Greenhouse Evaluation of Pinewood Biochar Effects on Nutrient Status and Physiological Performance in Muscadine Grape (*Vitis rotundifolia* L.)

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Abstract. Muscadine grape is a perennial crop that is highly responsive to local environmental factors and viticulture practices. Biochar is a promising soil amendment used to improve soil water and nutrient retention and promote plant growth. The present study aimed to assess the effects of different pinewood biochar rates on nutrient status and vegetative parameters of muscadine grape cv. Alachua grown on a nutrient-poor sandy soil, Ultisols (97.2% sand, 2.4% silt, and 0.4% clay), and mixed with five different rates (0%, 5%, 10%, 15%, and 20%) of biochar based on weight. Variations in soil moisture, temperature, and leaf greenness value [soil plant analysis development (SPAD) reading], net photosynthesis rate, and plant root and shoot dry weights were measured. In addition, the nutrient status of the soil, plant root, and shoot were determined. The results indicated that the higher rate of biochar could significantly ($P < 0.05$) improve soil moisture. Biochar can also decrease soil temperature, although there were no significant differences among treatments. Regarding the nutrient status, the biochar amendment increased the nutrient content of phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca), as well as the soil organic matter content and cation exchange capacity. Higher nutrient contents in soil lead to increased P and Mg in both aboveground and belowground muscadine plant tissues and decreased nitrogen (N), iron (Fe), and copper (Cu) in the root part. There were no significant differences observed in SPAD values, net photosynthesis, or dry weights of the root and shoot. This study demonstrates that the addition of biochar may enhance the soil water and nutrient status as well as improve plant P and Mg uptake; however, it showed no significant differences in the physiological performance of muscadine grape plants.

The Muscadine grape (*Vitis rotundifolia* L., Vitaceae) is the predominant grape cultivar commonly grown in the southeastern United States, with current markets existing for juice, wine, and fresh fruit (Duarte Alonso and O'Neill, 2012). Most Florida vineyard soils are characterized by poor soil fertility; the soil is highly susceptible to erosion (Collins, 2017; Gillette and Walker, 1977; Marchi et al., 2016) and has low organic matter contents (Brown et al., 2018), nutrient

and water retention (Githinji, 2014), and cation exchange capacity (CEC) (Marx et al., 1996; Reichert et al., 2016). Rapid drainage leads to nutrient leaching through sand-based root zones (Bigelow et al., 2001; Mohamed et al., 2016; Petri and Petrovic, 2001). Therefore, plant growth is strongly limited by root growth and fewer available nutrients in sandy soil (Bruun et al., 2014). Undesirable soil conditions influence the annual nutrient consumption and total nutrient

content of grapevine leaves as well as fruit quality.

Biochar is a pyrolysis product of organic materials, which means thermal degradation under anaerobic and hypoxic conditions (Kan et al., 2016; Lehmann and Joseph, 2015). It is also called black gold because it can improve soil fertility and increase agricultural crop production. There are three main reasons for improving soil fertility: increasing soil pH (Aamer et al., 2020; Rees et al., 2014; Weber and Quicker, 2018); improving nutrient retention through higher CEC (Gondek et al., 2019; Laghari et al., 2015; Nikravesch et al., 2019; Wang et al., 2019); and optimizing the physical properties, such as surface area and pore structure (Batista et al., 2018; Lehmann et al., 2011; Obour et al., 2019). All these directly affect the microbial biomass (Dempster et al., 2012; Silva et al., 2020), microbial community composition and abundance (Kaurin et al., 2018; Palansooriya et al., 2019; Zhu et al., 2017), and enzyme activities (Bailey et al., 2011; Irfan et al., 2019). Therefore, crop growth and production will be indirectly affected because of more fungi (Duan et al., 2019; Ohsowski et al., 2018; Schwartz et al., 2006) and rhizosphere bacteria (Aggangan et al., 2019; Compant et al., 2010).

