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Effects of Biochar on the Growth of *Vachellia etbaica* and *Faidherbia albida* Planted in Tigray, Northern Ethiopia

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Abstract

In northern Ethiopia, considerable enrichment planting is essential to enhance the regeneration of dry forests. However, planted seedlings suffer high levels of mortality in arid climates. Biochar can enhance seedling survival and growth. Therefore, this study aimed to determine the effect of biochar (derived from *Vachellia etbaica*) on the survival of *V. etbaica* and *Faidherbia albida* seedlings. We planted *V. etbaica* and *F. albida* into soil treated with 1 kg of biochar per seedling and into soil without biochar (control) using a randomized complete block design. We analyzed the soil nutrients, plant growth, and plant elements. The application of biochar did not increase the nutrient levels in the soil. However, the total dry mass of *V. etbaica* and *F. albida* increased following biochar application. Moreover, the total contents of several nutrients were higher in the leaves of biochar-treated *V. etbaica* and leaves and roots of biochar-treated *F. albida* than in the controls. These results suggest that biochar can alter the chemical properties of soil, resulting in the accelerated uptake of nutrients. In conclusion, the application of biochar enhances the uptake of nutrients, thereby enhancing the growth of *V. etbaica* and *F. albida*.

Discipline: Forestry
Additional key words: charcoal, enrichment planting, Fabaceae, forest restoration, nutrient physiology

Introduction

The Tigray region in northern Ethiopia has undergone widespread land degradation due to deforestation (Reubens et al. 2009), and thus has been targeted by the Ethiopian government as per its Climate Resilient Green Economy plan. As per this plan, the government has targeted 3 million ha of land to be afforested and reforested by 2030 (Federal Democratic Republic of Ethiopia 2011). Birhane et al. (2017) have proposed that exclosures, acting as a social fence, are an effective tool for landscape restoration in Ethiopia. However, as most of the soil in the Tigray region is severely degraded, the process of exclosure rehabilitation will be slow due to poor soil quality, seeds, and seedling banks (Birhane et al. 2017). Enrichment planting in exclosures has been frequently attempted to enhance the natural regeneration of vegetation (Hishe et al. 2020).

Vachellia etbaica (Schweinf.) Kyal. & Boatwr. is indigenous to the highland forests of the Tigray region, where it is an abundant part of the natural vegetation (Reubens et al. 2009, Mekuria et al. 2019). In contrast, *Faidherbia albida* (Del.) A. Chev. is a typical species in the farmlands of Tigray, where it is conserved as an important agroforestry species (Noulekoun et al. 2017). *V. etbaica* and *F. albida* belong to the polyphyletic genus of *Acacia s.l.* (Kyalangalilwa et al. 2013). Within this taxon, African species have been recently formalized into the genera *Senegalia* and *Vachellia* (Kyalangalilwa et al. 2013). *V. etbaica* can re-sprout, and thus is a good candidate species for recovering degraded land. Although it has been used previously for enrichment planting (Reubens et al. 2009), *V. etbaica* grown in exclosure face a crisis on the increasing of destruction by the selection felling of big trees (Mekuria et al. 2019). In contrast, *F. albida* is highly adaptable to harsh conditions and is valuable for use in land protection and reclamation (Reubens et al. 2011). However, seedlings of *F. albida* are

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rarely planted despite of lack of regeneration (Noulekoun et al. 2017, Sida et al. 2017). We expect that enrichment planting of *V. etbaica* and *F. albida* will enhance forest regeneration in northern Ethiopia. However, in this region, the rainy seasons are short and dry seasons are long (Reubens et al. 2009), and the latter will negatively affect the survival ratios of the *V. etbaica* and *F. albida* seedlings (Reubens et al. 2009, Sida et al. 2017). Therefore, new methods have to be developed to improve the survival of *V. etbaica* and *F. albida* seedlings.

Biochar is charcoal from plant material, produced for soil application. It reportedly improves the growth of seedlings (Makoto et al. 2011, Thomas & Gale 2015, Lefebvre et al. 2019, Zoghi et al. 2019, Tarin et al. 2020). Moreover, applying biochar to the seedlings can mitigate drought stress (Fujita et al. 2020) and increase seedling survival (Licht et al. 2014). Furthermore, biochar alters the physical and chemical properties and enhances microbial activity of the soil (Lehmann et al. 2011, Makoto & Koike 2021). Consequently, when applied to agricultural fields in Ethiopia, biochar enhanced the yields of crops (Abewa et al. 2014, Gebremedhin et al. 2015, Berihum et al. 2017). However, its effect on woody plant seedlings has not been studied in African forests.

Kayama et al. (2019) recently reported that although biochar application on seedlings of *Olea europaea* subsp. *cuspidata* and *Dodonaea angustifolia* in northern Ethiopia did not result in accelerated growth, it did increase the colonization of arbuscular mycorrhizal (AM) fungi and the uptake of several nutrients by the two species. *V. etbaica* and *F. albida* are Fabaceae species, and thus they can fix nitrogen (N) using rhizobia for N-uptake. In the case of *Samanea saman*, also a member of the Fabaceae, biochar application resulted in accelerated growth and N-uptake (Ghosh et al. 2015).

