

Soil Bacterial and Fungal Communities Response Differently to *Bombax Ceiba* (Malvaceae) in a Traditional Agroforestry System

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Abstract

Background Agroforestry system is one of promising directions for developing sustainable agriculture because the intercropping of crops and trees may facilitate resource cycling and avoid soil degradation. *Bombax ceiba* (Malvaceae), a tall tree with red flowers blooming in Spring, is traditionally planted in rice paddies in tropical Asia, while the roles of *B. ceiba* in the agroecosystem remain unexplored.

Methods In this paper, we investigated and assessed spatiotemporal variations of soil nutrient contents and soil bacterial and fungal communities along the distance gradients to *B. ceiba* during three reproductive stages of rice, i.e. booting, heading, mature, in a typical *Bombax*-dominated rice paddy on Hainan Island, South China.

Results *B. ceiba* in rice paddy could improve the soil nutrient conditions, particularly available K and soil organic carbons. The relative abundance of Chloroflexi and Ascomycota increased while Actinobacteria decreased along the distance gradient to *B. ceiba*. In addition, the relative abundance of Firmicutes was highest at harvesting stage of rice, while Acidobacteria was richer in the early reproductive stage of rice. Soil potassium content was the principal driver in shaping soil bacterial diversity and composition, while fungal community was mainly affected by soil nitrogen.

Conclusions Our results provide evidences for positive influences of *B. ceiba* on biotic and abiotic traits of rice paddy soils and thus lend supports to the ecological basis of this tropical Asian endemic traditional Agroforestry system, which could increase resource cycling and paddy stability and have the potential to reduce carbon emission.

Introduction

Agroforestry system is an artificial ecosystem in which trees and crops simultaneously grow on the arable land (Kay et al. 2019; Udawatta et al. 2019; Zhu et al. 2020). The ecological interactions of trees and crops are very complex that could be complementary or competition (Querné et al. 2017; Hombegowda et al. 2020). For instance, trees take up nutrients from deep soil layers while crops drawing nutrients from topsoil layers, thereby shaping a complementary interaction in resource utilization (Hombegowda et al. 2020; Homulle et al. 2021). Furthermore, these nutrients absorbed by trees will return to the topsoil of arable land by litter decomposition (Isaac and Borden 2019). These processes will promote the circulation of nutrients and reduce the loss of nutrients in arable land. In addition, agroforestry system is recognized as a possible approach for maintaining biodiversity and decreasing soil erosion (Udawatta et al. 2019; Zhu et al. 2020). However, in order to produce sufficient food, the traditional agroforestry systems that were based on diverse species and species interactions have been abandoned in many places in the past few decades; while the modern agriculture that generally depends on monocultures and inputs of chemical fertilizers and pesticides was developed (Xie et al. 2011). Although this convert considerably increases the agricultural production, it leads to soil compaction, environmental pollution and a decreasing biodiversity and ecosystem stability in arable lands (Chen et al.

2019). Therefore, exploring the interactions of species and recognizing the ecological legacy in the traditional agroforestry systems may help us develop environmentally sustainable agriculture.

In agroforestry system, trees can bring different microhabitats through forming shade or inputting multiple different resources by their root, flower, and leaf litters (Lin 2007), which could further affect the diversity and composition of soil microorganisms in arable land. Soil bacteria and soil fungi are two important groups of soil microorganisms, vastly different morphological traits, environmental sensitivities and substrate utilization may lead to their different responses to microhabitats in agroforestry system (Araujo et al. 2012; Hannula et al. 2017). Banerjee et al. (2016) found that bacterial abundance and richness were greater in plots with trees in agroforestry systems in Canada. Additionally, temperate agroforestry system has been shown to increase diversity of soil fungal communities and promote community turnover with increasing agroforestry age (Beule and Karlovsky 2021a). In addition to spatial variations, soil microorganisms would vary along with rice reproductive stages, i.e. booting, heading, mature, due to changing conditions caused by combined actions of organic matter decomposition and nutrient absorption of rice. Previous study showed that soil soluble organic carbon was significantly higher in mature stage than in booting and heading stages (Yang et al. 2019). Such soil change would bring the temporal dynamics of soil microorganisms.

In the tropical Asia including Vietnam, Thailand, Bangladesh, Nepal, and South China, *Bombax ceiba* (Malvaceae), a tall tree with numerous red flowers in Spring, is widely distributed in local rice paddies (Koju et al. 2020; Fig. 1). In Hainan Island, China, many old *B. ceiba* trees are kept and grown in paddies by indigenous people and they start to transplant rice seedlings only when *B. ceiba* starts flowering, normally at the middle February (Tang et al. 2015). These *B. ceiba* trees have been proven to be a good habitat for Ciconiiformes birds in Nepal (Koju et al. 2020). Several studies also found that soil microbial diversity and composition were changed in agroforestry systems due to more and different organic matter inputs from trees (Liu et al. 2019; Beule and Karlovsky 2021b). However, no studies have considered the influence of the *B. ceiba* with buttress roots and spreading fine roots on soil microorganisms and nutrient contents in this Asian endemic agroforestry system.

In this study, we assessed the influence of *B. ceiba* on soil nutrient contents and bacterial and fungal community during three different stages of rice, i.e. booting, heading, mature, in *Bombax* – rice agroforestry system. We hypothesized that (1) *B. ceiba* in rice paddies would increase the soil nutrient content, such as nitrogen (N) and phosphorus (P); (2) soil bacterial and fungal community would have different spatiotemporal distribution patterns along the distance gradients to *B. ceiba* during different reproductive stages of rice; (3) soil nutrient content might differently affect distribution patterns of bacterial and fungal community in *Bombax* – rice agroforestry system.

Materials And Methods

Study site and soil sampling

The study site was located at Changjiang County (19°14'36"N, 108°58'58"E), Hainan Island, China, which is a typical area with the most complete preservation of *Bombax* – rice agroforestry system (Fig. 1). This area is characterized by a tropical island monsoon climate, with sufficient sunlight and precipitation. The mean annual temperature is 24.3°C, with a mean annual precipitation of 1676 mm.

