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FERTILITY AND MICROBIAL DIVERSITY IN RHIZOSPHERE SOIL OF *CAMELLIA OLEIFERA* UNDER DIFFERENT INTERCROPPING SYSTEMS

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Abstract

In Guizhou, China, a variety of intercropping systems involving *Camellia oleifera* have been established, yet comprehensive scientific evaluations of these systems remain scarce. Our study aimed to assess the differences among these systems by examining two key aspects: soil properties and microbial species diversity. We discovered that *Ascomycota* and *Proteobacteria* were the prevalent fungal and bacterial groups, respectively, across all *Camellia oleifera* intercropping systems. In the dual-factor correlation network, encompassing species and soil environmental factors, fungi *Satiozyma* and bacteria *Vicinambicharacters* exhibited high Degree Centrality. Urease and Alkaline nitrogen emerged as soil environmental factors strongly associated with these fungi and bacteria. Particularly noteworthy were the Alpha diversity indices (Shannon, PD, and Chao1), which were significantly higher for soil fungi in the *Camellia oleifera-Zea mays* system and for soil bacteria in the *Camellia oleifera-Glycine max* system. Furthermore, the *Camellia oleifera-Glycine max* soil displayed notably elevated levels of Microbial Biomass Phosphorus, Sucrase, Urease, and Catalase. In the *Camellia oleifera-Zea mays* system, the overall soil fertility was substantially higher, coinciding with a high degree of centrality for fungi *Phoma* and bacteria *SC-I-84* in the correlation network. Additionally, Urease and Available Mn were identified as key environmental factors influencing these fungi and bacteria. Strong correlations were observed between *Phoma* and TN, and *SC-I-84* and TK ($r=0.94, 0.99\ p<0.01$). Also, there was a significant association between fungal *Neocosmospora* and bacterial *AD3* with UE and AMn, respectively ($r=0.69, -0.99\ p<0.05$). Based on our findings, we recommend the *Camellia oleifera-Zea mays* intercropping system as a particularly promising option, given its advantages in fungal alpha diversity and soil fertility. Concurrently, the *Camellia oleifera-Glycine max* system demonstrated substantial benefits in terms of microbial biomass phosphorus, bacterial alpha diversity, and soil enzyme activities.

Key words: Agroforestry, Soil properties, Bacterium, Fungus and Diversity.

Introduction

Camellia oleifera, a predominant woody oil crop globally, is primarily found in the high mountainous and hilly regions of southern China's subtropical areas, with smaller distributions in Southeast Asia and Japan. The oil from *Camellia oleifera*, a pure, natural, high-grade oil, is extracted from the seeds of camellia trees belonging to the Theaceae family. Notably, it is free from erucic acid and cholesterol and is rich in proteins, vitamins A, B, D, E, and linolenic acid. As of 2021, *Camellia oleifera*'s cultivation in China spans an impressive 45,333.33 km², contributing to over 90% of the world's *Camellia* oil production (Tu *et al.*, 2019; Ma *et al.,* 2011; Zhu *et al.,* 2020; Zeng *et al.,* 2023). Intercropping, a practice of cultivating two or more crops in proximity, has been increasingly recognized for its beneficial impacts on soil fertility and microbial diversity. This agricultural technique enhances the efficient utilization of resources and promotes a more sustainable farming ecosystem (Peng *et al.,* 2009). Studies have consistently demonstrated that intercropping significantly enhances soil nutrient content and structure, thereby enriching and balancing the microbial ecosystem. This practice not only boosts soil organic matter, nitrogen levels, and soil aggregates but also augments microbial biomass, diversifies microbial community structures, and promotes the decomposition of organic matter, humus formation, and nutrient transformation and circulation (Zhang *et al.*, 2007; Inal *et al.*, 2007; Lithourgidis *et al.*, 2011; Dang *et al.,* 2020; Xiao *et al.,* 2023; Yang *et al.*, 2023b). For example, intercropping *Camellia oleifera* with peanuts has shown remarkable improvements in soil parameters compared to single cropping, including an 8.41% increase in soil porosity, a 16.90% rise in electrical conductivity, and substantial growth in rhizosphere fungal (15.38%) and bacterial populations (43.87%). Additionally, it enhances soil organic matter by 5.69%, available nitrogen by 14.51%, calcium by 7.39%, and magnesium by 20.00% (Lu *et al.*, 2019). Similarly, intercropping cucumber with onions or garlic not only boosts cucumber yield but also improves the soil environment (Zhou *et al.*, 2011). In tree-based systems, the presence of AMF fungi and other beneficial soil microorganisms becomes more pronounced (Lacombe *et al.*, 2009). Moreover, maize-potato intercropping has been found to enhance soil microbial abundance, positively affecting microbial activity and functional diversity (Qin *et al.*, 2017). The same positive impact is seen in legume-cereal intercropping, where it significantly enhances the diversity of rhizosphere bacterial communities (Qiao *et al.*, 2012). These improvements in soil fertility and microbial diversity are vital for the sustained health and productivity of soil, highlighting the significant role of intercropping in contemporary sustainable agriculture. However, the practice of intercropping with *Camellia oleifera* is a relatively recent development in the local context, with crop selection often based more on traditional knowledge than on scientific evidence. To address this gap, our study aims to conduct a rigorous scientific evaluation of these intercropping systems. We assessed soil microbial diversity, fertility, and related chemical properties to provide a scientifically grounded rationale for selecting intercropping crops.

