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RESEARCH ARTICLE

Investigating the impact of soil properties on the nutrient content and microbial community of sisal (*Agave sisalana* Perrine)

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ABSTRACT

Sisal (*Agave sisalana* Perrine), a crucial natural fiber source, exhibits varying yields in China's Guangdong and Guangxi regions. This groundbreaking study sampled soil and sisal leaves from these production hubs to explore the interplay among soil pH, enzyme activity, microorganisms, and sisal nutrition. Using PCR and high-throughput sequencing, results indicate Guangdong's soil is mildly acidic, contrasting with Guangxi's neutral to slightly alkaline soil. Both regions show a robust correlation between soil pH and nutrient availability (organic matter, P, K). Enhanced soil enzyme activity is observed at higher pH levels (6.09, 7.39). Dominant bacterial phyla Acidobacteria, Proteobacteria, and Actinobacteria peak at pH 7.39, 6.09, and 5.41, respectively. Fungal phyla Ascomycota, Basidiomycota, and Mortierellomycota thrive at pH 4.23, 7.39, and 6.48. Sisal leaves display optimal nutrient levels at specific pHs: N, P, and K at pH 6.09; Ca at pH 7.39; Mg at pH 6.48. Functional predictions reveal fungal abundance varies with pH, while bacterial functions like metabolism, disease resistance, and environmental adaptation are bolstered in Guangxi's alkaline soils compared to Guangdong's acidic ones. These findings provide a scientific basis for optimizing sisal cultivation management.

Key words: High-throughput sequencing, plant nutrient content, rhizosphere soil microorganisms, sisal, soil pH.

INTRODUCTION

Sisal (*Agave sisalana* Perrine) is a perennial leaf fiber plant belonging to the genus *Agave*. It is widely cultivated in tropical Asia, including China, where it has been farmed for over a century. Sisal fibers are used extensively in cable making, textiles, papermaking, and shipbuilding. Plants' leaf residue and juice contain various compounds, including crude proteins, fats, sugars, waxes, pectin, and saponins, which offer the potential for comprehensive utilization (Medina-Morales et al., 2017; Sneha et al., 2021; Chege et al., 2022). After over one hundred years of development, China's sisal industry has established two major planting bases in Guangdong Province and the Guangxi Zhuang Autonomous Region. These areas boast large-scale planting and high production concentrations, with the planting area and yield of sisal in Guangdong and Guangxi accounting for more than 95% of the national total (Sun et al., 2020). However, the investigation uncovered a significant disparity in production between the two regions, with the underlying reasons for this difference remaining unknown.

Soil is an essential resource for human survival and development, its primary function is to supply nutrients and water to crops while supporting root growth. Continuous efforts to enhance soil research are vital for repairing damaged soil and establishing a resilient soil ecosystem, which is imperative for the healthy growth of crops. Soil microorganisms are key to maintaining soil fertility and are principal indicators of soil ecosystem stability, metabolism, and fertility. They are pivotal in transforming organic and mineral matter, affecting plant yields (Prosser, 2015; Carini et al., 2016).

Soil microorganisms are among terrestrial ecosystems' most important and complex components. They are a general term for all soil microorganisms that are invisible or not visible to the naked eye and contain millions of species. Root-associated soil microorganisms, particularly those associated with plants, play crucial roles in nutrient cycling, energy flow, and plant growth processes, significantly influencing crop health, growth, and even resilience to external stressors (Tang et al., 2019). However, the structure and diversity of soil microbial communities are affected by environmental gradients. The sensitivity of soil microorganisms to many environmental factors, such as climate change, soil characteristics, and plant growth, varies greatly (Li et al., 2016a; Wang et al., 2017; Yang et al., 2017; Zhang et al., 2018). Several studies have suggested that soil pH is the most critical soil factor(Li et al., 2022).

Soil pH greatly influences bacterial richness, diversity, and community structure. For instance, it shapes the composition and structure of acid-bacteria communities(Guo et al., 2021), highlighting its role in bacterial assembly. High pH values were identified as a key factor distinguishing bacterial communities between Xiao Xingkai Lake and Xingkai Lake and were associated with reduced β diversity in sediments (Pu et al., 2023). Soil pH is also strongly associated with the composition and diversity of soil bacterial communities across Southeast Asia and is a significant predictor of bacterial community structure in various land use types. Bacterial diversity peaks when the soil pH is near neutral (Yavitt et al., 2021). Additionally, soil pH exerts an important indirect effect on sedimentary bacterial composition by influencing various biogeochemical processes and environmental conditions (Wang et al., 2022). Soil pH influences changes in fungal richness, diversity, and community structure (Siles and Margesin, 2016). Soil pH was identified as a key factor driving the differences in soil fungal community characteristics among various typical vegetation types in Huangshan Mountain (Man et al., 2021), exhibiting the strongest association with soil fungal community composition and diversity indices, and in a study of the fungal community structure in northern Michigan's broad-leaved forests, USA, soil pH emerged as the most significant environmental factor influencing the soil fungal community (Romanowicz et al., 2016).

Moreover, pH is an important factor affecting plant growth and development. Soil pH is a powerful predictor of the density of the invasive 1-yr-old plant *Microstegium vimineum* (Barlow et al., 2020). A low soil pH leads to a decrease in species diversity and seed mixture coverage. Increasing the pH from 3.29 to 5.32 was found to enhance antioxidant enzyme activity and photosynthetic capacity in tea leaves, thereby promoting the growth of tea plants (Jia et al., 2023).

Sisal is an important tropical fiber crop characterized by a wide range of uses and high comprehensive utilization value. However, its growth is restricted by the encroachment of industrial parks and competition from more efficient crops. To promote the sustainable development of sisal and address these challenges, it is vital to maximize the potential of current sisal production. Recent studies have shown that, as the primary sisal cultivation area in China, the average annual yield of sisal leaves in Guangxi's production regions exceeds 8 t, while Guangdong's yield is approximately half that of Guangxi. To investigate the factors contributing to this disparity, rhizosphere soil samples were collected from eight sisal plantations with varying pH levels across Guangdong and Guangxi in August 2023. The physical and chemical properties of these soils were analyzed, and the diversity and composition of their bacterial and fungal communities were assessed through 16S rRNA gene and internal transcribed spacer (ITS) amplicon sequencing, respectively. The purpose of this study was mainly to answer the following questions: 1. How do soil nutrients and enzyme activities change along the pH gradient? 2. What are the dominant groups of bacteria and fungi in sisal-producing areas with different pH levels, and how does their relative abundance change with soil pH? 3. What is the impact of pH on predicting gene function? 4. What is the relationship between sisal leaf nutrient content and soil pH?

MATERIALS AND METHODS

Soil samples

The soil used in this study was sourced from the primary cultivation areas of *Agave sisalana* Perrine in Guangdong Province and Guangxi Zhuang Autonomous Region, where no fertilizer had been applied for 2 yr. We further divided and randomly selected five sub-points at each selected location as specific sampling areas, ensuring even coverage. Soil samples were collected from a depth of 0-20 cm in the rhizosphere at the main sisal planting sites. Basic information on the sampling points is provided in Tables 1 and 2. Soil samples from

each point within the plot were thoroughly mixed and passed through a 2 mm sieve to remove visible animals, plants, and their residues. The pH values of these mixed samples were measured separately using a standard pH meter and a 1:2.5 soil-to-water ratio. Soil samples with significantly different pH levels (details provided in Table 3) were selected for the determination of soil nutrient indices and enzyme activities. Subsequently, the soil samples were sent to Shanghai Shenggong Biotechnology Co., Ltd., for soil microbial sequencing using Illumina technology. Additionally, leaves from the sisal plants in these plots were collected from mature leaves during the same period, and were processed to determine the total N, total P, total K, total Ca, and total Mg contents.

