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Biochar improves soil physical characteristics and strengthens root architecture in Muscadine grape (*Vitis rotundifolia* L.)

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Abstract

Background: Biochar is widely assumed as an effective soil amendment. It improves soil structure and fertility, thereby enhancing crop growth and development. There is still a knowledge gap in research on the beneficial impact of biochar on root growth and root architecture in perennial woody plants. Therefore, in our 14-week greenhouse study, pinewood-based biochar was applied as soil amendment for muscadine grape cultivation to investigate its effects on soil physical properties and crop root growth. Muscadine grape cv. Alachua was grown on Ultisols soil mixed with five rates of biochar on weight basis. Soil mixture properties and root attributes were determined.

Results: The soil bulk density decreased 40% and the total porosity increased 50% by adding 20% biochar into pure sandy soil. The soil water-holding capacity (WHC) of 20% biochar amendment soil was 1.9 times as pure as sandy soil. In addition, the incorporation of biochar did not only ameliorate soil acidity at the beginning but also increased soil pH buffering capacity, providing suitable soil pH a few months after application. Moreover, biochar induced woody plant finer roots development and significantly promoted root length, number of root forks, and crossings, while decreasing root average diameter.

Conclusions: Pinewood biochar significantly improved soil physical properties by moderating soil thermal properties, buffering soil pH, improving soil WHC, decreasing soil bulk density, and increasing soil porosity. In addition, biochar also strengthened the root architecture by improving root length, number of root forks, and crossings. Furthermore, roots from the amended treatment had longer root length with less average diameter than unamended roots, indicating that biochar may stimulate muscadine fine root development. The incorporation of biochar in soil enhanced woody plant root growth and development improved soil structure in sandy soils. It could potentially be a good strategy to tackle water loss, particularly in sandy soils.

Keywords: Biochar, Leachate pH, Root architecture, Ultisols soil, Soil water-holding capacity

Background

Biochar, also named "agrichar," is a relatively stable carbon compound formed by the thermal decomposition of various biomass materials under low oxygen conditions [1, 2]. The use of biochar as amendment can improve water and nutrient retention in soil and stimulate plant growth [3–5]. In the southeastern United States, sandy soil is a typical soil type of the "Ultisols." Its limited water-holding capacity (WHC) led to leaching of water and nutrients [6]. Recently, biochar-related research is increasing rapidly and many benefits of soil amendment on Ultisols soil have been intensively investigated, including enhancing chemical and physical characteristics as well as fertility of soils [7]. Therefore, biochar is considered as one of the potential solutions to improve soil fertility and crop production in sandy soils.

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Most of the work done on biochar amendment focused on plant aboveground growth and yield, but very few on root architecture [8–10]. Plant root systems play an important role in plant growth as they are the first contact point between biochar particles and growing plants when absorbing water and nutrients [11]. Thus, biochar application may change root morphology, functioning, and hence affect plant performance. Therefore, it is important to determine how root traits respond to biochar application in sustainable soil management practice [12, 13].

Biochar can improve root–soil interactions in distinct root–soil zones for increasing root activity [11, 14]. Biochar-induced improvement in root growth and development may be associated with at least two mechanisms: (i) enhanced nutrient supply by serving as a direct nutrient source and (ii) helped increase nutrient retention and availability in the rhizosphere and bulk soils [14]. Biochar incorporation rate is highly dependent of crop and soil type. An optimal amount of biochar in sandy soil for turfgrass is close to 10% (v/v) considering root depth and soil WHC. Incorporation of more than 10% biochar in sandy soils may detrimentally affect plant root depth. It may be because of anaerobic conditions which are due to higher soil water retention and less oxygen in pore space [15].

Soil amendment with biochar was found to be very effective in improving soil WHC [16–19], soil water infiltration [20, 21], soil water availability [22], nutrient retention [23, 24], soil hydraulic conductivity [25], and soil aeration [26, 27]. In addition, biochar can increase soil microbial activity [28, 29], cause shifts in microbial diversity [30], increase soil electrical conductivity [31], and immobilize contaminants, such as trace elements, like copper [21, 25, 32] or pesticides [33, 34]. Therefore, the use of biochar in grape production under sandy soils could facilitate nutrient and irrigation management practices.

