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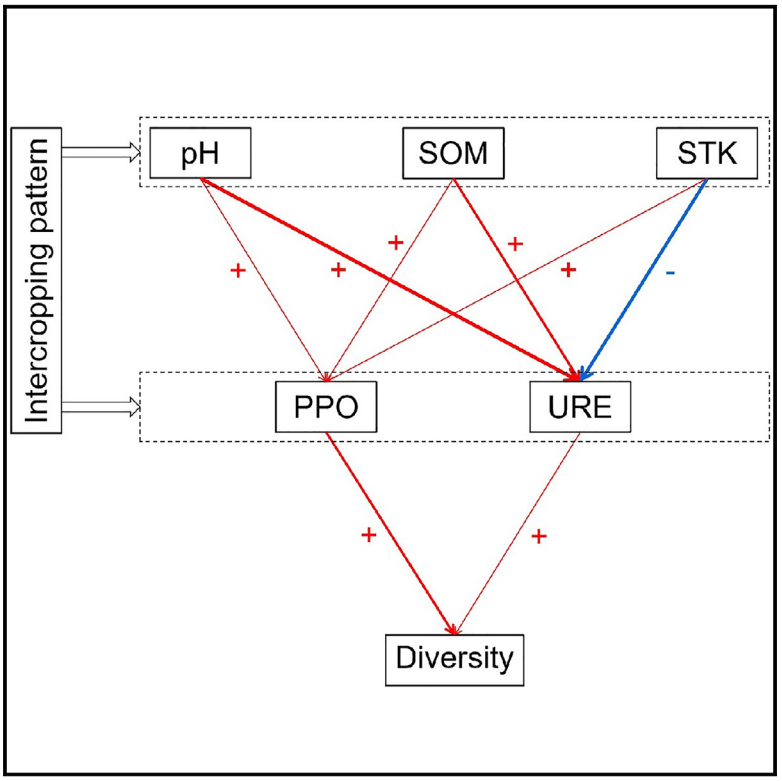
The effect of intercropping with *Pandanus amaryllifolius* Roxb. on rhizospheric microorganism of *Areca catechu* L.

Graphical abstract



Highlights

- Bacterial rather than fungal community diversity responded to intercropping pattern
- The reduction of SOM content or URE activity may inhibit bacterial community diversity
- Intercropping pattern does not affect the bacterial and fungal community structure
- Soil pH, SM, STK, and URE activity are potential factors for predicting specific taxa

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In brief

Soil science; Plant biology; Interaction of plants with organisms; Agricultural science; Soil ecology; Soil biology

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The effect of intercropping with *Pandanus amaryllifolius* Roxb. on rhizospheric microorganism of *Areca catechu* L.

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SUMMARY

The intercropping pattern promotes the sustainable development of efficient agriculture, especially cash crops, such as *Areca catechu* L. and *Pandanus amaryllifolius* Roxb. intercropping plantation in China. However, the mechanisms underlying intercropping pattern effects on soil microbial community diversity and composition are poorly understood. A monoculture and intercropping field experiment of the two crops was established to monitor the changes of soil physicochemical properties, enzyme activities, microbial (bacterial and fungal) diversity, and composition. Soil bacterial rather than fungal communities' diversity is more sensitive to intercropping pattern. The intercropping significantly decreased rhizospheric bacterial diversity of *Areca catechu* L. by 4.21%, and the decrease of soil nutrient content may be the main reason for the change of soil enzyme activity, bacterial community diversity, and composition structure under intercropping pattern. Supplementing nutrients to the soil of intercropping systems is conducive to maintain soil health and ecosystem functional stability in the tropical compound cultivation plantation.

INTRODUCTION

With the growth of global food demand and the progress of cultivated technology, intensive and efficient agricultural production pattern has become the main direction of agricultural development. As a cultivated pattern based on the principle of promoting and complementing ecology,[1] intercropping tradeoff the interspecies competition and spatial structure between two or more crops, optimizing utilization of resources,[2] promoting crop growth and acquiring considerable yield advantages.[3] Previous studies generally believe that tropical regions have sufficient light and heat resources, and strong ecological carrying capacity, which are more suitable for development of the intercropping pattern compared to temperate and cold regions. For example, Hainan Island is located in the tropical and subtropical transitional region of southern China, Due to its limited arable land, farmers in this region is usually intercrops perennial cash crops, such as *Areca catechu* L. (Ac) and *Pandanus amaryllifolius* Roxb. (Pa) intercropping, becoming a mainstream intercropping production model in the local area.[4] In order to maintain the economic benefits of Ac and Pa, the ecosystem stability of the intercropping plantation needs to be concerned. Current studies had indicated that soil nutrient availability and biodiversity are critical to crop growth and ecosystem stability in tropical plantation.[5] Maintaining the high efficiency of soil enzymatic reaction and stable microbial community structure is the prerequisite to ensure the sustainable development of plantation ecosystem in tropical regions.[6] Therefore, exploring soil properties, enzyme activities, and the response of microbial communities structure to Ac and Pa intercropping pattern is conducive to promote the sustainable development of high-efficiency agriculture in tropical areas.

As one of the most sensitive indicator groups indicating changes in soil properties, soil enzymes are an important power source in soil ecosystem metabolism. They directly participate in the dynamic cycling process of soil nutrients, such as decomposition of humus and biological residues, hydrolysis and transformation of organic compounds, and oxidation and reduction reactions of some inorganic compounds.[7] The activity of soil enzymes reflects the trend and intensity of various biochemical processes in the soil, and its catalytic efficiency is highly sensitive to planting management and soil nutrient characteristics.[8,9] On the one hand, previous studies show that soil enzyme activity is directly controlled by soil water, temperature, pH, and salinity.[10,11] On the other hand, the composition and content of soil organic matter and other nutrients can also have a feedback

Table 1. Results (*F* values) of repeated measures ANOVAs on the effects of cultivated patterns (C), experimental site (S), and their potential interactions on soil physicochemical properties

Treatments	pH	SM	BD	SOM	SAN	SAP	SAK	STN	STP	STK
C	10.29***	1.18	2.69`	6.64**	10.13***	1.94	13.84***	6.38**	3.36*	0.05
S	12.54***	54.69***	8.66***	5.80**	5.59**	3.09`	125.38***	15.20***	38.15***	8.12***
C×S	9.47***	0.84	0.94	4.62**	3.22*	2.92*	34.16***	5.93***	8.33***	0.39

"C" indicate different cultivated patterns (*Areca catechu* L. monoculture, intercropping and *Pandanus amaryllifolius Roxb*. monoculture), "S" indicate different experimental site (Qionghai, Wanning and Lingshui); SM: soil moisture, BD: soil bulk density, SOM: soil organic matter content, SAK: soil available potassium content, SAP: soil available phosphorus content, SAN: soil available nitrogen content, STN: soil total nitrogen content, STP: soil total phosphorus content, STK: soil total potassium content; Significant level: "`" indicate $p < 0.1$; "*" indicate $p < 0.05$; "**" indicate $p < 0.01$; "***" indicate $p < 0.001$.

effect on soil enzyme stability.[12] In addition, soil enzyme activity is closely related to soil microbial characteristics that improve ecosystem stability.[13] Soil enzyme activity reflects how the microbial community invests to obtain energy and nutrients under *in-situ* conditions. In turn, changes in soil microbial structure, function, and metabolic activity further affect the biochemical characteristics of soil enzymes and regulate enzyme secretion.[14] Therefore, soil enzyme activity is one of the indicators to characterize soil quality and health evaluation. Exploring the routine response of enzyme activity to intercropping patterns is one of the main contents of plantation health research.

Soil microorganisms are the key biological factors that drive the degradation of organic matter and nutrient cycling in plantation. The main functions of soil microorganisms are regulating the diversity of soil functions,[15] maintaining soil fertility and metabolic activity,[16] and inhibiting pathogen functions.[17] Previous studies indicate that soil microbial communities are sensitive to soil disturbance and changes in the external environment[18]; especially, transforming cultivated patterns can significantly alter soil ecological functions by regulating the composition and structure of soil microbial communities.[19] Studies have confirmed that the changes of spatial niche and biological activity of soil nutrients under intercropping pattern are the main reasons for the changes of soil microbial community structure.[2,20] For example, intercropping can promote the improvement of soil microbial community diversity and structural stability by improving soil physical and chemical properties, such as soil moisture and pH in various intercropping patterns,[21] increasing soil organic matter, nitrogen, phosphorus and potassium content,[22] and regulating soil enzyme activities such as acid phosphatase and β-glucosidase.[23,24] Therefore, clarifying the internal relationship among soil nutrient, enzyme activities, and microbial community under intercropping pattern would be an important step to evaluate the nutrient status and material cycle of soil microbial metabolism in Ac and Pa intercropping plantation, which will contribute to the stability and sustainable development of plantation ecosystem service function.