Table 1. Determination results of soil pH in Guangxi sisal plantation. Data are mean ± standard error. Different lowercase letters in the same column indicate significant differences between data according to Dunnett test ($P < 0.05$), while the same letter indicates insignificant differences between groups.

Abbreviation	Location	pH
WM1	23°20′52″ N, 108°14′41″ E	6.43 ± 0.01^{c}
WM2	23°16′44″ N, 108°17′19″ E	6.48 ± 0.03^{c}
WM3	22°16′50″ N, 109°88′19″ E	7.39 ± 0.04^{a}
DE1	23°18′44″ N, 108°15′18″ E	6.09 ± 0.05^{d}
DF1	21°98′70″ N, 109°47′17″ E	6.96 ± 0.06^{b}
SX1	22°26′51″ N, 107°56′5″ E	6.29 ± 0.10^{cd}
SX2	22°25′46″ N, 107°53′4″ E	6.21 ± 0.04^{cd}
SX3	22°24′53″ N, 107°55′18″ E	6.92 ± 0.08^{b}
PG1	23°61′71″ N, 107°62′3″ E	7.39 ± 0.04^{a}
PG2	23°61′99″ N, 107°62′61″ E	7.20 ± 0.04^{b}

Table 2 Determination results of soil pH in Guangdong sisal plantation. Data are mean ± standard error. Different lowercase letters in the same column indicate significant differences between data according to Dunnett test ($P < 0.05$), while the same letter indicates insignificant differences between groups.

Abbreviation	Location	pH
LM1	21°54′15″ N, 110°36′28″ E	4.89 ± 0.01^{bc}
LM2	21°32′42″ N, 110°21′46″ E	4.97 ± 0.02^{ab}
DS1	21°42′29″ N, 110°3′21″ E	5.41 ± 0.01^{a}
DS2	21°43′26″ N, 110°9′18″ E	4.87 ± 0.06^{bc}
WY1	20°29′39″ N, 110°9′3″ E	4.60 ± 0.03^{c}
WY2	20°28′44″ N, 110°8′48″ E	4.98 ± 0.04^{ab}
JX1	20°40′51″ N, 110°6′42″ E	4.23 ± 0.04^{d}
JX2	20°44′5″ N, 110°4′29″ E	4.26 ± 0.01^{cd}
DFH1	20°33′8″ N, 110°5′25″ E	4.76 ± 0.04^{bc}
DFH2	20°32′35″ N, 110°8′37″ E	4.93 ± 0.03^{b}

Table 3 Basic information about the sampling point.

Sampling points	Province/Autonomous region	Abbreviation	Location	pH	Annual output (t)
Jinxing Farm Team 13	Guangdong	JX1	20°40′51″ N, 110°6′42″ E	4.23	5
Wuyi Farm Team 18		WY1	20°29′39″ N, 110°9′3″ E	4.60	5
Dongfanghong Farm Team 7		DFH2	20°33′8″ N, 110°5′25″ E	4.93	5
Dongsheng Farm Team 18		DS1	21°42′29″ N, 110°3′21″ E	5.41	3
Dongfeng Farm	Guangxi	DE1	23°18′44″ N, 108°15′18″ E	6.09	10
Wuming District		WM2	23°20′52″ N, 108°14′41″ E	6.48	8
Dongfang Farm		DF2	23°16′44″ N, 108°17′19″ E	6.96	9
Pingguo City		PG1	23°61′71″ N, 107°62′3″ E	7.39	8

Determination of soil chemical properties and enzyme activity

The organic matter content was determined via the dilution heat method; the a alkali-N content via the alkaline hydrolysis diffusion method; available P content via molybdenum antimony scandium colorimetry after 0.5 M $NaHCO_3$ extraction; available K content via flame photometry after 1 M ammonium acetate extraction; soil pH via the potentiometric method; and Ca and Mg contents via EDTA titration. Soil catalase activity was determined by potassium permanganate titration, urease activity by phenol-sodium hypochlorite colorimetry, and sucrase activity by 3,5-dinitro salicylic acid colorimetry.

Extraction of total soil DNA and PCR amplification

Soil samples were processed using the OMEGA E.Z.N.A. Mag-Bind Soil DNA Kit (M5635-02, Omega Bio-tek, Norcross, Georgia, USA) following the manufacturer's protocol. The DNA integrity was assessed through agarose gel electrophoresis, while DNA concentration was quantified using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Primers specific to the hypervariable regions of the soil bacterial and fungal communities were synthesized for targeted PCR amplification, which served as a preparatory step for sequencing library construction. The PCR amplification focused on the V3-V4 region of the 16S rRNA gene for soil bacteria, utilizing primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'), and on the ITS1-ITS2 region for soil fungi, with primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3').

For the first round of amplification, 15 μL 2x Hieff Robust PCR Master Mix (10105ES03, Yeasen, Shanghai, China) was used. One forward primer and 1 reverse primer were added to each μL. The DNA template (10 ng) was supplemented with ddH_2O to 30 μL. The PCR conditions were as follows: Predenaturation at 94 °C for 3 min; denaturation at 94 °C for 30 s, annealing at 45 °C for 20 s, and extension at 65 °C for 30 s. These three steps were followed by 5 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s, and extension at 72 °C for 30 s. These three steps lasted for 20 cycles: Extension at 72 °C for 5 min and a final extension at 10 °C.

For the second round of amplification, 15 μL 2x Hieff Robust PCR Master Mix was used. One forward primer and 1 reverse primer were added to each μL. The DNA template (20 ng) was supplemented with ddH_2O to 30 μL. The PCR conditions were as follows: Predenaturation at 95 °C for 3 min; denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s, and extension at 72 °C for 30 s. These three steps lasted for 5 cycles, extension at 72 °C for 5 min, and extension to the end at 10 °C.

Illumina HiSeq sequencing and sequencing data processing

High-throughput sequencing was performed by Shanghai Shenggong Biotechnology (Shanghai, China), using the Illumina HiSeq sequencing platform. The original data obtained by sequencing were first spliced according to the overlap relationship to obtain the read data. Then, the samples were identified and distinguished according to the label sequence to obtain the sample data. The sample data were quality-controlled and filtered to obtain the effective date of each sample. Non-repetitive sequences were extracted from the optimized sequences of each sample, and single sequences without repetition were removed. The operational taxonomic unit (out) clustering was performed according to 97% similarity. Chimeras were removed during the clustering

process to obtain representative sequences of OTUs. The representative bacterial and fungal OTU sequences were classified by blasting the Ribosomal Database Project (RDP) database and the UNITE fungal internal transcribed spacer (ITS) database, respectively. The α diversity indices (including the Chao1, Simpson, and Shannon indices) were quantified according to the OTU richness. To evaluate the adequacy of the sample, a sparse curve of the observed number of OTUs was constructed, and all α diversity indices were calculated using Mothur software (version 3.8.31; Schloss et al., 2009). The OTU sparsity curve and rank abundance curve were plotted in R (version 3.6.0; R Foundation for Statistical Computing, Vienna, Austria). The diversity of the sample microbial community was estimated. The β diversity is used to evaluate the difference in the microbiome between samples, and visual representation is obtained by dimensionality reduction methods such as constrained principal component analysis (PCA). These analyses were performed using the R vegan package (version 2.5-6; McMurdie and Holmes, 2013), and finally, the distance between samples was represented as a scatter plot. Difference comparison was used to identify features with significantly different abundances between groups using STAMP (version 2.1.3; Parks et al., 2014) and LefSe (version 1.1.0; Segata et al., 2011). SparCC (version 1.1.0; Friedman and Alm, 2012) was used to calculate the correlation coefficient and p-value between the communities of OTUs, and the correlation matrix heatmap was drawn using the R corrplot software package (version 0.84; Conway et al., 2017).

