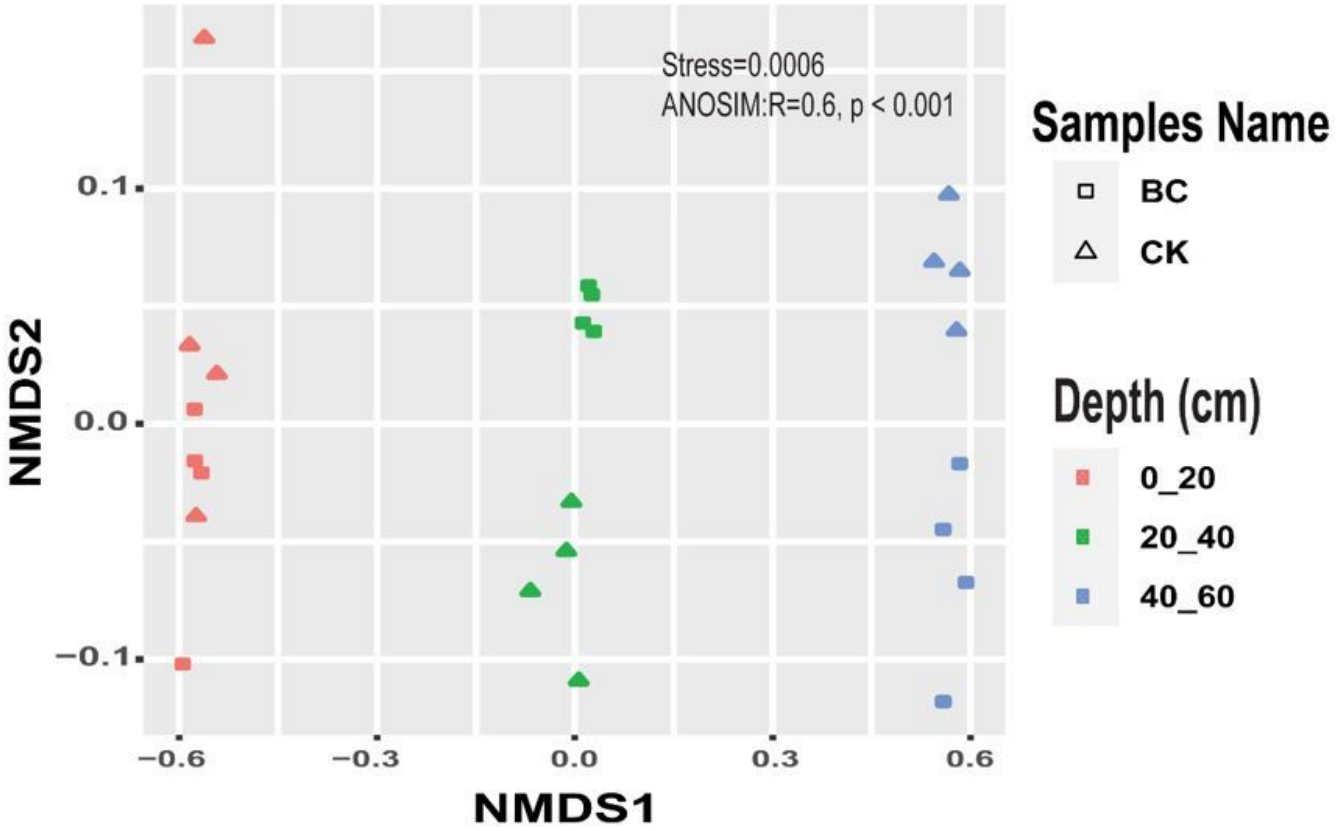

Figure 5

Analysis of nonparametric multidimensional scaling (NMDS) of the diazotrophic communities structure at 0-20 cm, 20-40 cm and 40-60 cm soil depths. control, (CK); BC, biochar amended soil.