Improving the growth of *V. etbaica* seedlings can increase their survival rate and dominance in dryland areas such as the Tigray region (Birhane et al. 2015). Moreover, the growth of seedlings of *V. etbaica* can increase by 14% by reducing the water deficit level (Birhane et al. 2015). In the case of *F. albida*, improvements in root growth and development at the seedling stage can determine the quality of the root system throughout the tree's entire life (Gachuiri et al. 2016). Through simulations, Sida et al. (2017) predicted that increasing the survival rate of seedlings by 30% will increase tree density by 15%. Based on this, we aim to rehabilitate the land by improving the growth of seedlings of *V. etbaica* and *F. albida*. We hypothesized that the application of biochar will enhance the uptake of nutrients by reinforcing the symbioses between plants, AM fungi, and rhizobia. Thus, biochar application will increase the survival rate and growth of *V. etbaica* and *F. albida* seedlings.

We tested our hypothesis in a field experiment with *V. etbaica* and *F. albida* seedlings grown in biochar-containing soil. After applying biochar, we examined the nutrients in the soil and the ecophysiological traits of the two species. In particular, we measured (1) the biomass of the seedlings and (2) the concentrations of elements in plant organs. These parameters were compared with seedlings planted in untreated soil. This study contributes to the establishment of new enrichment planting methods for landscape restoration in the Tigray region.

Materials and methods

1. Study site and land preparation

In July 2017, we prepared an experimental field measuring 24 m × 22 m on a farmland belonging to Mekelle University (13°48′N, 39°49′E, 2,210 m asl, Fig. 1). The site is located on a hill in Mekelle city, Tigray region. The experimental field was on a gentle, west-facing slope, at an inclination of 0.8%. The annual mean, maximum, and minimum temperatures in Mekelle city are 21°C, 27°C, and 15°C, respectively (weather data from 1988 to 2018; Weatherbase 2018). The mean annual precipitation of 705 mm y^{-1} is largely concentrated in July and August (Weatherbase 2018).

The experimental field was divided into six equally sized blocks (11 m × 6 m), with a 2 m distance between each block (Fig. 1). Two blocks were treated with biochar, whereas two other blocks were left untreated (control). Biochar and control blocks were randomly selected from the six blocks. The remaining two blocks were considered sub-blocks and were used for the cultivation of other woody species. At the Mekelle University nursery, we prepared 50 five-month-old seedlings of each tree species, i.e., *V. etbaica* and *F. albida*. The biochar, obtained from a market in Mekelle city, was produced from *V. etbaica*, a typical biochar material in the region (Gebrekidan et al. 2015). In general, biochar in Ethiopia is produced by slow pyrolysis, with a maximum kiln temperature of 400°C (Seitz et al. 2017). The same type of biochar produced positive effects for the seedlings of *Olea europaea* subsp. *cuspidata* and *Dodonaea angustifolia* (Kayama et al. 2019). The biomass of both of these tree species increased after applying biochar produced at 400°C (Makoto et al. 2011). Thus, we employed *V. etbaica* biochar for this experiment. The density, porosity, and pH of the biochar were 0.46 g cm^{-3}, 67.3%, and 8.9, respectively. Before use, the biochar was crushed into particles of < 1 cm in diameter. The mass-based particle size distribution of biochar was 38%, 50%,

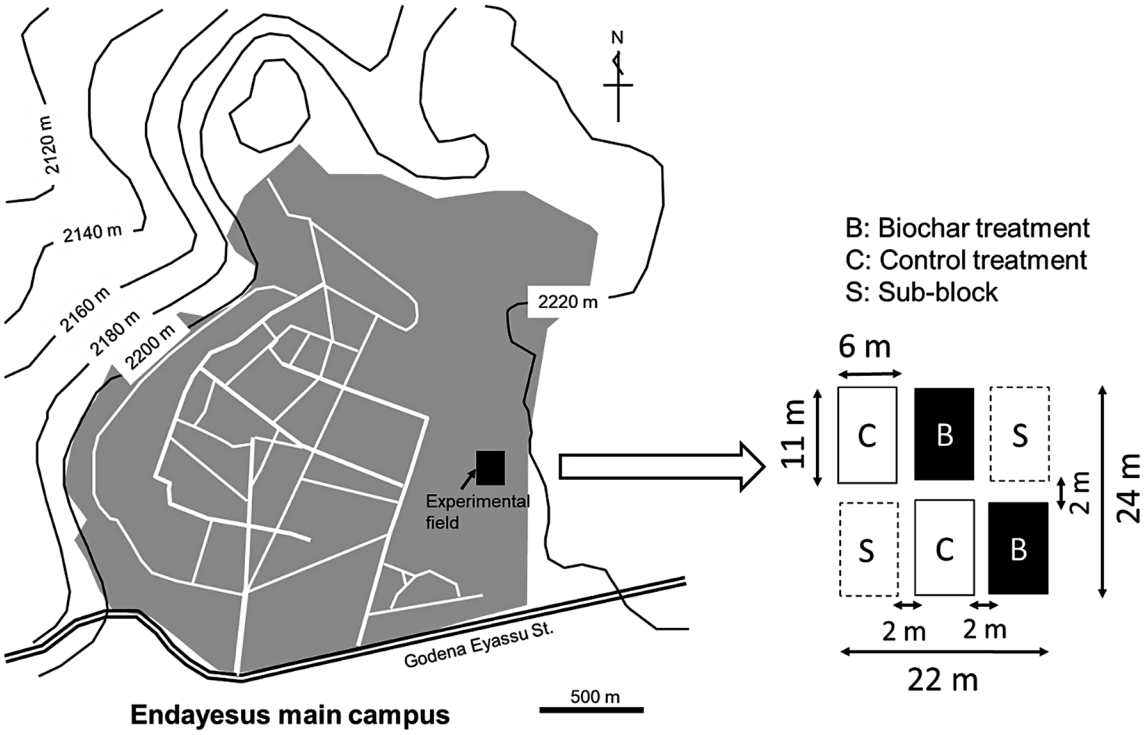

Fig. 1. Topographic map of Endayesus main campus of Mekelle University, Tigray region (gray area) and experimental field design
This map is modified from 20th International Conference of Ethiopian Studies (2017) and Map Tiler Team (2020).

and 12% for particle size ranges of 2.0-10, 0.02-2.0, and < 0.02 mm, respectively.

