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Article

Biochars Improve Nutrient Retention Capacity of Highly Weathered Tropical Soils

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Abstract: Highly weathered soils in the tropics are low in fertility, negatively affecting plant growth. The potential of biochar for improving soil nutrient retention is reportedly promising, triggering this study to assess the nutrient retention capacities of two biochars when applied at 2% in combination with two composts also applied at 2% to an Ultisol (Ustic Kanhaplohumult, Leilehua series) and an Oxisol (Rhodic haplustox, Wahiawa series) of Hawai'i. Chinese cabbage (*Brassica rapa* cv. Bonsai) was used as the test plant in two greenhouse plantings, which had a factorial completely randomized design with three replicates per treatment. The results indicated that the combined additions of biochar and compost significantly increased the pH, EC, P and K of the soils; improved Ca, Mg and Fe uptake; and increased shoot and total cabbage fresh and dry matter. Exchangeable aluminum in the Ultisol was decreased from 2.5 cmol+/kg to virtually zero. Extractable Mn and Fe in the high Mn-Oxisol were decreased by 55 and 42%, respectively. Chinese cabbage growth in the Ultisol amended with the lac tree (*Schleichera oleosa*) wood biochar and vermicompost was almost twice over lime at 2 cmol+/kg. Essential nutrients in the plant tissues, with the exception of N and K, were sufficient for the cabbage growth, suggesting increases in nutrients and reduced soil acidity by the additions of biochar combined with compost were the probable cause.

Keywords: biochar; compost; nutrient retention; highly weathered soil; Chinese cabbage.

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1. Introduction

Highly weathered soils are poor in nutrients mainly because of leaching. Low soil fertility necessitates fertilizer applications which increase cost of crop production, and can result in environmental issues such as algal bloom and contamination of water bodies [1]. Biochar reportedly has good capacity to retain nutrients due to its numerous pores and large surface area/charge [2,3]. Recent research has shown that additions of biochar reduced nutrient losses from the agricultural lands [4-10). Other observed benefits such as increased soil water retention [11,12], raised soil pH [13] and cation exchange capacity (CEC) [14,15], improved beneficial soil microbial population and activities [16,17], and consequently, enhanced plant growth. More specifically, biochar can reduce nitrate, ammonium, phosphorus, and cation concentration in the soil leachate [4,6,7,18]. For example, addition of a mixed hardwood biochar at 20 g/kg in combination with swine manure at 5 g/kg to a typical agricultural soil (a Hapludoll) of the Midwestern USA reduced total N and total dissolved P leaching by 11% and 69%, respectively, in a leaching column study [4]. Addition of biochars produced at higher temperature with large surface area may benefit sandy soils by

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increasing sorption sites and/or by improving the retention of nonpolar pollutant in soils and reducing nutrient leaching [19,20].

The nutrient retention capacity of biochar could be attributed to its large surface area, porosity, and surface charge, organic coating, and other factors, such as pH and ionic competition [2,3,21]. For example, NH4-N adsorption is due to cation exchange of the surface functional (e.g. phenolic and carboxylic) groups of biochar produced at relatively low (300-400°C) temperature [22] and physical entrapment in biochars pores [23]. In contrast, NO_3-N adsorption on the basic functional groups can be increased by increasing pyrolysis temperature [22]. Immobilization of N by microorganisms was also observed in low temperature-produced biochar [24]. Phosphorus ions were specifically adsorbed at certain sites of biochar or precipitated by Ca [25]. However, it appears that some biochars have no or only little effect on nutrient retention, and the degree of retention depended on biochar types, soil properties and other environmental conditions. The objective of this study was to evaluate the nutrient regulation or enhancement role of two biochars applied in combination with two composts to two highly weathered, tropical soils as measured by the nutrient uptake and growth of Chinese cabbage (*Brassica rapa*).

2. Materials and Methods

The nutrient retention of biochars were studied in a greenhouse, at the Magoon research facility, University of Hawai'i at Manoa, Honolulu, Hawai'i, using two acid soils, an Ultisol (Ustic Kanhaplohumult, Leilehua series) and an Oxisol (Rhodic haplustox,Wahiawa series). Soil samples were air dried, crushed and screened to pass a 4 mm sieve for the pot experiment. Finer (<0.5 mm) soil particles were used in chemical analysis. Wood-based biochars collected from Indonesia and Hawai'i, were oven dried at 70°C for 48 hours, ground and sieved to pass a 60 mesh (0.25 mm) sieve and stored before use. Selected properties of the biochars are listed in Table 1. Surface structure and porosity of biochars were measured with a scanning electron microscope (SEM) (HITACHI; Hitachi High-technologies, Corp., Tokyo, Japan). Pore diameters were measured from 2,000-time magnified SEM images. The pH (H_2O) of soils, biochars and composts were measured with a pH meter in a mixture of soil, biochar, compost and deionized water of 1:1, 1:5 and 1:5, respectively. The EC of soil, biochars and composts were measured using a pH/EC meter in a mixture of soil, biochar, compost and deionized water of 1:1, 1:5, and 1:10, respectively. The functional groups of biochar (carboxylic and phenolic) were quantified using the Boehm titration method [26]. Water soluble salts and carbonates in biochar were removed before the titration. Briefly, 0.50 g of fine biochar was added to 50 mL of each of the three 0.05M bases: $NaHCO_3$, Na_2CO_3, and NaOH. The mixtures, along with a control solution without biochar, was shaken for 24 h and then filtered to remove particles. Then, 5 mL of each filtrate were mixed with 10 mL of 0.05 M HCl and back-titrated with 0.05 M NaOH. The endpoint was determined using a pH meter and phenolphthalein color indicator. The total surface acidity was calculated as the quantity neutralized by NaOH, the carboxylic acid fraction as that neutralized by $NaHCO_3$, and the lactonic fraction as that neutralized by Na_2CO_3. The difference between quantity of NaOH and Na_2CO_3 is assumed to be the phenolic group content [27].

A locally produced vermicompost and a commercial thermocompost were collected from Honolulu, Hawai'i, oven dried at 70°C for 72 hours, screened to pass a 0.5 mm sieve for chemical analysis. Nutrient content of the composts were determined with an Inductively Coupled Plasma (ICP) spectrometer after a 0.20 g sample was burned at 500°C for 4 hours. The ash was dissolved with 15 mL of 0.1 M HCl. Soil exchangeable aluminum was measured with 1 M KCl leaching/titration method. The measured pH, EC and nutrient content in the soils, biochars and composts are listed in Table 1. Hydrated lime (Bandini®) with the $CaCO_3$ equivalent of 108 was dried and screened through a 60 mesh sieve before use.

Table 1. Selected properties of soils, biochars and composts used in the experiment

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Selected properties	Leilehua Ultisol	Wahiawa Oxisol	Lactree wood biochar	Hilo mixed wood biochar	Vermicompost	Thermocompost
PH (H_2O)	4.5	5.6	9.2	9.5	7.2	8.3
EC (dS/m)	0.08 ± 0.00	0.13 ± 0.00	1.93 ± 0.01	2.42 ± 0.01	2.28 ± 0.03	3.23 ± 0.02
CEC ($cmol_+$/kg)	16.8 ± 0.45	12.1± 0.23	18.0 ± 1.75	14.7± 0.20	44.8± 3.50	44.5± 1.00
N (%)	0.21 ± 0.00	0.15 ± 0.00	0.40 ± 0.02	0.50 ± 0.09	1.42 ± 00.0	1.90 ± 0.00
P (mg/kg)	1.95 ± 0.03	51.52 ± 0.08	0.06 ± 0.00	0.09 ± 0.00	1.48 ± 0.00	0.17 ± 0.01
K (mg/kg)	49.15 ± 0.53	140.70 ± 1.08	0.33 ± 0.00	0.47 ± 0.00	0.04 ± 0.00	1.37 ± 0.01
Ca (mg/kg)	111.84 ± 5.09	715.88 ± 5.68	3.13 ± 0.00	1.6 ± 0.01	2.11 ± 0.67	2.39 ± 0.05
Mg (mg/kg)	53.93 ± 1.06	232.60 ± 0.07	0.13 ± 0.00	0.22 ± 0.00	0.36 ± 0.00	0.36 ± 0.00
Fe (mg/kg)	98.14 ± 1.41	63.95 ± 0.59	684 ± 0.00	12259.5 ± 233.65	2407.90 ± 30.94	8345.20 ± 103.11
Mn (mg/kg)	11.41 ± 0.05	805.80 ± 5.35	55 ± 0.00	153.8 ± 2.32	606.00 ± 0.68	239.20 ± 1.23
Al (mg/kg)	2517.53 ± 5.48	1503.76 ± 12.67	448.1 ± 0.00	9766.9 ± 154.72	1832.00 ± 57.79	9933.30 ± 39.17
Exchangable Al ($cmol_+$/kg)	2.16±0.02	0.17±0.01	-	-	-	-
Total functional groups (mmol/g)	-	-	0.38 ± 0.02	0.58 ± 0.03	-	-
Carboxylic functional group (mmol/g)	-	-	0.12 ± 0.02	0.22 ± 0.02	-	-
Phenolic functional groups (mmol/g)	-	-	0.20 ± 0.04	0.27 ± 0.03	-	-
Lactonic functional group (mmol/g)	-	-	0.07 ± 0.02	0.10 ± 0.03	-	-

