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Effects of Empty Fruit Bunch Biochar and Nitrogen–Fixing Bacteria on Soil Properties and Growth of Sweet Corn

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ABSTRACT

Empty fruit bunch (EFB) biochar is being used as soil amendment to improve soil productivity of infertile soil for enhanced plant growth. Tropical soils are generally unfertile with, low organic matter, plant nutrients, acidic and low microorganisms that affect crop production. Addition of biochar such as empty fruit bunch (EFB) could improve the soil fertility. Laboratory and glasshouse studies were conducted to determine the effect of EFB biochar and nitrogen-fixing bacteria *Stenotrophomonas sp*. (Sb16) on soil microbial communities, enzyme activity, chemical properties and growth of sweet corn. Five rates of EFB biochar (0, 0.25, 0.5, 0.75 and 1%) were applied to soil either with or without bacteria Sb16 and incubated for 40 days under laboratory condition. Sweet corn were grown in pots containing 6 kg soil and applied with five rates of EFB biochar (0, 5, 10, 15 and 20 t/ha) with or without bacteria Sb16. The experiment was arranged in a randomized complete block design (RCBD), with 5 replications. Results of laboratory study showed that combination of EFB biochar at 0.5% without inoculation and 0.25% with bacteria Sb16 in both soil, significantly increased populations of soil bacteria, fungi, actinomycetes and N_2-fixing bacteria (NFB), enzymes (urease, acid phosphatase and fluorescein diacetate (FDA) hydrolysis activity), and soil chemical properties (pH, organic carbon, total N, available P and exchangeable K, Ca and Mg). The glasshouse experiment showed that application of EFB biochar at 5 t/ha with bacteria Sb16 significantly (p<0.05) improved growth of corn (shoot and root biomass, root length, root volume, plant height, leaf chlorophyll content and nutrient uptake). Addition of higher EFB biochar to soil negatively affected all the observed parameters. The studies showed that application of EFB biochar at 5 t/ha or 0.25% with N_2-fixing bacteria Sb16 and 10 t/ha or 0.5% without bacterial inoculation improved corn growth and the quality of soil for sustainable corn production.

Keywords: Empty fruit bunch (EFB) biochar. N_2-fixing bacteria *Stenotrophomonas sp*., sweet corn, soil enzymes, soil microbial properties.

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INTRODUCTION

Tropical soils are generally less fertile for crop growth. The soil can be effectively ameliorated by applying liming material that improves soil pH and organic matter or other amendments can be added to improve physical, chemical and biological properties of soil. Application of biochar such as empty fruit bunches (EFB) biochar can be an alternative to chemical fertilisers to improve soil fertility (Norazlina *et al.,* 2014). In general, biochar amendment which is alkaline in nature can improve soil pH for better plant growth. Biochar has the potential to offer multiple environmental benefits in that they do not only contribute to carbon storage, but at the same time act as a soil ameliorant (Cheng *et al.*, 2007). Biochar improves soil physical properties including pore-size distribution, total porosity, soil density, water holding capacity and soil moisture content (Atkinson *et al.*, 2010; Sohi *et al*., 2010). Addition of biochar to highly leached, infertile soils has been shown to increase the availability of basic cations (Glaser *et al*., 2002; Liang *et al.*, 2006), and significantly improve crop yields (Lehmann and Rondon, 2006). Enzymes are proteins produced by soil microbial community that increase reactions involved in soil organic matter and nutrient cycling (Lei *et al*., 2014). Soil enzymes, such as urease, phosphatase and FDA play an important role in the decomposition of organic matter, nutrient cycling for microbial activity and plant nutrient uptake (Lammirato *et al.*, 2011).

Biochar has the potential to release a wide range of organic and inorganic molecules and may provide a mechanism to protect these enzymes (Castaldi *et al.*, 2012; Lehmann *et al.*, 2011), but in general, there is little information on the possible impacts of biochar on soil enzymes. Biochar improves plant nutrients in soil and biological activity, thus enhancing soil microbial populations (Kim *et al.*, 2007; Unger and Killorn, 2012) through interactions with soil mineral, organic matter and microbial activities (Carson *et al.*, 2007; Nguyen *et al*., 2008). However, the relationship between chemical and physical properties of biochar and its influence on soil microbial activity and probable concomitant impacts on soil processes are poorly understood. Biochar can be applied as a soil ameliorant to enhance soil fertility and crop production in a wide range of soils (Blackwell *et al*., 2009). Addition of biochar is known to increase biomass of rice and cowpea (Lehmann *et al.*, 2003), sweet corn and soybeans (Singer *et al*., 2007), and maize grain (Major *et al.*, 2010). Increased nutrients retention by biochar may be the most important factor for increasing crop yields on infertile acid soils (Asai *et al*., 2009).

