

Article

Regulatory Mechanism of Phosphorus Tailings and Organic Fertilizer Jointly Driving the Succession of Acidic Soil Microbial Functional Groups and Enhancing Corn Yield

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Abstract

The continued acidification of red soil reduces phosphorus availability and microbial activity, which restricts corn growth. Phosphorus tailings, a waste product from phosphate mining, can neutralize soil acidity and supply controlled-release phosphorus, but their effects on the red soil-corn system remain unclear. A field experiment in Qujing, Yunnan (2023–2024), tested four treatments: CK (standard fertilization), T1 (CK plus phosphorus tailings), T2 (80% of standard fertilizer plus phosphorus tailings), and T3 (80% of standard fertilizer plus phosphorus tailings and organic fertilizer, both applied at 6.0 t·ha⁻¹). Using high-throughput sequencing, redundancy analysis (RDA), and structural equation modeling (SEM), the study evaluated impacts on soil properties, microbial communities, and corn yield and quality. Results showed: (1) Phosphorus tailings reduced soil acidification; T3 raised soil pH in the top 0–10 cm by 0.54–0.9 units compared to CK and increased total, available, and soluble phosphorus in the 0–20 cm layer to 952.82, 28.46, and 2.04 mg/kg, respectively. (2) T3 exhibited the highest microbial diversity (Chao1 and Shannon indices increased by 177.57% and 37.80% versus CK) and a more complex bacterial co-occurrence network (114 edges versus 107 in CK), indicating enhanced breakdown of aromatic compounds. (3) Corn yield under T3 improved by 13.72% over CK, with increases in hundred-grain weight (+6.02%), protein content (+18.04%), and crude fiber (+9.00%). (4) Effective nitrogen, ammonium nitrogen, available phosphorus, and soil conductivity were key factors affecting *gcd/phoD* phosphorus-reducing bacteria. (5) Phosphorus tailings indirectly increased yield by modifying soil properties and pH, both positively linked to yield, while *gcd*-carrying bacteria had a modest positive influence. In summary, combining phosphorus tailings with a 20% reduction in chemical fertilizer reduces fertilizer use, recycles mining waste, and boosts corn production in acidic red soil, though further studies are needed to evaluate long-term environmental effects.

Keywords: phosphorus tailings; acid red soil; maize yield and quality; soil properties; microbial community



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1. Introduction

The ongoing intensification of agricultural soil acidification poses a significant challenge to sustainable global development. This acidic soil condition is mainly caused by factors such as global climate change, acid rain, and heavy fertilizer use [1]. Currently, approximately 40% of the world's arable land is subject to acidification [2]. In China, this issue affects up to 21% of its farmland, and the extent of acidification continues to increase [1]. The fundamental cause of acidification is the accelerated loss of essential alkaline cations like calcium and magnesium from the soil, along with notable increases in hydrogen ions (H^+), exchangeable aluminum ions (Al^{3+}), and acidic compounds in the soil solution. This process reduces crops' ability to absorb and utilize nitrogen efficiently, decreases the availability of vital nutrients such as calcium and phosphorus, worsens soil physical conditions (leading to compaction), and ultimately severely limits crop yield and quality [3–5]. More importantly, acidification restricts the ecological niches of soil microbes, suppressing their activity and diversity, which further endangers plant health [6,7]. Additional studies show that soil pH is a crucial regulatory factor. When nitrogen is applied, decreases in pH primarily drive changes in microbial diversity and community structure in grasslands; higher pH levels are associated with phylogenetic clustering in bacterial communities [8,9]. At the same time, soil pH itself has a significant effect on crop growth and yield. Therefore, adopting remediation strategies such as applying lime, biochar, industrial by-products, manure, straw, or combinations of these is especially important. These approaches effectively raise soil pH and can boost crop yields by approximately 13% to 36% [10].