Soil amendment with biochar has been recommended as a new way to improve soil properties, including the water-holding capacity (Kammann et al., 2012; Marshall et al., 2019), water infiltration (Ippolito et al., 2012; Novak et al., 2016), soil water availability, nutrient retention (Clough et al., 2013; Sorrenti et al., 2016; Ventura et al., 2013), hydraulic conductivity (Buss et al., 2012; Liu et al., 2016), and soil aeration (Case et al., 2012; Obia et al., 2018). Significant increases in soil fertility, plant growth, and yield have been reported when biochar was applied to the soil surface in tropical and subtropical regions (Agegnehu et al., 2016a; Atkinson et al., 2010; Jeffery et al., 2017; Major et al., 2010). However, no studies have considered the effects of biochar on the intrinsic properties of the sandy soil in Florida vineyards.

Biochar can provide essential elements (Glaser et al., 2002) and promote nutrient retention in soil (Berek et al., 2018; Buss et al., 2018; Laird, 2008). Research of common beans showed that biochar can improve K, Ca, P, boron (B), and molybdenum (Mo) availability. However, under the high application rate of biochar, the available N and common beans biomass (although not yield) were decreased (Rondon et al., 2007). Moreover, research of other plants, such as grape, wheat, maize, rice, peanut, and tomato, indicated that, with an appropriate rate of biochar, the plant yield will be increased (Agegnehu et al., 2015, 2016b; Akhtar et al., 2014; Albuquerque et al., 2013; Ferreira et al., 2017; Liu et al., 2016; Vaccari et al., 2011; Zhang et al., 2012a, 2012b). The response of soil fertility and plant productivity to biochar application has been highly variable. Fertility responses can vary with the

nature of the biochar feedstock, total application rate, crop species, soil type, and other soil inputs, such as compost, as well as a combination of these factors (Gao et al., 2016).

No studies have reported the influences of pinewood biochar application on muscadine growth. This study aimed to examine whether pinewood biochar would: 1) improve soil nutrient and water retention; 2) increase nutrient uptake by muscadine grapes; and 3) promote muscadine grape growth.

Materials and Methods

Experimental site and materials. ‘Alachua’, a planted muscadine grape cultivar, was used for this experiment. One-month-old tissue-cultured vines were purchased from AgriStarts propagating nursery (Lakeland, FL). The activated biochar, produced by pyrolysis of southern yellow pine at 400 °C, was produced by Mirimichi Green Express, LLC (Castle Hayne, NC). The particle size is generally uniformly distributed between 0.6 and 10 mm. Table 1 shows the characteristics of the experimental soil and the pinewood biochar. The organic matter concentration of biochar was 616 g·kg⁻¹, which is 33.7-times that of sandy soil (<18.3 g·kg⁻¹). In addition, the CEC value of pinewood biochar is 20.13 meq/100 g, which is 7.1-times higher than that of sandy soil (2.83 meq/100 g). The surface morphology of the biochar sample was then studied using a FEI Nova 430 scanning electron microscope (SEM; FEI Company, Hillsboro, OR). Pinewood is one of the cheapest biochar feedstocks, and it is the most viable and suitable for growers; therefore, it was used during the present study.

Experimental design. This study was performed in a greenhouse at the University of Florida, Plant Science Research and Education Unit (PSREU; lat. 29.40°N, long. 82.17°W, altitude 21 m) in Citra, FL. To simulate the natural growth environment, the surface soil was taken from an assigned organic area (soil was free from herbicides and pesticide residuals) at a depth of 30 cm at the PSREU. This area is characterized by sandy soil (972 g·kg⁻¹ of sand, 2.4 g·kg⁻¹ of silt, and 4% g·kg⁻¹ of clay). This is a typical soil type in the southeastern United States, especially Florida. The limited water-holding

Table 1. Physiochemical properties of the tested soil and pinewood biochar in this study.

Characteristics	Sandy soil	Pinewood biochar
Active carbon (ppm)	182	526
Organic matter (%)	1.83	61.6
Total N (kg/ha)	1.04	16.27
Total P (kg/ha)	47.08	558.18
Total K (kg/ha)	19.05	1276.65
pH	5.7	6.1
CEC (meq/100 g)	2.83	20.13
Clay (%)	0.4	0.8
Silt (%)	2.4	20
Sand (%)	97.2	79.2

CEC = cation exchange capacity.

capacity of sand leads to increased leaching of nutrients (Yu et al., 2013). Biochar was applied and incorporated in the soil 3 d before planting in different proportions of 5%, 10%, 15%, and 20% on a dry weight basis. A control treatment comprising soil with unenriched biochar was used to compare the results with those of the other treatments incorporating different biochar rates. Then, the volume of the total potting media across biochar rate gradient was adjusted to a constant. Different percentages of sandy soil and biochar were thoroughly and gently mixed by hand to avoid damaging the biochar particle structure. This experiment had a randomized complete block design with five treatments and six blocks. Each replication plot consisted of two plants.