Beneficial bacteria such as nitrogen-fixing bacteria can be used to improve soil fertility by fixing N_2 and transferring the fixed N in soil. Several crops such as rice, wheat and maize need 20 to 40 kg soil N ha^{-1} to satisfy the N requirements for each tonne of grain produced (Peoples and Craswell, 1992). To fulfill such a demand for nitrogen, farmers must apply inorganic N fertilisers that have the potential to pollute the environment or rely on beneficial microbes such as biological nitrogen fixation (BNF) with the input of organic wastes, such as biochar. There are several bacteria that are capable of fixing atmospheric nitrogen.

They can be the free-living (non-symbiotic) bacteria associated with cereal crops and symbiotic bacteria associated with leguminous plants (Esawy *et al*., 2013). These bacteria transform atmospheric N_2 into ammonium (NH_4^+), a form of N that can be used directly by plants. Nitrogen cycling in natural ecosystems relies on N_2-fixing bacteria for agricultural production. N_2-fixing bacteria produce nitrogen, which is much more effective and less costly to improve plant growth. Free-living N_2 bacteria in the soil may provide substantial amounts of nitrogen (0 to 60 kg N ha^{-1} $year^{-1}$) (Burgmann *et al.*, 2004). This could be important in organically amended soils, which typically have a lower concentration of nitrogen in available forms. The EFB biochar can be applied with free-living N_2 fixing bacteria to improve soil fertility, microbial activity and plant growth. Therefore, this study was conducted to determine the effect of oil palm EFB biochar and N_2-fixing bacteria *Stenotrophomonas sp*. Sb16 on soil enzyme activity, microbial population, chemical properties and growth and nutrient uptake of sweet corn.

MATERIALS AND METHODS

Soil and EFB Biochar Preparation

Both laboratory and glasshouse experiments were conducted at the Faculty of Agriculture, Universiti Putra Malaysia (UPM). The soil samples were collected from the top soil (0 - 15 cm depth) of the UPM farm. Soil samples were air-dried for five days, ground and sieved through a 2.00 mm mesh for laboratory and 4.00 mm mesh for glasshouse study. The EFB biochar was made from empty fruit bunches of oil palm which was provided by a private company in Selangor. This biomass went through the pyrolysis process at a temperature of between 350-450^o C to produce EFB biochar. The chemical characteristics of soil and EFB biochar were analysed in the soil microbiology laboratory and the results are as shown in Table 1.

Preparation of Free- Living N_2-Fixing Bacteria

The bacterial culture of *Stenotrophomonas sp.* (Sb16) was used for soil inoculation (Radziah *et al.*, 2013). The bacteria were obtained from Faculty of Agriculture, UPM. The strain diversity of diazotrophs depends on the soil environment. In Malaysia, the tropical soils generally have low pH and thus favour low pH tolerant diazotrophs. Bacteria Sb16 is an endophyte which plays important roles in agricultural production as a plant growth-promoting bacteria or N_2-fixing bacteria. *Stenotrophomonas sp*. Sb16, formerly *Xanthomonas maltophilia* is widely found on or in plants and has a worldwide distribution. The strain was sub-cultured in 100 ml Erlenmeyer flask with Jensen's N-free broth and shaken continuously for 36 h (100 rpm at 28^o C) (Jensen, 1951), until reaching 10^8 (cfu / mL).

Laboratory Experiment

A factorial study was conducted using a completely randomised design (CRD) with three replications. Five rates of the oil palm EFB biochar (0, 0.25, 0.5, 0.75

and 1%) were applied to 150 g of sterilised soil in 250 mL conical flasks and the contents were mixed properly. Soils were inoculated with one milliliter of approximately 10^8 cfu mL^{-1} N_2-fixing bacteria (Sb16). Flasks were covered with aluminum foil and incubated for 40 days at room temperature (28 ± 2° C). The soils were analysed for microbial populations, enzyme activity and chemical properties.

Glasshouse Experiment

Six kilograms of sieved (4.00mm) soil was mixed thoroughly with EFB biochar at a rate of 0, 5, 10, 15 and 20 t ha^{-1} and placed in drained pots, either in the presence or absence of N_2-fixing bacteria (Sb16). The soil and EFB biochar mixture in pots were left to react for 20 days before planting with corn. Five uniform sweet corn seeds were planted 5.0 cm below the surface of soil in the pots and one mL of approximately 108cfu mL^{-1} of N_2-fixing bacteria (Sb16) was applied to each seed. All pots were watered daily. All pots were fertilised with urea (60 kg ha^{-1}), triple superphosphate (TSP) (60 kg ha^{-1}) and muriate of potash (MOP) (90 kg ha^{-1}) two weeks after sowing. The treatments were arranged in randomised complete block design (RC) with five replications. The corn plants were harvested at tasseling stage (55 days), separated into plant tops and roots, and were oven dried for 4 days at 65° C. The dry weights of plant parts were recorded and tissue was ground for chemical analysis.