Material and Methods

Camellia oleifera seedlings, with an age of 6 years, were transplanted in Tongren, Guizhou, China (109.04ºE, 27.77ºN) in March 2017. These seedlings originated from wild *Camellia oleifera* cuttings in Hunan, China. In the spring of 2022, additional intercropping crops were sown, with seeds sourced from China National SEED GROUP CO., LTD. The Soil Catalase (S-CAT) Activity Assay Kit, Soil Urease (S-UE) Activity Assay Kit, and Soil Saccharase (S-SC) Activity Assay Kit used in our study were all procured from Beijing Solarbio Science & Technology Co., Ltd.

Experimental design and sample collection: The 600 square meter *Camellia oleifera* forest area was segmented into six 100 square meter plots for planting *Cucurbita moschata*, *Glycine max*, *Arachis hypogaea*, *Zea mays*, *Vicia villosa*, and *Capsicum annuum* (designated as T1 to T6, respectively). These crops were intercropped between individual *Camellia oleifera* trees. In October, eight sampling points were established in an 'S' pattern within the *Camellia oleifera* rhizosphere for soil collection. The soil from these eight points was thoroughly mixed and then equally divided into eight samples. Three of these soil samples were allocated for Illumina MiSeq sequencing analysis, while the remaining five were used to assess soil fertility, enzyme activity, and other parameters.

DNA extraction and PCR amplification: According to the instructions of FastDNA® Spin Kit for Soil DNA, gradually extracted the total DNA of soil microbial community, used 1% agarose gel electrophoresis to detect the quality of DNA extraction, and used NanoDrop2000 to determine the concentration and purity of DNA. PCR amplification was performed on the V3-V4 variable region of the 16S rRNA gene using 338F 5'-ACTCCTACGGGAGGCAGCAG-3' and 806R 5'-GGACTACHVGGGTWTCTAAT-3', and ITS1 gene amplification was performed using ITS1F 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS2R 5'-GCTGCGTTCTTCATCGATGC-3' (Liu *et al.*, 2016; Chen *et al.*, 2018a). After mixing the PCR products of the same sample, used 2% agarose gel to recover the PCR products, used the AxyPrep DNA Gel Extraction Kit to purify the recovered products, and then used 2% agarose gel for electrophoresis detection, finally used the QuantusTM Fluorometer to detect and quantify the recovered products. Referenced the NEXTflex^{TM} Rapid DNA-Seq Kit manual for library establishment, and then used Illumina's Miseq PE300 platform for sequencing.

Determination of soil physical and chemical indicators: The comprehensive evaluation of soil fertility refers to the Chinese agricultural industry standard "Soil fertility diagnosis and evaluation method of farmland in Southern China" (NY/T 1749-2009). Use chloroform fumigation extraction method to determine microbial biomass carbon, microbial biomass nitrogen, and microbial biomass phosphorus in soil (Brookes *et al.*, 1985; Vance *et al.*, 1987). Disodium p-nitrophenyl phosphate ($C_6H_4NNa_2O_6P\cdot 6H_2O$, CAS: No.4264-83-9) was used as a substrate for the assay of soil acid phosphatase activity (Dick, 2020). The Evolution260 UV spectrophotometer (Thermo Scientific, USA) was used to detect the activities of soil urease, soil peroxidase, and soil sucrase (Johansson & Borg, 1988; Kandeler & Gerber, 1988; Witte & Escobar, 2001; Guo *et al.*, 2012; Gao *et al.*, 2013).

Data processing and statistical analysis: The merging and quality filtering of FASTQ files were conducted following the methods outlined by Chen *et al.*, 2018b. Subsequently, optimized sequences were subjected to cluster analysis using UPARSE 7.1, with a similarity threshold of ≥97% (Stackebrandt & Goebel, 1994; Edgar *et al.*, 2013). For index analysis, we employed mothur (version v.1.30.2, available at https://mothur.org/wiki/calculators/), using a 97% similarity level for OTU assessment (Schloss *et al.*, 2013). The beta diversity distance matrix was calculated using Qiime, followed by tree construction using R (version 3.3.1). Community column charts were generated based on the sequencing data table using R (version 3.3.1). Dual factor correlation network analysis was performed using Networkx (version 1.11). Variance analysis of soil physicochemical parameters and their graphical representation were conducted using Graph Pad Version 9.5.1 (528). Normality of the data was tested using four methods: Anderson Darling test, D'Agostino & Pearson test, Shapiro Wilk test, and Kolmogorov Smirnov test. In cases where normal distribution was not observed, non-parametric tests were employed. For multiple comparisons, the Turkey method was utilized.

