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ENHANCING GROWTH, SOIL CHEMISTRY, AND NUTRIENT UPTAKE IN *SONNERATIA CASEOLARIS* SEEDLINGS UNDER SALINITY STRESS THROUGH BIOCHAR APPLICATION

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Mangrove forests are well-known for their capability to withstand strong tides and play a crucial role in the aquatic ecosystem. However, anthropogenic factors combined with natural stressors have high chances of affecting the nutrient content, increased salinity and anoxic environment of mangroves, which influences their growth performances. *Sonneratia caseolaris* is a mangrove plant that resides in areas with fresh water due to its weak resistance against high saline waters. A six-months study was conducted to evaluate if biochar application alleviated salinity stress via modifying sediment properties and nutrient uptake of *Sonneratia caseolaris* seedlings. Five treatments were evaluated in this study: control; mangrove soil without biochar application (T1), 10% of *B. parviflora* biochar (T2), 20% of *B. parviflora* biochar (T3), 10% of *G. levis* biochar (T4), and 20% of *G. levis* biochar (T5). From the study, treatment 3 (20% B. *parviflora* biochar) proved the best improved growth performance which gave the highest plant height, stem diameter, biomass dry weight, root fresh weight, length of entire plant, length of primary root, and signifies a good impact for the growth of seedlings. In addition, application of biochar generally increase the exchangeable cations: K, Mg and Ca in sediment and the total P, K and Mg content in plant compared to control. This demonstrated the potential of the treatment as a soil amendment to not only improved soil properties, but also enhancing *Sonneratia caseolaris* plants' tolerance towards salinity stress. Hence, *B. parviflora* biochar proved as the best quality of biochar and can improve the growth of mangrove plant seedlings including root growth and development as well sediment chemical properties and nutrient uptake.

Keywords: Biochar, soil amendments, growth performance, nutrient uptake, salinity stress

INTRODUCTION

Mangrove forests are groupings of shrubs or trees found in harsh biotopes such as intertidal areas of estuaries, lagoons, rivers, and sheltered bays in tropical and subtropical regions, usually along coastline areas across the world (Kodikara et al. 2017). Mangroves also play a role in protecting against harsh weather and play an important role in the changing global climate. Mangroves support the livelihoods as well as provide aesthetic values to millions of coastal residents by supplying them with building materials, ecotourism and most importantly food and fodder (Friess et al. 2020). Unfortunately, since the 1980's, up to 35% of mangrove populations have declined throughout the whole world (Friess et al. 2019). In the early 21st century, the rates of mangrove deforestation worldwide have reduced compared to the 20th century, where between year 2000 and 2020, about 677,000 hectares of mangrove were lost mainly due to both human induced activities and natural retraction (FAO 2023). In the same research reported by Chowdhury et al. (2019), these changes have high chance of affecting the nutrient content, increased salinity and creating anoxic environment of mangroves which also represent significant dangers by contributing to the reduction of mangrove areas.

Due to the unique characteristics of their environment, salinity plays a significant role in regulating mangrove tree propagule germination, seedling growth, and reproduction (Naidoo 2016). A negative relationship between seedling emergence rate and salt content was obtained in *Avicennia marina* (Patel et al. 2010), meanwhile high salinity reduces photosynthesis in the leaves of *Bruguiera parviflora* (Parida et al. 2004). This is because, different species of mangrove plant prefers different salinity concentrations (Win et al. 2019). For example, *Avicennia* grows in the slightly elevated and mostly in adverse and frequently changing part of the intertidal habitat. Meanwhile, the low salinity environment provides the best condition for initial establishment and growth of seedling of *Rhizophora apiculata*, *Rhizophora mucronata*, *Avicennia marina*, *Avicennia officinalis*, *Bruguiera gymnorrhiza*, and *Bruguiera sexangular* until 15–20 weeks of age (Kodikara et al. 2017).

High salinity in mangrove soil can be a limiting factor for mangrove trees due to influences of climate, groundwater, soil texture, and other abiotic factors, and uncontrolled human activities (He et al. 2021). Findings in Bangladesh coastal showed a decrease in upstream discharge throughout the entire coastal zone might lead to an increase in saltwater intrusion, while an increase in salinity is produced by the rising sea levels in the shallower coastal regions (Akter et al. 2019). Salinity can affect the plant in several ways such as low water potential in root that may lead to water stress in crop plants, imbalance in Na^+ and K^+, nutrient imbalance (decreased uptake and distribution in upper parts of the plant), osmotic imbalance in plant cell, and regeneration of reactive oxygen species (ROS) (Patel at al. 2021).

In order to solve this issue, there is a need to mitigate and improve soil salinity problems. Soil amendments that were usually used for reclamation of salt-affected soils can be grouped into two categories: inorganic and organic. Inorganic amendments like gypsum, calcium chloride, and sulfuric acid are commonly used for salt-affected soils, while alkaline amendments are used for acid sulfate soils. However, inorganic amendments can be expensive and have negative effects on soil microflora. On the other hand, organic amendments such as biochar and compost can improve soil fertility and supply nutrients to plants (Nur-Hafiza et al. 2023). These organic amendments are derived from sustainable sources and are considered environmentally friendly because it reduces waste load while also reclaiming marginal areas (Gunarathne et al. 2020).

Biochar is a product of thermal degradation of organic materials in pyrolysis process (Lehmann et al. 2015) which can improve soil properties and enhance plant productivity in almost any soil (He at al. 2020). The benefits including increase in cation-exchange capacity, reduced leaching of nitrogen, increase water retention, increase number of beneficial soil microbes (Ajeng et al. 2023), moderating soil acidity (Huang et al. 2023) as well as mitigating drought and salinity impact on plant (Yang et al. 2020). Previously, biochar application significantly decreased Na^+ uptake and enhances the growth and productivity of wheat (Akhtar et al. 2015) and tomato (She et al. 2018) in a saline soil.

Presently, very few studies can be found in reported literature regarding the potential of biochar to mitigate salinity stress in mangrove seedlings. Hence, this study aimed to investigate the effectiveness of biochar in mitigating salinity stress in *Sonneratia caseolaris* seedlings by evaluating their growth performance and the effect on soil and plant properties. The outcomes of this study will contribute to the knowledge of the impacts of climate change (i.e. salinity) on the growth of *Sonneratia caseolaris* seedlings.

