Physicochemical Characterization and Stability of Biochar Obtained from 5 Species of Forest Biomass in Peru

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The maintenance of the extensive parks at National Agrarian La Molina University (UNALM) generates a large amount of forest biomass, with high treatment costs. For this reason, the pyrolysis of the forest biomass is an alternative for valorization, allowing proper management producing biochar. The aim of the research was to evaluate the stability of biochar at 300°C and 500°C from forest biomass of five predominant species at UNALM, *Grevillea robusta*, *Schinus molle*, *Casuarina cunninghamiana*, *Ficus benjamina* and *Eucalyptus spp*. To determine the stability of biochar produced, two methodologies were used (IBI and an adaptation of the López et al.(2010) method), for which organic, labile, oxidizable, recalcitrant carbon on biochar were determined. The results showed a decrease in biochar yield of 6.50% with an increasing pyrolysis temperature. Regarding physicochemical characteristics, the biochar produced was alkaline (pH above 8); besides, the pH and electrical conductivity increased with temperature, by 0.64 and 0.38 ds/m, respectively. However, in the case of nitrogen and ashes, the opposite occurred. It was concluded that all the biochar produced complied with the stability limits according to the applied methodologies, and BSM500 had the highest value of stability considering the applied methodologies. This may indicate that it could have the greatest sequestration of C once applied to the soil and also the reduction of emissions; for that reason, it would be important to carry out future research to estimate the potential for C sequestration in the long term.

Keywords: biochar, stability, characterization, *Grevillea robusta*, *Schinus molle*, *Casuarina cunninghamiana*, *Ficus benjamina*, *Eucalyptus spp*.

Introduction

In many countries, it is common for people and factories to burn straw and other agricultural, forestry, domestic and animal wastes in homes, fields and food processing factories (Joseph et al., 2017). This practice generates different environmental impacts on nature, including air pollution, as well as a large loss of nutrients in the soil. Forest residues and wood biomass can be generated from logging, pruning and natural disturbances (gravity fall, fires and insects) (Demirbas, 2010; White, 2010). However, these biomass residues can be converted into value-added resources, as they can provide benefits from an environmental point of view (Ulusal et al., 2020). The transformation of biomass into a carbon product is becoming increasingly popular to provide a sustainable material (Kwapinski et al., 2010), especially woody biomass, such as agricultural and forest biomass, as it can be used as a renewable and sustainable source of biochar (Ulusal et al., 2020).

Biochar is the product obtained from the pyrolysis of biomass under limiting conditions or in the absence of oxygen (Lehmann et al., 2011). This thermal decomposition process allows the components to become smaller structures and volatilize through heat (Stauffer, 2013). The benefits of biochar are multiple, such as climate change mitigation (Bruckman and Apydin Varol, 2016), energy production (Schnell et al., 2012), improvement of soil quality through its individual application or synergy with other amendments, such as compost (Virú-Vásquez et al., 2022), waste valorization (Guo et al., 2020) among others. Biochar can be produced by different ways, such as pyrolysis, gasification, hydrothermal carbonization (Wang and Wang, 2019). Qian et al. (2015) mentioned the weight yield of each type of production, with pyrolysis in general being the one with the highest yield of biochar (Yang et al., 2019).

The pyrolysis process can be divided into slow, fast and flash. The difference between the three is the process conditions, which involve heating speed, residence time, temperature (Canabarro et al., 2013) and the weight yield of biochar they generate. For the pyrolysis process, the factors that influence the process and will have effects on the physicochemical characteristics are: i) the pyrolysis temperature, which is one of the most important factors influencing the performance and the physicochemical and structural properties of the biochar (Tomczyk et al., 2020), which are pH, yield (%),

H/C, C/N, O/C, ash content (%) and surface area (m^2/g) (Nkoh et al., 2022); (ii) residence time (Sun et al., 2016), which determines the quality of the biochar given that the contact time between the steam and the solid part will generate a greater depolymerization of the structure, generating greater aromaticity (Zornoza et al., 2016); iii) heating rate, which, being faster, lower yield and greater loss of volatile components, is expected due to the more abrupt aromatization (Lehmann, 2009).

The decomposition of the biomass fractions during the process occurs depending on the type of pyrolysis performed, given that: i) slow pyrolysis, whith temperature varying approximately from 300°C to 500°C, presents a higher biochar content (30–40%) (Jahirul et al., 2012), for this stage is attributed to the evaporation of moisture (Tomczyk et al., 2020), which causes breakage of bonds and formation hydroperoxide groups, –COOH and –CO (Cárdenas-Aguiar et al., 2017); (ii) fast pyrolysis with temperatures within 500–800°C provides a higher percentage of bio-oil (40–50%) (Balat et al., 2009), and above 500°C, the degradation of lignin and other organic matter with stronger chemical bonds occurs (Cárdenas-Aguiar et al., 2017); (iii) flash pyrolysis at temperatures above 800°C shows a higher proportion of bio-oil (30–75%) (Balat et al., 2009; Jahirul et al., 2012).

The decomposition of biomass presents different stages. The first stage is associated with moisture (90°C), and then at 200°C, the decomposition of cellulose, hemicellulose and lignin begins to give way to CO, CO_2 and volatile compounds, such as alcohols, acids, phenols, among others. At 600°C, most of the material has decomposed, leaving fixed carbon as a residue, which corresponds to the organization of the non-volatile carbonaceous material (Ospina-Guarín et al., 2014). There are different ways to evaluate the quality of biochar, depending on the biomass, certain criteria and technical aspects of production and especially the application of biochar. For example, The International Biochar Initiative (IBI) indicates a guideline (IBI, 2015) with the intention to provide stakeholders and commercial entities with standards through physicochemical parameters to identify characteristics of biochar materials according to aspects, reliable for their application in the soil. Due to the great interest in biochar as a soil amendment and its potential ability to mitigate climate change, since its use means that stable carbon produced from biomass

can persist in the soil for hundreds or even thousands of years, some research has evaluated the stability of biochar (Chen et al., 2016; Leng et al., 2019; López et al., 2010). There are different indicators to determine the stability of biochar when applied to soil, for example, the O/C molar ratios, proposed by Spokas (2010), the H/C_{org} mole ratio as the indicator for biochar stability, proposed by IBI (2015), or indicators such H/C_{org} and O/C_{org} ratios to ensure biochar stability information (EBC, 2012); as well as, the ratio of recalcitrant organic carbon and total organic carbon expressed in percentage (López et al., 2010). Over the years, the stability of biochar has been determined by applying different methodologies (Almutairi et al., 2022; Wang et al., 2022). However, it still represents a challenge, and it is essential to investigate the physicochemical characteristics and stability to predict the potential implications for environmental and agricultural applications of biochar. In this sense, the aim of the research was to produce biochar at 300°C and 500°C, as well as to characterize it physicochemically, based on the standardized guidelines of IBI, being produced from 5 predominant species in the forest biomass at UNALM. Additionally, the study seeks to determine biochar stability and compare it using the methodology proposed by IBI (2015) and an adaptation of the methodology of López et al. (2010).