In July 2017, we planted ten seedlings of each species at 1 m × 1 m interval for each block. Total 40 holes were dug for planting. The remaining 10 seedlings of *V. etbaica* and *F. albida* were sampled at this time. Each hole measured 30 cm × 30 cm and was 30 cm deep. The soil that was dug up was collected and mixed thoroughly (using a hoe) with 1 kg of biochar for the biochar treatment. In total, 7.6 Mg ha^{-1} of biochar was applied for each hole, and the ratio of biochar to dry mass was approximately 4%. This ratio was determined during a previous pot test that showed the addition 4% biochar was effective in accelerating the growth of woody species (Kayama et al. 2016). Seedlings were planted into the holes of each block and were covered according to the treatment. All of the steps performed in the biochar treatment were implemented for the control treatment, except for biochar. After planting, each block was periodically weeded by hand.

Randomly selected unplanted seedlings of *V. etbaica* and *F. albida* were sampled for 2 g of fine roots (diameter, < 2.0 mm). Roots were fixed in a formaldehyde-acetic acid-alcohol (FAA) solution and then stored at 4°C until subsequent analysis for AM fungi. Moreover, we observed the nodules of rhizobia from the roots of *V. etbaica* and *F. albida*. The leaves, stems, and roots of the tree seedlings were oven-dried at 70°C for 72 h, and then their dry weights were measured.

2. Soil analysis

After biochar application (July 2017), we collected surface (0 cm-5 cm) soil samples from four seedlings located at the four corners of each replicate block. The soil samples were collected at a distance of 10 cm from the stem of the seedling. We assessed the soil texture and chemical properties of these samples. Before drying, the soil pH was determined via the glass electrode method using a 1:2.5 mass ratio of soil and water. Then, the soil samples were air-dried and sieved (mesh size, 2.0 mm) before further measurements. Soil texture was analyzed using the hydrometer method, which measures the amounts of sand (0.02 mm-2.0 mm), silt (0.002 mm-0.02 mm), and clay (< 0.002 mm) (Klute 1986). Soil chemical properties were analyzed using the methods of Sparks et al. (1996). The organic carbon (OC) concentration was analyzed using the loss-on-ignition method, and the total N concentration was determined using the Kjeldahl method. The amount of exchangeable phosphate was determined from soil extracts generated by a sodium

bicarbonate solution adjusted to pH 8.5. Soil extracts were analyzed using spectrophotometric methods. The exchangeable cations and cation exchange capacity (CEC) of the soil were determined via the ammonium acetate method using ammonia solution buffered at pH 7. The exchangeable cations—calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na)—were determined from the leachate using an atomic absorption spectrophotometer.

3. Analysis of growth, biomass, soil-plant analysis development (SPAD), and AM fungi

At the initial (time 0) and the 2nd, 5th, and 14th months after planting, we measured the survival rate, height, and diameter at the ground level of each plant. After we completed all measurements (end of September 2018), we harvested 10 seedlings of *V. etbaica* and eight seedlings of *F. albida* from each treatment. We collected 2 g of fine roots (< 2.0 mm diameter) from five seedlings of each species within each treatment, fixed them in FAA, and then stored them at 4°C until subsequent microscopic examination for levels of AM colonization. Root samples were cleared by boiling in 1 M KOH for 1 h. Clarified root samples were stained by soaking in 0.05% trypan blue in lactic acid and glycerol (1:1) and boiling for 15 min. After staining, samples were de-stained using lactic acid and glycerol (1:1). Next, the root samples were placed in a Petri dish placed on top of a ruled grid divided into 5 mm × 5 mm squares. We counted the intersections between grid lines and roots under a stereomicroscope at 7-45× magnification. Considering only intersections that numbered > 200, we calculated the proportion of AM fungi colonization of the roots using the gridline intersect method described by Giovannetti & Mosse (1980). Moreover, we observed the nodules of rhizobia on the roots of *V. etbaica* and *F. albida*.

We measured the SPAD value, which is an index of relative chlorophyll content in the leaves of the seedlings using SPAD-502 (Konica Minolta Co., Tokyo, Japan). After SPAD measurements, the roots of harvested seedlings were washed twice with tap water and then oven-dried at 70°C for 48 h. The plants were divided into leaf, stem, and root components, and each component's dry mass was measured. Using the data from July 2017 and September 2018, we calculated the relative growth rate (RGR; Hoffmann and Poorter 2002) using the following equation:

$$\mathrm{RGR} = (\ln M_2 - \ln M_1) / (t_2 - t_1)$$

where $\ln M_1$ and $\ln M_2$ are the natural logarithm-transformed total dry mass at times t_1 and t_2, respectively.

4. Analysis of element concentrations in plant organs

The concentrations of N, P, K, Ca, and Mg in the leaves and roots of seedlings were measured by first grinding the dried plant organs to a fine powder using a sample mill. Then, the pulverized samples were digested with sulfuric acid and hydrogen peroxide in a Digesdahl digestion apparatus (Hach Company, Loveland, OH, USA). The levels of all the elements mentioned above were measured spectrophotometrically using the following methods: N concentrations were determined using the indophenol method (Weatherburn 1967); P concentrations were determined using the molybdenum blue method (Sparks et al. 1996); and K, Ca, and Mg concentrations were determined using the tetraphenylboron, o-cresolphthalein complexone, and xylidil blue methods, respectively (Nishiguchi et al. 2007). The total amounts of the elements accumulated in each plant organ were calculated by multiplying the dry mass of leaves or roots with the concentrations of elements in the respective organs.