Chemical Analysis of Soil and Biochar

Soil and EFB biochar pH were determined using the Beckman Digital pH meter in a 1:2.5 (w/v, soil: water) for soil and 1:10 (w/v, biochar:water) for EFB biochar (Gaspard *et al.*, 2007). Nelson and Sommers (1982). Total N was determined according to the Kjeldahl method (Bremner and Mulvaney, 1982) and available phosphorus using Bray and Kurtz no. 2 method (Bray and Kurtz, 1945) and analysed by the auto analyser (Lachat instruments, Quik Chem® FIA+ 8000 series). The CEC, K, Ca and Mg in soil were determined using the NH_4OAc, pH7.0, leaching method (Dawid and Dorota, 2014) and analysed by an atomic absorption spectrophotometer (AAS) (Perkin Elmer, 5100 PC). The nutrients in EFB biochar were determined by digestion technique and analysed by AAS.

Measurement of Leaf Chlorophyll Content Using SPAD-502 Meter

Chlorophyll concentration measurements by SPAD meter makes simple, rapid, and non-destructive measurements, providing a relative indication of leaf chlorophyll concentration compared to the extraction methods. The measurements for sweet corn leaf tissue were determined by taking the average of three readings for each leaf.

Determination of Plant Growth Parameters

Plant height was measured before harvesting by measuring the plant from the soil surface to the tip of the main stem. Five plants were harvested from each

treatment and separated into roots and shoot (stem and leaves). The plant parts were then oven dried at 65° C until the weight was stable and the dry weight was recorded using digital balance (QC 35EDES- Sartorius- Germany). Root volume was determined using a water displacement method.

Root Measurements

The root length was measured by using a root scanner (Model Epsom Expression 1680) which was connected to a computer program Win RHIZO 2007.

Soil Microbial Populations

The populations of soil microbial community (bacteria, fungi, actinomycetes and N_2-fixing bacteria) were determined using 10 g of fresh soil following the dilution plate technique (Parkinson *et al.,* 1971). An amount of 100 µl of sample at selected dilutions was transferred onto Nutrient Agar (NA) for bacteria, Rose Bengal Streptomycin Agar (RBSA) for fungi (Martin, 1950), Actinomycete Isolation Agar (A.A) for actinomycetes and N_2-free media for N_2-fixing bacteria, respectively. After incubation at room temperature (28° C ± 2) for 2 days, the colonies were counted and population determined as colony forming unit (cfu) g^{-1} dry $soil^{-1}$ and then transformed to $\log_{10}$ values for statistical analysis.

Determination of Soil Enzyme Activity

Soil samples were air-dried and sieved through a 2 mm sieve. Soil phosphatase activity was determined using the method described by Tabatabai and Bremner (1969). Soil urease activity was determined as described by Tabatabai and Bremner (1972). Fluorescein diacetate hydrolysis Assay (FDA) was conducted for measuring the enzyme activity of microbial populations which can provide an estimate of overall microbial activity in an environmental sample (Schnurer and Rosswall, 1982).

Chemical Analysis of Plant Tissue

Plant tissue (0.25 mm sieve) was digested with concentrated sulfuric acid and 50% hydrogen peroxide and analysed for P, K, Ca and Mg concentrations (Thomas *et al.*, 1967). Total plant N was done according to the Kjeldahl method (Bremner and Mulvaney, 1982). Plant nutrient uptake was obtained by multiplying the concentration of nutrients in plant tissue with the total plant dry matter weight divided by 100 (Jones, 1985).

Statistical Analysis

The laboratory study was conducted using CRD with five levels of EFB biochar, two bacterial treatments with and without N_2-fixing bacteria (Sb16) and replicated three times. The glasshouse experiment was carried out using RCBD, five rates of soil EFB biochar with and without of N_2-fixing bacteria with five replications. The data were recorded and analysed using two way analysis of variance (ANOVA) by Statistical Analysis System (SAS) version 9.3 for Windows. The significant

difference of treatment means was checked by the Tukey's General Linear Model Test (GLM) at the 5% level of confidence.

