

EFFECTS OF DIFFERENT BIOCHARS AMMENDMENTS ON PHYSIOCHEMICAL PROPERTIES OF SOIL AND ROOT MORPHOLOGICAL ATTRIBUTES OF *FOKENIA HODGINSII* (FUJIAN CYPRESS)

TARIN, M. W. K.¹ – FAN, L. L.¹ – SHEN, L.¹ – LAI, J. L.¹ – TAYYAB, M.² – SARFRAZ, R.² – CHEN, L. Y.³ – YE, J.³ – HE, T. Y.³ – RONG, J. D.¹ – CHEN, L. G.¹ – ZHENG, Y. S.^{1,3*}

¹College of Forestry, Fujian Agriculture and Forestry University
Fuzhou, Fujian 350002, People's Republic of China

²Fujian Agriculture and Forestry University, Fuzhou 350002, China

³College of Arts & College of Landscape Architecture, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, People's Republic of China

*Corresponding author

e-mail: zys1960@163.com; phone: +86-50-397-265

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Abstract. Biochar (BC) is the carbon-rich material, increasingly as a soil additive for carbon sequestration and soil improvement. Various studies have shown that BC have a profound effect on root morphological traits on agricultural systems, however the effect of BC amendments on the root traits of forest species is scant. The current study attempts to evaluate the effects of different BCs as a soil amendment on the growth and root morphological traits of *Fokienia hodginsii*. For that reason, three different types of BCs; bamboo (BB), hardwood (BH), and rice straw (BR) with four levels (0, 5, 20, and 80 g kg⁻¹ of soil) collectively ten treatment combination including control (Bo) were incorporated to soil and seedlings were allowed to grow in pots in greenhouse for one year. After one year uprooted seedlings roots were scanned and analyzed by using WinRHIZO software for comparison. Bamboo and rice straw BC amendments were found to significantly improve root traits particularly root volume, average diameter, and root length compared to Bo. Additionally, the seedlings treated with BR80, BB80, and BH80 attained 36%, 29%, and 25% biomass, respectively compared to Control (Bo). Soil physiochemical properties particularly soil pH also varied between different BCs types and levels. Consequently, BC alkalinity is an important feature to correct soil acidity and improve soil fertility by altering soil nutrient content. Root establishment in acidic soils can be enhanced by BC amendments but selection of BC and soil is crucial to maximizing soil improvement and seedlings roots traits.

Keywords: *Fokienia hodginsii*, root traits, biomass, winRHIZO, alkalinity, soil improvement

Introduction

Fokienia hodginsii (Fujian cypress) is an important conifer; native to China and Vietnam. Currently, on the basis of its distribution, this conifer is globally threatened, protected species (B2/second degree) in China, and as vulnerable in red list of threatened species; generated by international union for conservation of nature (IUCN) (Luu and Thomas, 2000; Ding et al., 2017; Tarin et al., 2019). Timber of this conifer is valuable because of its straight grain and different aroma and is also being exported to Europe and elsewhere in Asia (Osborn, 2004). Moreover, siam-wood essential oil or pemou oil is extracted from its stumps and roots which contains high sesquiterpene content; which can sanitize and purify the air, kill bacteria, and resist insects. Furthermore, extract oil can be used to improve emotional balance and the mental clarity in aromatherapy (Paluch, 2009). As a result of, certain increase in human

population has put enormous pressure on this conifer community by over logging for utilization of timber wood (Nguyen et al., 2015; Tarin et al., 2017). These anthropogenic (human activities) and pedogenic or natural factors (parent material) has resulted infertile soils (Lal, 2015). Consequently, high rates of deforestation, rise in global temperature (approximately $0.85 \pm 0.20^\circ\text{C}$) during last 130 years will lead to unfavorable and harsh conditions for plants such as drought and flood etc. globally (Alexander et al., 2006; Tarin et al., 2019) Additionally, intensive forest management for conversion of natural forests into artificial plantations has been shown to deteriorate the soil physical health, like, reducing pH and porosity, and increasing bulk density (Tonks et al., 2017).

