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Original Research

Role of Coconut Shell Biochar on Soil Properties, Microbial Diversity and Nitrogen Mineralization in Tropical Latosol

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Abstract

Biochar is a promising material to improve soil quality. Effects of coconut shell biochar on tropical soil nitrogen mineralization and microbial community were less reported. The incubation experiment was conducted to observe the required effects. Biochar rates in latosol were 0%, 1%, 2% and 5% (w/w), respectively. Results showed: (1) soil pH and CEC improved with biochar rate; (2) urease activity was increased at the lower biochar rate (<2%) but reduced at the 5% rate, while acid phosphatase activity was not changed by biochar; (3) biochar addition reduced soil microbial diversity. The bacterial population like Proteobacteria, Gemmatimonadetes, Planctomycetes, Verrucomicrobia and Bacteroidetes enhanced by more biochar addition, while Actinobacteria and Chloroflexi were found less at the high biochar rate. Fungal strains like Ascomycota decreased by biochar addition, but Basidiomycota and Chytridiomycota increased at high biochar rates. Genus of Haematonectria, Chaetomium, Gibberella, Aspergillus, Fusarium and Eupenicillium decreased effectively under biochar addition; (4) soil nitrogen mineralization with biochar amendment was described well by the exponential model (R^2>0.95, P<0.01), but soil nitrogen mineralization potential was found to be less due to biochar application. Thus, the addition of biochar derived from coconut shells had positive effects on soil characteristics, microorganisms, and nitrogen mineralization.

Keywords: coconut shell biochar, latosol soil, nitrogen mineralization, soil enzyme, soil microbial community

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Introduction

Nitrogen (N) is an essential and limiting element for the proper growth of crops and biomass production. Plenty of nitrogen in synthetic form has been used for high crops for the last few decades in China along with a major source of nitrogen already stored in the soil by different inputs [1]. The majority of nitrogen in the soil is stored in organic form, which is useless unless it is converted to available forms. It is necessary to convert this organic form of nitrogen into inorganic forms (NH_4^+ and NO_3^-) through the process of microbial mineralization, which can make it available directly for the plants. This process of nitrogen transformation is essential for the sake of availability of nutrients for plants and microorganisms, as well as it can also generate repercussions on the loss of nitrogen by leaching [2], emission of nitrogen as nitrous oxide (N_2O) and nitrogen (N_2) through denitrification or ammonia volatilization [3-4].

However, according to studies of Hanan et al. [5] and Urakawa et al. [6], soil nitrogen mineralization can be influenced by changes in soil properties and conditions such as pH, nutrients, or temperature. In this regard, biochar can be helpful for changing the soil properties as Chintala et al. [7] observed that biochar application in six acidic soils had significant effects on soil pH and cation exchange capacity (CEC) (P<0.05). Similarly, Gamage et al. [8] found a significant increase in soil pH and CEC in the sandy loam soil by the addition of rice-husk biochar at the rates of 0.5% and 1%, respectively. Fan et al. [9] also recorded the increase in soil pH together with high enzymatic activity and microbiological diversity over six years of biochar addition. According to Futa et al. [10], soil application of 20 t ha^{-1} biochar significantly increased the phosphatase and urease activities. Gao et al. [11] found the highest relative abundance *Bacteroidetes* and *Proteobacteria* due to the addition of biochar to the soil, which agreed with the previous findings of Xu et al. [12] reported that soil application of biochar improved bacterial diversity.

Due to its unique characteristics, such as its high adsorption capacity and pH, biochar can also be used to monitor the migration and transformation of nitrogen. Singh et al. [13] reported that increased biochar concentration reduces N_2O emissions and ammonium leaching. In a similar report Zhao et al., [14] found that soil application of coconut shell biochar reduced nitrogen leaching by 34.0%. Several other authors like Sun et al. [15] and Ding et al. [16] examined the reduction of NH_3 volatilization by increasing its half-life due to the addition of biochar. Dempster et al. [17] and Zhu et al. [18] also reported that net nitrogen mineralization in the coarse-texture soil was decreased due to increase of Jarrah biochar concentration. However, the research on the impacts of coconut shell biochar on latosol soil, one of the main soil types of Hainan Island in the southernmost part of China, has been reported much less so far. Both latosol and coconut shell biochar contain strong relationships due to having some special characteristics, because the latosol is always produced by the process of aluminization in the tropical regions with less fertility and strong acidity [19]. Similarly, coconut shell biochar has high porosity and pH [20], hence we assume that application of coconut shell biochar on latosol can affect soil properties as well as nitrogen mineralization.

