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Article in *Journal of Soils and Sediments* · January 2020

DOI: 10.1007/s11368-019-02547-5

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# Soil organic carbon storage and quality are impacted by corn cob biochar application on a tropical sandy loam

Emmanuel Amoakwah<sup>1</sup> • Emmanuel Arthur<sup>2</sup> • Kwame A. Frimpong<sup>3</sup> • Sanjai J. Parikh<sup>4</sup> • Rafiq Islam<sup>5</sup>

Received: 21 July 2019 / Accepted: 18 December 2019

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## Abstract

**Purpose** Humic substances, which are integral components of total organic carbon (TOC), influence soil quality. The study aimed to investigate whether humic and non-humic fractions exhibit early, consistent, and measurable changes and affect TOC sensitivity and storage in a tropical sandy loam soils amended with corn cob biochar.

**Materials and methods** There were four treatments with four replicates established in a randomized complete block design. Composite soil samples were taken from plots without biochar (CT), from plots incorporated with 15 t biochar ha<sup>-1</sup> (BC-15), and 30 t biochar ha<sup>-1</sup> without or with phosphate fertilizer (BC-30 and BC-30+P). The TOC, and humin, humic acid (HA), and fulvic acid (FA) fractions of soil organic carbon were determined for each treatment. The optical densities (400–700 nm) were measured on the soil-free extracts by spectrophotometry; the densities measured at 465 and 665 nm were used to calculate the  $E_{465}/E_{665}$  ratios.

**Results and discussion** The BC-30 and BC-30+P plots recorded the highest TOC, humin, humic acid (HA), and fulvic acid (FA) contents with respect to the lowest in the CT. The total exchangeable carbon stratification was significantly higher in all the biochar-treated plots relative to the CT. Spectral analysis showed higher values of  $E_{465}/E_{665}$  (5.02 and 5.15) in the CT and BC-15-treated soils, respectively, compared with the BC-30 and BC-30+P-amended soils with  $E_{465}/E_{665}$  ratios of 2.76 and 2.98, respectively.

**Conclusions** Corn cob biochar applied to a tropical sandy loam:

- increased the concentrations of HA and FA and led to increased stratification of TOC, with a stronger effect on HA compared with FA;
- significantly lowered  $E_{465}/E_{665}$  at the high biochar application rate of 30 t ha<sup>-1</sup>, implying the dominance of high molecular weight humic acid-like substances, and increased degree of aromaticity of the TOC.

**Keywords**  $E_{465}/E_{665}$  · Fulvic acid · Humic acid · Humification index · Total organic C

Responsible editor: Hailong Wang

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## 1 Introduction

Soil is considered to be an essential natural resource that renders several important ecosystem functions such as a medium for plant growth, regulation, and partitioning of water and gaseous flow in the environment and an environmental buffer in the formation, attenuation, and degradation of natural and xenobiotic compounds (Larson and Pierce 1991). Poor agricultural and other environmental practices that cause a decline in soil quality potentially reduce these soil functionalities.

Given the resiliency of nature, appropriate soil management interventions can be largely expected to restore ecosystem functions of degraded soils. The soil organic contents are crucially important in providing energy, substrates, and the

biological diversity (Franzluebbers 2002) necessary to sustain numerous soil functional abilities. Soil total organic C (TOC) is the composite indicator of healthy soil to support food security and enhance ecosystem resilience (Don et al. 2011). The TOC is composed of both humic and non-humic fractions, and each of these fractions makes its particular contribution towards soil quality based on its chemical composition and lability, physico-chemical stability, and turnover rates (Stevenson 1994). The humified portion of TOC based on classical extraction procedures and solubility in alkaline and acidic solutions is categorized into three fractions, namely, humic acids (HA), fulvic acids (FA), and humin (Stevenson 1994). These compounds originate during and/or after plant and animal residue decomposition and subsequent polymerization of metabolites into complex and heterogenic nature of TOC with distinct functional groups, elemental composition, and physico-chemical properties (Hayes 1989).

The functionality of the humified C compounds is associated with their physical structures, polymeric nature, and chemical composition (Stevenson 1994). There is continual humification processes undergoing in TOC mediated through microbial and biochemical reactions and consequently, labile C compounds are converted into recalcitrant compounds or vice-versa (Zech et al. 1997). In contrast, the non-humic C pool is composed mainly of polysaccharide and protein-like compounds (Flaig et al. 1975).

Several studies have reported that TOC is not a consistent, sensitive, and early indicator to detect short-term changes (within 1 to 2 years) in soil C cycling, due to its inherent biochemical complexity and physico-chemical interactions with clays and calcium (Stevenson 1994; Salinas-Garcia et al. 1997). Thus, a better understanding on both humic- and non-humic C contents and their characteristics induced by the impact of biochar amendment may serve as a guide to evaluate soil's capacity as a sink or source of atmospheric CO<sub>2</sub> in a tropical agro ecosystem. Zhao et al. (2018) reported a significant increase in the content of soil organic carbon and humic fractions following an incorporation of biochar pyrolyzed at 300 and 500 °C to Earth-cumuli-Orthic Anthrosol. Other authors have equally reported significant increases in soil labile organic carbon and humic fractions following biochar application (Hairani et al. 2016; Rodríguez-Vila et al. 2016). Maintaining a steady labile C pool rather than TOC content is far more important to support soil biodiversity and efficiency, improve soil structural stability and water infiltration, and recycle essential nutrients for plant growth (Islam and Weil 2000; Franzluebbers 2002).