MATERIALS AND METHODS

Study area

The nursery study was carried out in a mangrove area at Taman Rekreasi Paya Bakau Kampung Sijangkang, Telok Panglima Garang, Selangor (2.9400831°N, 101.4219476°E). In this study, a mangrove species of *Sonneratia caseolaris* seedlings was used as the test plant to investigate and compare their reaction in saline soil. The seedlings of *S. caseolaris* were obtained from Kampung Kuantan, Kuala Selangor and on-site soil was used for each treatment. For each treatment, the soil was placed in a 9’ × 12’ polybag, kept at the mangrove bed (Nguyen et al. 2016) and left for natural irrigation (Figure 1). The tidal schedule was obtained from https://www.tide-forecast.com/locations/

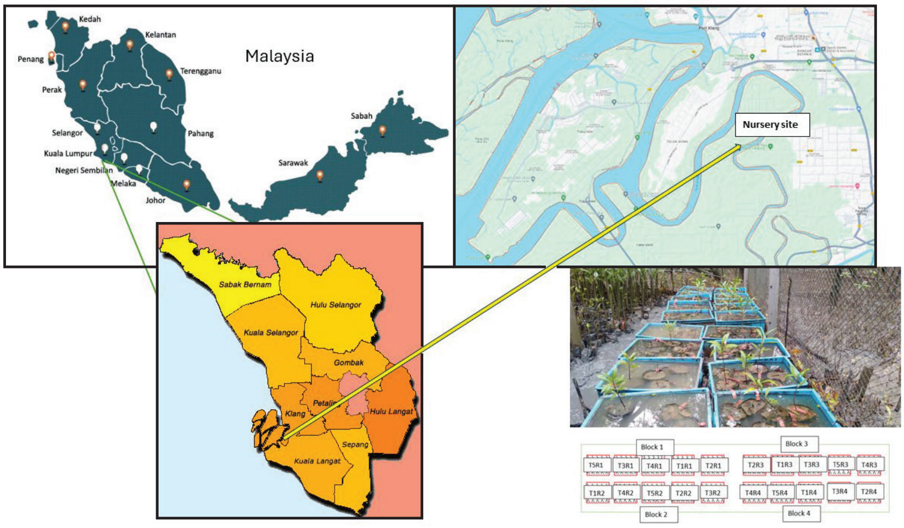

Figure 1 Mangrove nursery site at Taman Rekreasi Paya Bakau Kampung Sijangkang, Telok Panglima Garang, Selangor and the overall image of seedings in nursery site

Pelabuhan-Kelang-Malaysia/tides/latest, where the normal tide hour was at each 12 hours. Approximately 4 hours were taken by each tidal cycle to completely submerge the transplanted *S. caseolaris* seedlings in water.

Application of treatments and transplantating

Two different feedstocks of biochar were used in this study, which were biochar produced from the mangrove plant namely *Bruguiera parviflora* and biochar produced from the forestry waste of *Gigantochloa levis*. The biochar was selected from the previous optimization study which showed the best biochar characteristic when produced at 600 °C for two hours (Halim et al. 2023). The treatments were arranged in a randomized complete block design with four replications. Each plot of treatment was placed with 12 plants of *Sonneratia caseolaris* seedlings. The mangrove soil with treatments was mixed thoroughly before transplantation. After mixing, the three-month-old *S. caseolaris* seedlings were transplanted into each polybag. The treatments are listed in Table 1.

Growth performance measurement

The monthly growth parameters of *Sonneratia caseolaris* seedlings were measured, observed, and recorded for total of six months. Measurement of plant height was taken from the surface of soil in polybag up to the shoot using a measuring ruler. The stem diameter was measured at 5 cm above the soil using vernier caliper. The number of leaves was counted, and the leaf width was measured across the midline of the leaves. The relative chlorophyll content (RCC) was measured using a chlorophyll meter (SPAD-502, Konica Minolta, Japan).

During harvesting, the whole *Sonneratia caseolaris* seedlings roots were cleaned from mangrove soil and wrapped in aluminum foil and transported back to the Soil Science Laboratory, Universiti Malaya for processing. The length of the whole plant, leaf, root and weight were measured and recorded. All of

Table 1 List of treatments in the nursery study

Treatment	Content
T1	Control (100% mangrove soil)
T2	10% of biochar *Bruguiera parviflora* (90% mangrove soil: 10% biochar)
T3	20% of biochar *Bruguiera parviflora* (80% mangrove soil: 20% biochar)
T4	10% of biochar *Gigantochloa levis* (90% mangrove soil: 10% biochar)
T5	20% of biochar *Gigantochloa levis* (80% mangrove soil: 20% biochar)

samples including stems and roots were used for root analysis and plant nutrient analysis. For root analysis, the root surface area, average diameter, and root volume of *Sonneratia caseolaris* seedling was measured using the WinRHIZO root scanner.

Sediment chemical properties and nutrient analysis

Soil samples for pH and electrical conductivity (EC) determination were collected each month using a 16-inch stainless steel soil sampler. The soil was taken at 20 cm depth of the soil from each polybag, placed in air-tight plastic and transported back to the glasshouse at Rimba Ilmu, Universiti Malaya. The soil samples were air-dried for one month in the glasshouse, sieved by 2.0 mm and kept in closed container for analysis. The pH value of the soil was determined according to the method described by Rajkovich et al. (2012). The ratio of the dilution used was 10 g of soil and 25 mL of distilled water (1:2.5), and placed on Digital Orbital Shaker (DAIHAN Scientifc, Korea) for 60 minutes at 245 rpm. The pH meter electrode Starter 300 pH meter (OHAUS Corporation, US) was placed in the supernatant solution and the pH value was recorded. The electrical conductivity (EC) values of the samples were taken by diluting 10 g of sample with 50 mL of distilled water (ratio of 1:5). The solution was then shaken on an orbital shaker at 245 rpm for 60 minutes and the EC electrode HI 2315 Conductivity Meter (Hanna Instrument, Woonsocket, USA) was placed into the supernatant solution to obtain the EC value.

Laboratory analyses were carried out to determine nutrients in the sediment. The nitrogen (N) content determination was done using Kjedahl method, Bray and Kurtz method for determining the P element (Sims 2000), and the leaching method for determining exchangeable K, cation exchange capacity (CEC) and exchangeable Na, Ca, Mg (Schollenberger & Simon 1945). The content of exchangeable K, Ca, Mg and Na were determined using ICP-OES (Perkin Elmer Avio 200 ICP-OES, USA) and CEC were determined using the FIAstar TM 5000 Analyzer (FOSS Analytical, Sweden).

Plant Nutrient Analysis

During harvesting, the whole plant was measured and weighted. After the data collection, all samples were dried in oven for 48 hours at 65 °C. After oven dried, the plant samples were ground and sieved with 2.0 mm sieve and analysed. Plant samples were digested by using microwave digestion method (Sun et al. 1997) for N, P, K, Na, Ca, Mg element analysis.

Statistical analysis

Data were analysed using Analysis of Variance followed by Duncan's Multiple Range Test as the post-hoc analysis on SPSS. All data were presented as mean ± standard error of mean. The relationships between the plant growth performance, soil properties and soil nutrients were determined using a multivariate approach, namely the Partial Least Squares Regression (PLSR) using XLSTAT version 2021.1 (Addinsoft Inc., Paris, France). The scores for Variable Importance in Projection (VIP) were calculated to assess the importance of the soil properties and soil nutrient contents in influencing plant growth performance. A VIP score of more than 0.8 (90% confidence interval; CI) was deemed as important, whereas a VIP score of more than 1 (95% CI) was deemed as the most important.

RESULTS

Growth performance

Based on Figure 2 (a), the application of biochar to *Sonneratia caseolaris* showed a positive effect to its mean plant heights (cm). T3 (20% *B. parviflora*) and T5 (20% *G. levis*) showed a significant increase in heights which were 39.24 cm and 37.68 cm, compared to T2 (10% *B. parviflora*) with height 26.10 cm. Treatments that showed highest in stem diameter (mm) was T3 (20% *B. parviflora*) in Figure 2 (b) with a reading of 5.58 mm meanwhile the highest in leaf number was T5 (20% *G. levis*), with a reading of 13 in Figure 2 (c).The results have shown that only T3 (20% *B. parviflora*) and T5 (20% *G. levis*) gave the most favourable data and the most significant differences compared to other treatments.