We set three 20m × 20m plots, with one big *B. ceiba* tree at a corner of edge of a rice paddy, as three independent replicates in *Bombax* – rice agroforestry system, and the distance between every two plots is about 50 m. The *B. ceiba* tree in each plot has a diameter at breast height no less than 0.5m, a height about 5m and a crown width about 4m × 4m, to ensure the tree is at least five-year-old in the rice paddy.

In each plot, soil surface (0-10 cm depth) samples were collected from three different sites, i.e. **D0** (distance to *B. ceiba* is 0m), **D1** (distance to *B. ceiba* is 1m), and **D5** (distance to *B. ceiba* is 5m) to represent under, near and far from the *B. ceiba* tree. Each replicate consisted of three subsamples (three directions centered on *B. ceiba*), which were collected and saved separately for subsequent analysis.

Sampling was carried out in three reproductive stages of rice, respectively in middle April, early May and late May, namely booting stage (**BS**), heading stage (**HS**) and mature stage (**MS**). Each soil samples were divided into two portions: one was air-dried for soil nutrient analysis, and the other was stored in sterile centrifuge tube at -80°C for further DNA extraction.

Measurement of soil nutrient contents

Soil organic carbon (SOC) was measured by potassium dichromate - external heating method. Available nitrogen (AN), available phosphorus (AP), available potassium (AK), total nitrogen (TN), total phosphorus (TP) and total potassium (TK) were measured with Soil Nutrient Analyzer (YT-TRX03). In order to reduce the sampling error, we take the mean value of three subsamples in each treatment as representative for further analysis.

DNA extractions, PCR amplifications, Illumina Miseq-based sequencing and Bioinformatics analysis

Soil DNA were extracted using PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) following the manufacture instructions. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified using the forward primer 338F (5'-ACTCCTACGGGAGGGCAGCAG-3') and the reverse primer 806R (5'-GGACTACNNGGGTATCTAAT -3') (Li et al. 2018; Mwaheb et al. 2017), and the fungal ITS region was amplified using the forward primer ITS1 (5'- CTTGGTCATTTAGAGGAAGTAA -3') and the reverse primer ITS2 (5'- TGC GTTCTTCATCGATGC -3') (Liu et al. 2015; Chen et al. 2020). These primers were added a set of 8-digit barcode sequence unique to each sample (provided by Allwegene Technology, Beijing). PCR amplifications were performed following the procedure described by (Wang et al. 2019; Wang et al. 2021). PCR products were pooled and purified using the Agencourt AMPure XP Kit (Beckman Coulter, United States). Then purified PCR products were pooled in equimolar solution and paired-end sequenced (2 × 300) on an Illumina MiSeq platform by Allwegene Technology (Beijing).

We processed the sequence data in the QIIME 2 (Bolyen et al. 2019). Chimera sequences were removed with USEARCH, then the highly accurate sequences were clustered into operational taxonomic units (OTUs) with 97% identity using UPARSE. The taxonomy of each 16S rRNA gene sequence was assigned against the Silva138 16S rRNA database (Quast et al. 2012), while the taxonomy of each ITS gene sequence was assigned against the Unite database (Nilsson et al. 2019) using UCLUST. The bacterial and fungal OTU tables were rarefied to 17,893 and 24,045 sequences per subsample, respectively. Both non-bacteria and non-fungi in the OTU tables were removed, then we take the sum value of each OTU in three subsamples as soil sample value to obtain the final bacterial and fungal OTU tables. Based on these two OTU tables, we calculated the richness index and Shannon index of bacteria and fungi.

The bacterial and fungal DNA sequences in this paper have been submitted to SRA in the NCBI database under accession number SRP352234 and SRP352669, respectively.

Statistical analysis

One-way Analysis of Variance (One-way ANOVA) was used to compare the differences in soil nutrient contents, relative abundance of the dominant phyla (with relative abundance > 1.0%) and alpha diversity (richness and Shannon index) of bacterial and fungal community among distance gradients to *B. ceiba* for each reproductive stage of rice. We performed Two-way Analysis of Variance (Two-way ANOVA) to test the influence of *B. ceiba* and reproductive stage of rice on the soil nutrient contents.

The relationship between bacterial and fungal alpha diversity and soil nutrient properties were explored using Pearson's correlation analysis. Multiple regression analysis was used to evaluate the relative influence of *B. ceiba*, reproductive stage of rice and soil nutrient properties on bacterial and fungal alpha diversity based on all-subsets regression (nbest = 2) in "leaps" package (Lumley 2020). Then, we determined the best model based on the Mallows' Cp and Residual Sum of Squares for each model. Finally, the independent contribution of each variable in the best model was assessed through hierarchical partitioning analysis in "hier.part" package (Nally and Walsh 2004).

We generated non-metric multidimensional scaling (NMDS) plots to visualize the effect of *B. ceiba* and reproductive stage of rice on bacterial and fungal community composition, while tested these effects with ANOSIM and PERMANOVA methods in the "vegan" package (Oksanen et al. 2017). Function of "venn.diagram" in "VennDiagram" package (Chen 2021) was used to depict the number of common bacterial or fungal OTUs in different distances to *B. ceiba* for each reproductive stage of rice. Mantel test was used to explore the relationship between bacterial and fungal community composition (Bray-Curtis dissimilarity) and individual soil nutrient property (Euclidean distance) based on 10,000 permutations (Oksanen et al. 2017). Redundancy Analysis (RDA) was employed to evaluate explanation of all variables for bacterial and fungal community variations. Independent effect of each variable was evaluated by r-squared values using "rdacca.hp" package (Lai et al. 2021) of R 4.1.1.