Determination of leaf nutrient content

In general, dry ash extraction, H_2SO_4-H_2O_2 digestion, Nessler's colorimetric method were used to measure total N content, molybdenum antimony colorimetric method was used to measure total P content, total K content was measured by a flame photometer, and total Ca and total Mg contents were determined via the EDTA titration method.

RESULTS

Effects of different pH values on soil nutrients and sisal leaf nutrients

Comparisons of soil pH and nutrient analyses were conducted across eight distinct pH gradients: 4.23, 4.60, 4.93, 5.41, 6.09, 6.48, 6.95, and 7.39. As depicted in Table 4, available K (AK) and available P (AP) initially increased and then decreased with increasing pH. At pH 7.39, the alkali-N (AN) and soil organic matter (SOM) levels were significantly greater than those in the other treatments. Similarly, at pH 6.48, the concentrations of AK were significantly elevated relative to those in the other treatments. Furthermore, the AP content was significantly greater in the pH 6.95 treatment group than in the other treatment groups.

Table 4. Nutrient content of soils at different pH values. AN: Alkaline hydrolyzable N; AP: available P; AK: available K; SOM: soil organic matter. Data are mean ± standard error. Different lowercase letters in the same column indicate significant differences between data according to Dunnett test ($P < 0.05$), while the same letter indicates insignificant differences between groups.

pH	AN	AP	AK	SOM
	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	g kg^{-1}
4.23	58.33 ± 2.33^{bc}	1.69 ± 0.01^{f}	40.20 ± 0.66^{f}	14.50 ± 0.35^{c}
4.60	72.33 ± 6.17^{ab}	1.48 ± 0.00^{g}	58.71 ± 0.66^{de}	12.37 ± 1.40^{cd}
4.93	56.00 ± 4.04^{bc}	1.63 ± 0.01^{fg}	62.30 ± 0.58^{d}	11.70 ± 0.13^{cde}
5.41	44.33 ± 4.67^{cd}	2.01 ± 0.01^{e}	58.30 ± 2.64^{de}	9.44 ± 0.13^{de}
6.09	67.67 ± 5.09^{ab}	3.25 ± 0.14^{a}	87.22 ± 0.65^{b}	25.91 ± 0.61^{b}
6.48	45.50 ± 2.02^{cd}	2.76 ± 0.01^{c}	115.74 ± 2.97^{a}	14.45 ± 1.19^{c}
6.96	35.00 ± 2.02^{d}	2.96 ± 0.07^{b}	55.47 ± 1.12^{e}	8.94 ± 0.40^{e}
7.39	84.00 ± 10.69^{a}	2.36 ± 0.05^{d}	80.74 ± 2.97^{c}	35.08 ± 1.99^{a}

Figure 1 shows that the leaf Mg content increased and then decreased with increasing pH, peaking significantly at pH 6.48 compared to other pH levels. In contrast, the levels of TN, TP, TK, and Ca in leaves varied without a clear pattern as the pH increased. Overall, the nutrient content in leaves was notably greater at pH 6.09, 6.48, and 7.39 than at other pH levels.

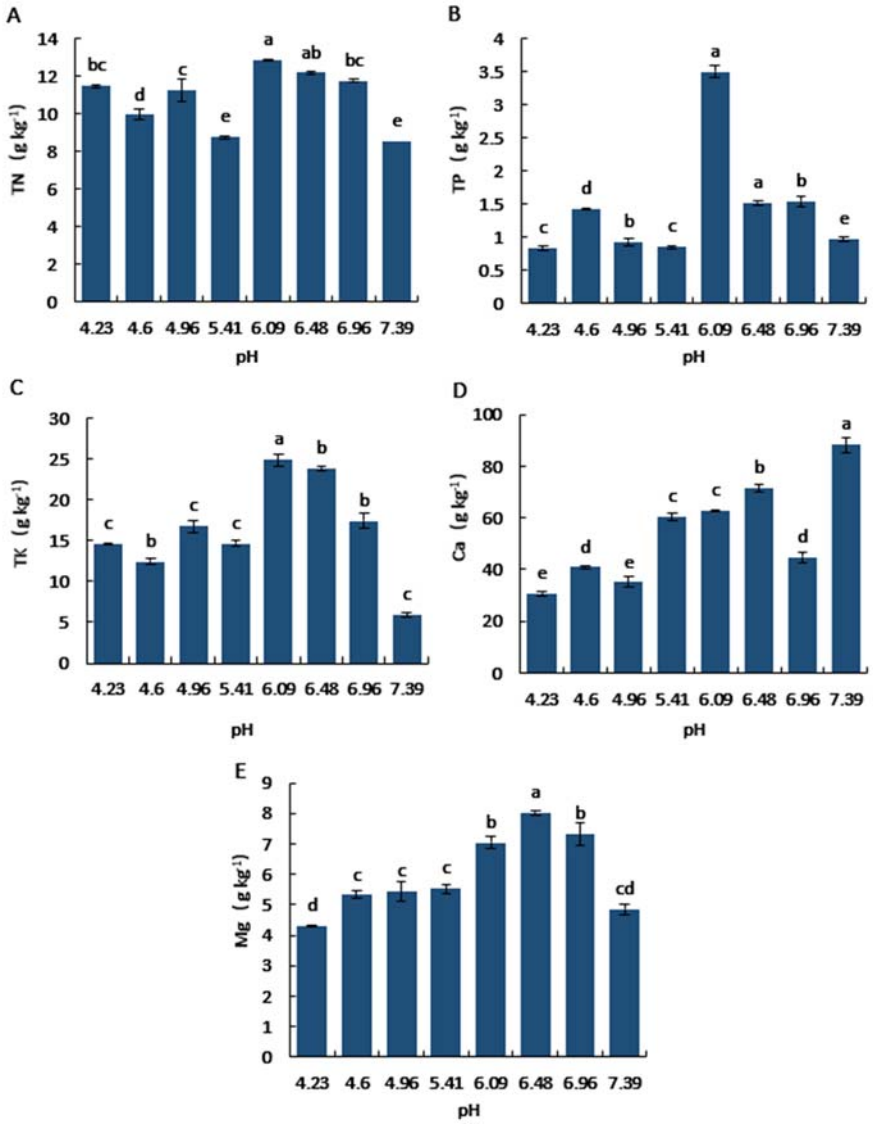

Figure 1. Nutrient content of leaves at different pH values. According to Dunnett's test, the different letters on the bar chart indicate significant differences in leaf nutrients ($P < 0.05$). The vertical bar corresponds to the standard error. Sampling point n = 120. TN: Total N; TP: total P; TK: total K.

