Original Research Paper

Bacterial Diversity and Community Structure Characteristics in Rhizosphere Soil of Coconut (*Cocos nucifera* L.) under Intercropping Pineapple

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Abstract: To explore the bacterial diversity and community structure characteristics of the rhizosphere soil of fruit type coconuts of different varieties under the intercropping pineapple model, the Illumina high throughput sequencing technology was used to detect the composition and diversity of soil microorganisms. Furthermore, the correlation between the physical and chemical properties of soil and the characteristics of the rhizosphere soil community was analyzed. The contents of OM, EC, AN, AP, AK, ACa, and AMg in coconut rhizosphere soil under intercropping were significantly higher than those under coconut monocropping. However, the soil pH value of the intercropping mode decreased significantly. The diversity of bacteria in the rhizosphere soil of coconut intercropped pineapple was higher than that of coconut monocropped. The diversity of bacteria in the rhizosphere soil of different varieties of coconuts was also different: The order of bacterial diversity was JG > JYT > JYO > JH. The dominant bacteria of the rhizosphere soil, in order, were *Proteobacteria*, *Acidobacteriota*, *Bacteroidota*, *Verrucomicrobiota*, *Actinobacteria*, *Planctomycota*, Firmicutes, *Gemmatimonadota*, *Myxococcota*, *Nitrospirota*, which indicated that the composition of bacterial species between intercropping and monocropping was very different. JG, JYO, and JYT had a high similarity of bacterial community through principal component analysis and cluster analysis, and the bacterial community composition of these three treatments was significantly different from that of CH and JH. In addition, JH had significant differences in bacterial community composition compared to CH. This also showed that different varieties of coconut intercropping pineapple have a great impact on the bacterial composition of coconut rhizosphere soil.

Keywords: Coconut, Illumina High-Throughput Sequencing, Rhizosphere Soil, Microbial Diversity, Intercropping

Introduction

Microbial diversity and abundance in soil are important indicators for judging soil quality. The study of soil ecology and rhizosphere soil microorganisms has attracted much attention. Intercropping can increase crop yield, improve the composition of bacterial and fungal communities in the soil and ameliorate the soil environment (Kong *et al*., 2020). Rotation increases the ability of microorganisms to use carbon sources, thereby improving the soil environment of continuous cropping crops (Peng *et al*., 2020). intercropping with different crops improved the flora composition of microorganisms around the root of continuous cropping watermelon and alleviates the adverse results of continuous cropping (Jiang *et al*., 2012). As for potato intercropping in maize, the inferior crop is potato and the chlorophyll in potato leaves decreased, the photosynthetic rate and the yield also declined (Xiao *et al*., 2011). Reasonable intercropping can increase the nutrient absorption of crops, promote the availability of nutrients in the soil and improve leaf area index and photosynthetic rate (Wang *et al*., 2019). In the mode of potato intercropping in kidney beans, the yield of potatoes increased by 17 times

compared with the monoculture and 5 times compared with the potato intercropping in corn (Wang *et al*., 2022). Intercropping potatoes and broad beans can improve potato tuber quality and change crop microcirculation (Li *et al*., 2012). Rotation of cabbage-type rape and potato can increase the number of bacteria, improve microbial activity, reduce the number of fungi in soil and reduce potato stem canker (Benitez *et al*., 2021; Wang, 2014). Young coconut trees were intercropped with leguminous grasses (Feng *et al*., 2007) and it was found that the growth rate of coconuts was 20~30% higher than that of monoculture. Compared with coconut monocropping, coconut intercropping pasture can increase the content of soil nutrient-active components (Longxiang *et al*., 2004). Different planting modes had different effects. Reasonable crop combinations can improve soil quality, provide a good soil environment, reduce planting costs and increase income (Wang *et al*., 2013).

Coconut, is an important cash crop in Hainan Province, because of its high tree type, it reduced the land utilization rate and was easy to breed weeds, which not only increased the management cost of the coconut garden but also affected the growth and economic benefits of the coconut. The short-term economic benefits were not good, which seriously restricted the development of the coconut industry (Yu *et al*., 2012; Liu *et al*., 2001) and also restricted the economic income of farmers (Tang *et al*., 2003). Coconuts were planted in the same place for a long time and the soil fertility was easy to degrade, which was attributed to the root exudates and root microorganisms. Allelochemicals secreted by roots will accumulate in the soil and affect root growth, reduce the efficiency of the coconut to absorb nutrients, and finally reduce the growth of the coconut and its resistance. Reasonable intercropping can increase root secretion, improve the structure of the microbial community, make soil quality healthier, generate adaptive microflora, and enhance root effects according to the cross of roots (Wang *et al*., 2013; Yu *et al*., 2012; Mao and Fu, 2011; Gong *et al*., 2019). Therefore, coconuts were often intercropped as short-term crops to increase the income of farmers (Hou *et al*., 2020; Zainuddin *et al*., 2022; Su *et al*., 2020). The interaction effect of coconuts and short-term cash crops can increase soil fertility. In the coconut garden, the development of multi-layer cultivation can take full advantage of the coconut garden space, improve short-term economic income and develop the enthusiasm of coconut farmers (Longxiang *et al*., 2004; Wang *et al*., 2013; Yu *et al*., 2012; Mathes and Li, 1989; Chen, 2000). Pineapple was one of the famous tropical fruits and one of the important cash crops (Guo, 2019). Different coconut varieties had different characteristics in root growth and development, so there may be differences and similarities in rhizosphere exudates, interactions between rhizosphere and nutrients, and interactions with intercropped crops. At present, there was no research on the effects of coconuts intercropping with pineapples on soil nutrients and microorganisms in the rhizosphere of coconuts and whether there are differences in soil microbial communities and structural characteristics in the rhizosphere of different varieties of coconuts. Therefore, this study intended to carry out the impact of different coconut varieties intercropping pineapple on the microbial and bacterial community and diversity of coconut rhizosphere soil and to conduct a correlation analysis between soil physical and chemical properties and the characteristics of the rhizosphere soil bacterial community. It provided the scientific basis for the management of coconut intercropping cultivation mode. To realize the healthy and sustainable development of microecology in coconut orchards under the cultivation mode of coconuts intercropping short-term cash crops.

Materials and Methods

Study Area and Soil Sampling

The sampling point of this study was located in the coconut planting base (19°45′ N, 109°21′E) of Team 1 Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences (CRICATAS), The location was flat and belongs to tropical and subtropical climate and belonged to tropical monsoon island climate. The annual average temperature is 23.9°C, the annual average sunshine was 1953.8 h and the annual rainfall was 1721.6 mm. The soil texture was sandy or lateritic clay silt sandy soil and tropical cash crops such as coconut, pineapple, and papaya were planted (Fig. 1).