Methods

Sampling and site of biochar production

The sampling place and biochar production was located at UNALM, with coordinates as follows: 287875 m E / 8663767 m S, shown in *Fig. 1*.

Fig. 1. *Location and production of biochar from different forest biomass*
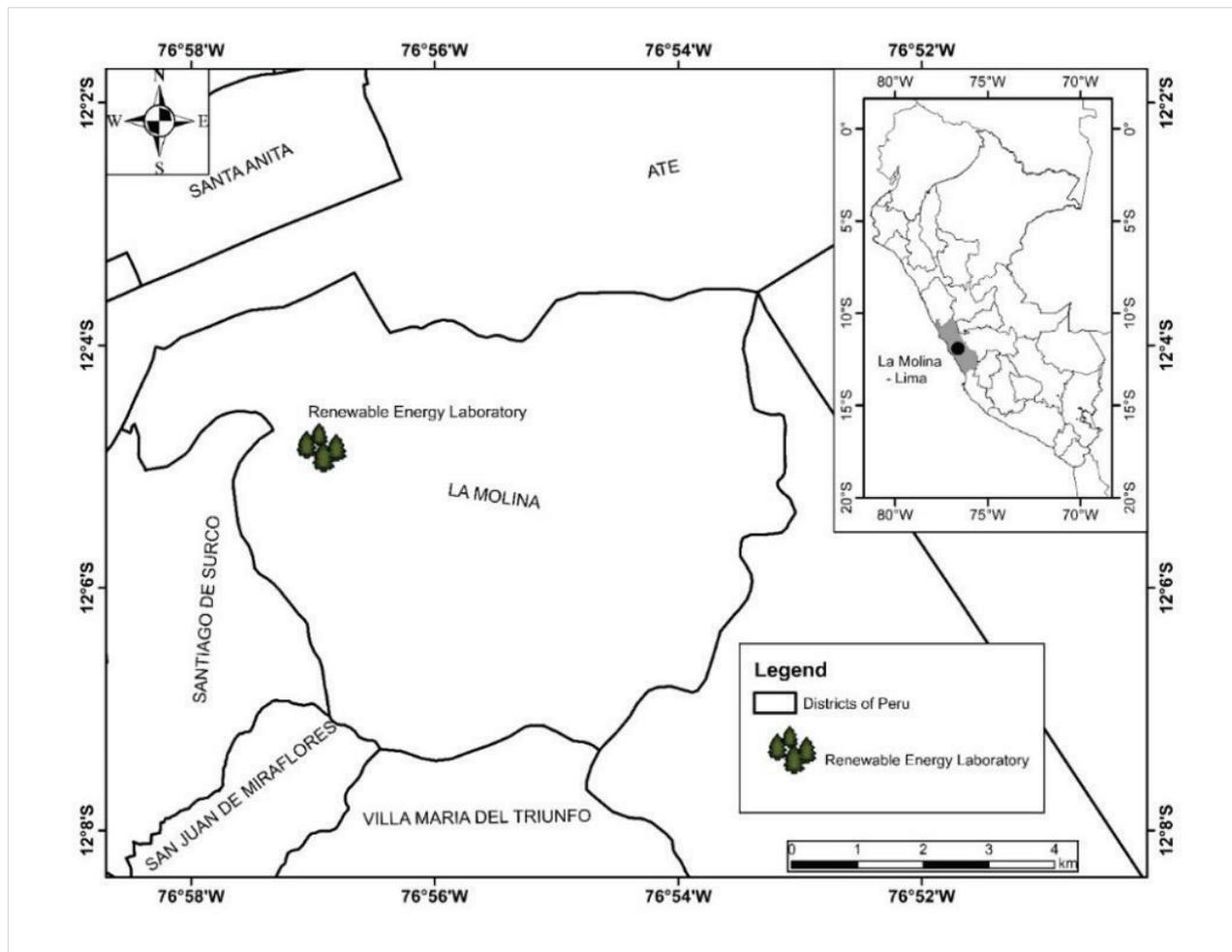
Biochar production

The five most abundant species at UNALM were selected. These species are shown in *Table 1*. This table also shows the total number of individuals (NI) by species from the entire university campus. Prior to the pyrolysis process, the collected samples were dried in an oven at 105°C for 24 hours (Chen et al., 2020) to remove moisture from the pruned matter and thus homogenize the sample.

Table 1. *Species for biochar production and number of individuals at UNALM*

Scientific name	NI	Percentage
Grevillea robusta	198	10.80%
Schinus molle	177	9.60%
Casuarina cunninghamiana	144	7.80%
Eucalyptus spp.	132	7.20%
Ficus benjamina	126	6.90%
Other species	1061	57.70%

The biochar was produced in a pyrolytic oven by own design (*Fig. 2*). This oven provided heat through a two-chamber interface. The final temperature was obtained by measuring with a thermostat. Each of the species was subjected to the pyrolysis process at two temperatures, which were 300°C and 500°C for 5 hours. The biochar produced from the species shown in *Table 1* received the following terminology: biochar from

Grevillea robusta (BGR), biochar from *Schinus molle* (BSM), biochar from *Casuarina cunninghamiana* (BCC), biochar from *Eucalyptus spp.* (BEE), and biochar from *Ficus benjamina* (BFB). The following were categorized according to the production temperature: BGR300, BGR500, BSM300, BSM500, BCC300, BCC500, BEE300, BEE500, BFB300 and BFB500.

After pyrolysis, the yield was measured, using the following equation:

$$Yield\ (\%) = \frac{W_{final}}{W_{start}} * 100 \qquad (1)$$

Where: W_{final} – weight in g at final or after production of biochar; W_{start} – weight in g at start or before production of biochar.

Physicochemical characterization of biochar

For the characterization, the biochar was subjected to a milling process followed by sieving with a 50 mm mesh. To determine the characterization parameters, it was based on the IBI (2015) guidelines, which requires: hydrogen (H), carbon (C), nitrogen (N), ash (%), electrical conductivity (EC), pH, organic carbon (C_{org}), and moisture (%).

For moisture and ashes, gravimetric analysis was performed using TGA 701 with the ASTM D7582 reference method, which consists of introducing the sample in small crucibles with a minimum volume of 0.8 g to 1.2 g.

Fig. 2. *The pyrolysis reactor to produce biochar*

The sample was subjected to a temperature increase and a specific atmosphere, as recommended in the methodology for moisture and ashes calculation of LECO Corporation (2015).

Elemental analysis was performed, which determines the percentage of C (%), H (%) and N (%). These components are determined following the ASTM D5373 reference method (LECO Corporation, 2013), which specifies the necessary analysis for each of the components when using CHN 628.

Likewise, complementary tests were performed for the determination of the indicated parameters, for the calculation of pH and conductivity. This was carried out in a 1:20 solution in deionized water according to Rajkovich et al. (2011). All tests were performed in triplicate to reduce experimental error.