Figure 2 (d) displayed the relative chlorophyll content (µmol m^{-2}) in a decreasing trend, except for T3 (20% *B. parviflora*) which showed an increasing trend and gave the highest mean relative chlorophyll content (60.87 µmol m^{-2}). In Figure 2 (e), the results showed a decreasing trend in electrical conductivity (mS cm^{-1}) in the early phases, followed by a sharp increased trend in between 5 to 6 months after transplantation (MAT). T3 (20% *B. parviflora*) showed significantly higher EC value while T1 (control) had the lowest EC value compared to other treatments. Figure 2 (f) exhibited that application of biochar showed an increasing trend in pH value of soils where T5 (20% *G. levis*) showed the significantly highest pH value of 7.09 compared to T1 (control) with pH 6.55.

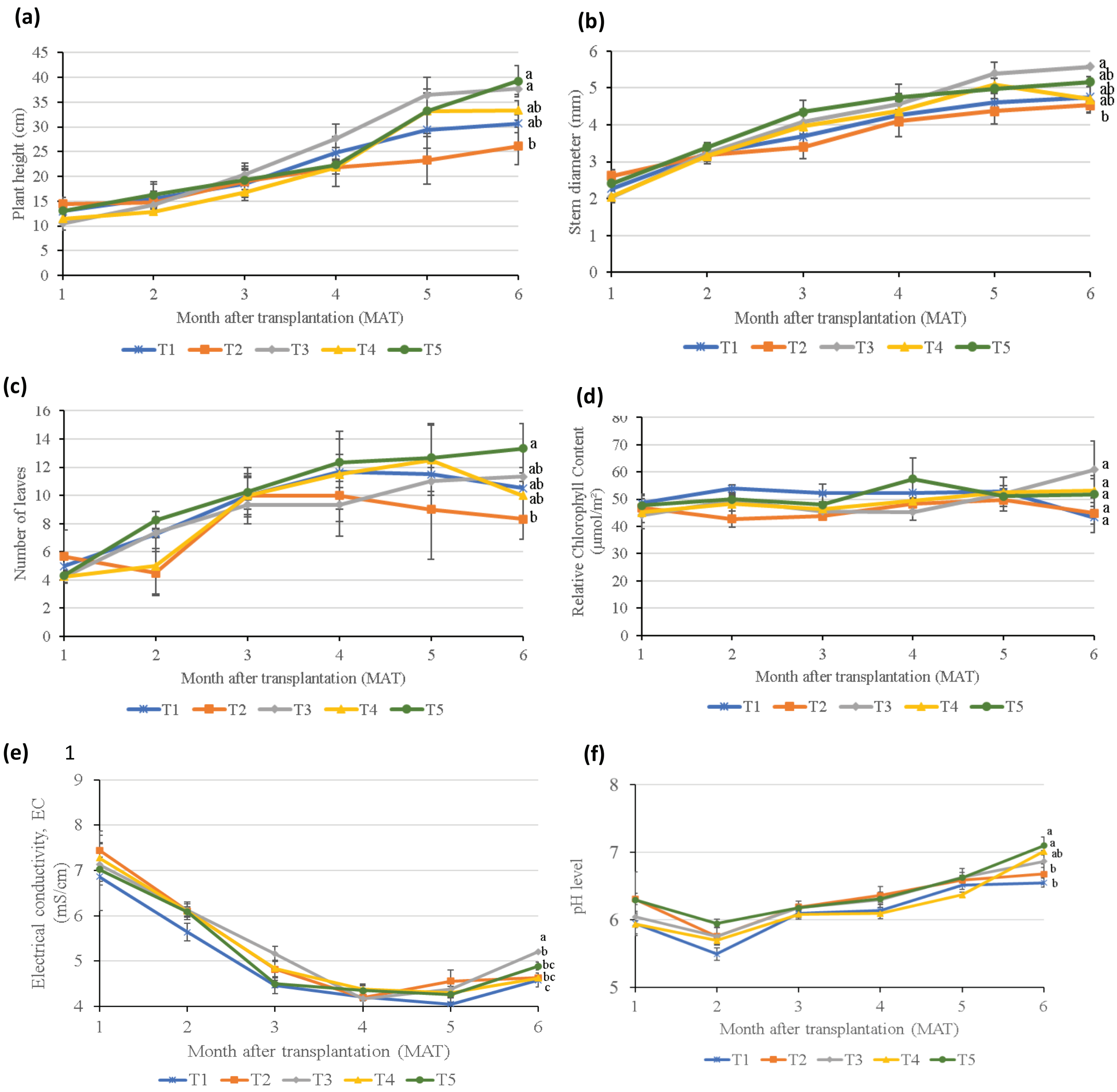

Figure 2 Effect of different biochar treatments on (a) plant height, (b) stem diameter, (c) leaf number, (d) relative chlorophyll content (e) electrical conductivity and (f) pH level, throughout five months after transplantation (MAT)

(T1) = Control, (T2) = 10 % *Bruguiera parviflora* biochar, (T3) = 20 % *Bruguiera parviflora* biochar, (T4) = 10 % *Gigantochloa levis* biochar, (T5) = 20 % *Gigantochloa levis* biochar. The vertical bars represent the standard error of the means. The different letters represent significant differences at $p<0.05$

Based on Figure 3 (a), T3 (20% *B. parviflora*) exhibited significantly the highest length of entire plant (65.25 cm) and primary root (21.60 cm) compared to other treatments. Meanwhile, Figure 3 (b) showed that T5 (20% *G. levis*) gave the highest value, in average leaf lengths of 16.12 cm compared to the lowest reading of T4 (10% *G. levis*) with average leaf lengths of 8.29 cm.

In Figure 3 (c), the root surface area (cm^2) in T3 (20% *B. parviflora*) showed a significant reading of 142.75 cm^2 compared to control by 120% higher. Figure 3 (d) referred to the average root diameter (mm) whereby T3 (20% *B. parviflora*) displayed the significant highest reading of 1.57 mm. Figure 3 (e) showed that T3 (20% *B. parviflora*) had the highest reading and was significantly elevated compared to control by 196%. The comparison of length between plants before harvest was shown in Figure 4.

The effects of biochar treatments on biomass fresh weight, biomass dry weight, water content, above-ground fresh biomass weight, and root fresh weight of *Sonneratia caseolaris* seedlings were analysed and compared. According to Table 2, the fresh biomass weight of seedling under T1 (control) with a reading of 23.46 g is 2 folds significantly higher against T2 (10% *B. parviflora*). Meanwhile, the dry biomass weight of T4 (10% *G. levis*) is significantly highest with a reading of 6.77 g. As for plant water content, T1 (control) significantly has the highest water content of plant (15.61 g) compared to other treatments.