The treatments, consisting of soil, biochar and compost, were arranged in a 2 x 2 x 2 factorial completely randomized design with 3 replicates, and a 2 cmol+/kg lime treatment was included for comparison. Two biochars, namely lac tree (*Schleichera oleosa*) wood and Hilo mixed wood, were applied at 0 (control) and 2% (w/w). The compost treatments were 0 and 2 % (w/w). After three weeks of incubation, all pots (1 kg soil/pot) were planted with Chinese cabbage (*Brassica rapa*) cv. Bonsai Chinensis group, which was harvested after 34 days of growth. The cabbage planting was repeated once (second planting). Shoots and roots were carefully removed from the soil, washed, and the fresh weight was measured before oven-drying at 70^{o}C for 48 hours. Soil samples were collected from each pot at 16 days after addition of biochar and compost (a week before the first planting) and 52 days (a week after harvest of the first planting), air-dried, crushed, and passed through a 0.5 mm sieve before analysis. Soil pH and EC were measured using a pH meter in a 1:1 mixture of soil and deionized water. Total carbon and nitrogen content was measured by dry combustion in a LECO CN-2000 elemental analyzer (Leco Corp., St. Joseph, MI). Soil nutrients quantified with an ICP Spectrometer after extraction with the Mehlich 3 solution [28]. Dry weights of shoots and roots were recorded. Plant nutrients were quantified with an ICP Spectrometer after dry digested and dissolved in 0.1 M HCl.

3.1. Statistical Analysis

Means and standard errors were calculated using a descriptive analysis from two or three replicates of the measured soil pH, electrical conductivity, plant dry weight, and nutrient content in soils and plant tissues. The relationship between nutrient uptake and plant dry matter was analyzed using regression analysis in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA). Histogram figures of soil aluminum changes and plant growth differences resulting from the biochar application were drawn using Microsoft Excel 2010 software. The effect of treatments on soil properties and cabbage growth were analyzed by a two-way analysis of variance using PROC ANOVA GLM of the SAS 9.2 software, and Tukey tests at $P \leq 0.05$ were performed to test the significant differences.

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3. Results

3.1. Soil pH, EC and Exchangeable Al

The soil pH significantly increased from 4.5 to 5.9 in the Ultisol and from 5.6 to 6.9 in the Oxisol, two weeks after treated with biochars in combination with composts (Table 2). They were further increased to 6.5 (Ultisol) and 7.2 (Oxisol), 7 weeks after treatment applications. The pH increase of the Ultisol at two weeks after treatment was significantly affected by the interaction of biochar and compost ($P<0.01$); however, the pH increases at 7 weeks were due to the effect of compost or biochar alone ($P<0.001$). Among the treatments, vermicompost in combination with either biochar increased pH the most. The pH increases in the Oxisol in both sampling times were affected by the interaction of biochar and compost ($P<0.05$). The data strongly demonstrate the liming capacity of both biochar and compost, especially for low pH soils. For comparison, the lime treatment raised soil pH to 5.6 and 6.8 in the Ultisol and Oxisol, respectively, two weeks after treated.

Table 2. Means and standard errors pH and EC of Leilehua Ultisol 2 and 7 weeks after being incubated with biochars and composts (n=3)

Treatments	pH 2 weeks	pH 7 weeks	EC 2 weeks	EC 7 weeks
			dS/m	
Ultisol soil				
Lac tree wood biochar 2%	5.8 ± 0.07 bc	5.8 ± 0.22 de	0.20 ± 0.01 d	0.26 ± 0.02 c
Hilo mixed wood biochar 2%	4.8 ± 0.01 e	4.8 ± 0.07 g	0.22 ± 0.00 d	0.25 ± 0.01 c
Vermicompost 2%	5.7 ± 0.02 c	6.3 ± 0.07 bc	0.51 ± 0.01 ab	0.38 ± 0.01 a
Thermocompost 2%	5.1 ± 0.03 e	5.5 ± 0.09 f	0.39 ± 0.02 c	0.27 ± 0.02 c
Lac tree wood 2% + vermicompost 2%	5.9 ± 0.04 ab	6.5 ± 0.71 ab	0.51 ± 0.04 ab	0.38 ± 0.01 a
Lac tree wood 2% + thermocompost 2%	5.8 ± 0.08 bc	6.1 ± 0.04 cd	0.43 ± 0.02 bc	0.29 ± 0.01 bc
Hilo mixed wood 2% + vermicompost 2%	5.8 ± 0.02 bc	6.5 ± 0.02 ab	0.55 ± 0.02 a	0.36 ± 0.02 ab
Hilo mixed wood 2% + thermocompost 2%	5.4 ± 0.01 d	5.8 ± 0.11 de	0.46 ± 0.02 abc	0.29 ± 0.03 bc
Lime 2 $cmol_+/kg$ + vermicompost 2%	6.1 ± 0.03 a	6.6 ± 0.07 a	0.50 ± 0.02 ab	0.38 ± 0.03 a
Lime 2 $cmol_+/kg$ + thermocompost 2%	5.6 ± 0.03 c	6.1 ± 0.07 cd	0.47 ± 0.01 abc	0.25 ± 0.01 c
Oxisol soil				
Lac tree wood biochar 2%	6.5 ± 0.03 b	6.6 ± 0.09 d	0.27 ± 0.00 e	0.34 ± 0.01 b
Hilo mixed wood biochar 2%	6.0 ± 0.03 d	6.0 ± 0.08 e	0.26 ± 0.01 e	0.35 ± 0.04 b
Vermicompost 2%	6.4 ± 0.11 cd	6.9 ± 0.09 bc	0.47 ± 0.02 bcd	0.42 ± 0.02 ab
Thermocompost 2%	6.3 ± 0.16 cd	6.5 ± 0.03 d	0.47 ± 0.01 bcd	0.49 ± 0.03 a
Lac tree wood 2% + vermicompost 2%	6.8 ± 0.05 ab	7.2 ± 0.09 ab	0.43 ± 0.00 d	0.40 ± 0.02 ab
Lac tree wood 2% + thermocompost 2%	6.7 ± 0.09 abc	7.1 ± 0.12 abc	0.46 ± 0.01 bcd	0.46 ± 0.01 a
Hilo mixed wood 2% + vermicompost 2%	6.9 ± 0.09 ab	7.2 ± 0.03 ab	0.49 ± 0.00 bc	0.44 ± 0.01 ab
Hilo mixed wood 2% + thermocompost 2%	6.3 ± 0.02 cd	6.8 ± 0.01 bcd	0.58 ± 0.02 a	0.44 ± 0.03 ab
Lime 2 $cmol_+/kg$ + vermicompost 2%	7.1 ± 0.02 a	7.3 ± 0.01 a	0.45 ± 0.01 cd	0.44 ± 0.01 ab
Lime 2 $cmol_+/kg$ + thermocompost 2%	6.8 ± 0.08 ab	7.1 ± 0.02 abc	0.52 ± 0.01 ab	0.41 ± 0.02 ab

Means within a column followed by the same letter(s) were not significantly different by Tukey's test at α = 5%.