Global climate change and intensive forest management both have adversely affected the features of forest soils via decrease in soil organic carbon, acidification, biological properties, and biodiversity (Li et al., 2018). Vast areas of tropical and subtropical regions of Southern China, soils are acidic where; soil fertility is the major constraint to plant growth (Haynes and Mokolobate, 2001; Baquy et al., 2017). However, mineral lime has been conventionally used to raise the soil pH (Adams, 1984) nevertheless, because of its high costs, much attention has been given to replacements like incorporation of crop straw in soil which also can increase soil pH (Xu and Coventry, 2003). Besides, direct addition of such plant residues into the soils do not stay longer because of decomposition (Yuan and Xu, 2011). Recently, biochar (BC) has received much attention as an alternate to these soil organic amendments and is considered as a potential fertilizer due to partial combustion of several organic components. It has mostly alkaline nature subject to pyrolysis temperature over 400°C (Gaskin et al., 2008) and can raise the pH of acidic soils by decreasing exchangeable Al from soil (Chan et al., 2008b). Its amendments into the soil can improve soil fertility (Topoliantz et al., 2005), stimulate the plant growth (Jeffery et al., 2011a) and enhance the sequestration of CO_2 from the atmosphere (Lehmann et al., 2006).

Numerous studies on agriculture and horticultural system have been shown to examine, how BC effects on plant growth and root morphological parameters. Inappropriately, research on effects of BCs amendments on growth of trees particularly on root morphological parameters is rare. BC application into the soil has significant effects on growth and root morphology as plant roots contact directly to its particles for uptake of soil nutrients and water (Prendergast-Miller et al., 2014). Therefore, measurement of roots morphological parameters, such as root length, root diameters etc. are essential to understand plant physiological functions. It is necessary to determine how root morphological traits respond to different BC application under multiple levels for better management of forest species.

In the context of previous studies, responses of root traits like root morphology and biomass (Brennan et al., 2014; Keith et al., 2015), root-associated microbes, and nutrient concentration (Rillig et al., 2010; Vanek and Lehmann, 2015) can be influenced by incorporation of BCs. Moreover, root morphological traits play diversified roles in plant growth and development (Nie et al., 2013). For instance, root length is possibly one of the important parameter, mainly because of general indicator of plant response to environmental factors and also controls the acquisition of nutrients or water (Clothier and Green, 1997; Edwards et al., 2004) whereas for the biomass increase, root diameter is considered as beneficial (Eissenstat and Yanai, 1997). To date, BC effects on root morphological traits are highly variable and controversial. For instance, because of various factors, root biomass remains relatively stable, decrease or increase under BCs

application (Macdonald et al., 2014; Prendergast-Miller et al., 2014; van de Voorde et al., 2014). The BC type (different materials or pyrolysis temperature) is an important factor because it varies significantly in chemical composition and pH (Lehmann et al., 2011). Consequently, the variation in characteristics of BCs and their application rate may also influence the soil physiochemical properties and ultimately alter the root morphological traits (Reibe et al., 2015; Reverchon et al., 2014). For that reason, it is important to know how root traits respond under different BCs application. In the current study, it was hypothesized that addition of BC into the soil may affect the biomass and root traits of the *F. hodginsii* seedlings by improving the soil physiochemical properties. Therefore, one year pot experiment was conducted to examine the effects of three different BCs under four levels (i) to identify the effects of different BCs on the physiochemical properties of soil, (ii) to investigate the effects of different BCs on growth (biomass) of *F. hodginsii* seedlings, and root morphological traits.

Material and methods

Study area and design

The present study was started in March, 2017 at Bamboo Institute of Fujian Agriculture and Forestry University Fuzhou, Fujian Province, P. R. China (lat. 26° 5' N, long. 119° 13' E, elevation 12 m; *Fig. 1*).