TABLE 1
The chemical analysis of soil and EFB biochar

Properties	**Soil**	**EFB Biochar**
pH water w/v	4.6	9.39
Carbon (%)	2.01	52
Total N (%)	0.1	1.58
Available P (mg/kg)	34	--
Total P (%)	--	0.22
Exch K (cmol $_+$ /kg)	0.2	--
Total K (%)	--	4.9
Exch Ca (cmol $_+$/kg)	2.3	--
Total Ca (%)	--	0.11
Exch Mg (cmol $_+$/kg)	0.8	--
Total Mg (%)	--	0.14
CEC (cmol $_+$ /kg)	8.1	63.2

RESULTS

Laboratory Study

Effects of EFB biochar and N_2-fixing bacteria (Sb16) on soil microbial populations

The total soil bacteria, fungi, actinomycetes and NFB increased significantly (p<0.05) with EFB biochar and N_2-fixing bacteria Sb16 application (Figure 1). EFB biochar amended soil was observed to stimulate the soil microbial community compared to control. Addition of EFB biochar at 0.25% positively improved soil microbial populations compared to non-amended soil. A lower microbial population was observed with increasing EFB biochar rates in both soils with or without inoculation. Application of EFB biochar at 0.75% and 1% with or without bacteria Sb16 inoculation adversely affected soil microbial activity. Enhancement of microbial population could be due to available nutrients in soil, which was affected by EFB biochar and bacteria Sb16 inoculation. Biochar may also provide a suitable habitat to protect beneficial microbes from predators in soil. The abundance of bacteria in biochar amended soils might be attributed to the beneficial properties and characteristics of EFB biochar (Ingram *et al*., 2005).

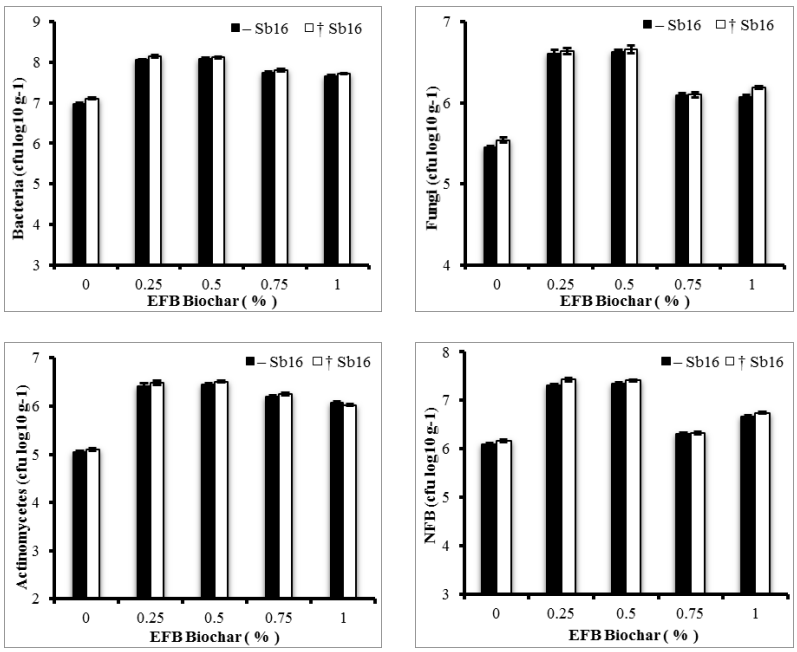

Figure 1: Effect of soil sterilisation, EFB biochar and N2-fixing bacteria (Sb16) on soil bacteria, fungi, actinomycetes and N2-fixing bacterial populations. Vertical bars represent standard error (S.E), n=3.

Effects of EFB biochar and N_2-fixing bacteria (Sb16) on soil enzyme activity

EFB biochar and N_2-fixing bacteria, Sb16, significantly enhanced (p<0.05) soil urease, phosphatase and fluorescein diacetate hydrolysis (FDA) activity in the soil. The effect of EFB biochar and bacteria Sb16 on soil enzymes activity is as shown in Figure 2. Addition of EFB biochar at 0.25% EFB biochar with and without bacteria inoculation significantly increased ($p \leq 0.05$) the urease and FDA activities, while 0.5% EFB biochar and bacteria Sb16 significantly improved soil phosphatase. In general the presence of bacteria Sb16 increased the production of soil enzymes. The enzymes activities were observed to decrease with increased EFB biochar rates. Presence of organic carbon from the EFB biochar may improve growth of soil microorganisms which can subsequently influence enzyme activities in soil (Anderson *et al*., 2011).