Therefore, the main objective of this study was to evaluate the impacts of coconut shell biochar on (I) changes in soil properties including pH and CEC of tropical latosol under different rates of biochar; (II) soil enzymatic activities (urease and acid phosphatase) and soil microbial diversity (bacterial and fungal community structure); (III) tendency of soil nitrogen mineralization and transformation under different rates of biochar. The aforementioned information will help in evaluating the effectiveness of biochar in latosol and enhancing nitrogen use effectiveness.

Material and Methods

Preparation of Soil and Biochar

The soil was sampled after the harvest of banana from a field (109°54′28.0″E, 19°56′16.5″N, and 35 m in altitude) in Chengmai county, Hainan province, P. R. China. The climate of this area is tropical monsoon with an annual average air temperature of 23.8ºC, 2059 sunshine hours and mean yearly rainfall of 1786 mm. The rainy season is from May to October. This field has been used for banana plantations for five years. According to the field survey, the annual input amount of chemical fertilizer for each banana plant was recorded as 3.9 kg of compound fertilizer (15-15-15), 0.6 kg of potassium chloride and 3.0 kg of organic fertilizer (sheep manure), respectively. Organic fertilizer was applied twice per year (mainly in the seedling and vegetative stages), and chemical fertilizers once in a month. According to the national standard of classification and codes for Chinese soil (GB/T 17296-2009), the soil type from that region belongs to latosol soils (Ferralsols), which develop from basalt. Some basic soil properties are given in Table 1.

The coconut shell was first cut into pieces and dried in the oven at 105ºC, then the dried coconut shell was ground and sieved through a 2 mm sieve. The coconut shell powder was pyrolysed in a vacuum environment (-0.07Mpa) at 600ºC for one hour with a heating rate of 10ºC per min from 0ºC to 600ºC. The properties of coconut shell biochar are also shown in Table 1.

Incubation Experiment

Firstly, the collected soil samples were air dried, sieved through 2 mm mesh and pre-incubated under field conditions for two weeks at 25ºC with a constant

Table 1. Basic properties of soil and coconut shell biochar.

Properties	Soil	Properties	Biochar
pH	5.4	pH	9.7
EC (µs cm^{-1})	135	EC (µs cm^{-1})	950
SOM (g kg^{-1})	24.0	C (%)	62.1
Total N (g kg^{-1})	1.33	N (%)	0.58
Total P (g kg^{-1})	0.84	P (g kg^{-1})	0.99
Total K (g kg^{-1})	3.93	K (g kg^{-1})	12.7
Available N (mg kg^{-1})	108.5	Ca (g kg^{-1})	23.7
Available P (mg kg^{-1})	36.4	Mg (g kg^{-1})	3.69
Available K (mg kg^{-1})	775.9	CEC (cmol kg^{-1})	8.2
CEC (cmol kg^{-1})	9.7	BET surface area (m^2 g^{-1})	5.84
Texture	Clay	Zeta potential (mV)	-8.76

humidity of >95%. Secondly, coconut shell biochar at the rates of 0%, 1%, 2% and 5% (w/w) was added to 100 g of the pre-incubated soil, respectively then mixed and put in a plastic container (500 mL) covered by a lid. For each biochar rate, fifteen individual soil samples were prepared and then incubated aerobically at 25ºC for 35 days under a constant humidity of >95%. During the incubation period, three random soil samples were collected from each treatment at seven-day intervals to determine soil water content, soil ammonium nitrogen, and nitrate nitrogen, respectively. At the end of incubation, soil pH, CEC and enzymatic activity (urease and acid phosphatase) of the incubated and air-dried soil samples were determined and part of soil samples were stored at -80ºC for soil microbial community structure and diversity measurement.