The use of alkaline substances to raise soil pH is widely acknowledged as an effective method to counteract acidification [11,12]. Recent studies have concentrated on applying lime, biochar, and organic materials to enhance acidic soils [13–15]. Lime quickly neutralizes hydrogen ions (H^+) and replenishes calcium, but its extensive long-term use can cause soil compaction and the risk of acid rebound [14]. Biochar has shown promise in regulating acidity and stimulating microbial activity [15], yet its high cost limits widespread use. The effectiveness of organic materials like straw is still uncertain: some research suggests that humic substances within these materials, containing carboxyl and hydroxyl groups, provide good buffering capacity against metal ions and protons. Soil organic matter (SOM), a key factor in neutralizing soil acidity, is positively linked to improved acid-neutralization capacity as its content increases [16]. However, there is evidence that the decomposition of added organic materials may produce carbon dioxide and organic acids, which could accelerate the loss of salt-based cations and impede pH improvement [17]. Overall, these approaches face challenges related to cost-efficiency, resource sustainability, durability of soil improvements, and environmental concerns [13,17]. Consequently, there is a pressing need to develop new environmentally friendly solutions that can simultaneously neutralize acidity, provide phosphorus, and remain economically feasible.

Phosphate tailings, which are concentrated calcium-magnesium materials resulting from phosphate ore processing, are usually stored in tailings ponds. This method not only uses up land but also creates environmental hazards due to long-term buildup and leaching caused by rainfall [18,19]. These tailings contain minerals like dolomite ($CaMg(CO_3)_2$) and fluorapatite ($Ca_5(PO_4)_3F$) [20], which are rich in soil-beneficial elements such as CaO, MgO, and P_2O_5 . Dolomite, the main mineral phase, has been shown to effectively neutralize hydrogen ions (H^+) in acidic soils and replace aluminum ions (Al^{3+}) by releasing calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions [21,22]. At the same time, fluorapatite gradually releases phosphate ions (PO_4^{3-}) into the soil, supplying crops with essential phosphorus and nitrogen [23]. Considering their mineral properties and the need to manage environmental risks, transforming phosphate tailings into materials for improving acidic soils offers

a promising approach to both recycle industrial solid waste and enhance farmland fertility simultaneously. Corn (*Zea mays* L.) is one of the world's most important crops for food, feed, and energy, and the stability of its production system plays a vital role in global food security [24,25]. Corn roots are highly sensitive to acidic conditions, with low soil pH potentially causing a 20–40% decrease in yield, making acidic soils a major limitation to its productivity [26–28]. Although initial research has shown that phosphorus tailings can improve soil chemical properties [29], the exact mechanisms by which they stabilize yields and improve quality in highly acidic red soil-corn systems—particularly through regulating the “soil-microbe-crop” interaction network—are not yet fully understood. There is a notable lack of quantitative studies examining the causal links between soil microbial community structures, functional genes (such as the *gcd* and *phoD* genes of phosphoregulative bacteria), and corn yield and quality under various application methods. This study seeks to clarify the fundamental relationships between dynamic changes in soil chemical and biological characteristics and corn productivity. Based on this, two main scientific hypotheses are proposed: (1) Moderate application of phosphorus tailings can effectively neutralize soil acidity and optimize nutrient availability, thereby sustaining corn production in highly acidic soils; (2) Changes in the soil environment induced by phosphorus tailings selectively promote the growth of specific functional microorganisms (e.g., phosphoregulative and aromatic degradation bacteria), which in turn enhance crop productivity through microbiota-driven nutrient cycling. Confirming these hypotheses will not only advance the scientific understanding of how phosphorus tailings improve soil but also provide theoretical guidance for assessing their value in sustainable agricultural land management.

2. Materials and Methods

2.1. Test Site Overview

The experimental location is situated in Malong District, Qujing City, Yunnan Province (N 25°21'17.0", E 103°22'57.0"), at an elevation of 1885 m in the northeastern hilly area of Yunnan. This region experiences a low-latitude plateau monsoon climate, characterized by clearly defined dry and wet seasons. The average annual temperature is 13.4 °C, with total yearly rainfall of 1032 mm, most of which occurs between July and September. The frost-free period extends for 241 days. The soil is identified as red soil, noted for its acidic properties, clay-rich and poorly structured texture, low nutrient retention, and high levels of iron and aluminum oxides. According to the IUSS WRB (2022) international soil classification, the soil is classified as Ferralsols. Before the experiment began, the topsoil contained 41.7 g·kg^{−1} of organic matter, 174.2 mg·kg^{−1} of alkaline hydrolyzed nitrogen, 14.4 mg·kg^{−1} of available phosphorus, 207.5 mg·kg^{−1} of available potassium, and had a pH of 5.21.