Inorganic, labile, oxidizable and recalcitrant carbon

To determine C_{org}, the following procedures were performed: the total carbon was determined, following the ASTM D5373 methodology. Then inorganic carbon was subtracted to determine the organic carbon. Inorganic carbon was determined directly (Wang et al., 2014) by its decomposition via an acid, HCl (2 M), which releases carbonates that are captured in a strong base NaOH (0.5 M). The reactions that occurred were as follows (Singh et al., 2017):

$$MCO_3 + 2HCl \rightarrow MCl_2 + CO_2 + H_2O$$

$$2\,NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$

$$Na_2CO_3 + BaCl_2 \rightarrow 2\,NaCl + BaCO_3$$
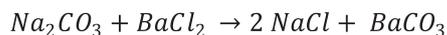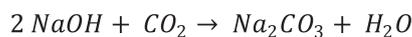
The determination of inorganic carbon is made indirectly, by titrating the unreacted sodium hydroxide. This titration is carried out with a strong acid in lower concentration (HCl). To avoid interference, an excess of $BaCl_2$ is added to precipitate the carbonate in form of barium carbonate. Additionally, procedures were performed to determine labile carbon, oxidizable carbon and recalcitrant carbon.

Labile carbon is an indicator to evaluate the oxidative stability of biochar, since it is the main source of soil organic matter, and it is what really determines the organic carbon available as nutrients for soil organisms (Chen et al., 2016).

The labile carbon was measured as a function of chemical oxidation, using the method of determining the oxidized organic carbon with $K_2Cr_2O_7$ by the spectrophotometric method. A total of 150 mg of biochar was weighed and placed in a beaker, 55 mL of 1 M $K_2Cr_2O_7$ / 11 M H_2SO_4 reaction solution was added and mixed homogeneously. The mixture was then heated at 135°C using oil for 30 min. Finally, the reaction solution and oxidized biochar were separated by centrifugation and the oxidized biochar was washed twice with deionized water and dried in an oven at 105°C for 12 hours (Chen et al., 2016). The standard ratio curve between absorbance and concentration of $K_2Cr_2O_7$ was measured as a function of the absorbance difference between the initial and calculated concentration.

Oxidizable carbon would correspond to the less active organic matter but which, due to microbiological processes, is integrated into the soil in the form of humus. It would correspond to the second recalcitrance because it needs to be synthesized by microorganisms to ensure its stability in the medium (Lützow et al., 2006). The carbon method of Walkley and Black (1934) was used to determine the oxidizable carbon, consisting of oxidation with 0.4 M potassium dichromate in an acid medium and subsequent titration with 0.2 M ferrous ammonium sulfate. The high organic content of the samples led to a modification of the initial weight of the sample, adjusting it to 0.01 grams of biochar. In order to determine recalcitrant carbon, the method of López et al. (2010) was used to determine lignin in wood and pulp. Since lignin is highly insoluble in mineral acids, it can be measured gravimetrically after hydrolyzing cellulose and hemicellulose with sulfuric acid. The determination consisted of two consecutive hydrolyses with sulfuric acid. The first stage was carried out with cold concentrated sulfuric acid, destroying cellulose and labile carbon compounds; the second stage was carried out with 0.7 N sulfuric acid diluted and heated to 350°C, hydrolyzing the remaining polysaccharides, as well as hemicellulose and proteins. The remainder is lignin and humic substances, which constitute the most resistant fraction. It was weighed to determine the substances most resistant to the process.

Biochar stability

To determine the stability, the IBI (2015) method and an adaptation of López et al.'s (2010) method were used.

Taking into account the IBI method, the stability of the biochar was determined with a division between the content of hydrogen and organic carbon ($H:C_{org}$). The $H:C_{org}$ molar ratio is recommended to distinguish biochar from other thermochemically altered organic matter for several reasons. H:C ratios change substantially with thermochemical treatment. For example, in Keiluweit et al. (2010), O:C ratios have been shown to correlate well with biochar stability (Spokas, 2010). H is determined directly in most laboratories, whereas O is calculated by subtraction. According to the values required for IBI, the biochar with high quality and stability should have a $H:C_{org} < 0.7$. Also, biochar that obtains $H:C_{org} > 0.7$ is not considered to be biochar, as these materials would not meet the definition of biochar as defined by the IBI Standards.

On the other hand, an adaptation of the López et al.'s (2010) methodology was used, since this methodology consisted of analyzing the stability in compost, while for this research it was used for biochar. To determine to stability, it was based on the ratio of recalcitrant organic carbon to organic carbon (López et al., 2010), and the degree of stability should be greater than 0.50 to be qualified as a good biochar to be applied in the soil as an amendment. The stability of biochar is important, since it is the responsible factor in C sequestration and also the emission reduction; therefore, understanding the factors of biochar stability in soils is important for estimating its potential in long-term C sequestration (Wang et al., 2022). Finally, a comparison was made between the two methods with the results obtained.

Statistical analysis

Data were measured in triplicate. All data were processed with JMP 16 Software. The Tukey test was applied, and means were also determined by two-way ANOVA, where temperature and species were the factors, as well as their interaction. Statistical tests were considered significant at $P < 0.05$.

Results and Discussion

Biochar production

In the case of biochar production from forest biomass at 300°C, there was a variation of ± 10°C, while at 500°C, there was a variation of ± 20°C. The biochar produced at different temperatures shows a yield variation that when the temperature increases, the yield decreases, as shown in *Fig. 3*. The average yield at 300°C is 39.03%, while at 500°C, it is 32.55%. This obtained yield (within the range of 30% to 40%) supports a correct slow pyrolysis process (Jahirul et al., 2012). The difference in yield production with respect to temperature is due to the fact that, during the pyrolysis process, the cell wall of the biomass is destroyed and then gives way to the destruction of cellulose and hemicellulose, which, due to their simpler parts compared to lignin, decompose as the temperature increases (Chen et al., 2016). In relation to the species, the higher lignin content produces a higher yield of biochar, but it is the volatile compounds that determine its variation as a function of temperature (Ospina-Guarín et al., 2014).

BCC presents a higher yield at both temperatures; according to Saleh and El-Lakany (1979), it is considered a hardwood due to its high lignin content. The volatile compounds of this species would only constitute less than 5% compared to other species, so it does not present a significant variation by pyrolysis temperature. In contrast, it is the case of BSM that, despite having a yield similar to BCC, at 300°C, presents a significant variation at 500°C, due to its high content of phenolic compounds, which would be easily volatilized in the pyrolysis process (Machado et al., 2019).

In the case of BEE and BFB, the percentage variation in yield is due to the fact that their structure has a greater amount of hemicellulose, so that, as the temperature increases, the variation in yield becomes more significant (Oliveira and Albert, 2009). The same occurs with BGR, which in torrefaction studies at temperatures below 250°C already presents a significant variation in yield (Mburu et al., 2008).