Determination of Soil and Microbial Parameters

Soil was oven-dried to a constant weight at 105ºC, and water content was calculated by the ratio of the lost mass to the dried soil mass. Soil pH was determined in a suspension with a soil-water ratio of 1:2.5 by a pH meter (PHS-3C, INESA, China). Soil samples (3.5 g) were extracted with 50 mL of 1.66 cmol L^{-1} hexamine cobalt trichloride, shaken for one hour and then filtered by Whatman-1. The filtrates were analyzed by an ultraviolet visible spectrophotometer (DR 6000, HACH, USA) at the wavelength of 475 nm by following the method of Ciesielski and Sterckeman [21] to determine soil CEC. Similarly, 10 g of soil samples were added to 50 mL of 2 mol L^{-1} potassium chloride solution, shaken for one hour and then filtrates were used for the determination of ammonium nitrogen and nitrate nitrogen by using the indophenol blue colorimetry and dual wavelength spectrophotometry (DR 6000, HACH, USA) methods, respectively. Soil enzymatic activity was measured through enzyme test kits (Suzhou Comin Biotechnology Co. Ltd, China). According to the instruction of enzyme test kits, urease activity was determined by the release of ammonium nitrogen as a result of the hydrolysis of urea by using the Indigo colorimetry (DR 6000, HACH, USA) method under the wavelength of 625 nm [22-23]. Acid phosphatase activity could also be measured by how much phenol was made when disodium phenyl phosphate was broken down into phenol and disodium hydrogen phosphate by water under the wavelength of 660 nm using microplate reader (Synergy H1, Bio Tek, USA) [22, 24]. Microbial parameters were observed by the extraction of DNA from the sampled soil by using E.Z.N.A® Soil DNA Kit (D4015, Omega, lnc., USA). For bacteria, primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) were used, while for fungal strains, primers ITS1F12 (5′-GTGARTCATCGAATCTTTG--3′) and ITS2 (5′-TCCTCCGCTTATTGATATGC--3′) were used. The amplified PCR products were treated with 2% agarose gel electrophoresis, purified by AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified by Qubit (Invitrogen, USA). The sequencing was done by using an Illumina NovaSeq platform provided by LC-BIO Technologies Co., Ltd (Hangzhou, China), while paired-end reads were assigned to the samples based on their unique barcode and primer sequence. Paired-end reads were also merged using FLASH. Quality filtering on the raw reads was performed under specific filtering conditions to obtain the high-quality clean tags by using fqtrim (v0.94). Chimeric sequences were filtered using Vsearch software (v2.3.4) and de-replication was done using DADA2 to obtain the feature table and sequences. Alpha and beta diversity were analyzed by the QIIME2 process and pictures were drawn by R (v3.5.2). The sequence alignment of species annotation was performed by QIIME2 plugin feature-classifier while the alignment database was done through SILVA and unites.

Curve Fitting of Soil Nitrogen Mineralization

The change of soil net accumulated mineralized nitrogen (sum of soil ammonium nitrogen and nitrate nitrogen) with time was fitted by first-order dynamic model (Equ. 1) [25]. The performance of the model was evaluated by coefficient of determination (R^2) and a better fitted model was produced with a high value of R^2;

$$N_{net} = N_0 \times [1 - \exp(-k \times t)] \quad (1)$$

Where N_{net} is the soil net accumulated mineralized nitrogen (mg N kg^{-1}) during the incubation time of t (days), N_0 denotes soil organic nitrogen mineralization potential (mg N kg^{-1}) while k stands for mineralization rate constant (day^{-1}). The *exp* refers to an exponential function.

Data Collection and Statistical Analysis

The data was primarily organized by Microsoft Excel 2017 and analyzed statistically using IBM SPSS statistics (v19.0) for performing analysis of variance along with Duncan's multiple range test. For the parameters of the first-order dynamic model for organic nitrogen mineralization, mineralization potential (N_0) and rate constant (k), SigmaPlot v12.5 (Systat Software Inc., California) was used. Histograms were drawn in Excel 2017. R-software was used to fit curves for soil nitrogen mineralization.

Results

Effect of Biochar on Soil Properties

As shown in Table 2, after the incubation, soil pH and CEC in the three biochar treatments were increased significantly (P<0.05) compared to the control, and with more rate of coconut shell biochar applying to soil (e.g., 5%), these properties improved more significantly, especially for soil pH. As concerned soil enzymatic activities, the biochar amendment showed a non-significant effect on soil acid phosphatase, although more biochar application increased acid phosphatase activity. Compared to the control, urease activity was significantly higher in the treatments that used 1% and 2% biochar, but it was significantly lower in the treatments that used up to 5% biochar, which meant too much coconut shell biochar application might have an inhibitory effect on urease activity.