Muscadine grapes (*Vitis rotundifolia.* L., Vitaceae) are the predominant grape cultivar commonly grown in the southeastern United States, with current markets existing for juice, wine, and fresh fruit. The *Vitis* genus is divided into two subgenera: Euvitis (the European grapes, *Vitis vinifera* L. grapes, and the American bunch grapes, *Vitis labrusca* L.) and Muscadinia (the Muscadine grapes *Vitis rotundifolia* L.) [35, 36]. Muscadine grapes are round, having either bronze- or purple-colored leather-like thick skin, and are well adapted to warm, humid climates, which are not suitable for growing table grapes (*Vitis Vinifera* L.) [37]. Native muscadine grapes have a distinct benefit compared with table grapes. Firstly, muscadines need fewer chilling hours than other grape cultivars so that they can thrive on the summer heat [38]. Secondly, they have a natural resistance to Pierce's disease, which is caused by the bacterial pathogen, *Xylella fastidiosa* [39]. This pathogen prevents the extensive cultivation of *V. Vinifera* in Florida.

A high correlation between developments of the underground and aboveground parts of grapevines has been demonstrated [40]; thus, studying the impact of biochar on root morphological attributes would be helpful for understanding plant–soil interactions and their ultimate impact on plant growth. We tested the hypothesis that biochar incorporation in sandy soils can improve soil physical properties and make root architecture stronger in muscadine grape.

Materials and methods

Site and description of the experimental materials

The research was conducted in a greenhouse at the University of Florida Plant Science Research and Education Unit (Latitude 29.40 N, Longitude 82.17 W, Altitude 21 m) in Citra, FL. Soil for this study was collected from the organic area in Plant Science Research and Education Unit at 0–30 cm depth, which means the soil is free from herbicides and pesticide residuals. The soil type at the site is characterized as Ultisols, which is acidic and strongly leached. The soil texture was classified as sandy soil with 972 g kg^{-1} of sand, 24 g kg^{-1} of silt, and 4 g kg^{-1} of clay. "Alachua," a widely planted muscadine grape cultivar, was used in this experiment. One-month-old tissue-cultured vines were provided by AgriStarts propagating nursery (Lakeland, FL, USA). The activated biochar produced by pyrolysis of southern yellow pine at 400 °C was provided by Mirimichi Green Express, LLC (Castle Hayne, NC, USA). This biochar had a pH of 6.2 and its particle size was evenly distributed between 0.6 to 10 mm with less than 4% ash. It had active carbon of 526 mg Kg^{-1}, C/N ratio of 30:1, and cation exchange capacity (CEC) of 20.13 meq 100 g^{-1}. In addition, it had surface area of 366.14 m^2 g^{-1}, and density of 1.89 g cm^{-3}.

Experimental design

Biochar was applied and incorporated into the soil, three days before planting, at rates of 5%, 10%, 15%, and 20% dry weight basis. A control treatment using soil with no biochar load was conducted to compare with the other treatments which incorporated different rates of biochar. Afterward, the volume of total potting media across biochar rate gradient was adjusted to a constant. Each of the five ratios of sandy soil and biochar was thoroughly and gently mixed by hand to avoid damaging biochar particle structure. Each rate has six replications, with two plants in each replication.

Experiment 1: Muscadine plants were planted individually in 5.7-L pots filled with the five different proportions of biochar–soil mixtures. The experiment was laid out in randomized complete block design with five treatments, and each treatment was replicated six times (two plants per replicate).

Experiment 2: In the column study, twenty soil columns (10 cm internal diameter by 100 cm length = 31,400 cm^3) made of white polyethylene pipes supported by a wooden table were used. A piece of fine wire mesh was attached to the column bottom to prevent losing soil mixtures and to let water drain down freely. Columns were filled up with five rates of biochar mixtures (0%, 5%, 10%, 15%, and 20%, based on weight) in four replicates. Each column was planted with one muscadine plant. The environmental conditions as well as the irrigation and fertilization schedules were all the same with the pot experiment. At the end of the experiment, all muscadines were destructively harvested for root and aboveground analysis, 16 weeks after transplant.

The greenhouse study in pots was conducted to determine the optimum biochar amendment rate, which also provided wider and shallower space. The column study provided longer and narrower space for grape root development, allowed easier collection of leached water, and reduced overlapping and competition of roots. In this way, the research gap on biochar in relation to root architecture can be minimized.