Based on previous studies related to soil properties, enzyme activities and microbial communities in other plantation ecosystems, the scientific hypotheses of this study are as follows: (1) The intercropping pattern may improve soil properties and promote some soil enzyme activities in tropical plantations; (2) intercropping pattern may significantly improve the richness and diversity of soil microbial (bacteria and fungi) community by regulating some soil properties and enzyme activities; and (3) various soil properties and enzyme activities may drive the changes in soil microbial (bacteria and fungi) community composition and structure under intercropping pattern.

RESULTS

Changes in soil physicochemical properties

Cultivated patterns, experimental site, and their interactions had significant influences on soil pH, SOM, SAN, SAK, STN, STP during the experimental period (all $p < 0.05$, Table 1), shows that there are significant differences in soil properties not only among the

Table 2. Biochemical and physical properties of the soil under different cultivated patterns

Soil physico-chemical properties	A	I	P
Soil pH	6.43 ± 0.16a	6.35 ± 0.12a	5.78 ± 0.19b
Soil moisture (SM, %)	20.82 ± 4.67a	23.05 ± 3.74a	26.15 ± 4.54a
Soil bulk density (SBD, g m^{-3})	1.49 ± 0.06a	1.63 ± 0.04a	1.50 ± 0.06a
Soil organic matter (SOM, g kg^{-1})	18.69 ± 0.48a	15.49 ± 0.90b	20.16 ± 0.86a
Soil available potassium (SAK, mg kg^{-1})	39.86 ± 5.28a	18.37 ± 2.05c	27.13 ± 3.84b
Soil available phosphorus (SAP, mg kg^{-1})	17.36 ± 1.27a	18.05 ± 2.55a	21.54 ± 1.20a
Soil available nitrogen (SAN, mg kg^{-1})	75.31 ± 5.28b	66.26 ± 5.72c	84.51 ± 9.61a
Soil total nitrogen (STN, g kg^{-1})	1.19 ± 0.05a	1.01 ± 0.06b	1.27 ± 0.10a
Soil total phosphorus (STP, g kg^{-1})	0.74 ± 0.07b	0.81 ± 0.10ab	0.90 ± 0.08a
Total soil potassium (STK, g kg^{-1})	5.17 ± 0.52a	5.00 ± 0.70a	5.25 ± 0.61a

Different letters indicate significant differences (ANOVA, $p < 0.05$, Tukey's HSD post hoc analysis) among different cultivated patterns; Data are represented as mean ± SE. Please refer to Table 1 for abbreviations of different cultivation patterns and experimental site.

Table 3. Results (*F* values) of repeated measures ANOVAs on the effects of cultivated patterns (C), experimental site (S), and their potential interactions on soil enzyme activities, soil microbial (bacterial and fungal) community richness and alpha diversity (Shannon index)

Treatments	CAT activity	POD activity	PPO activity	ACP activity	URE activity	Bacterial richness	Bacterial diversity	Fungal richness	Fungal diversity
C	1.26	0.69	6.16**	1.22	2.99*	0.35	2.50*	1.15	0.52
S	14.38***	22.35***	3.29*	3.50*	7.84***	2.39˙	10.07***	0.64	6.87**
C×S	2.60*	8.68***	0.55	1.14	3.70**	0.70	0.71	0.47	1.44

CAT: catalase, PPO: polyphenol oxidase, POD: peroxidase, ACP: acid phosphatase, URE: urease; Significant level: please see Table 1. Please refer to Table 1 for abbreviations of different cultivation patterns and experimental site.

three cultivated patterns, but also under three experimental regions. Compared with Ac monoculture, intercropping significantly decreased SOM by 17.10%, SAK by 53.90%, SAN by 12.02%, or STN by 15.14%, respectively (all $p < 0.05$, Table 2). The soil pH under Pa monoculture was significantly lower than that under the Ac monoculture by 0.65 ($p < 0.05$), but the STP was significantly stimulated by 22.56% ($p < 0.05$), when compare with Ac monoculture. The soil nutrient content in intercropping pattern was generally lower than that in monoculture in this experiment.

Changes in soil enzyme activities

Cultivated patterns, experimental site, and their interactions had significant influences on CAT, POD, PPO, ACP and URE over the experimental period (all $p < 0.01$, Table 3). There are significant differences in soil enzyme activities between different experimental areas. The PPO and URE activities have significant differences among three cultivated patterns, despite CAT, POD and ACP activities had significant influences on the interactions of cultivated patterns and different region. Compared with Ac monoculture and Pa monoculture, intercropping pattern significantly decreased activities of PPO by 36.41% and 30.76%, and decreased URE activities by 46.48% and 31.54%, respectively (all $p < 0.05$, Figure 1).

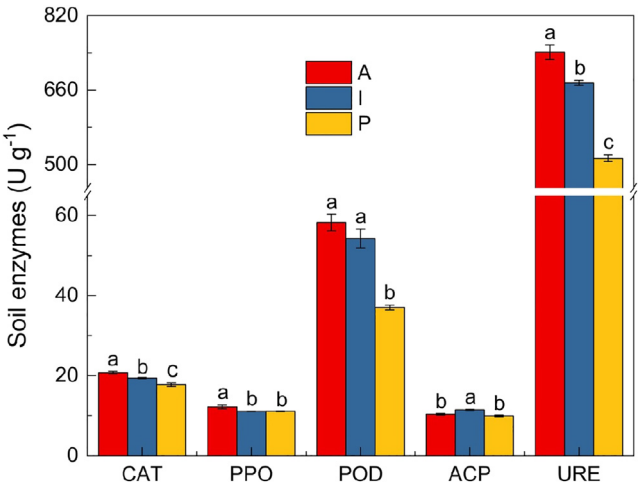

Figure 1. Effects of intercropping patterns on soil enzyme activities across the experimental period

n = 6. A: Ac monoculture, I: intercropping, P: Pa monoculture. CAT: Catalase, PPO: Polyphenol oxidase, POD: Peroxidase, ACP: Acid phosphatase, URE: Urease. Data are represented as mean ± SE; Different letters represent significant levels as $p < 0.05$.

The effects of cultivated patterns on soil enzyme activities were mainly concentrated in PPO and URE activities, although the soil enzyme activities were affected by regional differences rather than cultivated patterns in this experimental area.

Changes in soil microbial community richness and diversity

Cultivated patterns, experimental site, or their interactions did not affect bacterial and fungal richness over the experimental period (Table 3). The bacterial and fungal diversity had significant difference among three experimental sites, but bacterial rather than fungal diversity was significantly different among three cultivated patterns (Table 3). Intercropping significantly decreased bacterial diversity by 4.21% or 4.76%, when compared with Ac and Pa monoculture, respectively (all $p < 0.05$, Figure 2). In contrast, intercropping did not affect fungal diversity neither compared with Ac nor Pa monoculture.

Nonmetric multidimensional scaling analysis (NMDS) was conducted to reflect soil microbial beta diversity (Figure 3). The soil bacteria and fungi characteristics under the three cultivated patterns were nearly the same, indicated that the soil microbial characteristics did not affected by the intercropping pattern.