2.2. Experimental Design and Sample Collection

Between 2023 and 2024, a two-year field experiment was carried out in the strongly acidic red soil area of Malong District, Qujing City, Yunnan Province. The study utilized a randomized block design with four treatment groups: (1) Conventional full fertilizer application (CK, control); (2) Conventional full fertilizer plus phosphorus tailings (T1); (3) 80% of conventional fertilizer plus phosphorus tailings (T2); (4) 80% of conventional fertilizer plus phosphorus tailings combined with organic fertilizer (T3). Each treatment was replicated three times ($n = 3$). Each plot measured 46.2 m² (6.6 m × 7 m), totaling 12 plots. A 2 m-wide buffer zone surrounded the experimental area. The corn variety “Xingyu 101” was planted with 40 cm spacing between plants and 80 cm between rows. Phosphorus tailings, provided by Yunnan Phosphorus Chemical Group Co., Ltd. of Yun-

nan, China., contained 29.14% CaO, 12.26% MgO, 5.67% P₂O₅, 4.78% SiO₂, and had a pH of 8.3. The total heavy metal contents of Hg, As, Pb, Cd, and Cr were 0.2, 12.5, 49.6, 0.03, and 21.0 mg·kg⁻¹ respectively, all within the permissible limits for soil conditioners. After drying and grinding, the materials were passed through 100-mesh sieves and evenly applied during plowing at a rate of 6.0 t·ha⁻¹. Conventional fertilization involved both basal and top-dressing applications. The basal fertilizer was a compound fertilizer (N-P₂O₅-K₂O = 13-5-7, total nutrient content 35%) applied at 0.75 t·ha⁻¹. During the large trumpet stage, additional applications of urea (46% N, 0.3 t·ha⁻¹) and potassium sulfate (51% K₂O, 0.15 t·ha⁻¹) were made. The T3 treatment also received organic fertilizer (organic matter ≥ 40%, total N+P₂O₅+K₂O ≥ 4%) at 6.0 t·ha⁻¹ alongside the compound fertilizer. All plots were managed uniformly, with field operations focusing on weed control and pest and disease prevention.

Corn is planted every April and harvested in October. During harvest, two central rows from each plot were chosen to measure fresh weight yield. Ten corn ears were randomly and consecutively sampled from each plot, bagged for air-drying, and their ear rows and 100-kernel weights were recorded. Protein and crude fiber contents were also analyzed. After harvesting, soil samples were collected using a five-point sampling method at depths of 0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm in each plot. One kilogram of fresh soil was taken from each quadrat for analysis, totaling 48 soil samples. The homogenized soil samples were air-dried in a cool environment, ground into powder, and passed through a 2-mesh sieve for storage. The samples were then transported on ice to the laboratory and stored in a –80 °C ultra-low temperature freezer for later microbial community sequencing. For the 2024 harvest, 12 soil samples were collected from the 0–20 cm layer per plot. After sieving through a sterile 2-mesh soil sieve, these samples were placed into sterile centrifuge tubes, transported on ice to the laboratory, and stored at –80 °C for subsequent microbial community sequencing.

2.3. Assessment of Quality Indicators in Corn Samples

For the yearly corn harvest, two main sampling locations were chosen within each plot to record the fresh weight yield. Following this, ten consecutive corn ears were gathered from each plot. These samples were bagged and dried in the air. The number of cobs and the weight of one hundred grains were then measured [30]. Cellulose content was analyzed using the hydrochloric acid hydrolysis technique [31], while protein content was determined by the Kjeldahl method [32].

2.4. Chemical Analysis of Soil Samples

Soil organic matter was measured using the potassium dichromate oxidation method with external heating. Total nitrogen (TN) was determined by the semi-micro Kjeldahl technique. Total phosphorus (TP) was analyzed using the sodium hydroxide fusion followed by the molybdenum-antimony colorimetric method. Available nitrogen (AN) was assessed through the alkali diffusion method. Available phosphorus (AP) was measured using extraction with 0.05 mol·L⁻¹ HCl and 0.025 mol·L⁻¹ (half-strength) sulfuric acid. Available potassium (AK) was determined by ammonium acetate extraction combined with flame photometry. Water-soluble phosphorus (WSP) was extracted with 0.03 mol/L sodium chloride solution and analyzed by the molybdenum-antimony colorimetric method. Ammonium nitrogen (NH₄⁺-N) was measured using 2 mol·L⁻¹ potassium chloride extraction followed by indophenol blue colorimetry. Nitrate nitrogen (NO₃⁻-N) was determined by 2 mol·L⁻¹ potassium chloride extraction and ultraviolet spectrophotometry. Soil pH was measured with a pH meter (Metro-pH 320; Mettler-Toledo Instruments Ltd., Shanghai, China). Cation exchange capacity (CEC) was determined using the 1 mol·L⁻¹ ammonium