Results

Alpha diversity of fungi and bacteria: The Chao1 index is commonly employed in ecology to estimate the total species richness, indicating the diversity within a community. A higher Chao1 value signifies more Operational Taxonomic Units (OTUs) in the community, and thus, greater species richness. In this study, we utilized the Shannon index to assess soil microbial diversity, where a higher Shannon value correlates with increased community diversity. Phylogenetic diversity (PD) represents the total branch lengths occupied by all species within a local community on a phylogenetic tree. Generally, the PD and Shannon indices for bacteria in intercropped soil were higher than those for fungi, whereas the Chao1 index demonstrated the reverse trend. Notably, the Shannon, PD, and Chao1 indices for T5 fungi were significantly the lowest observed ($p<0.01$) (Fig. 1). Differences in the PD and Chao1 indices of fungi and bacteria across various intercropping systems were observed ($p<0.01$ and $p<0.05$). The fungal PD and Chao1 indices were highest in T4 ($p<0.01$) (Fig. 1b and c). The Shannon index for T6 fungi was higher than that for T5, yet lower than T3 and T4 ($p<0.01$); the bacterial Shannon index in T2 surpassed that in T5 ($p<0.01$) (Fig. 1a).

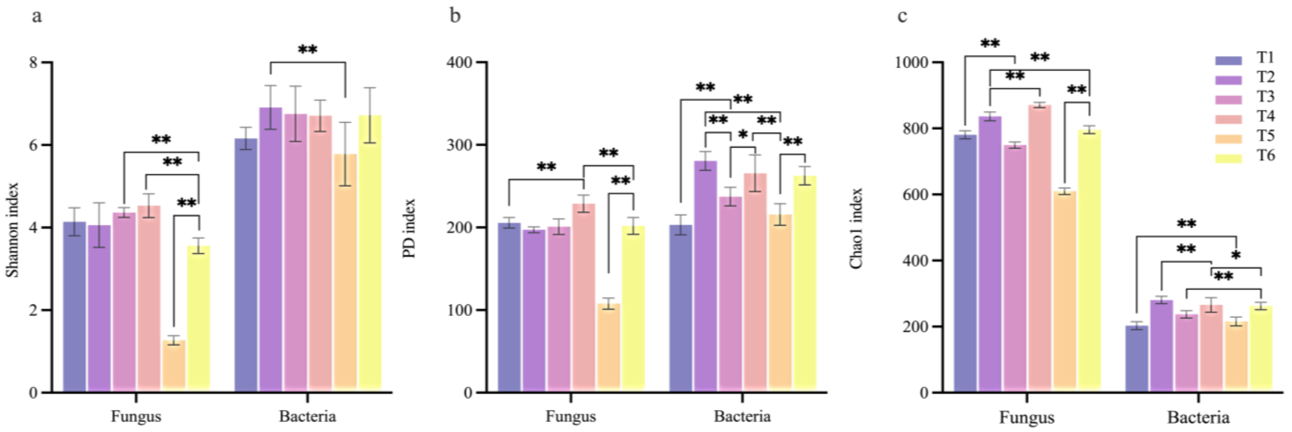

Fig. 1. Alpha diversity of fungi and bacteria in rhizosphere soil of different *Camellia oleifera* intercropping patterns
Note: PD Phylogenetic Diversity (mean ± SD, n = 5).***, p<0.01 ;*, 0.01<p<0.05.* Two bars with similar numbers without marked symbols indicated insignificant differences.