The above-ground fresh biomass weight showed that T1 has the highest above-ground fresh biomass weight (16.91 g), almost 2-folds compared to T2 (10% *B. parviflora*). Besides, the root fresh weight of T5 (20% *G. levis*) showed a significantly highest meanwhile the lowest reading was found in T2 (10% *B. parviflora*) with reading of 2.05 g. The root fresh weight of T5 (20% *G. levis*), T3 (20% *B. parviflora*) and T4 (10% *G. levis*) were all significantly higher than T1 (control) and T2 (10% *B. parviflora*).

Soil chemical properties and nutrient level of sediment

The physicochemical properties of soil at harvest in this study were shown in Table 3. Highest soil pH was observed in T5 (20% *G. levis*) with a reading of 7.10 compared to control after six months of biochar application. The results show that T1 (control) has the lowest soil electrical conductivity at (5.396 mS cm^{-1}) as compared to other treatments. While T4 (10% *G. levis*) has the highest electrical conductivity (6.273 mS cm^{-1}). Highest result of CEC was shown for T3 (20% *B. parviflora*) and T5 (22.790 cmol kg^{-1} and 21.195 cmol kg^{-1}), which are 20–28% significantly higher compared to control. Meanwhile T4 (10% *G. levis*) and T5 (20% *G. levis*) showed significantly higher result for organic carbon content compared to control.

Based on the overall data analysis, soil amendments were found to influence the mineral composition of the soil as shown in Table 4. This study shows no significant difference in nitrogen and available P content between the treatments. Generally, elevated levels of K, Ca

Table 2 The effects of biochar treatments on biomass (fresh weight and dry weight), and water content of Sonneratia caseolaris seedlings

Biochar treatment	Whole plant FW (g)	Whole plant DW (g)	Water content (g)	Above-ground FW (g)	Root FW (g)
T1	23.46 ± 2.43^{a}	5.92 ± 0.19^{ab}	15.61 ± 2.25^{a}	16.91 ± 1.56^{a}	2.23 ± 0.07^{b}
T2	11.59 ± 1.39^{b}	5.63 ± 0.25^{b}	5.80 ± 1.06^{b}	9.21 ± 0.63^{a}	2.05 ± 0.55^{b}
T3	14.77 ± 0.84^{ab}	6.67 ± 0.27^{a}	8.37 ± 0.94^{ab}	10.37 ± 1.47^{a}	5.76 ± 0.73^{a}
T4	16.65 ± 4.23^{ab}	6.77 ± 0.09^{a}	9.88 ± 4.14^{ab}	11.55 ± 4.40^{a}	5.10 ± 0.18^{a}
T5	14.19 ± 0.79^{b}	6.05 ± 0.23^{ab}	4.35 ± 2.56^{b}	9.87 ± 1.62^{a}	5.98 ± 0.83^{a}

The values are shown by mean ± standard error and different letters represent a significant difference ($p<0.05$, n=4). All the parameters above were measured after harvest. T1 = control, T2 = 10 % *Bruguiera parviflora* biochar, T3 = 20 % *Bruguiera parviflora* biochar, T4 = 10 % *Gigantochloa levis* biochar and T5 = 20 % *Gigantochloa levis* biochar. FW = fresh weight, DW = dry weight

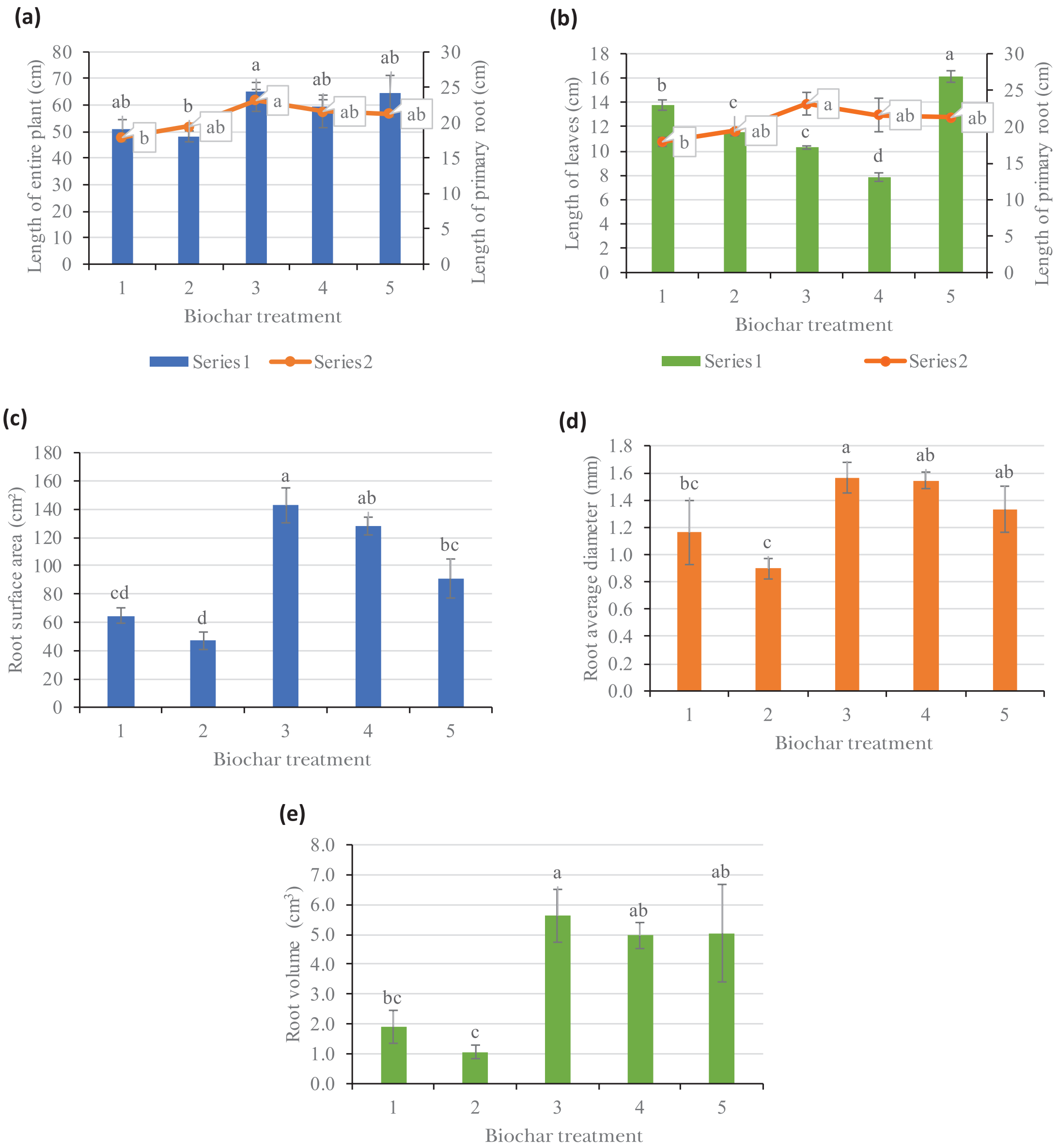

Figure 3 Effect of different biochar treatments on (a) whole plant length, (b) leaf lengths, (c) root surface areas, (d) root average diameters (e) root volumes after harvest