5. Statistical analysis

All parameters were statistically analyzed using Stat View 5.0 (SAS Institute Inc.). Differences between parameter means were evaluated by analysis of variance, and the parameters were compared between the biochar and control treatments of *A. etbaica* and *F. albida*. Moreover, we analyzed the correlations between RGR values and the contents and concentrations of the measured elements (see Table 4). The primary elements affecting the RGR were determined using stepwise multiple regression analysis.

Results

1. Soil properties

The pH of the soil in both treatments was > 7 (Table 1). The sand and clay contents of the biochar treatment were significantly higher and lower, respectively, than the control treatment ($P < 0.05$). In contrast, the pH, CEC, and levels of all the elements in the two treatments did not differ significantly.

2. Survival and growth characteristics

All of the *V. etbaica* seedlings survived in 2017. However, in 2018, 10 seedlings (25% of the total) were grazed on by animals during the dry season. In September 2018, the survival rates for the two blocks of *V. etbaica* seedlings in the biochar treatment were 30% and 90%, respectively. For the control treatment of *V. etbaica*, the survival rate in both the blocks was 90%.

Two *F. albida* seedlings in the control treatment

Table 1. Texture and chemical properties of the soils treated with and without biochar (mean ± SE, n = 8)

Treatment	Texture (%)			pH
	Sand	Silt	Clay	
Biochar	58.3 ± 1.1	27.0 ± 1.1	14.7 ± 1.0	07.8 ± 0.2
Control	53.5 ± 1.2	27.0 ± 0.7	19.5 ± 1.2	07.4 ± 0.1
Statistical test	*	n.s.	**	n.s.
	CEC (cmol kg^{-1})	OC (g kg^{-1})	N (g kg^{-1})	P (mg kg^{-1})
Biochar	17.7 ± 1.2	11.6 ± 0.8	1.41 ± 0.19	6.05 ± 1.21
Control	15.8 ± 1.8	13.1 ± 1.7	1.86 ± 0.21	3.91 ± 0.84
Statistical test	n.s.	n.s.	n.s.	n.s.
	Ca (mg kg^{-1})	Mg (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})
Biochar	37.0 ± 6.2	7.65 ± 1.74	5.13 ± 0.18	1.55 ± 0.33
Control	27.2 ± 4.6	9.24 ± 1.23	5.38 ± 0.62	1.93 ± 0.41
Statistical test	n.s.	n.s.	n.s.	n.s.

The mean values of each parameter were analyzed using analysis of variance. ** $P < 0.01$; n.s., not significant

Abbreviations: CEC, cation exchange capacity; OC, organic carbon; N, nitrogen; P, phosphorus; Ca, calcium; Mg, magnesium; K, potassium; Na, sodium

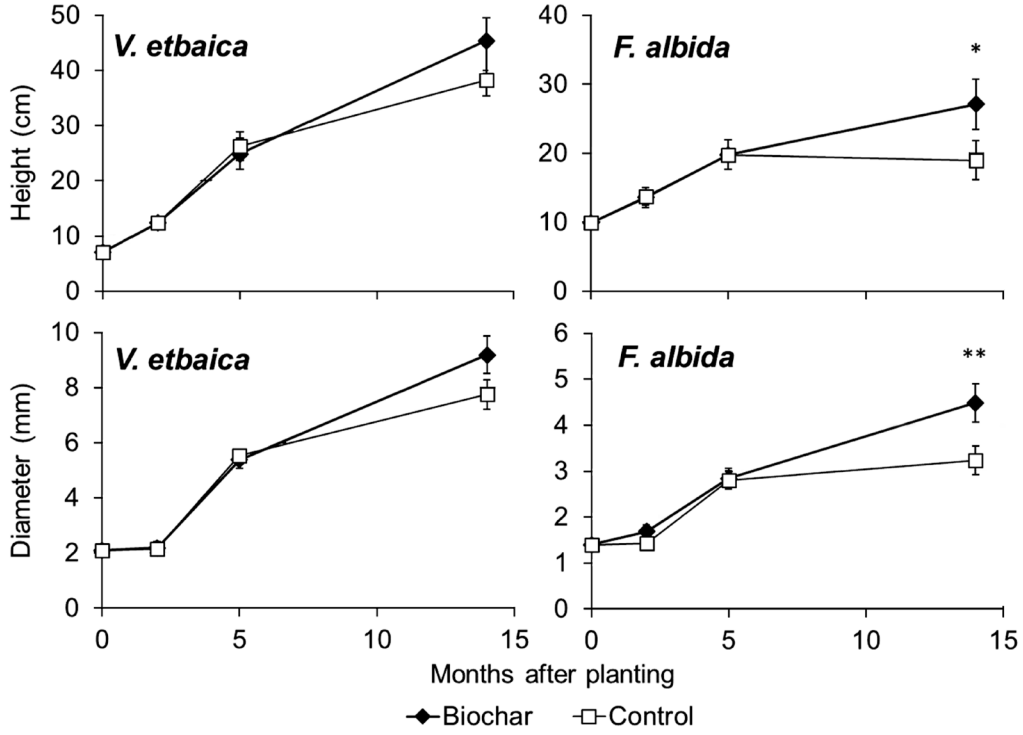

Fig. 2. Tree height and basal diameter of *V. etbaica* and *F. albida* (n = 20) treated with biochar (mean ± SE)

The mean values of each parameter were analyzed using analysis of variance.