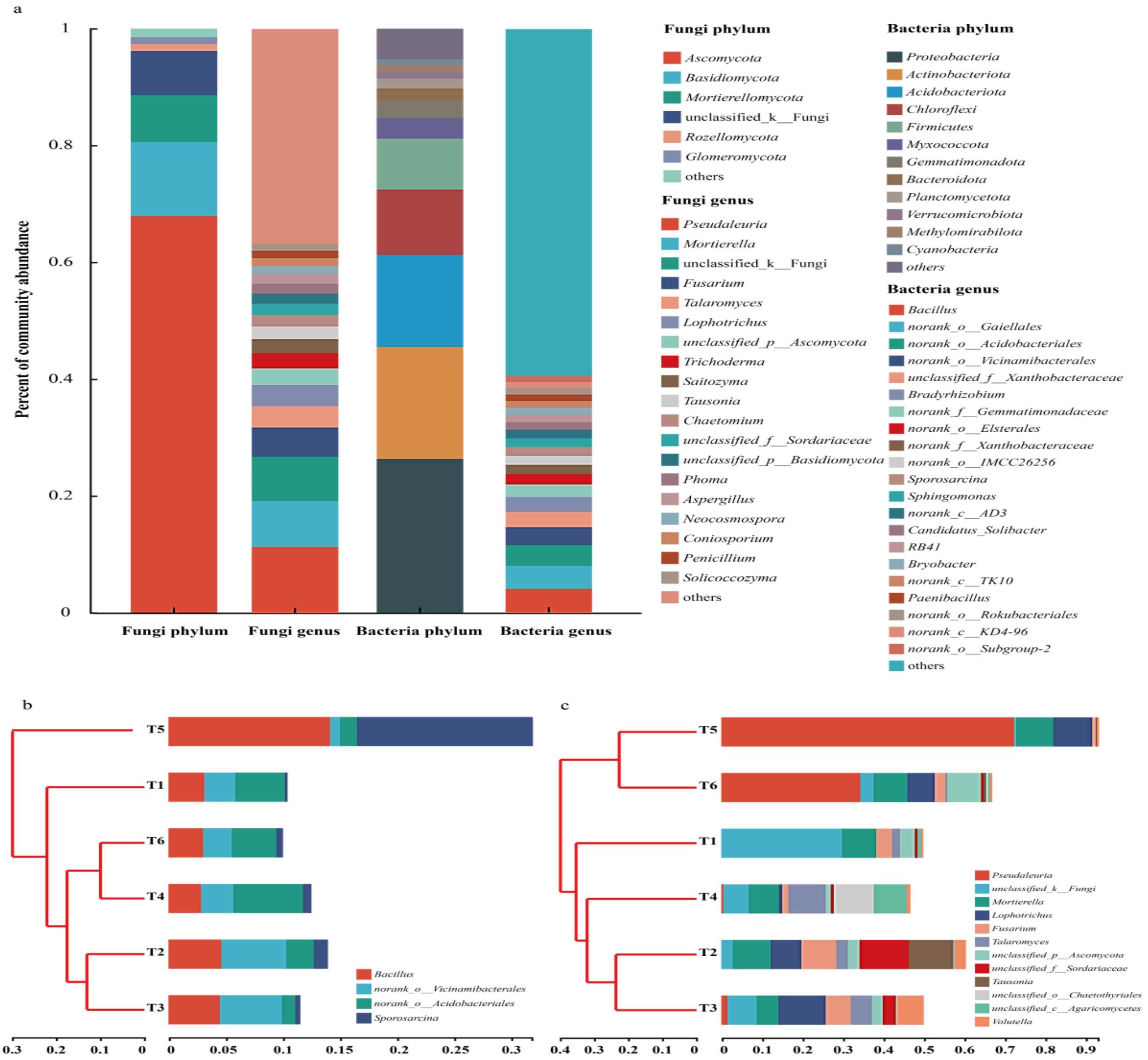

Fig. 2(a). The species structure of soil fungi and bacteria in all intercropping systems. (b) Hier-archical clustering tree on Bacterium genus level. (c)Hierarchical clustering tree on Fungus genus level.
Note: The length between branches represents the distance between different intercropping systems, and different dominant genera were represented by different colors.

