

Simple method of coffee-shrub biochar-ozonolysis^{1,2}

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J. Agric. Univ. P.R. 106(1):91-108 (2022)

ABSTRACT

Biochar, the pyrolysis product of carbon-rich biomass, renders climate benefits because it helps to sequester carbon in soil. Biochar also improves soil health because it increases the nutrient retention capacity in topsoil, improving aggregate stability and water holding capacity. These benefits contribute to agricultural production because biochar provides a good substrate for nourishing root growth and plant health, thereby contributing to the nation's food security. Biochar's contribution depends on the quantity and type of oxygen-containing functional groups. These functional groups are determinants in biochar interactions with nutrients and redox reactions. This study aims to develop a simple and economical method to improve biochar's agronomic traits through ozonolysis. We evaluated ozonolysis reaction time to oxidize a washed and unwashed coffee shrub biochar (WCSB and CSB, respectively). After the exposure of WCSB and CSB to ozone at different intervals, data from both collected by the FTIR-ATR spectra showed that the bands increased in intensity from 3331 to 3441 cm^{-1} (O-H band) and 1585 cm^{-1} (carbonyl functional group band). Besides, we observed a decrease in pH and an increase in specific conductance and soluble organic carbon content with the elapsing time of ozonolysis, demonstrating the effectiveness of ozonolysis in the oxidation of the biochar surface. The increase in the E_4/E_6 ratio suggests that the saturated products from the ozonolysis process increase with time due to the breakdown of the labile organic carbon and the formation of the functional groups of soluble acidic oxygen bonds through the breakdown of the double bonds of carbon. Furthermore, the results indicated that it is unnecessary to wash the biochar before subjecting it to the ozonolysis process.

Key words: biochar, oxidation, ozonolysis, functional groups, FTIR, soluble organic matter, redox

¹Manuscript submitted to the Editorial Board 2 September 2021.

²This work was supported by the USDA National Institute of Food and Agriculture project award number 2018-69002-27936. We thank the AFRI-NIFA initiative for providing encouragement and funding for this research.

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RESUMEN

Método simple para la ozonólisis de biocarbón producido de cafetos

El biocarbón, producto de la pirólisis de biomasa rica en carbono, brinda beneficios climáticos porque ayuda a secuestrar carbono en el suelo. El biocarbón, además, mejora la salud del suelo al aumentar la retención de nutrientes en la capa superficial, mejorando la estabilidad de los agregados y la capacidad de retención de humedad. Estos beneficios contribuyen a mejorar la producción agrícola, porque proporcionan un buen sustrato para nutrir el crecimiento de las raíces y la salud de las plantas, y por ende contribuyen a la seguridad alimentaria. La contribución del biocarbón es dependiente de la cantidad y tipo de grupos funcionales que contienen oxígeno. Estos grupos funcionales son determinantes en las interacciones del biocarbón con los nutrientes y las reacciones redox. Este estudio tiene como objetivo desarrollar un método sencillo y económico para mejorar las características agronómicas del biocarbón a través de la ozonólisis. Se evaluó el tiempo de ozonólisis para oxidar un biocarbón preparado de cafetos, lavado y sin lavar (WCSB y CSB, respectivamente). Los espectros FTIR-ATR mostraron bandas crecientes en el rango de 3331 a 3441 cm^{-1} (banda O-H) y 1585 cm^{-1} (banda del grupo funcional carbonilo), con el tiempo de exposición del biocarbón al ozono. Además, se observó una disminución del pH, y un aumento de la electroconductividad y el contenido de carbono orgánico soluble con el tiempo de ozonólisis. Con estos resultados se demostró la efectividad del método de ozonólisis para oxidar la superficie del biocarbón. El aumento en la relación E_d/E_g sugirió que los productos saturados del proceso de ozonólisis aumentaron con el tiempo debido a la ruptura del carbono orgánico lábil y a la formación de los grupos funcionales de enlaces de oxígeno ácido solubles mediante la ruptura de dobles enlaces de carbono presentes en el biocarbón. Además, los resultados encontrados indican que no es necesario lavar la superficie de carbono previo a someterlo al proceso de ozonólisis.