Results

Influence of *B. ceiba* on soil nutrient contents

The values of whole soil nutrient properties were highest at D0 among all reproductive stages of rice, suggesting *B. ceiba* may not compete with rice for soil nutrients (Fig. 2). Results of One-way ANOVA showed that *B. ceiba* had a significant influence on soil nutrient properties in rice paddy, and this influence varied with different reproductive stages of rice (Fig. 2 & Table 1). During booting stage of rice, both SOC ($P < 0.05$) and TN ($P < 0.05$) were significantly higher at D0 than D5 but not D1. Similarly, this pattern could be found on SOC ($P < 0.05$) and TP ($P < 0.05$) during heading stage of rice. Further, we found that AN ($P < 0.05$) and AK ($P < 0.05$) were significantly higher at D0 than D1 and D5 during mature stage of rice (Fig. 2). These results indicated that rice paddy closer to the *B. ceiba* would have higher soil nutrients.

Table 1

Two-way ANOVA of soil nutrient properties between distance gradients to *Bombax ceiba* and reproductive stages of rice.

Variance sources	SOM	AN	AP	AK	TN	TP	TK
Distance to <i>B. ceiba</i> (DB)	6.577**	1.604	2.825	6.549**	4.314*	4.489*	0.187
Reproductive stage of rice (RSR)	0.856	10.147**	4.825*	1.047	1.977	53.160**	17.028**
DB × RSR	0.189	2.324	0.813	0.856	0.352	1.417	0.204
Values in the table are F value, and “*” represent $P < 0.05$ while “**” represent $P < 0.01$							

Influence of *B. ceiba* on soil bacterial and fungal alpha diversity and its links with soil nutrient properties

One-way ANOVA showed that *B. ceiba* significantly affected bacterial richness at booting stage of rice and bacterial Shannon index at the whole reproductive stages of rice (Fig. 3). Specifically, these alpha diversity indices were higher at D1 than D0 (Fig. 3), suggesting that *B. ceiba* may promote the increase of bacteria in rice paddy soil. In contrast to bacteria, fungal richness and Shannon index were not obviously different among distance gradients to *B. ceiba* (Fig. 3). These results indicated that *B. ceiba* had different effect on rice paddy soil microorganisms.

Correlation analysis showed that bacterial richness was negatively correlated with AP and AK while Shannon index was negatively correlated with AP and TN. In addition, both the bacterial alpha diversity indices positively correlated with TK (Table S1). However, no significant relationship was found between fungal alpha diversity and soil chemical properties (Table S1).

Furthermore, multiple regression analysis showed that bacterial richness was mainly predicted by distance to *B. ceiba*, AP and TK ($R^2 = 0.177, 0.132$ and 0.193 , respectively; $P < 0.05$, Table 2), whereas the bacterial Shannon index was mainly predicted by distance to *B. ceiba*, reproductive stage of rice, and AP ($R^2 = 0.223, 0.381$ and 0.175 , respectively; $P < 0.05$, Table 2). In contrast, both richness and Shannon index of fungi were principally explained by reproductive stage of rice and AN ($R^2 = 0.256, 0.129$ and $0.177, 0.178$, respectively; $P < 0.05$, Table 2).

Table 2
Explanatory variables retained in the best models for explaining bacterial and fungal alpha diversity

	Alpha diversity index	Variables	Individual contribution of variable (%)	P	Model R^2_{adj}	Model P
Bacteria	Richness	Distance to <i>B. ceiba</i>	17.72	<0.05	0.496	<0.01
		AN	5.67	<0.05		
		AP	13.19	<0.1		
		SOM	5.28	<0.05		
		TK	19.33	<0.01		
	Shannon index	Distance to <i>B. ceiba</i>	22.28	<0.001	0.825	<0.0001
		Reproductive stage of rice	38.09	<0.01		
		AP	17.54	<0.05		
		AK	4.36	=0.102		
		SOM	3.00	<0.05		
Fungi	Richness	Reproductive stage of rice	25.58	<0.01	0.379	<0.01
		AN	17.72	<0.01		
		AP	4.12	=0.462		
	Shannon index	Reproductive stage of rice	12.93	<0.05	0.217	<0.05
		AN	17.78	<0.01		

The relative roles of *B. ceiba*, rice stage and soil nutrient properties in determining soil bacterial and fungal community composition

NMDS plot showed that bacterial community composition was different among distance gradients to *B. ceiba* (Fig. 4A), which was further demonstrated by ANOSIM ($R = 0.521$; $P = 0.0001$) and PERMANOVA ($F = 7.992$; $P = 0.0001$) methods (Table 3). Based on a relative abundance $> 1\%$, Proteobacteria, Acidobacteria, Firmicutes, Chloroflexi, Actinobacteria, Myxococcota, Verrucomicrobia, Desulfobacteria, Gemmatimonadetes, Bacteroidetes, Planctomycetes, Nitrospira, Methyloirabilota were identified as dominant bacterial phyla in the studied plots (Fig. 4B). The relative abundance of most of these phyla were significantly different among distance gradients to *B. ceiba* (Table S2). For example, the relative abundance of Chloroflexi phylum was significantly higher at D1 and D5 than D0, while the relative abundance of Actinobacteria phylum was significantly higher at D0 than D1 and D5 during the whole reproductive stages of rice (Table S2). In addition, it cannot be ignored that rice stages also significantly affected some bacterial dominant phyla. For instance, the relative abundance of Firmicutes phylum was significantly higher at heading and mature stages than at booting stage, while the relative abundance of Acidobacteria phylum was significantly higher at booting stage than at heading and mature stages (Table S2).