(T1) = Control, (T2) = 10 % *Bruguiera parviflora* biochar, (T3) = 20 % *Bruguiera parviflora* biochar, (T4) = 10 % *Gigantochloa levis* biochar, (T5) = 20% *Gigantochloa levis* biochar. The vertical bars represent the standard error of the means. The different letters represent significant differences at $p < 0.05$

Table 3 Effects of different soil amendments on soil physico-chemical properties after six months

Treatments	pH	EC (mS/cm)	CEC (cmol/Kg)	OC%
T1	6.533 ± 0.066^{c}	5.396 ± 0.256^{b}	17.735 ± 0.855^{b}	1.290 ± 0.080^{c}
T2	6.700 ± 0.152^{bc}	5.860 ± 0.336^{ab}	21.463 ± 0.763^{a}	1.666 ± 0.034^{b}
T3	6.866 ± 0.088^{ab}	5.523 ± 0.073^{b}	22.790 ± 1.226^{a}	1.810 ± 0.025^{ab}
T4	7.000 ± 0.00^{ab}	6.273 ± 0.164^{a}	18.013 ± 0.192^{b}	2.030 ± 0.120^{a}
T5	7.100 ± 0.115^{a}	5.930 ± 0.130^{ab}	21.195 ± 0.515^{a}	2.040 ± 0.088^{a}

The values are shown by mean ± standard error and different letters represent a significant difference ($p < 0.05$, n = 4). All the parameters above were measured after harvest. T1 = control, T2 = 10 % *Bruguiera parviflora* biochar, T3 = 20 % *Bruguiera parviflora* biochar, T4 = 10 % *Gigantochloa levis* biochar and T5 = 20 % *Gigantochloa levis* biochar; EC = electrical conductivity; CEC = cation exchange capacity, OC = organic carbon

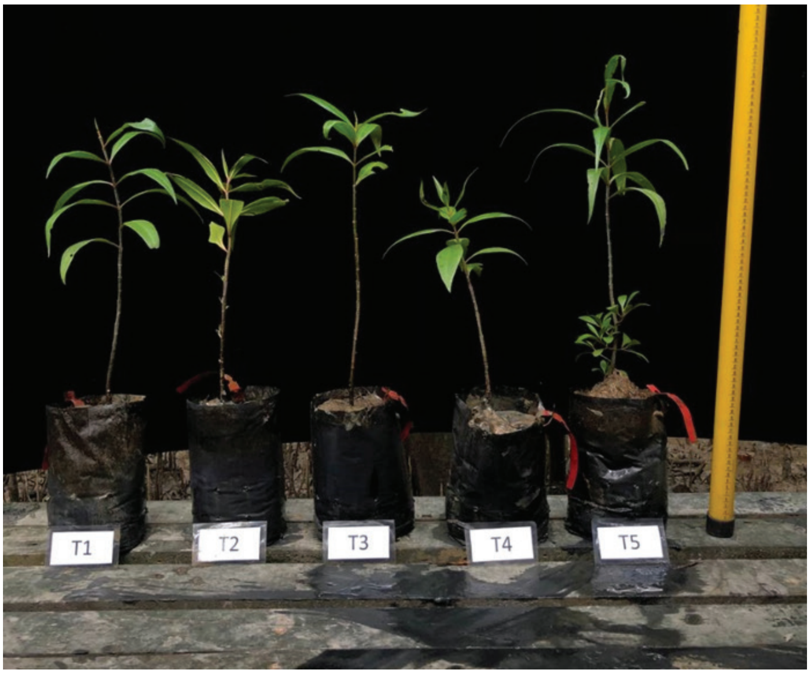

Figure 4 *Sonneratia caseolaris* saplings during 5 months after transplantation (MAT)

Table 4 The effects of different biochar treatments on the macronutrients of the soil

Treatments	Total N	Available P	Exchangeable cations K	Ca	Mg	Na
	%	mg/kg	cmol/kg			
T1	0.176^{a}± (0.008)	4.210^{a}± (0.294)	1.836^{b}± (0.116)	3.786^{b}± (0.274)	11.476^{b}± (0.661)	31.109^{c}± (1.314)
T2	0.203^{a}± (0.008)	4.116^{a}± (0.138)	2.470^{a}± (0.076)	4.726^{ab}± (0.461)	12.993^{ab}± (0.294)	36.463^{ab}± (1.536)
T3	0.206^{a}± (0.003)	4.060^{a}± (0.030)	2.470^{a}± (0.077)	4.360^{ab}± (0.235)	13.763^{a}± (0.193)	39.645^{a}± (0.259)
T4	0.200^{a}± (0.005)	4.303^{a}± (0.243)	2.190^{ab}± (0.217)	3.930^{b}± (0.565)	11.513^{b}± (1.024)	34.349^{bc}± (0.745)
T5	0.223^{a}± (0.029)	4.016^{a}± (0.150)	2.033^{b}± (0.092)	4.763^{a}± (0.329)	12.320^{ab}± (0.644)	36.327^{ab}± (0.812)

The values are shown by mean ± standard error and different letters represent a significant difference ($p<0.05$, $n=4$). All the parameters above were measured after harvest. T1 = control, T2 = 10 % *Bruguiera parviflora* biochar, T3 = 20 % *Bruguiera parviflora* biochar, T4 = 10 % Gigantochloa levis biochar, T5 = 20 % *Gigantochloa levis* biochar; N = nitrogen, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, Na = sodium

and Mg were seen in T2 and T3 compared to control results were significantly different.

Plant nutrients uptake of Sonneratia caseolaris seedlings

Table 5 shows the plant nutrient analysis in this study. It was found that, the *Sonneratia caseolaris* seedlings treated with biochar amendment did not give significant differences in total nitrogen content. However, values for P, K, and Mg were significantly elevated for T2 (10% *B. parviflora*) compared to control. T3 showed significantly different values compared to control for elements K and Mg whereas T4 (10% *G. levis*) shows the highest total P content (0.195%) and Mg content (0.457%). Na values were highest for control (without biochar) and T3 and T5 showed the least values for Total Na.

Correlation analysis

The relationships between the growth performance of *Sonneratia caseolaris* seedlings, soil properties and soil nutrients after harvest were elucidated using a PLSR analysis. As observed from the PLSR correlation biplot in Figure 5 (a), it can be observed that the control

