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Linking Soil Microbial Properties with Plant Performance in Acidic Tropical Soil Amended with Biochar

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Abstract: Soil microbial properties are frequently used as indicators of soil fertility. However, the linkage of these properties with crop biomass is poorly documented especially in biochar amended soil with high carbon:nitrogen (C:N). A short-term field trial was conducted to observe the growth response of maize to biochar treatment in a highly weathered Ultisol of humid tropics and to observe the possible linkage of the measured microbial properties with maize biomass. Soil microbial biomass (carbon (C), nitrogen (N), phosphorus (P)), enzyme activity (β-glucosidase, urease, phosphodiesterase) and gene abundance (bacterial *16S rRNA*, fungal ITS) were analyzed. For comparison, total soil C, N, and P were also analyzed. The data revealed no significant linkage of soil C, N, and P with maize biomass. A significant association of enzyme activity and gene abundance with maize biomass was not recorded. Strong positive correlation between maize above ground biomass with microbial biomass N was found ($r = 0.9186$, $p < 0.01$). Significant negative correlation was recorded between microbial biomass C:N with maize biomass ($r = -0.8297$, $p < 0.05$). These statistically significant linkages observed between microbial biomass and maize biomass suggests that microbial biomass can reflect the soil nutrient status, and possibly plant nutrient uptake. Estimation of microbial biomass can be used as a fertility indicator in soil amended with high C:N organic matter in the humid tropics.

Keywords: gene abundance; highly weathered soil; quantitative polymerase chain reaction; soil enzyme; soil indicator

1. Introduction

The recent strong interest in the study of biochar—a thermal degradation product of organic matter—as a soil amendment is largely influenced by the intensive research of the anthropogenic dark earths of Terra Preta in the Amazon basin [1]. Although most of the native soil in the basin is of low crop potential due to its acidic nature, when compared to the infertile adjacent soil of this basin, the anthropogenic soils demonstrate high fertility due to the high degree of pyrogenic organic matter [2]. The addition of pyrogenic organic matter has been documented to increase crop yield in different soil types in different climatic regions [3,4]. The amendment of biochar in the acidic weathered soil of the tropical humid region has been documented in many studies with an ameliorant effect of soil fertility [5,6] and improvement in plant productivity [7,8]. However, the study of soil microbiota in this soil upon biochar amendment is still poorly investigated in the humid tropics.

Soil microbial community plays essential roles in key biogeochemical cycles and is considered as an indicator of soil quality for different land uses and management in an agroecosystem [9]. Soil microbial community structure and function play crucial parts in the turnover of soil nutrients [9]. The changes in microbial properties such as biomass size, enzyme activity, diversity, and abundance in soil amended with biochar are frequently reported in many studies. Nevertheless, microbial properties data are rarely associated with the agronomic outcome of the crop. The microbial community responds quicker than other abiotic soil parameters to various soil conditions [10]. Therefore, measurable shifts in microbial properties reflect temporal deviations in soil nutritional status.

Although the application of biochar has been proved to increase crop productivity, a few studies reported the detrimental effects of biochar addition towards agronomic performance [11,12]. Inconsistent results across different biochars has been mainly attributed to the variations of biochar heterogeneity and physico-chemical properties due to different in pyrolysis conditions and feedstock origin [13]. Therefore, a sensitive indicator for soil fertility monitoring is essential in order to verify the effect of plant growth following biochar treatment. On the other hand, unravelling the connection between soil microbial community and plant agronomic performance in the biochar amended soil may not only enable the development of a reliable biological indicator for assessing soil fertility but also enhance future judgement for incorporating appropriate agricultural strategies to ameliorate infertile soil in the humid tropics.

This study evaluates the effect of biochar amendment on soil microbial properties in the humid tropics and, to verify the potential relationship of these properties with the yield of maize biomass, in tropical acidic soil treated with biochar. It is hypothesized that soil microbial properties will be greatly impacted following biochar amendment and this impact can be observed by the nutritional status of soil as reflected by the maize biomass yield. Therefore, it may serve as a potential candidate for soil fertility indicator.

2. Materials and Methods

2.1. Experimental Site and Design

The field trial was conducted in an uncultivated land at Pusat Penyelidikan Bioteknologi Glami Lemi (PPBGL), Negeri Sembilan, Malaysia (3°03′ N, 102°03′ E). The experiment site is located within a tropical rainforest climatic zone or equatorial climate with an annual precipitation and mean temperature of 2500 mm and 27 °C, respectively. The highly weathered soil was previously described with pH of 4.98, total organic carbon of 5.11 g C kg^{-1} soil, total nitrogen of 0.06 g N kg^{-1} soil [14], and classified as a sandy loam textured Udults Ultisol.