To monitor the effects of biochar on muscadine seedlings growth, 60 tissue-cultured muscadine plants were planted individually in 1.5-gal pots filled with the five different proportions of biochar/soil mixtures. Then, the pots were used for a short-term study lasting 16 weeks. Two data loggers were installed in the greenhouse to record the temperature and humidity values every 15 min during the experiment.

Environmental conditions. The temperature inside the greenhouse was set between 18 and 26 °C. Fertilizer was applied weekly through the irrigation system with standard N–P–K (10–10–10) plus micronutrients. One dripper with a flow rate of 40 mL·min⁻¹ was installed in each pot. The irrigation system worked three times per day for 2 min at 8:00 AM, 2 min at 2:00 PM, and 2 min at 8:00 PM, resulting in a total of 240 mL·d⁻¹ of water received. The trellis system was installed to support grape growth 2 weeks after transplanting.

Soil characteristic measurements. The soil moisture was measured biweekly with a double-probe soil moisture meter (Hydro-Sense II Handheld Soil Moisture Sensor; Campbell Scientific, Logan, UT). The soil temperature was measured monthly by a digital laboratory thermometer with a stainless-steel probe (DT301LAB stem thermometer; General Tools, New York, NY). Soil moisture and temperature were both measured at a depth of 15 cm, which is the main root zone. The measured soil moisture content should be based on the gravimetric water content percentage (GWC%) because the percentages of biochar in the mixtures

were considered weight-based. However, the data from the soil moisture meter were recorded as the volumetric water content percentage (VWC%). The conversion equation was performed using the following formula (El-Dine and Hosny, 2000):

$$VWC\% = GWC\% \times \left(\frac{BD}{\rho_w} \right), \quad [1]$$

BD refers to the bulk density of soil and ρ_w refers to water density equal to 1 g·cm⁻³. In this case, the following formula was used:

$$GWC\% = \frac{VWC\%}{BD}. \quad [2]$$

Nutrient content analysis. At the end of the experiment, the nutrient contents, including N, P, K, Ca, Mg, sulfur (S), zinc (Zn), Fe, Cu, Mn, and B, as well as the organic matter (OM) in soil, root, and shoot tissues were analyzed using an ICAP-Open vessel wet digestion system (Digi Block 3000; Waters Agricultural Laboratories Inc., Camilla, GA; <https://watersag.com/services/>). The nutrient measurements followed the protocol described by Isaac and Johnson (1985).

The base saturation percentage (BS%) represents the CEC value occupied by the basic cations, such as Ca²⁺, Mg²⁺, and K⁺. The base saturation percentage was expressed as follows:

$$\%BS = \frac{Ca^{2+} + Mg^{2+} + K^{+}}{CEC} \times 100\%. \quad [3]$$

Muscadine grape physiological performance. The chlorophyll values of the first four mature and healthy leaves from the growing point of each plant were recorded 35 and 54 d after transplanting with a portable SPAD 502 chlorophyll fluorimeter (Konica Minolta, Tokyo, Japan). The net photosynthetic rate was determined by using a LI-COR 6400 XT Portable Photosynthesis System (LI-COR, Lincoln, NE) 16 weeks after transplanting and before harvesting. LI-COR readings were obtained between 11:00 AM and 2:00 PM. After a destructive harvest, all the plants in each pot were collected and separated into roots and shoots. Dry weights of roots and shoots were determined using a digital scale with a precision of 1 g after 72 h at 55 °C in a drying oven.

Statistical analysis. All collected data were analyzed using the GLIMMIX procedure with SAS statistical software (ANOVA

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SAS 9.1; SAS Institute, Cary, NC). The mean analysis was performed with the Tukey-Kramer test at $P \leq 0.05$.

Results and Discussion

SEM characterization and soil physical properties. The SEM micrographs of the

pinewood biochar particles at magnifications of $\times 100$, $\times 500$, and $\times 1000$ are shown in Fig. 1. These SEM images represent the porous structure of the biochar samples used. Also, the SEM images show that raw biochar had smooth particles with a polygonal shape. The large channel-like structures proliferate the surface of biochar, showing at least par-

tial retention of the morphology of the initial input biomass.