Physicochemical characterization of biochar

Table 2 shows the physical and chemical characterization of the biochars obtained (C, H, N), moisture, ashes, pH and EC of the biochar from forest residues produced at 300°C and 500°C. Furthermore, this table shows that

Fig. 3. *Biochar yield*
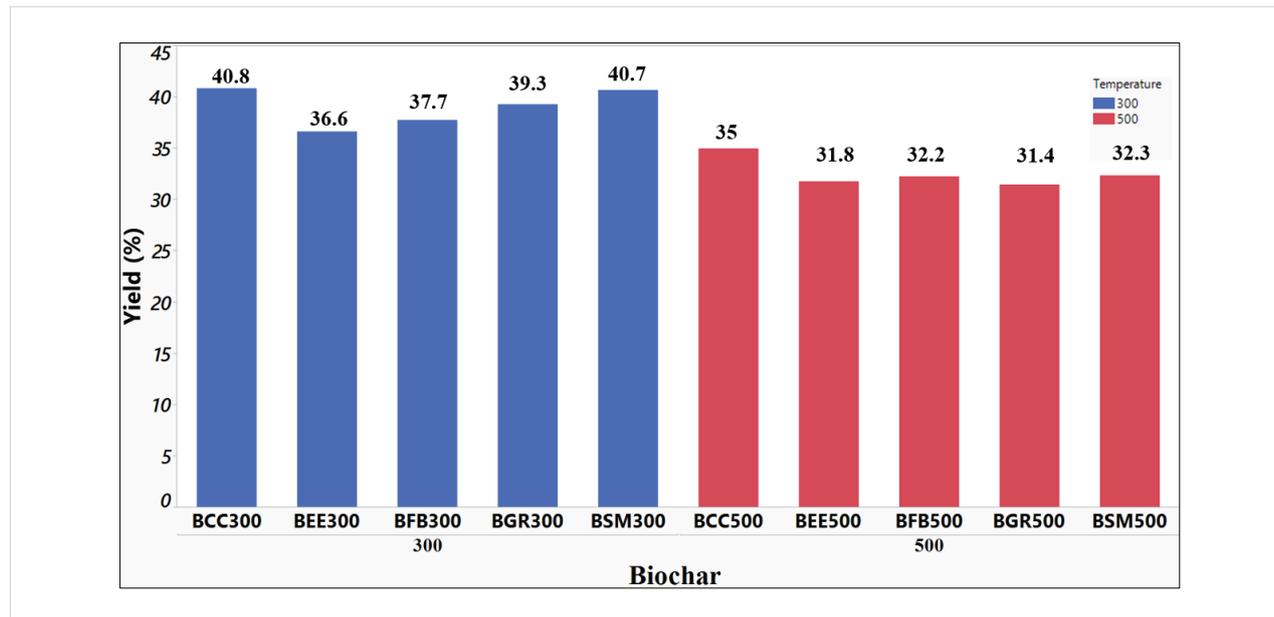
the error and the levels not connected by the same letter are significantly different, using the Tukey test at 5% significance, which was applied in the interaction of the species and the temperature.

In addition, it is shown that as the production temperature increases, the C (%) increases, except for BFB. In the case of H (%) when the temperature increases, its content decreases.

BCC had the highest N (%) content, with an average of 1.31%. The second was BSM with 1.045%, followed by BFB with an average of 0.82%, BEE with 0.79% and BGR with 0.78%. The raw material determines the initial content of the component and the form in which it will be presented to determine its degree of conversion to another state. BCC stands out since, in addition to its structure, according to Diagne et al. (2013), it would form ectomycorrhizas with which it helps to fix nitrogen in the soil and for the same species in a similar magnitude to legumes, which would allow it to adapt to nitrogen poor soils. Furthermore, due to the low nitrogen content, it will not interfere negatively with soil properties.

The average moisture of biochar produced at 300°C was 3.31%; while at 500°C, it was 3.58%. Each of the species showed the trend of a moisture increase at higher temperatures, except for BGR, which showed the opposite trend. Moisture increased as the pyrolysis temperature increased in each species, due to the fact that lignocellulosic components volatilize to a greater extent, leaving pores for moisture (Chen et al., 2016). Such a gradual decrease in the percentage of moisture could be explained by the breaking of the hydrogen bonds between water molecules in the raw materials and the subsequent release into the air as volatile material, which also supports a steady decrease of volatile compounds in the raw materials. The biochar produced was affected by the combustion of raw materials and increased heat treatment (Rafique et al., 2019; Usman et al., 2015).

The results showed a high ash content in BCC, BEE and BFB, which may be the result of the silica and mineral content of the raw material and the gradual loss of C (%), H (%), and O (%) during pyrolysis (Demirbas, 2004). The cellulosic and non-cellulosic components of the raw material were the ones that would determine the compounds that pass to another state, and the mineral components, carbonates and others are the ones that will remain in the structure of the product due to their high boiling point (Jahirul et al., 2012). High concentrations of nutrients in biomass can generate biochar with higher ash content and alkalinizing capacity (Deenik et al., 2011). Therefore, biochar can be used in soils to correct acidity, increase the cation exchange capacity (CEC) of the soil, as well as water retention (Namgay et al., 2010; Singh et al., 2010).

The most alkaline biochar was BFB, due to its high content of volatile material, so its thermal decomposition leaves a more alkaline biochar (Pérez-Arévalo and Velázquez-Martí, 2018). The second was BEE, which behaves similarly to BFB since the *Ficus benjamina* species contain a high hemicellulose content, which volatilizes (Oliveira and Albert, 2009). In the case of BSM, it also had a high content of this type of material (Machado et al., 2019). In the fourth place, there was BCC, which would be explained by the low content of volatile material (Saleh and El-Lakany, 1979), while in the case of BGR, it would be due to the elimination of the acids of galactose, xylose, arabinose, rhamnose and uranic acid present in hemicellulose (Mburu et al., 2008). The variation in pH of each of these species as a function of temperature was due to the different boiling points of the compounds to be volatilized, as it was the case of *Grevillea robusta*, which possesses uranic acid, which changes its state at temperatures above 300°C (Mburu et al., 2008).

It was estimated that with an increasing temperature all the species increased their pH. This is demonstrated since the alkalinity and pH variation of the biochar is statistically due to the pyrolysis temperature. For example, Nkoh et al. (2022) performed an analysis for 112 researches where the correlation between temperature and pH ($r^2 = 0.53$) was determined. On the other hand,

Mohan et al. (2006) affirmed that the carboxylic acids, alcohols, phenols, aldehydes and ketones, amines, amides and other heterocycles that give the raw material its acid character are the compounds that volatilize in the pyrolysis process, passing to the liquid phase or bio-oil, thus leaving a more alkaline biochar.

The type of raw material and pyrolytic temperature significantly influenced the EC (dS/m), of all biochars produced. In general, the EC values increased with increasing pyrolytic temperature for all biochars. BFB500 has the highest conductivity (2.77 dS/m), the second is BGR500 (2.42 dS/m), the third is BSM500 (2.22 dS/m) and the last ones are BEE500 and BC500 (1.35 dS/m and 1.31 dS/m, respectively). The last two species have a low salt content, as they are salt-tolerant species, which grow in saline environments without incorporating salt into their structure (Sun and Dickinson, 1995).

Table 3 shows the test of the effects of temperature, species and the interaction of both on the physicochemical characteristics of biochar produced from forest biomass. It is shown that the determining factor for moisture, ashes, nitrogen, and EC, is "Species", while for the case of pH, C (%), and H (%), it is temperature (F ratios). In addition, the *P* value is shown with values lower than $P < 0.05$.