The field study was carried out from 22 September 2015 until 6 December 2015 during the plantation of maize (*Zea mays* L.). The palm kernel shell and rice husk biochar used in this study were purchased from the Malaysian Palm Oil Board (MPOB) and Padiberas Nasional Berhad (BERNAS), respectively. The physico-chemical properties of biochars used were previously characterized in the previous study [14] and are shown in Table 1. The soils were treated with: (1) 20 t ha^{-1} palm kernel shell biochar (PK); (2) 20 t ha^{-1} rice husk biochar (RH); (3) 20 t ha^{-1} palm kernel shell biochar + fertilizer (FPK); (4) 20 t ha^{-1} rice husk biochar + fertilizer (FRH); (5) fertilizer (F); and (6) control without amendment (C), each with three replications on a completely randomized block design. Treatment was done a week before sowing. Each 4 × 4 m^2 plot consisted of 75 crops (5 rows × 15 crops). The commercial fertilizer applied consisted of 60 kg N ha^{-1} urea, 60 kg P_2O_5 ha^{-1} triple phosphate and 40 kg K_2O ha^{-1} muriate of potash.

Table 1. Selected properties of pyrolytic palm kernel and gasified rice husk biochar used in this study [14].

Properties	Palm Kernel Shell Biochar	Rice Husk Biochar
Production Process	**Slow Pyrolysis at 400 °C**	**Gasification at 800 °C**
pH	8.67	10.24
Total C (g kg^{-1})	434.1	101.7
Total N (g kg^{-1})	5.07	2.52
Total P (g kg^{-1})	1.56	2.11
C:N	85.62	40.37
NH_4^+—N (mg kg^{-1})	21.05	25.48
NO_3^-—N (mg kg^{-1})	40.88	92.17
Ash (g kg^{-1})	180	750
BET surface area (m^2 g^{-1})	184.2	1.6
Particle size range (mm)	5–10	1–3

C = carbon; N = nitrogen; P = phosphorus; BET = Brunauer–Emmett–Teller.

2.2. Soil Sampling and Physico-Chemical Analysis

Sampling of soil from each treatment plot was conducted during the harvesting stage of *Zea mays* L., which was on the 75th day of planting by collecting soil of 0–20 cm depth from five random sites in each plot using an auger (Eijkelkamp, Giesbeek, The Netherlands). Soil was air-dried at room temperature and passed through a 2 mm mesh sieve prior to physico-chemical analysis. Soil pH was determined using 1:2.5 soil water suspension ratio. Cation exchange capacity (CEC) of soil was measured by the $BaCl_2$ compulsive exchange method [15]. Total organic carbon was analyzed spectrophotometrically [16]. Total N was measured using TruSpec CHN analyser (LECO, St. Joseph, MI, USA) while total P was determined using an Optima 8300 ICP-OES (Perkin Elmer, Waltham, MA, USA). Soil ammonium, NH_4^+—N and nitrate, NO_3^-—N concentration were analyzed by colorimetric determination [17,18]. Available P was determined using the Bray II extraction method [19].

2.3. Microbial Biomass Analysis

Soil microbial biomass was extracted by the fumigation extraction method [20]. A portion of soil was fumigated with CH_3Cl in a vacuum desiccator for 24 h in 25 °C. For microbial biomass C and N, fumigated and an equal portion of non-fumigated soils were extracted with 1:4 ratio of soil to 0.5 M K_2SO_4 prior to filtration with Whatmann no. 42 filter paper. The filtrate obtained was subjected to dichromate oxidation [21] and persulfate digestion [22] for microbial biomass C and N analysis, respectively. Fumigated and non-fumigated soils were extracted with 0.5 M $NaHCO_3$ on 1:20 ratio [23] for microbial biomass phosphorus. Filtrate obtained was then subjected to the spectrophotometric measurement of phosphate [24].

2.4. Soil Enzyme Assay

β-glucosidase (EC 3.2.1.21) assay was done based on the recovery of p-nitrophenol released after 1 h incubation of soil enzyme with p-nitrophenyl glucopyranoside solution at 37 °C [25]. Assay for urease (EC 3.5.1.5) activity was performed based on the determination of the recovered ammonium after 2 h incubation of the soil enzyme with an urea solution at 37 °C [26]. Phosphodiesterase (EC 3.1.4.1) activity was done based on the determination of p-nitrophenol produced following 1 h incubation of soil enzyme with tris-p-nitrophenyl phosphate solution at 37 °C [27]. The moiety released for each assay was conducted spectrophotometrically by using a V-630 spectrophotometer (Jasco, Utrecht, The Netherlands) at their respective optimum wavelength.