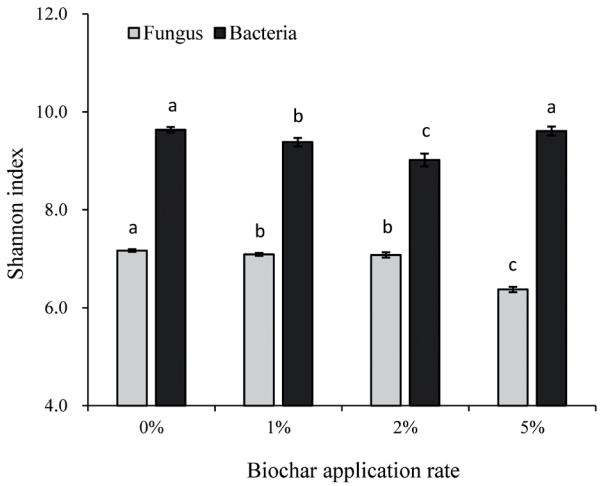

Fig. 1. The change of soil microbial diversity for the treatments with different application rates of biochar (0%, 1%, 2% and 5% *w/w*). The lowercase letters among different treatments indicate the significance of difference. The same letters show no significant difference, and different letters show significant difference (P<0.05).

Effect of Biochar on Soil Microbes

Biochar amendment had a significant effect on soil microbial diversity. For bacteria, 1% and 2% of biochar addition compared to that of control significantly reduced soil bacterial Shannon index (P<0.05), but when biochar rate increased to 5%, the bacterial diversity increased and returned to a similar level as the control (Fig. 1). The structure of the soil bacteria

Table 2. Soil pH, CEC and enzyme activity for the treatments with different application rates of biochar..

Biochar rates	Soil properties			
	pH	CEC	Urease	Acid phosphatase
	(-)	(cmol kg^{-1})	(μg d^{-1} g^{-1})	(μmol d^{-1} g^{-1})
0%	5.86±0.02 d	8.12±0.01 c	726±12.0 c	15.8±2.10 a
1%	6.02±0.03 c	8.45±0.06 b	884±17.4 b	16.4±1.50 a
2%	6.09±0.02 b	8.69±0.02 a	1003±16.5 a	17.0±2.25 a
5%	6.41±0.03 a	8.80±0.11 a	678±22.3 d	18.1±0.28 a

Note: CEC refers to cation exchange capacity (cmol kg^{-1}). Same letters show no significant differences, and different letters show significant differences (P<0.05).

community was also changed by addition of coconut shell biochar. The major bacterial communities in the soil amended with biochar consisted of *Proteobacteria, Actinobacteria*, *Acidobacteria, Gemmatimonadetes* and *Chloroflexi*. At phylum level, the relative abundance of *Proteobacteria*, *Gemmatimonadetes, Planctomycetes, Verrucomicrobia* and *Bacteroidetes* increased with a rate of 1% or 2% biochar addition. The relative abundance of *Actinobacteria* and *Chloroflexi* reduced with a 5% rate of biochar addition (Fig. 2). At genus level, the relative abundance of *Sphingomonas, Lysobacter* and *Gemmatirosa* increased with a rate of 1% or 2% biochar application, while only *Gemmatimonas* increased significantly when the biochar application rate was up to 5%. For fungal diversity, the Shannon index for the treatments with a rate of 1% and 2% of biochar