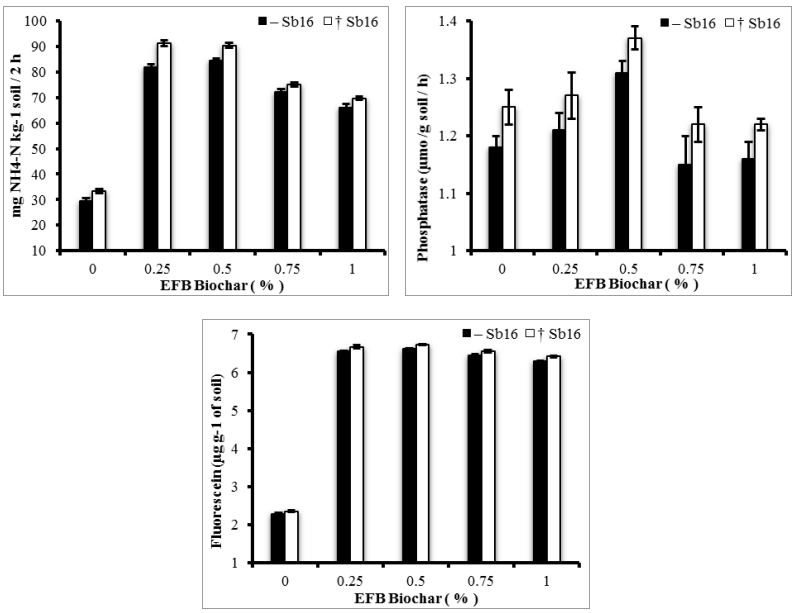

Figure 2: Effect of EFB biochar and N_2-fixing bacteria in (a) soil urease, (b) phosphatase, and (c) FDA activity. Vertical bars represent standard error (S.E), n=3.

Effects of EFB biochar and N_2- fixing bacteria (Sb16) on soil chemical properties
Application of EFB biochar and bacteria Sb16 in soil did not significantly improve soil chemical properties except for soil P (Table 2). Among all EFB biochar rates with or without bacteria Sb16, the treatments 0.25% and 0.5% showed the highest value of selected soil chemical properties. There was no significant interaction between EFB biochar and bacteria Sb16 on soil pH, organic carbon, total N and exchangeable K. In general, bacterial inoculation was better than non-inoculated treatment. The increase in acid soil chemical composition may be due to the presence of these elements in EFB biochar.

Glasshouse Study
Effects of N_2-fixing bacteria Sb16 and EFB biochar on growth of sweet corn
Application of N_2-fixing bacteria Sb16 and EFB biochar significantly improved growth of corn at tasseling stage (as indicated by root and shoot biomass, root length, root volume, plant height and leaf chlorophyll content) and nutrient uptake (Figure 3). Plant growth with EFB biochar was better compared to without EFB biochar. Among the EFB biochar treatments, 5 t ha^{-1} showed highest shoot biomass (48.5 g/plant). However, growth decreased with a further increase in biochar levels. Significant improvement in plant height and leaf chlorophyll content was observed at 10 t ha^{-1} EFB biochar. Inoculation of soil with N_2-fixing bacteria further improved growth of corn compared to non-inoculated plants. Higher shoot biomass (61.4 g/plant) was found at 5 t/ha EFB biochar with N_2-

TABLE 2
Effect of EFB biochar and N2-fixing bacteria Sb16 on soil chemical properties

EFB Biochar (%)	Bacterial Inoculation	pH	C (%)	N (%)	P(mg kg^{-1})	K(cmol $_{(+)}$ kg^{-1}
0	-Sb16	4.5 ± 0.03	1.87 ± 0.01	0.004 ± 0.01	28 ± 0.91 g	0.17 ± 0.01
0	+ Sb16	4.7 ± 0.03	1.92 ± 0.01	0.006 ± 0.01	31 ± 0.93 g	0.20 ± 0.01
0.25	– Sb16	5.5 ± 0.03	2.71 ± 0.01	0.060 ± 0.01	81 ± 0.90 c	0.80 ± 0.01
0.25	+ Sb16	5.6 ± 0.03	2.78 ± 0.01	0.090 ± 0.01	96 ± 0.57 a	0.85 ± 0.01
0.5	– Sb16	5.6 ± 0.03	2.73 ± 0.02	0.080 ± 0.01	85 ± 0.57 c	0.75 ± 0.02
0.5	+ Sb16	5.7 ± 0.03	2.75 ± 0.02	0.110 ± 0.01	91 ± 0.96 b	0.81 ± 0.01
0.75	– Sb16	5.4 ± 0.03	2.20 ± 0.01	0.060 ± 0.01	60 ± 0.98 e	0.65 ± 0.01
0.75	+ Sb16	5.5 ± 0.03	2.23 ± 0.02	0.080 ± 0.01	69 ± 0.95 d	0.69 ± 0.01
1.00	– Sb16	5.3 ± 0.03	1.96 ± 0.01	0.040 ± 0.01	50 ± 0.97 f	0.44 ± 0.02
1.00	+ Sb16	5.4 ± 0.03	2.01 ± 0.01	0.006 ± 0.01	56 ± 0.95 e	0.47 ± 0.01
Biochar		0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Sb16		0.0224*	0.0001*	0.0085*	0.0001*	0.0006*
Biochar*Sb16		0.8452^{ns}	0.2124^{ns}	0.1747^{ns}	0.0133*	0.8252^{ns}