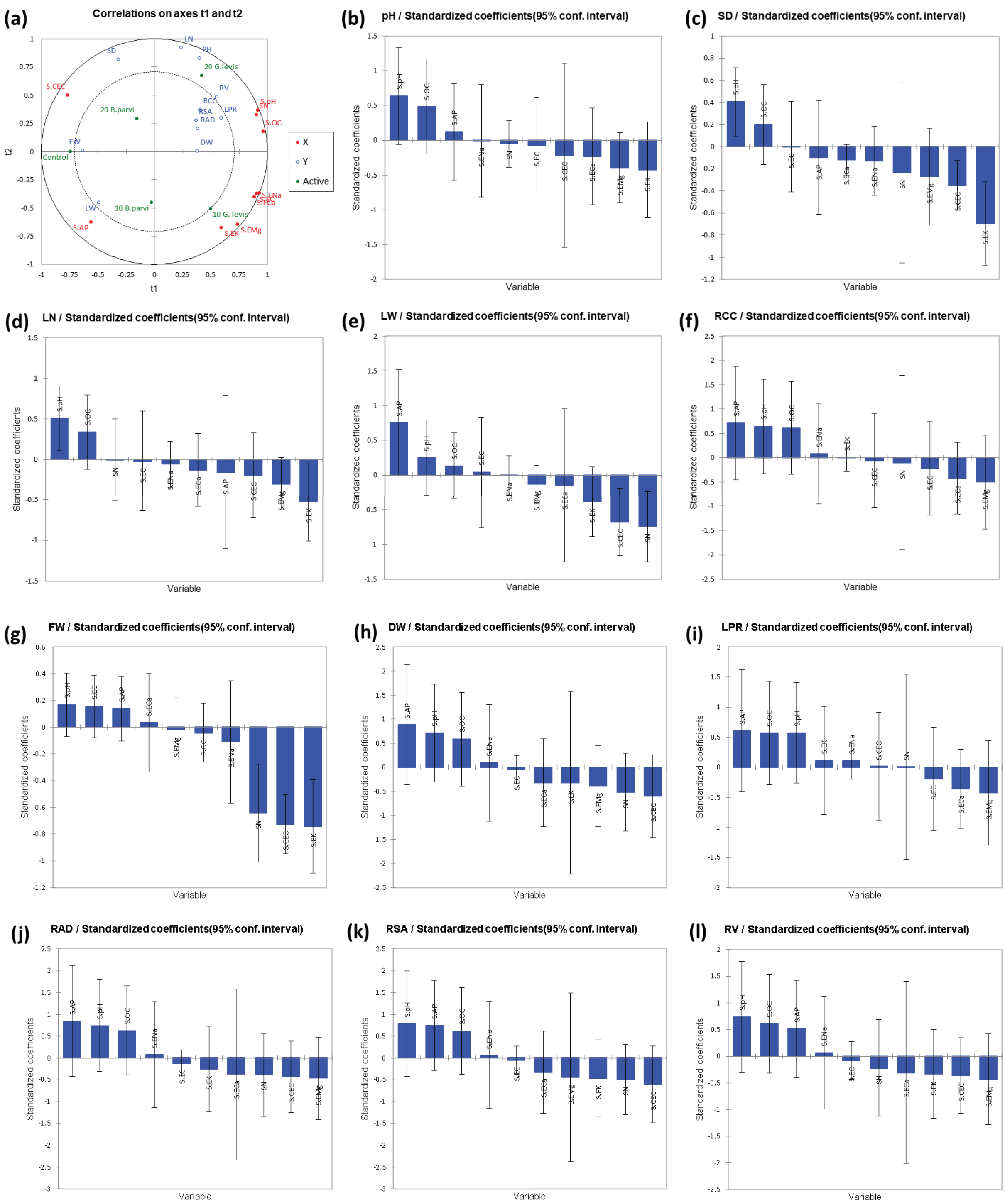

Figure 5 Partial Least Squares Regression (PLSR) analysis to determine the relationship between the plant growth performance, soil properties and nutrients: (a) PLSR correlation plot, (b–i) standardsed coefficients of variables (soil properties and soil nutrients) corresponding to plant height (PH), stem diameter (SD), number of leaves (LN), leaf width (LW), relative chlorophyll content (RCC), plant fresh weight (FW), dry weight (DW), length of primary root (LPR), root average diameter (RAD), root surface area (RSA), and root volume (RV)

plants and plants supplemented with 10–20% *Bruguiera parviflora* biochar were separated from the plants grown with 10–20% *Gigantochloa levis* biochar. Along Dimension 1, the control plants and plants supplemented with 10–20% *Bruguiera parviflora* biochar exhibited negative scores, while plants grown with 10–20% *Gigantochloa levis* biochar exhibited positive scores. Meanwhile, most of the X variables including soil pH (S.pH), soil nitrogen (S.N), soil organic carbon (S.OC), soil EC (S.EC), soil exchangeable Na (S.ENa), soil exchangeable Ca (S.ECa), soil exchangeable Mg (S.EMg), and soil exchangeable K (S.EK) were positively loaded along Dimension 1, while only soil CEC (S.CEC) and soil available P (S.AP) were negatively loaded along Dimension 1. It can also be observed that the treatment with 10% *G. levis* biochar (T4) was associated with higher levels of soil EC, soil exchangeable Na, soil exchangeable Ca, soil exchangeable Mg, and soil exchangeable K. On the other hand, the treatment with 20% *B. parviflora* biochar (T3) was associated with higher soil CEC, while the treatment with 20% *G. levis* biochar (T5) would result in higher soil pH, soil nitrogen and soil organic content (Figure 5 (a)). These observations further confirmed earlier results of the ANOVA analysis (Table 3 and Table 4).

Furthermore, soil pH, soil organic content and soil available P were found to be positively correlated to plant height (Figure 5 (b)), whereas soil pH and soil organic content were positively correlated to stem diameter (Figure 5 (c)) and number of leaves (Figure 5 (d)). In addition, soil available P, soil pH, soil organic content and soil EC were positively correlated to leaf width (LW) (Figure d (f)). Soil available P, soil pH, soil organic content and soil exchangeable Na were positively correlated to plant's relative chlorophyll content (Figure 5 (f)), plant dry weight (Figure 5 (h)), root average diameter (Figure 5 (j)), root surface area (Figure 5 (k)), and root volume (Figure 5 (l)). Meanwhile, soil pH, soil EC, soil available P and soil exchangeable Ca were positively correlated to plant fresh weight (Figure 5 (g)), and soil available P, soil organic content, soil pH, soil exchangeable K, as well as soil exchangeable Na were positively correlated to the length of primary roots (Figure 5 (i)).

Nevertheless, based on the computed VIP values (Table 5), only soil pH, soil organic carbon, soil nitrogen and soil exchangeable Na recorded VIP scores of >1.0, which implied that these four soil properties were the most important variables that significantly influenced the overall growth performance of the *Sonneratia caseolaris* seedlings.

DISCUSSION

The results of this study indicated that the highest plant height was observed in the *S. caseolaris* seedlings applied with 20 % *B. parviflora* biochar and 20 % *G. levis* biochar. Meanwhile, 20 % *B. parviflora* biochar allowed increased for stem diameter as well as relative chlorophyll content, biomass dry weight and highest length of entire plant. All the results stated above showed that these two treatments improved the growth (including biomass production) of *S. caseolaris* seedlings. This is attributed to the addition of biochar, which improves soil properties that favours the plant growth (Ajeng et al. 2023, Huang et al. 2023).