Changes in soil microbial community structure

Among all sequences, the dominant bacterial phyla (relative abundance >1%) were *Proteobacteria*, *Acidobacteriota*, *Actinobacteriota*, *Chloroflexi*, *Firmicutes*, *Bacteroidota*, *Myxococcota*, *Methylomirabilota*, *Gemmatimonadota*, *Planctomycetota*, *Verrucomicrobiota*, *Desulfobacterota and Cyanobacteria* with contributions of 23.91%, 18.52%, 15.57%, 12.75%, 9.39%, 2.86%, 3.10%, 2.18%, 1.73%, 1.36%, 1.27%, 1.00% and 1.16%, respectively (Figure 4; Table S1). The most dominant classes, such as *Proteobacteria*, *Acidobacteriota*, *Actinobacteriota*, *Chloroflexi*, *Firmicutes* (relative abundance >5%) did not affected by different cultivated patterns, although some of *Bacteroidea* and *Myxococcota* were influenced by intercropping patterns. Compared with Ac monoculture, the quantities of *Gemmatimonadota* was significantly increased by 0.36% (absolute value, $p < 0.05$), while the quantities of *Myxococcota* was significantly decreased by 0.76% (absolute value, $p < 0.05$) under intercropping pattern. Compared with Pa monoculture, the quantities of *Methylomirabilota* and *Gemmatimonadota* were significantly increased by 1.38% and 0.55%, respectively (absolute value, $p < 0.05$), whereas the quantities of *Planctomycetota* was significantly decreased by 0.62% (absolute value, $p < 0.05$) under intercropping pattern. The dominant fungal phyla (relative

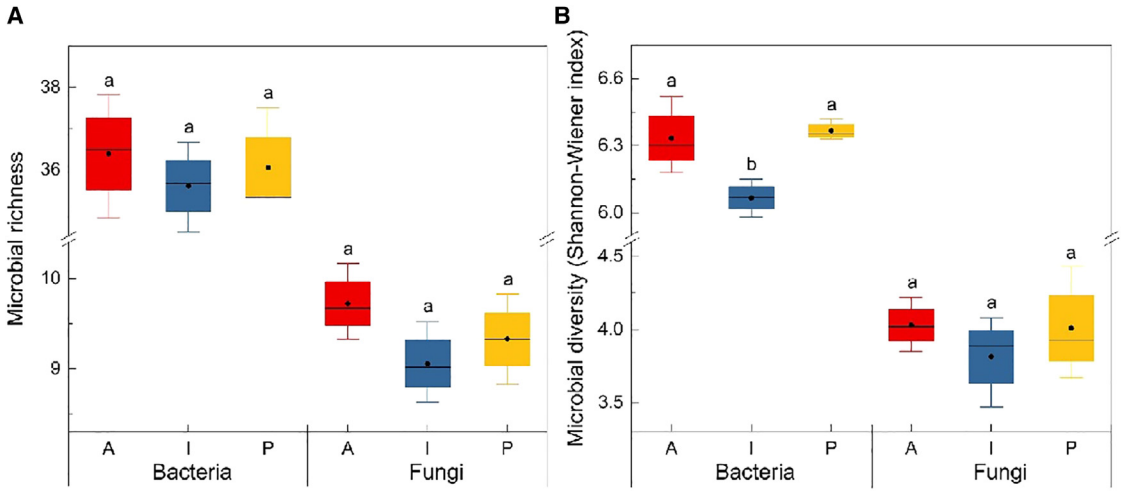

Figure 2. Effects of intercropping patterns on soil microbial diversity

Effects of intercropping patterns on soil microbial (bacterial and fungal) richness (A) and alpha diversity (Shannon index, B) across the experimental period. $n = 6$. Error bars indicate the maximum and minimum value of indicators, data are represented as mean ± SE; Box scope indicate SE; Black horizontal line indicate mean value of indicators; Different letters represent significant levels as $p < 0.05$.

See Figure 9 for treatment abbreviations.

abundance >1%) were *Ascomycota*, *Basidiomycota*, *Mortierellomycota* and *Rozellomycota* with contributions of 72.03%, 12.70%, 1.61% and 1.77%, respectively in this study (Figure 4; Table S1). All sequences of fungal phyla did not affected by cultivated patterns (Figure 4; Table S1).

Correlation between soil enzyme activities and soil physico-chemical properties

There was a significant negative correlation between the soil BD (R = 0.59, $p < 0.01$), SM (R = 0.72, $p < 0.01$) and CAT, in contrast, STP had positive correlation with CAT (R = 0.44, $p < 0.05$). Soil BD (R = 0.51, $p < 0.01$) and SM (R = 0.46, $p < 0.01$) were negatively correlated with PPO, whereas it was also stimulated by SOM (R = 0.53, $p < 0.01$), SAN (R = 0.52, $p < 0.01$), STN (R = 0.82, $p < 0.001$) and STK (R = 0.53, $p < 0.001$). There was a significant negative correlation between the SOM (R = 0.46, $p < 0.05$), SAK (R = 0.64, $p < 0.01$), SOP (R = 0.79, $p < 0.01$), STN (R = 0.47, $p < 0.05$), STK (R = 0.42, $p < 0.01$) and ACP. SM rather than other soil physical and chemical properties was significantly decreased POD (R = 0.54, $p < 0.01$). ACP was stimulated by soil BD (R = 0.69, $p < 0.01$), while it were also inhibited by SOM (R = 0.50, $p < 0.01$), SAK (R = 0.64, $p < 0.01$), SAP (R = 0.79, $p < 0.01$), STN (R = 0.47, $p < 0.05$) or STK (R = 0.42, $p < 0.05$). There was a significant positive correlation between the soil pH (R = 0.82, $p < 0.01$), SOM (R = 0.51, $p < 0.01$), STK (R = 0.67, $p < 0.01$) and URE, but SM was negatively correlated with URE (R = 0.70, $p < 0.01$, Figure 5).

Correlation between soil microbial community diversity, soil enzyme activities and soil physico-chemical properties

There were significant negative correlation between the soil BD (R = −0.45, $p < 0.05$), STP (R = −0.44, $p < 0.05$), ACP (R = −0.43, $p < 0.05$) and bacterial diversity, in contrast, SOM (R = 0.64, $p < 0.001$), SAK (R = 0.41, $p < 0.05$), STN (R = 0.47, $p < 0.05$), STK (R = 0.70, $p < 0.001$), PPO (R = 0.50, $p < 0.01$), URE (R = 0.52, $p < 0.01$) had positive correlation with bacterial diversity (Table S2). There was a significant negative correlation

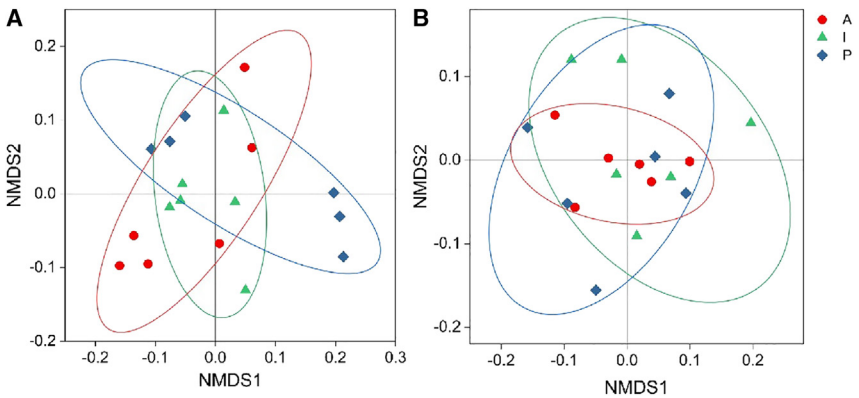

Figure 3. Effects of intercropping patterns on soil microbial structure

Effects of intercropping patterns on soil bacterial (A) and fungal (B) beta diversity (NMDS) across the experimental period.

See Figure 1 for treatment abbreviations.