Cultural practices

The air temperature of the greenhouse was set between 18 and 26 °C. Fertilizer was applied weekly through the irrigation system at 1.5% rate with nitrogen-phosphorous-potassium plus trace elements (15-5-15 + TE, elements, Peters, PA, USA). For muscadine plants in pots, there was one dripper in each pot, with a flow rate of 40 mL min^{-1}. The irrigation system was set to irrigate three times a day, at 8:00 am, 2:00 pm, and 8:00 pm. The plants were irrigated one minute each time (120 mL day^{-1}) in the first 8 weeks, two minutes each time (240 mL day^{-1}) in weeks 9 and 10, and three minutes each time (360 mL day^{-1}) starting from week 11 to the end of the trial (week 14). The pH of irrigation water is around 8. The trellis system was installed to support grape growth two weeks after transplanting.

Measurement of soil properties

Soil temperature in pots was measured monthly at a depth of 15 cm using a digital lab thermometer with stainless steel probe (DT301LAB stem thermometer, General Tools, NY, USA). Additionally, in the column study, the irrigation was turned on manually for half an hour and the leachate was collected to measure pH value monthly, lasting for three months. Exudates (50 mL) collected at the bottom of each column were taken to the lab and their pH was measured at room temperature with a digital pH meter (FE20-Basic benchtop digital pH meter, Mettler Toledo, OH, US).

The other soil physical properties, including soil WHC, soil bulk density, and soil porosity, were measured with soil samples from pots. Soil moisture was determined gravimetrically from the mixed samples from each plot at the end of the study. All samples were air-dried and evenly mixed. The soil samples (50g) were weighed and packed into funnels covered with filter paper to prevent the loss of soil or biochar. All filter papers were saturated with deionized water. Next, 50 mL of water was gradually added to each soil sample. A beaker was used to collect the leachate water for 30 min until there was no drain at room temperature. The top of the funnel was covered by a plastic wrap to prevent the water loss by evaporation [41, 42]. Finally, with a known leachate water volume, soil WHC was calculated as follows [6]:

$$\text{WHC} = \frac{\text{Total water added - Volume of water run down}}{\text{Dry weight of soil sample}} \times 100\%, \tag{1}$$

where WHC represents the water-holding capacity.

Soil bulk density was determined at the end of the experiment from one pooled sample from each treatment. Total porosity was calculated from soil bulk densities by the following expression:

$$P = \frac{\text{Volume of voids}}{\text{Total volume}} \times 100\% \tag{2}$$

where P represents the total porosity. This equation can also be transformed into:

$$p = \left(1 - \frac{\text{BD}}{\text{PD}}\right) \times 100\%, \tag{3}$$

where BD is the bulk density and PD is the particle density. The soil particle density (PD) can be calculated from as follows:

$$PD = \frac{\text{Mass of sample}}{\text{Volume of sample}} \times 100\%. \tag{4}$$

However, the soil particle density is always assumed to be 2.65 g cm^{-3} for any soil sample [43]. Based on this, we calculate total porosity as follows:

$$P = \left(1 - \frac{\text{BD}}{2.65\text{g/cm}^3}\right) \times 100\%. \tag{5}$$

Determination of root architecture

In the pot study, the roots were separated by breaking the pots from the two sides. In the column study, the columns were laid down and the roots were gently pulled out by hand. The roots were then submerged in tap water, rinsed with mild water flow, and soaked in deionized water until scanned. Root scanner (Epson 12000XL, Seiko Epson Corporation, Nagano, Japan) and WinRHIZO software (WinRHIZO Pro, Regent Instruments, QC, Canada) were used to measure total root length, average root diameter, specific root length, root volume, and number of crossings.

Statistical analysis

All collected data were analyzed using Proc GLIMMIX (General Linear Mixed Model) in SAS statistical software (ANOVA SAS 9.1, SAS Institute, Cary, NC, USA). We assumed a Gaussian response distribution, and the default covariance matrix format was used to compare treatment means. Type III tests of fixed effects were used to examine main effects for each treatment. Mean separation was accomplished with the Tukey–Kramer honest significant difference test ($\alpha = 0.05$). In all cases, assumptions of normal distribution and equal variance were validated by diagnostic plots generated from the GLIMMIX procedure.

Results and discussion

Evaluation of soil properties

Data on soil temperature from the first two measurements depicted a negative correlation between soil temperature and rate of biochar incorporation (Table 1). However, the third-time measurement showed a down trend, although no statistically significant difference was found among the groups. The results corroborated with a previous study in which the thermal conductivity of soil decreased significantly by 3.5% and 7.5%, with 4.5 and 9.0 Mg ha^{-1} yr^{-1} in response to increment in biochar rate, respectively [44]. This was also consistent with the decrease in soil bulk density. Authors [44] also reported that biochar treatment may regulate the extreme soil temperature, reducing the temperature when the soil temperature is high and raising it when soil temperature is low. The adjustment capability of soil daily average temperature and diurnal range was mostly within ± 0.4 °C and ± 0.8 °C, respectively. Regulation of soil temperature by biochar can be understood by the synergistic reaction of changes in soil thermal conductivity and reflectance [44].