Effect of different pH values on soil enzyme activities

Enzyme activity measurements for catalase (SCAT), sucrase (SSC), and urease (SUE) were conducted across eight sisal-producing areas characterized by varying soil pH levels. Catalase and sucrase activities both showed an initial increase followed by a decrease with increasing pH, reaching their highest levels at pH 6.09. In contrast, urease activity was significantly greater at pH 7.39 than in the other treatments (Table 5).

Table 5. Enzyme activity in soils at different pH values. Data are mean ± standard error. Different lowercase letters in the same column indicate significant differences between data according to Dunnett test ($P < 0.05$), while the same letter indicates insignificant differences between groups.

pH	Soil urease	Soil sucrase	Soil catalase
	U g^{-1}	U g^{-1}	U g^{-1}
4.23	0.18 ± 0^{b}	104.36 ± 4.69^{d}	2.57 ± 0.22^{e}
4.60	0.17 ± 0^{cd}	131.68 ± 19.36^{cd}	3.55 ± 0.29^{d}
4.93	0.17 ± 0^{bc}	145.16 ± 14.19^{cd}	5.25 ± 0.52^{c}
5.41	0.15 ± 0^{e}	120.45 ± 17.06^{d}	8.50 ± 0.51^{b}
6.09	0.17 ± 0^{d}	451.75 ± 11.92^{a}	9.91 ± 0.27^{a}
6.48	0.15 ± 0^{e}	328.59 ± 13.24^{b}	3.16 ± 0.11^{de}
6.96	0.15 ± 0^{e}	229.01 ± 1.50^{c}	4.90 ± 0.32^{c}
7.39	0.19 ± 0^{a}	171.36 ± 3.94^{cd}	3.05 ± 0.17^{de}

Changes in microbial community diversity and composition

In our current study, 76320 OTUs were collected from eight rhizosphere soil samples of sisal under different pH conditions for bacterial community analysis. The alpha diversity (Ace, Shannon, and Chao1 indices) of bacterial communities in samples with pH 4.60-5.41 was significantly higher than those in samples with pH 4.23 (strong acid) and higher pH (Figure 2A). Furthermore, no similar microbial communities were observed in various soil samples, they all formed a distinct cluster, as shown in the principal component PCA based on Bray Curtis differences (Figure 3A). The histogram composed of phylum level classification shows that compared to pH 7.39, the relative abundance of Acidobacteria decreased by 53.38%, 77.89%, 138.51%, 62.79%, 47.81%, 26.70%, and 108.59% at pH 4.23, 4.60, 4.93, 4.41, 6.09, 6.48 and 6.96 respectively. The relative abundance of Proteobacteria was highest at pH 6.09. In contrast, the relative abundance of Actinobacteria is highest at pH 5.41 (Figure 4A). In addition, 15, 18, 27, and 41 specific genera were detected in the bacterial communities of four acidic soil samples in Guangdong, with pH values ranging from 4.23 to 5.41 (Figure 5A).

For fungal, a total of 25 440 OTUs were collected from all rhizosphere soil samples. Overall, the alpha diversity (Ace, Shannon, and Chao1 indices) of fungal communities in soil samples with pH 6.09-7.39 were significantly higher than those at pH 4.23-5.41 (Figure 2B). In addition, except for the pH 6.09 and 7.39 treatments, highly similar microbial communities were observed in all other treatments, forming a distinct cluster, as shown in the principal component PCA based on Bray Curtis differences (Figure 3B). According to the histogram composed of phylum level classification, the relative abundance of Ascomycota in pH 4.23 increased by 1.52%%, 56.39%, 64.44%, 33.68%, 9.30%, 34.36% and 40.82% at pH 6.96, 4.60, 4.93, 5.41, 6.09, 6.48, and 7.39 respectively. The relative abundance of Basidiomycota is highest at pH 7.39. The relative abundance of Mortierellomycota is highest at pH 6.48 (Figure. 4B). In addition, 23, 22, 29, 26, 10, 17, 11, and 17 specific genera were detected in fungal communities of eight soil samples with pH values ranging from 4.23 to 7.39. (Figure. 5B).

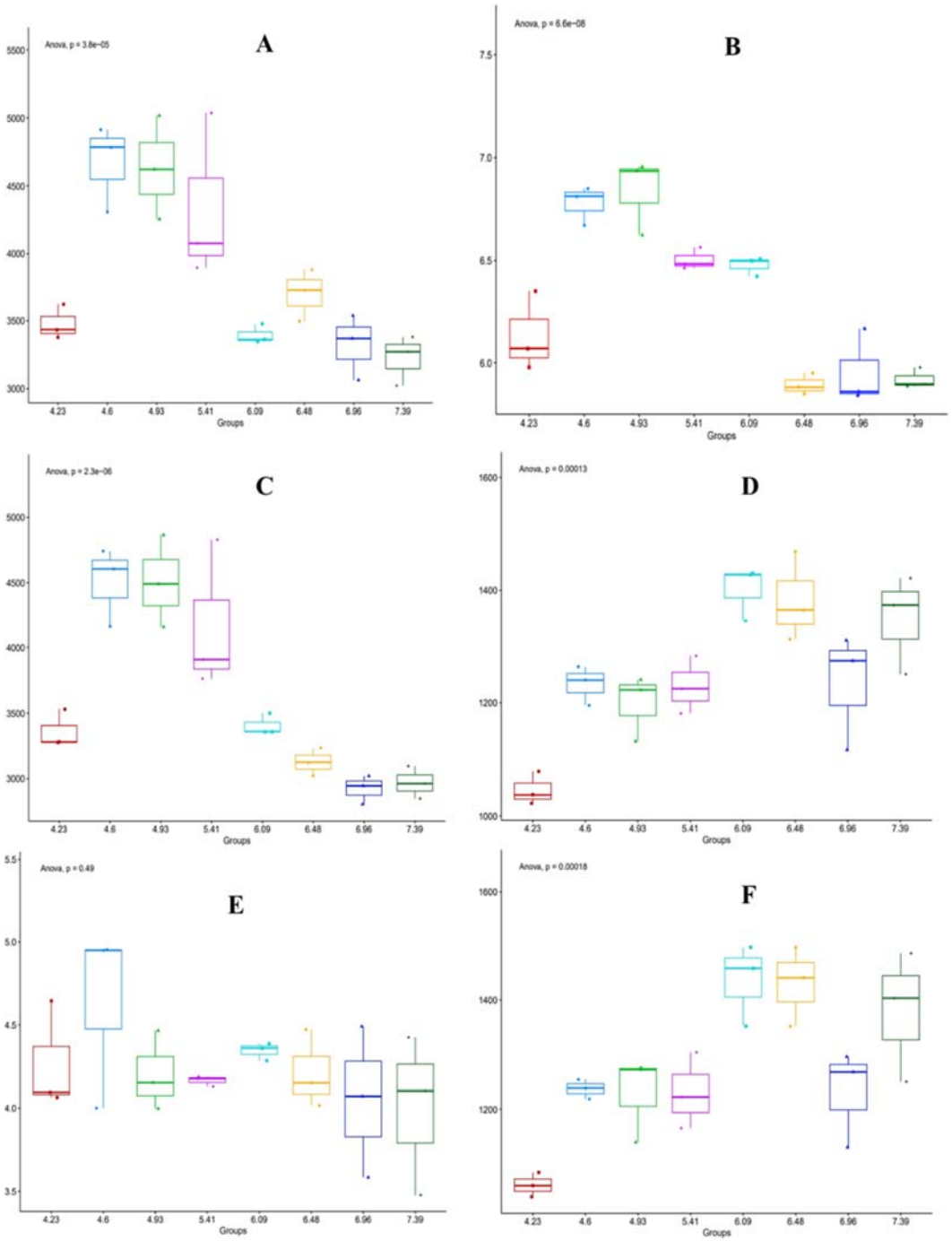

Figure 2. α-Diversity analysis for bacteria and fungi, Ace index for bacteria (A), Shannon index for bacteria (B), Chao1 index for bacteria (C), Ace index for fungi (D), Shannon index for fungi (E), Chao1 index for fungi (F). Different colors are used to distinguish different pH groups.