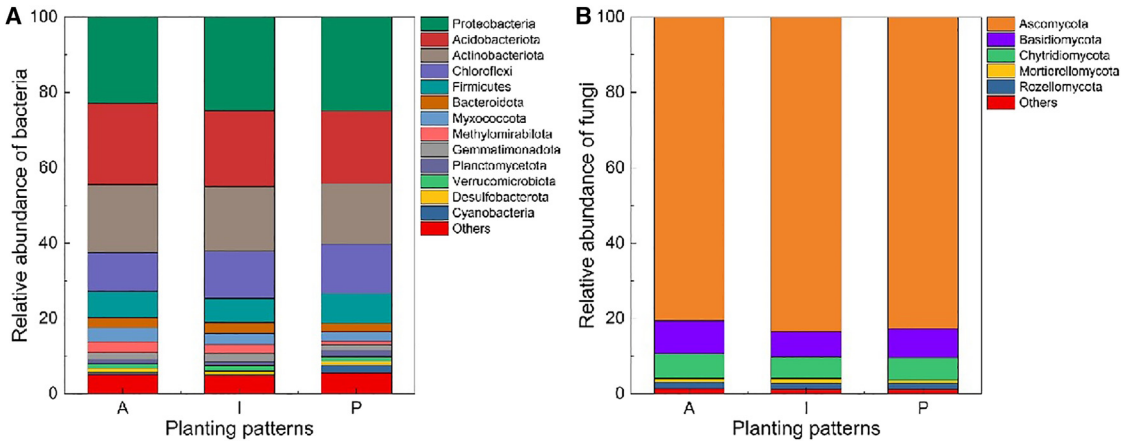

Figure 4. Effects of intercropping patterns on soil microbial composition

Changes in soil bacterial (A) and fungal (B) taxonomic composition at the phylum level under intercropping patterns. The abundance of each taxon was calculated as the percentage of sequences per gradient for a given microbial group.

See Figure 1 for treatment abbreviations.

between the SAN (R = −0.45, $p < 0.05$), STN (R = −0.44, $p < 0.05$), PPO (R = −0.43, $p < 0.05$) and fungal diversity. On the contrary, SM had positive correlation with fungal diversity (R = 0.57, $p < 0.01$, Table S2).

The stepwise multiple linear analyses revealed that the combination of soil pH, SOM, STK, PPO and URE explained the 57.99% variations of soil bacterial diversity ($F = 16.57$, $p < 0.001$). Thus, the above five indicators were considered as key indicators for regulating bacterial diversity, and were input into the SEM model. The fit between the Ac model and data were adequate for intercropping pattern ($\chi^2 = 3.65$, $p = 0.30$, Figure 6A). This model was accepted because it explained 66.0% of the variation in bacterial diversity. Compare with Ac monoculture, intercropping had direct negative effects on SOM or STK, respectively. Intercropping had indirect negative effect on PPO by regulating SOM and STK. Intercropping significantly inhibited URE, owing to the indirect negative effects by decreasing SOM and STK reverse the direct positive effect of intercropping on URE. Soil pH or STK were not one of the indicators of regulatory bacterial diversity, because soil pH and STK did not directly affected bacterial diversity in this study.

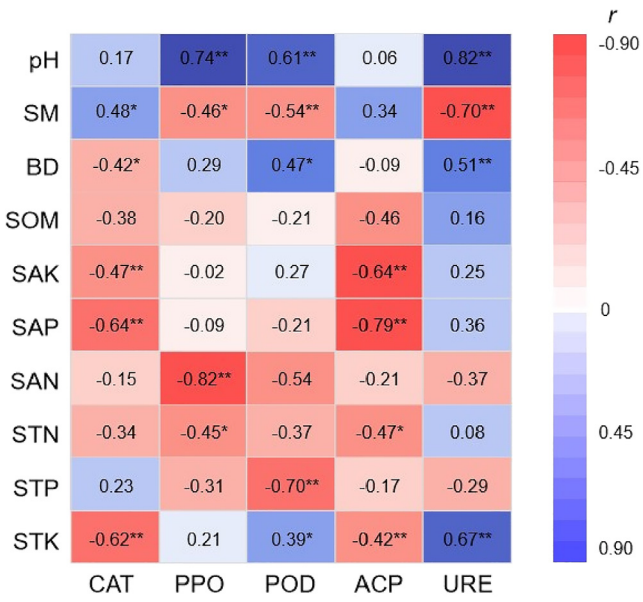

Figure 5. Relationships between the soil properties and soil enzyme activities

Significant level: "^" indicate $p < 0.1$; "*" indicate $p < 0.05$; "**" indicate $p < 0.01$; "***" indicate $p < 0.001$.

See Table 1 and Figure 1 for soil properties and enzyme abbreviations, respectively.

The fit between the Pa model and data were adequate for intercropping pattern ($\chi^2 = 3.36$, $p = 0.34$, Figure 6B). This model was accepted because it explained 86.0% of the variation in bacterial diversity. Compare with Pa monoculture, intercropping had direct positive effects on soil pH, whereas it had negative effects on SOM and STK, respectively. The fitting between the Pa model and data were adequate under intercropping pattern treatment. Intercropping pattern indirectly excited URE owing to the direct and indirect positive effects on URE. Intercropping significantly reduced bacterial diversity because the indirect negative effects by regulating SOM and STK concealed the indirect positive effects by increasing URE on bacterial diversity. Soil pH and PPO were not one of the indicators in regulating bacterial diversity because they had no effect on bacterial diversity in this study. Therefore, compared with Ac monoculture, intercropping reduced bacterial diversity by inhibiting SOM, PPO, or URE; while compared with Pa monoculture, intercropping reduced bacterial diversity by debasing SOM and STK.

Correlation between soil microbial community composition, soil enzyme activities and soil physico-chemical properties

Network analysis was used to determine the co-occurrence patterns of soil properties, enzyme activity, and microbial community based on strong and significant correlations

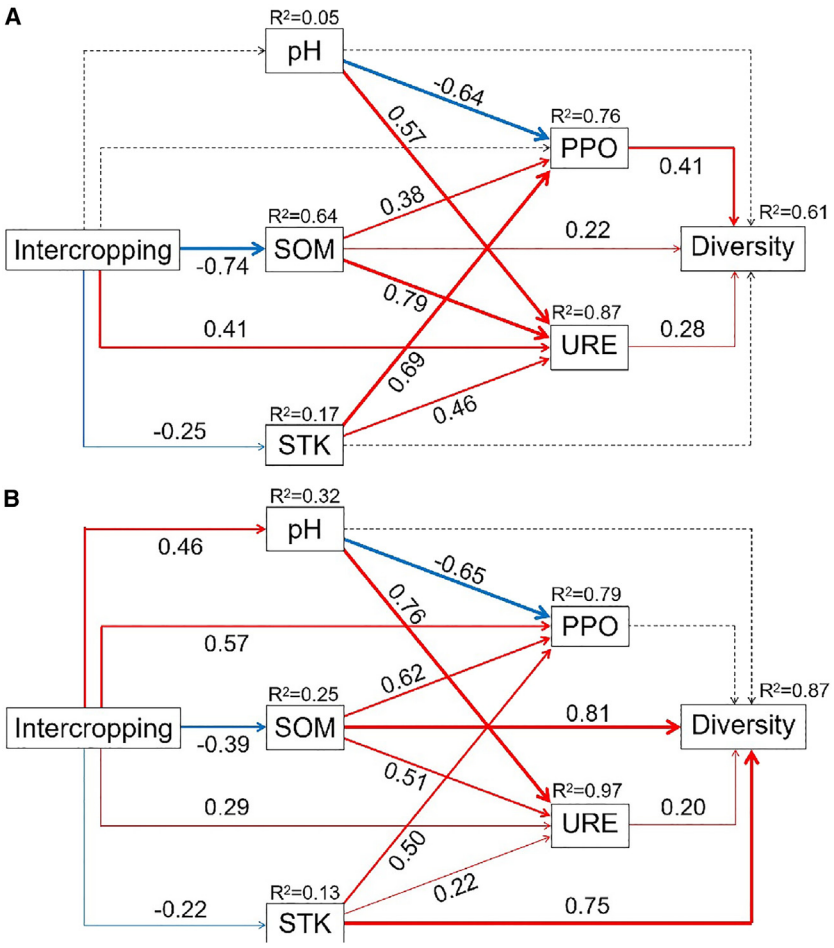

Figure 6. Key factors affecting soil microbial diversity

Structural equation models (SEM) for intercropping effects on bacterial alpha diversity, when compared with Ac (A) and Pa (B) monoculture. Non-significant paths are indicated by dotted arrows. The thickness of the solid arrows reflects the magnitude of the standardized SEM coefficients. Standardized coefficients are listed beside each significant path. Diversity (bacterial Shannon index), pH (Soil pH), SOM (soil organic matter content), STK (soil total potassium content), PPO (polyphenol oxidase activity), URE (Urease activity).