Table 5 Effects of different biochar treatments on plant nutrient content

Treatments	Total N	Total P	Total K	Total Ca	Total Mg	Total Na
	%	%	%	%	%	%
T1	1.606^{a}± (0.038)	0.168^{c}± (0.010)	1.356^{b}± (0.023)	0.398^{b}± (0.010)	0.376^{b}± (0.022)	3.525^{a}± (0.133)
T2	1.673^{a}± (0.071)	0.187^{ab}± (0.004)	1.539^{a}± (0.034)	0.395^{b}± (0.007)	0.416^{ab}± (0.008)	3.250^{ab}± (0.050)
T3	1.646^{a}± (0.086)	0.184^{ab}± (0.005)	1.608^{a}± (0.060)	0.509^{a}± (0.0.23)	0.358^{b}± (0.004)	3.082^{b}± (0.065)
T4	1.510^{a}± (0.075)	0.195^{a}± (0.003)	1.291^{b}± (0.074)	0.384^{b}± (0.024)	0.457^{a}± (0.040)	3.260^{ab}± (0.206)
T5	1.273^{a}± (0.012)	0.170^{b}± (0.002)	1.349^{b}± (0.015)	0.401^{b}± (0.015)	0.377^{b}± (0.004)	2.736^{c}± (0.025)

The values are shown by mean ± standard error and different letters represent a significant difference ($p < 0.05$, n = 4). All the parameters above were measured after harvest. T1 = control, T2 = 10 % *Bruguiera parviflora* biochar, T3 = 20 % *Bruguiera parviflora* biochar, T4 = 10 % *Gigantochloa levis* biochar and T5 = 20 % *Gigantochloa levis* biochar; N = nitrogen, P = phosphorus. K = potassium, Ca = calcium, Mg = magnesium, Na = sodium

Research in this study showed that application of 20% biochar v/v was beneficial for escalated seedling growth in mangrove soil. According to Tomczyk et al. (2020), biochar is highly biodegradable, contains abundant amounts of total and organic carbon, and ideal amounts of micro- and macro-elements. Due the natural nutrients that are available in the biochar itself, biochar is able to provide the nutrients directly to the plants (Zheng et al. 2018). Besides that, biochar also improved the mangrove soil properties to become more favourable for plant growth. Biochar is known to possess high cation exchangeable capacity such as calcium ions (Ca^{2+}) and potassium ions (K^+) that are able to displace the sodium ions (Na^+) at the exchange site; thus, reduce the Na^+ uptake by plants. Accumulation of Na^+ in leaves can cause reduction in photosynthetic activity and necrosis (Parkash & Singh 2020, Doganlar et al. 2010). Biochar also helps to increase the soil pH and alleviate aluminium toxicity in plants; thus, enhancing the growth of the plants (Halim et al. 2018).

Interestingly in this study, the highest numbers of leaves were observed in the 20% *G. levis* biochar treatment. This is attributed to the stress tolerance mechanism performed by the seedlings in order to withstand the conditions of low soil salinity and hot temperature. According to Sharmin et al. (2021), increase of salinity causes the reduction of transpiration rate due to the reduction of stomatal conductance. The reduction of stomatal conductance is caused by the decrease of major nutrient contents such as calcium ions (Ca^{2+}), potassium ions (K^+), and magnesium ions (Mg^{2+}) in roots, which resulted in the reduction of (Ca^{2+}) and Mg^{2+} in the leaves. However, biochar was proven to reduce salinity in this study as biochar treated seedlings showed lesser Na compared to control. Thus, its shows that biochar application can help *S. caseolaris* photosynthetic activity and productivity of the plant.

Electrical conductivity (EC) is the indicator of the ability and its capacity to conduct electric (Tan et al. 2021). As biochar can take up Na^+ from the soil and promote salt leaching event, soil amended with biochar will have lower EC values. However, it is in contrast with the results obtained, whereby soil supplemented with 20 % *B. parviflora* biochar (T3) showed the highest EC value. The result obtained can be explained by the release of cation (weakly bound ions) from the biochar to the solution of soil sample. The same justification was also given by Chintala et al. (2014) when an increase of soil EC was observed in the acidic soils when incorporated with biochar. The release of Na^+ from biochar may be due to the grinding process of soil samples, where biochar combined with soil samples was broken down, triggering the release of Na^+ that was initially bound inside the biochar. Thus, from another point of view, the high amount of Na^+ release from the biochar to the soil sample solution indicates the high amount of Na^+ was taken up by the biochar from the mangrove. In contrast, control treatment did not have biochar that helped to absorb Na^+ thus showing elevated levels of Na^+.

In this study, the mangrove soil was slightly acidic (pH 5.9). Mangrove soil applied with 10 % *G. levis* biochar and 20 % *G. levis* biochar showed a significantly higher soil pH after six months (pH 7.01 and 7.10). This is attributed to the addition of biochar which has an alkaline pH (7.97) as well several functional groups like carbonyls (COO-), phosphates (PO_4^{3-}), and carbonates ($CaCO_3$) and other alkaline compounds that can neutralize the soil acidity and raise the pH (Dai et al. 2017). As biochar has large surface area, loose and porous structure, the toxicity of Al^{3+} can be immobilized by biochar to mitigate the soil aluminium toxicity (Qian & Chen 2013).

Due to the presence of acidic clays and sulphur-reducing bacteria, mangrove soil typically ranges from neutral to slightly acidic (Arianto et al. 2015). However, in Malaysia, certain mangroves have extremely acidic brackish waters due to the aeration of soil sulphates, which resulted in the formation of sulphuric acid. Aluminium toxicity often thrives in acidic soil (Zhao & Shen 2018). In acidic soil particularly, soil pH is lower than 5, the abundant aluminium (Al) in soil will transform to ionic forms (Al^{3+}) (Panda et al. 2009, Zheng 2010) due to the presence of hydrogen ions (H^+). The Al^{3+} in soil is toxic to plants as it can penetrate the root tip cell and hinders the plant's roots from developing (Panda et al. 2009), causing the plants unable to take up the essential nutrients effectively and limits the plant productivity (Chintala et al. 2014).

In addition, the control treatment seedlings were observed to have the highest water content. This is attributed to the frequent osmotic adjustment done by the plant as they faced higher osmotic stress compared to other seedlings which supplemented with biochar. Based on a study by Kul et al. (2021), under saline conditions, biochar amendments on tomato caused a significant improvement in root fresh and dry weights. The same study suggests that the application of biochar may have increased plant performance because it enhanced soil porosity and decreased bulk density, which improved conditions for growth and root proliferation in salt-stressed conditions. Plants usually undergo osmotic adjustment when they face water stress (osmotic stress) by accumulating solutes (Girma & Krieg 1992). Due to the saline environment, halophytes like mangrove undergo osmotic adjustment to take up water from the surrounding soil. Halophytes accumulates energetically cheap inorganic ions like Na^+ and Cl^- as well as osmolytes (organic solutes with low molecular weight) in their body during osmotic adjustment (Slama et al. 2015) in order to lower the total water potential inside their anatomy.

From the result, 20 % *B. parviflora* biochar treatment and 20 % *G. levis* biochar treatment showed significantly highest root fresh weights after harvest. This can be attributed to the ability of biochar to enhance root growth and development by improving soil conditions. Furthermore, the porous structure of biochar can help to enhance the air and water filtration in soil, which creates a soil condition that favours the root growth and development (Zheng et al. 2018).

Biochar improves soil conditions and boosts root growth through two mechanisms; providing nutrients directly and enhancing nutrient retention and availability in both the rhizosphere and bulk soils (Prendergast Miller et al. 2014). According to the linear regression and structural equation modelling analysis by Zou et al. (2021), total nitrogen and available phosphorus are the primary factors influencing root biomass production. Nitrogen-fixing microorganisms thrive within biochar's porous structure, benefiting from its nutrient-rich environment.(Zhang et al. 2021), increasing soil nitrogen.