Doane et al. (2003) reported that the humic- and non-humic compounds have a variable turnover of C based on input quality and changes in land use systems. Science-based information of both humic- and non-humic C fractions is needed to determine consistent and early changes in TOC content in response to soil management practices. There is a lack of

information to evaluate the impact of biochar on the quality of TOC content in a highly weathered soil of the humid tropics. The objectives of the study were to (1) investigate whether humic- and non-humic characteristics exhibited early, consistent, and measurable changes in TOC sensitivity to biochar application and (2) determine if differences translated into C storage by different rates of biochar application under similar soil and climatic conditions.

## 2 Materials and methods

### 2.1 Experimental site

The study was conducted at the University of Cape Coast teaching and research farm, in the coastal agro-ecological zone of Ghana (5° 07' N, 1° 17' W). The area is characterized by two seasons: a rainy season where most rainfall events occur between April and October, and June being the month when precipitation mostly occurs. Long-term records showed the relatively high rainfall events (1400 mm per annum) in the area. The area also has a dry season where a long dry spell occurs between November and March, with March being the hottest month (maximum temperature of 31 °C). The mean monthly temperatures range from 24 °C to 28 °C.

The soil has a well-drained sandy loam texture (18, 9, and 73% by weight of clay silt and sand, respectively) developed on sandstones, shales, and conglomerates and classified as a *Haplic Acrisol* (IUSS Working Group WRB 2015). Soil chemical properties prior to establishment of the biochar experiment were 9.3 g kg<sup>-1</sup> soil organic carbon, 0.73 g kg<sup>-1</sup> total nitrogen, with total phosphorus, potassium, and magnesium contents of < 0.4, 11.9, and 9.3 mg 100 g<sup>-1</sup>, respectively, pH of 6.1, and electrical conductivity of 200 μS cm<sup>-1</sup> (Amoakwah et al. 2017a).

### 2.2 Biochar field experiment

The biochar was produced from corn cob feedstock pyrolyzed at 500–550 °C in a reactor (Lucia stove) for 48 h. The biochar particles were homogenized by milling the biochar to a < 2 mm particle size. Biochar chemical properties include the following: 853 g kg<sup>-1</sup> dry matter, 388 g kg<sup>-1</sup> total carbon, 9 g kg<sup>-1</sup> total nitrogen, pH of 10.2, 3.31 mg kg<sup>-1</sup> polycyclic aromatic hydrocarbons, 3150 mg kg<sup>-1</sup> phosphorus, with calcium, magnesium, potassium, and sodium of 8.69, 4.51, 31.8, and 2.16 g kg<sup>-1</sup>, respectively (Amoakwah et al. 2017b), and 1.07 and 2.33 g kg<sup>-1</sup> fulvic acid (FA) and humic acid (HA), respectively.

The experiment was laid out in November 2015 by following the randomized complete block design with four biochar treatments replicated four times. Biochar treatments were 0 (control, CT), 15 t ha<sup>-1</sup>, (BC-15), 30 t ha<sup>-1</sup> (BC-30), and

30 t ha<sup>-1</sup> (BC-30+P) with phosphate fertilizer (P-enriched biochar), respectively. The biochar application rates corresponded to 0, 0.51, and 1.03%, respectively, on an oven-dried weight basis. Biochar was broadcasted followed by incorporation to 20 cm depth by using a hoe. Each replicated plot was 3 m wide × 6 m length. The plots were separated by 60-cm buffer strips.

### 2.3 Soil sampling and processing

In mid-January 2017, composite soil samples at 0 to 20 cm depth were randomly collected by using a soil auger (5 cm internal diameter) from each plot in sealable plastic bags and placed on ice in a cooler. A portion of the field-moist soil from each bag was air-dried at room temperature (~25 °C) for 15 days and ground with a porcelain mortar and pestle to pass through a 2-mm sieve prior to chemical and physical analyses.

### 2.4 Extraction, fractionation, and analysis of humic and non-humic C fractions

The extractions of HA and FA of the soil total organic C (TOC) were carried out by a modified procedure outlined by Ghosh and Schnitzer (1979) and Navarrete et al. (2011). Briefly, a 1.0 g oven-dried equivalent (ODE) sample of the air-dried soil was placed in a 50-mL plastic tube containing 15 mL of 0.1 M NaOH solution (~pH 12.2) under continuous shaking at 250 rpm for a 24-h. period. After shaking, the soil suspension was centrifuged at 3000 rpm for 10 min and filtered to obtain soil-free extracted C (TEC) aliquot. The procedure of obtaining soil-free extracted C (TEC) was repeated thrice. The three TEC aliquots were combined (~45 mL) and slowly acidified with concentrated H<sub>2</sub>SO<sub>4</sub> to ~pH 2 and allowed to stay overnight for coagulation and precipitation of HA from the FA. The tubes were then centrifuged at 3000 rpm for 10 min for complete precipitation and separation of the HA from the FA. The FA in the solution was separated from the precipitated HA by filtration using 0.4 μm Millipore membrane (filter paper). The precipitated HA was re-dissolved in 45 mL of 0.1 M NaOH and purified from silica and ash contents by repeated dialysis in water. A 5-mL sample of filtered HA or FA aliquot was diluted to 20 mL volume in a glass tube with 15 mL of distilled deionized water. The total C in the diluted HA and FA samples was determined by using the Shimadzu® total dissolved organic C and N analyzer. The above-mentioned protocol was used to extract HA and FA from the biochar.