(Figure 7). Overall, different cultivated patterns showed a remarkable effect on association networks of soil properties and microbial community. More negative co-occurrence relationships between soil properties and dominant bacterial and fungal phyla, but positive co-occurrence relationships between soil enzyme activity and dominant soil microbial community were found under three cultivation patterns. Strikingly, more negative co-occurrence relationships among dominant bacteria and fungi communities were found in the soil under three cultivation patterns indicating that the intensity of interspecific competition is greater than the synergistic effect under different cultivated patterns. However, the positive correlation between different microbes indicates that there is a potential balance between individual and collective survival of different microbes, and they dominate in the rarest soil microbes. Altogether, there was no significant difference in the assembly of soil microbial community structure among the three cultivated patterns. Compared with monoculture, the microbial community composition under the intercropping pattern still remained relatively stable.

Redundancy analysis (RDA) showed that bacterial and fungal taxa responded differently to changes in the soil properties and enzyme activities at phylum levels (Figure 8A). The soil properties (pH, SM, and STK) and soil enzyme activity (URE) were considerably related to changes in *Gemmatimonadota*, *Myxococcota*, *Methylomirabilota*, *Gemmatimonadota*, *Planctomycetota* for bacteria (Figure 8A; Table S3). Similarly, soil properties (pH, SM, STK, and STP) and soil enzyme activities (URE and PPO) were associated with fungal structure variation, although none of the fungal phylum was affected by intercropping pattern in this study (Figure 8B; Table S3).

DISCUSSION

Responses of soil properties and enzyme activities to intercropping patterns

Soil physical and chemical properties are generally defined as key indicators reflecting soil quality, which are used to maintain crop productivity, ensure soil ecological security and promote animal and plant health.[25] Previous studies showed that intercropping pattern could significantly improve the physical and chemical properties of soil, thereby promoting the stability of plantation ecosystem and elevating the sustainable as well as resources use efficiency.[26] First, studies suggested that the increase of organic matter input after root decomposition is related to the increase of organic acid content during the decomposition process.[27] Therefore, the pH of intercropping soil was similar to that of Ac monoculture, but significantly higher than that of Pa monoculture, which may be caused by the higher root biomass of Ac (Table 2). Second, previous studies on wheat and corn intercropping patterns indicated that compared with monoculture, intercropping management significantly reduced soil BD and SM.[28] However, the significant increase of BD and SM may be related to the change of physical properties and soil structure caused by the distribution and infiltration of roots in rhizosphere soil under intercropping pattern in this study (Table 2). Third, studies on intercropping of wheat and corn showed that intercropping significantly increased the contents of soil organic matter, total carbon, total nitrogen, and total phosphorus.[26] However, intercropping significantly reduced organic matter, total nitrogen and potassium, and available nitrogen and potassium, which may be caused by the increase of crop density after intercropping (Table 2). The crops in this study

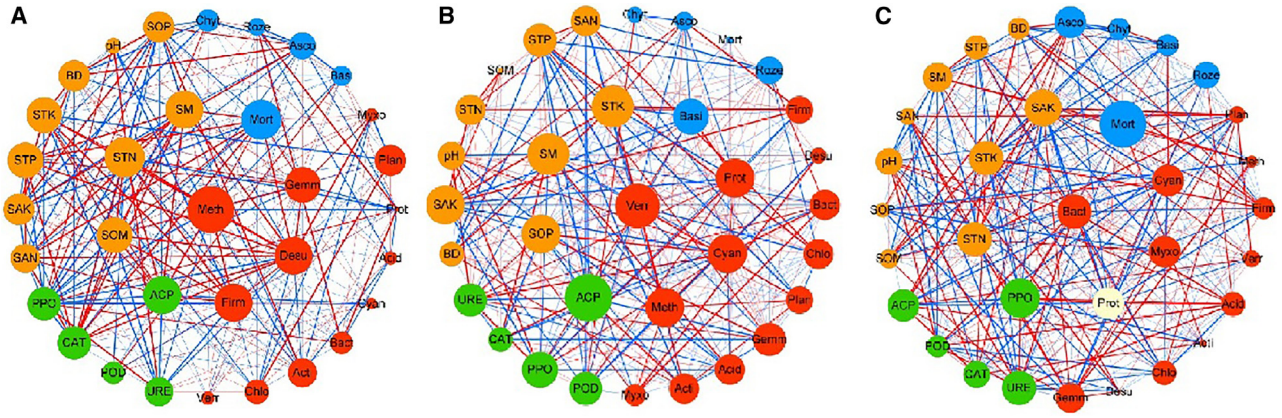

Figure 7. Interaction networks among soil properties, enzyme activities and microbial (bacterial and fungal) phyla in different cultivated patterns

A connection stands for a strong (Spearman's rho >0.4) and significant ($p < 0.05$) correlation for the *Areca catechu* L. monoculture (A), intercropping (B) and *Pandanus amaryllifolius* Roxb. monoculture (C). For each panel, the node size is proportional to the number of node connection across all the samples, and the thickness of each connection between two nodes (i.e., edge) is proportional to the value of Spearman's correlation coefficients. Red lines indicate a positive correlation while the blue lines show a negative correlation. Bacterial taxa: *Proteobacteria* (*Prot*), *Acidobacteriota* (*Acid*), *Actinobacteriota* (*Acti*), *Chloroflexi* (*Chlo*), *Firmicutes* (*Firm*), *Bacteroidota* (*Bact*), *Myxococcota* (*Myxo*), *Methylomirabilota* (*Meth*), *Gemmatimonadota* (*Gemm*), *Planctomycetota* (*Plan*), *Verrucomicrobiota* (*Verr*), *Desulfobacterota* (*Desu*), *Cyanobacteria* (*Cyan*), *Others*. Fungal taxa: *Ascomycota* (*Asco*), *Basidiomycota* (*Basi*), *Unclassified* (*Uncl*), *Mortierellomycota* (*Mort*), *Rozellomycota* (*Roze*), *Others*.

See Table 1 and Figure 1 for soil properties and enzyme abbreviations, respectively.

belong to tropical perennial economic crops, and their management methods are different from grain crops. Both crops only apply fertilizer once a year during the nutritional growth period. Intercropping pattren promotes the absorption and utilization of soil nutrients, but did not supplement soil fertility, resulting in insufficient exogenous organic matter added to the soil to supply both crops. This may be one of the reasons for the reduction of soil organic matter under intercropping treatment in this study.[29] It is generally believed that in the global biochemical cycle, phosphorus is mainly coupled with the water cycle, the movement and migration of phosphorus mainly occur in the soil and water in the plantation ecosystem.[30] The accelerated loss of soil water under Ac monoculture may be the main reason for aggravating phosphate leaching, and ultimately resulting in a significant decrease in soil total phosphorus content in this study (Table 2). The ACP activity with a tendency to enhancement under intercropping and the promotion of the transformation of organic phosphorus to available phosphorus may be the main reason why the content of available phosphorus is not different from that of Ac monoculture (Table 2; Figure 9). In addition, the disintegration of aggregate structure caused by the massive decomposition of soil organic matter under intercropping may also be one of the main reasons for the increase of BD (Table 2).

Soil enzyme activity represents the metabolic capacity of soil ecosystem and directly participates in the dynamic cycle of soil nutrients.[8] Soil enzyme activity is generally believed as the index to evaluate soil fertility depends on its positive correlation with soil nutrient dynamics, and the soil enzyme activity under intercropping pattern may be affected by the change of soil microclimate and nutrient supply.[23,26] Most previous studies have observed that intercropping pattern could significantly improve some soil enzyme activities (i.e., urease, peroxidase, catalase and acid phosphatase), mainly due to the positive feedback effect of soil nutrient accumulation on higher enzyme activities.[31] There is a significant synergy between soil nutrients and enzyme activities was Confirmed in previous studies.[32] The rich substrate from soil could stimulate soil enzyme synthesis and then accelerate the change of soil enzyme activity.[33] The positive correlation between SOM, STK and PPO, URE activities confirmed the above argument that the reduction of PPO and URE activities under intercropping pattern is mainly attributable to the decreasing of soil nutrient content the current study (Table 2; Figures 5 and 9). However, intercropping had neutral effects on CAT and POD activities in this study (Figure 1), which did not agree with previous studies.[31] The reason for this inconsistency may be related to the changes of soil microclimate and nutrient conditions under intercropping plantation. Studies suggested that soil enzyme activity may contribute to improving soil physical properties and soil structure,[34] but the variety of soil properties may have feedback effect on soil enzyme activity. The neutral effect of intercropping pattern on SM and BD may be the main reason for the non-difference of CAT and POD activities, although he negative correlation between SM, BD and CAT and POD in this study indicates that the decrease of soil porosity and the anaerobic environment may have potential negative effects on enzymes under intercropping pattern (Table 2; Figure 5). Unexpectedly, the constant ACP activity under intercropping plantation may be due to the complex interaction between acid phosphatase and soil phosphorus content,[35,36] despite soil phosphorus content being conspicuously decreased by intercropping in this study. The accumulation of phosphorus in tropical soil may also be completed through other regulatory channels, and the change of soil organic matter, available nutrients, and total