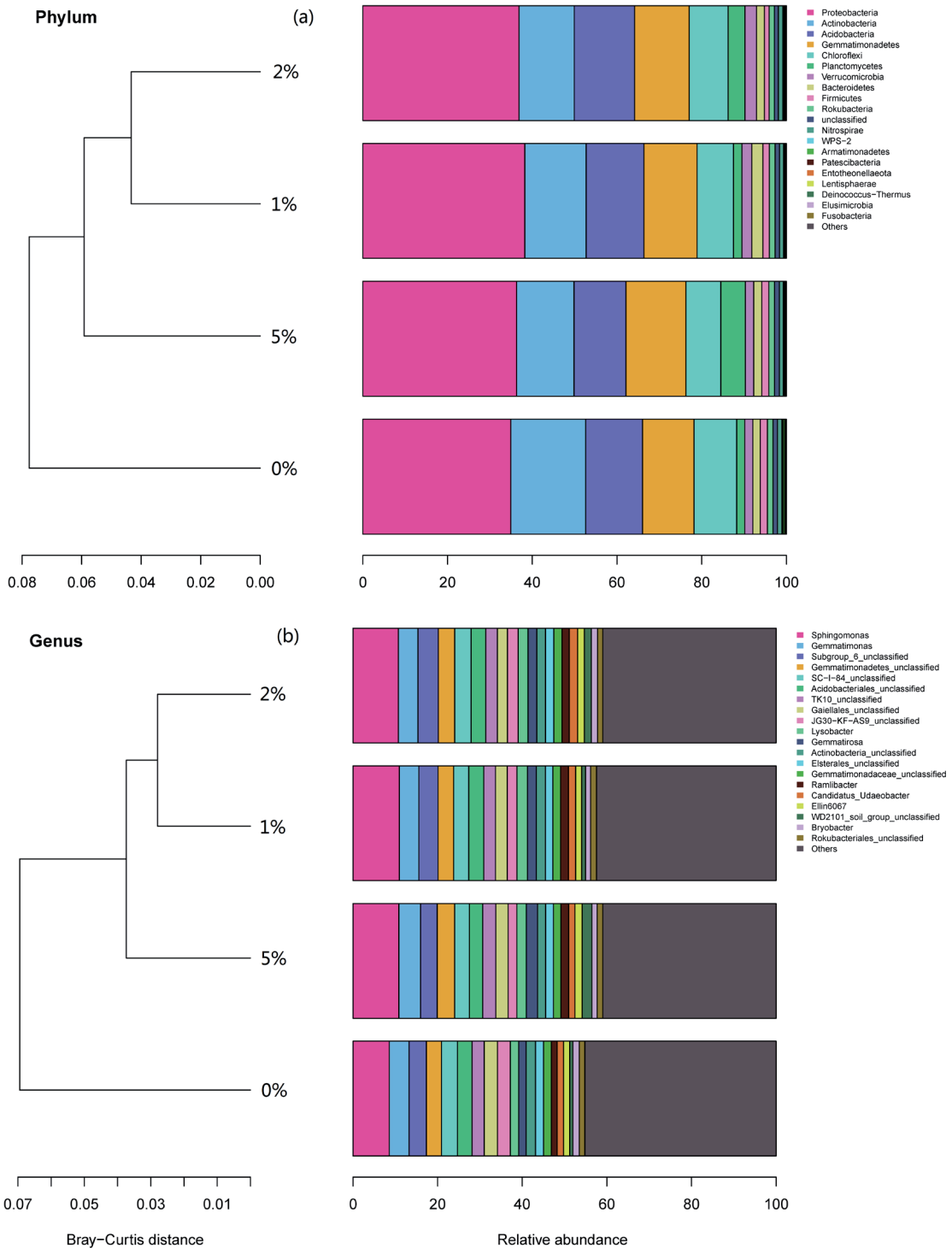

Fig. 2. The cluster analysis of soil bacteria community structure in phylum a) and genus levels b), respectively. 0%, 1%, 2% and 5% refer to the biochar addition rate (*w/w*).

application decreased slightly, while it decreased significantly when the biochar rate was up to 5%. At phylum level, the relative abundance of *Ascomycota* for the treatments with 1% and 2% rates of biochar decreased, while *Basidiomycota* and *Chytridiomycota* increased significantly. The abundance of *Ascomycota, Basidiomycota* and *Chytridiomycota* for the treatment with 5% biochar increased, but *Zygomycota* abundance decreased. At the genus level, the relative abundance of *Haematonectria, Chaetomium, Gibberella, Aspergillus, Fusarium* and *Eupenicillium* for all the biochar treatments decreased effectively, especially the *Fusarium* species with more than 80% decrease compared with the control, but *Humicola* and *Curvularia* abundance for the treatment with 5% biochar increased greatly (Fig. 3).