The non-humic glucose equivalent C was determined in both HA and FA aliquots by the anthrone-sulfuric acid method (Brink et al. 1960). Briefly, 1 mL of diluted HA or FA aliquot was taken into a 25-mL plastic tube with 5 mL of cold anthrone-sulfuric acid reagent mixture. The mixture in the tube was slowly vortexed to mix thoroughly followed by heating in a boiling water bath for 10 min. After cooling, the

absorbance of the aliquot as against the glucose C standard solutions was measured at 607 nm using a Shimadzu® spectrophotometer. The concentration of the anthrone reactive glucose equivalent C was subtracted from both HA and FA fractions. Humic, the most stabilized residual fraction of TOC, was calculated after subtracting the TEC concentration from the TOC concentration.

Several quotients as measures of humification characteristics of TOC, such as humification index (HI), humification ratio (HR), and degree of humification (DH), were calculated (Saviozzi et al. 1994; Aziz and Islam 2014).

$$\text{HI}(\%) = \left[ \frac{\text{Glucose equivalent total non-humic C}}{\text{TOC}} \right] \times 100 \quad (1)$$

$$\text{HR}(\%) = \left[ \frac{\text{Glucose-free HA} + \text{FA}}{\text{TOC}} \right] \times 100 \quad (2)$$

$$\text{DH}(\%) = \left[ \frac{\text{Glucose-free HA} + \text{FA}}{\text{TEC}} \right] \times 100 \quad (3)$$

For spectroscopic analyses, the HA-C and FA-C fractions were adjusted to pH 7 by adding aqueous solution of NaOH or H<sub>2</sub>SO<sub>4</sub> and their optical densities were measured by a Shimadzu® spectrophotometer at visible wavelengths (400–700 nm) with 50-nm intervals. The optical densities and wavelength values were log-transformed for log-log relationship, and the slope of each relationship was calculated (Ghosh and Schnitzer 1979). The optical densities of soil-free extracts measured at 465 and 665 nm were used to calculate the  $E_{465}/E_{665}$  ratios (Chen et al. 1977). The  $E_{465}/E_{665}$  ratio serves as an indicator of total organic carbon (TOC) humification process which influences TOC quality in soils. Thus, it indicates the degree with which TOC is dominated by high molecular weight humic acid-like substances. A smaller ratio (gentle slope) suggests an increasing proportion of aromatic chain structures and higher degree of aromatic condensation (Khan 1959).

### 2.5 General soil properties

Soil pH in 1:2 soil-distilled deionized water suspensions was determined by the glass electrode method. Electrical conductivity (ECe) in 1:1 soil-water suspensions was measured by the electrical conductivity meter. The TOC and total N contents were determined on finely ground (<200 μm) soil using the CE Elantech® automated dry combustion CNS analyzer. Soil bulk density (ρ<sub>b</sub>) was determined by the core method. Soil-particle size analysis was conducted by using the Bouyoucos hydrometer method.

### 2.6 Calculation of carbon stocks and stratification

The TOC, TEC, HA, FA, humin, and non-humin C stocks were calculated by multiplying their concentration with the

concurrently measured soil bulk density ( $\rho_b$ ) values and depth. Stratification of extracted C fractions was calculated by dividing their C values under various treatments with the respective values of that C fraction in the control treatment (reference) soil (Franzluebbers 2002). The use of the reference soil to calculate for C stratification assessment is important, so that similar soils under various treatments can be compared systematically.

## 2.7 Statistical analysis

Statistical analyses were performed using SigmaPlot 11 (Systat Software Inc., San Jose). All data obtained were checked for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). Significant differences in the concentration and stocks of C fractions and their stratification and spectral characteristics influenced by the biochar treatments were evaluated using one-way analyses of variance (ANOVA). The Holm-Sidak post-hoc test was used to differentiate between any two given treatment means. The statistical significance among treatment means was evaluated at  $p \leq 0.05$ , unless otherwise mentioned. Statistical significance is indicated by lower case letters beside the mean values. Results are given as mean  $\pm$  standard error (SE) in both tables and figures. All the results were expressed based on ODE (105 °C) weight basis of soil.

## 3 Results

### 3.1 Concentration of total and extracted organic carbon fractions

Significant differences were observed in the concentrations of TOC and extracted C fractions by the impact of corn cob biochar application (Table 1). Soils amended with 30 t ha<sup>-1</sup> of biochar (BC-30 and BC-30+P) had the highest concentration of TOC (57 to 62%) compared with the unamended soil (CT) ( $F = 4.26$ ,  $p = 0.03$ ). Among the biochar treatments,

humic C concentration was highest in the BC-30 (32.4%) and BC-30+P-treated plots (38.0%) than that in BC-15-treated plots (17.87%). Among the treatments, the CT plots had the lowest humin C concentration (0.79%), with no significant difference in humin C concentrations observed between CT and BC-15-amended plots. Application of corn cob biochar significantly ( $F = 8.14$ ,  $p = 0.03$ ) increased the TEC concentration by 88.8, 116.8, and 131.0% in the BC-15, BC-30, and BC-30+P-treated plots relative to the CT (Table 1).