acetate exchange method. All these soil chemical analysis methods were referenced from “Soil Agricultural Chemistry Analysis” [33].

2.5. Soil DNA Extraction and High-Throughput Sequencing

Soil samples from the 0–20 cm layer collected in 2024 were subjected to DNA extraction using the E.Z.N.A.[®] Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). The V3-V4 region of the bacterial 16S rRNA gene was amplified with primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR mixture had a total volume of 20 µL, containing 4 µL of 5× FastPfu buffer, 5 µM of each forward and reverse primer, 2.5 mM dNTPs, 0.4 µL FastPfu polymerase, and 10 ng of template DNA. The PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen, Union, CA, USA) and quantified with the Qubit[®] 3.0 fluorometer (Thermo Fisher, Waltham, MA, USA). Finally, the prepared libraries were sequenced on the Illumina MiSeq platform (PE250 mode) at Biozeron Biological Technology Co., Ltd. (Shanghai, China) following standard protocols (Accessed on 20 October 2024. <https://www.illumina.com/>).

Following sequencing, the raw fastq files were processed using the QIIME2 pipeline (Accessed on 5 November 2024. <https://qiime2.org/>). The cutadapt plugin was utilized to trim primers and index barcode sequences, after which the reads were merged into paired-end sequences. Subsequent steps included quality filtering, noise reduction, and removal of chimeric sequences. The refined bacterial ASVs were then clustered against the Silva 138.1 database at a 97% similarity cutoff. Samples containing only unique ASVs were excluded, and the remaining data were normalized based on the lowest read count across all samples. The raw sequencing data are available in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA1328694.

2.6. Calculations and Statistical Analysis

The five treatment groups were analyzed using SPSS 27.0 through one-way ANOVA, followed by Duncan's multiple comparison test ($p < 0.05$) to determine significant differences. Microbial alpha and beta diversity were assessed using the “vegan” package in R version 4.1.0. Coexistence network analysis was conducted to explore interactions among microbial phyla. Correlations between phyla were calculated using the “psych” package in R 4.5.0 to build microbial coexistence networks [34]. p -values were adjusted via the Benjamini–Hochberg method, with networks considered stable when correlation coefficient $r > 0.6$ and $p < 0.05$. Network properties (MENA Network Reports) were computed based on predefined similarity thresholds [35]. The final network visualization was created using Gephi 0.10.1 (Accessed on 12 July 2025. <https://gephi.org/users/download/>), and its topological characteristics, including node count (species number) and edge connectivity (inter-species relationships), were analyzed. Spearman correlation heatmaps were generated to identify key factors influencing corn yield and quality. The importance of factors was assessed by measuring the decline in prediction accuracy or increase in mean squared error (MSE) between observed and predicted values. Redundancy analysis (RDA) of soil microbial *gcd* and *phoD* genes was performed using Canoco 5.0 software to examine their relationships with soil physicochemical properties. Significant predictors identified from the correlation heatmap were used in subsequent structural equation modeling (SEM) to evaluate the direct and indirect effects of soil chemical properties, physical characteristics, and microbial communities on corn yield and quality. SEM was conducted using the “plspm” package in R 4.5.0 through partial least squares path modeling (PLS-PM). Model validation included multiple criteria such as the chi-square test ($p > 0.05$), goodness-of-fit in-

dex ($GFI > 0.6$), and R^2 , which represents the proportion of variance in crop yield explained by the model [36].