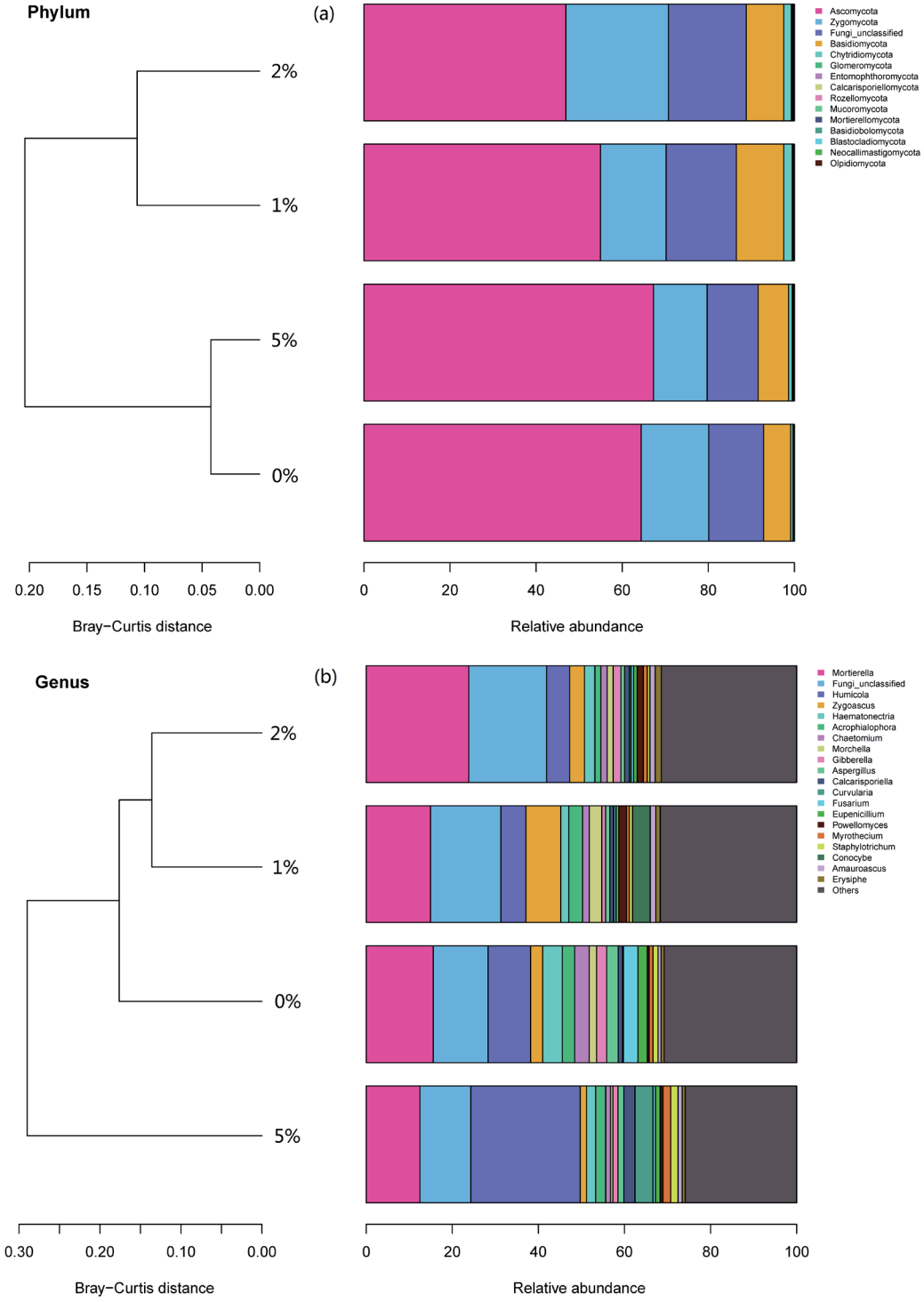

Fig. 3. The cluster analysis of soil fungal community structure in phylum a) and genus levels b), respectively. 0%, 1%, 2% and 5% refer to the biochar addition rate (*w/w*).

Table 3. The fitted parameters of nitrogen mineralization model for the treatments with different application rates of biochar.

Biochar rates	R^2	N_0	k	P
0%	0.98	15.62	0.17	<0.01
1%	0.98	9.56	0.15	<0.01
2%	0.97	5.68	0.12	<0.01
5%	0.98	6.19	0.10	<0.01

Note: R^2 refers to the coefficient of determination of model; N_0 was nitrogen mineralization potential (mg N kg^{-1}); k was the constant of mineralization rate (day^{-1}). P was the P-value for the fitted model.