* = significant (P<0.05) NS = not significant (P>0.05)
Means in each column with the same letter (s) for each variable are not significantly different according to Tukey 's test at 5%.

fixing bacteria Sb16 as compared to 0 t ha^{-1} EFB biochar. Leaf chlorophyll (SPAD reading) increased by144% compared to non-amended control. The EFB biochar which is an alkaline product could improve soil properties and thus, improve soil nutrient for plant growth. EFB biochar provides a conducive environment for beneficial microbes, especially the N_2-fixing bacteria to increase N in available form that can be absorbed by plants.

Effects of EFB biochar and N_2-fixing bacteria Sb16 on plant nutrient uptake

Addition of EFB biochar and N_2-fixing bacteria Sb16 positively affected plant nutrients concentration (Table 3) and uptake (Table 4). Plant nutrients content was higher in EFB biochar treatments. Application of EFB biochar at 5 t/ha and N_2-fixing bacteria significantly increased plant nutrients concentration and uptake compared to control. Bacterial inoculation showed better nutrient content than non-inoculated plants. Higher EFB biochar content of more than10 t ha^{-1} in treatments with or without bacteria reduced plant nutrients uptake.

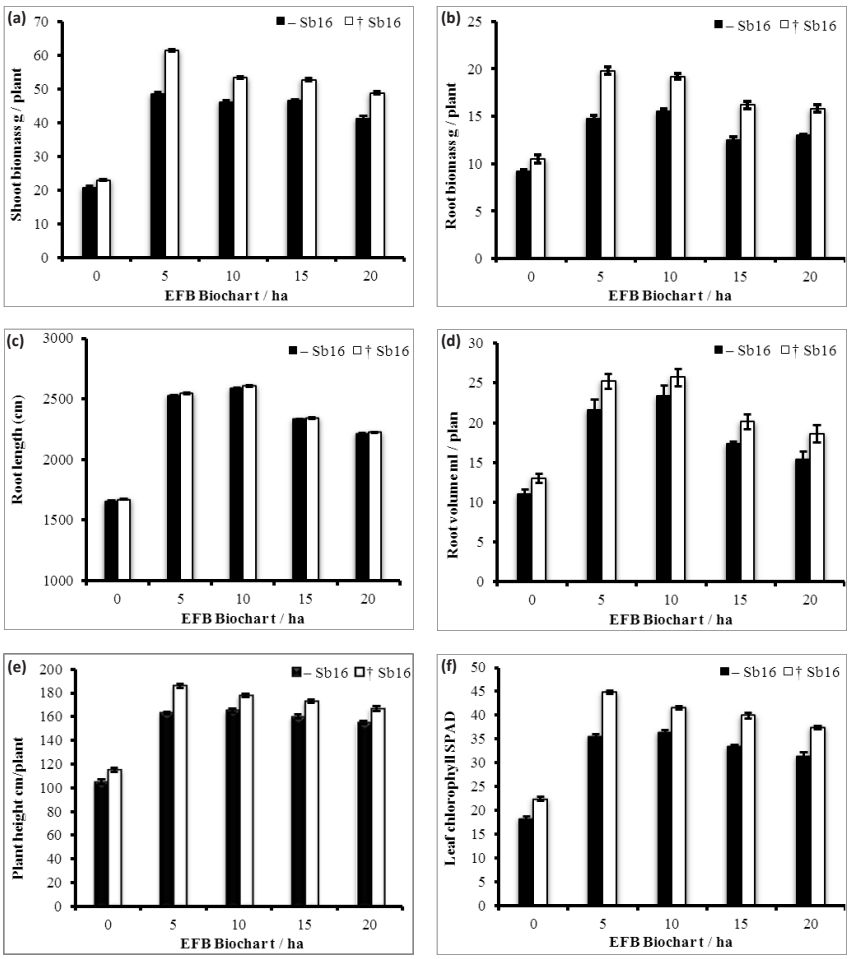

Figure 3: Effect of EFB biochar and N_2- fixing bacteria Sb16 on (a) shoot biomass, (b) root biomass, (c) root length, (d) root volume, (e) plant height and (f) leaf chlorophyll content. Vertical bars represent standard error (S.E), n=5.