3. Results

3.1. Corn Yield and Quality

Figure 1a–d illustrate that applying phosphorus tailings has a significant impact on corn yield and quality. In terms of yield, treatment T3 (80% conventional fertilizer combined with phosphorus tailings and organic fertilizer) achieved a 13.72% higher yield compared to CK (full conventional fertilizer application) under the 80% phosphorus tailings condition ($p < 0.05$). However, no significant differences were detected among CK, T1 (full conventional fertilizer plus phosphorus tailings), and T2 (80% conventional fertilizer plus phosphorus tailings) (Figure 1a). For the 100-kernel weight, both T2 and T3 treatments significantly exceeded CK, with increases of 8.10% and 6.02%, respectively ($p < 0.05$), though no significant difference was observed between T2 and T3 (Figure 1b). Regarding corn quality, treatment T3 exhibited a significant increase in protein content by 18.04% compared to CK ($p < 0.05$), along with a 9.00% rise in crude fiber content ($p < 0.05$) (Figure 1c,d). No significant differences in protein or crude fiber content were found between CK and T1. Likewise, crude fiber content did not differ significantly between T1 and T2 treatments.

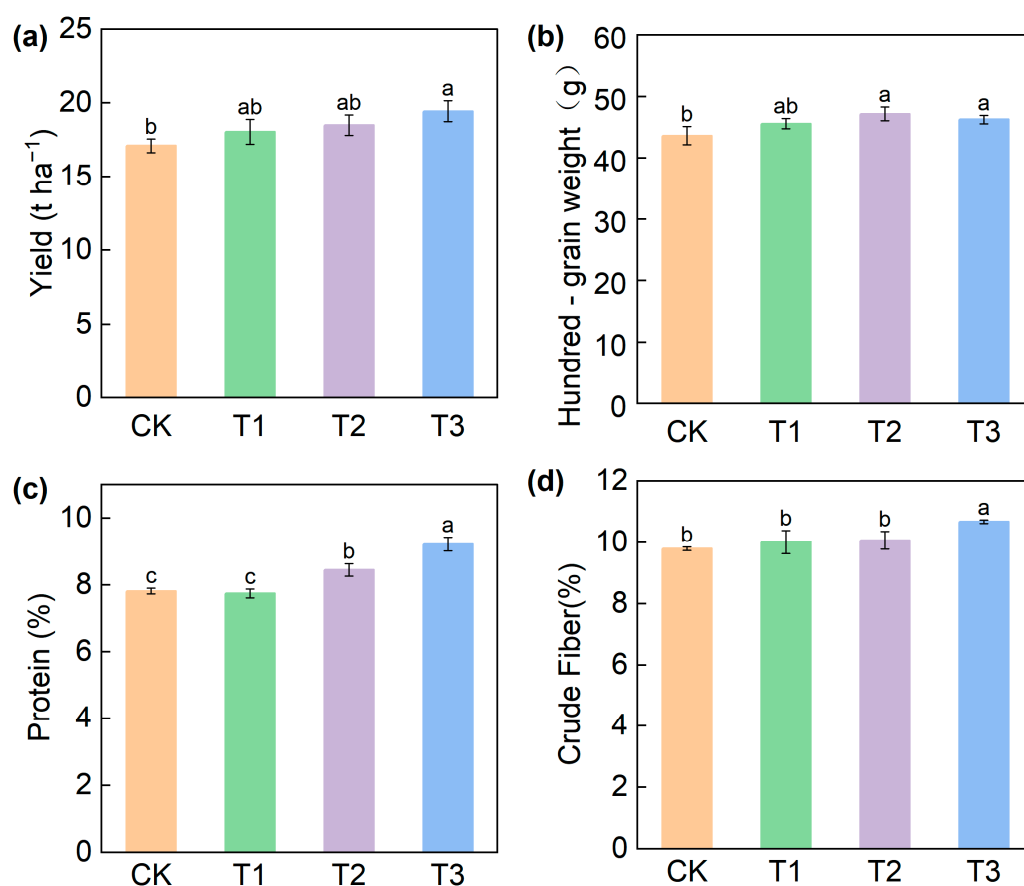


Figure 1. Impact of various phosphorus tailings treatments on corn yield and quality. (a) Grain yield; (b) Weight of one hundred grains; (c) Protein content; (d) Crude fiber content. Different lowercase letters above the bars denote significant differences between treatments (Duncan's test, $p < 0.05$). Data are shown as mean $\pm$ SD ($n = 3$).