2.5. DNA Extraction and Real-Time Quantitative Polymerase Chain Reaction (qPCR)

Total soil genomic DNA was extracted using a PowerSoil® DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) following the manufacturer's instruction. Thermal reaction of qPCR was conducted using

a QuantStudio™ 12K Flex Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). DNA from each sample was subjected to qPCR cycle using the primer set Eub338f/Eub518r for bacteria and ITS1f/5.8s for fungi [28] with the following thermal profile; 95 °C for 15 min, 40 cycles of 95 °C for 10 s, 53 °C for 10 s, and 70 °C for 20 s, followed by melting curve analysis. Gene amplification was done in a final reaction volume of 20 µL, consisting 4 µL of template DNA, 10 µM of each primer and 10 µL of SensiFAST™ SYBR® Lo-ROX Kit (Bioline, London, UK). Standard curves were constructed using a ten-fold serial dilution of plasmid containing known copy numbers of the target gene (respectively amplified from *Bacillus subtilis* and *Fusarium solani*). The linear coefficient correlations of both standard curves obtained were >0.99. Bacterial and fungal abundance were expressed as gene copy numbers per g of soil.

2.6. Plant Sampling and Performance Analysis

Harvest was performed when the crop physiologically matured. Five plants from each plot were randomly collected and were subjected to oven-drying at 60 °C, until a constant dry above-ground biomass was obtained. Total N and P in plant tissue were determined by LECO FP528 (LECO, St. Joseph, MI, USA) and Optima 8300 ICP-OES (Perkin Elmer, Walthamcity, MA, USA), respectively. The amount of N and P uptake by the plant were calculated by multiplying N and P concentration with respective biomass measured.

2.7. Statistical Analysis

All statistical analysis was done using XLSTAT (Addinsoft, New York, NY, USA). Normality of data was tested using the Shapiro–Wilk test. The dissimilarity of treatment was examined by performing a one-way ANOVA, and the Tukey's Honest Significant Difference (HSD) test was conducted to assess the mean separation at 0.05 level of significance. Pearson's correlation coefficient was conducted to indicate the significant connection between each parameter. Principal component analysis (PCA) was conducted to evaluate the difference among soil microbial biomass and plant performance in all treatments.

3. Results

3.1. Effect of Biochar on Soil Properties.

Table 2 tabulated the soil properties after 75 days of field exposure. The incorporation of biochar showed a significant increase ($p < 0.05$) of soil pH, ranging from 6.14 to 6.31. CEC was significantly improved ($p < 0.05$) following the application of both biochars compared to the C plot. Total organic C was significantly ($p < 0.05$) affected by a biochar amendment where all soil added with biochar recorded higher values ranging from 8.39 to 9.41 g kg^{-1} soil compared to the C and F plots. A significant increase of total N was observed in all biochar treated soil and soil treated with fertilizer. Incorporation of fertilizer to biochar, however, did not significantly increase total N compared to biochar amendment without fertilizer incorporation. No significant increase was detected for total P in all treatment compared to the control soil. Conversely, a significant change ($p < 0.05$) was observed for available P in FPK when the palm kernel shell biochar was added together with the fertilizer.

Amendment of biochar in PK, RH, and FRH resulted in a significantly greater total soil C:N ratio ($p < 0.05$) than the untreated soil. A similar observation was also recorded for the soil C:P ratio ($p < 0.05$) in these three treatments. The soil N:P ratio was also improved greatly ($p < 0.05$) in these treatments, including F. The co-application of palm kernel biochar with fertilizer in FPK showed insignificant changes in all molar ratios compared to the control soil. Significant modification of NH_4^+—N ($p < 0.05$) was recorded compared to the control soil when biochar is supplemented with fertilizer in FPK and FRH. A significant increase of NO_3^-—N ($p < 0.05$) was detected in RH and FRH while the increases in PK and FPK are not significantly different from F and C. Only FPK recorded significantly higher P availability compared to the control soil.

Table 2. Soil properties after the 75 day of planting.

Treatment	pH	Cation Exchange Capacity (CEC) ($cmol_c$ kg^{-1} soil)	Total			Available		
			Organic C (g kg^{-1} soil)	N (g kg^{-1} soil)	P (mg kg^{-1} soil)	NH_4^+—N (mg kg^{-1} soil)	NO_3^-N (mg kg^{-1} soil)	P (mg kg^{-1} soil)
C	4.97 ^{b}	4.11 ^{b}	5.96 ^{b}	0.61 ^{b}	189.0 ^{ab}	9.86 ^{b}	20.93 ^{b}	37.33 ^{b}
F	5.03 ^{b}	4.21 ^{b}	5.83 ^{b}	0.83 ^{a}	147.6 ^{ab}	10.52 ^{b}	21.74 ^{b}	46.33 ^{b}
PK	6.31 ^{a}	4.94 ^{a}	8.39 ^{a}	0.77 ^{ab}	130.9 ^{b}	10.03 ^{b}	21.04 ^{b}	42.64 ^{b}
RH	6.21 ^{a}	4.88 ^{a}	9.41 ^{a}	0.93 ^{a}	134.4 ^{ab}	10.15 ^{b}	35.11 ^{a}	40.73 ^{b}
FPK	6.18 ^{a}	4.93 ^{a}	8.85 ^{a}	0.91 ^{a}	238.2 ^{a}	12.31 ^{a}	22.33 ^{b}	65.26 ^{a}
FRH	6.14 ^{a}	4.89 ^{a}	9.19 ^{a}	0.87 ^{a}	145.7 ^{ab}	12.88 ^{a}	38.42 ^{a}	42.53 ^{b}

Mean values indicated by different alphabets within a column are significantly different at $p < 0.05$ by Tukey's HSD test.