The coconut collected on August 15, 2020, in this study includes three varieties of coconut intercropping pineapple (hybrid pineapple) models: Single red dwarf coconut wenye '3' (CH), perfume coconut wenye '4' intercropping pineapple (JG), red dwarf coconut wenye '3' intercropping pineapple (JH), yellow dwarf coconut wenye '2' intercropping pineapple area 1 (JYO), yellow dwarf coconut wenye '2' intercropping pineapple area 2 (JYT), coconut planting model 6×6 m, pineapple pattern at 50×50 cm in two rows is intercropped between two rows of coconuts. CH, JG, JH, and JYO are distributed in the same area, while JYT was distributed in the other area. The straight-line distance between the two areas was 1 km. All treatments had the same fertilization amount, type, and management mode. Coconut was planted for 3 years and pineapple was intercropped for 3 consecutive years. Soil samples were collected when pineapple was planted for 5 months each year. Sampling method: Randomly selected 3 areas (each area was about 40 m^2) for each mode and collected three coconut rhizosphere soil samples from each area to mix one sample. Rhizosphere soil collection: Picked out the coconut root system, collected the rhizosphere soil by shaking the root method, took the soil

sample back to the laboratory, and stored it in the refrigerator at 4°C. The rhizosphere soil samples were collected for air drying and preservation for 3 consecutive years to determine the physical and chemical indexes of the soil and the average value was taken. In the third year, a part of fresh rhizosphere soil from coconuts intercropped with pineapple was collected to extract total microbial DNA for high throughput sequencing.

Determination of Soil Physical and Chemical Properties

The air-dried and screened soil samples were analyzed for the following Physical and Chemical Properties (PCP): Soil pH (soil: Water = 2.5:1) was measured with a portable soil pH meter, Electrical Conductivity (EC) was measured with a conductivity meter and Organic Matter (OM): Potassium dichromate volumetric method-external heating method. Alkali hydrolyzed Nitrogen (AN): Alkali hydrolyzed diffusion method. Available Phosphorus (AP): Molybdenum antimony anti-colorimetry. Available potassium (AK): NH4Ac extraction Atomic Absorption Spectrophotometry (AAS) (AA280FS) (Bao, 2000). Available Calcium (ACa) and magnesium (AMg) contents: DTPA extraction Atomic Absorption

Spectrophotometry (AAS) (SHIMADZU AA6300F). Physical and Chemical Properties (PCP) were calculated as follows:

$$PCP(meanvalue) = (PCPi + PCPi + ...PCPn]n1$$
$$+ [PCPi + PCPi + ...PCPn]n2 + /[PCPi + PCPi + ...PCPn]n3)/3)$$

Extraction, PCR Amplification, and Sequencing of Soil Microbial Genomic DNA

The total DNA of soil microorganisms was extracted with the soil genomic DNA rapid extraction kit of shanghai San gong Bioengineering Co., Ltd; the extracted DNA samples were sent to BMX (Biotechnology Co., Ltd.) with dry ice for sequencing. The whole process included PCR amplification, sample mixing and purification of PCR products, and library construction. The V3-V4 region of 16S rDNA of bacteria was amplified by two rounds. The upstream primer 338F: 5'-ACTCCTACGGGGAGGCAGCA-3'. Downstream primer 806R: 5'-GGACTACHVGGGTWTCTAAT-3'. Reaction system 10 μ. There was 5-50 ng genomic DNA, *Vn F (10 μm) 0.3 μL, *Vn R (10 μm) 0.3 μL, KOD FX Neo Buffer 5 μL, dNTP (2 mm each) 2 μL, KODFXNe 0 0.2 μL.
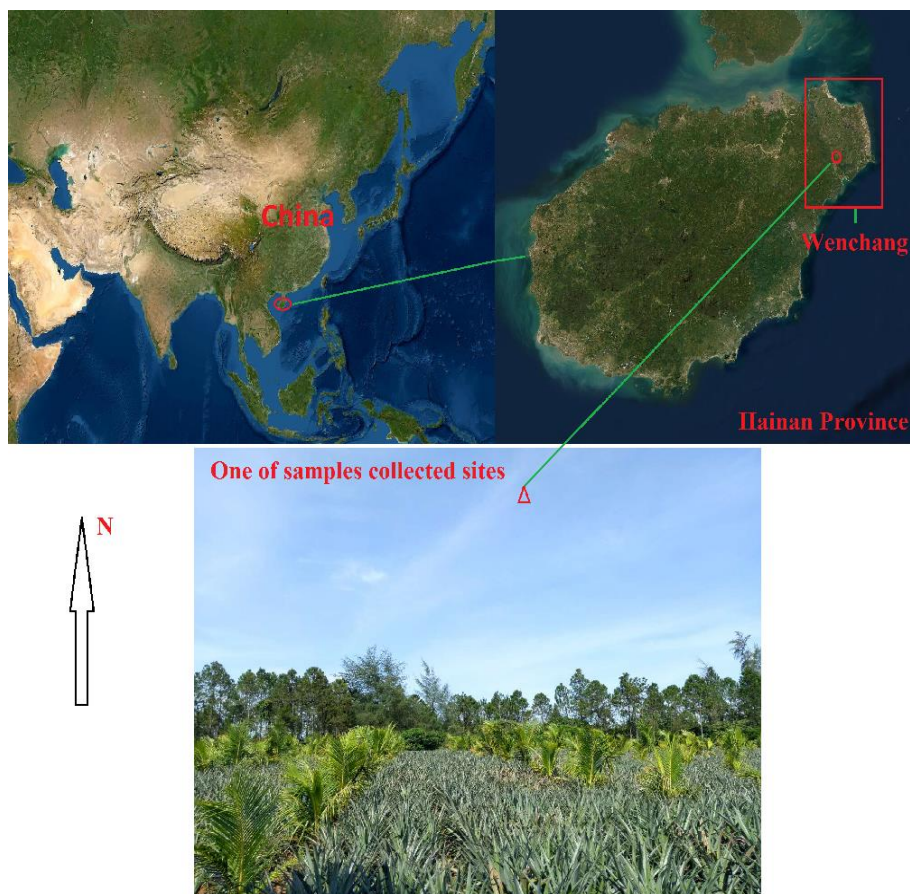

Fig. 1: Information and sites about collected samples

Amplification conditions: 5 min at 95°C, 30s at 95°C, 30s at 50°C, 40s at 72°C, 25 cycles of amplification and 7 min at 72°C. Then, take the first PCR product as the template, use Illumina bridge PCR compatible primer and the reaction system was 20 μL, PCR purified primer 5 μL, *MPPI-a (2 μm) 2.5 μL, *MPPI-b (2 μm) 2.5 μL, 2 × Q5HF MM10 μL. Amplification conditions: 98°C 30s, 98°C 10s, 65°C 30s, 72°C 30s, 10 cycles of amplification, 72°C extension for 5 min. PCR products were recovered and quantified by the magnetic bead method. Mix the products of repeated individual PCR of each sample to ensure sufficient sample detection concentration. Send 15 mixed samples to BMX (Biotechnology Co., Ltd.) for high throughput sequencing using the Illumina MiSeq platform.