TABLE 3
Effect of EFB biochar and N_2-fixing bacteria (Sb16) on plant nutrient concentrations

EFB biochar t/ha	Bacterial inoculation	Nutrient Concentration (%)				
		N	P	K	Ca	Mg
0	- Sb16	1.16 h	0.08 e	0.55 g	0.12	0.09
0	+Sb16	1.22 g	0.11 e	0.61 f	0.16	0.11
5	- Sb16	2.81cd	0.25 bc	1.55 b	0.34	0.20
5	+ Sb16	3.03 a	0.37 a	1.74 a	0.45	0.28
10	- Sb16	2.85 c	0.28 b	1.58 b	0.35	0.22
10	+ Sb16	2.94 b	0.35 a	1.69 a	0.42	0.25
15	- Sb16	2.68 e	0.22 cd	1.41d e	0.32	0.14
15	+ Sb16	2.79 d	0.27 b	1.48 c	0.38	0.16
20	- Sb16	2.62 f	0.21 d	1.39 e	0.31	0.15
20	+ Sb16	2.73 e	0.26 b	1.46 cd	0.36	0.17
EFB Biochar		0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Sb16		0.0001*	0.0001*	0.0001*	0.0001*	0.0024*
EFB Biochar*Sb16		0.0021*	0.0101*	0.0062*	0.1871^{ns}	0.2647^{ns}

* = significant (P<0.05) NS = not significant (P>0.05)
Means in each column with the same letter (s) for each variable are not significantly different according to Tukey 's test at 5%.

TABLE 4
Effect of EFB biochar and N_2-fixing bacteria Sb16 on plant nutrient uptake

EFB Biochar t/ha	Bacterial inoculation	Nutrient uptake (g/plant)				
		N	P	K	Ca	Mg
0	– Sb16	0.24 ± 0.01 g	0.02 ± 0.01 f	0.11 ± 0.01 g	0.02 ± 0.01 g	0.02 ± 0.01 e
0	+ Sb16	0.28 ± 0.02 g	0.03 ± 0.01 f	0.14 ± 0.01 g	0.04 ± 0.01 g	0.03 ± 0.01 e
5	– Sb16	1.36 ± 0.02 d	0.12 ± 0.01cd	0.75 ± 0.03 cd	0.16 ± 0.01 de	0.10 ± 0.01 c
5	+ Sb16	1.86 ± 0.04 a	0.23 ± 0.01 a	1.09 ± 0.03 a	0.28 ± 0.01 a	0.18 ± 0.01 a
10	– Sb16	1.32 ± 0.03 de	0.13 ± 0.01 c	0.73 ± 0.02 cd	0.16 ± 0.01 de	0.10 ± 0.01 c
10	+ Sb16	1.57 ± 0.02 b	0.19 ± 0.01 b	0.9 ± 0.02 b	0.22 ± 0.02 b	0.13 ± 0.01 b
15	– Sb16	1.25 ± 0.04 e	0.10 ± 0.01 de	0.65 ± 0.01 e	0.15 ± 0.01 ef	0.07 ± 0.01 d
15	+ Sb16	1.47 ± 0.01 c	0.14 ± 0.01 c	0.78 ± 0.04 c	0.20 ± 0.01 bc	0.09 ± 0.01cd
20	- Sb16	1.08 ± 0.03 f	0.09 ± 0.01 e	0.57 ± 0.01 f	0.13 ± 0.01 f	0.06 ± 0.01 d
20	+ Sb16	1.33 ± 0.04 de	0.13 ± 0.01 c	0.71 ± 0.01 d	0.18 ± 0.02 cd	0.08 ± 0.01cd
EFB Biochar		0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Sb16		0.0001*	0.0001*	0.0001*	0.0001*	0.0024*
EFB Biochar*Sb16		0.0001*	0.0001*	0.0001*	0.0002*	0.0061*

* = significant (P<=0.05) NS = not significant (P>0.05)
Means in each column with the same letter (s) for each variable are not significantly different according to Tukey 's test at 5%.

DISCUSSION

Soil acidity is a serious constraint for crop production in tropical regions. Plants require sufficient quantities of elements for healthy growth. To compensate for nutrients deficiency in soil, EFB biochar with bacteria Sb16 were used as an alternative to improve soil properties and plant growth. The current studies showed that oil palm EFB biochar, and application of N_2-fixing bacteria significantly improved soil chemical compositions, microbial populations and enzyme activity and growth of sweet corn. In an incubation study, the combination of bacteria Sb16 and EFB biochar at 0.25% significantly improved soil microbial populations. Earlier studies also observed that addition of biochar released nutrients into the soil and thus stimulated microbial growth (Rutigliano *et al.*, 2014; Rondon *et al.,* 2007). Biochar has a high surface area and porosity that enables it to retain nutrients and also provide a suitable habitat for beneficial microorganisms to flourish (Tejada *et al.*, 2006). Biochar provides a suitable habitat, where indigenous microorganisms may escape from predators, as well as provide carbon, energy, and nutrients (Thies and Rillig, 2009). The alkaline nature of EFB biochar (pH 9.39) alleviates soil acidity by increasing the pH for increased microbial activity. Organic carbon rich EFB biochar and free living N_2-fixing bacteria significantly improve soil enzyme activities. Biochar has the capacity to adsorb a wide range of organic and inorganic molecules which may provide a mechanism to protect enzyme activity (Bailey *et al.*, 2010).