Table 3. Soil microbial biomass, enzyme activity and gene abundance after the 75 day of planting.

Treatment	Microbial Biomass			Enzyme Activity			Microbial Gene Abundance	
	C (mg kg^{-1} Soil)	N (mg kg^{-1} Soil)	P (mg kg^{-1} Soil)	β-glucosidase (μmol pNP g^{-1} Soil h^{-1})	Urease (μmol NH_3 g^{-1} Soil h^{-1})	Phosphodiesterase (μmol pNP ^{-1}g Soil h^{-1})	Bacterial *16S rRNA* (log_{10} Gene Copies g^{-1} Soil)	Fungal ITS (log_{10} Gene Copies g^{-1} Soil)
C	97.1 ^{c}	8.33 ^{c}	0.79 ^{b}	228.3 ^{b}	2.46 ^{c}	95.1 ^{b}	6.35 ^{b}	5.46 ^{b}
F	93.9 ^{c}	26.67 ^{b}	0.85 ^{b}	222.2 ^{b}	6.93 ^{a}	167.5 ^{a}	8.23 ^{a}	6.03 ^{b}
PK	140.5 ^{b}	23.33 ^{b}	0.82 ^{b}	295.9 ^{a}	5.62 ^{b}	152.9 ^{a}	8.57 ^{a}	7.62 ^{a}
RH	160.9 ^{a}	21.68 ^{b}	0.81 ^{b}	287.1 ^{a}	5.24 ^{b}	165.3 ^{a}	9.78 ^{a}	7.54 ^{a}
FPK	137.2 ^{b}	38.34 ^{a}	0.91 ^{a}	256.4 ^{a}	7.24 ^{a}	181.7 ^{a}	9.11 ^{a}	7.23 ^{a}
FRH	154.8 ^{a}	36.71 ^{a}	0.84 ^{b}	265.1 ^{a}	7.47 ^{a}	175.2 ^{a}	9.02 ^{a}	7.42 ^{a}

pNP = p-Nitrophenol. Mean values indicated by different alphabets within a column are significantly different at $p < 0.05$ by Tukey's HSD test.

3.2. Effect of Biochar on Soil Microbial Properties

The variability of soil microbial biomass in all treated soil is shown in Table 3. Biochar significantly increased microbial biomass C by 41% to 66% when compared to the control ($p < 0.001$) with RH and FRH both recorded the highest significant values. A significant increase of microbial biomass N was also observed ($p < 0.001$). Soil microbial biomass N was significantly increased by the application of biochar and fertilizer. Across amendment types, microbial biomass N in FPK and FRH appeared inferior to other treatments with the greatest significant increase of 360% and 341%, respectively over the control. The co-application of fertilizer with palm kernel biochar (FPK) showed significant differences in soil microbial biomass P, which was significantly different ($p < 0.05$), registering the highest microbial biomass P by 16% over the control.

The data of the microbial biomass molar ratio is tabulated in Table 4. It revealed variable results. Biochar amendment significantly impacted the soil microbial biomass C:N ratio ($p < 0.05$) with a major decrease in FPK and FRH. A similar decrease was also observed in soil treated with fertilizer alone. For the microbial biomass C:P ratio, a significant shift ($p < 0.05$) was recorded for all biochar amended soils compared to F. Nevertheless, a microbial biomass C:P ratio in PK and FPK were not significantly different from the control soil, although these two treatments recorded higher values. The value of the microbial biomass N:P ratio was significantly higher ($p < 0.05$) in all biochar treatments amended with fertilizer (FPK and FRH) than that in the control and soil treated with biochar alone.

Table 4. Molar C:N:P ratios in soil and microbial biomass.