Data Analysis

The OTU representative sequences with 97% similarity were taxonomically analyzed (USEARCH, version 10.0) and the bacterial and fungal community composition of each sample was counted at the level of the kingdom, phyla, class, order, family, and genus. Draw a bar chart of species classification and a heat map of species abundance. Using 16S and 18S sequences, the diversity index (chao1, Shannon, Simpson index, and coverage) of alpha was calculated after resampling and unified sequencing depth. Draw the dilution curve (based on the abundance of OTUs) for dilution analysis. Beta diversity analysis: The diversity matrix of species was presented based on binary_jaccard. Draw the Principal Component Analysis (PCA), Non-Metric Multi-Dimensional Scaling (NMDS), environmental factors, and Correlation Analysis (CCA) of sample composition according to R language. Analyze OTU division, alpha diversity index calculation, and dilution analysis through mother software; Using GraphPad 6 software to complete paired t-test and mapping; CCA was analyzed by vegan software package of R. After the OUT data was standardized (logarithmic), the largest number of species was selected and the heatmap diagram clustering analysis was conducted based on R language tools. The samples were hierarchically clustered using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to judge the similarity of species composition among the samples. Lefse Line Discriminant Analysis (LDA) effect size (Segata *et al.*, 2011) Biomarker was used for analyzing statistical differences.

Results

Soil Physical and Chemical Properties of Different Fruit Type Coconuts under Intercropping Mode

Compared with the coconut monoculture (Table 1), the models of coconuts intercropping with pineapple significantly ($p < 0.05$) increased the contents of EC, OM, AN, AK, AP, ACa, and AMg. That was, the coconut intercropping models can increase the nutrient content of coconut rhizosphere soil, of which JYO treatment has the highest nutrient content, followed by JYT, JG, and JH, it may be caused by the cross-influence of coconut and pineapple root exudates. There was little difference in pH change among the five treatments, but the pH of the mode of coconut intercropping with pineapple was lower than that of the coconut monoculture treatment. In the intercropping treatment, the interaction between coconut and pineapple affected the absorption and transformation of soil nutrients, which may cause a difference in nutrient content in the soil.

Microbial Bacterial Diversity and Community Structure in Rhizosphere Soil

Sequencing Data Preprocessing Results

Figure 2A, the dilution curves of the 15 samples were finally flat and the sequencing was close to saturation, indicating that the sampling was basically reasonable and could correctly reflect the microbial communities in the 15 soils. Combined with the coverage of each sample (0.96-0.99) (Table 2), it was concluded that the sequencing included all microbial groups and the microbial community structure composition could be obtained. Comparing the dilution curves of bacteria, it can be found that the dilution curve of bacteria is more gentle and the coverage rate is higher, indicating that almost all bacterial species have been found, while a small number of bacterial species have not been found.

Diversity of Rhizosphere Soil Microbial Bacterial Communities

Different intercropping model treatments had the same change trend in soil bacteria chao1 and ACE values, namely JG>JYT>JYO>CH>JH. Among them, JG had the largest abundance of soil bacteria, and JH had the smallest abundance of soil bacteria. The ACE values of JG, JYT and JYO were 30.06, 27.09, and 15.28% higher than that of CH, while the ACE values of JH were 18.78% lower than those of CH and the chao1 values of JG, JYT, and JYO were 24.85, 21.27 and 9.20% higher than that of CH, the value of chao1 in JH was 21.36% lower than that in CH. The Shannon index of bacteria in CH was the lowest, the Simpson index was the highest and the bacterial diversity was the lowest; The Shannon index of bacteria in JG was the largest, the Simpson index was the smallest and the diversity of soil bacteria was the highest. The Simpson values of bacteria in JG, JYT, JYO and JH were 82.13, 65.46, 71.02, and 53.33% lower than those in CH and the Shannon values of bacteria in JG, JYT, JYO, and JH were 20.37, 17.64, 13.65 and 5.81% higher than those in CH. Except for JH, the abundance of bacteria in the other 3 intercropping modes was greater than that in the control treatment and the diversity of bacteria in all intercropping models is greater than that in the control treatment, indicating that the intercropping models can significantly improve the abundance and diversity of bacteria

(Table 2). From the Venn chart analysis results, the OTU numbers of bacteria unique to CH, JYT, JYO, JH, and JG are 71, 48, 19, 58, and 32 respectively and the total bacterial OTU numbers were 352. The five treatments overlap each other and the common OTU numbers account for a large proportion. Therefore, it can be stated that the consistency of rhizosphere soil bacterial groups of the five treatments was very high (Fig. (2B) and 3).

Composition of Rhizosphere Soil Microbial Bacterial Community

After high-throughput sequencing of 15 samples, except for unclassified and others, the top 10 bacteria accounted for more than 64%. Although the relative content was different, the top 10 bacteria in the five coconut cultivation treatments were the same Fig. (4A), including proteobateria (26.02-34.08%), *Acidobacteria* (10.02-20.45%), *Bacteroidota* (4.45-14.02%), *Verrucomicrobiota* (5.25-8.25%) *Actinobacteriota* (1.75-6.58%), *Planctomycetota* (2.54-6.25%), *Firmicutes* (4.12-5.24%), *Gemmatimonadota* (0.89-6.25%), *Myxococota* (2.01-4.05%), *Nitrospirota* (1.25-4.02%). At the genus level, *Vicinamibacter*, *Pedosphaera*, *Acidibacter*, and *Burkholderia* are the dominant genera of rhizosphere bacterial community in CH soil (abundance >1%); *Terrimonas*, *Vicinamibacter*, *Pedosphaera*, Candidatus, *Nitrospira*, *Acidibacter*, and *Gaiella* are the dominant genera of JG soil rhizosphere bacterial community; *Terrimonas*, *Vicinamibacter*, *Pedosphaera*, Candidatus, *Gemmatimonas*, *Sphingomonas* are the dominant genera of JH soil rhizosphere bacterial community; *Terrimonas*, *Vicinamibacter*, Candidatus, *Gemmatimonas*, *Nitrospira*, and *Gaiella* are the dominant genera of JYO soil rhizosphere bacterial community; *Terrimonas*, *Vicinamibacter*, *Pedosphaera*, Candidatus, *Nitrospira* are the dominant genera of JYT soil rhizosphere bacterial community (Fig. 4B). The bacteria clustering heat map analysis based on the phyla level showed that the bacterial communities of JYO, JYT,

JG, and JH were clustered into one group and were at the same level. The bacterial community composition of CH was quite different from JYO, JYT, JG, and JH, which were grouped into one (Fig. 5).