Palabras clave: biocarbón, oxidación, ozonólisis, grupos funcionales, FTIR, materia orgánica soluble, redox

INTRODUCTION

The management of soil to improve soil health is vital to preserving the nation's agricultural food security and production (Lal, 2016). Soil health assessment includes physical, chemical and biological properties (Guo, 2020; Kibblewhite et al., 2008; Lal, 2016; Raghavendra et al., 2020). Soil structure changes caused by traditional farming methods usually cause soil degradation; thus, infiltration and water retention problems arise as soil porosity changes (Sá et al., 2013). Soil structure changes are usually due to soil organic matter losses impacting soil respiration processes and root penetration (Almendro-Candel et al., 2018; Martínez et al., 2008). These changes usually have concomitant problems (e.g., erosion and fertility loss), further decreasing soil organic carbon and impairing soil health (McBratney

et al., 2014). Soil organic carbon strongly impacts land functionality and health (Lal, 2016) because soil organic carbon is the primary energy source of the natural ecosystems. Implementing sustainable agricultural practices that increase organic carbon content in the soil improves soil health and promotes a farming system with high production capacity, decreasing carbon dioxide emission and moderating climate change (Aisosa et al., 2019; Hu et al., 2020; McBratney et al., 2014; Schmidt et al., 2011; Tivet et al., 2013). Biochar can enhance the soil's physical, chemical and biological properties, reducing bulk density, increasing water and nutrient retention, and providing porosity (Guo, 2020), which enhances microbial respiration. Biochar (especially when rich in functional groups) used as an organic soil amendment improves the cation exchange capacity (Escalante et al., 2016; Guo, 2020; Huff et al., 2018; Kharel et al., 2019; Liang et al., 2006).

Biochar is a carbonaceous material with agronomic purposes obtained from the thermal decomposition of biomass by pyrolysis (Aisosa et al., 2019; Escalante et al., 2016; Guo, 2020; Huff et al., 2018). This process is carried out in the absence of or with a limited amount of oxygen, and the carbon produced is a recalcitrant material, which gives it the ability to sequester carbon in soil and help mitigate climate change (Escalante et al., 2016; Huff et al., 2018). The characteristics of biochar may vary according to the feedstock, pyrolysis temperature, humidity and residence time, extinction process, post-production management and storage (Escalante et al., 2016; Guo, 2020; Li et al., 2013; Yuan et al., 2017). These parameters are considered determinants for the aromatic to aliphatic biochar ratio and nutrient content (Spokas et al., 2012). When biochar is prepared under high temperatures, it usually has a few functional groups, such as carboxylic and hydroxyl moieties (Guo, 2020).

Through biochar ozonolysis, we can incorporate oxygen-derived functional groups during a post-production process with the aim of increasing the cation exchange capacity (CEC) through functional groups, such as carboxyls and hydroxyls (Huff et al., 2018; Kawamoto et al., 2005; Kharel et al., 2019) that bear a negative charge that can attract and hold oppositely charged ions. Several research groups found a significant increase in the CEC of biochar with the incorporation of functional groups (Huff et al., 2018; Kharel et al., 2019; Yuan et al., 2017). Cation exchange capacity is a key property for nutrient and water retention, a desirable agronomic trait that is present in an oxidized biochar (Huff et al., 2018; Kharel et al., 2019). Ozonolysis is an oxidation process in which ozone breaks down the carbon-carbon double bonds producing carbonyl, hydroxyl and carboxylic moieties

in the biochar carbonaceous skeleton. These oxygen content moieties, rich in lone pair electrons, enhance the biochar cation retention capacity.

This study aims to develop a simple and economical method to improve biochar's agronomic traits through post-production oxidation (Aisosa et al., 2019; Suliman et al., 2016). We evaluated the ozonolysis reaction time to significantly enhance the washed and unwashed biochar chemical properties by increasing functional groups containing oxygen in the biochar carbonaceous skeleton (Aisosa et al., 2019). We quantified the oxidized biochar's redox, acidity and electrical conductivity (EC). In addition, we analyzed soluble labile organic carbon (LOC) and the optical density ratio at 465 and 665 nm (E_4/E_6) of soluble organic layers in biochar surfaces to assess their degree of unsaturation. Finally, we evaluated the effect of ozonolysis time on biochar to determine the minimum time required to obtain biochar with the desirable agronomic traits.

MATERIALS AND METHODS

Experimental setup (ozone treatment)

The experiment, established in July 2020, comprises two types of biochar [unwashed and washed coffee shrub biochar (CSB and WCSB)] with eight treatments of ozone exposure times. We prepared four ozone treatment systems consisting of a 68-L plastic box each with a lid, an ozone generator (Model 5000-OG, 5000 mg/h)⁷ and two biochar samples (CSB and WCSB). The plastic box was inverted, and the lid was the bottom of the system. Two 100-g samples of each type of biochar were spread over paper towels and placed randomly on the lid in front of the system. The ozone generator was placed behind the samples and each system was closed with the plastic box inverted. The samples were treated at room temperature with ozone at intervals of 0, 1, 2, 4, 8, 12, 24, and 36 h, taking aliquots of 10 g after each exposure. To take these aliquots, each system was opened and allowed to stand for 5 min for safety reasons. Then, each sample was mixed to guarantee even ozone exposure and closed for the next ozone exposure.