Table 2. *Physicochemical characteristics of biochar*

Parameter	BEE300	BEE500	BFB300	BFB500	BGR300	BGR500	BCC300	BCC500	BSM300	BSM500
C (%)	59·88 ± 0.050h	71.84 ± 0.252d	63.27 ± 0.094g	63.08 ± 0.104g	73.20 ± 0.143c	79.89 ± 0.153a	64.07 ± 0.231g	68.12 ± 0.401 f	69.47± 0.162e	78.28 ± 1.088b
H (%)	3.09 ± 0.087 c	1.67± 0.018g	3.34 ± 0.013b	1.82± 0.008f	3.60 ± 0.009a	1.98 ± 0.007e	2.96 ± 0.011d	1.71 ± 0.072g	3.43 ± 0.014b	1.74f ± 0.034g
N (%)	0.089 ± 0.019 e	0.68± 0.009g	0.93± 0.006e	0.71 ± 0.004g	0.77± 0.003 f	0.78 ± 0.001f	1.45 ± 0.004a	1.17 ± 0.037b	1.1 ± 0.007c	0.99 ± 0.016d
Moisture (%)	2.86 ± 0.092g	3.27 ± 0.032e	3.44 ± 0.038d	3.87 ± 0.081 b	3.71 ± 0.015 c	3.24 ± 0.057e	3.06 ± 0.035f	3.37 ± 0.011de	3.47 ± 0.015d	4.12 ± 0.047a
Ashes (%)	19.36 ± 0.01b	16.08± 0.040d	17.6 ± 0.198c	25.78 ± 0.046 a	10.21 ± 0.04g	12.65 ± 0.026e	17.99 ± 0.512c	19.17 ± 0.011b	11.89 ± 0.07f	12.12 ± 0.038f
pH	9.33 ± 0.015d	9.77± 0.036b	9.54 ± 0.01c	10.02 ± 0.073a	8.6 ± 0.01f	9.05 ± 0.117e	8.26 ± 0.035g	9.63 ± 0.040 bc	9.16 ± 0.06e	9.57 ± 0.017c
EC (dS/m)	1.27 ± 0.029d	1.35± 0.006d	2.26 ± 0.015bc	2.77 ± 0.063a	2.02 ± 0.36 c	2.42 ± 0.113ab	1.2 ± 0.040d	1.31 ± 0.0152d	1.42 ± 0.05d	2.22 ± 0.025bc

Levels not connected by the same letter are significantly different (the Tukey test at a 5% significance)

Table 3. *Testing the effects of species, temperature and their interaction on the physicochemical properties of biochar*

Parameter	Interaction	SS	F ratio	P value
C (%)	Species	797.8339	1281.775	< 0.001
	Temperature	294.30778	1891.303	< 0.001
	Species*Temperature	128.58866	206.5866	< 0.001
H (%)	Species	0.805515	134.4876	< 0.001
	Temperature	16.819995	11232.96	< 0.001
	Species*Temperature	0.172142	28.7405	< 0.001
N (%)	Species	1.26636664	1444.654	< 0.001
	Temperature	0.1993131	911.4386	< 0.001
	Species*Temperature	0.0845169	96.6217	< 0.001
Moisture (%)	Species	2.17788	217.788	< 0.001
	Temperature	0.533333	213.3333	< 0.001
	Species*Temperature	1.0975333	109.7533	< 0.001
Ashes (%)	Species	470.60079	3738.487	< 0.001
	Temperature	22.93376	728.75	< 0.001
	Species*Temperature	104.47219	829.9348	< 0.001
pH	Species	3.88669867	349.4265	< 0.001
	Temperature	2.9704533	1073.658	< 0.001
	Species*Temperature	1.0136133	91.5916	< 0.001
EC (dS/m)	Species	7.36458	118.937	< 0.001
	Temperature	1.0792033	69.716	< 0.001
	Species*Temperature	0.5262467	8.4988	0.0004

SS: Sum of squares.

Organic, labile, oxidizable, recalcitrant carbon on biochar

The results of organic, labile, oxidizable and recalcitrant organic carbon are shown in *Table 4*. Also, this table shows that the error and the levels not connected by the same letter are significantly different, using the Tukey test at a 5% significance, which was applied in the interaction of the species and the temperature. According to the percentage content of organic carbon (C_{org}) (IBI, 2015), the biochar produced qualifies as first-class biochar as they have a C_{org} content higher than 60%, except for BEE300, as it had 59.42%, which will qualify as second-class biochar. The variation of C_{org} as a function of temperature (*Fig. 4*) was due to

the fact that the aromaticity of the structure fixed in the biochar is a function of the compounds that volatilize due to the pyrolysis process (Chen et al., 2016). Of these compounds, the more sensitive ones volatilize at higher temperatures and the more resistant ones are transformed into more aromatic substances, which fix organic carbon (Zhao et al., 2013).

BGR500 had a higher content of organic carbon, since there is a rapid modification of its structure, becoming more stable as temperature increases (Mburu et al., 2008). In the second place, BSM500 was found, with an average value of 76.43%, which was due to its high content of volatile substances (Machado et al., 2019), so it would generate that the aromatization of the com-

pound occurs much faster. In the case of BEE (BEE300 and BEE500), the great variation of organic carbon by temperature was due to its high hemicellulose content (Oliveira and Albert, 2009), which continues to decompose with increasing temperature. The opposite case was observed in BCC, which, due to its low volatile material content (Saleh and El-Lakany, 1979) easily stabilizes at low temperature. As noted in Diagne et al. (2013), it was one of the species that would give a high-quality charcoal, due to its low ash content and its high calorific value. As an atypical case, BFB was shown, which minimally decreases in C_{org} content as temperature increases. This was due to the fact that carbon is unstable and can still continue to decompose, as this species has a higher content of volatile material, compared to other species studied by Pérez-Arévalo and Velázquez-Martí (2018). It is shown that as temperature increases, labile carbon content decreases. The variation is due to the fact that organic matter volatilizes, generating the change of state of hemicellulose and cellulose (Chen et al., 2016).

There was a significant difference between species, with the highest labile carbon being BFB300 and BFB500. This species has a large amount of hemicellulose (Pérez-Arévalo and Velázquez-Martí, 2018), which volatilizes as a function of temperature, so it presents a high variation with temperature, i.e., when performing the pyrolysis process at 300°C, 21.75% is obtained, while at 500°C it decreases to 14.73%.

As for the highest loss of labile carbon when the biochar production temperature increases (*Fig. 5*), BGR and BEE were found with a loss of 60.28% and 34.08%, respectively. On the other hand, the lowest losses of labile carbon are found in BFB and BSM, with values of 32.27% and 8.12%, respectively. Labile carbon is important because it can be utilized by soil microbes (Dong et al., 2017). However, over time this labile carbon may dissolve or leach out during aging, so this will influence the structure and functional groups of the biochar (Jiang et al., 2019). Considering that the labile carbon content of the produced biochar is between 6.73% and 21.75%, it would be said that it does not benefit or affect the soil with the negative priming effect, as it would not alter the aggregation in the soil or the excess of available carbon (Jiang et al., 2019).