The positive effects of biochar on soil enzyme activities may be due to the high pH, surface area, pore size distribution, and charge properties (Nannipieri *et al.,* 2012). Tejada *et al.* (2006) reported that the application of biochar stimulated soil microbes to produce some enzymes. Soil enzymes may positively influence soil quality and play important roles in maintaining soil health and fertility management in ecosystems. Addition of EFB biochar and bacteria Sb16 increased most nutrients in the soil that are responsible for improved plant growth.

Addition of biochar to agricultural soil has recently received much attention due to the apparent benefits it accords to soil functions. Besides increasing plant nutrients and soil enzymes, EFB biochar was able to decrease soil acidity which reduces liming requirements in soil. Improvement in soil pH by biochar addition may be due to several mechanisms, including proton consumption by functional groups associated with the organic materials (Wu *et al*., 2014), decarboxylation of organic acid anions during residue decomposition (Sarah *et al*., 2013) and through beneficial bacterial hydrolysis of organic nitrogen or nitrogen fixation that release NH_4^+ and decomposition of organic residues (Afeng *et al.*, 2012). An increase in soil pH and CEC may reduce the activity of Fe and Al and thus contribute to available plant nutrients in soil. A similar finding was observed by Abebe (2012) who observed an improvement in soil chemistry by application of alkaline biochar. The increased value of soil organic C may be explained by the carbon and energy substrates provided by biochar itself (Sander *et al.*, 2010). The initial increase in essential plant nutrients in the soil could be due to the abundance of macronutrients in the biochar, N_2 fixation by beneficial bacteria and mineralisation of organic

nutrients in the biochar (Amonette and Joseph, 2009). The light fraction organic matter in the soil and microbial biomass could have resulted in better nutrient release from EFB biochar (Carson *et al.*, 2007). Application of EFB biochar with and without bacteria Sb16 significantly enhanced a few chemical and biological properties and enzyme activities in acidic soil. EFB biochar may have potential as a beneficial soil amendment up to a certain limit for enhancing soil properties but at higher rates, was found to have a negative effect on soil properties.

Application of EFB and bacteria Sb16 to sweet corn under glasshouse study significantly improved shoot and root biomass, root development and nutrients uptake. Biochar has been known to improve soil properties, plant growth and nutrient uptake (Atkinson *et al.,* 2010; Lehmann *et al.*, 2011). Application of biochar improves soil acidity, pore structure, surface area, essential nutrients and changes in microbial populations, thus increasing crop productivity. The combination of EFB biochar with bacteria Sb16 improved leaf chlorophyll which is essential for photosynthesis, N uptake and plant productivity. Solubilisation and porosity of ash-biochar may control the release of soluble nutrients available for plant absorbtion (Brockhoff *et al*., 2010). Amending soil with EFB biochar at 5 t/ha and bacteria Sb16 affected soil microbial activities. Biochar application to poor fertile soil has been found to provide longer-lasting improvements in soil fertility (Xu *et al.*, 2013). Biochar has a positive effect on plant nutrients in the soil, available for plants in two common ways: nutrient addition and nutrient retention.

Several groups of microorganisms in biochar are known to be able to regulate plant growth through nutrient cycling (Rutigliano *et al.*, 2014). Production of enzymes and other compounds in biochar could also enhance plant growth. Application of biochar has been observed to generally increase plant root hairs and effective root surface areas beyond common root absorption zones causing higher nutrient transfer for plant production and nutrient uptake (Steiner *et al.*, 2008). The study also suggests that EFB biochar with beneficial bacteria Sb16 could be a good soil buffer providing suitable conditions for soil microbial populations, enzyme activity and essential nutrients for plant growth. Addition of EFB biochar with or without bacterial inoculation improved growth of sweet corn, nutrient uptake and soil chemical properties. However, addition of higher levels (> 10 t/ha rate) of EFB biochar resulted in low plant growth and decreased the soil microbial and chemical properties.

Addition of EFB biochar may be an alternative solution in enhancing the quality of acid soils. Inoculation of bacteria Sb16 further improves release of nutrients from EFB biochar. Incorporation of EFB biochar and bacteria Sb16 can induce changes to chemical and biological properties and enzyme activity of acidic soils and improve crop production.

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