Biochar contributed immensely to the concentrations of FA and HA in the treated soils. The FA concentration recorded in the biochar was 1.07 g kg<sup>-1</sup>, out of which 0.3 g kg<sup>-1</sup> (28%) was released to the BC-15-treated soils, with 0.6 g kg<sup>-1</sup> (56.1%) of the total FA in the biochar released to the plots treated with 30 t ha<sup>-1</sup> biochar. The total HA concentration in the biochar was 2.33 g kg<sup>-1</sup>, out of which 1.3 g kg<sup>-1</sup> (55.8%), 1.5 g kg<sup>-1</sup> (64.4%), and 1.6 g kg<sup>-1</sup> (68.7%) were released to the BC-15, BC-30, and BC-30+P-treated soils, respectively. Comparatively, higher concentrations of HA were observed in all the treatments than FA concentrations. Among the treatments, the biochar-amended soils significantly increased the HA concentrations compared with the CT ( $F = 3.89$ ,  $p = 0.16$ ). The FA concentration was highest ( $F = 35.28$ ,  $p < 0.01$ ) in the BC-30+P (193.3%) and BC-30 (183.3%) treated soils than the rest of the treatments. Among the biochar treatments, concentration of  $T_{\text{glucose}}$  was significantly higher in the 30 t ha<sup>-1</sup>-treated plots (0.37 to 0.43 g kg<sup>-1</sup>), with a lower concentration (0.22 g kg<sup>-1</sup>) observed in the 15 t ha<sup>-1</sup>-amended soils ( $F = 12.74$ ,  $p < 0.01$ ). Though the lowest concentration of  $T_{\text{glucose}}$  was recorded in the CT, no significant difference was observed between the BC-15-treated soils and the CT (Table 1).

Corn cob biochar application impacted positively on the proportion of FA to HA ( $F = 4.34$ ,  $p = 0.01$ ), with the highest percentage FA:HA ratio of 53% observed in the plots treated with 30 t ha<sup>-1</sup> of biochar. The control plot recorded the smallest FA:HA ratio, with no significant difference between the CT and the BC-15-treated soils.

**Table 1** The impact of biochar on bulk density and the concentrations of total extracted, fulvic acid, humic acid, humin, and glucose equivalent non-humic C fractions at different rates

Treatment	TOC (g kg <sup>-1</sup> )	TEC (g kg <sup>-1</sup> )	Humin (g kg <sup>-1</sup> )	FA (g kg <sup>-1</sup> )	HA (g kg <sup>-1</sup> )	$T_{\text{glucose}}$ (g kg <sup>-1</sup> )	Bulk density (g cm <sup>-3</sup> )
CT	8.7 $\pm$ 0.8b*	2.0 $\pm$ 0.1b	6.7 $\pm$ 0.2b*	0.3 $\pm$ 0.01c	1.6 $\pm$ 0.1b	0.1 $\pm$ 0.02b	1.53 $\pm$ 0.01a
BC-15	11.7 $\pm$ 1.1ab	3.7 $\pm$ 0.6a	8.0 $\pm$ 0.3b	0.6 $\pm$ 0.1b	2.9 $\pm$ 0.6a	0.2 $\pm$ 0.02b	1.44 $\pm$ 0.02b
BC-30	13.6 $\pm$ 1.1a	4.4 $\pm$ 0.3a	9.2 $\pm$ 0.9a	0.9 $\pm$ 0.04a	3.1 $\pm$ 0.3a	0.4 $\pm$ 0.04a	1.38 $\pm$ 0.03b
BC-30+P	13.5 $\pm$ 0.8a	4.5 $\pm$ 0.4a	9.6 $\pm$ 1.4a	0.9 $\pm$ 0.1a	3.2 $\pm$ 0.4a	0.4 $\pm$ 0.1a	1.38 $\pm$ 0.03b

TOC, total organic carbon; TEC, total exchangeable carbon; FA, fulvic acid; HA, humic acid;  $T_{\text{glucose}}$ , total glucose equivalent carbon

\* Means separated by same lower case letter in each column were not significantly different among the treatments at  $p < 0.05$



A significantly higher proportion of TEC/TOC was observed in the BC-30+P-treated soils (39%) compared with a lower proportion in CT plots (Table 2) ( $F = 2.06$ ,  $p = 0.04$ ). Among the biochar treatments, there was no significant difference in the proportion of TEC/TOC. Similarly, no significant difference in TEC/TOC was observed in BC-30, BC-15, and the CT. While the FA/TEC was highest in the 30 t ha<sup>-1</sup>-treated soils, the HA/TEC was highest in 15 t ha<sup>-1</sup>-treated soil and the CT. No significant difference was observed in  $T_{\text{glucose}}/\text{TEC}$  in all the treatments.

The incorporation of biochar significantly influenced the distribution of C when expressed on a mass per unit area basis (Table 3). The TOC stocks were significantly higher ( $F = 2.60$ ,  $p = 0.01$ ) in the plots amended with 30 t ha<sup>-1</sup> of biochar (by 10.9 Mg/ha) than that in the CT and BC-15-treated plots. The TOC stocks in the BC-15-treated plots were not significantly different from the CT. In contrast to TOC, application of biochar at all rates significantly increased the TEC stocks, with an increasing trend observed with increasing the rates of biochar application ( $F = 6.45$ ,  $p < 0.01$ ). Like TEC, the humin stocks showed a similar pattern in response to biochar amendment (Table 3). Similarly, HA stocks were impacted significantly by biochar application at all rates ( $F = 3.06$ ,  $p = 0.07$ ). Corn cob biochar application significantly increased the FA stocks; however, the increases in FA stocks in the BC-30 and BC-30+P-treated soils were significantly higher than that of the BC-15-treated soils ( $F = 30.59$ ,  $p < 0.01$ ). Amendment with 30 t ha<sup>-1</sup> of biochar increased the  $T_{\text{glucose}}$  stocks, with an increase of 194 and 243% in the BC-30 and BC-30+P-treated plots, respectively, compared with the CT ( $F = 5.12$ ,  $p = 0.03$ ). The increase in  $T_{\text{glucose}}$  stocks in the BC-15-amended soils was statistically similar to that of the CT plots.