⁷Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

Preparation of unwashed (CSB) and washed biochar (WCSB)

Air-dried coffee shrub residues of ca 1 m long and ca 0.13 m diameter obtained from the pruning of coffee trees (common agricultural practice) were carbonized in a modified Kon-Tiki apparatus (Chong, J.A., C. Rivera and J.A. Dumas, 2021. Bottomless Biochar Open Kiln. Unpublished data; Schmidt and Taylor, 2014) at the Agricultural Experiment Substation of Adjuntas. The pyrolytic temperature ranged from 500 to 700 °C for ca 5 h. Biochar was then completely wetted using well water until it was quenched.

First, we used sieves to homogenize the biochar particles, obtaining sizes smaller than 2.34 mm and larger than 1 mm. The biochar used for experimentation (1,500 g) was crushed and sieved, and the fraction <1 mm was oven-dried at 105° C for 44 h. Then, two subsamples were prepared; one was stored in a sealed plastic bag and labeled as CSB. A biochar slurry was made with the second subsample by mixing 750 g of the biochar sample with distilled water for 5 min and then filtering the mixture through a glass fiber filter (Whatman GF/B). Next, the biochar residues in the glass fiber filter were washed continuously with distilled water until reaching a constant value of electrical conductivity of the leachate. Afterwards, the water-washed biochar was vacuum drained, oven-dried at 105° C for 28 h and labeled WCSB.

Fourier-Transform Infrared Spectroscopy (FTIR)

Approximately 1 g of CSB and WCSB were ground using an agate mortar and sieved to 500 µm before FTIR analysis. All the spectra were collected using an IRTracer-100 spectrophotometer (Shimadzu Corp.) equipped with a high-luminance ceramic source and a DLATGS detector with a temperature control system. Spectra were corrected applying baseline and attenuated total reflectance (ATR) correction protocols. The ATR sampling device was a QATR-10 single reflection with a diamond sample port. Aliquots of the samples were placed over the diamond sample port and pressed over the surface using a stainless-steel rod. Each spectrum consisted of forty-five scans in the range of 4000 to 400 cm⁻¹ using a resolution of 4 cm⁻¹.

Soluble Labile Organic Carbon (LOC)

We assessed labile organic carbon in treated biochar using the modified procedure developed by Wang et al. (2007). Briefly, we weighed 2.00 g of biochar (particles <1 mm) in 50 mL plastic test tubes. Then, 30 mL of distilled water was poured into the test tubes, cap partially

closed, and biochars were extracted at 70° C for 18 h using an Auto Clave (Yamato SN510C). After extraction, the samples were allowed to cool, stirred, and centrifuged at 9,000 rpm for 10 min. Finally, we decanted the supernatant into a 40 mL Total Organic Carbon (TOC) vial, and the amount of carbon was determined using a TOC analyzer (Model Torch). This solution was used to determine the optical density ratio between 465 and 665 nm (E_4/E_6 ratio) using a UV/Vis spectrophotometer (Shimadzu model UV-1800). The E_4/E_6 ratio gives information about the recalcitrance and aromaticity of the soluble organic component of the biochar.

pH, electrical conductivity and redox potential

Biochar pH, EC, and ORP were determined by mixing 2.00 g of biochar with 20 mL of distilled water (1/10 w/v) into a 50-mL test tube. The biochar-water mixture was shaken for one hour, then let stand for 30 min. The CSB slurry was then measured for both pH and EC using calibrated meters (Mettler Toledo pH meter with a LE438-IP67 pH probe and Mettler Toledo EC meter with a LE703 EC probe, FiveEasy Plus model). Finally, the oxidation-reduction potential was measured using an ORP meter (HI 2215 pH/ORP Meter) with an Orion combination ORP electrode. The ORP electrode combines a platinum electrode with an internal Ag/AgCl reference electrode calibrated against a Zobel solution.

Statistical analysis

The experimental design was a randomized split-plot design with four replications. The type of sample (two pre-treatments: washed and unwashed) was randomly applied, and then was split plot by ozonolysis time (eight levels: 0, 1, 2, 4, 8, 12, 24 and 36 h). The explanatory variables were pre-treatments and ozonolysis elapsing times, and the response variables were LOC, pH, EC and ORP. An analysis of variance (ANOVA) was carried out to evaluate the effects of the type of sample and ozonolysis time, both being fixed effects. Fisher's least significant difference (LSD) was performed to evaluate significant differences between means using the SAS statistical program with a significant level of 0.05. After carrying out a Q-Q plot and a residual versus fits plot, the EC and E_4/E_6 ratio were transformed as $\ln(x)$ prior to statistical analyses to homogenize and normalize the variance in the data. We use a variance adjustment technique for redox potential analysis at each ozone exposure time to accomplish the homogeneity of variance assumption.