Soil EC increased after 2 weeks of incubation and then decreased after the first harvest (Table 2). It increased from 0.35 to 0.47 dS/m (Ultisol) and from 0.30 to 0.37 dS/m (Oxisol). It then, however, decreased after the first harvest particularly in the Ultisol soil perhaps due to the removal of nutrients from the soil by cabbage plants. The increased EC in the Ultisol was significantly affected by compost alone, while the increased EC of the Oxisol 2 weeks after treatment was affected by the interaction of biochar and compost ($P<0.01$). Further increases of soil EC after 2 weeks of incubation were attributed to the compost alone. Such soil EC increases were attributed mainly to the basic cations (K, Ca, Mg) enrichment by both biochar and compost in the Oxisol and mostly by compost in the Ultisol (Tables 3a-b).

Soil exchangeable Al of acid Ultisol was reduced from 2.16 cmol+/kg to 1.27 and 0.17 cmol+/kg by the additions of the Hilo mixed wood and Lac tree biochars alone, respectively. Thermo-compost and Vermi-compost reduced the exchangeable Al to 0.14 cmol+/kg and undetected level, respectively. Combination of biochars and compost eliminated the exchangeable Al to undetected level (Fig. 1a). Exchangeable Al in the Oxisol was reduced from 0.12 cmol+/kg to undetectable level

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by additions of biochars or composts alone at 2% or in combination of both (Fig. 1b). For comparison, lime at 2 cmol+/kg in combination with composts reduced exchangeable Al to undetectable level in both soils.

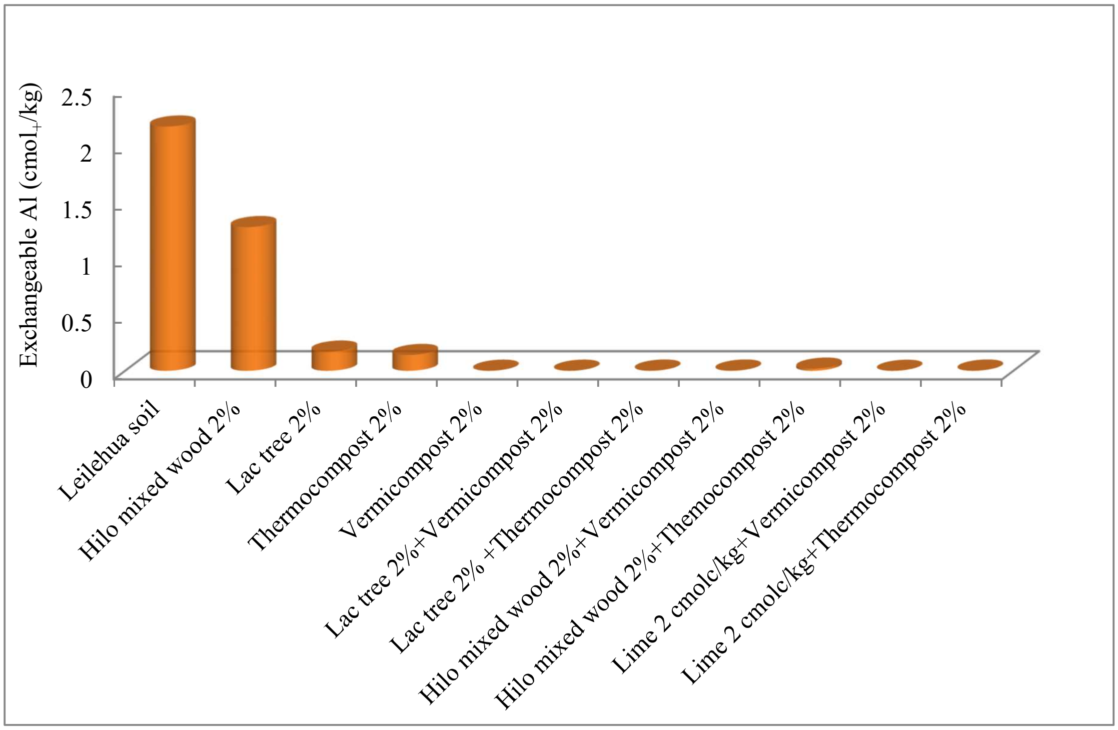

Figure1a. Exchangeable Al in the Leilehua Ultisol as affected by biochar and compost additions

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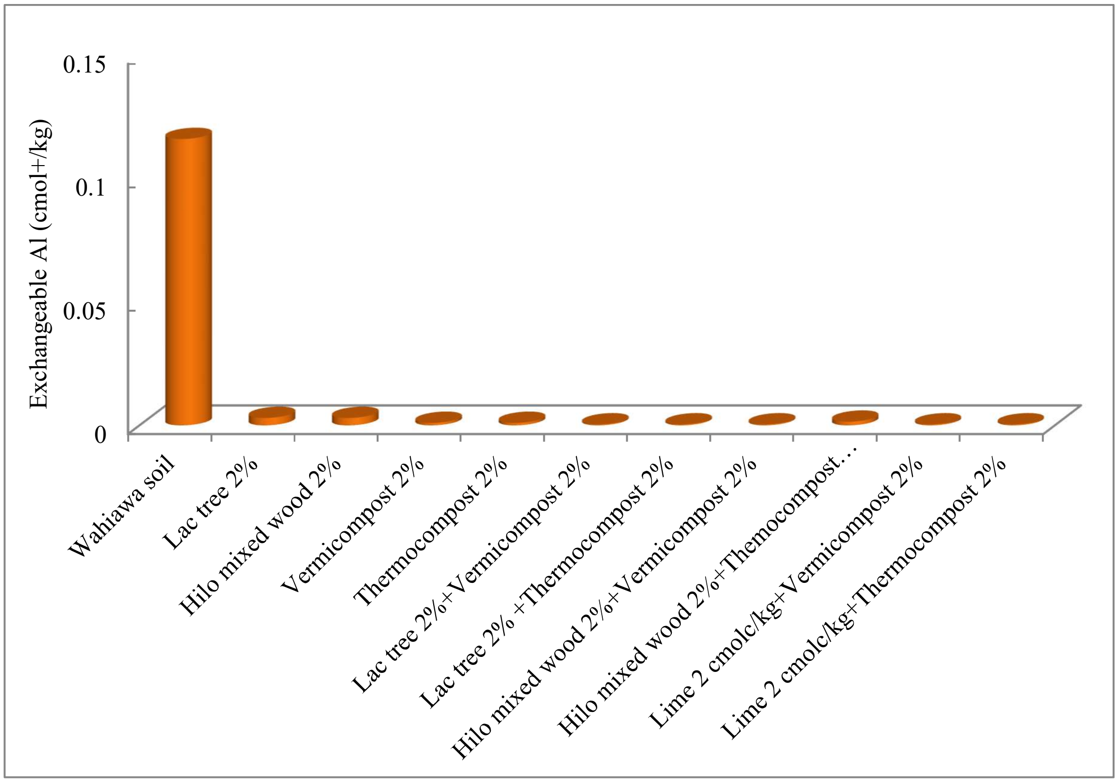

Figure 1b. Exchangeable Al in the Wahiawa Oxisol as affected by biochar and compost additions

3.2. Nutrient Content in Soils

Effects of added biochar and compost on soil nutrients were varied, depending on the nutrient, soil, compost and biochar types (Tables 3a-b). Nutrients in the Ultisol (with exception of Fe) were significantly enhanced by compost ($P<0.01$) alone, and only K, Ca, Mg, and Fe were increased by biochars in combination with composts ($P<0.01$), although there was no interaction effect between biochar and compost ($P>0.05$) on soil nutrients. Nutrients (with exception of Mn and Fe) in the Oxisol were significantly enriched by the added biochars or composts alone, and only P and K were significantly increased ($P<0.01$) by the interaction between biochar and compost. Increasing nutrient content by biochar and compost indicated that these amendments contain nutrients or make nutrients more available. For example, Ca was increased by the application of lac tree wood biochar alone at 2% from 111.8 mg/kg to 816.9 mg/kg in the Ultisol and from 715.9 mg/kg to 1514.4 mg/kg in the Oxisol. Such increases in Ca were more than twice of those increases resulted from the Hilo mixed wood biochar. This might be related to the the higher Ca content in the lac tree wood than in the Hilo mixed wood biochar. The combined lac tree wood biochar and vermicompost increased soil Ca the most. In contrast to Ca, K increased more than twice by the thermocompost in combination with either biochar than by the vermicompost, probably due to the higher K content of the thermocompost (Table 1). Mn and Fe in the Oxisol soil were sharply decreased by the lac tree wood biochar in combination with vermicompost from 805.8 mg/kg and 63.9 mg/kg to 361.2 mg/kg and 36.9 mg/kg, respectively, perhaps due to the higher liming potential of lac tree wood biochar that raised pH of the Ultisol, and precipitated Mn and Fe.