During the first two measurements, the soil temperature at a depth of 15 cm decreased with the increased biochar rate. The third measurement showed a downward trend; however, no statistically significant difference was found between groups

Fig. 1. Scanning electron microscopy (SEM) images (bar, 50 μm ; $\times 1000$ magnification) of the pinewood biochar particles at magnifications of $\times 100$ (A, D, J), $\times 500$ (B, E, H), and $\times 1000$ (C, F, I).

Fig. 2. Soil temperatures measured in May, June, and July, respectively. (A) Measured on transplanting day. (B) Measured 33 d after transplanting. (C) Measured 52 d after transplanting. Bars with the same letters indicate that the means are not significantly ($P \leq 0.05$) different according to the Tukey-Kramer test. Error bars show the SE ($n = 12$).

(Fig. 2). According to Zhang et al. (2013), the thermal conductivity of the soil significantly decreased by 3.48% and 7.49% with 4.5 and 9 Mg·ha⁻¹·year⁻¹, respectively, following the application of biochar. This was also consistent with the decrease in soil bulk density. The same author reported that biochar treatment might regulate the extreme soil temperature, thus reducing the temperature when the soil temperature is high and increasing it when the soil temperature is low. The adjustment capabilities of the daily average temperature and diurnal range of the soil were mostly within ± 0.4 and ± 0.8 °C, respectively. Zhang et al. (2013) assumed that the regulation of soil temperatures by biochar might be explained by the combined action of changes in soil thermal conductivity and reflectance.

Figure 3 illustrates the soil moisture content in terms of the gravimetric water content percentage. Apparently, biochar significantly improved the soil moisture content. The first soil moisture measurement was performed 35 d after transplanting. The second measurement was performed 54 d after trans-

Fig. 3. Soil moisture in gravimetric water content (GWC%) in the bag and pot in June and July. (A) Measurements of the plants in a pot 33 d after transplanting. (B) Measurements of the plants in a pot 54 d after transplanting. Bars with the same letters indicate that the means are not significantly ($P \leq 0.05$) different according to the Tukey-Kramer test. Error bars show the SE ($n = 12$).

planting, after the soil was treated with drought stress by stopping irrigation for 24 h. The first measurement indicated that the moisture of the amended soil with the highest biochar rate (20%) was 17-times higher than that of the control group. The second measurement indicated that the average soil moisture rates were 0.03% and 20.9% in the control group and 20% in the biochar-amended group, respectively. In this case, a moisture rate of 20% in the biochar-amended soil is 847-times higher than that of pure sandy soil during the bag experiment and pot experiment. Therefore, it has been concluded that biochar can significantly ($P < 0.05$) increase soil moisture, especially under drought stress conditions.

The application of biochar can improve negative effects of drought and salt stress on plants. According to a recent study by Ali et al. (2017), the application of biochar improved the growth, biomass, and yield of the plants, and it enhanced photosynthesis, nutrient absorption, and gas exchange characteristics of treated plants exposed to drought salt stress. In addition, it indicated that biochar improved the soil water-holding capacity and physical and biological properties, as well as decreased the absorption of Na⁺ and increased the absorption of K⁺ by plants under salt stress (Ali et al., 2017). The results of a biochar experiment performed in a non-irrigated vineyard in central Italy also indicated that an increase in grape productivity was inversely proportional to precipitation

during the vegetative period, thus confirming the vital role of biochar in managing plant water availability (Genesio et al., 2015). Similarly, a biochar experiment conducted in southwest Spain for sunflower (one of the most important nonirrigated crops in Southern Europe) showed better growth of amended plants during the drought period. A higher reduction of stomatal conductance, indicating an improvement in water use efficiency, is the main reason for better crop performance following biochar treatment (Paneque et al., 2016).