### 3.2 Stratification of total and extracted organic carbon fractions

A significantly higher stratification (by 55 to 56%) of TOC was observed in the 30 t ha<sup>-1</sup> of biochar-treated soils followed by the plots treated with 15 t ha<sup>-1</sup> (34%) when compared with the CT (Table 4) ( $F = 4.88$ ,  $p = 0.02$ ). Like TOC, a similar pattern on humin and  $T_{\text{glucose}}$  stratification was observed. The total exchangeable carbon (TEC) stratification was significantly higher in all the biochar-treated plots relative to the CT ( $F = 8.14$ ,  $p < 0.01$ ). Though no significant difference was observed in the TEC stratification among the biochar treatments, an increasing TEC stratification trend was observed in the order of BC-30+P > BC-30 > BC-15. The  $T_{\text{glucose}}$  stratification was highest in the BC-30 (by 209%) and BC-30+P (by 262%) treated soils compared with the CT ( $F = 13.51$ ,  $p < 0.01$ ).

### 3.3 Characteristics of total and extracted organic carbon fractions

The humification index (HI), one of the indicators of TOC quality, was significantly increased by the application of corn cob biochar at a rate of 30 t ha<sup>-1</sup>, with an increase of 50 and 69% in the BC-30 and BC-30+P-amended soils, respectively, compared with the CT ( $F = 2.19$ ,  $p = 0.14$ ). Among the biochar treatments, the lowest HI was observed in the plots treated with BC-15 with no difference between this treatment and the CT. The humification ratio was significantly ( $F = 1.57$ ,  $p = 0.25$ ) impacted by the incorporation of biochar at all rates, with an increase of 35, 31, and 41% in the BC-15, BC-30, and BC-30+P-treated plots respectively relative to the CT. In contrast, the degree of humification (DH) of TOC was impacted differently by the biochar treatments. The DH was significantly higher ( $F = 1.34$ ,  $p = 0.14$ ) in the CT and BC-15-amended soils than that in soils treated with BC-30 and BC-30+P (Fig. 1). There was no difference in DH in the BC-30 and BC-30+P-treated plots.

The optical densities measured for both HA-C and FA-C fractions decreased progressively with increasing wavelength. The slope of log optical density vs. log wavelength was significantly smaller in the BC-30 ( $p = 0.032$ ) and BC-30+P ( $p = 0.031$ ) treated soils, compared with the CT and BC-15-amended soils. No significant difference ( $p = 0.23$ ) was observed in the slope between the CT and BC-15-treated soils (Fig. 2).

Spectral analysis showed higher values of  $E_{465}/E_{665}$  for the FA (5.02 to 5.15) in the CT and BC-15-treated soils than that of the HA (2.76 to 2.98) in the BC-30 and BC-30+P-amended soils (Fig. 3).

## 4 Discussion

### 4.1 Effects of corn cob biochar on the concentration of total and extracted organic carbon fractions

The significant increase in TOC in the plots treated with 30 t ha<sup>-1</sup> of biochar could be attributed to the higher aromatic character and high C concentration (Domingues et al. 2017) of the added biochar. Similarly, Zhang et al. (2017) reported a significant increase in TOC when straw-derived biochar pyrolyzed at 350–550 °C was applied at a rate of 16 t ha<sup>-1</sup> to a silty clay loam. Through its interactions with minerals and other organic compounds in soil (Fernández-Ugalde et al. 2017), the applied biochar promotes macro aggregate formation which protects the native and applied C from microbial decomposition (Hartley et al. 2016), hence, increasing the TOC content. Furthermore, the increase in the TOC content could be ascribed to a slower decomposition of the relatively high amount of biochar in the BC-30 and BC-30+P-treated

**Table 2** The impact of biochar on the percent distribution of total extracted, fulvic acid, humic acid, and glucose equivalent non-humic carbon fractions

Treatment	TEC/TOC	FA/TEC	FA/HA	HA/TEC	$T_{\text{glucose}}/\text{TEC}$
CT	0.3 ± 0.03b*	0.2 ± 0.05b	0.2 ± 0.02b	0.8 ± 0.03a	0.06 ± 0.1 <sup>ns</sup>
BC-15	0.3 ± 0.03ab	0.2 ± 0.06ab	0.2 ± 0.02ab	0.8 ± 0.05ab	0.06 ± 0.1
BC-30	0.3 ± 0.02ab	0.2 ± 0.09a	0.3 ± 0.04a	0.7 ± 0.06b	0.09 ± 0.1
BC-30+P	0.4 ± 0.05a	0.2 ± 0.06a	0.3 ± 0.03a	0.7 ± 0.07b	0.10 ± 0.2

TOC, total organic carbon; TEC, total exchangeable carbon; FA, fulvic acid; HA, humic acid;  $T_{\text{glucose}}$ , total glucose equivalent carbon

\* Means separated by same lower case letter in each column were not significantly different among the treatments at  $p < 0.05$

<sup>ns</sup> Denoted no significant difference among the treatments

soils. This is primarily due to the high amount of recalcitrant C in biochar and the dominance of energy efficient fungal food webs (Aziz et al. 2013) as a result of the high CN ratio of biochar. Consequently, transformation of the biochar-derived C through humification into TOC is believed to be far more efficient in the plots treated with 30 t ha<sup>-1</sup> of biochar.