* and ** indicate $P < 0.05$ and $P < 0.01$, respectively.

died in 2017. In 2018, seven and nine seedlings died in the biochar and control treatments, respectively. In September 2018, the survival rates of the two blocks of *F. albida* seedlings in the biochar treatment were 60% and 70%, respectively. In the control treatment, the survival rates of the two blocks were 30% and 60%, respectively.

Fourteen months after planting, the average height and root collar diameter measurements of the biochar-

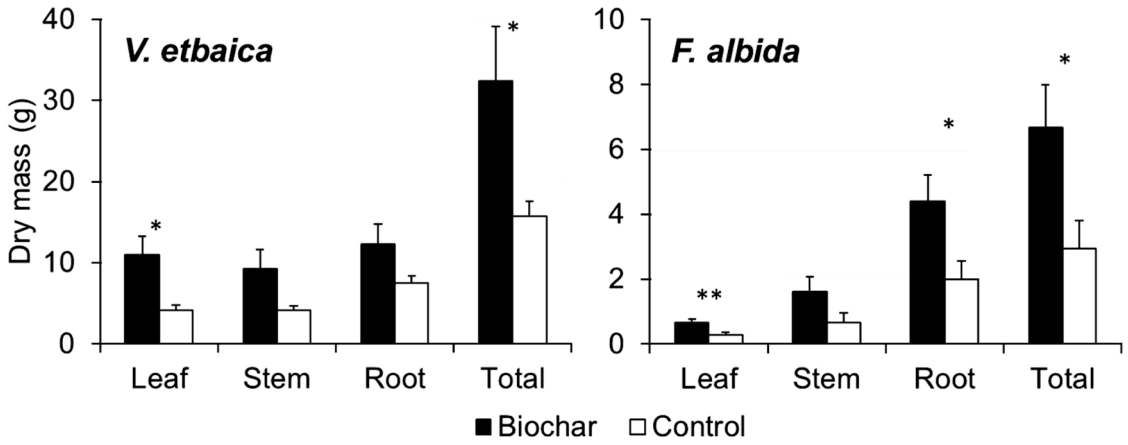

Fig. 3. Dry mass of each organ and total dry mass of *V. etbaica* (n = 10) and *F. albida* (n = 8) grown with and without biochar

The mean values of each parameter were analyzed using analysis of variance.
* and ** indicate $P < 0.05$ and $P < 0.01$, respectively.

treated *F. albida* were significantly higher than those of the control ($P < 0.05$, Fig. 2). In contrast, in the control treatment, the tops of some *F. albida* seedlings dried up in 2018, resulting in a lower average height 14 months after planting. In *V. etbaica*, plant height and basal diameter measurements did not differ significantly between the biochar and control treatments.

At the beginning of the experiment, the averages of leaf, stem, and root dry mass of *V. etbaica* were 0.19 g, 0.09 g, and 0.2 g, respectively, whereas those of *F. albida* were 0.06 g, 0.06 g, and 0.11 g, respectively. Upon analyzing 10 sampled seedlings from each species, we observed nodules of rhizobia on eight and three seedlings of *V. etbaica* and *F. albida*, respectively. The average numbers of nodules with rhizobia in *V. etbaica* and *F. albida* were 3.9 and 2.3, respectively. Moreover, 96% of the roots were colonized by AM fungi in *V. etbaica* and *F. albida.*

After 14 months, the total dry mass of the seedlings of *V. etbaica* and *F. albida* increased significantly (Fig. 3, $P < 0.05$). In *V. etbaica*, compared with the control treatment, biochar treatment resulted in significantly higher leaf dry mass ($P < 0.05$), whereas in *F. albida*, compared with the control treatment, biochar treatment increased the dry mass of leaves and roots ($P < 0.05$). At this point, we did not determine the levels of rhizobia nodulation. However, 100% of the roots of both species were colonized by AM. Furthermore, the levels of AM colonization in both species did not differ between the biochar and control treatments. The RGR values for the biochar and control treatments of *V. etbaica* were 3.46 and 2.95 g y^{-1}, respectively, whereas those for *F. albida* were 2.80 and 2.02, respectively. The RGR values of the biochar-treated *V. etbaica* and *F. albida* were significantly larger than those of the controls ($P < 0.05$).

3. Concentrations of elements in plant organs

The concentrations of N and K in the leaves of biochar-treated *F. albida* were significantly higher than those in the controls (Table 2, $P < 0.05$). For biochar-treated *F. albida*, the concentration of K in the roots was also significantly higher than that in the control ($P < 0.05$). In contrast, biochar treatment in *V. etbaica* did not result in significant differences in the concentrations of elements. However, the leaf SPAD values for the biochar treatments of both species were significantly higher than those for the control treatments ($P < 0.01$).

The total contents of N, P, Ca, and Mg in the leaves of biochar-treated *V. etbaica* were significantly higher than those in the control treatment (Table 3, $P < 0.05$). The content of Ca in the roots of biochar-treated *V. etbaica* was also significantly higher than that in the control treatment ($P < 0.05$). For *F. albida*, the contents of N, P, K, and Ca in the leaves and roots of the biochar treatment were significantly higher than those in the control treatment ($P < 0.05$).

Regression analysis of the concentrations or contents of elements in the leaves and roots of *V. etbaica* and RGR (Table 4) found no significant relationship. In contrast, the concentrations of P in the leaves and roots of *F. albida* significantly negatively correlated to the RGR ($P < 0.05$). The total contents of each element in the leaves and roots of both species, except for K in the roots of *V. etbaica*, were significantly positively correlated with RGR ($P < 0.05$). For *V. etbaica*, Ca in the leaves was most strongly correlated to RGR, whereas for *F. albida*, the N content of the roots was most strongly correlated to RGR.