RESULTS AND DISCUSSION

Effect of ozonolysis treatment time on washed and unwashed biochar functional group generation

Figures 1 and 2 show the FTIR-ATR spectra of CSB and WCSB at the treatment of 0 and 36 h. In the IR spectra, we observed band shifts of 100 cm^{-1} to the right from the frequency of 3500 to 1585 cm^{-1} . The difference in the IR spectrum via ATR compared to the transmission spectrum is documented elsewhere (Grdadolnik, 2002). Unfortunately, most IR library bands, which assign a band to a specific functional group, are from the transmission spectrum; thus, it is essential to corroborate band

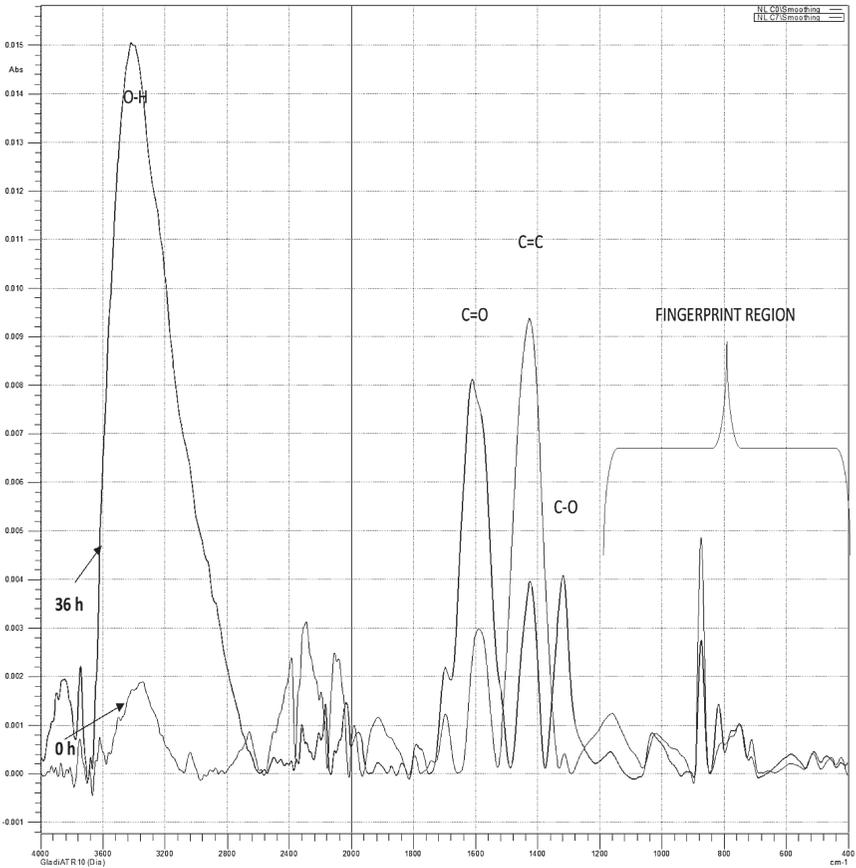


FIGURE 1. FTIR-ATR spectra of CSB sample and its product after 36 hours of ozone exposure. The fine black line represents the NOE spectra, and the bold black line shows the spectra after 36 hours of ozone exposure.