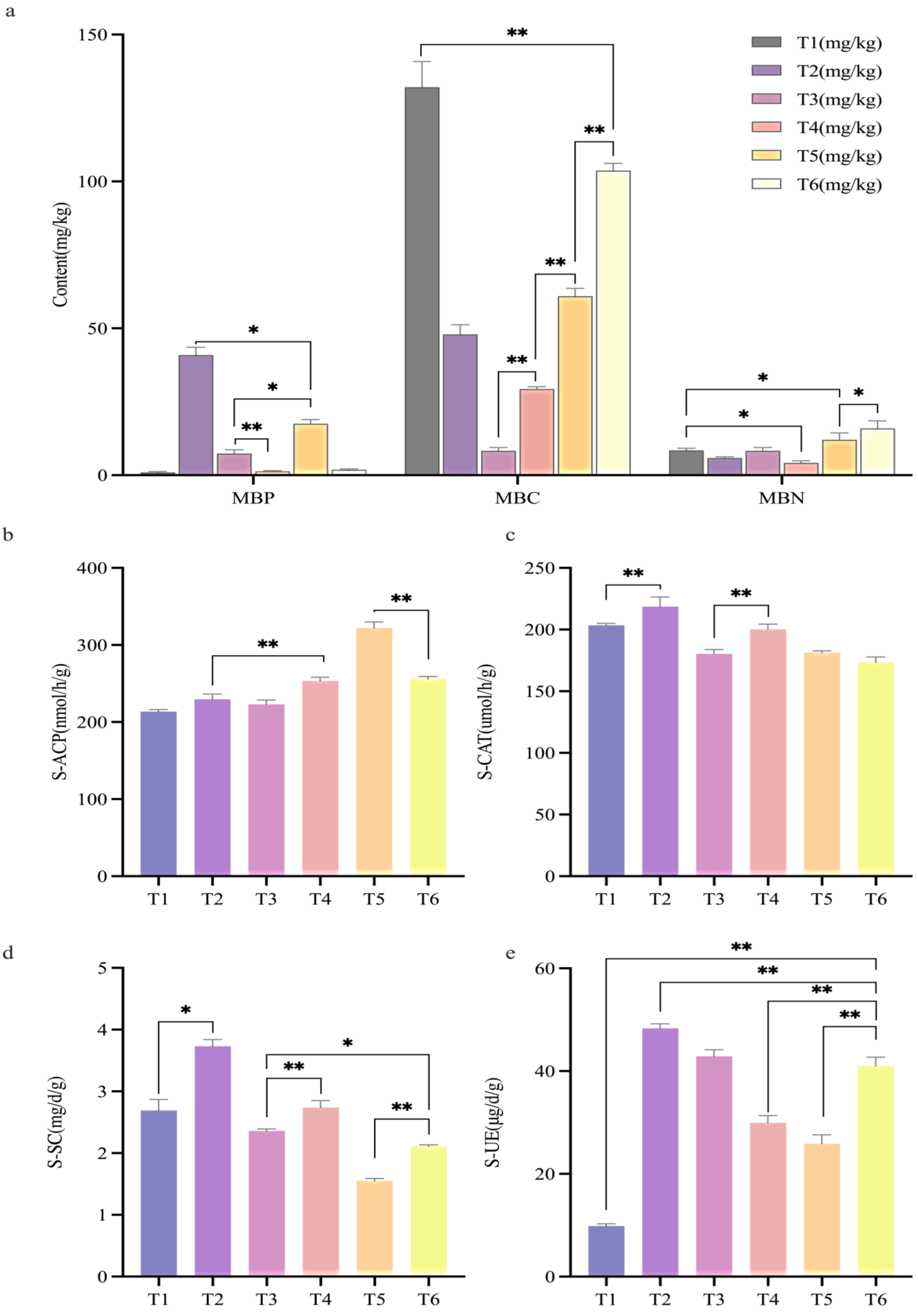

Fig. 3. Analysis of differences in soil enzyme content and microbial biomass C&N&P among different intercropping systems.
Note: mean ± SD, n = 5.

Beta diversity of fungi and bacteria: In intercropped soils, *Ascomycota* emerges as the dominant fungal phylum with a relative gene abundance of 67.91%, surpassing *Basidiomycota* and other phyla ($p<0.01$). The collective abundance of fungal genera, each with a relative gene abundance below 1%, totals 36.89%, significantly higher than *Pseudoleucia* ($p<0.01$) and holding a clear advantage. For bacteria, *Proteobacteria* is the predominant phylum in intercropped soil, accounting for 26.33% of relative gene abundance and exceeding that of *Actinobacteriota* ($p<0.05$). The total abundance of bacterial genera, each with less than 1% abundance, stands at 59.41%, signifying a substantial majority (Fig. 2a). At the genus level, predominant soil bacteria include *Bacillus* and *Sporosarcina*, while key fungal genera comprise *Pseudaleuria*, *Mortierella*, *Lophotrichus*, *Fusarium*, among others. Notably, the bacterial species structure in T5 is distinct from other treatments due to its relatively high gene abundance of *Bacillus* and *Sporosarcina*, at 14.15% and 15.42% respectively. Furthermore, the soil fungal species structure in T5 and T6 aligns closely, yet differs from other treatments, attributed to their higher relative gene abundance of *Pseudaleuria*, recorded at 72.38% and 34.36% respectively (Fig. 2b and c).

Soil enzyme content and microbial biomass C&N&P in different intercropping systems: In intercropped soils, the total microbial biomass carbon (MBC) was higher than microbial biomass phosphorus (MBP) and microbial biomass nitrogen (MBN). Notable differences in MBP, MBC, and MBN were observed among various intercropping systems ($p<0.01$ & $p<0.05$). The coefficient of variation (CV) for MBP was the highest, reaching 122.21%. Specifically, T2 showed the highest MBP (40.95 mg/kg), T1 the highest MBC (132.11 mg/kg), and T6 the highest MBN (15.97 mg/kg) (Fig. 3a). Differences in the activities of soil acid phosphatase (S-ACP), soil catalase (S-CAT), soil saccharase (S-SC), and soil urease (S-UE) were also evident among different intercropping systems ($p<0.05$) (Fig. 3b, c, d, & e). Among these, the activities of S-CAT and S-SC in T2 were significantly higher ($p<0.01$ & $p<0.05$). T2 and T3 exhibited higher S-UE activity ($p<0.01$), with no significant difference between them (Fig. 3e). T5 had a notably higher S-ACP activity compared to other systems (Fig. 3b). The CV for S-UE was the greatest at 39.03%, suggesting that the variability in S-UE activity across different intercropping systems was more pronounced than that of other enzymes (Fig. 3e).

Comprehensive evaluation of soil fertility in different intercropping systems: Following the Chinese agricultural industry standard 'NY/T 1749-2009,' we conducted a comprehensive fertility evaluation of the soil in intercropping systems. This evaluation included 15 soil physicochemical parameters such as pH, organic matter (OM), cation exchange capacity (CEC), among others, with each parameter's corresponding standard value denoted as Si. The results revealed that the comprehensive fertility index of *Camellia oleifera* rhizosphere soil across different intercropping systems varied from 0.47 to 4.62, exhibiting a coefficient of variation (CV) of 77.70%. The rankings of comprehensive soil fertility, from highest to lowest, were T4, T5, T6, T3, T1, and T2. Among these, available boron (AB) and available phosphorus (AP) showed the greatest variation across different intercropping systems, with CVs of 40.10% and 40.02% respectively. T4's high comprehensive soil fertility was attributed to elevated levels of OM, AP, available iron (AI), available manganese (AMn), and available zinc (AZ), with AP making the most significant contribution, being 28.60 times higher than its standard value (Table 1).