Nutrient content analysis. The contents of P, K, Mg, Ca, BS-K (the value of CEC occupied by K⁺), BS-Mg (the value of CEC occupied by Mg²⁺), and organic matter significantly increased with a higher rate of biochar amendment, whereas the contents of BS-H (the value of CEC occupied by H⁺) decreased significantly (Table 2). There was no difference in the BS-K content, which was confirmed by another biochar experiment using sugarcane seedlings (Yang et al., 2015). These basic cations are different from acid cations H⁺ and Al³⁺. Therefore, soils with a high percentage of base saturation generally have more fertility because soils with a high percentage of base saturation show lower BS-H and higher pH (Gaspar and Laboski, 2016). Therefore, they have a greater buffering effect on acid cations from plant roots and soil acidification. Furthermore, they contain greater amounts of essential plant nutrient cations Ca²⁺ and Mg²⁺ for plant use.

The concentrations of nutrients in root tissues are presented in Table 3. The contents of P and Mg increased 4% and 20%, respectively, with the 20% biochar rate compared with the control group. In contrast to the trend observed for P and Mg, N significantly decreased following the 20% biochar amendment. However, Fe and Cu significantly decreased on soil amendment with 10% biochar. The K, Ca, S, B, Zn, and Mn contents were not significantly affected by the treatments. The concentrations of nutrients in shoot tissues are presented in Tables 4. Biochar treatment had no significant effects on N, K, S, B, Zn, Ca, Mn, Fe, and Cu contents; however, it did have an effect on the P and Mg contents in shoot tissues.

Muscadine roots responded to soil improvement because biochar can serve as a nutrient source through the addition of soluble P and biochar N retention and by changing the soil nutrient content (Prendergast-Miller et al., 2014). Although the soil nutrients were significantly increased after biochar amendment, only a few mineral nutrients, such as P and Mg, increased in plant tissues. Decreases in Fe and Cu may have been attributable to an increase in soil pH caused by the liming effect of biochar. A higher soil pH was negatively correlated with the availability of Fe and Cu, leading to reductions in their uptake by the plant (Bravo et al., 2017). Decreased N can be explained by the porous structure of biochar because its high surface area and CEC determine the higher

Table 2. Nutrient content in postharvest soil as influenced by the biochar treatments.

Biochar rate %	0	5	10	15	20
P (%)	125.83 c ^z	178.00 b	166.00 b	224.50 a	222.17 a
K (%)	242.50 b	248.83 b	250.00 b	348.50 a	375.33 a
Mg (%)	108.67 c	176.50 b	200.33 b	272.17 a	301.50 a
Ca (%)	707.33 c	1487.00 b	1505.33 b	2116.00 a	2219.17 a
pHw ^y	5.40 b	5.82 a	5.78 a	5.68 a	5.68 a
pHb ^y	7.79 a	7.78 ab	7.74 bc	7.70 cd	7.68 d
CEC (%)	4.22 c	6.57 b	7.00 b	9.25 a	9.87 a
BS-K (%)	7.32 a	4.88 a	4.60 a	4.83 a	4.88 a
Mg (%)	10.73 c	11.27 bc	11.95 abc	12.22 ab	12.72 a
BS ^x -Ca (%)	42.00 a	56.58 a	53.83 a	57.03 a	56.08 a
BS-H (%)	39.95 a	27.23 b	29.63 b	25.93 b	26.32 b
OM ^y (%)	0.41 c	0.68 b	0.86 b	1.20 a	1.37 a

^zThe means followed by the same letters are not significantly different ($P \leq 0.05$) according to the Tukey-Kramer test.

^ypHw, pHb, BS, and OM refer to pH measured from dissolving soil in the water, pH measured from dissolving soil in the buffer solution, base saturation, and organic matter, respectively.

Table 3. Nutrient content in root tissues as influenced by the biochar treatments.

Biochar rate %	0	5	10	15	20
N (%)	1.58 a ^z	1.62 a	1.57 ab	1.47 ab	1.39 b
P (%)	0.23 b	0.23 b	0.24 b	0.25 ab	0.26 a
K (%)	0.72 a	0.70 a	0.72 a	0.74 a	0.73 a
Mg (%)	0.25 c	0.26 bc	0.28 abc	0.29 ab	0.30 a
Ca (%)	1.77 ab	1.80 a	1.70 ab	1.65 b	1.66 b
S (%)	0.15 a	0.15 a	0.15 a	0.15 a	0.15 a
B (ppm)	16.42 ab	17.25 ab	16.17 b	18.50 b	19.33 a
Zn (ppm)	65.67 b	66.67 ab	67.33 ab	77.08 a	75.75 ab
Mn (ppm)	38.75 a	32.00 a	31.75 a	34.42 a	35.08 a
Fe (ppm)	526.75 a	425.42 ab	391.58 b	372.00 b	361.00 b
Cu (ppm)	17.00 a	14.50 ab	12.92 b	12.67 b	12.00 b

^zThe means followed by the same letters are not significantly different ($P \leq 0.05$) according to the Tukey-Kramer test.