It was observed that the oxidizable carbon content decreased as the pyrolysis temperature increased (*Fig. 6*). Also, it should be noted that the average variation as a function of temperature in each of the species was similar. In addition, the average of biochar produced was between 13.35% and 34.10%, so that being a higher average than that observed in labile carbon, the thermal modification of the structure of the raw material was evident. This means that the structure polymerizes forming a more stable one as organic carbon, which was the second most resistant part of carbon (Ospina-Guarín et al., 2014). With respect to biochar, the species with in the lowest labile carbon, such as BEE and BCC, were those with the highest percentage of oxidizable carbon, similar to the study conducted by Chen et al. (2016). For BFB, its high oxidizable carbon content would be due to the high content of hemicellulose and cellulose. Due to its structure prone to thermal decomposition and low aromatization, its higher composition is concentrated in oxidizable carbon (Oliveira and Albert, 2009).

Table 4. *Organic, labile, oxidizable and recalcitrant carbon on biochar*

Parameter	BEE300	BEE500	BFB300	BFB500	BGR300	BGR500	BCC300	BCC500	BSM300	BSM500
Organic carbon (%)	59.42 ± 0.244g	70.80 ± 0.865c	62.92 ± 0.195f	62.46 ± 0.346f	71.61 ± 0.273c	79.67 ± 0.304a	63.24 ± 0.43f	66.71 ± 0.786e	68.91 ± 0.454d	76.43 ± 1.170b
Labile carbon (%)	10.21 ± 3.703cde	6.73 ± 2.091e	21.75 ± 0.709a	14.73 ±3.273bc	18.96 ± 2.328ab	7.53 ± 1.911 de	10.23 ± 1.359cde	7.17 ± 1.765de	13.29 ± 2.248bcd	12.21 ± 0.623cde
Oxidizable carbon (%)	34.10 ± 0.960a	18.15 ± 0.515d	28.49 ± 0.344bc	15.64 ± 0.2252e	27.18 ± 1.066c	13.28 ± 0.327f	29.95 ± 1.215b	18.05 ± 0.549d	28.34 ± 0.544bc	13.35 ± 0.639f
Recalcitrant carbon (%)	43.68 ± 0.934f	62.49 ± 0.520b	50.46 ± 0.244e	54.71 ± 0.215d	58.91 ± 1.097c	70.82 ± 0.370a	49.00 ± 1.383 e	59.42 ± 0.56 c	56.30 ± 0.491d	70.41 ± 0.680a

Levels not connected by the same letter are significantly different (the Tukey test at a 5% significance).

Fig. 4. *Organic carbon in different biochar obtained from forest biomass*
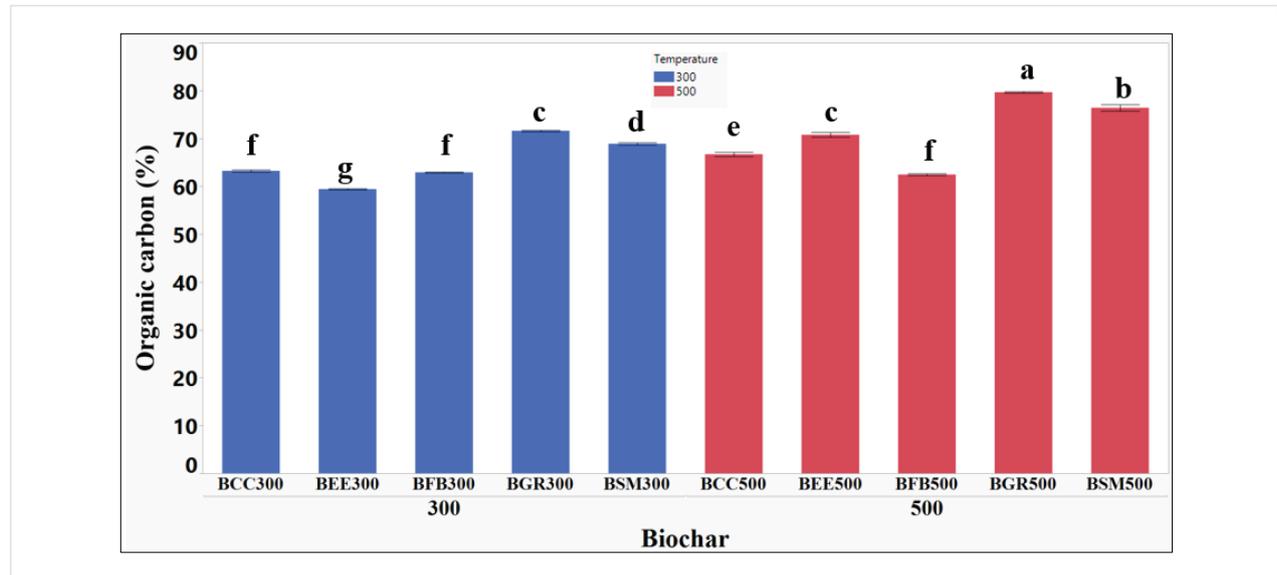
Fig. 5. *Labile carbon in different biochar obtained from forest biomass*
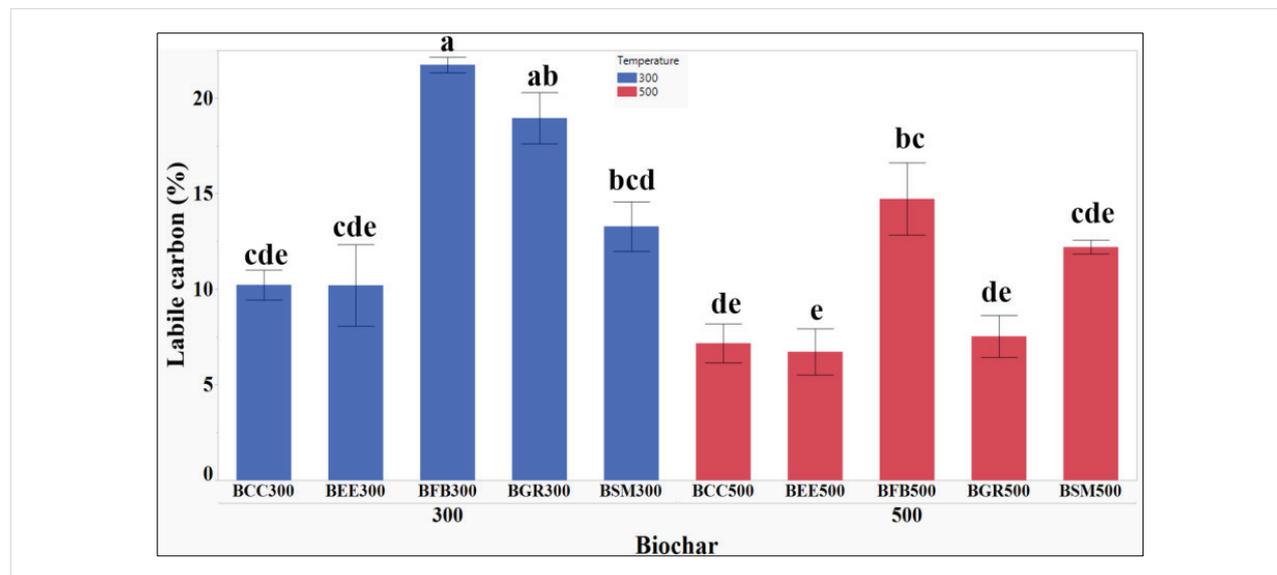
Oxidizable carbon in the soil is important because when biochar is applied to the soil, it transports easily oxidizable matter, which stimulates microbial activity in a short time, the intensity and direction of the priming effect, and this can vary according to the species with which the biochar was produced, in addition to other factors, such as soil texture, soil organic matter composition, among others (Li et al., 2021).