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Table 3a. Means and standard errors of P, K and Ca in the soils as affected by biochars and composts additions (n=3)

Treatments	P 2 weeks	P 7 weeks	K 2 weeks	K 7 weeks	Ca 2 weeks	Ca 7 weeks
				mg/kg		
Ultisol						
Lac tree wood biochar 2%	0.8±0.1 b	1.0±0.1 b	25.5±0.8 c	70.2±2.1 d	816.9±40.7 bc	1112.7±8.6 cd
Hilo mixed wood biochar 2%	1.1±0.2 b	0.9±0.3 b	8.7±8.3 cd	48.2±2.9 e	315.5±8.5 c	411.3±89.9 e
Vermicompost 2%	75.6±3.5 a	133.4±5.4 a	0.0 d	0.0 e	4038.5±28.6 a	4408.9±339.4 a
Thermocompost 2%	3.4±0.7 b	4.10±0.2 b	82.1±4.3 b	204.2±4.5 c	1044.9±39.3 bc	1076.5±67.4 c
Lac tree wood 2% + vermicompost 2%	68.0±13.9 a	146.2±14.1 a	0 d	63.2±7.9 d	4166.5±600.6 a	5021.0±227.6 a
Lac tree wood 2% + thermocompost 2%	3.1±0.2 b	5.8±0.1 b	140.7±16.9 a	312.6±8.9 a	1679.9±107.1 b	2009.9±51.94 b
Hilo mixed wood 2% + vermicompost 2%	61.8±3.5 a	125.4±4.5 a	0 d	50.0±1.5 d	3548.6±350.8 a	4615.8±127.7 a
Hilo mixed wood 2% + thermocompost 2%	3.7±0.4 b	6.5±0.6 b	114.8±17.3 ab	274.6±5.6 b	1230.1±34.6 bc	1337.4±38.5 bc
Lime 2 cmol+/kg + vermicompost 2%	72.9±0.8 a	128.5±1.6 a	0.0 d	0.0 e	4171.2±32.8 a	5004.5± 164.1 a
Lime 2 cmol+/kg + thermocompost 2%	2.3±0.2 b	5.5±0.1 b	93.7±0.1 ab	209.4±1.4 c	1529.1±56.4 bc	1899.6±1.6 bc
Oxisol						
Lac tree wood biochar 2%	37.7±1.6 b	40.2±1.2 d	106.3±14.0 d	136.5±2.4 ef	1514.4±8.7 cd	1695.1±0.6 d
Hilo mixed wood biochar 2%	35.8±0.7 b	36.7±0.0 d	64.3±2.9 d	119.9±0.6 f	864.2±5.8 d	939.3±58.8 e
Vermicompost 2%	285.1±16.8 a	365.4±3.9 a	65.6±11.9 d	117.4±4.2 f	4367.9±260.3 a	4609.8±28.9 b
Thermocompost 2%	56.8±1.7 b	60.7±1.3 c	241.5±4.5 bc	356.2±1.8 b	1556.4±6.3 cd	1962.2±178.5 d
Lac tree wood 2% + vermicompost 2%	327.4±31.2 a	341.6±2.3 b	73.9±4.0 d	162.5±0.2 de	4869.6±45.8 a	5504.8±131.8 a
Lac tree wood 2% + thermocompost 2%	60.5±3.7 b	68.8±1.1 c	313.9±16.2 a	406.7±11.4 a	2528.5±263.9 b	2770.6±57.9 c
Hilo mixed wood 2% + vermicompost 2%	270.9±11.1 a	367.9±7.2 a	93.6±11.4 d	174.7±1.4 d	4178.1±218.8 a	4682.6±155.9 b
Hilo mixed wood 2% + thermocompost 2%	64.6±2.0 b	70.2±1.1 c	271.6±20.9 ab	419.3±0.3 a	1708.2±41.0 c	1896.0±103.4 d
Lime 2 cmol+/kg + vermicompost 2%	295.6±13.1 a	353.0±4.2 ab	52.9±8.5 d	76.1±3.4 g	4738.2±47.3 a	5512.5±157.1 a
Lime 2 cmol+/kg + thermocompost 2%	57.5±7.2 b	62.7±0.1 c	186.5±4.62 c	304.7±8.5 c	2138.3±19.5 cb	2608.4±96.4 c

Means within a column followed by the same letter(s) were not significantly different by Tukey's test at $\alpha = 5\%$.
2 and 7: weeks after the soils treated with biochars and composts

Table 3b. Means and standard errors of Mg, Fe and Mn in the Leilehua Ultisol as affected by biochars and composts additions (n=3)

Treatments	Mg 2 weeks	Mg 7 weeks	Fe 2 weeks	Fe 7 weeks	Mn 2 weeks	Mn 7 weeks
				mg/kg		
Ultisol						
Lac tree wood biochar 2%	136.2 ± 4.4cd	92.5 ± 2.6 f	106.1 ± 2.1 a	109.19±3.01 a	8.1 ± 0.2 b	8.1 ± 0.3 d
Hilo mixed wood biochar 2%	134.6 ± 4.4d	70.9 ± 3.5 g	103.9 ± 2.5 a	103.94±2.73 a	7.7 ± 0.5 b	8.8 ± 0.3 d
Vermicompost 2%	211.1 ± 7.1ab	185.8 ±7.7cd	108.9 ± 0.7 a	97.36±0.98 a	24.0 ± 3.9 a	19.6 ± 1.6 ab
Thermocompost 2%	211.9 ± 9.2ab	156.1 ± 4.9 e	115.7 ± 3.9 a	103.61±0.29 a	18.8 ± 1.1 ab	14.6 ± 0.2 c
Lac tree wood 2% + vermicompost 2%	183.1±18.3bc	209.5 ±5.3bc	113.0 ± 4.5 a	115.07±13.05 a	23.1 ± 0.7 a	20.3 ± 1.3 a
Lac tree wood 2% + thermocompost 2%	209.9 ± 7.7ab	183.9 ±3.0cd	109.30 ± 6.5 a	118.65±1.27 a	24.9 ± 3.1 a	14.7 ± 0.1 c
Hilo mixed wood 2% + vermicompost 2%	195.6 ± 1.4ab	192.4 ±3.3cd	105.9 ± 1.2 a	111.34±0.54 a	26.9 ± 0.9 a	18.4 ± 1.5 a
Hilo mixed wood 2% + thermocompost 2%	198.4 ± 7.3ab	168.9 ±4.8de	122.4 ± 12.0 a	117.16±2.13 a	24.2 ± 1.1 a	14.4 ± 0.0 c
Lime 2cmol+/kg + vermicompost 2%	240.0 ± 2.4 a	248.0 ±5.9 a	99.0 ± 0.4 a	99.02±2.73 a	23.0 ±1.6 a	18.4 ± 0.0 bc
Lime 2 cmol+/kg + thermocompost 2%	230.6 ±11.6ab	232.0 ±2.2ab	101.0 ± 0.8 a	103.55±8.97 a	16.9 ± 3.0 ab	14.4 ± 0.3 c
Oxisol						
Lac tree wood biochar 2%	284.6 ± 7.1 cd	250.8 ± 4.1c	47.3 ± 10.0 a	40.92±0.19 b	517.2 ±92.2a	428.7±24.9bcd
Hilo mixed wood biochar 2%	266.2 ± 3.6 d	232.1 ± 3.5 c	47.0 ± 0.1 a	46.36±1.77 b	526.3 ±14.4a	506.1±30.4abc
Vermicompost 2%	351.6± 4.9 abc	358.3 ± 9.1 b	43.4 ± 1.3 a	39.16±0.46 b	422.7 ±1.2a	366.3±4.3 cd
Thermocompost 2%	343.9 ± 7.9 bc	357.5 ± 8.8 b	54.6 ± 2.9 a	45.93±1.03 b	526.3 ±14.3a	449.0±0.4 bcd
Lac tree wood 2% + vermicompost 2%	369.1 ± 6.8 ab	365.3 ± 0.4 b	44.8 ± 2.3 a	40.47±1.78 b	419.3 ±4.4a	360.9 ±7.0 d
Lac tree wood 2% + thermocompost 2%	401.1± 40.8 ab	353.8 ± 5.4 b	60.8 ± 3.6 a	47.26±0.42 b	634.0 ±68.1a	481.6 ±8.3 bcd
Hilo mixed wood 2% + vermicompost 2%	335.2±3.2bcd	361.5 ± 5.5 b	49.0 ± 0.1 a	41.60±0.64 b	464.0 ±13.8a	425.9±10.5bcd
Hilo mixed wood 2% + thermocompost 2%	350.0 ±1.2abc	342.0 ± 5.9 b	58.5 ± 2.1 a	54.97±0.20 b	593.4 ±10.1a	557.1 ± 2.4 ab
Lime 2 cmol+/kg + vermicompost 2%	422.0 ± 2.0 a	406.8 ± 0.1 a	47.1 ± 1.4 a	48.11±5.59 b	467.4 ±9.5a	415.2±44.1bcd
Lime 2 cmol+/kg + thermocompost 2%	391.4 ± 3.6 ab	402.8 ± 1.4 a	59.9 ± 3.4 a	73.18±7.68 a	611.4 ±47.9a	636.6 ± 54.1 a