Soil bulk density decreased, while WHC and total porosity increased gradually with the increase in biochar rate (Table 2) and a significant positive correlation between WHC and biochar application rate is observed when the biochar application rate was below 20%. The WHC of 20% biochar-amended soil reached 52%, while pure sandy soil only had WHC of 28%. Biochar significantly increased WHC by more than 50%, compared to the controls. Soil WHC was increased by 1.2% by mass for each 1% addition of biochar over the agriculturally relevant range up to 20% biochar concentrations. Yu et al. reported that WHC of loamy sand soil mixed with different proportions of yellow pinewood biochar had a positive correlation between WHC and percent of biochar amendment for biochar concentrations below 10%. In addition, the increase in WHC per unit of biochar amendment was increased when biochar concentrations were below 10%, but was constant at 12% when biochar concentration was beyond 10%. Thus, it was suggested that 10% amendment maximizes the water holding value

Table 2 Soil properties measured from remaining soil after harvesting

Biochar rate %	Water-holding capacity (%)[a]	Bulk density (g cm^{-3})	Total porosity (%)
0	28	1.47	45
5	34	1.21	54
10	40	1.08	60
15	46	1.02	62
20	52	0.88	67

[a] Water-holding capacity (WHC) ($n = 3$) and bulk density were determined from unsieved soil samples at the end of the experiment. Total porosity was calculated from bulk densities assuming a 2.66 g/cm^3 particle density for soil minerals. Three replicate samples from the pool of each treatment were used to determine statistical uncertainty

Table 1 Soil temperature measured in May, June, and July, respectively

Biochar rate %	19-May		19-Jun		19-Jul	
0	26.1	a[a]	29.7	a	31.2	a
5	26.0	ab	29.1	ab	30.9	a
10	25.2	c	29.1	ab	30.7	a
15	25.6	bc	28.8	b	30.7	a
20	25.3	C	29.0	ab	30.8	a

[a] Least square means followed by the same letters are not significantly different ($P \leq 0.05$) according to the Tukey–Kramer test