The total contents of each element in the leaves and roots of both species were highly correlated to RGR

Table 2. Concentrations of elements (N, P, K, Ca, and Mg) in the leaves and roots and leaf SPAD values for *V. etbaica* (n = 10) and *F. albida* (n = 8) grown with and without biochar

Treatment		*V. etbaica*		*F. albida*	
		Leaf	Root	Leaf	Root
N	Biochar	1,533 ± 100	712 ± 54	3,014 ± 64	1,175 ± 67
(μmol g^{-1})	Control	1,679 ± 82	18 ± 51	2,677 ± 118	1,168 ± 81
	Statistical test	n.s.	n.s.	*	n.s.
P	Biochar	303 ± 67	1,456 ± 115	353 ± 11	1,685 ± 79
(μmol g^{-1})	Control	345 ± 9	1,540 ± 117	578 ± 105	1,808 ± 166
	Statistical test	n.s.	n.s.	n.s.	n.s.
K	Biochar	631 ± 103	147 ± 37	2,404 ± 670	920 ± 148
(μmol g^{-1})	Control	580 ± 110	234 ± 26	908 ± 155	539 ± 84
	Statistical test	n.s.	n.s.	*	*
Ca	Biochar	759 ± 10	407 ± 29	762 ± 21	338 ± 28
(μmol g^{-1})	Control	791 ± 17	390 ± 12	877 ± 225	336 ± 21
	Statistical test	n.s.	n.s.	n.s.	n.s.
Mg	Biochar	199 ± 23	207 ± 43	158 ± 30	171 ± 32
(μmol g^{-1})	Control	198 ± 20	136 ± 29	305 ± 96	161 ± 33
	Statistical test	n.s.	n.s.	n.s.	n.s.
SPAD	Biochar	39.2 ± 0.9		35.0 ± 1.0	
	Control	35.1 ± 0.9		28.6 ± 1.9	
	Statistical test	**		**	

The mean values of each parameter were analyzed using analysis of variance.
*$P < 0.05$; **$P < 0.01$; n.s., not significant
Abbreviations: N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium

Table 3. Total contents of elements (N, P, K, Ca, and Mg) in the leaves and roots of *V. etbaica* (n = 10) and *F. albida* (n = 8) grown with and without biochar

Treatment		*V. etbaica*		*F. albida*	
		Leaf	Root	Leaf	Root
N	Biochar	17.5 ± 4.5	8.57 ± 1.56	2.01 ± 0.28	5.37 ± 0.77
(mmol $plant^{-1}$)	Control	7.4 ± 1.4	5.89 ± 0.55	0.77 ± 0.23	2.21 ± 0.47
	Statistical test	*	n.s.	**	**
P	Biochar	3.38 ± 0.77	18.9 ± 4.1	0.234 ± 0.033	8.00 ± 1.57
(mmol $plant^{-1}$)	Control	1.45 ± 0.24	11.3 ± 1.4	0.116 ± 0.024	3.12 ± 0.51
	Statistical test	*	n.s.	*	*
K	Biochar	8.17 ± 2.92	1.99 ± 0.81	1.65 ± 0.46	4.02 ± 0.72
(mmol $plant^{-1}$)	Control	2.60 ± 0.66	1.63 ± 0.20	0.23 ± 0.06	0.95 ± 0.20
	Statistical test	n.s.	n.s.	**	**
Ca	Biochar	8.19 ± 1.60	4.92 ± 0.88	0.504 ± 0.068	1.56 ± 0.26
(mmol $plant^{-1}$)	Control	3.27 ± 0.51	2.91 ± 0.33	0.150 ± 0.025	0.62 ± 0.14
	Statistical test	**	*	***	**
Mg	Biochar	2.12 ± 0.56	2.28 ± 0.62	0.111 ± 0.033	0.740 ± 0.163
(mmol $plant^{-1}$)	Control	0.78 ± 0.12	0.99 ± 0.21	0.055 ± 0.016	0.325 ± 0.107
	Statistical test	*	n.s.	n.s.	n.s.

The means of each parameter were analyzed using analysis of variance.
*$P < 0.05$; **$P < 0.01$; ***, $P < 0.001$; n.s.; not significant
Abbreviations: N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium

(Table 4). Using stepwise multiple regression analysis, we identified, Ca in the leaves ($F = 81.9$) and K in the roots ($F = 16.5$) of *V. etbaica* as the most effective elements for modeling RGR. The equation for RGR in *V. etbaica* is given as follows:

$$RGR=2.48+1.00\times10^{-4}Ca_{(leaf)}+8.30\times10^{-5}K_{(root)}, R^2=0.91***$$

Regarding *F. albida*, N in the roots ($F = 42.6$) and P in the leaves ($F = 19.0$) modeled RGR by the following equation:

Table 4. Regression coefficients of relationships between the relative growth rate (dependent variable) and concentrations or contents of elements in the leaves or roots (independent variable) of *V. etbaica* (n = 20) and *F. albida* (n = 16)