Table 3

Variations of bacteria and fungi community composition among distance gradients to *Bombax ceiba* or in different reproductive stages of rice, as tested with ANOSIM and PERMANOVA methods

Variables		ANOSIM		PERMANOVA	
		R	P	F	P
Bacteria	Distance to <i>B. ceiba</i>	0.5211	0.0001	7.9924	0.0001
	Reproductive stage of rice	0.1483	0.0133	2.0714	0.0325
Fungi	Distance to <i>B. ceiba</i>	0.5861	0.0001	5.7872	0.0001
	Reproductive stage of rice	0.0440	0.1714	1.1771	0.2220

Similarly, fungal community composition also varied with distance to *B. ceiba* (Fig. 5A), which was confirmed by ANOSIM ($R = 0.586$; $P = 0.0001$) and PERMANOVA ($F = 5.787$; $P = 0.0001$, Table 3). Of the identified phyla, Ascomycota, unidentified fungi, Basidiomycota, Mortierellomycota, Rozellomycota, Chytridiomycota and Glomeromycota phyla dominated the fungal communities in this study plots (Fig. 5B). The relative abundance of Ascomycota phylum was significantly higher at D5 than D0 and D1 at heading stage of rice while higher at D5 and D1 than D0 at mature stage of rice (Table S2), which suggesting that soil fungal community in rice paddy closer to *B. ceiba* would be simultaneously affected by *B. ceiba* and rice. Furthermore, Venn diagram showed that more bacterial and fungal OTUs were shared at D0 and D1 rather than D0 and D5 (Fig. S1).

Mantel correlation analysis showed that both bacterial and fungal community composition were significantly correlated with AN, AP, AK, SOC and TN (Table S3). In RDA, the whole variables respectively explained 52.4% and 39.8% of the variations of bacterial and fungal community (Fig. 6). Furthermore, the relative contribution of each variable was quantified by “rdacca.hp” function. Distance to *B. ceiba* ($R^2 = 0.217$, $P < 0.01$) and AK ($R^2 = 0.076$, $P < 0.05$) were revealed to be factors significantly affecting bacterial community composition, while distance to *B. ceiba* ($R^2 = 0.194$, $P < 0.01$), SOC ($R^2 = 0.050$, $P < 0.05$) and TN ($R^2 = 0.037$, $P < 0.05$) significantly affected fungal community composition (Fig. 6).

Discussion

Influence of *B. ceiba* on soil nutrient contents

Our results revealed that soil nutrient content was highest at D0, and decreased from D1 to D5 (Fig. 2), indicating that *B. ceiba* may increase soil nutrient contents rather than competing with rice for soil resources. The root system of rice is mainly distributed at the topsoil of 0-20cm, while the fine roots of *B. ceiba* were distributed at 0-40 cm of soil (Das and Chaturvedi 2008). Based on such distribution characteristics of these two plant roots, resources competition should be found (Yun et al. 2012; Wu et al. 2020). However, our investigation results of soil nutrients could not confirm this prediction. Two scenarios could explain such observed pattern. First, it may be due to good nutrient conditions in this arable land since soil background nutrient is high in this tropical island (Tang et al. 2015). Second, the *B. ceiba* is in blooming during the entire rice stages and it might need limited growth and even this tree could increase soil nutrient contents through decomposition of its dropped flowers and dead roots (Hassan 2018). Therefore, *B. ceiba* may promote the accumulation and cycling of nutrients in the *Bombax* – rice agroforestry system. Soil nutrient content was higher at D1 closer to *B. ceiba* than D5 far from *B. ceiba*, especially on the early reproductive stage of rice (Table 1), which could support this idea.

We also found that changes of soil nutrient contents among the distance gradients to *B. ceiba* were different among rice stages. For instance, SOC was significantly different between D0 and D5 during booting and heading stages of rice, whereas it showed no difference during mature stage of rice (Fig. 2). This point out that it is necessary to consider temporal dynamics when exploring the ecological processes in *Bombax* – rice agroforestry system. Previous study in cocoa agroforestry systems has reported that nutrient return through leaf litter followed a seasonal pattern (Asigbaase et al. 2021).

Changes in diversity and composition of soil bacterial and fungal community in *Bombax* – rice agroforestry system

To our knowledge, this is the first study simultaneously investigating spatiotemporal variations of soil bacterial and fungal community and their response towards the tree in *Bombax* – rice agroforestry system. Our results showed that bacterial alpha diversity changed among distance gradients to *B. ceiba*, and it was lowest at D0 (Fig. 3). However, fungal alpha diversity was not different along distance

gradients to *B. ceiba* (Fig. 3). The possible explanation for this difference could be attributed to the fact that fungi is generally more resistant than bacteria to environment change (de Vries and Shade 2013), thus the microhabitats resulted from *B. ceiba* would increase the bacterial rather than fungal alpha diversity in rice paddies.

The compositions of soil bacterial and fungal community differed among the distance gradients to *B. ceiba* (Fig. 4 & Fig. 5), and this indirect variable of distance to *B. ceiba* explained most variations of bacterial and fungal communities (Fig. 6). The underlying reason maybe the different nutrient available arose from *B. ceiba* for microorganisms. Specifically, we found that relative abundance of Chloroflexi phylum was higher in rice paddy soil far away from *B. ceiba* during the whole reproductive stages of rice, whereas relative abundance of Actinobacteria phylum occupied a greater advantage at D0 (Table S2). Previous studies have reported that Chloroflexi phylum commonly exist in the topsoil of rice fields (Osman et al. 2017; Li et al. 2019), which play a critical role in polysaccharide degradation in the anoxic zones of rice fields (Podosokorskaya et al. 2013). Actinobacteria play an important role in decomposition of soil organic materials, such as cellulose and chitin (Stevenson and Hallsworth 2014). Dead roots and dropped flowers of trees generally contain more complex compound, which make it more difficult to be decomposed by microorganisms than herbs. Therefore, dead roots and dropped flowers of *B. ceiba* that is more difficult to decompose around the *B. ceiba* may attract more members of Actinobacteria.