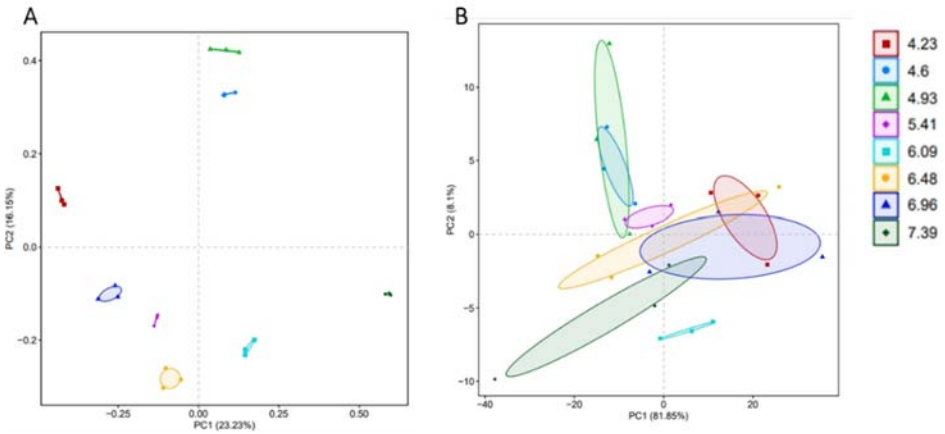

Figure 3. Principal component analysis of soil bacterial (A) and fungal (B) communities.

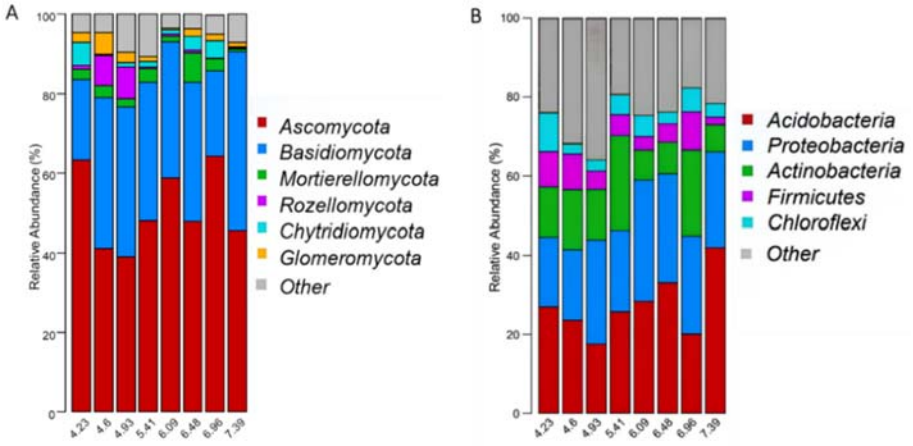

Figure 4. Relative abundance of bacteria (A) and fungi (B) at different pH and community compositions at the phylum level. Listing the top five dominant microbial populations in Guangdong and Guangxi production areas.

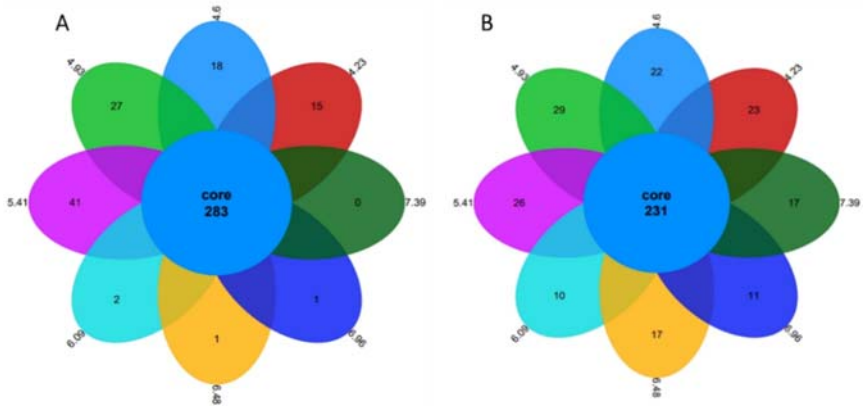

Figure 5. Venn analysis of the bacterial (A) and fungal (B) communities influenced by different pH levels.

Relationships between the soil microbial community and soil factors

To study the existing relationship between the soil microbial community and soil factors, we did a correlation analysis of soil physicochemical properties using paired Spearman rank correlation coefficients, as shown in Figure 6. The results revealed the dominant bacterial communities, of Acidobacteria, Proteobacteria, and Actinobacteria which constitute 56.53% to 72.98% of the total bacterial community. Specifically, Acidobacteria was significantly positively correlated with pH ($|r| > 0.8$, $P < 0.001$), Proteobacteria was significantly positively correlated with SSC ($|r| > 0.8$, $P < 0.001$), and Actinobacteria exhibited a significantly positive correlation with pH and AP ($|r| > 0.8$, $P < 0.001$). Among the dominant fungal communities, which account for 78.69% to 94.42% of the total fungal community, Ascomycota is positively correlated with AP, Basidiomycota is positively correlated with AK, and Mortierellomycota also exhibits a positive correlation with AP.