Elements	Leaves		Roots	
	r	*F*	*r*	*F*
		V. etbaica		
Concentration				
N	0.125	0.29	−0.329	2.19
P	−0.086	0.13	0.058	0.06
K	0.404	3.51	−0.293	1.69
Ca	−0.394	3.31	0.026	0.01
Mg	−0.244	1.14	0.061	0.07
Content				
N	0.867	54.5***	0.827	39.1***
P	0.885	65.0***	0.845	44.9***
K	0.826	38.6***	0.378	3.00
Ca	0.905	81.9***	0.899	76.0***
Mg	0.747	22.7***	0.590	9.61**
		F. albida		
Concentration				
N	0.410	2.83	−0.210	0.65
P	−0.600	7.87*	−0.548	6.01*
K	0.416	2.93	0.193	0.54
Ca	−0.369	2.20	−0.371	2.23
Mg	−0.482	4.25	0.046	0.03
Content				
N	0.848	35.8***	0.868	42.6***
P	0.852	37.0***	0.825	29.8***
K	0.654	10.5**	0.588	7.38*
Ca	0.755	18.6***	0.796	24.2***
Mg	0.583	7.22*	0.694	13.0**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$
Abbreviations: N, nitrogen; P, phosphorus; K, potassium; Ca, calcium, Mg, magnesium

$$RGR=1.28+1.48\times10^{-4}N_{(root)}+3.00\times10^{-3}P_{(leaf)},R^2=0.90***$$

Discussion

The application of biochar can alter the physical and chemical properties of soils (Glaser et al. 2002). Considering its physical properties, biochar can decrease soil bulk density and increase its water retention (Glaser et al. 2002). The density of biochar used for our experiment was 0.46 g cm^{-3}, a low value compared with that used for experiments involving other species (0.47 g-0.85 g cm^{-3}; Brewer et al. 2014, Suliman et al. 2017). The soil bulk density in Mekelle ranges from 1.10-1.59 g cm^{-3} (National Soil Research Center 2005). At a 4% application rate, biochar decreased bulk density by 3% in our experiment. However, to accelerate the growth of seedlings, bulk density should decrease to 8% (Zoghi et al. 2019). Thus, the 3% decrease in soil bulk density by the application of biochar is not sufficient to accelerate the growth of the seedlings of *V. etbaica* and *F. albida*. The application rate of 4% biochar increased water retention and root growth in sandy soil (Kayama et al. 2016). However, the soil in our experimental plots was sandy loam, based on USDA criteria (Klute 1986). For loamy soil, biochar produces no change in water retention (Glaser et al. 2002). In a previous experiment, we applied biochar to the soil of the Kilte Awlaero district, 25 km north of Mekelle University, to estimate water retention due to biochar (Kayama et al. 2019). The application of 4% biochar did not increase the moisture content of clay loam soil (Kayama et al. 2019). Therefore, we do not expect that biochar application in our experiment altered the physical properties of soil to the degree that affected the growth of *V. etbaica* and *F. albida*.

Biochar can alter soil chemical properties by increasing the retention of plant-available nutrients and decreasing the leaching of nutrients (Glaser et al. 2002). This capacity stems from the high porosity of biochar. In

our experiment, the porosity value of 67.3% compares well with those of other biochars made from wood (55%-81%; Brewer et al. 2014, Suliman et al. 2017). The application of 4% *V. etbaica* biochar resulted in slight increases in pH, CEC, and concentrations of P and Ca. However, these increased values were not significantly different from those of the control treatment (Table 1). However, in the leaves of *V. etbaica* and in the leaves and roots of *F. albida*, the application of biochar resulted in nutrient accumulations (Table 3). We did not specifically measure the retention and leaching of nutrients. However, we suggest that biochar-induced accumulation of nutrients may result from effective nutrient uptake due to improved chemical properties of the soil. Therefore, our results suggest that the application of biochar is important because it alters soil chemical properties.

The benefits of biochar are reflected by the higher survival rate of seedlings of biochar-treated *F. albida* (65%) than that of the controls (45%). In Ethiopia, the mortality of seedlings of *F. albida* is generally high during the long dry season, resulting in a survival rate of 48% (Sida et al. 2017). This value is similar to that observed for the controls in our experiment. These findings are in line with those of previous research, highlighting that the application of biochar can increase seedling survival (Licht et al. 2014). Therefore, the application of biochar to seedlings of *F. albida* is linked to a decrease in plant mortality. For *V. etbaica*, the results were confounded by the disappearance of seedlings, especially in the biochar treatment. We confirmed the invasion of the Abyssinian hare (*Lepus habessinicus* Hemprich & Ehrenberg) within our experimental plots. Previously, Kayama et al. (2019) reported evidence of scars due to grazing by hares in their experiment site. The scars were characterized by chisel-like 45° cuts on stems of seedlings (e.g., Bird et al. 2012).

Moreover, hares tend to graze on barks, twigs, and Acacia roots during the dry season (Dawson & Ellis 1994). Evidence of grazing on roots and digging was confirmed previously by Kayama et al. (2019). Therefore, the probability that entire seedlings of *V. etbaica* were grazed on by hare, leaving nothing but holes in the ground, is high.

The application of biochar accelerated the growth rates in height and basal diameter in *F. albida* (Fig. 2). At the end of the experiment, the total biomass of biochar-treated *V. etbaica* and *F. albida* was significantly higher than that of the controls (Fig. 3). Moreover, biochar-treated *V. etbaica* and *F. albida* had higher RGR values than the controls. Thus, biochar application accelerated the growth of *V. etbaica* and *F. albida*. Thomas & Gale (2015) reported that many woody species undergo accelerated growth in response to biochar application. However, most of these previous experiments were conducted in pots. Under field conditions, biochar accelerated the growth of woody species over 4 y (Sovu et al. 2011, Omil et al. 2013, Eyles et al. 2015, de Farias et al. 2016). However, within 1 or 2 y, the effects of biochar are not evident under field conditions (Sovu et al. 2011, Eyles et al. 2015, de Farias et al. 2016, Krapfl et al. 2016), except for fast-growing species in humid tropical regions (Lefebvre et al. 2019). In our experiment, the early growth of both species was verified, and we found evidence of growth acceleration in both species within 14 months. Previous work suggests that the effects of biochar differ depending on the production temperature, and biochar produced at 400°C generates higher growth rates in *Larix gmelinii* than biochar produced at 800°C (Makoto et al. 2011). The *V. etbaica* biochar was produced at a low temperature; thus, it may be suitable for enhancing the growth of *V. etbaica* and *F. albida*.