In addition, we found that temporal dynamic of soil bacterial community cannot be negligible. The relative abundance of Firmicutes phylum was higher during rice harvesting stage. Firmicutes were reported to be enriched in the soil with lower water content (Acosta-Martínez et al. 2010) and they could survive under stressful environment, such as desiccation (Battistuzzi and Hedges 2009). Generally, water depth in paddy field was about 3cm during the early reproductive stage of rice, which was unfavorable to Firmicutes and then resulting decrease of Firmicutes during booting stage of rice. Contrast to the Firmicutes, relative abundance of Acidobacteria phylum was higher during the early reproductive stage of rice. Acidobacteria belonged to the acidophilic bacteria, and its abundance is significantly higher under acidic conditions (Jones et al. 2009; Bardhan et al. 2012). The anaerobic environment because of above-ground water in paddy field may promote the accumulation of acid and further increase Acidobacteria during early reproductive stage of rice.

For fungal community, the relative abundance of Ascomycota phylum was higher in rice paddy soil far away from *B. ceiba*. Members of Ascomycota are normally common in extreme environments (Nara 2008), thus the less soil nutrient condition at D5 than that at D0 may be a possible reason (Fig. 2). At the same time, we found that bacteria and fungi shared more OTUs at D0 and D1 than at D0 and D5, suggesting soil microorganisms were jointly affected by *B. ceiba* and rice in the transition zone from *B. ceiba* to rice paddy.

Driving forces for bacterial and fungal community in *Bombax* – rice agroforestry system

In agreement with our third hypothesis, diversity and composition of bacterial and fungal community responded differently towards the soil nutrient properties investigated in this study. In a previous

phosphorus surplus study, phosphorus content had no significant effect on bacterial diversity while fungal alpha diversity decreased significantly along increasing phosphorus content (Liu et al. 2018). The possible explanation for this different response between bacteria and fungi is their different identity, environment sensitivity and substrate utilization (Hannula et al. 2017).

Alpha diversity of bacteria was negatively correlated with AP and positively correlated with TK (Table S1). Among all soil nutrient properties, AK was the main variable affecting the bacterial community composition (Fig. 6). These results suggested that potassium was the most important factor affecting bacterial community in *Bombax* – rice agroforestry system. Similarly, a study in tropical island aquifers confirmed that potassium had most significant relationship with bacterial community (Kirs et al. 2020). Another study confirmed that the higher bacterial richness observed in floodwaters may associate with higher concentration of nutrients (phosphorus and potassium) (Pittol et al. 2018). More potassium ions could adjust the osmotic pressure of the bacterial cell membrane, which importantly promoted the absorption of nutrients by bacteria.

Fungal alpha diversity has no correlation with all soil nutrient properties in Pearson's correlation analysis (Table S1). However, AN had significant explanation on fungal alpha diversity in the regression analysis. This may be due to significant temporal variation of AN. In this study, the value of AN reached more than 100mg/kg during booting stage of rice, whereas it below 100mg/kg during heading and mature stages of rice. The effect of one variable was under the control of other variables in multiple regression analysis, indicating AN had a significant impact on fungal alpha diversity under the control of rice reproductive stage variable. In addition, the composition of fungal community was significantly explained by SOC and TN. These results suggested that nitrogen was the most important factor affecting fungal community in *Bombax* – rice agroforestry system. This was expected given fungi cannot fix carbon and nitrogen and its growth mainly rely on external organic matter and nitrogenous compounds (Walker and White 2017), thus changes of soil SOC and TN may bring changes of fungal community. In addition, a previous study reported that saprotrophic fungal diversity was positively correlated with leaf nitrogen content (Nguyen et al. 2016), which suggested variations of fungal community in our study may be due to different responses of fungal guilds to soil microhabitat.

Conclusions

Knowledge of soil biotic and abiotic factors in the agroforestry ecosystem could help us improve the management of sustainable land-use. In our study, *B. ceiba* increased soil nutrient contents and changed soil bacterial and fungal community diversity and composition in the rice paddy. In addition, bacterial community would alter along different reproductive stages of rice. Soil potassium was the principal driver in shaping soil bacterial diversity and composition, while soil nitrogen largely determined fungal community composition. Our results may provide guidance for developing sustainable agriculture in less developed mountainous areas in tropical Asia, although the ecological basis of *Bombax* root exudations and underlying mechanism of nutrient cycling are still in need of further illumination in this traditional *Bombax* - rice agroforestry system.

Declarations

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Data Availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Geographical location of the study site (A) and a typical landscape view (B) of the *Bombax* – rice agroforestry system on Hainan Island, South China.