Difference Analysis of Soil Microbial Community

Based on binary_jaccard, the bacterial community composition of UPGMA cluster tree analysis was shown in Fig. (6C). The bacterial community composition of JG, JYT, and JYO soils had a high similarity. The Principal Component Analysis (PCA) showed that JG, JYO, and JYT treatments were close to each other, indicating that the bacterial community similarity of these three treatments was high. CH treatment was located in a separate area, JH treatment was also located in a separate area, and CH, JH, and the other three groups were located in three areas, indicating that CH, JH, and the other three groups had the largest differences in bacterial community composition. The contribution rates of Principal Component 1 (PC1) and Principal Component 2 (PC2) in the different analyses of bacterial communities in the rhizosphere soil of different coconuts intercropped with *pineapples* were 67.07 and 11.29% respectively Fig. (6A). *Bacteroidetes* (4.45%) and *Gemmatimonadetes* (0.89%) in the rhizosphere soil of coconut monoculture (CH) were significantly lower than those in the rhizosphere soil of intercropping (JG, JH, JYO, and JYT) ($p<0.05$). There was no significant difference ($p>0.05$) between the five cultivation treatments for other bacteria and *Proteobateria* accounted for the largest proportion in the five cultivation treatments Fig. (4A). In addition, Non-Metric Multi-Dimensional Scaling (NMDS) was conducted to reflect microbial diversity Fig. (6B). The soil microbial characteristics of bacteria, CH and JG, JH, JYO, and JYT treatments were significantly different and there were also differences between JG, JH, JYO and JYT treatments. Soil microbial biomass was greatly affected by the intercropping model, as well as coconut varieties and regions.

Table 1: Physical and chemical properties of different fruit-type coconut soils in intercropping mode

SN	pH	EC/ms·cm⁻¹	OM/g·kg⁻¹	AN/mg·kg⁻¹)	AK/mg·kg⁻¹	AP/mg·kg⁻¹	ACa/mg·kg⁻¹	AMg/mg·kg⁻¹
CH	6.89±0.21ᵃ	3.49±0.21ᶜ	0.85±0.01ᵇ	77.40±3.25ᵈ	67.05±2.36ᶜ	8.70±0.56ᵇ	561±10.26ᶜ	153.79±10.25ᶜ
JG	6.28±0.31ᵇ	6.39±0.15ᵇ	1.24±0.03ᵃ	108.40±7.56ᶜ	110.60±3.25ᵇ	12.13±0.75ᵃ	678±09.85ᵃᵇ	164.26±06.89ᵃ
JH	6.49±0.25ᵇ	6.31±0.24ᵇ	1.22±0.04ᵃ	114.71±8.12ᵇ	116.98±6.14ᵇ	11.47±0.68ᵃ	696±13.86ᵃ	173.32±08.56ᵃ
JYO	6.31±0.65ᵇ	7.00±0.16ᵃ	1.27±0.01ᵃ	123.86±8.02ᵃ	123.55±5.85ᵃ	12.16±0.78ᵃ	717±17.02ᵃ	177.54±11.36ᵃ
JYT	6.44±0.41ᵇ	6.84±0.25ᵃ	1.31±0.06ᵃ	125.79±9.14ᵃ	114.53±6.45ᵇ	11.79±0.52ᵃ	655±12.65ᵇ	163.69±10.96ᵇ

Note: φ Represents the mean value ± standard deviation of soil physical and chemical indexes in 3 years; different lowercase letters in the same column showed significant differences among different treatments (P<0.05) SN: Samples Number pH: pH value; EC: Electrical Conductivity; OM: Organic Matter; AN; Alkali Nitrogen; AP: Available Phosphorus; AK: Available Potassium; ACa: Available Calcium; AMg: Available Magnesium

Table 2: Sequencing data statistics and alpha diversity of soil samples

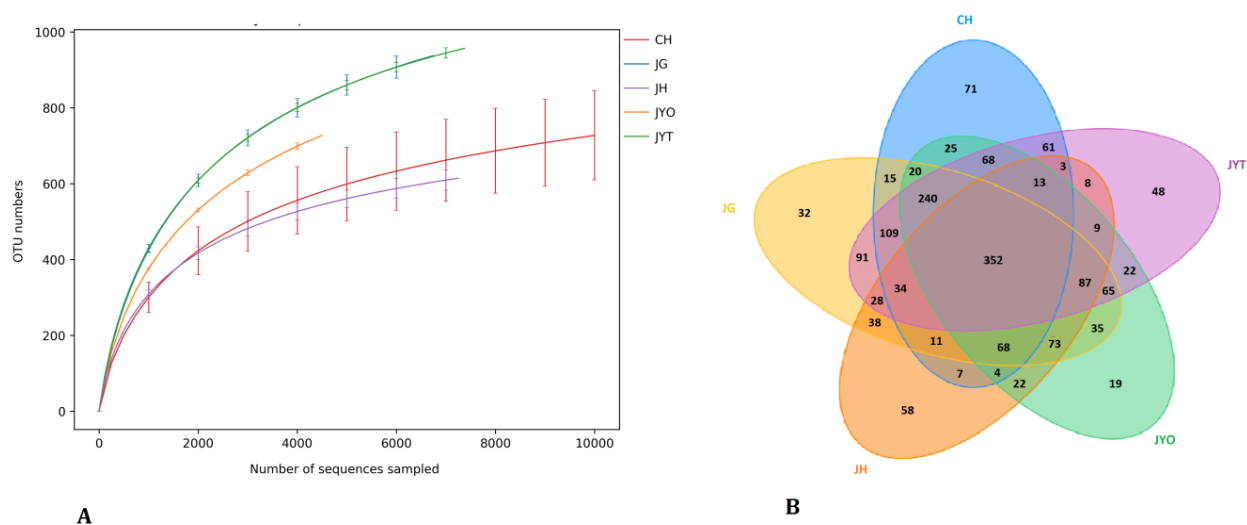| Items	SN	OTU	ACE	Chao1	Simpson	Shannon	Coverage
Soil bacteria	CH	745	889	941	0.0360	5.0002	0.9832
	JG	973	1157	1175	0.0064	6.0188	0.9682
	JH	621	722	740	0.0168	5.2907	0.9817
	JYO	849	1025	1028	0.0104	5.6828	0.9691
	JYT	969	1130	1141	0.0124	5.8822	0.9704