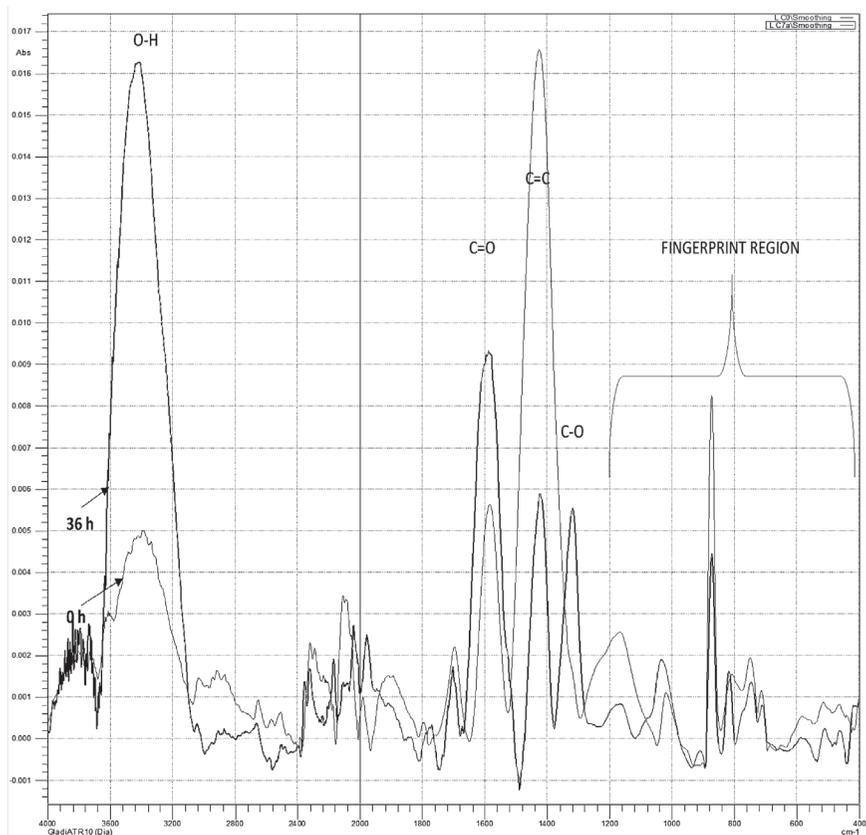


FIGURE 2. FTIR-ATR spectra of WCSB sample and its product after 36 hours of ozone exposure. The fine black line represents the NOE spectra, and the bold black line shows the spectra after 36 hours of ozone exposure.

shifts using ATR compared to the IR transmission spectrum with an infrared transmittance standard. We selected ethylenediaminetetraacetic acid (EDTA) to corroborate band skew to longer wavelength (lower wavenumber) of the functional groups. The FTIR-ATR spectra of EDTA show similar shifting in IR spectra via ATR compared to its transmission FTIR spectra, which corroborate ATR band assignments. In addition, we observed prominent band changes in the range of 3331 cm^{-1} to 3441 cm^{-1} , from the hydroxyl functional group, whose intensity increased with exposure time to ozone. The same pattern occurs for the absorption band in the 1585 cm^{-1} , corresponding to the carbonyl functional group. The hyperchromic effect on these bands was directly proportional to the ozone exposure time of the biochar, indicating higher surface oxidation. Thus, the spectra suggest losses in unsaturated carbon skeleton resulting in the formation

of oxygen-containing functional groups. We observed similar IR spectra between non-ozone exposed washed and unwashed biochar suggesting it is probably unnecessary to wash the biochar before ozonolysis (Figures 1 and 2). Also, the lack of or relatively low band in the carbonyl and hydroxyl regions in both spectra suggest we have a biochar with relatively low bio-oils content. We observed remarkable changes in the IR spectra after 36 h of ozone exposure compared to non-ozone exposed (NOE) samples for both sample types (Figures 1 and 2) and unremarkable differences for biochar from both sample types after 36 h of ozone time. In previous publications, the generation of these oxygen-containing group IR bands in biochars was concomitant with the increase in cation retention capacity because the associated negative surface charge increased (Huff et al., 2018; Kharel et al., 2019).

Biochar's ozonolysis treatment time and E_4/E_6 optical ratio of extractable organic carbon

Ozonolysis is an oxidation process that breaks down large unsaturated organic molecules into smaller molecules that have relatively increased oxygen concentration with higher polarity and usually greater water solubility. The LSD used to determine significant differences between soluble organic carbon indicated significant differences between sample types (CSB and WCSB) and ozonolysis time elapsed ($p < 0.0001$). The WCSB had significantly lower LOC than the CSB before the ozonolysis process began because the washing process removed them. In addition, the WCSB showed a significant difference in LOC after 8 h compared to untreated WCSB (control time); however, for the CSB, we only found a difference after 36 h compared to untreated CSB (control). This finding suggests more accessible spaces interact with ozone in WCSB than CSB because the washing process presumably removes ashes that coat the surface delaying the ozonolysis process on biochar surfaces. We observed that LOC from CSB and WCSB increases with time elapsed in ozonolysis, from 686 $\mu\text{g/g}$ to 1,089 $\mu\text{g/g}$ (an increase of 59%) after 36 h of ozonolysis for CSB. Similarly, for WCSB, LOC rose from 353 $\mu\text{g/g}$ to 892 $\mu\text{g/g}$ (an increase of 153%) after 36 h (Table 1). In both sample types, the breakdown of carbon-carbon double bonds during the ozonolysis treatments produced oxygenated soluble organic molecules, as reported by Kharel et al. (2019). These compounds are energy sources in the soil, which potentially can lead to more significant benefits upon biochar application to the soil (Kharel et al., 2019).