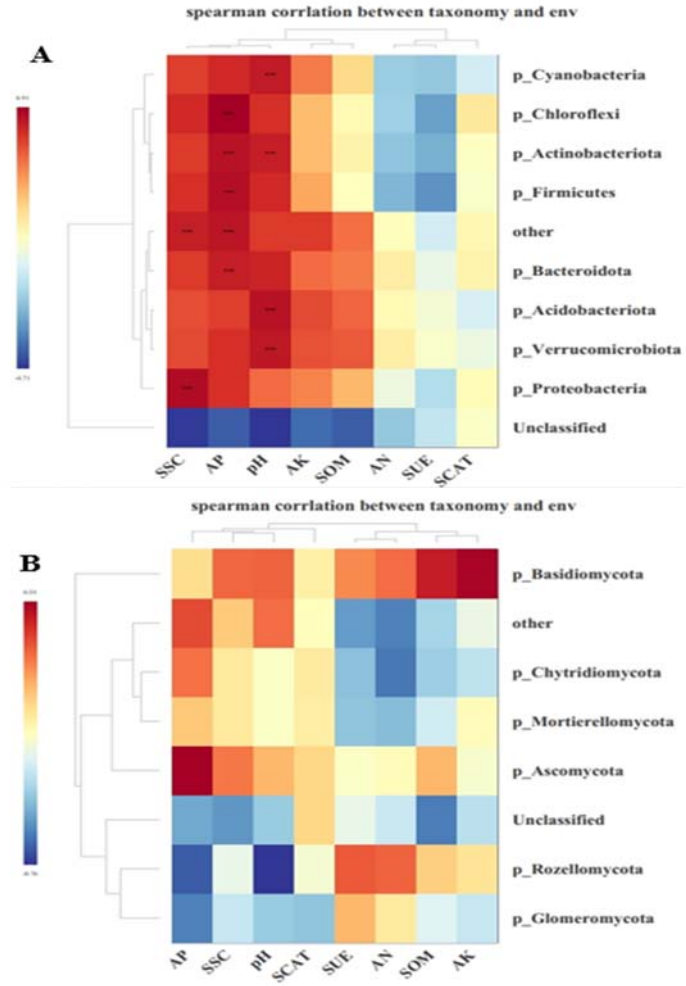

Figure 6. Correlation heatmap of soil bacterial (A) and fungal (B) phyla and soil environmental factors. The color intensity indicates the correlation strength: red indicates a strong positive correlation, whereas blue represents a strong negative correlation. The top tree illustrates the hierarchical clustering of environmental factors, and the tree on the left depicts the hierarchical clustering of species. Branch length signifies the level of dissimilarity, with shorter branches indicating greater similarity. The graph only shows the results with p-values less than 0.05 and absolute correlation values greater than 0.8. *When $|r| > 0.8$, $0.05 > P \geq 0.01$; **$0.01 > P \geq 0.001$, ***$P < 0.001$. AN: Alkaline hydrolyzable N; AP: available P; AK: available K; SOM: soil organic matter; SUE: soil urease; SSC: soil sucrase; SCAT: soil catalase.

Prediction of microbial function in different pH soil

According to the functional grouping of fungi based on the fine classification of Guild subclasses (Figure 7, Table 6), the top ten soil samples were: a 1 Unknown, a 2 undefined saprotroph, a 3 bryophyte parasite-dung saprotroph-ectomycorrhizal-fungal parasite-leaf saprotroph-plant parasite-undefined saprotroph-wood saprotroph, a 4 wood saprotroph, a 5 animal pathogen-endophyte-fungal parasite-lichen parasite-plant pathogen-wood saprotroph, a 6 endophyte-plant pathogen-undefined saprotroph, a 7 endophyte-litter saprotroph-soil saprotroph-undefined saprotroph, a 8 arbuscular mycorrhizal, a 9 animal pathogen, and a 10 endophyte-plant pathogen-wood saprotroph.

The functional fungal abundance of a 2 and a 10 is significantly higher than other treatments at pH 4.93; that of a 3 is significantly higher at pH 7.39; a 4 and a 7 show significantly higher abundance at pH 6.48; a 5 and a 9 exhibit significantly higher abundance at pH 6.96; a 6 displays significantly higher abundance at pH 4.23; both a 8 and a 9 have significantly higher abundance at pH 4.60. There is nonsignificant difference in the functional fungal abundance of a 1 across different pH levels in the sisal fields. Among them, a 5, a 6, a 9, and a 10 are animal and plant pathogens, indicating an increased risk of infection in sisal fields at pH levels of 4.23, 4.60, 4.93, and 6.96.

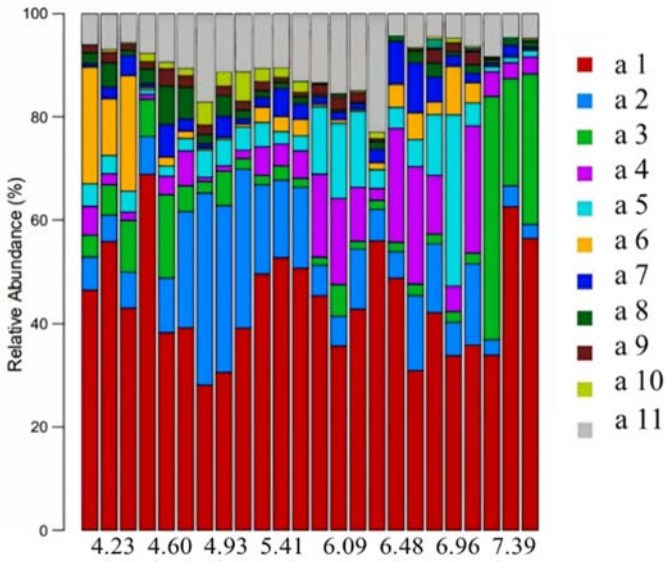

Figure 7. Functional groups of fungal Guild subcategories. a 1- Unknown, a 2- undefined saprotroph, a 3- bryophyte parasite-dung saprotroph-ectomycorrhizal-fungal parasite-leaf saprotroph-plant parasite-undefined saprotroph-wood saprotroph, a 4- wood saprotroph, a 5- animal pathogen-endophyte-fungal parasite-lichen parasite-plant pathogen-wood saprotro, a 6- endophyte-plant pathogen-undefined saprotroph, a 7- endophyte-litter saprotroph-soil saprotroph-undefined saprotroph, a 8- arbuscular mycorrhizal, a 9- animal pathogen, a 10- endophyte-plant pathogen-wood saprotroph, a 11-other.

Table 6. Comparison of relative abundance prediction of fungal functional groups by subdivision. Different lowercase letters in each row (i.e., for each functional group at different pH) indicate significant differences in data ($P < 0.05$), while identical letters indicate insignificant differences between groups.

pH	4.23	4.6	4.93	5.41	6.09	6.48	6.96	7.39
a1	48.48 ± 3.82^{a}	48.79 ± 10.03^{a}	32.64 ± 3.33^{a}	50.96 ± 0.92^{a}	41.34 ± 2.92^{a}	45.21 ± 7.43^{a}	37.28 ± 2.52^{a}	50.98 ± 8.71^{a}
a2	6.08 ± 0.51^{cd}	13.39 ± 4.61^{bc}	33.33 ± 1.93^{a}	16.00 ± 0.66^{b}	7.68 ± 1.91^{cd}	8.60 ± 2.99^{bcd}	11.77 ± 2.75^{bc}	3.26 ± 0.40^{d}
a3	6.70 ± 1.73^{b}	9.45 ± 3.41^{b}	3.63 ± 1.50^{b}	2.11 ± 0.32^{b}	3.03 ± 1.50^{b}	1.89 ± 0.10^{b}	2.01 ± 0.06^{b}	32.31 ± 7.76^{a}
a4	3.11 ± 1.25^{cd}	3.78 ± 1.68^{bcd}	1.17 ± 0.24^{d}	5.03 ± 0.41^{abcd}	14.42 ± 1.99^{ab}	15.71 ± 6.71^{a}	13.63 ± 5.83^{ab}	3.73 ± 0.53^{bcd}
a5	4.00 ± 0.24^{c}	1.78 ± 0.44^{c}	4.94 ± 0.24^{bc}	3.35 ± 0.70^{c}	14.14 ± 0.53^{ab}	4.32 ± 0.48^{c}	16.49 ± 8.62^{a}	1.14 ± 0.09^{c}
a6	18.68 ± 3.83^{a}	1.20 ± 0.37^{b}	0.48 ± 0.06^{b}	3.03 ± 0.09^{b}	0.52 ± 0.07^{b}	3.67 ± 1.17^{b}	5.25 ± 2.11^{b}	0.13 ± 0.01^{b}
a7	2.24 ± 0.93^{b}	3.11 ± 1.68^{ab}	1.96 ± 1.08^{b}	3.45 ± 1.06^{ab}	1.31 ± 0.14^{b}	6.84 ± 2.17^{a}	2.93 ± 0.96^{ab}	1.06 ± 0.55^{b}
a8	2.61 ± 1.11^{b}	5.48 ± 1.38^{a}	2.52 ± 0.73^{b}	1.27 ± 0.05^{b}	0.43 ± 0.04^{b}	1.68 ± 0.28^{b}	1.73 ± 0.58^{b}	1.17 ± 0.29^{b}
a9	1.58 ± 0.24^{ab}	2.31 ± 0.57^{a}	1.76 ± 0.06^{ab}	1.17 ± 0.29^{bc}	2.03 ± 0.18^{ab}	0.50 ± 0.05^{cd}	2.28 ± 0.29^{a}	0.24 ± 0.04^{d}
a10	0.57 ± 0.08^{cd}	1.52 ± 0.10^{bc}	4.39 ± 0.83^{a}	2.29 ± 0.21^{b}	0.36 ± 0.07^{d}	0.69 ± 0.37^{cd}	0.48 ± 0.25^{cd}	0.35 ± 0.08^{d}
a11	5.94± 0.23^{c}	9.17 ± 0.58^{bc}	13.18 ± 0.20^{ab}	11.34 ± 0.12^{b}	14.74 ± 0.03^{a}	10.88 ± 0.33^{b}	6.16 ± 0.17^{c}	5.64 ± 0.05^{c}