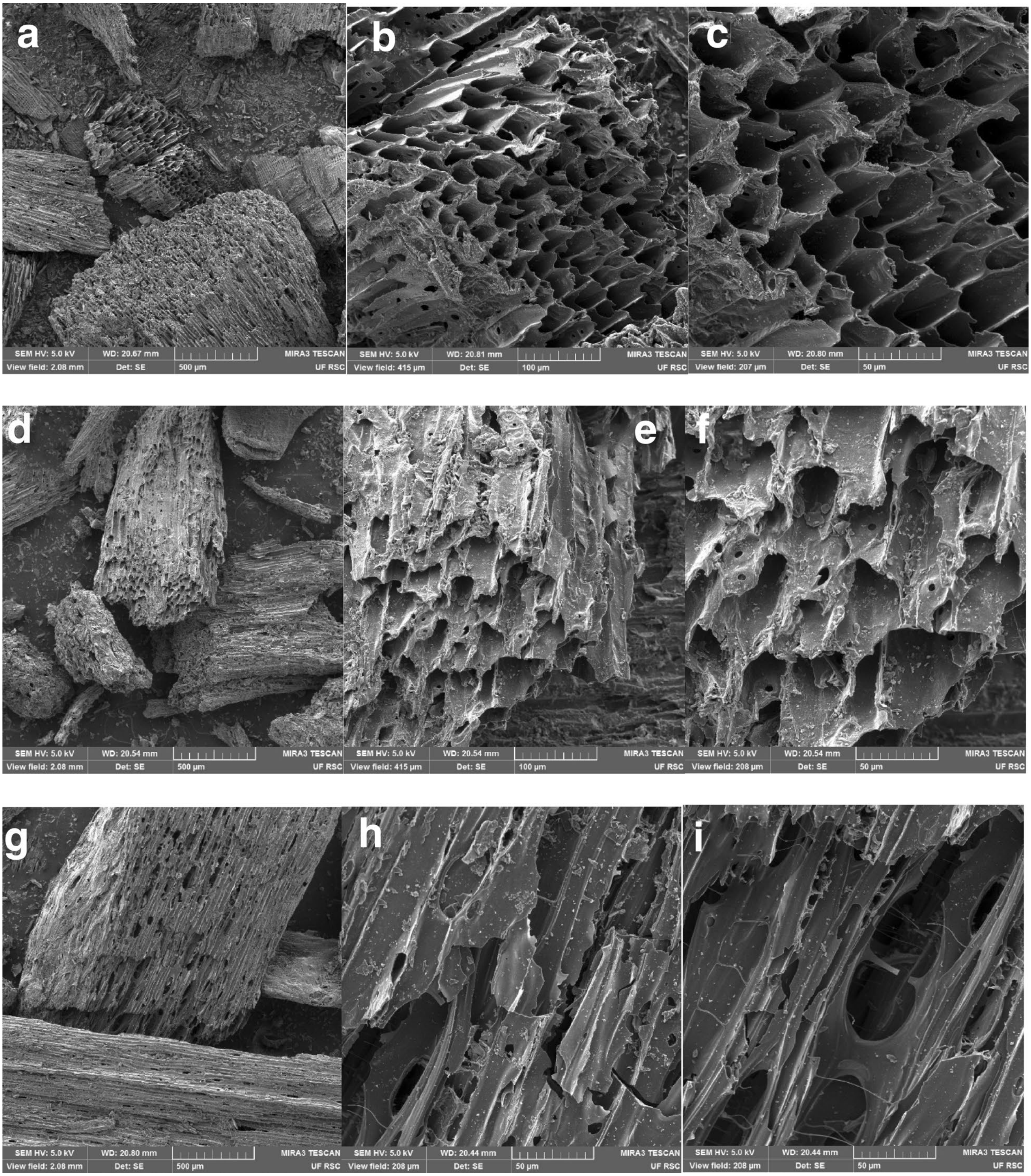

Fig. 1 SEM images (*bar* 50 μm; × 1000 magnification) of the pinewood biochar particles at 100× (**a**, **d**, **j**), 500× (**b**, **e**, **h**), and 1000× (**c**, **f**, **i**) magnifications

of single percent of biochar. It was also reported that the standard deviation showed very little variance in biochar rates up to 20%, but gradually increased as the biochar amendment rate increased [6].

The modification of biochar has two effects on soil WHC [45]. Firstly, biochar itself can retain water in its internal pores, thus directly increasing soil moisture content. The scanning electron microscopy (SEM) micrographs of the pinewood biochar particles at 100 ×, 500 ×, and 1000 × magnification are shown in Fig. 1. An earlier study on straw biochar confirmed that the presence of pores, with diameters between 0.1 and 10 μm, in the biochar enhances the percentage of plant available water. In addition, other factors, such as the total pore volume and hydrophilic functional groups on the surface of biochar, may also help improve soil WHC, although these effects may be limited considering the tiny amount of biochar added to the soil.

Biochar particles can reduce the overall bulk density of sandy soils (Table 2). Specifically, the bulk density of sandy soil was reduced more by small biochar particles, while the bulk density of loamy sand soil was reduced more by large biochar particles [46]. A decrease in biochar particle size can increase water retention, but may reduce saturated water flow [46]. Modulations in bulk density for the biochar treatments along the incubation period were not always significant [47]. The changes in mixed soil bulk density may result from the low bulk density of the biochar itself [48]. Biochar and soil physical property data mentioned in the study showed that biochar generally reduces soil bulk density by 3 to 31%, increases porosity by 14 to 64%, and increases wet aggregate stability by 3 to 226%. Biochar appears to improve soil properties of sandy soils more than clayey soils [46].

The influence of biochar on soil physical properties can directly affect muscadine growth because the penetration depth and accessibility of air and water in the root zone mostly depend on soil physical composition. It can further affect the ability of the soil to react to water, its aggregation, dynamics, and permeability at expansion, as well as its ability to maintain cations and respond to pH changes [3].