Treatment	C:N	C:P	N:P
	Soil		
C	9.77 ^{b}	31.52 ^{b}	3.22 ^{b}
F	7.02 ^{c}	39.49 ^{b}	5.62 ^{a}
PK	10.89 ^{a}	64.49 ^{a}	5.92 ^{a}
RH	10.12 ^{ab}	70.01 ^{a}	6.91 ^{a}
FPK	9.73 ^{b}	37.16 ^{b}	3.82 ^{b}
FRH	10.56 ^{a}	63.34 ^{a}	5.99 ^{a}
	Microbial biomass		
C	11.65 ^{a}	122.92 ^{bc}	10.54 ^{c}
F	3.52 ^{c}	110.52 ^{c}	31.37 ^{ab}
PK	6.02 ^{b}	171.35 ^{b}	28.45 ^{b}
RH	7.43 ^{b}	198.64 ^{a}	26.76 ^{b}
FPK	3.58 ^{c}	150.78 ^{bc}	40.13 ^{a}
FRH	4.22 ^{c}	173.65 ^{a}	41.05 ^{a}

Mean values indicated by different alphabets within a column are significantly different at $p < 0.05$ by Tukey's HSD test.

Table 3 shows the data of soil enzyme activity in all treatments. The addition of biochar to the soil leads to a significant increase ($p < 0.05$) of soil β-glucosidase activity compared to C, without significant change in F. A significant strong association of β-glucosidase activity with total soil C was found with a Pearson's correlation coefficient of 0.8303 ($p < 0.05$). Soil urease activity was significantly increased by the incorporation of fertilizer. The value of urease activity measured in soils applied with biochar was significantly higher ($p < 0.01$) than the value obtained from C, where FRH, FPK and F had the highest activity followed by RH and PK. Phosphodiesterase activity of the soil was significantly higher ($p < 0.05$) in all soils amended with biochar including soil treated with fertilizer alone. No significant correlation was found between urease activity and total soil N and phosphodiesterase activity with total soil P.

The abundance of bacterial and fungal gene copy numbers of the soil during the harvesting stage of maize are tabulated in Table 3. High bacterial *16S rRNA* gene ($p < 0.05$) abundance was recorded in all biochar treated soil and F plot in comparison to the control soil. The abundance of the fungal ITS

gene in all soils treated with biochar significantly increased ($p < 0.05$) as compared to the gene copy number recorded from C while the ITS gene abundance in F was insignificant.

The PCA biplot performed to soil microbial properties is displayed in Figure 1. The principal component 1 and 2 defined 69.9% and 24.3% of total variance, respectively. The microbial biomass N and urease activity contributes to 48.8% and 30.3% of the total variance on component 1, respectively. The biplot obtained revealed a distinct separation of biochar amended soil supplemented with an additional fertilizer regime (FPK and FRH) along component axis 1 with soil amended with biochar alone (PK and RH), including soil without a biochar amendment (C and F).