Fig. 2: Bacterial OTU dilution curve of each soil sample (A); Distribution of OTUs of bacteria in rhizosphere soil of different coconut intercropping patterns Venn chart (B)
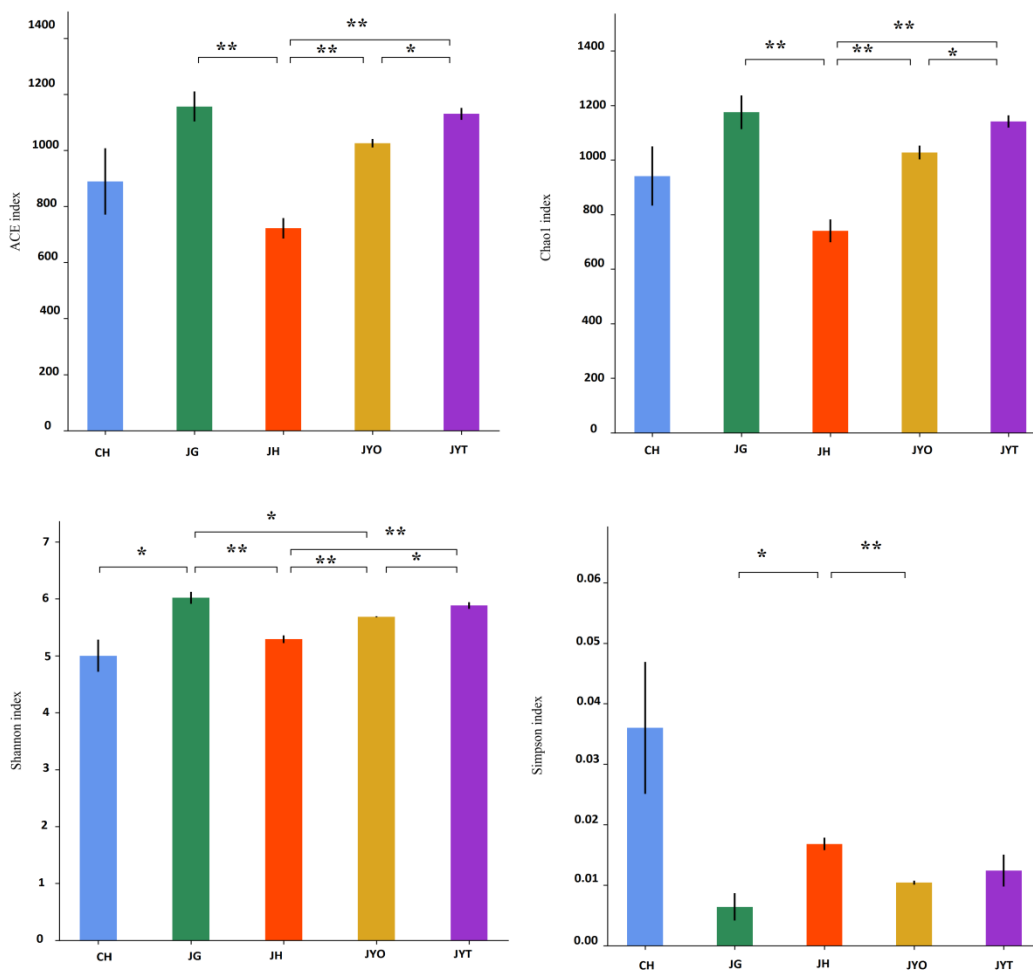

Fig. 3: Alpha diversity analysis. Significant difference level: * P<0.05, ** P<0.01
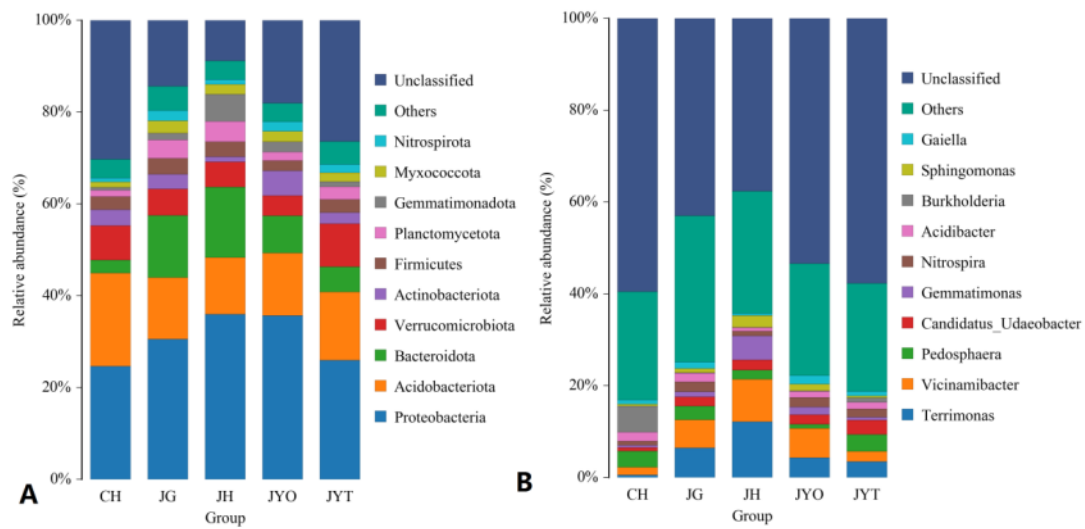
Fig. 4: Histogram (A: Phylum) and (B: Genus) of comparison of bacterial classification levels in rhizosphere soil of different coconut intercropping models
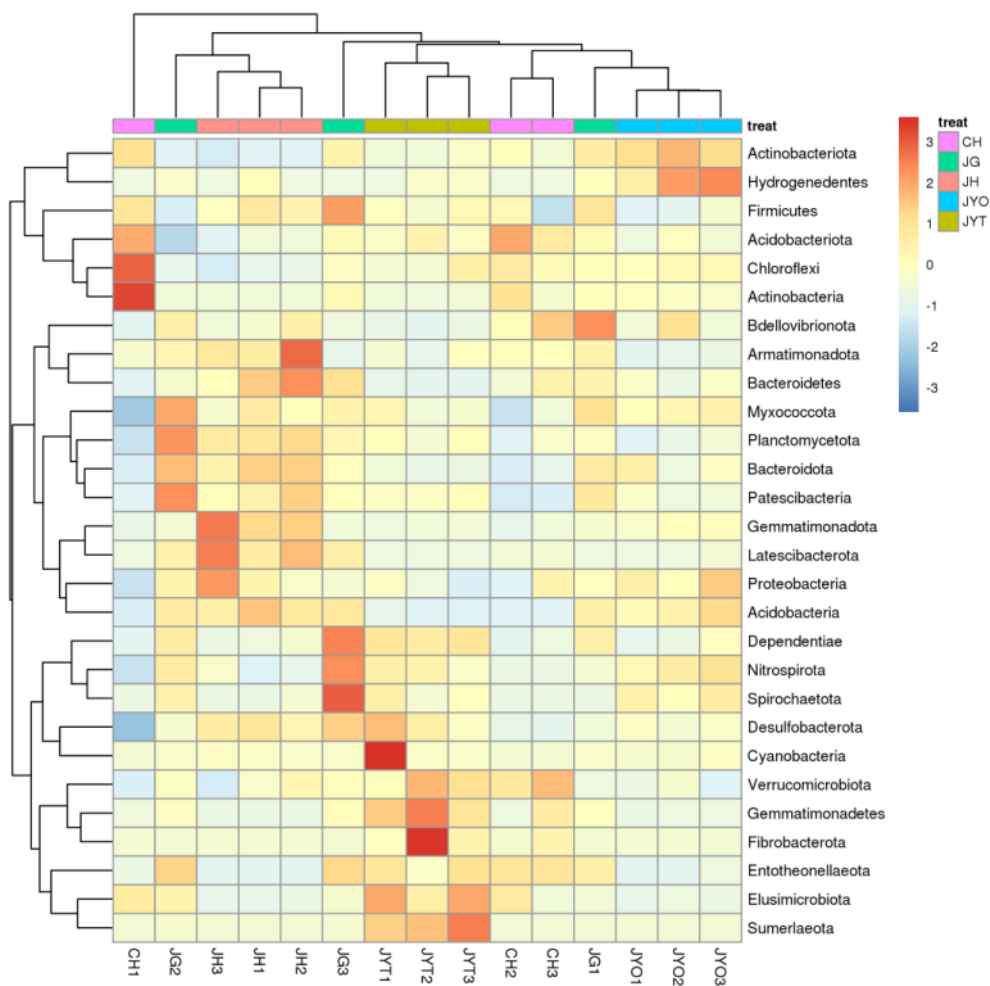