3.2. Soil pH

Data from a two-year integrated study showed that phosphorus tailings treatments had a significant effect on soil pH levels (Figure 2a,b). To reduce year-to-year variability, we analyzed the combined data from both years. Initial statistical tests indicated no significant differences between the years, so the year factor was not treated as an independent variable. The main analysis examined soil at depths of 0–10 cm and 10–20 cm. In the 0–10 cm layer, treatments T1, T2, and T3 all significantly raised soil pH by 0.54 to 0.9 units compared to the control (CK), with no significant difference between T2 and T3. In the 10–20 cm layer, treatments T2 and T3 also significantly increased soil pH relative to CK, but no significant difference was observed between these two treatments.

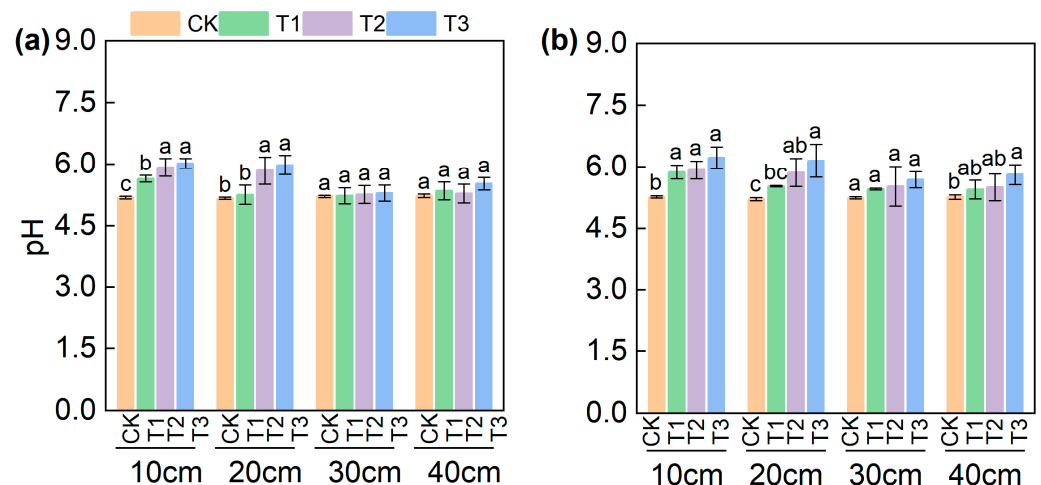


Figure 2. Changes in soil pH under phosphorus tailings treatments during 2023–2024. (a) Data from 2023; (b) Data from 2024. Soil depths are 10 cm (0–10 cm), 20 cm (10–20 cm), 30 cm (20–30 cm), and 40 cm (30–40 cm). Error bars show the mean $\pm$ SD ($n = 3$). Different lowercase letters denote significant differences between treatments ($p < 0.05$).

3.3. Spatial Distribution Characteristics of Phosphorus

Using data collected over two years, we examined how adding phosphorus tailings affected phosphorus distribution within different soil layers (see Figure 3). The findings indicated that total phosphorus (TP), available phosphorus (AP), and soluble phosphorus (SP) levels in the treatment groups CK, T1, T2, and T3 all decreased as soil depth increased. Significant differences in TP, AP, and SP were found between the 0–10 cm and 10–20 cm soil layers. Among the treatments, T3 exhibited the highest concentrations in both the 0–10 cm and 10–20 cm layers, with average values of 952.82 mg/kg for TP, 28.46 mg/kg for AP, and 2.04 mg/kg for SP over the two-year period. Conversely, T2 had the lowest phosphorus concentrations across all layers, even lower than the control group CK. In the deeper 20–30 cm and 30–40 cm layers, phosphorus levels remained relatively stable with only slight fluctuations. Overall, the addition of phosphorus tailings had a significant impact on phosphorus distribution and concentration, especially in the upper soil layers (0–10 cm and 10–20 cm). The T3 treatment, which combined 80% conventional fertilizer with phosphorus tailings and organic fertilizer, showed clear benefits in increasing total, available, and soluble phosphorus content.