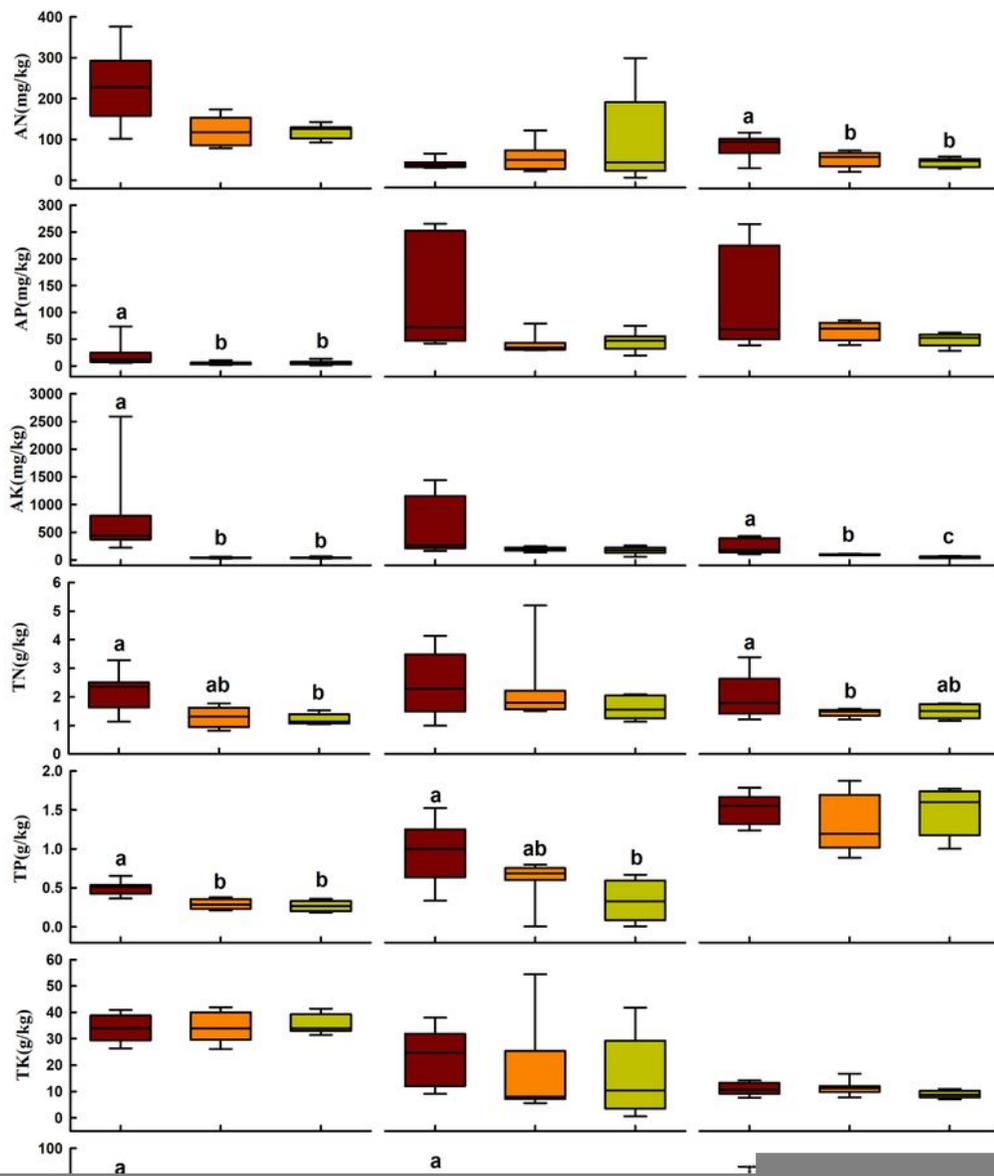


Figure 2

Soil nutrient contents in different distances to *B. ceiba* for each reproductive stage of rice. D0, distance to *B. ceiba* is 0m; D1, distance to *B. ceiba* is 1m; D5, distance to *B. ceiba* is 5m. Different letters indicate significant difference at P < 0.05.

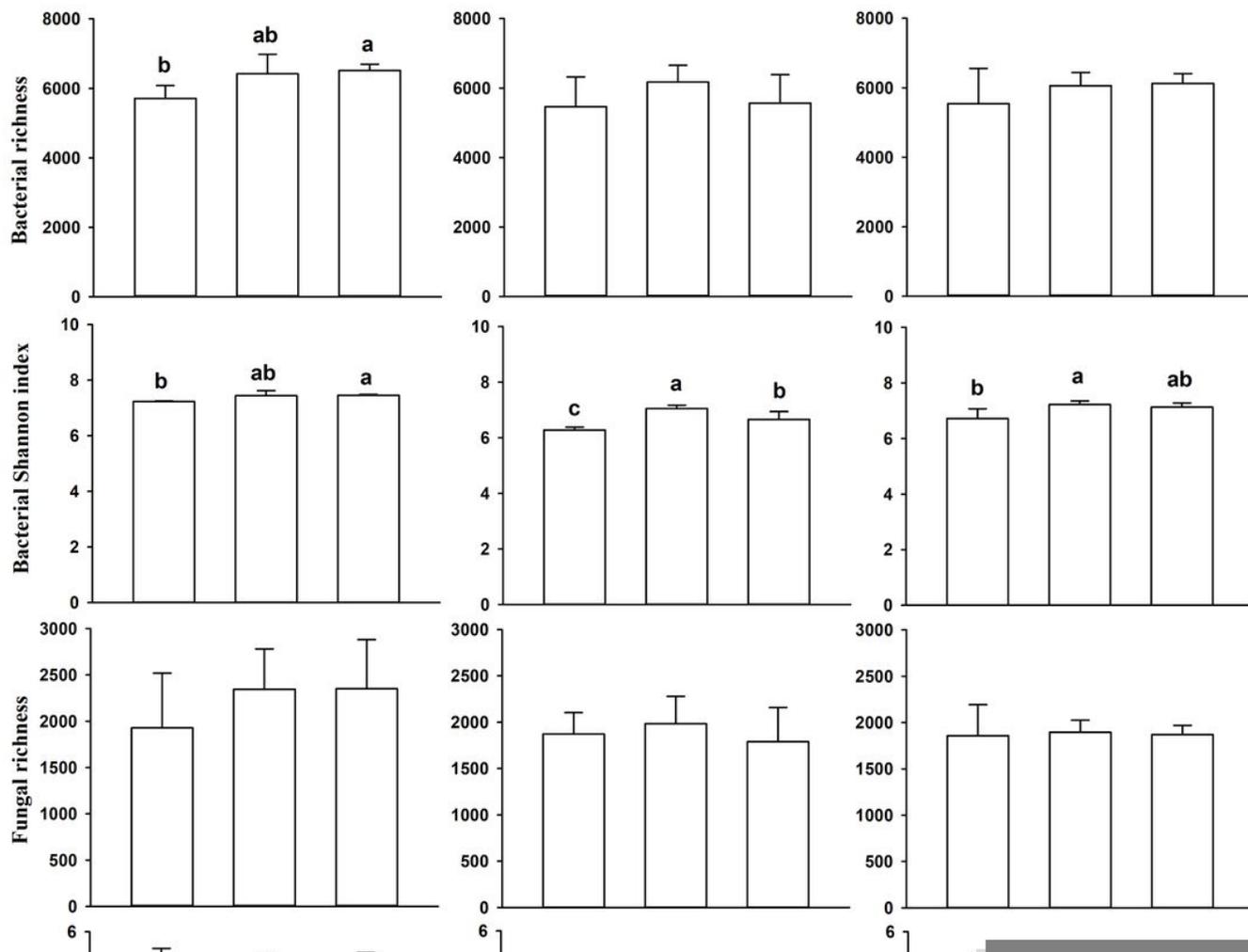


Figure 3

Richness and Shannon index of bacteria and fungi in different distances to *B. ceiba* for each reproductive stage of rice. D0, distance to *B. ceiba* is 0m; D1, distance to *B. ceiba* is 1m; D5, distance to *B. ceiba* is 5m. Different letters indicate significant difference at $P < 0.05$.