Means within a column followed by the same letter(s) were not significantly different by Tukey's test at $\alpha = 5\%$.

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2 and 7: weeks after the soils treated with biochars and composts

3.3. Plant Growth

Chinese cabbage (*Brassica rapa*) growth in the Ultisol expressed in shoot dry matter in the second planting was significantly (P<0.05) affected by the interaction of biochar and compost, while shoot fresh matter, total fresh and dry weights were significantly increased (P<0.05) by the lac tree wood biochar or compost alone. For example, the shoot fresh weights of cabbage at the first planting in the Ultisol ranged from 4.9 to 29.5 g, and the best growth expressed in shoot or total fresh and dry weights was obtained from the application of lac tree wood biochar in combination with vermicompost (Fig. 2). Shoot, root and total fresh and dry weights of the first planting in the Oxisol were significantly increased by the interaction between biochars and composts; however, there were o significant differences among the treatments.

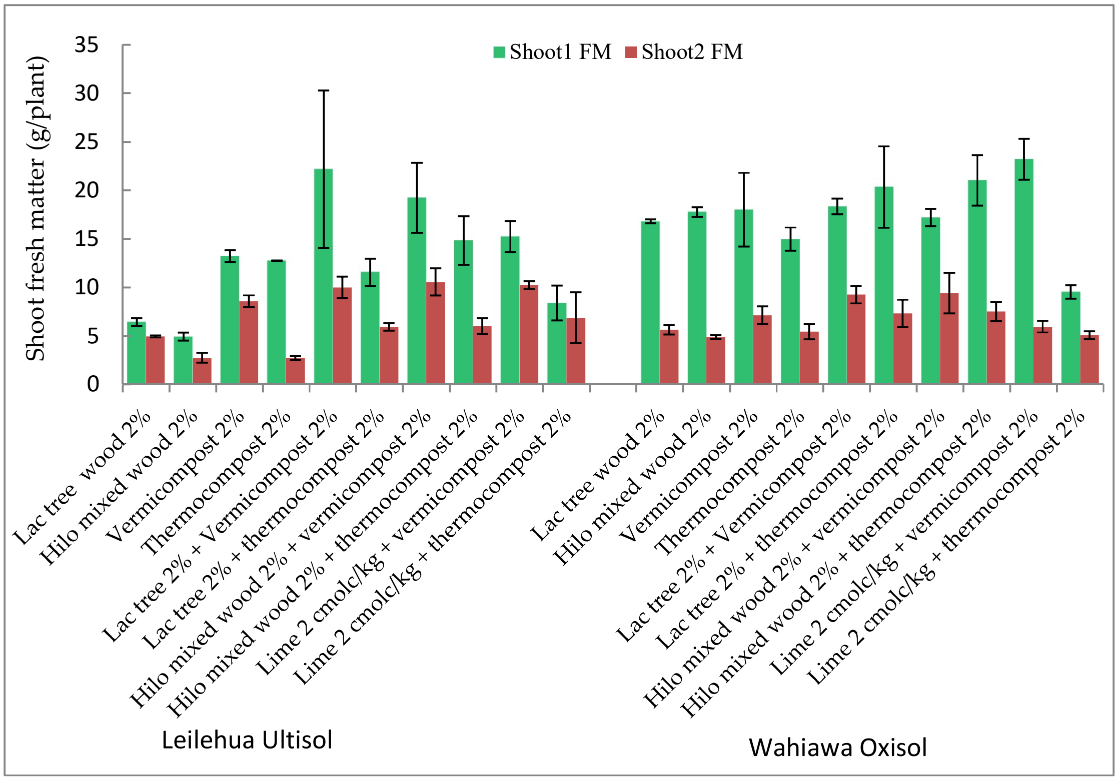

Figure 2. Chinese cabbage shoot fresh weight as affected by biochar and compost additions. FM = fresh matter, 1 and 2 : first and second plantings; Error bars : Se (n=3)

3.4. Plant Nutrients

Phosphorus, K, and Mn concentration in the Chinese cabbage tissues of both plantings in the Ultisol were significantly affected by biochars and composts additions (Table 4a); P and Mn in the Oxisol was significantly affected by biochars (Table 4b); and plant Ca in both soils was significantly increased by the interaction between biochar and compost. Plant P in treatments with compost alone or in combination with biochar was higher than P in plants treated with biochar alone, probably due to the lower P initial content in the biochars. This might be also the reason why P in plants treated with biochar alone was lower than 0.4% (a deemed critical level for cabbage growth). It was likely that the added biochar attributed more to their liming effects than P addition. Nutrient uptake by Chinese cabbage in the Ultisol for N, P, K and Mg was significantly improved by the lac tree biochar

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in combination with the vermicompost, while nutrient uptake in the Oxisol was not significantly different among the treatments (Table 5, Figs. 3a and 3b).

Table 4a. Means and standard errors of N, P and K in Chinese cabbage tissues as affected by biochar and compost additions (n=3)