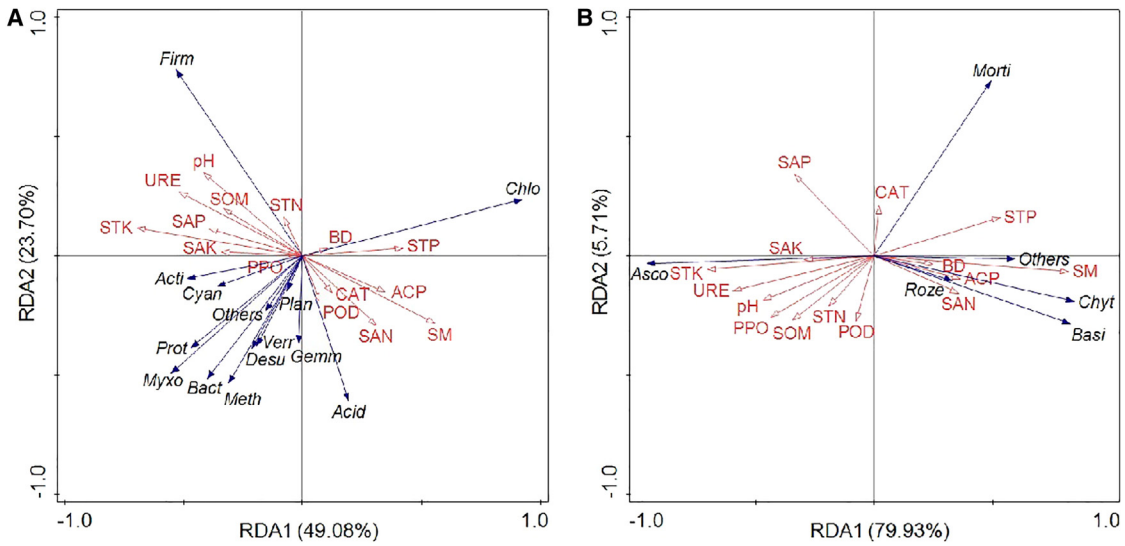

Figure 8. Key factors affecting soil microbial composition

Ordination plots of the results from the redundancy analysis (RDA) to identify the relationships among the soil bacterial (A) and fungal (B) taxa (blue arrows), the soil properties and enzyme activities (red arrows) at the phylum level.

See Figure 7 for abbreviation of microbial phylum name.

See Table 1 and Figure 9 for soil properties and enzyme abbreviations, respectively.

nutrients may be one of the main nutrient indicators for regulating soil phosphatase activity (Table 2; Figure 9).

Responses of microbial diversity to intercropping pattern depend on soil properties and enzyme activities

Soil microbial community is a key driving factor for the cycling and transformation of soil organic matter and nutrients in plantation, which plays an important role in maintaining the stability of plantation ecosystem, resisting stress interference and sustainable utilization of resources.[37] It is important to understand the diversity and succession of soil microbial communities for maintaining soil health and plantation ecosystem services.[38] It is well known that the most interference of plantation ecosystem comes from human management, and the crops cultivated pattern is one of the most important factor affecting the diversity of soil microbial community.[39,40] The rational space-time distribution among crops will improve the soil rhizosphere microclimate and nutrient accumulation, promote the activity of catalytic substances (i.e., soil enzymes), regulate the nutrient metabolism balance of microorganisms, and improve the diversity and relative stability of soil microbial communities.[41,42] The change of soil microbial diversity under intercropping pattern was mainly attributed to the change of interaction mechanism between soil properties, nutrient content and soil carbon enzyme activity in this study (Tables S2 and S3), which was consistent with previous studies.[43] Previous studies suggest that the response of bacterial and fungal communities to intercropping is generally significantly different, such as in wheat and faba bean mixed intercropping system, bacterial and fungal communities varied between crop species and plant compartments resulting in different responses of these communities toward cropping regimes.[44] Compared with bacterial community, there is little difference between planting patterns for fungal community in the current study, which indicating that bacteria are more sensitive to intercropping pattern than fungi in tropical plantation (Figures 1, 2, and 3).

Specifically, previous studies suggested that differences in soil physical and chemical properties could explain the soil bacterial community characteristics of each soil plot.[45] First, soil nutrient status plays an important role in the assembly of bacterial community structure and is an important factor that dominates bacterial community succession and diversity.[46] The contents of soil organic matter, phosphorus, and potassium are plant nutrient sources that regulate soil physical properties and improve soil structure, and their contents reflect the intensity of carbon source utilization and metabolic rate of soil bacteria.[43] The increase of the above soil nutrient content contributes to the enhancement of bacterial richness and diversity.[47,48] However, the negative correlation between phosphorus and bacterial diversity in this study (Figure 5; Table S2) indicates that the tropical farmland in this study is more sensitive to the variation of phosphorus content and the increase of soil phosphorus reduces the complexity of soil bacterial symbiosis network and the original metabolic level of soil bacteria, thus having a negative impact on bacterial diversity.[49] Second, soil microorganisms are sensitively influenced by the changes of soil environmental conditions, such as soil physical structure, nutrient content, pH, temperature, and moisture.[50] Soil pH significantly affects the bacterial diversity by affecting the chemical fertility of soil matrix and soil enzyme activity,[51] although the decoupling of the correlation between soil pH and soil microbial diversity in this study (Table S2) indicates that soil pH is not the factor to regulate microbial community under intercropping pattern. However, the increase of soil bulk density led to a significant decrease in soil gas diffusion rate, may induce the transformation of soil bacterial community to anaerobic, which may be

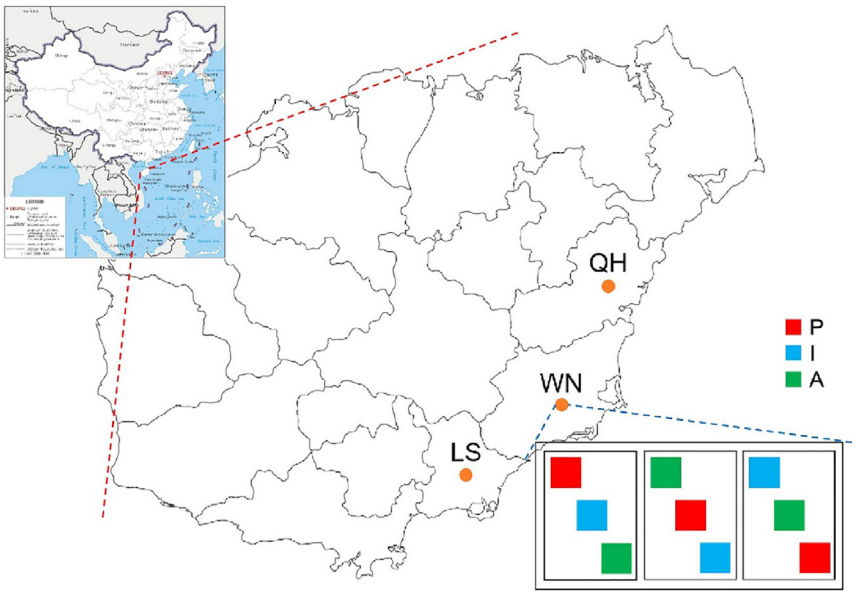

Figure 9. Location of experimental site on the Hainan island of China

one of the potential reasons for the reduction of soil bacterial diversity by intercropping (Table S2). Third, soil enzyme (i.e., URE, POD, CAT, ACP) activities improve the soil nutrient content by accelerating the decomposition of organic matter and high molecular organic nitrogen and the hydrolysis of phosphorus, and thus have a significant impact on bacterial diversity.[52] Moreover, PPO, as one of the key catalysts for degradation of soil phenolic toxic substances (allelopathy), contributes to the maintenance of soil microbial community stability and soil health.[53] The decline abundance of organic matter degrading microbial functional groups in the soil of Ac plantations after intercropping Pa, and the positive correlation between URE, PPO and bacterial diversity in this study confirmed the previous inference (Figure 6; Tables S2 and S4).