Table 4. Nutrient content in shoot tissues as influenced by biochar treatments.

Biochar rate %	0	5	10	15	20
N (%)	2.56 a ^z	2.53 a	2.53 a	2.49 a	2.45 a
P (%)	0.23 b	0.23 ab	0.23 b	0.23 ab	0.26 a
K (%)	1.10 a	1.13 a	1.07 a	1.06 a	1.09 a
Mg (%)	0.22 b	0.22 b	0.23 ab	0.24 ab	0.26 a
Ca (%)	1.80 a	1.80 a	1.83 a	1.77 a	1.79 a
S (%)	0.31 a	0.29 a	0.29 a	0.30 a	0.29 a
B (ppm)	22.42 a	22.00 a	23.08 a	22.67 a	24.00 a
Zn (ppm)	119.08 a	113.75 a	108.58 a	116.75 a	114.00 a
Mn (ppm)	959.33 a	888.25 a	858.92 a	927.08 a	926.75 a
Fe (ppm)	71.42 a	71.67 a	71.08 a	69.50 a	66.75 a
Cu (ppm)	24.83 a	21.75 a	20.58 a	22.17 a	20.83 a

^zThe means followed by the same letters are not significantly different ($P \leq 0.05$) different according to the Tukey-Kramer test.

absorption ability of biochar for ammonium (through cation exchange) and nitrate (in solution in biochar pores) (Prendergast-Miller et al., 2014). According to Olmo et al. (2016), biochar addition reduced N availability but increased available P to plants. They expected that the N availability reduction also must have decreased the plant aboveground and belowground N concentrations. However, they only observed significant effects in wheat aboveground tissue, not in underground tissue. Decreased N content in the root but not in the plant aboveground part was observed during the current study.

Phosphorus has a major role in the growth of new tissues and cell division of plants (Vance et al., 2003). It is the main component of the nucleic acid structure, which is responsible for regulating the synthesis and stability of membranes in plant cells. Furthermore, P is a critical component of nucleic acid syn-

thesis. The structures of both deoxyribonucleic acid and ribonucleic acid are linked by P (Huang et al., 2019). Third, plants perform complex energy transmissions that require P fertilization. Phosphorus is crucial for deoxy-sine-5'-triphosphate components. ATP exists in all plants from early growth to maturity and is essential for energy generation and carbohydrate metabolism (Bielecki and Ferguson, 1983).

During this experiment, interesting results were found regarding the absorption of P and Mg, which are the only elements that are increased in soil, root, and leaf exposed to biochar treatment. Similarly, in a biochar experiment involving wheat root development, Olmo et al. (2016) reported that the application of biochar reduced N and Mn availability in soil but significantly increased the available concentration of P. However, the present study further indicated that soil amendment with biochar could affect the

whole absorption pathway of P. At present, the global P demand for agricultural crop production is alleviated by a large amount of mineral P fertilizer that has more than 15,000,000 Mg (Wang et al., 2012). Phosphorus in fertilizer is divided into two parts. Only 5% to 30% of P is eventually consumed by crops. The rest of P is fixed, complexed, precipitated, and leached into the soil, thereby making the plants unable to use it (Pierzynski et al., 2005). In tropical and subtropical areas, such as Florida, most soils are acidic because of high weathering rates and heavy rainfall. Acidic cations, such as aluminum (Al) and Fe, are predominate in such soils, and they can fix a large amount of applied inorganic P depending on the soil pH (Adnan et al., 2003). It has been reported that biochar may improve P availability by minimizing P fixation in acid soils because of its high affinity to Al and Fe in soils (Chang et al., 2014). Their affinity enables the long-

Fig. 4. Leaf chlorophyll [soil plant analysis development (SPAD) reading] values of the newest six mature leaves of muscadine grape at (A) 35 d and (B) 54 d after transplanting. Bars with the same letters indicate that the means are not significantly ($P \leq 0.05$) different according to the Tukey-Kramer test. Error bars show the SE ($n = 12$).