Treatments	N		P		K	
	1st planting	2nd planting	1st planting	2nd planting	1st planting	2nd planting
				%		
Leilehua Ultisol						
Lac tree wood biochar 2%	1.13±0.03 a	1.74±0.25 a	0.28±0.05 b	0.22±0.02 cd	3.64±0.59 ab	3.46±0.08 ab
Hilo mixed wood biochar 2%	1.68±0.07 a	1.48±0.49 a	0.29±0.09 b	0.05±0.03 d	2.51±0.89 ab	1.53±0.17 b
Vermicompost 2%	1.04±0.10 a	1.51±0.02 a	0.34±0.03 ab	0.57±0.10 ab	3.04±0.35 ab	2.65±0.03 ab
Thermocompost 2%	1.86±0.43 a	1.66±0.16 a	0.65±0.18 ab	0.08±0.01 cd	2.67±0.16 ab	2.40±0.24 ab
Lac tree wood 2% + vermicompost 2%	1.28±0.09 a	1.72±0.16 a	0.55±0.07 ab	0.47±0.15 abc	2.87±0.58 ab	3.47±0.75 ab
Lac tree wood 2% + thermocompost 2%	1.12±0.14 a	1.63±0.10 a	0.57±0.01 ab	0.21±0.03 cd	3.57±0.12 ab	4.25±0.77 ab
Hilo mixed wood 2% + vermicompost 2%	1.64±0.46 a	1.39±0.04 a	0.78±0.01 a	0.57±0.01 ab	2.87±0.13 ab	3.11±0.65 ab
Hilo mixed wood 2% + thermocompost 2%	1.09±0.25 a	1.77±0.29 a	0.51±0.09 ab	0.19±0.04 cd	3.83±0.33 ab	3.46±0.35 ab
Lime 2 cmol$_{+}$/kg + vermicompost 2%	1.30±0.22 a	1.95±0.57 a	0.63±0.16 ab	0.69±0.04 a	2.11±0.32 b	3.24±0.13 ab
Lime 2 cmol$_{+}$/kg + thermocompost 2%	1.15±0.03 a	1.32±0.15 a	0.53±0.02 ab	0.29±0.12 bcd	5.04±1.01 a	4.72±0.57 a
Wahiawa Oxisol						
Lac tree wood biochar 2%	1.56±0.29 a	1.13±0.01 a	0.64±0.14 a	0.59±0.00 a	4.84±0.69 a	3.68±0.29 a
Hilo mixed wood biochar 2%	1.26±0.15 a	1.28±0.32 a	0.41±0.12 a	0.57±0.09 a	4.57±0.63 a	4.06±0.14 a
Vermicompost 2%	1.27±0.08 a	1.42±0.15 a	0.52±0.05 a	0.95±0.07 a	2.68±0.20 a	3.43±0.19 a
Thermocompost 2%	1.35±0.13 a	1.62±0.76 a	0.55±0.11 a	0.90±0.03 a	3.09±0.07 a	4.94±0.30 a
Lac tree wood 2% + vermicompost 2%	1.31±0.11 a	1.35±0.16 a	0.79±0.01 a	0.74±0.22 a	3.38±1.11 a	3.95±0.51 a
Lac tree wood 2% + thermocompost 2%	1.32±0.14 a	1.61±0.49 a	0.69±0.03 a	0.56±0.19 a	2.96±0.07 a	4.02±1.45 a
Hilo mixed wood 2% + vermicompost 2%	1.81±0.19 a	1.85±0.45 a	0.63±0.09 a	0.73±0.07 a	4.24±0.72 a	4.91±0.81 a
Hilo mixed wood 2% + thermocompost 2%	1.17±0.05 a	1.73±0.14 a	0.68±0.08 a	0.81±0.05 a	3.41±0.02 a	4.61±0.49 a
Lime 2 cmol$_{+}$/kg + vermicompost 2%	1.41±0.32 a	1.46±0.16 a	0.86±0.00 a	0.50±0.03 a	3.76±1.28 a	2.54±0.38 a
Lime 2 cmol$_{+}$/kg + thermocompost 2%	1.63±0.15 a	1.53±0.01 a	0.75±0.18 a	0.53±0.09 a	3.49±0.07 a	3.02±0.41 a
Sufficiency level for *Brassica rapa* (%)	3-4		0.4-0.7		4.5-7.5	

Table 4b. Means and standard errors of Ca, Mg, Fe and Mn in Chinese cabbage tissues as affected by biochar and compost additions (n=3)

Treatments	Ca		Mg		Fe		Mn	
	1st planting	2nd planting	1st planting	2nd planting	1st planting	2nd planting	1st planting	2nd planting
	%		%		mg/kg		mg/kg	
Leilehua Ultisol								
Lac tree wood biochar 2%	3.38±0.08 a	3.91±1.06 a	0.56±0.06 a	0.98±0.25 a	120.0±58.3 a	81.6±18.3 a	42.8±21.5 a	64.7±23.9 a
Hilo mixed wood biochar 2%	2.28±0.41 a	2.08±0.04 a	0.64±0.09 a	0.96±0.24 a	192.8±41.4 a	77.7±25.4 a	79.0±33.9 a	71.9±30.7 a
Vermicompost 2%	2.57±0.57 a	3.29±0.05 a	0.59±0.09 a	0.85±0.15 a	104.1±8.0 a	71.2±18.7 a	31.1±0.4 a	75.5±14.9 a
Thermocompost 2%	3.59±1.09 a	2.21±0.30 a	0.49±0.08 a	0.53±0.04 a	118.0±6.9 a	59.3±29.3 a	25.8±7.8 a	42.8±10.5 a
Lac tree wood 2% + vermicompost 2%	3.87±0.21 a	4.09±0.59 a	0.43±0.03 a	0.65±0.19 a	69.6±7.6 a	50.9±6.1 a	14.2±3.8 a	61.7±9.3 a
Lac tree wood 2% + thermocompost 2%	2.70±0.14 a	3.58±0.35 a	0.56±0.07 a	0.70±0.07 a	119.5±44.1 a	82.9±7.8 a	41.7±4.4 a	73.1±7.9 a
Hilo mixed wood 2% + vermicompost 2%	3.51±0.43 a	4.16±0.21 a	0.42±0.02 a	0.70±0.09 a	88.5±22.6 a	64.1±11.8 a	16.3±2.0 a	50.9±11.9 a
Hilo mixed wood 2% + thermocompost 2%	2.43±0.33 a	2.57±0.02 a	0.51±0.00 a	0.57±0.12 a	92.6±1.9 a	144.9±60.5 a	36.0±10.6 a	61.4±12.8 a
Lime 2 cmol$_{+}$/kg + vermicompost 2%	3.50±0.19 a	2.51±0.85 a	0.52±0.12 a	0.75±0.07 a	126.4±9.1a	69.6±22.3 a	26.4±1.4 a	82.8±0.7 a

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Lime 2 cmol$_+$/kg + thermocompost 2%	3.84±0.05 a	4.38±0.51 a	0.74±0.06 a	0.92±0.16 a	145.9±21.4 a	92.0±10.2 a	74.3±8.9 a	78.6±40.3 a
Wahiawa Oxisol								
Lac tree wood biochar 2%	3.06±0.32 ab	3.57±0.16 a	0.51±0.04 a	0.83±0.10 a	197.4±87.5 a	108.4±36.4 a	113.7±18.8 a	145.9±34.1 a
Hilo mixed wood biochar 2%	2.32±0.41 b	3.15±0.81 a	0.65±0.08 a	1.08±0.29 a	81.2±23.3 a	108.4±42.9 a	103.7±20.3 a	120.3±30.8 a
Vermicompost 2%	2.07±0.08 b	3.79±0.03 a	0.39±0.01 a	0.73±0.00 a	106.6±19.2 a	57.7±6.3 a	73.3±12.7 a	163.5±15.9 a
Thermocompost 2%	3.04±0.65 ab	3.56±0.86 a	0.64±0.12 a	0.94±0.15 a	165.1±8.4 a	230.9±61.3 a	75.8±3.4a	113.9±30.6 a
Lac tree wood 2% + vermicompost 2%	3.01±0.27 ab	2.35±0.92 a	0.49±0.04 a	0.92±0.09 a	133.4±17.8 a	74.1±6.6 a	115.8±10.5 a	196.7±3.0 a
Lac tree wood 2% + thermocompost 2%	2.71±0.21 ab	3.26±0.01 a	0.44±0.03 a	0.81±0.02 a	67.4±4.9 a	80.3±17.8 a	122.5±6.3 a	160.1±9.6 a
Hilo mixed wood 2% + vermicompost 2%	3.27±0.53 ab	3.88±0.60 a	0.47±0.07 a	0.69±0.04 a	120.1±80.2 a	83.4±4.5 a	113.2±25.9 a	181.3±27.1 a
Hilo mixed wood 2% + thermocompost 2%	2.18±0.19 b	3.73±0.42 a	0.45±0.05 a	0.83±0.03 a	80.29±1.5 a	100.9±44.0 a	80.9±2.2 a	108.2±0.3 a
Lime 2 cmol$_+$/kg + vermicompost 2%	4.35±0.15 a	3.36±0.26 a	0.62±0.03 a	0.63±0.00 a	140.1±62.3 a	70.7±26.9 a	125.9±29.4 a	159.7±25.3 a
Lime 2 cmol$_+$/kg + thermocompost 2%	2.75±0.04 b	3.02±0.04 a	0.48±0.00 a	0.83±0.15 a	119.6±14.3 a	64.5±21.3 a	125.2±13.3 a	116.9±2.4 a
Sufficiency level for *Brassica rapa*	1.9-6.0		0.23-0.75		40-300		25-200	

Means within a column followed by the same letter(s) were not significantly different by Tukey's test at α = 5%.