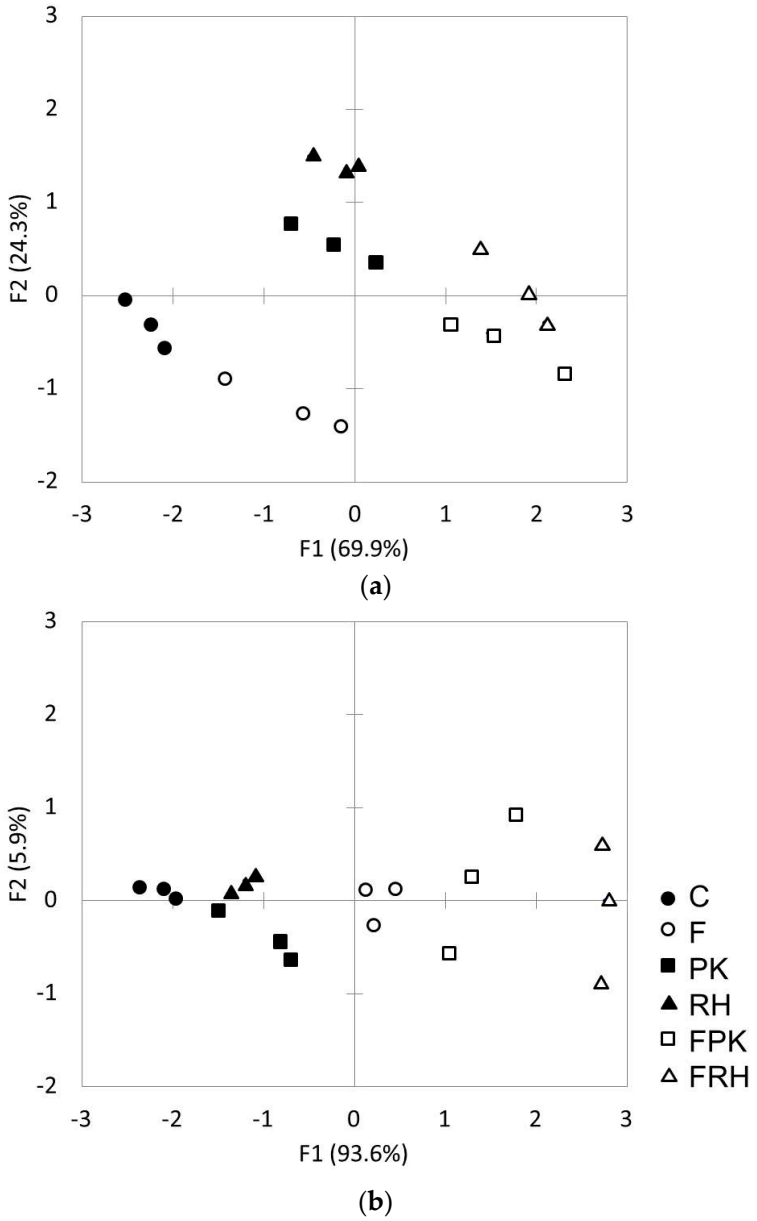

Figure 1. Principal component analysis (PCA) applied to soil microbial biomass (**a**) and *Zea mays* performance (**b**) after the 75 day of planting. C = control; F = fertilizer; PK = palm kernel biochar; RH = rice husk biochar; FPK = palm kernel biochar + fertilizer; FRH = rice husk biochar + fertilizer.

3.3. Effect of Biochar on Maize Growth Performance

Table 5 showed the effect of biochar amendments on the growth performance of maize which is manifested in terms of plant nutrient uptake and biomass. At 75th day of planting, differences of N and P uptake by the plant were significantly modified ($p < 0.05$). However, the amendment of biochar alone in PK and RH had no significant effect on the N uptake by a plant than the control soil. Higher N uptake was only observed when biochar was co-applied with fertilizer in FPK and FRH. Nevertheless, a significant increase of P uptake by the plant can be seen in soil treated with biochar either with or without additional fertilization.

Values of 195% to 520% biomass increase were observed in biochar treated soil compared to the C plot. Integrating the biochar with fertilizer resulted in more substantial biomass where maize grown in FRH significantly yielded the highest biomass. Fertilizer incorporation with biochar harvested greater biomass than F with 115% and 156% increases for FRH and FPK, respectively. An outcome of PCA conducted on maize performance is displayed in Figure 1. The total variance described by the first two principal component axes was 99.5% (93.6% principal component (PC) 1 and 5.9% PC 2). A clear separation was observed between soil amended fertilizers with and without fertilizer in component axis 1.

Table 5. Plant nutrient uptake and biomass after the 75 day of planting.

Treatment	Uptake		Biomass (t h^{-1})
	N (t ha^{-1})	P (kg ha^{-1})	
C	8.01 ^{c}	28.87 ^{e}	1.97 ^{d}
F	60.34 ^{bc}	102.77 ^{c}	6.56 ^{b}
PK	19.36 ^{c}	77.42 ^{cd}	4.41 ^{c}
RH	32.39 ^{c}	51.61 ^{ed}	3.84 ^{c}
FPK	94.69 ^{ab}	144.71 ^{b}	7.57 ^{b}
FRH	115.01 ^{a}	194.62 ^{a}	10.25 ^{a}

Mean values indicated by different alphabets within a column are significantly different at $p < 0.05$ by Tukey's HSD test.

3.4. Relationship of Microbial Properties with Maize Biomass

Table 6 tabulated the matrix of Pearson's correlation coefficients of maize biomass with soil properties and microbial biomass. No significant relationship between soil C, N, and P with plant biomass was detected. A strong positive connection between maize above ground biomass with microbial biomass N was found with a correlation matrix (Pearson *r*) value of 0.9186 ($p < 0.01$). Strong negative correlation was recorded between microbial biomass C:N with maize biomass ($r = -0.8297$, $p < 0.05$). The correlation calculated also revealed an insignificant relationship of all enzyme activities and microbial genes abundance with maize biomass.

Table 6. Matrix of Pearson's correlation coefficients of maize performance with soil and microbial properties.

Parameter	Biomass
Soil	
C	0.4016
N	0.5998
P	0.0705
C:N	−0.0443
C:P	0.1624
N:P	0.2204

Table 6. *Cont.*

Parameter	Biomass
Microbial biomass	
C	0.3449
N	0.9186 **
P	0.8105
C:N	−0.8297 *
C:P	0.2015
N:P	0.8061
Enzyme activity	
β-glucosidase	0.0131
Urease	0.8174
Phosphodiesterase	0.7686
Microbial gene abundance	
16S rRNA	0.5035
ITS	0.4075

* Significant at $p < 0.05$; ** significant at $p < 0.01$.

4. Discussion

4.1. Effect of Biochar on Soil Properties

Biochar addition clearly improved tropical soil properties in general. Improvements of soil pH and CEC on acidic tropical soil after biochar treatment were in agreement in many biochar experiments, either in a pot [29] or field study [30]. Increases of total organic C in all biochar treated soils revealed that biochar contribute to the organic C pool in the soil. The result of organic C is in agreement in many findings, which showed a significant increase of the total organic C pool in the soil environment [31]. The same observation on total soil N also matches those reported in earlier studies [8]. Despite the palm kernel shell biochar comprised of higher initial total C and N (Table 1) than rice husk biochar, the pairwise comparison of total organic C and N were not significant between soils treated with both biochar. On the other hand, greater organic C concentrations may lead to the elevation of soil microbial activity [32], causing higher N demand in soil, hence immobilized and reduced available N for plant.