Figure 1. Boundary of Fujian Agriculture and Forestry University (Bamboo Institute), experimental design and order

The metrological informations of the study site during the entire experiment period are presented in *App. 1*. Three different types of commercial biochars (BCs); bamboo

biochar (BB, produced at 450°C), hardwood biochar (BH, produced at 420°C), and rice straw biochar (BR, produced at 500°C) were used; which were purchased from Nanjing Qinfeng Straw Technology Co. Ltd. China. Before application to soil the physiochemical characteristics of these BCs and basic soil were determined (Table 1).

Table 1. Physiochemical properties of BCs and soil used in the experiment, BB: bamboo biochar

Characteristics	BB	BH	BR	Basic soil
pH	9.687	5.703	9.833	4.685
EC (m Scm ⁻¹)	3.140	6.343	4.070	106.667
TC (g Kg ⁻¹)	311.220	330.530	250.053	3.358
OM (g Kg ⁻¹)	536.543	569.834	431.092	5.790
TN (g Kg ⁻¹)	6.243	8.297	5.153	0.497
AP (mg Kg ⁻¹)	32.381	24.318	29.076	10.245
TP(g Kg ⁻¹)	0.399	0.296	0.311	0.256
Ext. K (g Kg ⁻¹)	0.335	0.321	0.371	0.356
C:N Ratio	49.975	39.846	48.641	6.864

BB; Bamboo biochar, BH: hardwood Biochar, BR, rice straw biochar TN: total nitrogen, TC: total carbon, TP: total phosphorus, AP: available phosphorus, OM: organic matter, Ext. K: extractable potassium, C: N: carbon nitrogen ratio

These three BCs were incorporated to soil with four different levels; 0, 5, 20, and 80 g kg⁻¹ of soil (Tarin et al., 2019). One-year old seedlings of *F. hodginsii* (uniform size) were collected from forest nursery (Anxi County, Fujian, China), transplanted to poly vinyl pots (height = 18 cm, top circumference = 62 cm, bottom circumference = 52 cm, d = 22 cm), and established in greenhouse in completely randomized design (four replication) for one year till the end of March, 2018. These seedlings were allowed to grow under greenhouse condition (natural light) and usual irrigation practice in order to produce healthy seedlings (Wang et al., 2014; Razaq et al., 2017). Additionally, after 15 days of establishment, all the seedlings were treated 10 g each pot with NPK fertilizer (Tarin et al., 2019). After one year; March 2018, seedlings were uprooted to determine plant fresh/dry weights and morphological parameters of roots.

Determination of physiochemical properties of soil

To determine the soil physiochemical properties, all the analysis were conducted in the Bamboo Institute, College of Forestry, Fujian Agriculture and Forestry University, China. At the start of the experiment (March, 2017), physiochemical properties of soil and all three types of BCs were assessed (Table 1). After one year, at the end of experiment (March, 2018), soil samples from 0-15 cm depth from each replicates were collected, mixed to produce composite samples and were sealed in clean, air-tight polythene bags. Four samples from each treatments were air-dried and then passed through a 2-mm/0.149-mm mesh to assess the physiochemical properties of soil. Soil pH (1:2.5 soil/water suspensions) was measured by using glass electrode meter as described by Tarin et al. (2019). Elemental analyzer (Thermo Scientific™, Waltham, MA, USA) was used for determination of soil Total N and C (Carbon) and then calculated as organic matter (OM), whereas for determination of total Phosphorus (TP) and available Phosphorus (AP) alkali fusion-Mo-Sb anti-colorimetry method as described by Sarfraz et al. (2019). Ammonium acetate solution was used for the measurement of extractable Potassium (ext. K) (Pansu and Gautheyrou, 2007).