Compared with the Pa and Ac, the regulation mechanism of soil bacterial diversity reduction after intercropping is significantly different in current study (Figure 7). Soil pH, SOM, STK content and PPO, URE activity were selected as the key factors to regulate bacterial diversity in different models by stepwise regression (Figure 5; Table S2). Compared with Ac monoculture, intercropping pattern significantly reduced bacterial community diversity by indirectly reducing SOM content, PPO and URE activities. However, compared with Pa monoculture, the decreased SOM, STK and URE activities were the main reasons for the reduction of soil bacterial diversity (Figure 6). Furthermore, this study suggests that soil pH plays an important role affecting regulating soil bacterial community diversity by indirectly regulating soil polyphenol oxidase and urease activities (Figures 5 and 6; Table S2).

Responses of microbial compositions to intercropping patterns depend on soil properties and enzyme activities

Soil microbial composition and structure are closely related to basic soil processes,[54] which is regulated by the feedback of soil nutrient content and structural characteristics to a certain extent. Moreover, the feedback regulation process is also affected by the change of the relationship between soil enzyme activity and microbial function.[55] Therefore, the intercropping pattern could affect the composition and structure of plantation ecosystem by regulating the interaction among soil nutrient content, enzyme activities, microbial community structure.

All fungal phyla and the most dominant bacterial classes, such as *Proteobacteria*, *Acidobacteriota*, *Actinobacteriota*, *Chloroflexi*, *Firmicutes* (relative abundance >5%) were not sensitive to the environmental conditions, except other specific taxa, especially *Gemmatimonadota* was significantly increased, while *Myxococcota* was significantly decreased under intercropping pattern, when Pa was intercropped under Ac plantation in current study (Figure 3). The changes of the above two bacteria are closely related to soil pH, water content and total potassium, which may be attributed to their ecological strategies.[56] Among them, the increase of *Gemmatimonadota* is regulated by soil properties (pH and SM) (Figure 3; Table S3), while the decrease of *Myxococcota* under intercropping is not only affected by the significant decrease of STK, but also closely related to the decrease of URE activity, because the change of *Myxococcota* is stimulated by the higher URE activity and is well adapted to soil through its physiological metabolism.[57] Therefore, these results suggested that the differential response of soil bacterial community composition to intercropping pattern depends largely on environmental changes (pH and SM) and soil nutrient parameter dynamics (STK), but the influence of soil enzyme activity on community composition cannot be ignored.

Furthermore, the reasonable intercropping could improve the functional diversity of beneficial microorganisms and inhibit the metabolic activities of harmful microorganisms, such as anaerobic bacteria and denitrifying bacteria, which is conducive to maintain the stability of soil microbial community structure.[51] The results of this study believe that intercropping maintain the relative balance of soil microbial community structure is beneficial to the continuity of soil functional diversity, and provides potential guarantee for improving the productivity of tropical plantation ecosystem.

Limitations of the study

This study is an intentional attempt to explore the soil microbial community in the intercropping ecosystem. However, this study only currently analyzes the microbial community at the phylum level and further classification research still continues. This study focused on analyzing the changes in soil microbial community structure without predicting microbial functions, which is another regret of this manuscript. Moreover, the root system interaction mechanism mediated by root exudates is one of the main driving

mechanisms of crop reciprocity in intercropping ecosystems. Therefore, further research on this scientific issue will be conducted in the future.

Conclusions

The soil microbial diversity and structure of Ac plantation were significantly changed after intercropping with Pa, wherein the bacterial rather than fungal community diversity responded to intercropping pattern in the current study. Intercropping pattern markedly inhibited the bacterial community diversity by decreasing nutrient contents of SOM, STK or reducing the enzyme activities of PPO, URE, with significant correlations being observed. The intercropping pattern has a neutral effect on the dominant bacterial phyla, except significantly increases *Gemmatimonadota* and decreases *Myxococcota*, and the changes in soil pH, SM, STK and URE activities are considered as potential factors for predicting specific taxa. The findings of this study highlight the different responses of bacterial communities to different planting patterns, and further clarify the decrease of soil nutrient content may be the main reason for the change of soil enzyme activity, bacterial community diversity and composition structure under intercropping pattern. Supplementing soil nutrients during the transition from monocropping to intercropping systems in the tropical plantation is conducive to maintain soil health and functions.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by Ang Zhang, angzhang_henu@163.com.

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Raw and analyzed data have been uploaded to figshare: [https://doi.org/10.17632/xfckg48dk2.1]. These data are publicly available as of the date of publication.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the first author upon request.

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AUTHOR CONTRIBUTIONS

A.Z.: Writing-original draft, Writing-review and editing, Project administration; F.S: Investigation, Resources; X.Q.: Conceptualization; H.Y.: Funding acquisition, Project administration; Y.Z.: Investigation, Resources; X.J.: Investigation, Project administration; S.H: Investigation, Resources; Y.Z.: Investigation, Resources; N.A.: Investigation; L.L.: Investigation; S.C.: Investigation. A.Z. and F.S. have equal contributions to this study and should be considered as co first authors.

DECLARATION OF INTERESTS

The authors declare no competing interests or personal relationships that could have appeared to influence the work reported in this paper.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Rhizosphere soil samples were collected from *Pandanus amaryllifolius* Roxb. and *Areca catechu* L. under monoculture and intercropping treatment	Collected in Jun. 2021 at the eastern plain of Hainan Island, China	N/A
Critical commercial assays		
Soil DNA Extraction Kit	Omega, Norwalk, CT, USA	N/A
Deposited data		
Raw and analyzed data	This paper	https://doi.org/10.17632/xfckg48dk2.1
Software and algorithms		
QIIME 1.9.1	Zhong et al.[4]	https://qiime.wordpress.com/
UPARSE 7.0.1090	Robert C Edgar	http://drive5.com/uparse/
MOTHUR 1.30.2	Zhong et al.[4]	https://www.mothur.org/wiki/Download_mothur
IBM SPSS 23.0	IBM, Armonk, NY, USA	https://www.ibm.com/cn-zh/spss
SAS V8	SAS Institute Inc, Kerry, USA	https://www.sas.com/en_us/home.html
IBM SPSS Amos 21.0	IBM, Armonk, NY, USA	https://www.ibm.com/cn-zh/spss
Canoco 4.5	Informer Technologies, Inc.	https://canoco.software.informer.com/4.5/
Cytoscape 3.5.0	Zhong et al.[4]	http://cytoscape.org
Origin 2021b	OriginLab Corporation	https://www.originlab.com/2021b
Other		
Supplemental information	This paper	https://doi.org/10.17632/xfckg48dk2.2

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study site

The study was located in the eastern plain of Hainan Island, China. Hainan Island is about 230 km long from the north to south, and 290 km wide from the east to west. The coastal plain in the east of Hainan Island is mainly used for agricultural cultivation, Ac and Pa intercropping pattern has become a common cultivated pattern in this area. Based on the purpose of reducing errors, three sites were chose to employ those cultivated pattern, and all of which had similar geographical conditions and vegetation composition: Zhongyuan Town, Qionghai City (QH, 110°25' E, 19°07' N, a.s.l. 65 m), the mean annual temperature is 23.7°C and mean annual precipitation is 2043 mm, the soil pH is 5.31, soil organic carbon (SOM) content is 18.18 g/kg, the alkalihydrolyzed nitrogen content (SAN) is 142.33 mg/kg, the soil-available phosphorus content (SAP) is 15.28 mg/kg, and the soil-available potassium content (SAK) is 52.37 mg/kg; Xinglong Tropical Botanical Garden, Wanning City (WN, 109°56' E, 18°31', a.s.l. 36 m), the mean annual temperature is 24.5°C and mean annual precipitation is 2201mm, the soil pH is 5.49, SOM is 15.19 g/kg, SAN is 77.47 mg/kg, SAP is 16.28 mg/kg, and SAK is 32.86 mg/kg; Wenluo Town, Lingshui County (LS, 109°56' E, 18°31' N, a.s.l. 38 m), the mean annual temperature is 25.4°C and mean annual precipitation is 1809 mm, the soil pH is 6.14, SOM is 22.26 g/kg, SAN is 36.87 mg/kg, SAP is 27.21 mg/kg, and SAK is 39.56 mg/kg (Figure 9). The soil type of the three sites is sandy argillaceous yellow latosol (China Soil System Classification).