The pH value of leachate is shown in Table 3. The soil used in the current study had pH of 5.7. The data of the measurement in April demonstrated that the leachate pH increased with the increase of biochar percentage. In the measurement in June, leachate pH decreased, which was due to the application of nitrogen fertilizer. In the measurement in August, the leachate pH increased, which was mainly due to irrigation. After four months, leachate pH of the soils receiving biochar was between 6.4 and 7.1. It was very close to the optimum pH for muscadine growth, which is 6.5. After five months, the leachate pH of soil from control treatment began to reach 6.3, while the leachate pH of modified soil stabilized at about 6.9. This is consistent with numerous studies in which the use of biochar increased the pH of acidic soils [49]. Furthermore, soil pH can affect nutrient availability. For example, maximum phosphorous (P) availability is near pH 6.5 [50, 51]. P becomes insoluble phosphorous/aluminum minerals at low pH and forms calcium-phosphate solid mineral at high pH. Besides, potassium, calcium, and magnesium are less available in acid soils because of leaching [52]. Moreover, the three measurements showed that the leachate pH of the control group increased over time, while that of the biochar treatments remained at similar value. It indicated that the incorporation of biochar did not only ameliorate soil acidity but also increased soil pH buffering capacity. The principal mechanism for the increase of pH buffering capacity of acid soils with the incorporation of biochar is the protonation and deprotonation of oxygen-containing functional groups in biochar. These oxygen-containing functional groups can absorb and provide protons through an association reaction of low pH and dissociation reaction of high pH, thus buffering the change of soil pH [53].

Evaluation of root architecture

The results of the pot experiment showed that biochar application significantly increased not only root length, root project area, and surface area but also root

Table 3 Soil pH measured from leachate monthly in column study from April to August in 2019

Biochar rate %	19-Apr		19-Jun		19-Aug	
0	5.75	D[a]	4.89	c	6.27	b
5	6.47	c	6.43	b	6.82	ab
10	6.73	b	6.81	ab	6.94	a
15	6.73	b	7.16	a	6.99	a
20	6.87	a	7.11	a	6.92	a

[a] Least square means followed by the same letters are not significantly different ($P \leq 0.05$) according to the Tukey–Kramer test