Biomass and morphological parameters

Above ground morphological parameters like plant height and basal diameters of seedlings were measured by using the ordinary measuring tape (cm) and vernier caliper (mm), respectively. Whereas for biomass determination, seedlings were uprooted from pots with intensive care to avoid any damage to the roots, washed with deionized water and similarly, for roots morphological traits, root samples were carefully taken in each treatment, using the procedure described by Guo et al. (2008). The root samples were scanned with Expression 10000XL 1.0 scanner (dpi = 400; Epson Telford, Ltd., Telford, UK). Finally, the seedlings were oven dried to constant mass at 65°C, in order to determine dry mass. Later the images of roots were analyzed by using WinRHIZO (Pro2004b) software (Instruments Regent Co., Ville de Québec, QC, Canada) in order to determine the root surface area, root volume, total root length, and average root diameter.

Statistical analysis

Data was statistically analyzed with SPSS-19 statistical software. All the data were expressed as means and standard errors (SE) with four replications. Comparisons of different means were analyzed by Duncan's multiple range tests; α 0.05 probability level. For tables and figures Origin lab 8.5 and Microsoft excels were used.

Results

Effects of biochar amendments on physiochemical properties of soil

At the final harvest, soil nutrient dynamics under different BCs amended treatments were changed (Fig. 2). The change in soil pH after one-year under different BCs (BB, BH, and BR) incorporated samples is shown in Figure 2a.

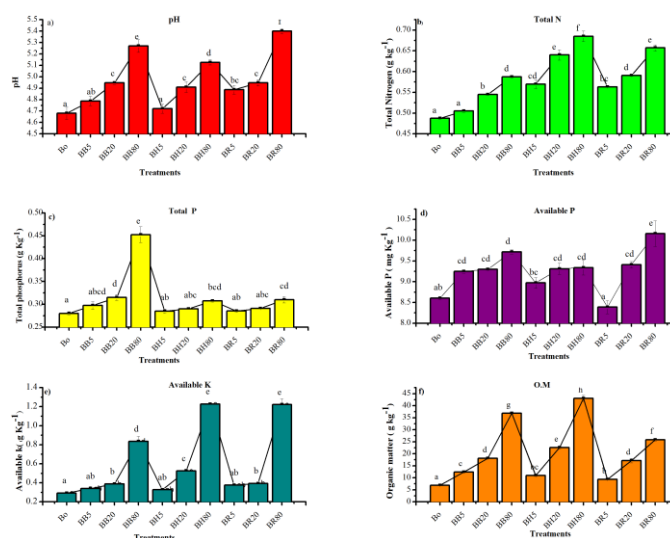


Figure 2. Comparison of soil physiochemical properties under different biochars amendments. Treatments include; control as Bo, bamboo biochar as (BB; 5, 20 and 80 representing different doses g kg⁻¹ of soil), hardwood biochar as (BH; 5, 20 and 80 representing different doses g kg⁻¹ of soil), rice straw biochar as (BR; 5, 20 and 80 representing different doses g kg⁻¹ of soil). Different letters indicate significant difference ($P \leq 0.05$) among different treatments with error bars

The liming effects of BB and BR were significant for rising soil pH ($P \leq 0.05$) as compared to BH. All BCs amended soil presented higher macronutrients (NPK) concentrations compared to Bo. Available P concentrations were significantly higher under BR80 compared to all other treatments, whereas total N concentration was highest in BH80. Overall, the concentrations of macro-nutrients increased under higher doses of BCs depending upon their type. Total N, Total P, available P, extractable K, and organic matter showed significant difference than that of Bo.

Effects of biochar amendments on biomass and morphological parameters of seedling

Application of BC significantly increased the morphological parameters of the seedlings. *Figure 3* shows the complete comparison of treatments combination for above and below ground attributes.



Figure 3. Complete view of seedlings showing the above and below ground comparison of morphological traits for all the treatments.