Using the PICRUSt method, we predicted functional genes related to the root zone soil (as shown in Figure 8), encompassing metabolism, human diseases, environmental information processing, organic systems, genetic information processing, and cellular processes. Among the soil samples, those from Guangdong production areas (with pH values of 4.23, 4.60, 4.93, and 5.41) showed nonsignificant relative abundance of these predicted functions. In contrast, the highest proportions of these functions were exhibited by those from Guangxi production areas (with pH values of 6.09, 6.48, 6.96, and 7.39) among all samples. Notably, the proportion of metabolic functions in soil with a pH of 7.39 was higher than that observed in other soil samples from Guangxi production areas.

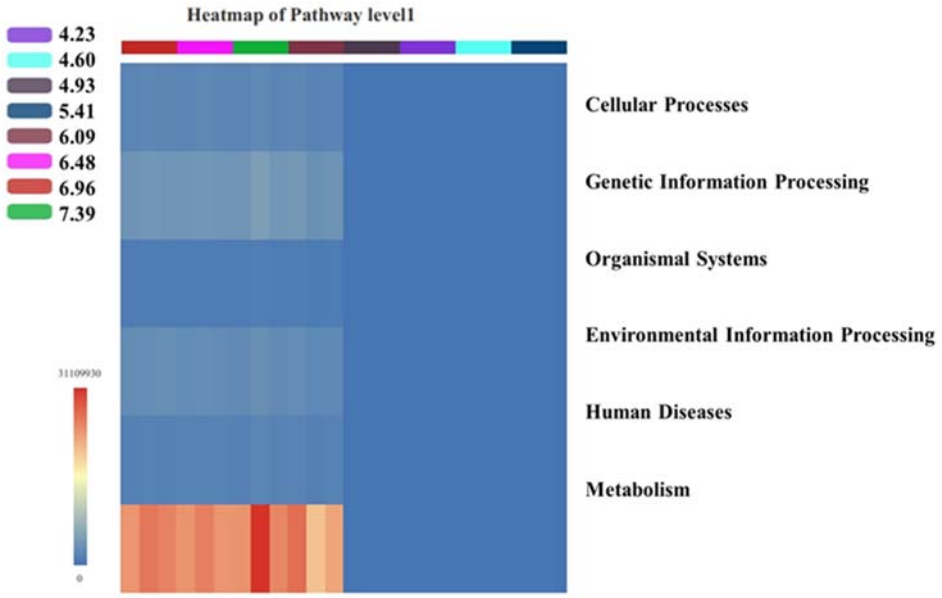

Figure 8. Thermal map of relative abundance predicted by bacterial PICRUSt function.

DISCUSSION

The diversity index of a soil microbial community reflects its richness and evenness of its species distribution. This study analyzed the diversity of bacterial and fungal communities in soils with varying pH levels. Our findings revealed that these microbial communities exhibited distinct diversity patterns in response to different pH conditions. Specifically, fungi achieved peak richness and diversity at pH 6.48 and 4.60, respectively, whereas bacteria reached their highest richness and diversity at pH 4.60 and 6.09, respectively. This pattern is consistent with previous research. For instance, earlier studies have indicated that bacterial species diversity generally increases from acidic to neutral pH levels, while fungi tend to predominate in acidic soils (Wang et al., 2023). Moreover, Zhang and Li (2020) research indicated that microbial activity might be heightened in high-pH soil conditions, potentially shaping microbial diversity and distribution. These results support the view that soil pH significantly influences microbial diversity and community composition. This may be because pH impacts key physiological and biochemical activities, such as enzyme synthesis, enzyme activity, metabolic pathways, and cell membrane permeability, which consequently influences diversity of entire community (Akinola et al., 2023).

The PCA based on the Bray-Curtis similarity index revealed that when the soil pH changed within a certain range, the fungal community species abundances under other pH conditions were highly similar, in addition to the specific pH values (pH 6.09 and 7.39). This indicates that the composition of the fungal community is relatively stable across this pH range. Conversely, the species composition of the soil bacterial community samples varied greatly, suggesting that bacteria are more responsive to environmental fluctuations (Luu et al., 2019). In this study area, the dominant fungal phyla in both Guangdong and Guangxi were Ascomycota, Basidiomycota, unclassified, Rozellomycota, unclassified Fungi, Mortierellomycota, Chytridiomycota, and Glomeromycota. The main bacterial phyla in Guangdong were unclassified, unclassified, and Proteobacteria, while the dominant bacterial phyla in Guangxi were Acidobacteria, Proteobacteria, Actinobacteria, Chloroflexi, Bacteroidota, Verrucomicrobiota, Firmicutes, and Cyanobacteria. Different dominant bacterial phyla exhibit varying degrees of variation under different pH conditions.

In this study, we observed distinct differences in the fungal community composition based on soil pH. Specifically, the relative abundances of Ascomycota, Basidiomycota, and Mortierellomycota were significantly greater under weakly acidic-alkaline conditions (6.09-7.39) than under weakly acidic-alkaline conditions (4.23-5.41). Among these dominant groups, Ascomycota and Basidiomycota alone accounted for 60.51%-86.09% of the total fungal phyla. Although less abundant, Mortierellomycota also contributes to soil health by participating in soil C cycling and nutrient transformations. These research results highlight the importance of understanding how different fungal communities, particularly Ascomycota, Basidiomycota, and Mortierellomycota, interact with each other and with their environment to enhance nutrient uptake and utilization in weak acid-to-alkaline soil environments (de Araujo et al., 2017). This knowledge is crucial for developing sustainable management practices that maintain the productivity and resilience of agricultural ecosystems. These findings highlight soil pH's profound influence on shaping microbial communities' structure. Furthermore, the varying relative abundances and functional differences observed within dominant bacterial phyla across different pH levels reflect their diverse adaptive strategies to the environment.