The amount of FA and HA recorded in the treated plots relative to the total concentrations of FA and HA in the biochar suggests that corn cob biochar is a slow releaser of FA and HA. This observation can be ascribed to the slow degradation of HA due to its high molecular weight. The relatively lower concentrations of FA recorded in the treated plots relative to the total concentrations of FA in the biochar may be attributed to the loss of some of the released FA via surface runoff. This effect was quite prominent in the BC-15-treated soils than that in the plots treated with 30 t ha<sup>-1</sup> biochar.

The relatively low FA/TEC in the CT as compared with the BC-30 and BC-30+P-treated plots may be ascribed to significant losses of labile FA. This is substantiated by the lower FA concentrations observed in the CT soils, which was expected primarily due to greater utilization of more labile FA by inefficient bacteria-dominated food webs and concomitant loss of soluble labile FA by leaching and runoff (Schnitzer 2000). Earlier results from the plots showed significantly improved stability, friability, and tensile strength of the aggregates in the BC-30 and BC-30+P-treated plots compared with the CT (Amoakwah et al. 2017a) and this may have enhanced the resistance of the soil against erosive forces to have subsequently minimized the loss of labile FA through leaching and runoff.

The humified C fractions of soil organic matter (SOM) have been reported to undergo variable stabilization processes through complex and close physico-chemical interactions with calcium or clays, or by physical protection within soil aggregates (Stevenson 1994; Salinas-Garcia et al. 1997). Comparatively, among the humified C fractions, the FA concentration is greatly influenced by agronomic practices (e.g., application of organic amendments) than concentrations of the HA, humin, and TOC (Zalba and Quiroga 1999). The

relatively higher concentrations of the FA in the BC-30 and BC-30+P-treated soils is related to an accumulation of low molecular weight aliphatic C compounds from the added biochar that are perhaps weakly associated with cations or clays (Stevenson 1994). The FA has lower molecular weight than HA, and therefore, it is more polar and labile than the HA, and due to its sensitivity to agronomic practices, it can be used as an early indicator when evaluating changes in TOC quality than the HA.

The substantial increase in HA-C in the biochar-treated soils gives an indication of a significant accumulation of aromatic C in the soil. Humic acid has a high molecular weight and therefore degrades slowly in the soil. An accumulation of HA-C in the biochar-amended soils therefore implies that C is sequestered in passive form. Consequently, nutrient accumulation will be potentially enhanced through an increase in the cation exchange capacity (Wang et al. 2017), soil structure will be improved (Daoyuan et al. 2017), water holding capacity will increase (Liu et al. 2017; Yu et al. 2017), and erosion will be reduced (Li et al. 2017). The significantly higher concentrations of glucose equivalent non-humic C compounds in the BC-30 and BC-30+P-treated soils are possibly ascribed to the increased release and translocation of nonstructural carbohydrates from the applied biochar.

#### 4.2 Effects of biochar on the stratification of total and extracted organic carbon fractions

The stratification of TOC and extracted C fractions generally increased with increasing the biochar application rate, primarily due to the relatively high amount of C input (Franzluebbers 2002; Yousaf et al. 2017) from the applied biochar. Also, the high stratification of TOC and C fractions in the BC-30 and BC-30+P-treated soils could be attributed to high respiration quotient and low specific maintenance respiration rate in the biochar-treated plots which indicates high ecosystem functionality and a less stressed ecosystem (Islam and Weil 2000). The 30 t ha<sup>-1</sup> of biochar-amended soils would be expected to provide greater and diverse quantities of crop

**Table 3** The impact of biochar on the total stocks of total extracted, fulvic acid, humic acid, humin, and glucose equivalent non-humic C fractions measured at a soil depth of 0–20 cm

Treatment	TOC (Mg ha <sup>-1</sup> )	TEC (Mg ha <sup>-1</sup> )	FA (Mg ha <sup>-1</sup> )	HA (Mg ha <sup>-1</sup> )	<i>T</i> <sub>glucose</sub> (Mg ha <sup>-1</sup> )	Humin (Mg ha <sup>-1</sup> )
CT	26.8 ± 2.4b*	6.0 ± 0.3b	0.9 ± 0.04c	4.8 ± 0.3b	0.4 ± 0.1b	20.7 ± 2.6b
BC-15	33.7 ± 3.2ab	10.7 ± 1.8a	1.7 ± 0.17b	8.4 ± 1.7a	0.6 ± 0.1b	23.0 ± 2.0a
BC-30	37.7 ± 3.0a	11.8 ± 0.9a	2.3 ± 0.10a	8.4 ± 0.8a	1.0 ± 0.3a	25.9 ± 2.4a
BC-30+P	37.3 ± 3.8a	12.5 ± 1.2a	2.4 ± 0.16a	8.9 ± 1.1a	1.2 ± 0.2a	24.7 ± 3.8a

TOC, total organic carbon; TEC, total exchangeable carbon; FA, fulvic acid; HA, humic acid; *T*<sub>glucose</sub>, total glucose equivalent carbon

\* Means separated by same lower case letter in each column were not significantly different among the treatments at  $p < 0.05$

residues to the soils, which would subsequently increase the content and lability of TOC, and consequently enhance soil health.