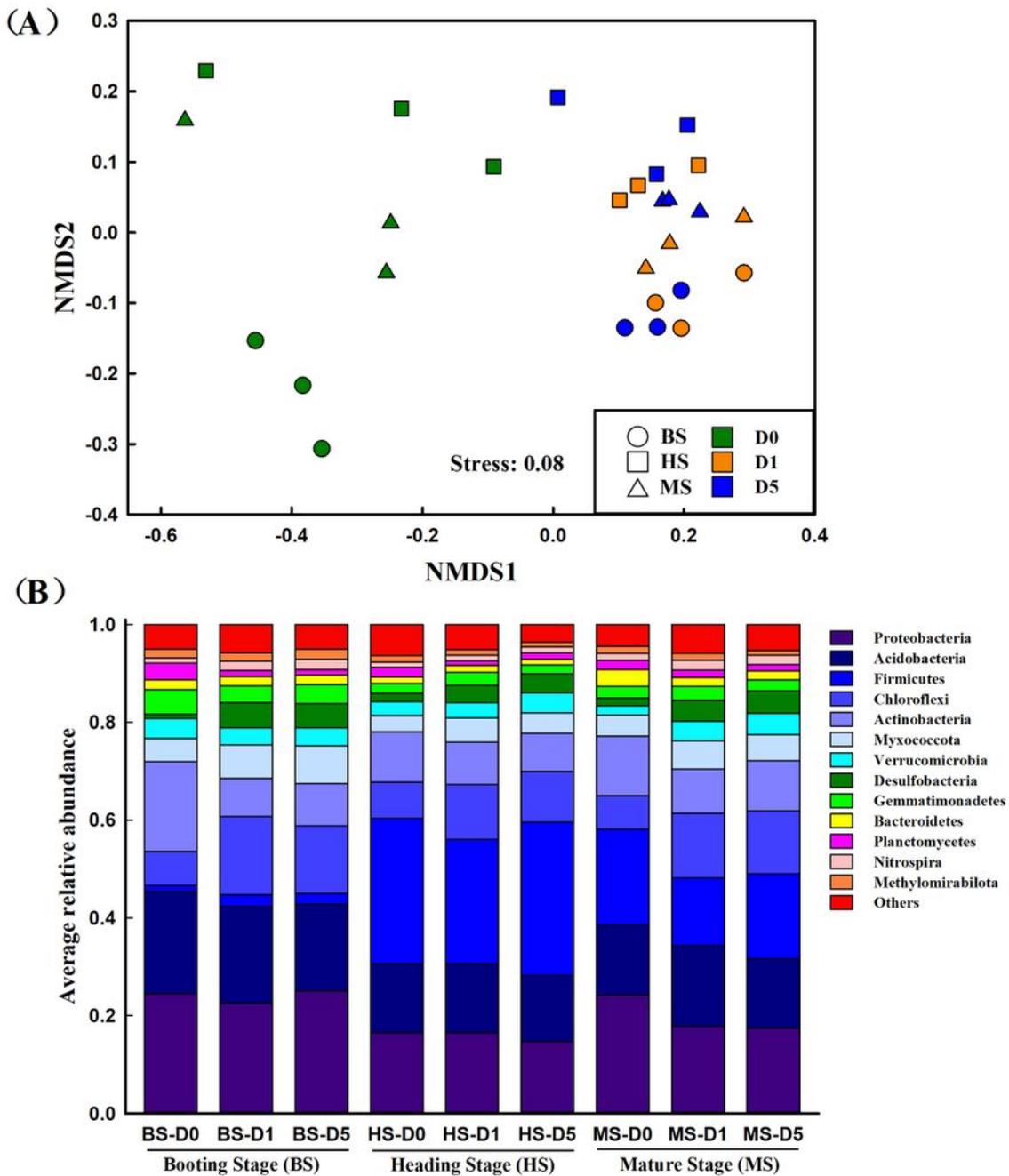


Figure 4

Non-metric multidimensional scaling ordination (NMDS) of bacterial communities (A) and the relative abundance of dominant bacterial phyla in different distance to *B. ceiba* for each reproductive stage of rice (B). D0, distance to *B. ceiba* is 0m; D1, distance to *B. ceiba* is 1m; D5, distance to *B. ceiba* is 5m.