Treatments include; control as Bo, bamboo biochar as (BB; 5, 20 and 80 representing different doses $g\ kg^{-1}$ of soil), hardwood biochar as (BH; 5, 20 and 80 representing different doses $g\ kg^{-1}$ of soil), rice straw biochar as (BR; 5, 20 and 80 representing different doses $g\ kg^{-1}$ of soil)

Our results showed that BCs application increased the plant height, particularly seedlings treated with BB20, BH20 and BR20 were taller and showed significant difference than that of Bo (*Fig. 4a*). Moreover, within different BCs treatments, no significant difference was observed for seedlings basal diameter except BR80 amendments (*Fig. 4b*). Under different BC amendments, biomass of seedlings, treated with BB80, BH20, BH80, BR20, and BR80 were significantly increased than that of Bo (*Fig. 4c*). Likewise, for dry weight, seedlings treated with BB20, BB80, BR20, and BR80 showed significant differences compared to Bo (*Fig. 4d*).

Effects of biochar amendments on root morphological traits

Results from the current study suggested that BC amendment promoted the morphological traits of the roots. Under different BC types and levels, the roots morphological characteristics varied in terms of root volume, surface area, diameter, and length. BC application significantly ($P \leq 0.05$) increased root volume in all the BCs

treated seedlings except BH5 and BR5 as compared to Bo. For all BCs treatment combinations, highest root volume was observed in seedlings treated with BB80 and BR80 (Fig. 5a).

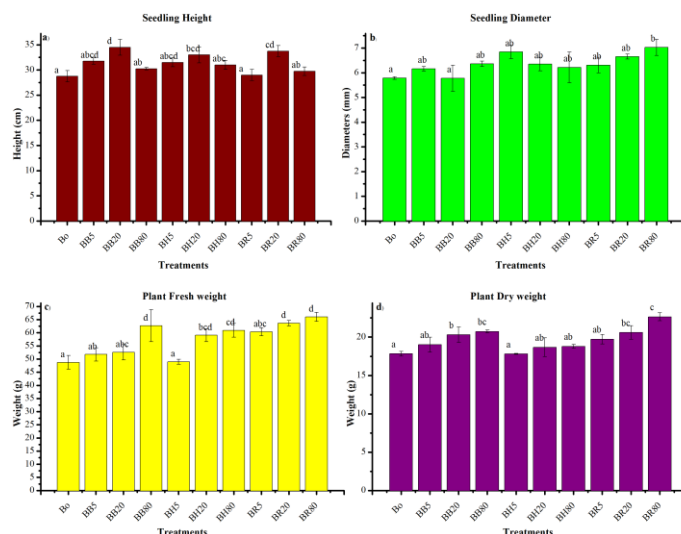


Figure 4. Comparison of above ground parameters of seedlings treated with different biochars, a) seedling height b) seedling basal diameter, c) plant fresh weight, d) dry weight. Treatments include; control as Bo, bamboo biochar as (BB; 5, 20 and 80 representing different doses $g\ kg^{-1}$ of soil), hardwood biochar as (BH; 5, 20 and 80 representing different doses $g\ kg^{-1}$ of soil), rice straw biochar as (BR; 5, 20 and 80 representing different doses $g\ kg^{-1}$ of soil). Different letters indicate significant difference ($P \leq 0.05$) among different treatments with error bars