Moreover, the SPAD values of biochar-treated *V. etbaica* and *F. albida* were higher than those of the controls (Table 2). This is in line with the findings of Kayama et al. (2019), who reported an increased SPAD value in *Olea europaea* subsp. *cuspidate*, resulting from the application of biochar. Because SPAD value is an indicator of chlorophyll concentration, it is positively correlated with the photosynthetic rate (Enríquez et al. 1996). Several researchers have reported increases in chlorophyll contents and photosynthetic rates after applying biochar (Zoghi et al. 2019, Tarin et al. 2020). Thus, biochar probably increases the photosynthetic rates of both species, resulting in increased productivity.

In *F. albida*, biochar treatment increased the concentrations of K in the leaves and roots and the concentration of N in the leaves (Table 2). *F. albida* may have the capacity to respond to the biochar-induced changes in the chemical properties of soil by accelerating the uptake rates of these nutrients. Experimental evidence suggests that the application of biochar can increase the uptake of nutrients (Lehmann et al. 2003, Kayama et al. 2019, Zoghi et al. 2019). Moreover, biochar alters the activities of microorganisms such as mycorrhizal fungi and rhizobia (Lehmann et al. 2011). For example, the application of biochar increases the root colonization of AM fungi, resulting in accelerated uptake of nutrients (Kayama et al. 2019). In our experiment, all the roots in both treatments were colonized by AM fungi. Therefore, we cannot conclude that biochar application accelerates the uptake of nutrients by increasing mycorrhizal colonization. We did not confirm nodulation by rhizobia at the end of the experiment. However, the rhizobia-induced nodulation of *Vachellia* seedlings has been

confirmed using a soil suspension collected near non-nodulating roots of *F. albida* (Boukhatem et al. 2016). Therefore, rhizobia may have established nodules on the roots of *F. albida* in our experiment. The addition of biochar is known to enhance biological N_2 fixation by rhizobia (Rondon et al. 2007, Lehmann et al. 2011). Therefore, the increased uptake of nitrogen in biochar-treated *F. albida* may be due to the enhanced N_2 fixation by rhizobia.

The levels of several nutrients positively correlated with RGR (Table 4). Therefore, higher levels of uptake and accumulation of nutrients are associated with the accelerated growth of *V. etbaica* and *F. albida*. Using stepwise multiple regression analysis, we identified Ca in the leaves as the most effective element for accelerating the growth of *V. etbaica* treated with biochar. The total contents of Ca in the leaves and roots of *V. etbaica* were also significantly higher in the biochar treatment (Table 3). These results suggest that the uptake of Ca is important for the growth of *V. etbaica*. In general, the application of biochar improves nutrient availability and increases the uptake of Ca (Lehmann et al. 2003, Kayama et al. 2019). Information on the characteristics of nutrient uptake in *V. etbaica* is insufficient. However, several *Acacia* species actively absorb Ca, resulting in the formation of crystals of calcium oxalate in cells (He et al. 2012). Calcium oxalate plays a role in many processes such as regulation, plant protection, and detoxification (Franceschi & Nakata 2005). Therefore, one positive effect of the application of biochar was the increase of the Ca uptake of *V. etbaica*, which grew faster as a result of biochar application. In the case of *F. ablida*, the most effective nutrient affecting growth acceleration was N content in the roots. Moreover, the total N content in the leaves was significantly higher for the biochar treatment (Table 3). The total N content of the roots of *F. albida* is an essential source of soil N fertility and it positively correlated to total dry mass (Gueye et al. 1997). Thus, increasing N in the roots of *F. albida* through the application of biochar is important for growth acceleration.

Kayama et al. (2019) applied biochar to *Olea europaea* subsp. *cuspidata* and *Dodonaea angustifolia* during the same period as our experiment at a site located in Kilte Awlaero. However, in that experiment, biochar did not accelerate the growth of the two species. The level of precipitation at Kilte Awlaero (Wukro town) during the experimental period was very similar to that at Mekelle city (National Meteorological Agency 2018). However, Kayama et al. (2019) reported higher concentrations of N, K, and Ca in soil than those for this experiment. In Ethiopia, soil fertility is negatively affected during drought conditions. For example, *Senegalia senegal* undergoes marked decreases in stem water potential when grown using a high fertility substrate (Merine et al. 2015). Thus, compared with our experiment, drought stress during the dry season could have had more substantial effects on the experiment conducted at Kilte Awlaero (Kayama et al. 2019). Tree growth tends to be suppressed due to water deficits, even with biochar (Zoghi et al. 2019). Thus, biochar application failed to accelerate the growth of *Olea europaea* subsp. *cuspidata* and *Dodonaea angustifolia* because of a severe water deficit during the dry season.

Conclusion

Our experiment has clarified the growth acceleration effects of application 4% biochar on *V. etbaica* and *F. albida*. Biochar application increases the levels of certain nutrients, and these nutrient levels are positively correlated with RGR. However, we were unable to confirm whether biochar application reinforces the symbioses between plants and microorganisms. In conclusion, the application of biochar alters soil chemical properties and enhances the uptake of nutrients, resulting in increased growth of *V. etbaica* and *F. albida*.

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