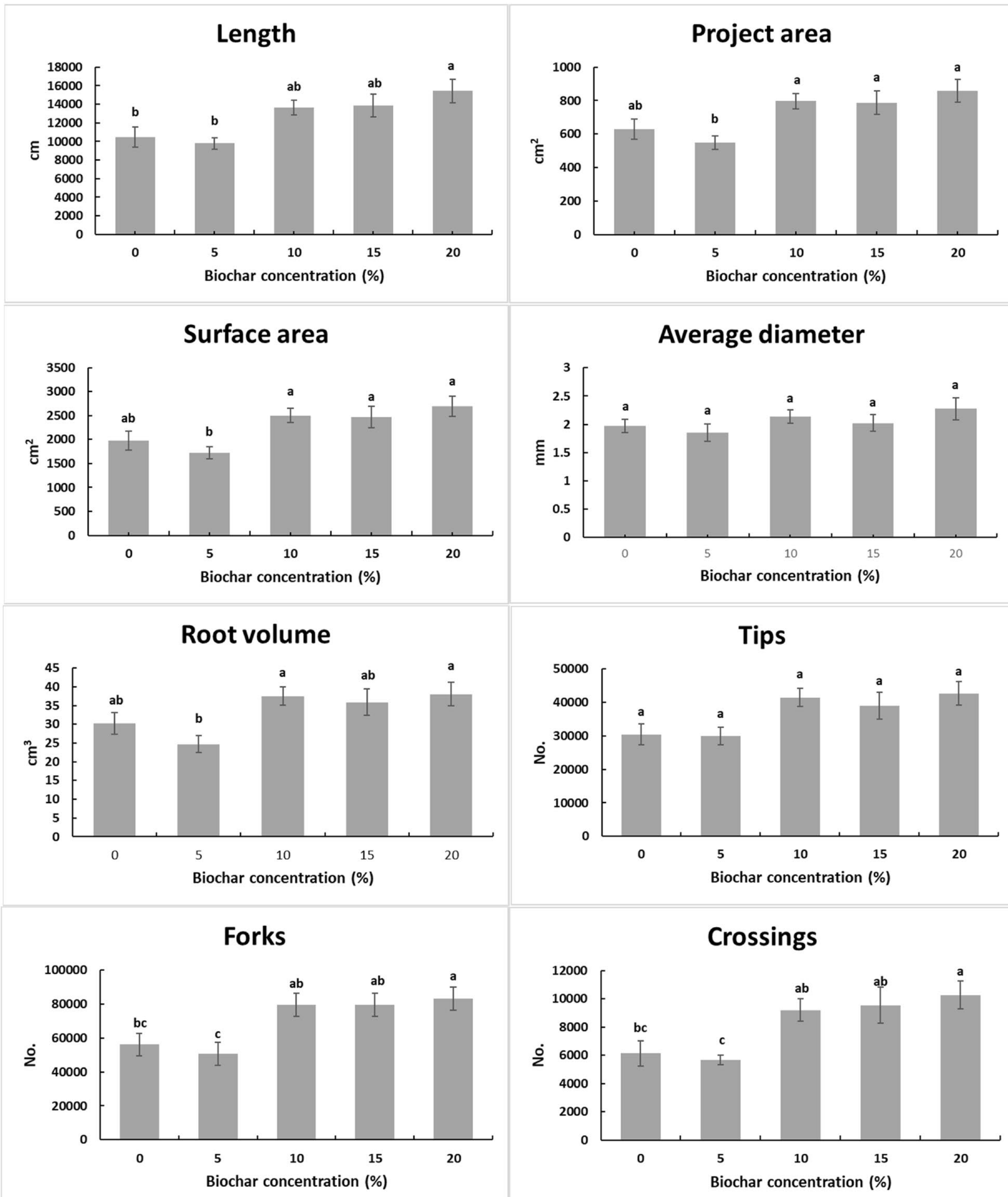

Fig. 2 Root traits from pot experiment obtained from WinRhizo software (WinRHIZO Pro, Instruments Regent, QC, Canada). Bars with the same letters indicate that the least square means are not significantly different ($P \leq 0.05$), according to the Tukey–Kramer test

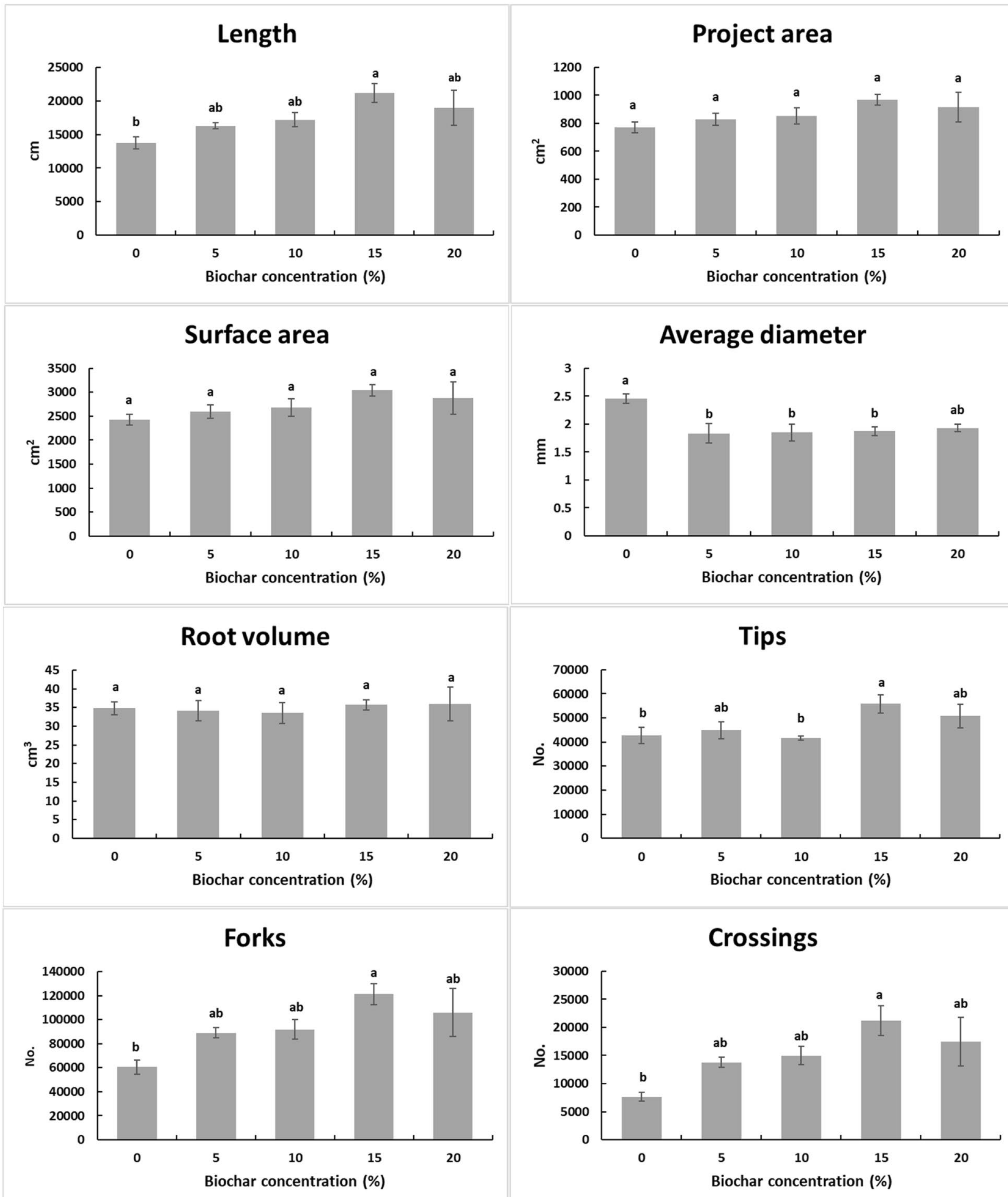

Fig. 3 Root traits of muscadine from columns. Bars with the same letters indicate that the least square means are not significantly different ($P \leq 0.05$) according to the Tukey–Kramer test