In the ozonolysis process, both samples increased the amount of LOC on the biochar surface, which should improve its agronomic characteristics as a soil amendment. These findings denote ozonolysis treatment for enhancing biochar agronomic properties in labile organic carbon based on

TABLE 1.—Soluble labile organic carbon (LOC) content and E_4/E_6 ratio of coffee shrub biochar as affected by the increase in ozonolysis time.

Samples	Parameters	Ozonolysis time elapsed (hours)							
		0	1	2	4	8	12	24	36
CSB	LOC ($\mu\text{g/g}$)	686 a ¹	768 a	794 a	811 a	743 a	745 a	849 a	1,089 b
	E_4/E_6 ^{2,3}	2.36 a	2.25 a	2.33 a	2.50 a	2.65 ab	4.01 bc	5.74 cd	7.33 d
WCSB	LOC ($\mu\text{g/g}$)	353 a	415 ab	482 abc	501 abc	566 bc	613 c	803 d	892 d
	E_4/E_6 ^{2,3}	2.07a	1.79 a	2.00 a	2.25 ab	3.81 cd	3.25 bc	5.13 de	5.62 e

¹Means followed by the same letter within a row are not significantly different according to LSD, $p < 0.05$.

² E_4/E_6 data was analyzed using a logarithmic transformation.

³ E_4/E_6 reported values are the back-transformed means after the logarithmic transformation statistical analysis.

a previous biochar treatment. The benefits of high LOC content in the soil are that they promote microbial activities because they serve as microbial energy sources promoting mineralization processes (Guo, 2020; Martínez et al., 2008). Additionally, the recalcitrant portion in the biochar helps to preserve soil physical structure and long-term chemical and biological properties related to soil health (Guo, 2020; Kibblewhite et al., 2008).

The high recalcitrance of biochar due to the aromatic nature of its carbon skeleton and its low content of LOC (Escalante et al., 2016) makes it a material with a reduced capacity to serve as microbial energy source. In our case, we found that the ozonolysis process promotes a labile organic layer in CSB and WCSB that should improve soil agronomic properties in the short term, with the recalcitrant portion promoting long-term benefits. The increase in labile “oxidated” organic carbon during ozonolysis elapsing time was evident by the increase in extractable organic carbon concentration. The increase in LOC concentration is due to losses in biochar’s carbon skeleton recalcitrance per se and possibly further oxidation of particulate organic matter from the first step ozonolysis products. The findings of LOC derived initially from recalcitrance carbon were also supported by the E_4/E_6 ratio increase with the ozonolysis time elapsed for both CSB and WCSB. We found no significant differences between sample types for E_4/E_6 ratio ($p=0.2508$) but significant differences between ozonolysis time elapsed $p<0.0001$). The E_4/E_6 ratio for CSB and WCSB not treated with ozone were 2.36 and 2.07, respectively. The ozone-treated samples of CSB and WCSB had an E_4/E_6 ratio of 7.33 and 5.62 after 36 h of ozonolysis, respectively (Table 1). This increase with ozone exposure time suggests further unsaturated moieties losses (Chen et al., 1977), implying that the labile organic compounds of the first ozonolysis reaction could react further with ozone, producing molecules with higher aliphatic nature.

Effect of ozonolysis treatment time on CSB and WCSB pH

The biochar pH is an important parameter that governs the release of nutrients and water from and into its porous carbonaceous structure (Ding et al., 2016). When biochar is mixed with soil, its pH changes the available nutrient species affecting soil fertility (Ding et al., 2016) According to the LSD analysis, there were significant differences in biochar pH between CSB and WCSB prior to ozonolysis treatments, and until 4 h of ozonolysis (Table 3). This finding indicates that the washing process removes basic-soluble compounds from biochar surfaces. The pH of the CSB was significantly higher (8.28) than that of WCSB (7.54) because washing removes ashes, which are rich in carbonates from the biochar surface. After eight hours of ozonolysis there were no significant differences between CSB and WCSB, which indicates that biochar acidity depended on ozonolysis time for both biochar types; and the effect of biochar washing