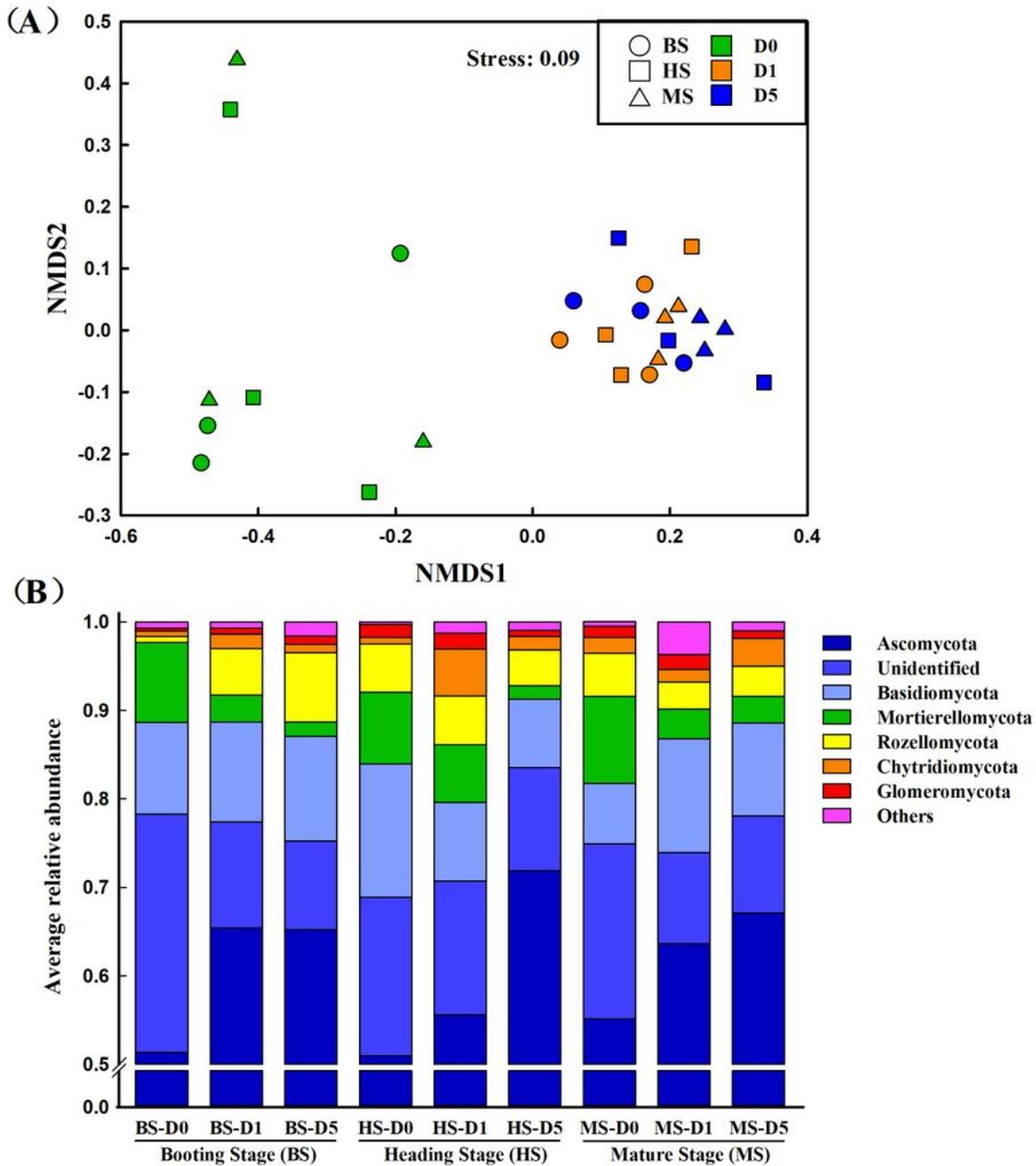


Figure 5

Non-metric multidimensional scaling ordination (NMDS) of fungal communities (A) and the relative abundance of dominant fungal phyla in different distance to *B. ceiba* for reproductive stages of rice (B). D0, distance to *B. ceiba* is 0m; D1, distance to *B. ceiba* is 1m; D5, distance to *B. ceiba* is 5m.

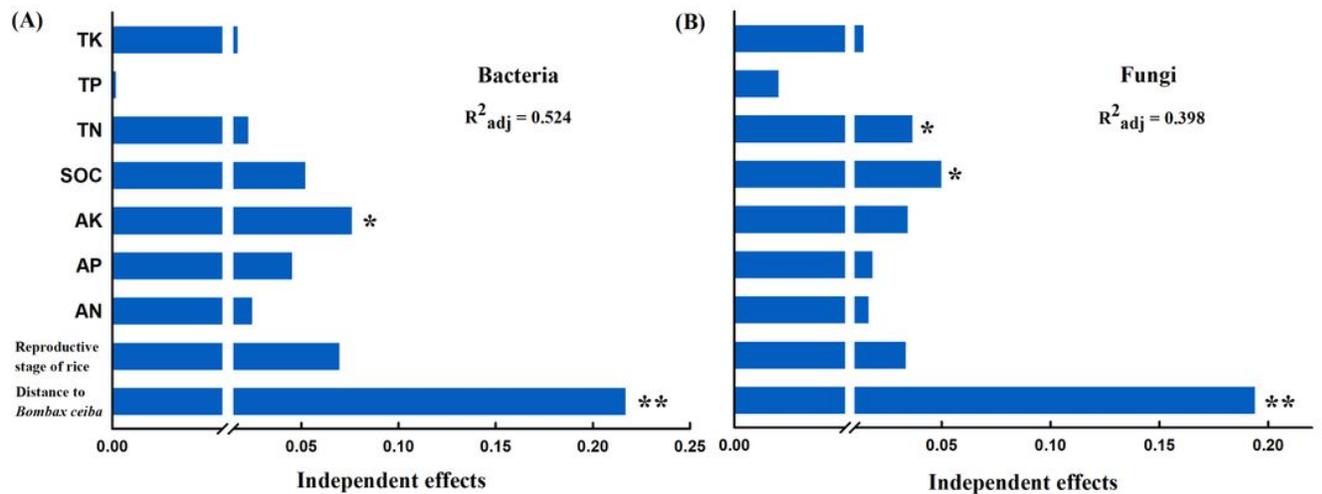


Figure 6

The independent effects of each variable on variations of bacterial community composition (A) and fungal community composition (B). Significance was determined by a permutation test with ** representing at 0.01 significance level while * at 0.05 level. TK, total potassium; TP, total phosphorus; TN, total nitrogen; SOC, soil organic carbon; AK, available potassium; AP, available phosphorus; AN, available nitrogen.

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