The porous structure of biochar helps to prevent leaching of nitrogen from fertilizer and holds it in the soil. Biochar also enhances the available phosphorus in soil by altering the phosphorus sorption characteristics (Wu et al. 2022). Biochar is known to promote "fine root turnover" mechanism by enhancing the root growth and development of the seedlings. The "fine root turnover" mechanism, is a salt tolerance strategy, involves continual production of young roots to replace older ones, preventing excess ion build-up (Ramoliya et al. 2004). Therefore, increasing young root production enhances stem tissue quantity, which inhibits Na^+ transfer to leaves. In conclusion, biochar addition shows promise for promoting seedling growth to help mitigate the adverse impacts of salinity stress on plant growth and physiology (Zhang et al. 2023a).

Application of biochar resulted in significant increase of pH, electrical conductivity, CEC and organic carbon compared to control. The increase in macronutrient content (N, P and K) may be due to the increase in the soil pH and EC from biochar itself. The increase in soil pH affects soil fertility, and hence the nitrogen content through nutrient cycling by beneficial soil microorganisms through their chemical and biochemical activities (Ajeng et al. 2021, Turner 2010). Nitrogen played a very important role for growth, reproduction and maintenance of photosynthetic capacity of plants (Zong-min et al. 2012, Crous et al. 2021).

Increased organic carbon content for biochar treatments supports its potential to sequester carbon in the long term because of its high stability (Gross et al. 2021, Wang et al. 2016). SOC addition due to biochar transpires into a long living C pool that would otherwise be emitted as CO_2 (Gross et al. 2021).

Additionally, soil supplemented with biochar can retain the nutrients contained and reduce leaching losses in the soil (Biederman et al. 2017). With reference to Table 4, the exchangeable cations of K, Mg, and Ca were significantly high for biochar treatments. The soil was influenced by the soil amendments compared to the control. The increase of soil pH occurred because of the soil's inherent Ca and Mg content being released from biochar itself. As the loss of basic cations by leaching is minimal, it is possible to increase soil pH through the accumulation of basic cations throughout the plant (Ch'ng et al. 2016).

In this study, the observed increase in mineralisation following soil amendment with different rates of biochar application could be attributed to the chemical composition of the biochar dose itself. Biochar has also been used as an organic amendment for reducing adverse effects of salinity on soil functions governed by their rates of addition (Zhao et al. 2020). Basic cations such as Na^+, Ca^{2+}, Mg^{2+} and K^+ in the form of oxides or carbonates can dissolve in water and produce OH^-, which in turn increases the soil pH (Berek & Hue 2016, Smider & Singh 2014). The carbonate content is responsible for the alkalinity of biochar (Mukome et al. 2013) and was positively correlated with basic cation (Berek et al. 2018).

From this study, the *S. caseolaris* seedlings treated with biochar amendment showed a significant difference in the total P, K, Ca, Mg and Na content. 20 % *B. parviflora* biochar treatment showed a significantly higher result in total K and Ca content (1.608% and 0.509 %) and low (3.082%) total Na content in plant compared to control. Improved P, K, Mg and Ca uptake by plant root systems (Awad et al. 2017) may be attributed to beneficial microorganisms despite microbial interactions were not accounted for in this study. Similar mechanism for improving the uptake of macronutrients by maize plants in soil treated with biochar were reported by Kim et al. (2017) and Rehman et al. (2016). Furthermore, biochar as an organic material has the ability to break down and release basic cation such as K^+, Mg^{2+} and Ca^{2+} contents (Ch'ng et al. 2019) which can dissolve in water (Smider & Singh 2014) thus, enhancing the nutrient uptake of plant.

Plant maintains higher K^+ and lower Na^+ concentrations in its cytoplasm when it is under salt stress. H^+ pumps provide the driving power for transport, which is maintained by K^+ and Na^+ transporters. Studies also have shown that biochar maintains ion balance and alleviates ion toxicity caused by Na^+/K^+ imbalance by absorbing Na^+ and releasing K^+ (Zhang et al. 2023b, Rezaie et al. 2019) where in this study, the Na^+ in plant is significantly reduced in 20 % *B. parviflora* biochar treatment meanwhile the Na^+ in soil increased. This study also demonstrated the critical function of Ca^{2+} plays in plants tolerance to salt. Moreover, it activates K^+/Na^+ transporters. Elevated salinity also causes a shift in the intracellular compartments and apoplast to higher levels of cytosolic Ca^{2+} (Patel et al. 2017). This could be attributed to the release of basic cations from biochar. The results confirmed the hypothesis that the use of 20% biochar (20 % *B. parviflora* and 20% *G. levis* biochar) as soil amendments improved the nutrient contents as biochar improved and enhance plant productivity (Patel et al. 2017).

The relationship between the growth and soil nutrient analysis after harvest was elucidated using a PLSR analysis. The correlation biplot

Table 6 Variable of Importance (VIP) coefficients for the different variables (soil properties and soil nutrients) corresponding to the growth performance of *Sonneratia caseolaris* seedlings

Variable	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)
S.pH	1.633	0.251	0.936	2.330
S.OC	1.631	0.418	0.471	2.791
SN	1.341	0.457	0.071	2.612
S.ENa	1.005	0.618	-0.710	2.720
S.EC	0.726	0.461	-0.553	2.006
S.ECa	0.587	0.334	-0.340	1.515
S.AP	0.581	0.948	-2.051	3.214
S.EK	0.556	0.855	-1.817	2.929
S.CEC	0.527	0.465	-0.764	1.819
S.EMg	0.259	0.745	-1.809	2.327

VIP coefficients above 1.0 (shaded grey) were identified as the most important; S.pH = soil pH, S.EC = soil EC, S.CEC = soil CEC, S.OC = soil organic carbon, SN = soil nitrogen, S.AP = soil available P, S.ENa = soil exchangeable Na, S.ECa = soil exchangeable Ca, S.EK = soil exchangeable K, S.EMg = soil exchangeable Mg

(Figure 5) showed that plant growth and soil nutrient exhibited a positive load in Dimension 1. Besides that, it can also be observed that all X variables (the soil nutrient) monitored in this study exhibited high positive loadings along Dimension 1 except for soil CEC and Soil Available P. Based on the computed VIP values, only soil pH, soil organic carbon, soil nitrogen and soil exchangeable Na recorded VIP scores of >1.0, which indicated that these variables were the most important factors that influenced the growth of the plants. The VIPs values were listed in Table 6.

CONCLUSIONS

The application of different biochar treatments positively gave significant effects on the growth performance, sediment chemical properties and plant nutrient uptakes of *Sonneratia caseolaris* when grown under salinity stress. From the study, it was found that treatment 3 (20% *B. parviflora* biochar) proved the best improved growth performance which gave the highest plant height, stem diameter, biomass dry weight, root fresh weight, length of entire plant, length of primary root. Other than that, the exchangeable cations of K, Mg and Na in soil sediment was increased whereas an increase in total K and Ca content in the plants were observed. *B. parviflora* biochar was able to improve the growth of mangrove plant seedlings, the sediment chemical properties and nutrient uptake of plant.

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