number of forks and crossings. However, there were no differences between 0 and 5% biochar-amended treatments. Similarly, there were no significant differences among 10%, 15%, and 20% biochar-amended treatments (Fig. 2). The results from the current study confirmed that pinewood biochar application can promote muscadine grape root growth in the early growth stage. Masahide et al. found that bark biochar significantly increased the root volume, or biomass, and colonization rate of arbuscular mycorrhizal (AM) fungi in maize [54]. Ishii and Kadoya [55] also reported an increase in root biomass after charcoal application. The reason may be that the biochar application increases soil water retention [56] and the gaseous phase [57]. Specifically, amelioration of the soil physical and chemical properties could be effective in enhancing root growth. It has been previously reported that biochar-type materials can promote root growth [58]. In addition, roots can even grow into biochar pores [59, 60]. Makoto et al. [61] showed a significant increase in both root biomass (47%) and root tip number (64%) within a carbonized layer from forest fire, with larch twigs, birch twigs, and shoots of dwarf bamboo. Furthermore, with the improvement of biochar, the root length of rice also increased [62]. Therefore, not only the abundance but also the growth behavior of roots may be changed by the presence of biochar [49].

Muscadine root traits measured in the column study are presented in Fig. 3. There were no differences among treatments regarding project area, surface area, or root volume. Overall, root length, average diameter, number of root tips, forks, and crossings reached the highest value at 15% biochar amendment and the lowest value without biochar application. Interestingly, in the control group, the root length had a similar surface area, but the average diameter was more substantial than other treatments. This means the roots were thicker in the control, while the roots from other treatments were finer. Fine roots, also known as feeder roots, are functional component and responsible for resource acquisition. Therefore, biochar can promote feeding root development, which is consistent with our observation. The reduced diameter of roots can be explained by more root occupancy of soil pore space [63]. The largest root length and the highest number of root tips, forks, and crossings were found at 15% biochar amendment. However, there was no significant difference in root length in other treatments. The root project area, surface area, and root volume were not affected by treatments. A similar study also reported that the incorporation of biochar can increase the root depth of barley in coarse sandy soil [64]. One of the major factors determining root growth is soil mechanical resistance, including soil texture, moisture, and bulk density [65]. Sandy soil shows high resistance of root growth due to large bulk density, poor structure, and low compressibility. However, biochar amelioration may offset some of these properties. From literature, biochar reduced soil bulk density and increased soil compressibility [66], making the soil easier for root development. In addition, biochar improved soil WHC, thus increasing the amount of soil moisture content. Higher soil moisture can also reduce mechanical resistance and increase the amount of available water for plants. Generally, the mechanism of how biochar improves crop root growth might be by modifying soil structure, especially for bulk density, and improving soil WHC to reduce mechanical resistance [67].

Conclusion

Biochar amendment had a strong impact on soil physical properties, such as moderating soil thermal properties, buffering soil pH, improving soil WHC, and decreasing soil bulk density. Since biochar lowered soil bulk density, thereby increasing soil porosity and soil aeration, it may have positive effects on roots. Furthermore, biochar improved root length, project area, surface area, the number of forks, and crossings significantly. Plants without biochar incorporation had shorter and thicker roots than those grown with biochar, suggesting that biochar could facilitate formation of finer and longer roots. If economically viable, biochar could potentially be used as the best management practice for muscadine production, particularly in sandy soils with low nutrient and WHC; however, more research is needed to explore the impact of biochar from different feedstocks with different incorporation rates at various growth stages under field conditions.

Abbreviations

WHC: Water-holding capacity; SEM: Scanning electron microscopy.

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Authors' contributions

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. YC was involved in formal analysis, investigation, writing––original draft, project administration. LR, LZ, BG, and MAS were involved in writing––review and editing, resources. AS was involved in supervision, writing––review and editing, resources, and validation. All authors read and approved the final manuscript.

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