### 4.3 Effects of biochar on the quality and characteristics of total and extracted organic carbon fractions

The significantly higher FA/HA values in the BC-30 and BC-30+P-treated soils indicate a relatively higher proportion of low molecular weight aliphatic C compounds with a higher degree of aromatization (Stevenson 1994). The high molecular weight aromatic C, heavily substituted with hydroxyl and carboxyl functional groups, is typical of higher FA/HA ratios and dominant in biologically diverse and active soils (Islam et al. 2000). In contrast, the lower FA/HA values in the CT (untreated soils) reflect leaching and loss of labile FA-C due to the susceptibility of these soils to erosion and leaching because of low TOC content compared with the BC-30 and BC-30+P-treated soils.

Humification index (HI) reflects the presence of non-humic organic carbon that is less condensed, less aromatic, easily metabolized, and therefore easily decomposable and labile. A significantly higher HI in the BC-30 and BC-30+P-treated soils therefore suggests that biochar amendment might have significantly increased the aliphatic TOC quality. However, the proportionate increase in the aliphatic C was far less than

that of the aromatic C. This observation is accentuated by the high humification ratio (HR) values in the plots treated with 30 t ha<sup>-1</sup> of biochar. The HR is directly related to the content of humified organic matter, and therefore, the high HR values in the BC-30 and BC-30+P-amended soils indicate a more humified nature of the TOC in these soils (Saviozzi et al. 1994). The application of biochar at a rate of 30 t ha<sup>-1</sup> resulted in a significant decrease in the degree of humification (DH). The degree of humification is a measure or indicator of the rate of decomposition of soil organic matter in the soil medium. The decrease in SOM humification degree may be attributed to the increase in aggregate stability (Amoakwah et al. 2017a) and enhanced macro aggregate-associated C that is protected in the macro aggregate colloidal particles from rapid decomposition by opportunistic soil microbes. In this sense, the incorporation of biochar at a rate of 30 t ha<sup>-1</sup> decreased the decomposition rate of SOM with positive effects on its degree of humification (Bayer et al. 2002). This has the potential to subsequently improve the physical, chemical, and biological properties of the soil (Milori et al. 2002). Zech et al. (1997) reported that the humification of SOM is characterized by increases in carboxyl C, alkyl C, and aromatic C (mainly phenolic groups) and a decrease in O-alkyl C. The changes that occur in SOM during the humification process are therefore related to the preferential preservation of more recalcitrant organic compounds such as phenolic structures (Milori et al. 2002) in the BC-30 and BC-30+P-treated soils.

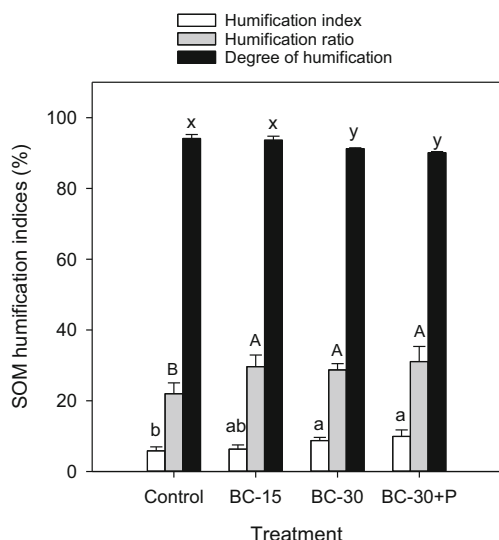
**Table 4** Stratification of total organic carbon and extracted carbon fractions as impacted on by biochar application at different rates

Biochar treatment	TOC	TEC	FA	HA	<i>T</i> <sub>glucose</sub>	Humin
	Values were divided by the values at 20 cm of the reference soil					
CT	1.0b*	1.0b	1.0c	1.0b	1.0b	1.0b
BC-15	1.3 ± 0.13ab	1.9 ± 0.31a	2.0 ± 0.2b	1.9 ± 0.37a	1.8 ± 0.20b	1.2 ± 0.10ab
BC-30	1.5 ± 0.12a	2.2 ± 0.16a	2.8 ± 0.1a	2.0 ± 0.20a	3.1 ± 0.30a	1.4 ± 0.13a
BC-30+P	1.6 ± 0.16a	2.3 ± 0.22a	3.0 ± 0.2a	2.1 ± 0.27a	3.6 ± 0.54a	1.3 ± 0.20a

TOC, total organic carbon; TEC, total exchangeable carbon; FA, fulvic acid; HA, humic acid; *T*<sub>glucose</sub>, total glucose equivalent carbon

\* Means separated by the same lower case letter in each column were not significantly different among the treatments at  $p < 0.05$