Fig. 5: Clustering heat map of rhizosphere soil bacteria (Phylum) in different coconut intercropping models
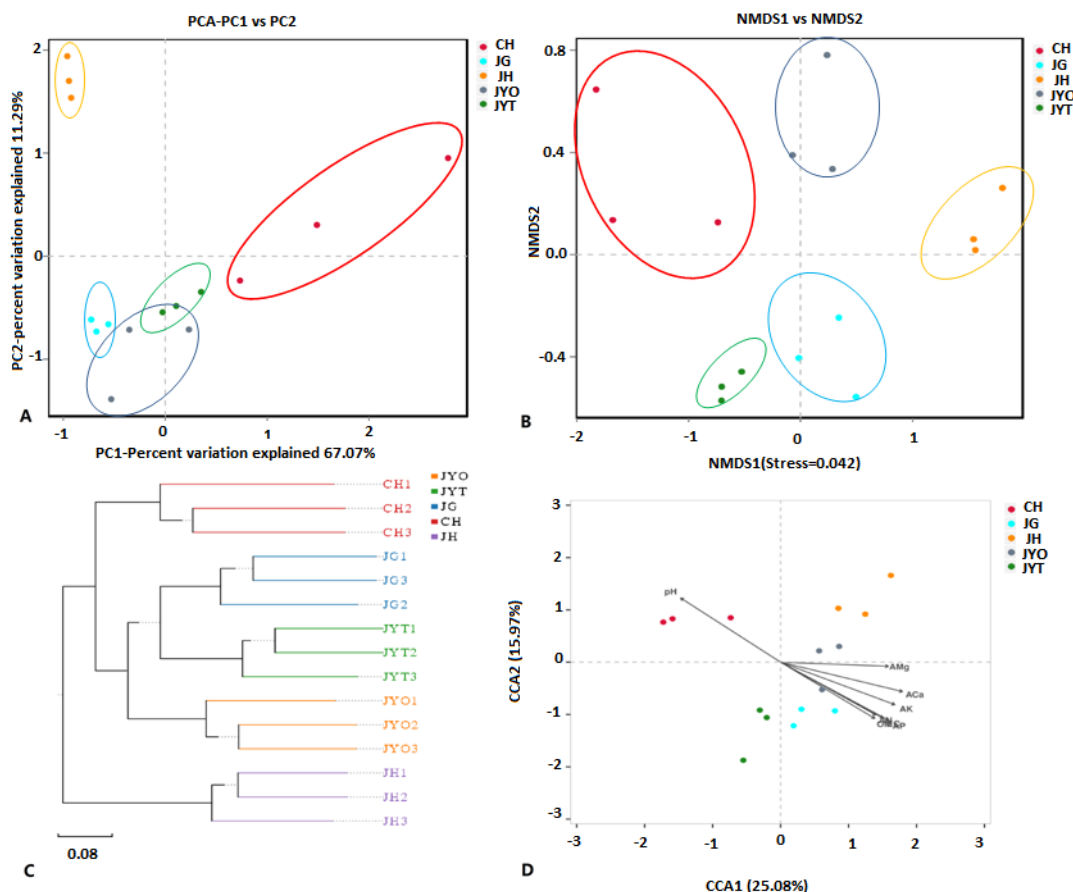
Fig. 6: Analysis chart of bacterial Principal Components (PCA) in rhizosphere soil of different varieties of coconut intercropping model (A); NMDS analysis (B); Based on binary_ Jaccard's UPGMA cluster tree analysis showed that different colors represented different groups (C). Redundancy analysis of bacterial community composition and soil physical and chemical properties (CCA1). Dots with different colors represent different treatments

The results of lefse were reflected in Fig. 7 (A-B), which showed the potential markers of bacteria in rhizosphere soil at each sampling point. At the phylogenetic level, 12 types of bacteria and 34 types of bacterial species were identified, excluding unidentified species. According to the results of LDA values (Fig. 7), the main indicator bacteria in rhizosphere soil bacteria in the CH area include *o_Acidobacteriales*, *c_Acidobacteriae*, *f_Acidobacteriae_Subgroup_1_*, *f_Burkholderiaceae*, *g_Burkholderia* and *s_Burkholderia_Sp*, JYO area includes *c_Bacteroidia*, *p_Bacteroidota*, *g_Terrimonas*, *o_Chitinophagales*, *f_Chitinophagaceae*, *s_Terrimonas_sp*, *f_Vicinamibacteraceae*, *g_Vicinamibacter*, *c_Vicinamibacteria*, *o_Vicinamibacterales*, *s_Vicinamibacter_silvestris*, *f_Gemmatimonadaceae*, *o_Gemmatimonadales*, *c_Gemmatimonadeles*, *p_Gemmatimonadota*, *g_Gemmatimonas*, *s_Gemmatimonas_sp*, *p_Planctomycetota*, *o_Bacillales*,

f_Dongiaceae, *o_Dongiales*, *f_Bacillaceae*. JH area includes *p_Actinobacteriota*, *g_Chujaibacter*, and *s_Chujaibacter_soli*. JG area contains *o_Xanthomonadales*, *c_ Bacilli*, *f_ Xanthomonadaceae*.