Table 1. Comprehensive evaluation of soil fertility under different intercropping conditions.

Evaluation index of soil fertility	T1	T2	T3	T4	T5	T6	CV/%	Si
pH	5.33±0.11c	6.07±0.04a	6.26±0.02a	6.07±0.20a	5.11±0.04d	5.85±0.10b	7.25	—
OM (g/kg)	19.24±0.36ab	16.33±0.40c	19.19±0.45ab	19.99±0.53a	15.84±0.19c	18.95±0.32b	8.62	12.50
CEC (cmol+/kg)	7.35±0.06e	11.17±0.07b	13.48±0.21a	9.88±0.07c	9.14±0.12d	7.50±0.29e	21.81	12.00
TN (g/kg)	1.13±0.04e	1.84±0.04a	1.74±0.04b	1.47±0.03d	1.51±0.02d	1.61±0.04c	14.62	1.00
TP (g/kg)	0.29±0.03c	0.39±0.02a	0.36±0.02ab	0.36±0.03ab	0.31±0.02bc	0.31±0.02bc	10.52	0.59
TK (g/kg)	14.66±0.06d	19.55±0.06a	19.58±0.40a	17.62±0.07c	18.27±0.06b	14.86±0.10d	11.51	16.00
AN (mg/kg)	199.35±0.37a	115.41±3.78d	124.95±2.55c	117.21±2.30d	120.67±1.62cd	150.57±3.08b	21.6	105.00
AP (mg/kg)	25.92±1.61d	175.28±1.88b	164.91±13.42b	214.51±8.32a	168.21±5.09b	136.20±2.30c	40.02	7.50
AK (mg/kg)	23.07±1.01b	29.95±1.14a	21.82±1.18b	23.18±0.74b	20.84±0.78b	21.69±0.66b	12.93	80.00
AC (mg/kg)	0.35±0.04d	0.91±0.03a	0.75±0.04b	0.79±0.08b	0.63±0.03c	0.59±0.03c	26.48	1.70
AI (mg/kg)	74.99±1.48d	102.10±1.06b	99.96±2.15b	120.47±1.24a	89.06±1.15c	77.20±1.36d	16.66	35.00
AMn (mg/kg)	21.61±0.74c	27.71±0.83b	28.55±0.53b	36.3±2.60a	22.37±0.46c	29.74±0.41b	17.7	35.00
AZ (mg/kg)	1.40±0.51b	2.24±0.12a	1.94±0.06a	2.38±0.03a	2.43±0.37a	2.15±0.05a	16.63	80.00
AB (mg/kg)	0.35±0.03a	0.13±0.02d	0.26±0.03b	0.13±0.02d	0.38±0.03a	0.21±0.02c	40.1	64.00
AMo (mg/kg)	0.19±0.02a	0.19±0.03a	0.09±0.03b	0.13±0.02b	0.13±0.02b	0.21±0.02a	27.25	0.15
P	0.62	0.47	0.68	4.62	2.93	2.43	—	—

OM Organic matter, CEC Cation exchange capacity, TN Total nitrogen, TP Total phosphorus, TK Total potassium, AN Alkaline nitrogen, AP Olsen-P, AK Available potassium, AC Available copper, AI Available Iron, AMn Available Mn, AZ Available zinc, AB Available boron, AMo Available molybdenum (mean ± SD, n = 5). Different lowercase letters within a line indicate means that are significantly different ($p < 0.05$). Si Evaluation standard value of an index i in soil, P Comprehensive index of soil fertility

Correlation analysis of main microecological factors in intercropping soil: In this study, we calculated correlations between species and environmental factors using dual factor network analysis, building a correlation network to analyze these relationships. The network graph illustrated the interplay between the top 25 genera in terms of abundance and soil environmental factors (Fig. 4). The fungal genera *Satiozyma* and *Albifimbria* showed extensive connections in the rhizosphere soil of *Camellia oleifera* across all intercropping systems, each with six nodes linked and a Degree Centrality of 0.158. *Satiozyma* exhibited a higher Closeness Centrality coefficient (0.302) than *Albifimbria*, whereas *Albifimbria* had a greater Betweenness Centrality coefficient (0.245). Among soil factors, urease (UE) demonstrated high Degree and Closeness Centrality coefficients of 0.132 and 0.295, respectively, while microbial biomass carbon (MBC) had a notable Betweenness Centrality coefficient of 0.163 (Fig. 4a). Seven soil factors showed significant associations with the unannotated genera in *Vicinambicharacters*, exhibiting a high Degree Centrality (coefficient: 0.184). The unannotated bacterial genera in the TK10 class displayed high Closeness and Betweenness Centrality coefficients (0.371, 0.285). Seven bacterial genera were significantly correlated with available nitrogen (AN), which had high centrality coefficients in all three measures (Fig. 4b). In T4, soil urease content was significantly correlated with four fungal genera ($p<0.05$), with *Neocosmospora* showing the highest correlation coefficient ($r=0.69$, $p<0.01$) and high centrality values. *Phoma* had high centrality measures in the network and was significantly associated with three soil factors, with the strongest correlation to total nitrogen (TN) ($r=0.94$, $p<0.01$) (Fig. 4c). Available manganese (AMn) in T4 soil correlated with four bacterial genera and showed high centrality scores in the network, with the strongest correlation to unannotated genera in AD3 ($r= -0.99$, $p<0.05$). The unannotated bacterial genera in SC-I-84 had high centrality scores and significant correlations with four soil factors, with the highest correlation to total potassium (TK) ($r=0.99$, $p<0.01$).