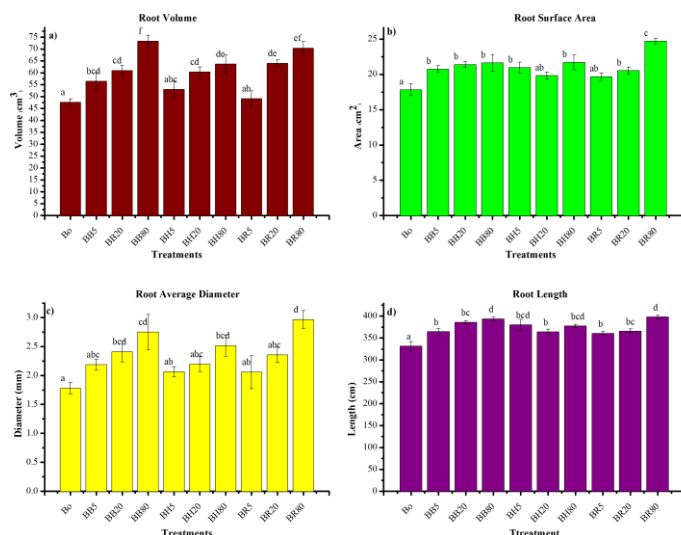


Figure 5. Comparison of root morphological features among all BC treatments; a) Root volume (cm^3), b) Root surface area (cm^2), c) Root average diameter (mm), d) Root length (cm). Treatments include; control as Bo, bamboo biochar as (BB; 5, 20 and 80 representing different doses $g\ Kg^{-1}$ of soil), hardwood biochar as (BH; 5, 20 and 80 representing different doses $g\ Kg^{-1}$ of soil), rice straw biochar as (BR; 5, 20 and 80 representing different doses $g\ Kg^{-1}$ of soil). Different letters indicate significant difference ($P \leq 0.05$) among different treatments with error bars

Root surface area of the seedlings was not much influenced within the different BC amendments, although there was significant difference among the seedlings treated with Bo, BH20 and BR5 compared to all other treatment combination with maximum value in BR80 (*Fig. 5b*). For average diameter, seedlings treated with BB5, BH5, BH20, BR5, and BR20 had no significant difference than that of Bo and under BR80 amendments, seedlings had the highest roots diameter followed by BB80 with significant difference compared to Bo (*Fig. 5c*). Similarly, BC treated seedlings significantly increased the root length compared to Bo but between different BCs treatment combinations, there was no significant difference, except BB80 and BR80 (*Fig. 5d*).

Discussion

Effects of biochars amendments on physiochemical properties of soil

Various studies have demonstrated that BC application into the soil can promote the plant growth by improving physiochemical properties of soil like essential nutrients (NPK), pH and cation exchange capacity (Lehmann et al., 2011). Therefore, the responses of root traits to BC incorporated soil depend on soil type and BC (Macdonald et al., 2014). In addition, BC often applied with fertilizer to the soil has interactively regulated the root growth of the plants (Alburquerque et al., 2015). The incorporation of all three BCs increased soil pH, however the rise in pH was evident under higher dose of BB and BR (*Fig. 2a*) because both of these two BCs have higher pH compared to BH (*Table 1*). Alkaline nature of BCs (BB and BR) to soil has resulted in an increase in pH. Our results are in line with the previous findings where incorporation of alkaline BC with higher dose raised the soil pH (Ishii and Kadoya, 1994). Moreover, the liming effect of BR was generally greater than that of other two types of BCs (BB and BH); our findings are in agreement with the previous study on acidic soil (Wang et al., 2009). Therefore, the pH of BC must be taken into account in order to control liming effects and consequently can be applied to acidic soil to raise the soil pH. In the current study the soil pH of Bo was 4.68, consequently, lower soil pH (highly acidic) always reduces the cation exchange capacity and also the bio-availability of nutrients (Verheijen et al., 2010). The rise in soil pH from 4.68 to 5.27 and 5.40 under BB80 and BR80, respectively altered soil physiochemical properties (*Fig. 2a*) which is in agreement with numerous studies (Vaccari et al., 2011; Yuan and Xu, 2011). Our results were further justified by Yuan and Xu (2011) that incorporation of BC not ameliorates soil acidity and but also improves the soil fertility. The total Nitrogen (TN) contents were highest in BH80 compared to other types of BCs (BB and BR). A potential mechanism for increased Nitrogen retention in soils mixed with BCs is the increased nitrates recycling because of higher availability of C (Verheijen et al., 2010). The increase in SOM and total N following BC application is similar to previous findings where soil C and N were increased because of application of different types of BCs (Stavi and Lal, 2013; Angst et al., 2014). Consequently, under BB80 and BR80 treatments, greater values were observed for available P compared to BH80 (*Fig. 3d*). Nevertheless, evidence suggests that BC can increase the nitrification if applied to acidic soils (Ball et al., 2010; Nelson et al., 2011). On the contrary, as described by few researchers that incorporation of BC to agricultural system, may decrease soil N just after addition, because N demand for agriculture crops has always been higher compared to forest plants (Tammeorg et al., 2012). At the start of our experiment