Effect on Soil Nitrogen Mineralization

As shown in Table 3 and Fig. 4, the dynamics of soil organic nitrogen mineralization (SONM) for all the treatments could be fitted well by a first-order kinetic model. The soil nitrogen mineralization potential (N_0) and mineralization rate constant (k) reduced significantly after biochar application compared to the control. However, biochar application rate 5% enhanced the mineralization potential from 5.68 to 6.19 mg N kg^{-1} of soil. It showed that 2% of biochar was optimal to inhibit soil nitrogen mineralization.

Discussion

Effects of Biochar Addition on Soil Property and Enzymatic Activity

In the present study, coconut shell biochar application in tropical latosol increased soil pH and CEC, and more amounts of biochar resulted in higher soil pH and CEC. Through a one-year incubation study, Yang et al. [26] observed rice straw biochar significantly (P<0.05) increased soil pH and CEC, especially at a 5% rate of biochar. Biochar could increase soil pH and CEC due to the alkaline feature and high cation exchange capacity of biochar [7]. As for soil enzyme activity, we found soil urease activity showed an increasing or decreasing trend with the fluctuating application rates of biochar, and 2% of coconut shell biochar addition was found to be a turning point, while biochar addition had non-significant effects on soil acid phosphatase activity. Various conclusions could be found in the literature. Futa et al. [10] suggested a rate of 20 t ha^{-1} of biochar addition to soil had the highest urease activity among the four treatments (0, 10, 20, and 30 t ha^{-1}). However, Yang et al. [26] demonstrated that urease activity increased after the addition of 5% rice straw biochar, and found that both bamboo and rice straw biochar applied in a sandy loam paddy soil had no significant impact on acid phosphatase activity, but Gao et al. [11] showed 2% wheat straw biochar amendment significantly enhanced soil acid phosphatase (P<0.05). As a result, we think that soil type, application rate, and biochar feedstock type all had an impact on soil enzyme activity. According to Acosta-Martínez's research [27], acid phosphatase activities was greater in Oxisols and Ultisols than in Inceptisols, and demonstrated that within the watershed, acid and low fertility soils such as Oxisols and Ultisols have in general higher enzyme activities than less weathered tropical soils of the order Inceptisols, probably due to their higher organic matter content and finer texture.

Effects of Coconut Shell Biochar Addition on Soil Microbes

Compared to the control, soil bacterial diversity increased when the biochar addition rate was less than 2%, but it significantly decreased with a 5% rate of biochar (Fig. 1). The fungal diversity continued to decline significantly with biochar addition. Xu et al.

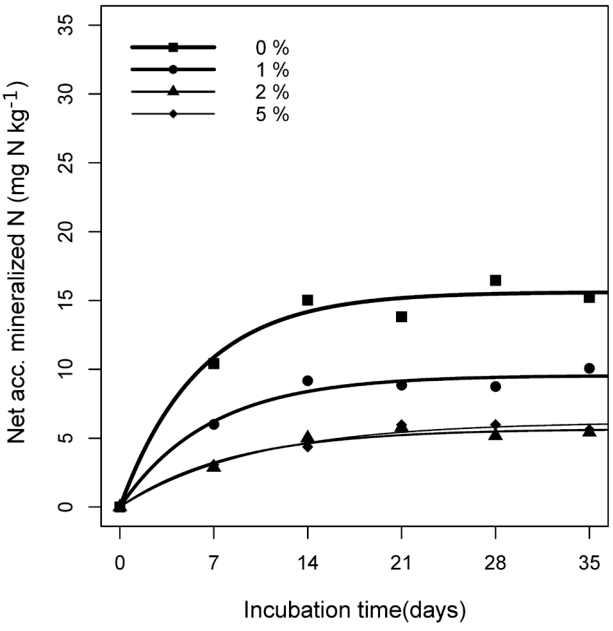

Fig. 4. The fitted soil nitrogen mineralization dynamic by first-order exponential model for the treatments with different application rates of biochar (0%, 1%, 2% and 5% *w/w*). The data points refer to the measured values, and the curve lines were the fitted models of soil nitrogen mineralization for different treatments.