Table 5.Total nutrient uptake by *Brassica rapa* in Leilehua Ultisol as affected by biochar and compost additions (n=3)

Treatments	N	P	K	Ca	Mg	Fe	Mn
				mg/plant			
Leilehua Ultisol							
Lac tree wood biochar 2%	20.7±0.1 bc	4.0±1.0 b	54.4±1.4 bcd	3.91±1.06 a	11.1±0.4 dc	0.17±0.06 a	0.08±0.01 a
Hilo mixed wood biochar 2%	11.4±4.2 c	11.4±4.2 b	17.6±9.2 d	2.08±0.04 a	5.7±0.6 d	0.1±0.01 a	0.06±0.04 a
Vermicompost 2%	35.9±0.8 abc	12.6±1.1 ab	85.6±4.4 abcd	3.29±0.05 a	20.5±0.0 ab	0.27±0.03 a	0.14±0.01 a
Thermocompost 2%	28.4±10.7 bc	8.5±4.1 ab	39.2±10.2 cd	2.21±0.30 a	7.2±0.6 d	0.16±0.05 a	0.05±0.01 a
Lac tree wood 2% + Vermicompost 2%	66.5±11.7 a	26.6±8.0 a	139.7±7.6 a	4.09±0.59 a	22.9±2.5 a	0.31±0.05 a	0.12±0.0 a
Lac tree wood 2% + Thermocompost 2%	28.1±1.5 bc	9.2±0.4 ab	82.4±12.2 abcd	3.58±0.35 a	13.2±0.8 bcd	0.23±0.07 a	0.12±0.01 a
Hilo mixed wood 2% + vermicompost 2%	52.9±0.6 ab	25.9±5.7 a	110.9±6.1 abc	4.16±0.21 a	19.8±0.2 abc	0.27±0.01 a	0.11±0.01 a
Hilo mixed wood 2% + Thermocompost 2%	39.7±4.5 abc	13.1±0.1 ab	122.2±18.2 ab	2.57±0.02 a	18.2±0.8 abc	0.29±0.06 a	0.14±0.02 a
Lime 2 cmol$_+$/kg + vermicompost 2%	30.8±6.5 bc	14.5±3.2 ab	55.7±18.5 bcd	2.51±0.85 a	12.7±3.1 bcd	0.24±0.05 a	0.09±0.04 a
Lime 2 cmol$_+$/kg + thermocompost 2%	25.6±2.9 bc	9.0±0.2 ab	104.2±23.7 abc	4.38±0.51 a	17.1±2.8 abc	0.26±0.04 a	0.15±0.06 a
Wahiawa Oxisol							
Lac tree wood biochar 2%	39.7±4.3 ab	17.2±2.0 a	124.7±6.5 a	88.7±4.4 ab	16.6±1.1 a	0.47±0.13 a	0.34±0.0 abc
Hilo mixed wood biochar 2%	32.8±0.4 ab	11.7±1.7 a	117.1±10.3 a	64.9±2.9 ab	19.4±0.2 a	0.22±0.02 a	0.28±0.02 bc
Vermicompost 2%	41.1±11 ab	19.6±4.2 a	88.0±16.4 a	80.2±20.7 ab	15.1±3.3 a	0.27±0.05 a	0.29±0.03abc
Thermocompost 2%	32.9±0.9 ab	15.0±1.2 a	85.3±8.1 a	71.6±1.4 ab	16.4±0.0 a	0.41±0.15 a	0.20±0.03 c
Lac tree wood 2% + Vermicompost 2%	43.9±1.3 ab	25.9±0.7 a	124.5±33.2 a	91.9±16.6 ab	20.9±0.1 a	0.37±0.01 a	0.47±0.02 a
Lac tree wood 2% + Thermocompost 2%	41.1±2.2 ab	20.9±0.4 a	105.9±9.6 a	88.2±2.3 ab	16.7±1.0 a	0.21±0.01 a	0.42±0.01 ab
Hilo mixed wood 2% + vermicompost 2%	58.2±2.4 a	21.8±2.7 a	148.5±2.8 a	113.7±17.6 ab	18.2±1.6 a	0.34±0.03 a	0.45±0.07 ab
Hilo mixed wood 2% + Thermocompost 2%	46.4±9.8 ab	25.5±6.7 a	134.2±30.9 a	93.7±29.1 ab	19.7±6.0 a	0.29±0.03 a	0.32±0.05abc
Lime 2 cmol$_+$/kg + vermicompost 2%	48.5±0.5 ab	27.7±5.8 a	117.5±6.7 a	144.5±23.2 a	22.2±5.3 a	0.41±0.10 a	0.45±0.01 ab
Lime 2 cmol$_+$/kg + thermocompost 2%	26.3±1.8 b	10.9±0.1a	55.6±9.3 a	47.7±6.4 b	10.2±2.1 a	0.17±0.05 a	0.20±0.01 c

Means within a column followed by the same letter(s) were not significantly different by Tukey's test at α = 5%.

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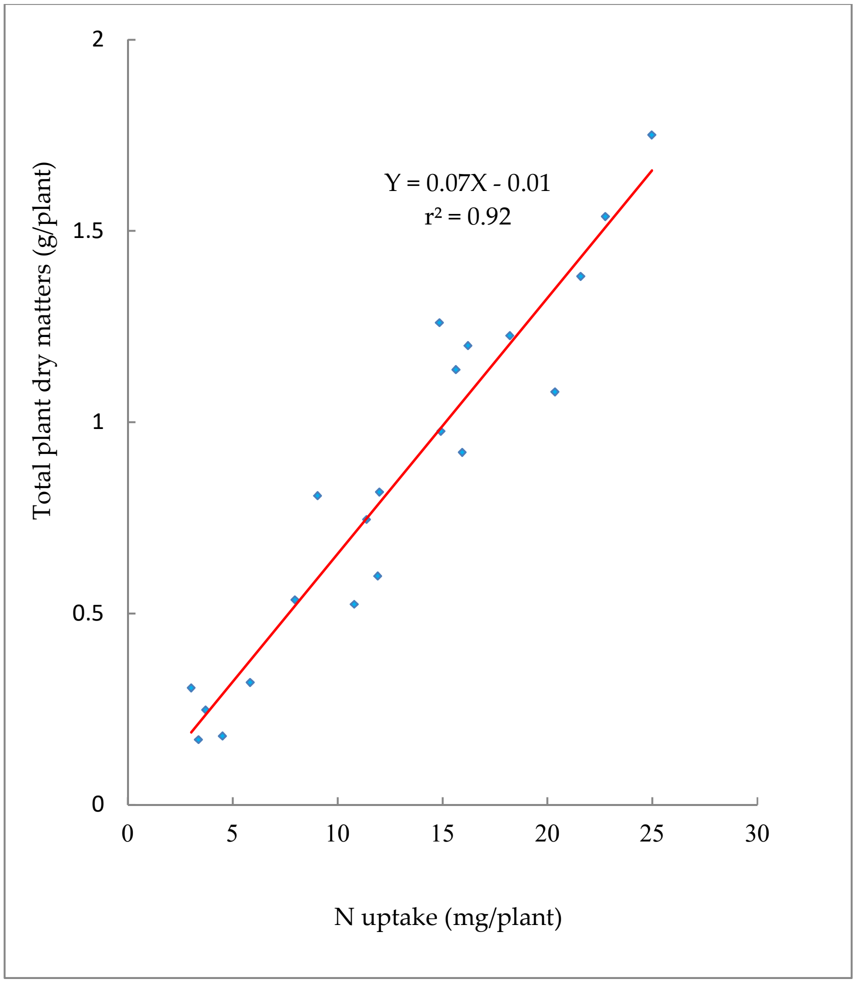