Insignificant changes of NH_4^+—N following biochar addition in PK and RH is consistent with the data reported from the tropical soils of Zambia and Indonesia [30]. Greater NH_4^+—N concentration in FPK and FRH than PK and RH indicate that ammonification of N does not occur when biochar is applied alone. On the other hand, the association between CEC and NH_4^+—N cation is not observed although the negatively charged biochar surfaces has been suggested to allow retention of cations such as NH_4^+—N [33]. This may due to the low concentration of native NH_4^+—N in the investigated tropical soil. Supplementation of additional fertilizer enhanced N mineralization through ammonification process. Incorporation of fertilizer to soil with biochar amendment significantly altered NO_3^-—N concentration. Significant increment of NO^{3-}—N in all biochar treated soil than F is likely due to the initial concentration of NO_3^-—N from the biochar itself (Table 1) and not due to mineralization of N through the nitrification of NH_4^+—N, while a greater concentration in soils treated with rice husk biochar (RH and FRH) than palm kernel shell biochar (PK and FPK) is due to the double fold initial concentration of NO_3^-—N. These findings further confirm the association of a high C:N ratio of organic input with N immobilization in soil.

Increased of P availability upon biochar treatment has been commonly reported, especially in the P deficient soil of the humid tropics [29]. Biochar is capable of increasing the soil P availability through either its direct inputs or its retaining potential of P applied [34]. However, the increment of soil P availability due to the biochar input itself is unlikely as the difference of available P in PK and RH is not significantly different. Greater P availability in FPK may suggest the retention of P from the fertilizer applied as a palm kernel shell biochar possess larger BET surface area (Table 1), hence able

to hold greater P in the soil. The surface property, such as the microporous structure of the biochar, has been demonstrated to determine its capacity in P adsorption [35].

4.2. Effect of Biochar on Soil Microbial Properties

Although made up recalcitrant C with high stability, biochar comprises small fractions of readily mineralized labile active C, which is bioavailable for microbial utilization and serves as a substrate [36]. Application of rice husk biochar with or without fertilization had a greater significant effect on soil microbial biomass C than the plot treated with palm kernel shell biochar. The greater microbial biomass size can be linked with the higher labile active C input of rice husk biochar compared than in the palm kernel biochar.

The PCA in Figure 1 suggested a variation of the soil microbial biomass when biochar is co-applied with fertilizer, especially for microbial biomass N as indicated in component axis 1. The enhancement of microbial biomass N status of soil in FRH and FPK treatment indicate a strong stimulation of biochar in combination with the fertilizer. This finding indirectly points out the mineralization of N by microorganisms due to the fertilization input. Amendment of fertilizer reduced the C:N ratio of soil and hence stimulated N mineralization as supported by the result of NH_4^+—N concentration in FPK and FRH (Table 2). Although Spohn and Kuzyakov [37] suggest that mineralization of P appears to have been driven by the microbial demand for C, in which organic moiety of phosphorylated organic carbon will be used as a carbon source, this reason however cannot be inferred for the microbial biomass P in this study as the carbon is not limited in the soil due to the abundant organic carbon pool of the biochar applied. The available P result however points out the possible association with microbial biomass P, which supports the possible mineralization of P (r = 0.9129, $p < 0.01$), hence, suggesting that the size of the microbial biomass P is directly associated with the availability of P to plants [38]. In fact, the microbial biomass P can be re-mineralized in a short turnover period [39], consequently serving as an available P for plant.

The microbial biomass ratio is considered as an important indicator of microbial nutritional balance [10]. The soil microbial biomass data showed that the priming effect after biochar addition was higher on microbial biomass N than C, subsequently decreasing the microbial biomass C:N. High microbial biomass C:N ratio in the control soil may indicate an unbalanced soil nutritional status in which the native soil microorganisms could possibly compete for available nitrogen with the plant [40]. This situation may interfere with plant nutritional requirements due to the nitrogen immobilization, which can become a limiting factor for the plant and the soil microorganisms. Previously, Li and co-authors [41] quantitatively showed a negative correlation of microbial biomass C:N with plant agronomic output, where a high ratio resulted in crop nitrogen deficiency. In this study, high reduction of microbial biomass C:N was achieved when biochar was co-applied with fertilizer. The microbial biomass C:N ratio in the plot treated with both biochar and fertilizer (FPK and FRH) proposed that fertilizer application is vital to create a nutritional balance in the soil due to the high C:N content of the applied biochar (Table 1). The statistically significant relationship found between microbial biomass N and maize biomass points out that microbial biomass can act as a significant sink and source of N [10]. However, the increment of microbial biomass N could not actually reflect the degree of soil fertility as the greater size of microbial biomass N may also indicates N immobilization [41], by which N is taken up by soil organisms and therefore inaccessible for crop uptake. On the other hand, the C:P and N:P are rarely systematically assessed in the agricultural soil, although the ecological stoichiometric relationships have been broadly studied in various terrestrial ecosystems [42]. Therefore, the significance of these two molar ratios in the agricultural soil cannot be ruled out in this short-term experiment. In fact, no significant correlation of these molar ratios with a plant biomass yield was observed (Table 6).