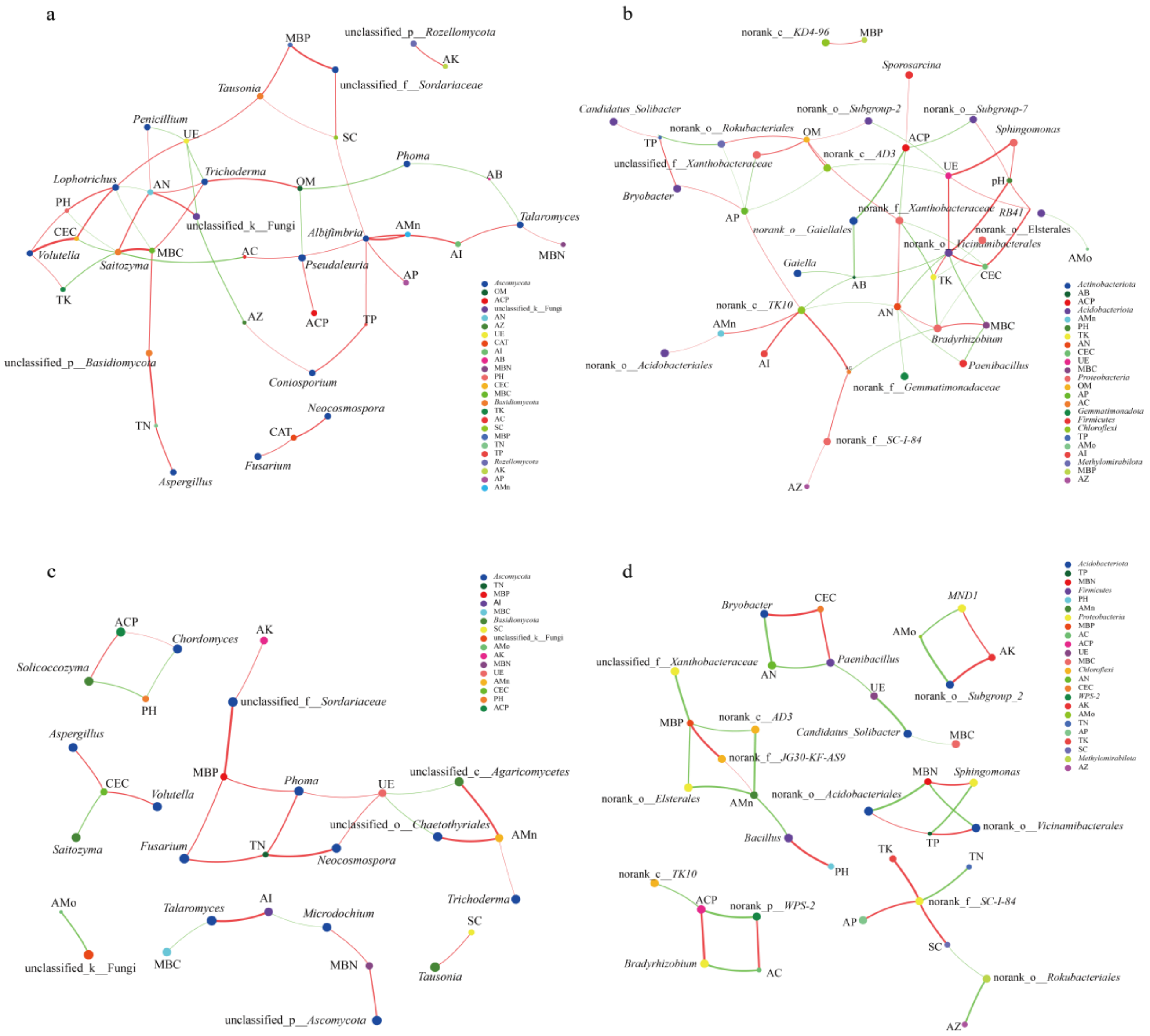

Fig. 4. Network Analysis of the relationship between soil microorganisms and environmental factors. (a. Soil environmental factors and fungal species. b. Soil environmental factors and bacterial species. c. Soil environmental factors and fungal species of T4. d. Soil environmental factors and bacterial species of T4).