Figure 3a. Relationship between N uptake and total dry matter of Chinese cabbage of second planting in the Leilehua Ultisol soil

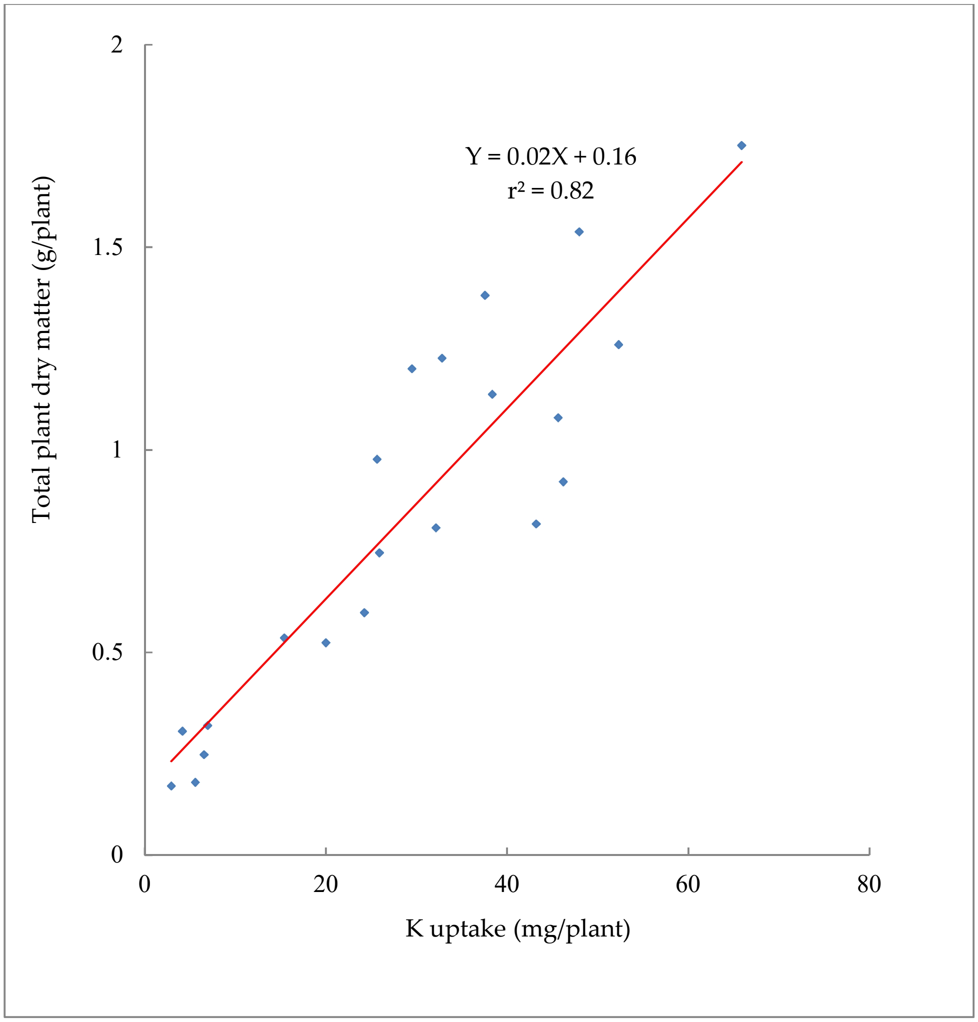

Figure 3b. Relationship between K uptake and total dry matter of Chinese cabbage of second planting in the Leilehua Ultisol soil.

4. Discussion

Additions of biochars in combination with composts increased acid soils pH and decreased exchangeable aluminum. Such effects could be attributed to the alkalinity or liming capacity of biochar. The basic cations, such as Ca^{2+}, Mg^{2+}, K^{+}, in the form of oxides or carbonates contained in the biochars can produce OH^{-} which in turn increase the soil pH and precipitated "active" Al [29, 30]. Carbonate content is responsible to the biochar alkalinity [31,32] particularly in biochars produced at high temperature [33]. The closed correlation (r^2 = 0.84) between alkalinity and basic cations in the biochars has also been shown by [34], and the carbonate content is closely correlated (r^2 = 90) with the basic cations [30]. Soil EC was also increased with biochars and composts. Increasing soil EC could be attributed to the release of basic cations from both biochars and composts. A similar result was reported by [35].

Incorporation of biochars and composts into acid soils enhanced nutrients in the soils The increase of nutrients in soils were attributed to: (1) the release of such nutrients directly from composts and biochars (as nutrient sources); nutrients such as N, P and K were released by composts and some of them were released by biochars [2,36]; (2) soil properties changes by biochar, e.g. the increase of soil pH that can solubilize nutrients such as P or precipitated Al and Fe [30]; (3)

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adsorption of nutrients by the surface charge or the micropore structure of biochar and the complexation of Al and Fe by organic acids and functional groups of composts and biochars [37]; (4) decreasing nutrient leaching such as NO_3^- and P by improving water-holding capacity of the soils [6,38,39], and (5) the formation of organic coating in the outer and inner pores of biochar when it was co-composted or added in combination with compost which then acted as a glue for water and nutrient retention [21,40].

The Chinese cabbage growth in the acid soils treated with biochars and composts was enhanced. The likely reasons for the increased growth especially in the Ultisol, would be the pH increase from 4.5 to 5.9, decreased Al from 2.17 cmol+/kg to 0.17 cmol+/kg, in addition to the release of nutrients into the soils and subsequently uptake by the plants as reported by one study [36] for ten composts. Similar result was reported by [41] who showed that application of biochar in combination with compost and low rate inorganic nitrogen fertilizer increased barley yield 54-60% compared to yield obtained from the maximum inorganic nitrogen fertilizer rate. In the second planting, the fresh and dry weights of cabbage in both soils decreased to almost 50% of the first planting (Fig. 2) perhaps due to the deficiency of nutrients such as N and K. The tight linear relationship between total dry matters and N and K uptake suggested such deficiencies (Figs. 3a and 3b).

Nutrients in plant tissues, except N and K (Tables 4a and 4b), were in the adequate range for the normal growth of cabbage in both plantings [42]. The sufficiency of P, Ca, Mg, Fe, and Mn in cabbage plant tissues in both plantings indicated an improvement of such nutrients --supply and retention-- in these highly weathered soils by the addition of biochars and composts.

5. Conclusions

The nutrient retention capacity of biochars in combination with composts was assessed in two highly weathered, tropical soils of Hawai'i (Oxisol, Wahiawa series; and Ultisol, Leilehua series). The interaction between biochar and compost additions has significantly increased the pH of both soils, EC, P and K in the Oxisol, cabbage shoot and total fresh and dry matter in the Oxisol, plant tissue Ca in both soils, Ca and Mg uptake in the Oxisol, and Fe uptake in the Ultisol. Chinese cabbage growth in the Ultisol was enhanced by the addition of the lac tree wood biochar and vermicompost as a source of nutrients. Increased nutrient content in soil by compost in particular and some nutrients such as Ca by biochar, increased soil pH and decreased exchangeable Al, and subsequently increased nutrient content in plant tissues, was likely the reasons for the better cabbage growth in the acid Ultisol. The Chinese cabbage growth in the Oxisol was also enhanced by application of biochars in combination with composts. However, there were no significant differences among the treatments with respect to the plant growth. The sufficiency of nutrients in the plant tissues, with exception of N and K, for the cabbage growth in both plantings indicated an improvement of nutrients supply and retention in these highly weathered tropical soils by a combination of biochar and compost.

Author Contributions: Conceptualization-A.K.B and N.V.H; Methodology-A.K.B and N.V.H.; Software-A.K.B, N.V.H.; Validation-N.V.H, T.J.K.R. and A.A.A.; Formal Analysis-A.K.B.; Investigation-A.K.B; Resources-A.K.B., N.V.H., A.A.A.; Data Curation-A.K.B.; Writing-Original Draft Preparation-A.K.B; Writing-Review & Editing-N.V.H., T.J.K.R., A.A.A; Funding Acquisition-N.V.H.

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Conflicts of Interest: The authors declare no conflict of interest.

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