Soil environmental factors are closely related to soil microbial communities. Studies have shown that nutrient status affects the composition of soil microbial communities (Herrmann et al., 2016). This study showed that soil pH, available P, soil sucrase, available K, organic matter, soil urease, alkali-hydrolyzed N, and soil catalase all had certain effects on the soil microbial community. In the fungal community, available P was positively correlated with Ascomycota and negatively correlated with Rhodomycetes and Glomeromycota. Available K and SOM were positively correlated with Basidiomycota. For the bacterial community, pH positively correlated with Cyanobacteria, Actinobacteria, Acidobacteria, and Verrucomicrobia. Available P was significantly positively correlated with Actinobacteria, Chloroflexi, Bacteroidetes, and Firmicutes. Soil sucrase (SSC) was significantly positively correlated with Proteobacteria. Studies have shown that SOM, pH, and AP are highly correlated with most groups of soil microorganisms (Li et al., 2016b), which is consistent with our findings. The profound influence of soil pH on microbial community diversity, growth, and reproduction underscores its vital role in modulating enzymatic processes, activities, and metabolic pathways essential for microbial life (Akinola et al., 2023). Furthermore, the primary constituent of organic matter, SOM, serves as a vital C source for soil microorganisms, fostering their proliferation and activity (Chakrawal et al., 2022). Additionally, Available P, a

crucial indicator of soil P availability, not only influences microbial communities but also plays a pivotal role in supporting plant growth and development (Zhu et al., 2021). Notably, the abundances of the phyla Acidobacteria, Verrucomicrobia, Cyanobacteria, and Actinobacteria were significantly positively correlated with soil pH. This implies that they might be key microbial groups in shaping soil acidity. For instance, Acidobacteria can degrade plant material, participate in the Fe cycle, and metabolize single-C compounds—all processes that can be sensitive to changes in pH. Similarly, Verrucomicrobia are integral in breaking down polysaccharides; they may be particularly effective at this task, and their growth could be favored within specific pH ranges (Orellana et al., 2021). Cyanobacteria, which are renowned for their diversity and adaptability, can survive in the absence of light and utilize it for photosynthetic C fixation when available. Nonetheless, their growth and metabolic activities might be more robust at certain pH levels. In addition, the metabolites generated by Actinobacteria bolster plant defense against diseases, and the production and efficacy of these compounds can be influenced by the pH of the soil (Zhang et al., 2020; Ouchene et al., 2021). The observed correlations between these bacterial phyla and soil pH reflect their unique physiological characteristics and ecological roles, leading to differing levels of activity and influence depending on the pH.

The fungal function prediction in this study indicated that the pathogenic fungi in soil samples from sisal orchards were significantly more abundant at pH values of 4.23, 4.60, 4.93, and 6.96, suggesting an elevated risk of infection at these pH levels. Conversely, at pH 6.48, there was an increase in various saprophytic fungi, including wood rot fungi, endophytic fungi, and soil-dwelling saprophytic fungi, which contribute to the decomposition of organic matter, enhancement of soil fertility, and positive impacts on soil and water conservation capacity and nutrient cycling in sisal plantations (Wang et al., 2022). Regarding bacterial function, the higher pH Guangxi soil samples exhibited significantly increased functional abundance in metabolism, environmental information processing, organic systems, genetic information processing, and cellular processes compared to slightly acidic Guangdong soil samples. This enhancement of bacterial metabolic function provides a richer energy source for bacterial communities, while improved genetic information processing capability strengthens gene expression, jointly promoting nutrient absorption efficiency and environmental adaptability of bacterial communities (Zhang et al., 2021). These changes accelerated bacterial reproduction and growth, further enriching the diversity of bacterial functional communities and underpinning the long-term health and stability of the jute orchard ecosystem.

Soil enzyme activity is a key measure of soil ecosystem health and sustainability and plays a vital role in maintaining soil fertility. Enzymes can be susceptible to extreme pH levels. Acidic or alkaline conditions can disrupt the enzyme's molecular structure or interfere with its active site, leading to reduced activity (Kompala-Baba et al., 2021). Peng et al. (2023) noted a negative correlation between soil pH and the activity of urease and sucrase in the middle reaches of the Heihe wetland. Our study revealed that catalase and sucrase activity peaked in weakly acidic environments, while urease activity was greater under alkaline conditions. Soil pH was negatively associated with catalase and urease activity but positively associated with sucrase activity, which contrasts with previous findings. This variation in results might be attributed to differences in the research subjects and their environments. To fully understand these discrepancies, further research is needed to explore the underlying mechanisms involved.

Soil pH levels significantly influence the efficiency of plant uptake of major nutrients, but this influence varies depending on both the plant species and the specific nutrients involved. In some instances, an increase in soil pH can lead to enhanced absorption of certain elements. For example, the leaf Mg content in this study peaked at a pH of 6.48. Conversely, the leaf contents of N, P, K, and Ca did not exhibit a consistent trend of change with increasing pH. This observation contrasts with previous research findings. Potential reasons for these discrepancies include variations in research materials, experimental conditions, or the differing abilities of plants to adapt to their environments. Under acidic conditions (pH < 6.5), some studies have suggested that the accumulation of N, P, and Mg tends to increase with increasing pH. However, when the pH falls below 4.0, the growth of plants, such as tea trees, becomes restricted, and their ability to absorb key nutrient elements is compromised. These findings indicate that an extremely acidic environment can negatively affect plant nutrient uptake. In summary, soil pH has a significant effect on the ability of plants to absorb N, P, K, Ca, and Mg, but the specific impact pattern varies depending on plant species, soil conditions, and the nature of the nutrient element. Therefore, in actual agricultural production, the soil pH should be reasonably adjusted according to the specific needs of crops and the actual situation of the soil to optimize nutrient absorption and improve crop yield and quality.

CONCLUSIONS

The results obtained from the study enable us to conclude our initial goals. The pH is significantly correlated with organic matter content and highly correlated with available P and available K content. At pH 6.09, the activities of catalase and sucrase were significantly higher than in other treatments, while urease activity was significantly higher at pH 7.39.

The relative abundance of the dominant bacterial phyla Acidobacteriota, Proteobacteria, and Actinobacteriota peaked at pH 7.39, 6.09, and 5.41, respectively. Similarly, the relative abundance of the dominant fungal phyla Ascomycota, Basidiomycota, and Mortierellomycota was highest at pH 6.96, 7.39, and 6.48, respectively.

The functional abundance of rhizosphere soil fungal communities of sisal in Guangdong and Guangxi varies with pH. In contrast, the bacterial functions such as metabolism, disease, and environmental information processing in Guangxi soil samples with higher pH are significantly improved compared to the acidic soil samples in Guangdong.

Overall, the nutrient content of leaves did not exhibit a consistent pattern of change with increasing pH. In this study, total N, P, and K in leaves were observed to be highest at pH 6.09. At the same time, Ca and Mg content peaked at pH 7.39 and pH 6.48, respectively.

Therefore, in actual agricultural production, the soil pH should be reasonably adjusted according to the specific needs of crops and the actual situation of the soil to optimize nutrient absorption and improve crop yield and quality.

Author contributions

Conceptualization: H.C., T.C., K.Y. Methodology: P.L., Z.H. Validation: S.T., T.C. Formal analysis: P.L. Investigation: J.X. Resources: W.W. Data curation: P.L., Z.H. Writing-original draft: P.L. Writing-review & editing: H.C. Visualization: P.L. Supervision: T.C., K.Y. All co-authors reviewed the final version and approved the manuscript before submission.

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