Plants

This study was conducted in Jun 2015. Randomized block design was executed in this experiment. This study consists of three cultivation treatments, Ac monoculture (A), Pa monoculture (P), and Ac and Pa intercropping (I) (Figure 9), each located in one plot (10 × 10 m), which were randomly selected in each block. At each experimental site, three repetitive block were chose, and the distance among all blocks were not exceed 50 m to ensure the uniformity of soil properties. The total cultivated area of the two crops at each site is 1∼5 hm2, and the cultivated density is the same, which is 2.5×2.5 m for Ac, and 50×50 cm for Hb. The cultivated period of Ac is about 5 years, and that of Pb is about 1 year. The Ac and Pa used in this study are both local mainstream single varieties.

The field management methods, such as water and fertilizer management, were consistent during the experiment. In order to reduce the interference of fertilization on soil physical and chemical properties and microbial community structure, all treatments of fertilization were completed in July of the previous year (i.e. 2020) and no topdressing was carried out. Each treatment fertilized with 24 kg/hm^2 of 46% urea ($CO(NH_2)_2$), 40 kg/hm^2 of 64% diammonium phosphate ($(NH_4)_2HPO_4$), and 36 kg/hm^2 of 50% potassium sulfate (K_2SO_4) in this study. All treatments were irrigate 4 times a month in dry season (November-May of the following year), with an average irrigation volume of 20mm (i.e. 10 t/ha), while did not irrigate during the rainy season (Jun-October) in 2021.

METHOD DETAILS

Sample collection and environmental information

The rhizosphere soil samples were collected in June 2021, when Ac and Pa were grew rapidly. Three replicate surface soil and root samples (upper 20 cm) 50cm away from monoculture plants were randomly collected and homogenized to provide one composite sample per replicated site. The same method was used for intercropping treatment, but the collection location was on the line connecting two plant of different species. After shaking the excavated plant roots and removing the bulk soil between them, collect the rhizosphere soil attached to the surface of the roots with a small brush. Among them, due to the inability of the roots obtained from intercropping to distinguish which crop they belong to, all rhizosphere soils obtained from intercropping were mixed in the actual experimental process to obtain a single sample in this study. Then, each rhizosphere soil sample was sieved through a 2 mm mesh to remove plant materials and rocks. The tools were disinfected between different soil samples to avoid contamination between treatments during this sample arrangement process. A portion of each soil sample was air-dried and stored at room temperature prior to physicochemical properties analysis. Subsamples for molecular and enzyme activity analyses were immediately homogenized and stored at -80°C and 4°C, respectively.

Soil moisture (SM) was assessed by oven drying to a constant mass at 75°C. Soil bulk density (BD) was calculated by sampling and gravimetry with a fixed volume ring-knife. The soil pH were measured by FE28 pH meter in a 1:5 soil/water suspension solution. The soil organic matter (SOM) is measured by the total organic carbon analyzer (multi n/c 3100). Soil alkali hydrolyzed nitrogen (SAN) was determined by alkaline digestion and diffusion method; Soil available phosphorus (SAP) was determined using the ascorbic acid reductant method; Soil available potassium (SAK) was assessed through atomic absorption; Soil total nitrogen (STN) was determined by semimicro-Kjeldahl method; Soil total phosphorus (STP) and total potassium (STK) were determined by the NaOH molten-molybdenum antimony colorimetric method.

Soil catalase (CAT) activity was determined by potassium permanganate titration; The activities of polyphenol oxidase (PPO) and peroxidase (POD) were determined by pyrogallol colorimetry; Acid phosphatase (ACP) and Urease (URE) activities were determined by sodium phenylene phosphate colorimetry and indophenol blue colorimetry, respectively.

QUANTIFICATION AND STATISTICAL ANALYSIS

Bioinformatics analysis

Soil microbial DNA was extracted from 0.5 g of fresh soil three times by using the EZNA® Soil DNA Extraction Kit (Omega, USA). The purity and quality of the genomic DNA were checked on 0.8% agarose gels. Barcode-labeled primer sequences bacteria: 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and fungi: ITS1F (5'-CTTGGTCATTTAGAG GAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') were used to amplify the corresponding soil bacterial 16S rRNA V3-4 region fragment and fungal ITS-1 sequence fragment. 2% agarose gel electrophoresis was used to detect the length of the amplified product fragments. According to the quantitative detection results, the amplified products were mixed into one sample, and then a clone library was constructed. The loading amount for each library was calculated based on the library search results, and the paired-end sequencing method was used on the Illumina MiSeq high-throughput platform for sequencing.

Paired-end reads of raw DNA fragments were merged using FLASH 1.2.11 software and quality filtered using QIIME 1.9.1 software. Valid sequences were obtained, and reads that could not be assembled were discarded. Unique sequences with 97% or greater similarity were clustered into operational taxonomic units (OTUs) using uPARSE 7.0.1090 software. MOTHUR 1.30.2 annotated each OTU using the small subunit rRNA SILVA database. The sample with the least data was used as the standard for normalization. Soil microbial community diversity and richness were calculated by using QIIME.

Statistical analysis

Taxonomic alpha diversity was calculated as the estimated bacteria and fungi community diversity by the Shannon index using the MOTHER v.1.33.3 software, respectively (Figure 2). Nonmetric multidimensional scaling (NMDS) was selected to illustrate the clustering of different samples and further reflect the bacteria and fungi community structure, while the changes in microbial structure under different cultivated patterns were referred to as bacteria and fungi beta diversity, respectively (Figure 3). Network interaction analysis of microbial composition was analyzed by SPSS 23.0 (Figure 7).

All the data were tested using a mixed-effects pattern with repeated measurements (Proc Mixed, SAS 8.1). Cultivated patterns was fixed factors, and experimental sites were assigned as random factors. Before the data analysis, all the data which input pattern were accord with the normality test. The data were analyzed using a one-way analysis of variation (ANOVA) for different cultivated patterns,

including soil properties (i.e., SM, BD, SOM, SAK, SAP, SAN, STN, STP, STK, Tables 1 and 2), soil enzyme activities (i.e., CAT, PPO, PPO, ACI, URE, Figure 1; Table 3) and the microbial characteristics (i.e., Bacterial richness, Bacterial diversity, Fungal richness, and Fungal diversity, Figure 2) in each experimental site. Data analysis was performed by using the Duncan test, the difference between mean values was determined by using the least significant difference (LSD, $P < 0.05$) as indicated by different letters.

The correlations between the soil enzyme activities and the soil properties were determined by Spearman's correlation analysis (SPSS 23.0, Figure 5). In order to determine the main influencing factors affecting bacterial diversity, stepwise multiple linear analyses were used to analyze the main factors affecting microbial alpha diversity (Table S2). Then structural equation model (SEM) was further conducted by the main influencing factors to quantify the direct and indirect effects of cultivation on soil bacterial alpha diversity (Zhang et al.,[58] Figure 6). A conceptual model of hypothetical relationship was created based on a prior and theoretical knowledge. Data were fitted to the model, using the maximum-likelihood estimation method. Adequate model fit is indicated if a Chi-square test is not significant ($P > 0.05$). The SEM model were divided into Ac and Pa model, due to the different effects of intercropping on Ac and Pa monoculture soil (Figure 6). SEM analysis was performed by using SPSS Amos 21.0. Redundancy analysis (RDA) was performed and mapped using the analysis of soil microbial community composition in relation to soil properties and enzymes activity; the model was assessed for 999 iterations based on Monte Carlo permutations (Figure 1; Table S3). The RDA was performed using the CANOCO 4.5 software package. The graphs were plotted by using Origin 2021 and Cytoscape 3.5.0.

ADDITIONAL RESOURCES

Supplemental information can be found online at https://doi.org/10.17632/xfckg48dk2.2.