(March, 2017) for better effect of BCs, we had applied minimal dose (10 g each pot) of NPK fertilizer to all treatments including Bo. In the view of this, earlier BCs studies have demonstrated that application of BC combined with fertilizers have been more effective for attaining better growth (Sarfranz et al., 2017). Besides, increased N retention under rice straw BC experiment is similar to our findings (Noguera et al., 2010). In our experiment BB80 and BR80 treatments, soil pH was relatively higher than that of other treatment combinations, rise in soil pH resulting availability of P, because pH of BH under three levels (BH5, BH20, and BH80) was not much increased. Our these findings are in the line with the previous findings, because BCs particles not only holds the soil N in the form of nitrates, but also supply P to the soil and plant but their affect varies under different feedstocks (Prendergast-Miller et al., 2014). Additionally BC incorporation increased the extractable potassium (Ext. K) under BC amendments because BC is a soil conditioner which might act like a fertilizer and it contains ash which in return adds nutrients like Potassium (K), Magnesium (Mg), and Calcium (Ca) to soil which in response raises the soil pH and provide essential nutrients to soil for better growth (Agegnehu et al., 2015).

Effects of biochars amendments on morphology and biomass

Various studies have confirmed that BC addition to soil increases the seedlings and roots growth both under natural and nursery conditions (Chan et al., 2008a; Noguera et al., 2010). Growth parameters of *F. hodginsii* seedlings under BC amended soils were improved as compared to Bo (Fig. 3) which is consistent with previous findings (Ali et al., 2017; Tayyab et al., 2018). Strong positive responses to BC additions were observed in biomass, resulting increase in plant fresh weight and height of (Fig. 4a and c), which is agreement with the previous studies (Caroline et al., 2016; Seehausen et al., 2017). Because BC is a soil conditioner which might act like a fertilizer and provide nutrients to soil for plant growth (Agegnehu et al., 2015). The availability of primary nutrients to soil for plants (Sarfranz et al., 2017) with improved water holding capacity is attributed to the BC structure (Jeffery et al., 2011). Better water retention is the consequence of BC amended soil, which increases the water use efficiency of a plant, thus enhancing plant height and fresh/dry weight (Liang et al., 2006; Uzoma et al., 2011; Younis et al., 2015). In general, seedling growth parameters (seedling height, Biomass, root traits) varied only within the BC types and with the highest doses. The results obtained from the current study suggested that BC application increased the above and below ground morphological attributes (Figs. 4 and 5). Although basic soil in this study was strongly acidic (pH 4.68), but the slight increase in pH by applying alkaline BCs (BB and BR) has resulted improvement in nutrient availability (or nutrient use efficiency) with successive growth which is in the line with the findings of Robertson et al. (2012). BCs application particularly under BB and BR (20 and 80 g kg⁻¹ of soil) increased the root volume, surface area, total root length, and average root diameter compared to Bo (Fig. 5). However, under BH (5, 20, 80 g kg⁻¹) the root morphological traits had greater or no significant difference compared to Bo. Previously for conifers seedling, there is no research data to compare the BC effects on root traits but our findings are attributed to change in the physiochemical properties of soil. Under higher dose of BCs (20 and 80 g kg⁻¹ of soil) the improved roots morphological traits are consistent with previous studies (Razaq et al., 2017). Similar to our findings, Guo et al. (2008) reported that BC incorporation to acidic soil has been known to influence the root surface area, length, and volume of the seedlings in acidic red soil. In another study by Ishii and Kadoya