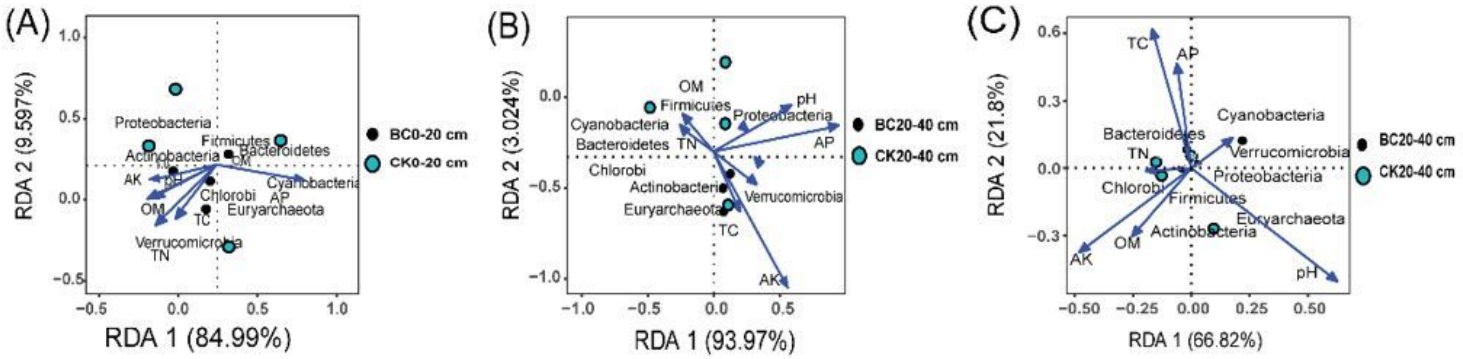

Figure 6

Redundancy analysis (RDA) of diazotrophic communities and soil environmental at the phyla (A) and genera (B) levels under and biochar amended soil at three different soil depths; BC0-20, BC20-40, BC40-60, and control; CK0-20, CK20-40 CK40-60.

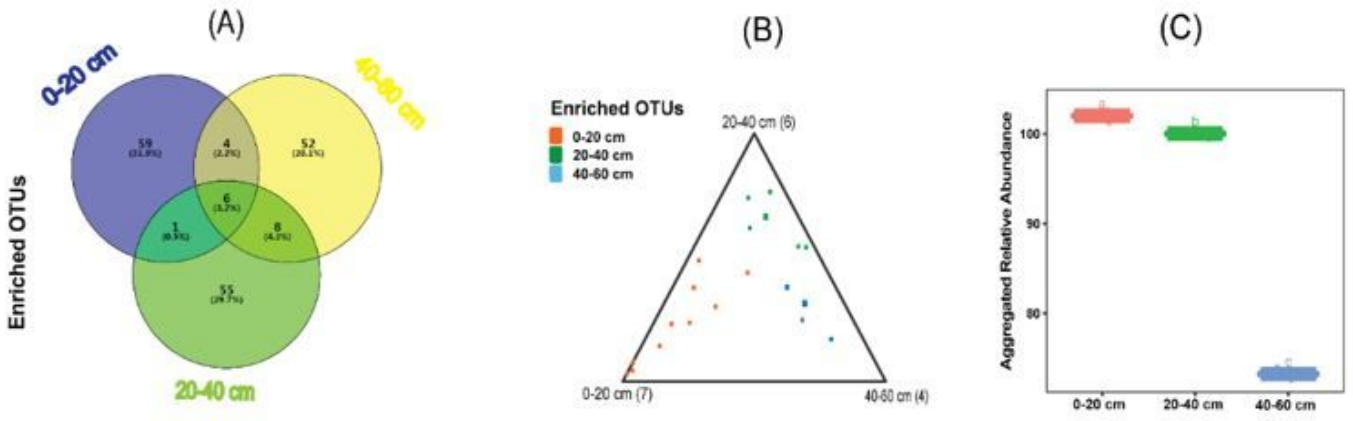

Figure 7

Diazotrophic enriched OTUs exhibited distinct distribution patterns in different soil depths (0-20, 20-40, and 40-60 cm) at the genera level. a-c, (a) Percentage of enriched diazotrophic OTUs in different soil profiles. b, Ternary plot depicting compartment specificity relative abundance of all enriched OTUs (> 5 %) for diazotrophic distribution patters in different soil depths. Each point corresponds with an OTU. Its position represents its relative abundance with regard to each compartment, and its size represents the average across all three compartments. Colored circles represent diazotrophic OTUs enriched in one compartment compared with the others (0-20, 20-40 and 0-60 cm). c, Aggregated relative abundance of each group of enriched OTUs in three soil depths are shown.