Relationship Between Microbial Community and Soil Physical and Chemical Factors

According to the correlation analysis results (Table 3), there was a significant positive correlation ($p < 0.05$) between the microbial bacterial diversity and the contents of EC, OM, AN, AK, AP, ACa, and AMg in coconut rhizosphere soil and a significant negative correlation ($p < 0.05$) with the pH of coconut rhizosphere soil. pH was negatively significantly correlated with EC, OM, AN, AK, AP, ACa, and AMg ($p < 0.05$). Except for pH, there was a positive significant correlation between the tested rhizosphere soil nutrients ($p < 0.05$).

CCA results showed that pH, ACa, AK, and AP were the key environmental factors of bacteria in

coconut rhizosphere soil and pH was the largest factor affecting soil microorganisms in CH treatment. In addition to the physical and chemical properties of ACa, AK, and AP, JYO treatment is also affected by the physical and chemical properties of AMg, OM, EC, and AN. JH, JYT, and JG treatments were less affected by the above soil's physical and chemical properties (Fig. 6D).
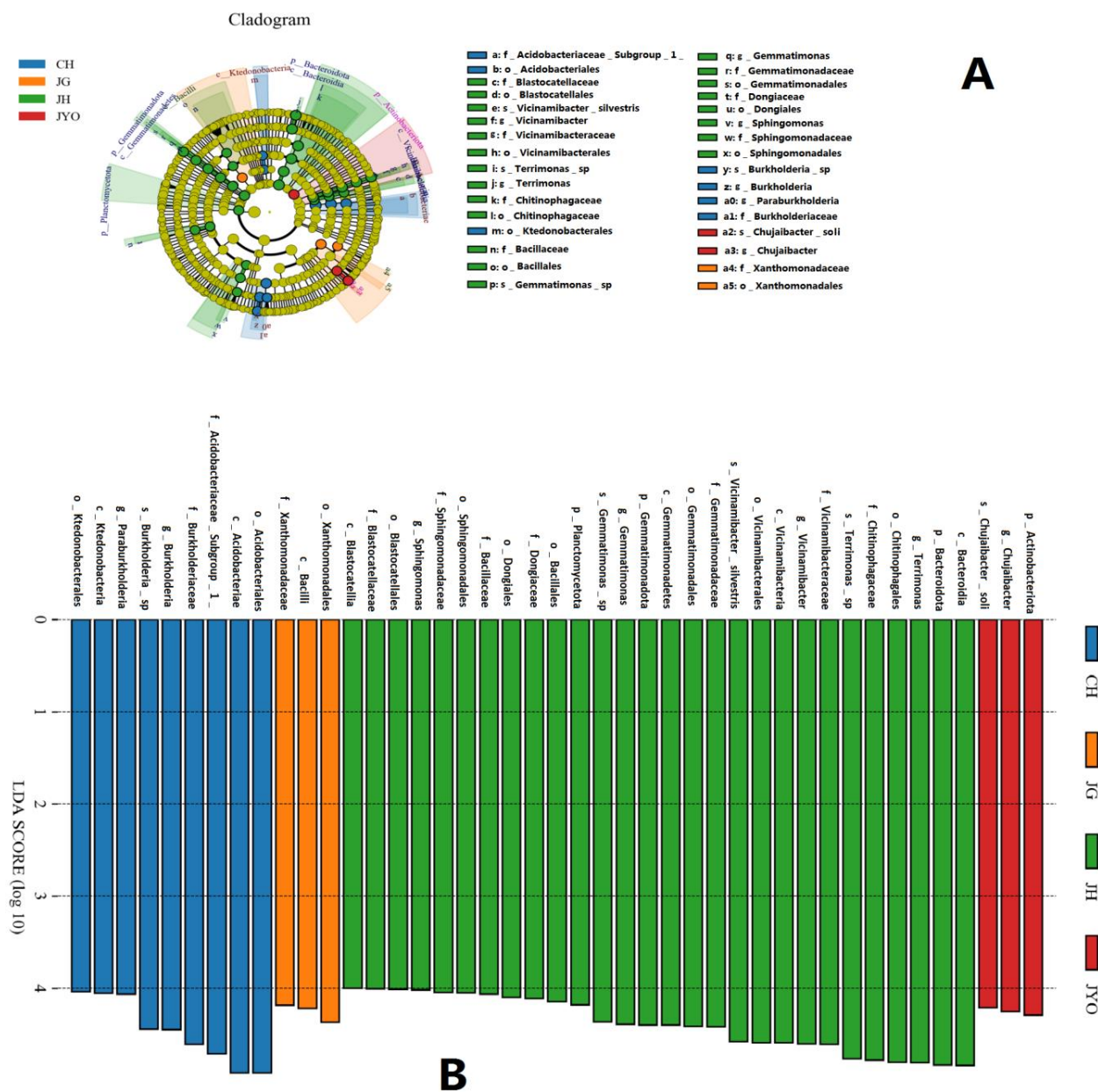

Fig. 7: Lefse analysis of samples between groups bacterial LDA value distribution histogram. It shows the species whose LDA score is greater than the set value (default setting is 4.0). The length of the histogram represents the impact of different species (i.e., LDA score) and different colors represent different groups of species; the evolutionary branching diagram of bacteria was analyzed by Lefse. The circle radiating from inside to outside of the evolutionary branching diagram represented the classification level from phyla to species; Each small circle at different classification levels represents a classification at that level and the diameter of the small circle is proportional to the relative abundance; The coloring principle is to uniformly color the species with no significant difference into yellow and color other species according to the group with the highest abundance of the species. Different colors represent different groups and nodes with different colors represent microbiota that plays an important role in the groups represented by the color. K: Kindom, P: Phylum, C: Class, O: Order, F: Family, G: Genus; S: Species

Table 3: Correlation coefficient analysis of soil physical and chemical indexes and bacterial community structure

Items	Bacterial Shannon index	pH	EC	OM	AN	AK	AP	ACa
pH	-0.758*	-						
EC	0.667*	-0.908**	-					
OM	0.660*	-0.867**	0.947**					
AN	0.611*	-0.817**	0.968**	0.906**	-			
AK	0.606*	-0.889**	0.975**	0.928**	0.945**	-		
AP	0.736*	-0.913**	0.947**	0.865**	0.886**	0.920**	-	
ACa	0.517*	-0.879**	0.911**	0.842**	0.858**	0.958**	0.902**	-
AMg	0.225	-0.647*	0.746*	0.678*	0.730*	0.845**	0.699*	0.897*