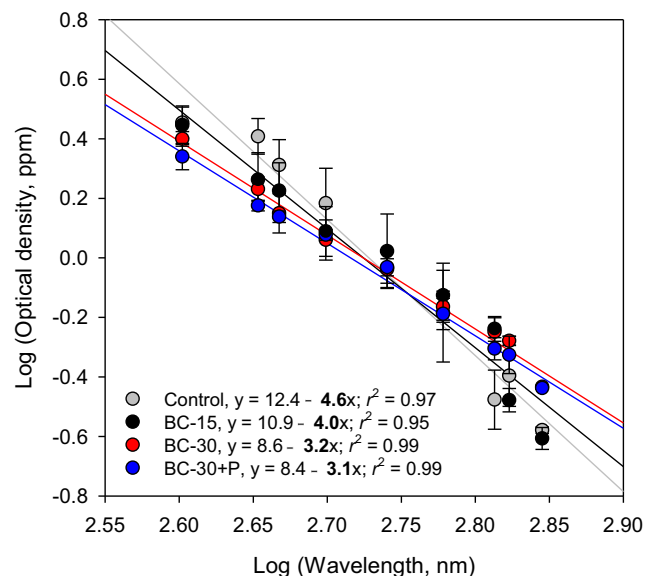




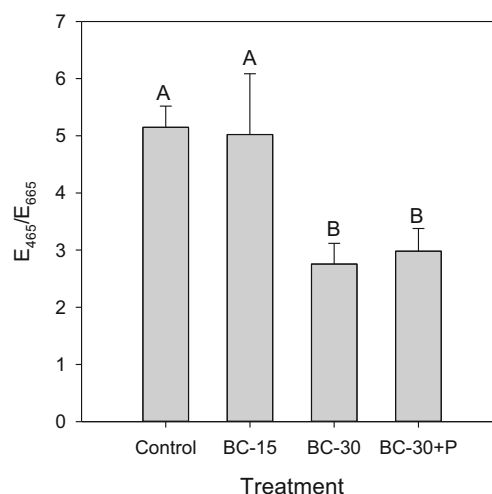
**Fig. 1** The impact of biochar on soil organic matter humification characterized by humification index, humification ratio, and degree of humification (BC-15, 30, and 30+P denote biochar treatments with 15 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 30 t ha<sup>-1</sup> + 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> respectively)

Conversely, the increase in the DH in the CT and BC-15-treated soils reflects the breakdown of soil aggregates (Balesdent et al. 2000) which exposes soil carbon to rapid microbial degradation, with subsequent loss of the labile components of SOM through catabolism and leaching processes, and reduces ecosystem services.

Spectroscopic analysis provides useful insight into the qualitative nature of the soil TOC in the HA-C and FA-C fractions (Islam et al. 1999). The slope of the log optical density vs. log wave length graphs serves as a direct index of the



**Fig. 2** Slopes calculated from the relationship between log wavelength and log optical densities of humic and fulvic acid C fractions combined across biochar treatments and the control (BC-15, 30, and 30+P denote biochar treatments with 15 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 30 t ha<sup>-1</sup> + 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, respectively)



**Fig. 3** The E<sub>465</sub>/E<sub>665</sub> ratios of humic and fulvic acids C fractions combined across the biochar treatments and the control (BC-15, 30, and 30+P denote biochar treatments with 15 t ha<sup>-1</sup>, 30 t ha<sup>-1</sup>, and 30 t ha<sup>-1</sup> + 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> respectively)

particle size or molecular weight of C compounds in TOC (Khan 1959). A relatively gentle (lower) slope suggests the dominance of high molecular weight aromatic HA-C fraction, and this supports the theory that states that the slope decreases with increasing molecular weight of organic C compounds (Khan 1959). The significantly lower slopes in the BC-30 and BC-30+P may be ascribed to the lower E<sub>465</sub>/E<sub>665</sub> ratio (1.95 ± 0.08) of the biochar which suggests that TOC in the biochar is dominated by high molecular weight humic acid-like substances with an increasing proportion of aromatic chain structures and higher degree of aromatic condensation (Chen et al. 1977). This subsequently resulted in significantly lower E<sub>465</sub>/E<sub>665</sub> ratios of the BC-30 and BC-30+P-treated soils, compared with the BC-15 and the CT. Thus, increasing the rate of biochar application resulted in a significant reduction in the E<sub>465</sub>/E<sub>665</sub> ratio, with subsequent significant increases in HA concentrations. The E<sub>465</sub>/E<sub>665</sub> ratio serves as an indicator of humification processes (Stevenson 1994) to influence TOC quality in soils. Higher proportions of aromatic phenolic acids and phenolic polymers are typical of the humic acid fraction of soils with relatively higher organic carbon content (Yavitt and Fahey 1985) as observed in the plots treated with 30 t ha<sup>-1</sup> of corn cob biochar. The above observations could suggest that the BC-30 and BC-30+P treatments may have enhanced the dominance of energy efficient biochemical pathways responsible for the increased aromatic TOC quality.

## 5 Conclusions

This study assessed how corn cob biochar applied at 0 [CT], 15 t ha<sup>-1</sup> [BC-15], and 30 t ha<sup>-1</sup> [BC-30] enriched with P [BC-

30+P] to a highly weathered tropical sandy loam influenced the quality of TOC pools. Results demonstrated the following:

- Increasing the rate of corn cob biochar increased the concentrations of both HA and FA; however, the concentrations of HA were comparatively higher than that of FA.
- Stratification of TOC and extracted C fractions increased with increasing biochar application rates.
- Though biochar increased both aliphatic and aromatic quality of TOC, the spectroscopic analysis from the comparatively gentle slopes of the log optical density vs. log wavelength, coupled with the lower  $E_{465}/E_{665}$  support a suggestion that the BC-30 and BC-30+P treatments favored high molecular weight aromatic quality of TOC in the treated soils.

In perspective, corn cob biochar applied in the right rate may potentially enhance the stability of soil organic carbon by improving the aromatic TOC quality. Biochar as a soil amendment has the potential to enhance C sequestration in a tropical agro ecosystem.

**Acknowledgments** This work was supported by Danida (Ministry of Foreign Affairs of Denmark) under the project “Green Cohesive Agricultural Resource Management, WEBSOC,” DFC project no: 13-01AU, and the Borlaug Leadership Enhancement in Agriculture Program (Borlaug LEAP) through a grant to the University of California-Davis by the United States Agency for International Development.

**Data availability statement** The data that support the findings of this study are available from the corresponding author upon reasonable request.

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