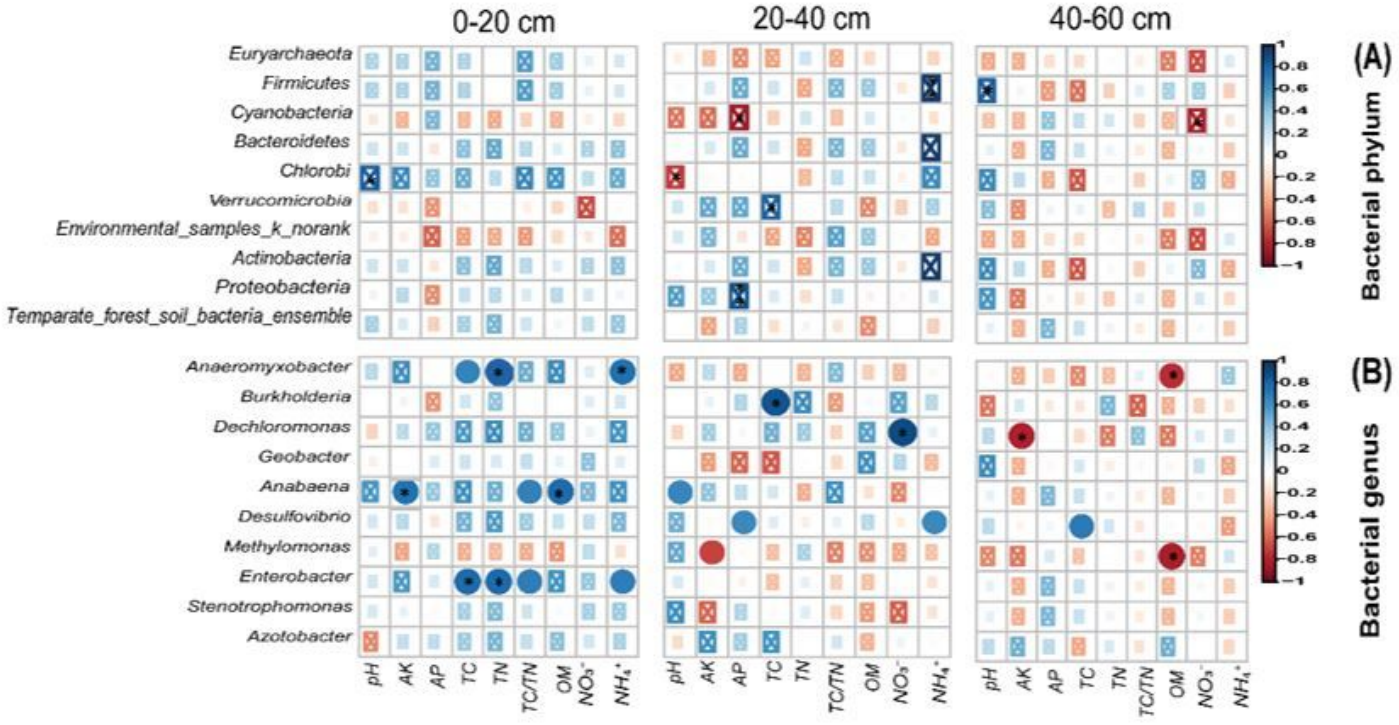

Figure 8

Pearson’s correlation coefficients for soil physio-chemical properties and the most abundant diazotrophic phyla at 0-20 cm, 20-40 cm and 40-60 cm (A); and diazotrophic genera at 0-20 cm , 20-40 cm and 40-60 cm (B) soil depths. The heatmap cells marked by “*” or “**” are statistically significant: * $p < 0.05$ and ** $p < 0.01$