Note: In the figure, the default display highlights species with significant correlations ($p<0.05$). The size of the nodes in the graph represents the abundance of each species, while different colors differentiate between species. The color of the connecting lines indicates the nature of the correlation: red signifies a positive correlation, and green indicates a negative correlation. The thickness of each line reflects the magnitude of the correlation coefficient – thicker lines denote a stronger correlation between species. Additionally, a greater number of lines between nodes suggests a closer connection.

Discussion

Our study revealed a robust alpha diversity of fungi in the rhizosphere soil of the *Camellia oleifera-Zea mays* intercropping system. Drawing from the theories of resource abundance and environmental energy, we found a positive correlation between fungal alpha diversity and plant productivity, as suggested by Whittaker (2006). Previous research indicates that intercropping modifies the structure and boosts the diversity of bacterial communities in crop rhizospheres, enriching dominant bacterial populations and influencing crop agronomic traits (Qiao *et al.*, 2012; Cao *et al.*, 2017; Yang *et al.*, 2023a). Beyond these findings, our study also notes higher soil bacterial diversity, microbial biomass phosphorus, and activities of catalase, sucrase, and urease in the C*amellia oleifera-Glycine max* system compared to other intercrops. However, current research does not establish a significant link between enzyme activity and bacterial community composition. Enzyme activity might be influenced more by specific functional bacterial communities than by the entire bacterial community, as Ling *et al.,* (2014) suggest. Thus, a rich bacterial diversity implies a high likelihood of functional bacterial communities in the soil, potentially enhancing the growth of intercropped plants. We observed that *Bacillus* and *Sporosarcina* are prevalent across all intercropping systems studied, particularly dominant in the *Camellia oleifera-Vicia villosa* system. These bacteria, abundant in the rhizospheres of intercropped leguminous plants, possess phosphorus solubilizing abilities. They secrete organic acids, increasing the solubilization of ineffective phosphorus, thus enhancing soil acid phosphatase activity and phosphorus availability, as shown in Song *et al*., (2022a). *Vicia villosa*, an annual herb of the *Leguminosae* family, serves as an excellent forage and green manure crop (Alam *et al*., 2015). The acid phosphatase activity in its rhizosphere soil was higher than in other systems, aligning with findings by Song *et al*., (2022b). The *Sporosarcina* genus, though less reported, includes many species isolated in China, some beneficial for soil improvement (Badiee *et al*., 2019). The fungal community structures in the rhizospheres of intercropped *Vicia villosa* and *Capsicum annuum* are similar, with *Pseudaleuria* as a predominant fungus. However, studies on *Pseudaleuria* are scarce, though some link it to soil-borne diseases (Sabri *et al*., 2018; Xiang *et al*., 2020).

Intercropping *Zea mays* with leguminous plants is a widely reported system, yet studies on intercropping with woody species are less common (Mucheru *et al*., 2010; Nyoki & Ndakidemi, 2018). The enhanced comprehensive soil fertility in the *Camellia oleifera-Zea mays* intercropping system is attributed primarily to increased levels of organic matter, available phosphorus, and various trace elements. The soil microbial biomass serves dual roles as both a reservoir and a source for labile nutrient pools during nutrient cycling (Griffiths *et al*., 2012). The microbial biomass carbon (C) and nitrogen (N) reflect the activity level of soil microorganisms, with their high content typically indicating vigorous microbial activity, beneficial for soil fertility enhancement (Egamberdieva *et al*., 2010). Microbial biomass phosphorus (P) represents a dynamic fraction of soil organic phosphorus, responsive to soil environmental changes and playing a crucial role in the efficient utilization of phosphorus by plants in both native and agricultural ecosystems (Katsalirou *et al*., 2016). The significant presence of microbial biomass P in the rhizosphere soil of the *Camellia oleifera-Glycine max* intercropping system represents a novel finding, suggesting its potential as an effective phosphorus source for *Camellia oleifera*.

In the *Camellia oleifera-Zea mays* intercropping system, characterized by its higher comprehensive soil fertility, we observed that the availability of iron in the rhizosphere can suppress the advantages of *Bacillus* and *Mortierella*. Conversely, in this system, the available zinc content appear to inhibit the growth benefits of *Saitozyme*. Similarly, in the *Camellia oleifera-Glycine max* system, known for its high soil enzyme activity, *Bradyrhizobium* has been found to inhibit urease activity in the soil, while *Fusarium* seems to enhance the activity of soil sucrase. These two novel observations might be incidental, as current literature provides no supporting evidence. Therefore, these findings warrant further investigation for validation.

Conclusion

Our research primarily focused on the variances in soil microbial diversity (including fungi and bacteria), fertility, microbial biomass of carbon, nitrogen, and phosphorus, as well as enzyme activity. In the *Camellia oleifera-Zea mays* intercropping system, we observed a diverse range of fungal species and notably high comprehensive soil fertility in the rhizosphere. Otherwise, the rhizosphere soil of the *Camellia oleifera-Glycine max* system exhibited a rich diversity of bacterial species coupled with elevated enzyme activity. We identified potential linkages between certain microbial species (both fungi and bacteria) and soil environmental factors (such as fertility and enzyme activity) in these intercropping systems. However, further research is necessary to substantiate these connections more conclusively in the future.

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