on the biochar pH is shown by losses during the oxidation process (Table 3). Chemical changes occur on the biochar surface because of its carbon skeleton nature, rich in unsaturated aromatic and non-aromatic moieties, which are susceptible to oxidation. The oxidation of unsaturated moieties with ozone is recognized elsewhere (Grosjean and Grosjean, 1999; Huff et al., 2018; Zhong et al., 2017). The oxidation pathways are pH-dependent through molecular ozone direct reactions and hydroxyl radical indirect reactions, the last pathway forming hydroxylation products (Zhong et al., 2017). The hydroxyl radicals attack most of the organic and inorganic molecules due to their high oxidizing potential, which confers a nonselective oxidative nature on the hydroxyl radicals (Gu et al., 2021; Kappenol and Liebman, 1984; Westerhoff et al., 1999; Zhong et al., 2017). The hydroxylation products eventually are further oxidated to carboxylic acids. Thus, decreases in pH depend on the time elapsed in ozone exposition. As observed in our study, the oxidation process increases carboxylic moieties, increasing hydronium concentration during the dissociation process in an aqueous solution. Table 2 shows that the pH of the CSB and WCSB samples decreases with ozone exposure time. There were significant pH decreases for untreated samples of CSB and WCSB from 8.28 and 7.54, respectively, to 6.18 and 6.33, respectively, after 36 h of ozonolysis. The observed decrease in pH of the biochars with ozone exposure concurs with the results of Huff et al. (2018) and Kharel et al. (2019), which the authors attributed to the generation of acidic oxygen functional groups on the surface during the ozonolysis.

The decrease in pH of biochar could also be associated with an increase of oxygen rich labile organic carbon (ORLOC) from recalcitrant carbon skeleton breakdown with the ozonolysis time, but more with a further breakdown pathway of ORLOC during ozonolysis. The ORLOC further breakdown proposed is according to the reported enhancement in nucleophilicity induced by the hydroxyl and carboxylate moieties in aromatic molecules that promote its electrophilic ozone attack (Zhong et al., 2017). The breakdown pathway of ORLOC could help to explain the significant changes in pH after 4 h of ozonolysis for CSB without substantial increases in total extractable organic carbon until after 36 h. These findings probably stem from the number of organic molecules increasing because of ORLOC's further breakdown, while the total carbon content in the solution does not. The suggestion is in accordance with the WCSB findings, where we found significant changes in pH and labile organic carbon after 8 h of ozonolysis because the washing treatment removed easily oxidable pretreatment ORLOC before ozonolysis (Tables 1, 2 and 3). The thin film layer of bio-oils that covers the surface of the CSB should be more susceptible to ozonolysis than the recalcitrant carbon skeleton of the inner layer. Therefore, extractable organic carbon

TABLE 2.—*The pH, EC and redox potential values of coffee shrub biochar as affected by the increase in ozonolysis time.*

Samples	Parameters	Ozonolysis time elapsed (hours)								
		0	1	2	4	8	12	24	36	
CSB	pH	8.28 a ¹	8.17 a	8.03 ab	7.89 b	7.34 c	7.09 c	6.69 d	6.18 e	
	EC (µS/cm) ²	383.1 a	433.2 ab	442.3 ab	486.9 b	789.5 c	1,097.9 d	1,990.3 e	3,464.8 f	
	Redox	164.4 a	171.7 a	167.7 a	171.6 a	204.7 b	207.0 b	214.6 b	225.7 b	
WCSB	pH	7.54 a	7.51 a	7.48 a	7.32 a	6.99 b	6.87 bc	6.62 c	6.33 d	
	EC (µS/cm) ²	158.9 a	185.1 ab	211.2 b	260.0 c	476.9 d	807.7 e	1,790.8 f	3,232.0 g	
	Redox	181.3 a	205.8 b	221.9 b	220.7 b	219.8 b	213.4 b	216.8 b	194.5 ab	

¹Means followed by the same letter within a row are not significantly different according to LSD, $p < 0.05$.

²EC reported values are the back-transformed means after the logarithmic transformation statistical analysis.

is generated sooner in CSB than WCSB, and its significant effects on pH are observed sooner. Furthermore, Table 3 shows no significant differences in pH between CSB and WCSB after 12 h of ozonolysis, which could be due to the generation of oxygen-containing functional groups and their corresponding buffering capacity, which delay significant pH changes (Huff et al., 2018).

Effect of ozonolysis treatment time on CSB and WCSB EC

The changes in EC, an indicator of changes in the ionic conductivity, were significantly different between sample types ($p < 0.0001$) and between ozonolysis time elapsed ($p < 0.0001$). We found that the EC of the CSB and WCSB samples increased with ozone exposure time (Table 2). The significant difference between untreated CSB and WCSB (Table 3) was probably because the ionic nature of the soluble material was removed during the washing process. We found significant differences in the CSB after 2 h of ozone exposure compared to unexposed samples. Similarly, we found significant differences in the WCSB after 4 h of ozone exposure compared with the unexposed sample. These findings indicate losses of a highly reactive organic layer during the biochar washing process, explaining the delay in unsaturated disruption of WCSB compared with CSB. The unexposed CSB had a mean electrical conductivity of 383.1 $\mu\text{s}/\text{cm}$ that significantly increased to 3,464.8 $\mu\text{s}/\text{cm}$ after 36 h of ozonolysis (Table 2). The untreated WCSB had a mean electrical conductivity of 158.9 $\mu\text{s}/\text{cm}$ that significantly increased to 3,232.0 $\mu\text{s}/\text{cm}$ after 36 h of ozonolysis (Table 2).