[12] suggested that the farmland bacterial diversity improved with corn-straw biochar (addition ratio: 2%, 4% and 8%) and urea addition, and was positively related to the biochar addition ratio. However, Liu et al. [28] observed a decrease trend in the bacterial diversity after the addition of wheat straw biochar (the ratio: 0.5%, 1% and 2%) in an agricultural soil. Tarin et al. [29] found that the red soil amended with bamboo biochar exhibited a substantial ability to increase the fungal richness and diversity, and showed that soil pH was the most influential factor in shaping the soil fungal communities. Li et al. [30] suggested that long-term and low-rate addition of biochar always increases total microbial diversity, and the increases in total microbial diversity with biochar addition were greater in acidic and sandy soils with low soil organic carbon content and in laboratory incubation studies. The difference in the impacts might be due to the biochar rates and the nature of soils. Zhang et al. [31] suggested that the effects of biochar and chemical fertilizers on soil fungi may vary due to the soil type. Lehmann et al. [32] supported the arguments about the amount of biochar addition, soil conditions and pyrolysis conditions of biochar.

Soil microbial community structure was also affected by biochar addition. In the present study, for bacteria, the relative abundance of *Proteobacteria*, *Gemmatimonadetes, Planctomycetes, Verrucomicrobia* and *Bacteroidetes* increased with a rate of 1% or 2% biochar addition. The relative abundance of *Actinobacteria* and *Chloroflexi* reduced with a 5% rate of biochar addition. The main reasons in the decrease of *Actinobacteria* might be due to increase in the soil pH after the addition of a higher amount of biochar [11]. Gao et al. [33] and Zheng et al. [34] also revealed that *Actinobacteria* decreased with biochar addition, but Prayogo et al. [35] and Xu et al. [12] found that *Actinobacteria* increased after corn-straw biochar addition. Xu et al. [12] also showed that the relative abundance of *Proteobacteria* and *Chloroflexi* decreased. *Chloroflexi* decreased, which may have been caused by an increase in soil electrical conductivity and salinity following charcoal application [36]. Zhang et al. [37] reported that *Chloroflexi* were more prevalent with low soil salinity. For fungi, the relative abundance of *Ascomycota* decreased with 1% and 2% rates of biochar addition, while *Basidiomycota* and *Chytridiomycota* increased. The abundance of *Ascomycota, Basidiomycota* and *Chytridiomycota* with 5% biochar increased, but *Zygomycota* abundance decreased. The results were consistent with Gao et al. [33] and Zheng et al. [34], showed that *Ascomycota* decreased with the increasing application rates of biochar. The variations in the soil chemical properties after applying biochar, such as pH, SOC and C/N, were found to be important factors for shifting communities [34].

Effects of Biochar Addition on Nitrogen Mineralization

The addition of biochar affected soil chemical properties and microbial activity [32], and thus influenced soil nitrogen transformation [35]. In this study, it was found that the addition of coconut shell biochar suppressed soil nitrogen mineralization (Fig. 4). Prayogo et al. [35] also examined that willow biochar with the application rates of 0.5% and 2% significantly reduced soil nitrogen mineralization, and Dempster et al. [17] suggested the net nitrogen mineralization in the coarse-texture soil decreased with the increase of Jarrah biochar application. Lentz et al. [38] found that soil nitrogen mineralization after applying biochar was reduced by 33%. Biochar could significantly decrease NH_4-N and NO_3-N due to nitrogen immobilization in the soil [39]. Yang et al. [40] found that rice straw biochar applied in Gleyi-Stagnic Anthrosols and Argi-Udic Ferrosols reduced soil ammonium nitrogen by 9-35% and 5-22% less than that of un-amended soils, respectively. Chemical sorption was the main reason for the inhibition of nitrogen mineralization in the biochar-amended soil [10, 40]. Another reason might be the high soil pH after applying biochar, which stimulated ammonia volatilization [41]. Free ammonia can slow down the process of nitrification, which is a key step in mineralization [42].

Conclusions

Through an incubation experiment in tropical latosol, coconut shell biochar showed its capability to increase soil pH, cation exchange capacity and urease activity, but had no impact on acid phosphatase activity. The microbial diversity for biochar-amended soil decreased and microbial community structure also showed a significant response. Moreover, it was also observed that soil nitrogen mineralization with biochar addition could be fitted well by the first-order exponential model, and biochar addition decreased soil nitrogen mineralization. It was suggested that the special characteristics of coconut shell biochar such as porosity and alkalinity, could change the soil properties by altering its enzymatic activity, microbial community shifting and nitrogen transformation.

Acknowledgments

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Conflict of Interest

The authors declare no conflicts of interest.

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