(1994) who demonstrated that application of BC increases the plant and root biomass. Our these results were further justified by Yang et al. (2015) who confirmed at the seedling stage, incorporation of BC improves the root morphological traits because of improved water holding capacity and soil physiochemical properties ultimately effects the root growth (Piccolo et al., 1996). Even though there are only few other comparable studies like, review by Lehmann et al. (2011) has demonstrated that BC has positive effect on root growth. BC application has significant effects on morphological traits and functioning of roots because its particles contact the roots directly for uptake of nutrients. The increased root length in our experiment (*Fig. 5d*) suggests that BC application increases the plant rhizosphere enabling plants to absorb water and nutrients freely (Prendergast-Miller et al., 2014). BC addition to soil, also enhances the microbe's community, reduces soil pathogen, and boosts plant defenses which consequently improve the seedlings health with minimal damage to the environment (Elad et al., 2010). Overall, these findings demonstrate the potential of BC application for improved seedling growth.

Conclusion

Our results confirmed that positive response of *F. hodginsii* biomass and root growth was attributed to BC amendment at seedling stage because it can serve as a direct nutrient supply through addition of soluble P and biochar-N retention (Prendergast-Miller et al., 2014). But the positive effects varied under different level and BC feedstock used. The seedlings treated with BR80, BB80, and BH80 attained 36%, 29%, and 25% biomass, respectively compared to Bo. Our these findings are in consistent with previous studies suggesting increase in biomass of woody plants summarized by a meta-analysis with an average 41% increase in biomass (Thomas and Gale, 2015). This is the first report on the effect of BC application on *F. hodginsii* morphological traits of root. Biochar application improved the physiochemical properties of soil, biomass and roots morphological attributes at seedling stage in greenhouse experiment. The current experiment was focused on the influence of different BCs application on *F. hodginsii* root traits at the early stage in a pot experiment. It is suggested that further field studies are needed to assess the complete effect of BC application on root system changes, soil microbial and nutrients dynamics.

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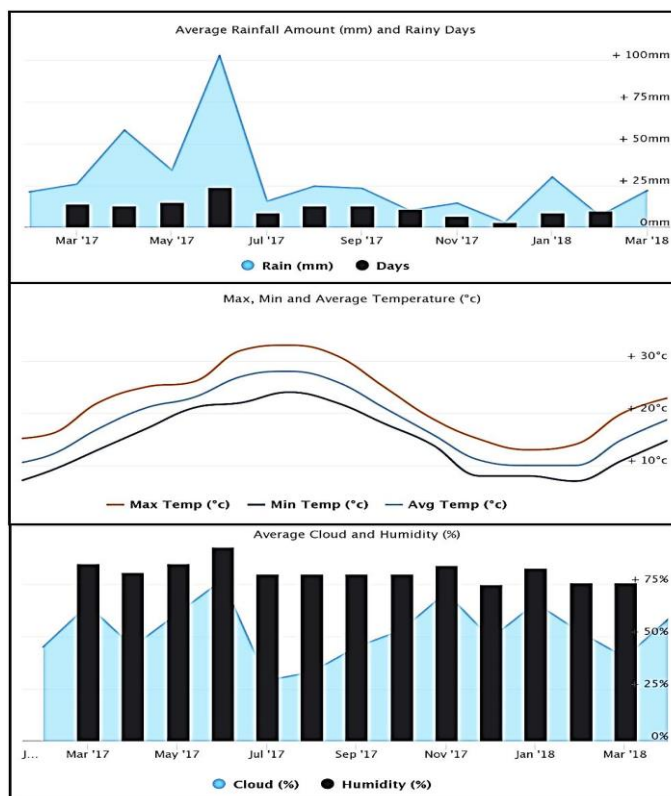
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APPENDIX



A-I. One-year metrological information of study area, during the entire experiment period
(Ref: <https://www.worldweatheronline.com>)