As the temperature increased, the carbon content was higher (*Fig. 7*). The percentage of recalcitrant carbon in

this research ranged from 43.68% and 70.82%, which was an indicator of the formation of more aromatic and stable carbon (Keiluweit et al., 2010) because there was a higher transformation of lignin, cellulose and hemicellulose compounds (Suárez-Abelenda et al., 2017). BGR500 had a higher recalcitrant carbon content; however, when the production temperature was increased from 300°C to 500°C, there was only a 7.77% variation. The variation is a function of the temperature and in fact with the large amount of hemicellulose that this material has, it has a

Fig. 6. *Oxidizable carbon in different biochar obtained from forest biomass*
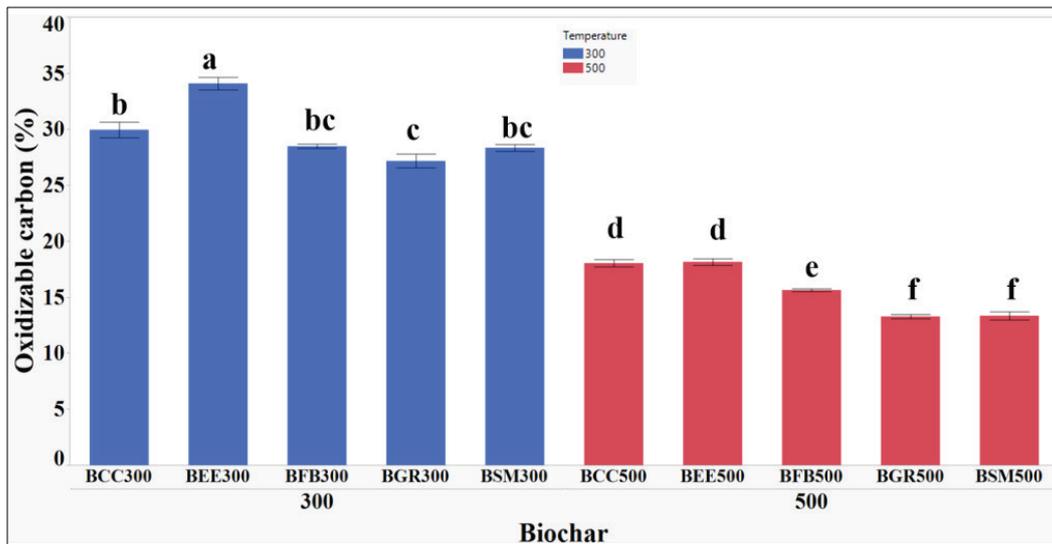
Fig. 7. *Recalcitrant carbon in different biochar obtained from forest biomass*
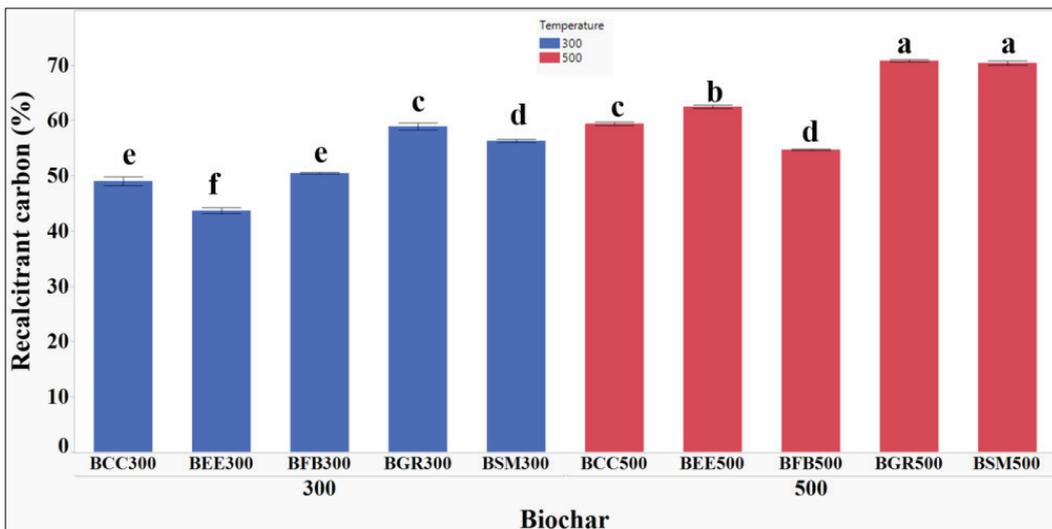
greater amount of mineralizable carbon and its degree of recalcitrance is lower (Oliveira and Albert, 2009). Aller et al. (2017) mention that the variation is due to the structuring left by pyrolysis. The first temperature of 300°C is characterized by a transition carbon; it is the result of the dehydration of volatile materials and depolymerization of biopolymers. The second temperature of 500°C is characterized by an amorphous carbon since small aromatic units are found in various structures that are almost completely depolymerized.

Table 5 showed the effects of the species, temperature and their interaction on organic, labile, oxidizable and recalcitrant carbon of biochar. As shown in this research, the temperature factor had a greater effect on all the carbons (F ratio) with a sample *P* value less than 0.05 ($P < 0.05$).

Table 5. *Test of the effects of species, temperature and their interaction on carbons of biochar*

Parameter	Interaction	SS	F ratio	P value
Organic carbon (%)	Species	753.65683	536.5888	< 0.0001
	Temperature	269.64012	767.9138	< 0.0001
	Species*Temperature	125.3485	89.2423	< 0.0001
Labile carbon (%)	Species	384.01835	19.6808	< 0.0001
	Temperature	203.73708	41.7658	< 0.0001
	Species*Temperature	99.89642	5.1197	0.0053
Oxidizable carbon (%)	Species	140.0922	68.9191	< 0.0001
	Temperature	1452.4129	2858.087	< 0.0001
	Species*Temperature	15.7078	7.7275	0.0006
Recalcitrant carbon (%)	Species	857.2952	388.2771	< 0.001
	Temperature	1062.194	1924.311	< 0.001
	Species*Temperature	169.8873	76.9436	< 0.001

Stability of biochar

The stability of biochar is shown in *Table 6*. Also, this table shows the error and the levels not connected by the same letter are significantly different, using the Tukey test at a 5% significance, which was applied in the interaction of the species and the temperature estimated. For the adapted method of López et al. (2010), BSM500 had the highest stability (0.920). Meanwhile, in the case of production temperature at 300°C, BGR300 had the highest stability (0.823). In addition, the average stability of biochar estimated at 300°C was 0.79 and at 500°C it was 0.89.The stability of the evaluated biochar is between 0.733 and 0.920 (*Fig. 8*, *Table 6*). All of them constitute a stabilized amendment for soil application, having a stability higher than 0.500 (López et al., 2010). Also, when temperature increased (from 300°C at 500°C), the stability tended to increase. Stability correlated with low nitrogen content, high moisture concentration, presence of carbonates and high pH. On the other hand, according to the results, stability had an inverse relationship with labile carbon and oxidizable carbon content; it would be the matter most susceptible to be mineralized by soil biota. Stability was directly related to the recalcitrant carbon; since, both would indicate the degree of aromatization of the biochar.