The EC of biochars in solution increases with the increase in pyrolysis temperature. Higher pyrolysis temperature decreases the biochar oxygen content and increases ashes with a concomitant increase

TABLE 3.—*Statistical comparison between WCSB sample and CSB sample at each ozonolysis exposure.*

Ozonolysis elapsed time (hours)		p-value				
CSB	WCSB	pH	CE	Redox	LOC	E4/E6 ratio
0	0	<0.0001	<0.0001	0.0181	0.0005	0.5646
1	1	<0.0001	<0.0001	0.0047	0.0003	0.2450
2	2	<0.0001	<0.0001	0.0013	0.0011	0.4646
4	4	<0.0001	<0.0001	<0.0001	0.0012	0.6747
8	8	0.0080	<0.0001	0.1344	0.0530	0.1827
12	12	0.0790	0.0025	0.1125	0.1467	0.3116
24	24	0.5654	0.2089	0.8974	0.6067	0.9913
36	36	0.2296	0.4869	0.1261	0.0326	0.4792

in EC (Rehrah et al., 2014). Our findings suggest that increasing the oxygen content in the biochar increased the material's electrical conductivity, which seems contrary to the generally reported observation that the oxygen content in the biochar decreased its electrical conductivity (Kane et al., 2021; Rehrah et al., 2014). The discrepancies exist because the EC reported is the solid-state material capacity to conduct an electrical charge (Kane et al., 2021); our values are the EC in a solution that results from ORLOC and the capacity of the soluble salt to conduct an electrical charge, not the solid conduction capacity. The changes in biochar EC follow the same pattern shown by LOC and pH in ozonolysis. Biochar inorganic salt content and electron conductivity properties resulting from pyrolytic temperature must produce a conductive material. The ozonolysis treatment of biochar modifies its surface, increasing the electron conductive properties of the soluble materials, which increases the electron and proton transfer process (Tables 1 and 2). The findings previously mentioned occur because biochar electrical conductivity depends on the type of feedstock, pyrolytic conditions and ozonolysis treatment.

Effect of ozonolysis time periods on CSB and WCSB redox properties

The redox potential was significantly different between sample types ($p=0.0002$), denoting the pre-treatment effect on the biochar properties of electron storage, mobility and transfer. The CSB shows an increasing trend in redox potential with ozonolysis time elapsed and shows significant differences after 8 h of ozone exposure compared to the unexposed samples (Table 2). On the other hand, the WCSB did not show a trend in oxidation-reduction potential (ORP), probably because the oxygen dissolution during the sample measurement makes it difficult to get stable values to allow a discernable trend (Joseph et al., 2015). We observed a significant change for the ozone exposure samples from 1 to 24 h compared to the unexposed WCSB. The ORP increased constantly from 1 to 24 h of ozone exposure but dropped significantly at 36 h of exposure, where we did not observe a significant difference between the control sample (unexposed WCSB) and the exposed samples. These findings confirm the oxidation of the organic molecules during the ozone exposure and support the above findings of losses of highly reactive organic molecules during the biochar pre-treatment washing process. Our findings denote the significant differences between sample types, but more conclusive studies are needed to characterize the ozonolysis process and better understand biochar surface electron transfer processes.

CONCLUSION

Soil organic amendments that help preserve and increase soil organic matter are needed everywhere. Biochar surface modification renders benefits to the soil's chemical, physical and biological properties. Surface modification of biochar by oxidation has been widely studied on a small scale, but an economic protocol for ozonolysis is needed. This study demonstrates the effectiveness of an ozonolysis method to change the chemical and physical properties of unwashed and washed biochar of coffee shrubs. As the ozonolysis time increased, we observed a decrease in biochar pH and an increase in LOC content and electrical conductivity. These changes may be due to the formation of acidic oxygen bond functional groups from the carbon double bond breakdown on the biochar surface, the further breakdown of the LOC products from ozonolysis, and the release of nutrients deeper in the biochar structure because of the biochar ozonolysis process. The results demonstrate that it is unnecessary to wash the biochar before the ozonolysis process, but the exposure time for ozonolysis of the unwashed biochar needs to be extended. The use of ozone as an oxidizing agent does not produce harmful by-products and is an economical method to improve biochar benefits to the farmer. The proposed ozonolysis method to improve the properties of biochar could be easily implemented by farmers because it does not require expensive or hard to acquire equipment. Furthermore, this experiment demonstrates that ozonolysis does not require a costly and time-consuming washing process and could easily be implemented in pit or Kon-tiki systems.

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