Fig. 5. Leaf photosynthesis net of muscadine grape plants measured 16 weeks after transplanting with the three newest mature leaves. Bars with the same letters indicate that the means are not significantly ($P \leq 0.05$) different according to the Tukey-Kramer test. Error bars show the SE ($n = 12$).

term chelation of Al and Fe instead of P. The mechanisms behind soil P availability with biochar treatment are not fundamentally understood (Mukherjee et al., 2019). It has also been speculated that soil amendment with biochar may affect the availability of soil P by regulating the activities of microbial enzymes in soil (Chen et al., 2011; Zhai et al., 2015).

Muscadine grape physiological performance. There was no significant difference in SPAD readings among the treatments (Fig. 4). Similarly, Quilliam et al. (2012) reported that biochar had no significant effect on SPAD measurements or plant height of dwarf bean during a 3-year field

Fig. 6. (A) Shoot and (B) root dry weight of muscadine grapes determined after 72 h at 45 °C in a drying oven. Bars with the same letters indicate that the means are not significantly ($P \leq 0.05$) different according to the Tukey-Kramer test. Error bars show the SE ($n = 12$).

experiment in the United Kingdom. However, another field experiment in northern Laos showed that the application of biochar reduced the SPAD value of rice leaves; it was reported that biochar application can potentially improve soil productivity of upland rice production in Laos, but the effect is highly dependent on fertilizer management (Asai et al., 2009).

The net photosynthesis data collected 16 weeks after transplantation are shown in Fig. 5. The results illustrated that pinewood biochar did not affect the leaf net photosynthesis in muscadine grape. Similarly, Xu et al. (2015) reported that peanut shell biochar application to an acidic (pH 5.6) sandy loam soil with low contents of many mineral nutrients generally did not affect the photosynthesis of peanut leaves. Other studies also suggested no detectable differences in the physiological variables of leaves, such as photosynthesis, under various salinity and fertilization treatments (Albuquerque et al., 2013; Thomas et al., 2013). However, some other research showed the opposite results. Wang et al. (2014) found rice husk biochar applied to replant soil at 80 g·kg⁻¹ can enhance plant height, fresh weight, and photosynthetic parameters (Wang et al., 2014). In addition, biochar application enhanced the in situ leaf photosynthetic rate of tomato and grape plants under drought stress (Akhtar et al., 2014). In contrast, Kammann et al. (2011) found that crop photosynthesis declined in quinoa on biochar-ameliorated soil when the soil moisture remained constant (Kammann et al., 2011).

Figure 6 shows that 20% biochar had a negative effect on the dry stem weight during the pot experiment. In general, there was no significant difference in the stem and root dry weights among treatments. Other studies confirmed no significant differences in the aboveground biomass and levels of foliar nutrients (Quilliam et al., 2012). In addition, Peng et al. (2016) found that soil amendment with biochar did not significantly improve the peanut yield and aboveground biomass, and they suggested that the application of organic manure was a more appropriate practice for hillslope Ultisols management than biochar. According to Abrishamkesh et al. (2015), the biochar type, interaction between biochar type, and biochar application rate had no significant effects on the aboveground and belowground biomass of lentils. Biochar application had no significant effect on the aboveground dry biomass, but it had a significant effect ($P < 0.01$) on the root dry biomass.

Conclusion

This is the first report of the effect of biochar application on muscadine grape growth. Soils in Citra, FL (Central Florida) are dominated by sandy soils, which have a naturally high leaching capacity and limited water-holding capacity. According to the results, biochar amendment had a strong impact on improving soil physical properties, explaining its positive effects on root traits. Pinewood biochar reduced soil temperature and soil bulk density and increased soil moisture, CEC, organic matter, and pH. Another interesting result of this experiment was that pinewood biochar increased the nutrient contents of P, K, Mg, and Ca in the soil. However, when the nutrients were transferred to plant root and leaf successively, a significant increase was only detected in the P content rather than the contents of other nutrient elements.

On the contrary, biochar did not affect leaf greenness, photosynthesis, or plant biomass. However, during this greenhouse experiment, our work focused on the influence of biochar application on muscadine grape growth during the early stage only. Further studies are needed to assess the long-term effects of biochar application on plant nutrient uptake and physiological performance, and especially on the nutrient absorption pathway and utilization efficiency.

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