Regarding the proposed method to determining stability according to the IBI method (IBI, 2015), all values of the $H:C_{org}$ ratio constituted adequate biochar, as they did not exceed the limit of 0.7. Since this threshold is imposed to ensure abundant fused aromatic ring structures and to distinguish biochar from raw materials or other partially or sparsely carbonized materials. It is a conservative value, based on several incubation experiments and their model results, to ensure that 50% of biochar carbon (95% confidence) can remain stable in soil after 100 years (Budai et al., 2013). At higher temperatures, a lower $H:C_{org}$ ratio was obtained (*Fig. 8*) for the IBI method, which represented the degree of final aromaticity of the product, i.e., the lower this value, the greater the aromatic structure; and therefore, the more stable it will be (Manyà et al., 2014). This index decreases because the lignocellulosic compounds present, which pyrolyze easily, release -H radicals in the gas phase (Lehmann, 2009); thus, the degree of aromaticity can be inferred from the unsaturation of these radicals. On the other hand, *Table 7* shows the effects of species, temperature and their interaction on H: C_{org} and stability. In this case, temperature is the determining factor (F ratio = 7997.538), in addition to the *p* value less than 0.05 ($P < 0.05$).

Table 6. *Stability of biochar*

Method	BEE300	BEE500	BFB300	BFB500	BGR300	BGR500	BCC300	BCC500	BSM300	BSM500
IBI method	0.623 ± 0.023 ab	0.28 ± 0.00fg	0.64 ± 0.000a	0.350 ± 0.00e	0.603 ± 0.006bc	0.300 ± 0.000f	0.563± 0.006 d	0.303 ± 0.0153f	0.593 ± 0.006c	0.270 ± 0.000g
Lopez et al. (2010) adapted method	0.733 ± 0.028e	0.883 ± 0.015ab	0.803 ± 0.006cd	0.877 ± 0.058b	0.823 ± 0.015c	0.890 ± 0.000ab	0.777 ± 0.023d	0.890 ± 0.01ab	0.817 ± 0.006cd	0.920 ± 0.017a

Levels not connected by the same letter are significantly different (the Tukey test at a 5% significance)

Fig. 8. *Comparison of the stability of different biochar obtained from forest biomass*
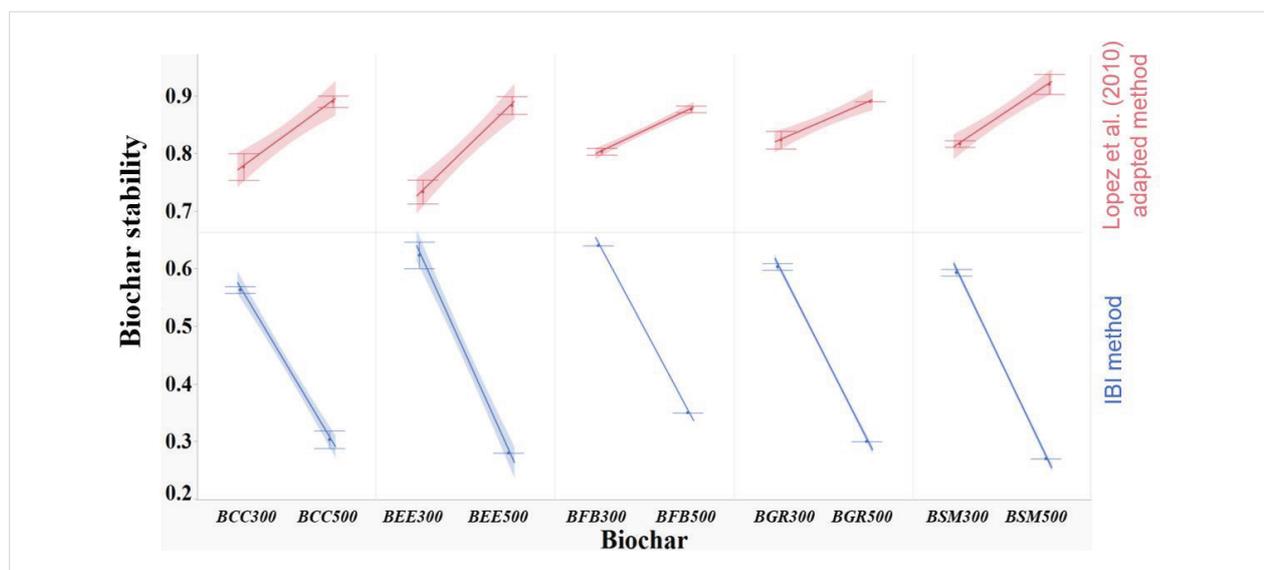
Table 7. *Stability of biochar*

Method	Interaction	Sum of Squares	F ratio	p value
IBI method	Specie	0.01565333	45.1538	< 0.0001
	Temperature	0.69312	7997.538	< 0.0001
	Specie*Temperature	0.00608	17.5385	< 0.0001
Lopez et al. (2010) adapted method	Specie	0.0127133	16.4397	< 0.0001
	Temperature	0.0770133	398.3448	< 0.0001
	Specie*Temperature	0.0067533	8.7328	< 0.0001

Conclusions

The biochars obtained are considered amendments alkaline (pH greater than 8), highly porous that could contribute to soil carbon content without affecting other soil macronutrients. UNALM forest biomass shows a higher yield at lower temperatures (300°C), with a percentage difference of 6.5% compared to pyrolysis at 500°C. From the data obtained, temperature was a determining factor (F value) instead of the species, in the physicochemical properties of the biochar for the following parameters: C (%), H (%), pH. Meanwhile, the species was a determining factor for N (%), moisture (%), ashes (%) and EC (dS/cm). For the content of carbons in biochar, the following were obtained: the temperature was a determining factor for organic carbon (%), labile carbon (%),

oxidizable carbon (%) and the recalcitrant carbon (%). Regarding stability of biochar, based in the IBI method all the biochar was less than H:C_{org} < 0.7, and for the adapted method of López et al. (2010), the ratio of recalcitrant organic carbon to organic carbon was greater than 0.50. Therefore, considering both methodologies to determine stability, biochars comply with established limits, which make them excellent amendments to be applied to the soil. On the other hand, BSM500 had the highest value of stability considering both methodologies described previously, and this could indicate that it has the greatest sequestration of C once applied to the soil, and also the emissions reduction; for that reason, it would be important to carry out future research to estimate the potential for C sequestration in the long term, taking into account the stability of biochar with the methods described in this research.

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