An available nutritional substrate found in the soil environment is utilized by the soil microorganisms through the hydrolytic degradation of the microbial enzymes. This hydrolytic process is widely regarded as a proximate indicator of elemental mineralization and nutrient cycling [9].

The three enzymes selected in this study (β-glucosidase, urease, phosphodiesterase) are related to the transformation of soil C, N, and P [43]. Higher β-glucosidase activity in all biochar treated soils can be linked to the boost of organic C pool in the soil and therefore may result in the increment of a distinctive subset of cellulolytic microorganisms from the soil environment. Urease activity change in response to treatment appears to result from fertilizer incorporation. Incorporation of fertilizer had a significant effect on the soil urease activity compared to when biochar was added alone, suggesting that the addition of urea in the fertilization regime enhanced the activity of many enzymes catalyzing N. However, single application of biochar without fertilization still showed significant difference with the control soil and this result is consistent with urease and phosphodiesterase activity data obtained in many previous studies [44–46]. However, a significant difference of phosphodiesterase activity due to the phosphate addition in the biochar amended soil co-applied with fertilizer (FPK and FRH) was not observed.

Quantitative PCR has been widely used as a promising approach to quantify soil microbial community structure and function at broad taxonomic levels. The *16S rRNA* and ITS are two commonly used conserved regions in enumerating the population density of the soil bacteria and fungi, respectively [28]. Data on the abundance of the gene copy number of both bacteria and fungi shows a strong stimulation of soil microbial population density by palm kernel shell biochar. The heterogeneous characteristic of biochar, such as the porosity, has been suggested to serve as a colonizing site for microbiota, hence, acting as a potential habitat [47]. Biochar was also discovered to be an ideal ecological niche by not only allowing microorganisms to establish themselves on the pores and surfaces, but also may retain readily metabolized nutrient [48] and substrate [49], therefore, stimulating the total size of soil microbial population. A number of researchers reported an increment in the abundance of these two conserved genes after the application of biochar to the soil [50,51], however, there is no research available reporting the effect of a biochar addition on microbial gene abundance in the acidic soil of the humid tropic.

4.3. Effect of Biochar on Maize Biomass Yield and its Relationship with Microbial Properties

Enhanced maize growths upon biochar addition in the tropical soil have been reported in a controlled glasshouse [7] as well as in a field trial [30]. In fact, biochar effects on crops planted in this climatic region are found to be more profound from those tested in temperate regions [52].

Insignificant N uptake in PK and RH compared to the control is supported by the higher microbial biomass C:N in which limited available N is revealed. Enhancement of the N uptake in FPK and FRH can be reflected by the reduction of the microbial biomass C:N upon fertilizer supplementation. For P uptake, although FPK recorded a higher available P in the soil, the result showed that the P uptake in FRH is more significant. The increased microbial biomass P in the present study may indicate the immobilization of some of the P in the soil. However, most of the immobilized P may be available due to the short-term turnover of microbial biomass P [53]. This observation is reflected by the fact that both available P and microbial biomass P did not show significant correlation in the maize biomass during the harvest stage.

Soil organic C and total N properties are commonly regarded as the main soil fertility factors in the context of crop productions [54]. The insignificant correlation between plant biomass and all measured soil parameters (C, N and P) suggests that significant changes cannot precisely indicate the effect of the soil organic matter dynamic due to soil management practices within a short time. In the present study however, we demonstrated that no significant correlation of these two soil properties can be established with crop biomass. The microbial biomass C:N ratio was shown to be a sensitive indicator capable of assessing plant growth. Negative correlation of maize biomass with microbial biomass C:N points out that the high C:N ratios of biochar lead to nitrogen immobilization in the soil and are disadvantageous to agricultural production. This matches those observed in earlier studies, which used other types of amended organic material [41,55].

5. Conclusions

In conclusion, this present study revealed a significant linkage of soil microbial mass with maize biomass. Therefore, we proposed the estimation of soil microbial biomass as an indicator for soil monitoring in soil amended with high C:N organic matter for crop production in the humid tropics. Future works need to be done to qualitatively establish the acceptable range of microbial biomass ratio values, which could reflect the degree of soil fertility for optimum crop production.

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