Note: Significant difference level * $p<0.05$; ** $p<0.01$

Discussion

Previous studies have shown that for soil nutrients, intercropping was more effective than monocropping. Intercropping increased soil fertilizer supply, improved the nutrient absorption environment of roots (Wei *et al*., 2017; Yang *et al*., 2021; Yan *et al*., 2020), changed the physical and chemical properties of soil, and altered the rhizosphere soil microbial community (Cui and Huang, 2021; Mao *et al*., 2021; Weng *et al*., 2021; Qiao *et al*., 2020). In this study, intercropping significantly increased soil water content, electrical conductivity, organic matter, nitrogen, potassium, phosphorus, available calcium, and available magnesium. Correlation analysis also showed that the diversity and composition of microbial bacteria ($p<0.05$) were positively correlated with these soil nutrients. This shows that intercropping pineapple model can improve soil nutrients and intercropping had an important impact on soil microbial community structure. Therefore, the intercropping model is a measure that can increase rhizosphere soil nutrients and improve soil microorganisms.

Crop systems with high organic carbon content promote soil microbial diversity (Lin *et al*., 2021). In terrestrial ecosystems, soil microorganisms, as an important component unit, played a key role in many ecological processes (Esmaeilzadeh-Salestani *et al*., 2021). In this study, compared with the CH treatment, the OM content of JYO, JYT, JG and JH increased and bacterial diversity increased. JG treatment had the highest bacterial abundance and diversity, while the CH treatment had the lowest bacterial diversity, which indicated that coconut intercropping pineapple improved the diversity of soil bacteria. In this study, the wenye '4' coconut intercropping pineapple area (JG) was most conducive to the growth of the bacterial community, while JH treatment had the lowest bacterial abundance and is lower than the control (CH), which indicated that wenye '3' coconut intercropping pineapple models reduced the abundance of soil bacteria, which is not conducive to the growth of bacterial quantity, In addition, other intercropping models can increase the abundance of rhizosphere soil bacteria compared with the control treatment. Therefore, coconut varieties have a certain impact on the abundance of soil rhizosphere bacteria. This

is consistent with the research on the effect of plant varieties (Mao *et al*., 2021) on bacteria in the soil rhizosphere. The number of microorganisms can indicate soil fertility. Generally, the higher the fertility, the higher the corresponding number of bacteria (Ding *et al*., 2021; Kang *et al*., 2017). The more the number of fungi and actinomycetes in the soil, the lower the soil fertility (Li *et al*., 2022) and the limited crop growth. In this study, JG treatment may provide the most favorable microecological environment for the growth of coconuts. On the contrary, monoculture treatment may provide the most unfavorable environment for coconuts.

Bacteria were the main microorganisms in the rhizosphere soil of different fruit types of coconuts. JG had the most abundant bacterial groups, JH had the lowest OTU number of bacteria and the poorest microbial groups and the microbial composition was also significantly different from the other four groups. Compared with the control, the intercropping models can significantly increase the abundance and diversity of bacteria in the soil, which was consistent with the study of (Qiao *et al*., 2020). If the habitats were different, then the microbial communities around the roots were naturally different (Li *et al*., 2018; Yinglan and Ting, 2020). The soil microbial community of the monoculture model was mainly affected by the physical and chemical properties of pH, while that of the intercropping model was mainly affected by soil nutrients such as ACa, AK, AP, AMg, OM, EC, AN, etc. Compared with coconut monoculture, the proportion of *Proteobacteria* and *Bacteroidota* in the intercropping mode increased, while the proportion of *Acidobacteriota* decreased. That was, the intercropping models were conducive to the development of *Proteobacteria* and *Bacteroidota*, but not conducive to the growth of *Acidobacteriota*. Compared with JYO and JYT, JG and JH were conducive to the development of *Bacteroidota*. The possible reason was mainly due to the coconut variety, namely, the root system of wenye '4' is more conducive to the growth of *Bacteroidota*.

The bacterial community similarity of JG, JYO, and JYT samples was high, indicating that the composition of the rhizosphere soil microbial community of wenye '4' and wenye '2' was consistent. Because of coconut varieties and intercropping patterns, JH had the largest difference

from the other four groups in bacterial community composition. PCA analysis also confirmed that Principal Component 1 (PC1) and Principal Component 2 (PC2) were the two most different characteristics that led to the microbial and bacterial communities of different varieties of coconut intercropping and their contributions to bacterial diversity were 67.07 and 11.29%, respectively. It showed that it was not only one factor that affected soil microorganisms in coconut intercropping of the same variety, but multiple factors (Yanxia *et al.*, 2018; Stefan *et al.*, 2021).

The bacterial community similarity of JG, JYO, and JYT samples was high, indicating that the composition of the rhizosphere soil microbial community of wenye '4' and wenye '2' was consistent. Because of coconut varieties and intercropping patterns, JH treatment has the largest difference from the other four groups in bacterial community composition. PCA analysis also confirmed that Principal Component 1 (PC1) and Principal Component 2 (PC2) were the two most different characteristics that led to the microbial and bacterial communities of different varieties of coconut intercropping and their contributions to bacterial diversity were 67.07 and 11.29%, respectively. It shows that it is not a factor that affected soil microorganisms in coconut intercropping of the same variety, but multiple factors (Dang *et al.*, 2020; Jia-En *et al.*, 2009).

Conclusion

Coconut intercropping pineapple models significantly increased EC, AN, AP, AK, ACa, and AMg contents. Moreover, intercropping pineapple improved the abundance and diversity of bacteria in the coconut rhizosphere soil and the structure and diversity of microorganisms in the rhizosphere of different varieties of coconuts intercropped with pineapple were also different. In the intercropping models, there was a correlation between the bacterial diversity in the rhizosphere soil of different varieties of fruit-type coconut and the physical and chemical properties of the soil (p<0.05). The increase in soil bacterial richness and diversity can reflect the improvement of the soil microbial community and microbial environment to a certain extent. Therefore, intercropping pineapple can improve soil nutrients and microbial community structure and diversity to a large extent.

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Author's Contributions

Lilan Lu: Designed and performed the experiments, analyzed the data, and prepared the paper. Designed the experiments and revised the manuscript.

Hui Zhu and Yingying Liu: Designed and performed the experiments, analyzed the data, and prepared the paper. Participated to collect the materials related to the experiment.

Yi Wu: Participated to collect the materials related to the experiment.

Siting Chen: Designed the experiments and revised the manuscript.

Ethics

The authors declare their responsibility for any ethical issues that may arise after the publication of this manuscript.

Conflict of Interest

The authors declare that they have no competing interests. The corresponding author affirms that all of the authors have read and approved the manuscript.

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