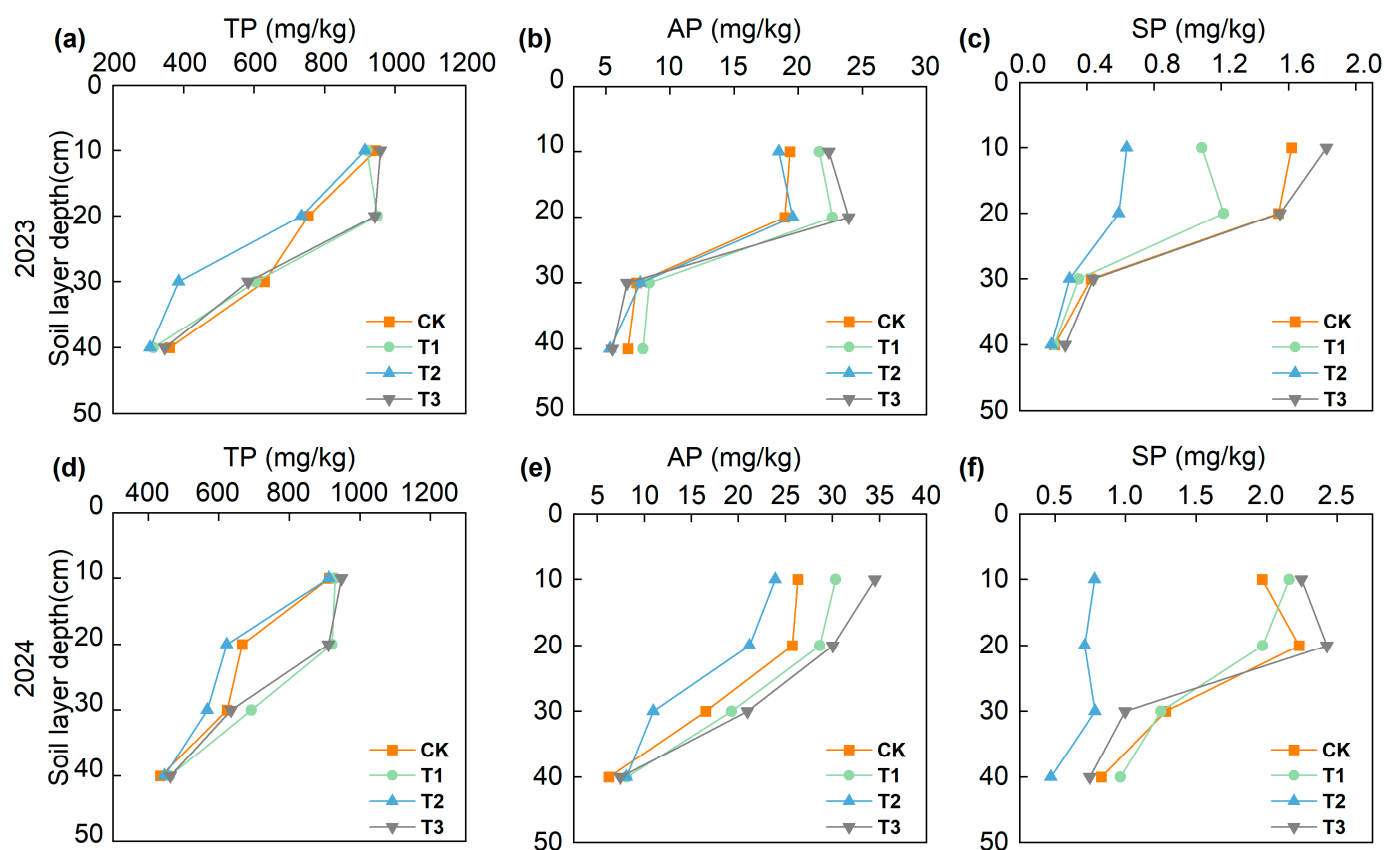


Figure 3. Phosphorus content distribution across soil layers under various phosphorus tailings treatments, 2023–2024. (a) Total phosphorus in soil in 2023; (b) Available phosphorus in soil in 2023; (c) Soil soluble phosphorus in 2023; (d) Total phosphorus in soil in 2024 (e) Available phosphorus in soil in 2024 (f) Soluble phosphorus in soil in 2024; TP refers to total phosphorus; AP to available phosphorus; and SP to soluble phosphorus.

3.4. Soil Microbial Community and Functional Characteristics

3.4.1. Variations in Soil Bacterial α - and β -Diversity Following Phosphorus Tailings Treatment

The impact of phosphorus tailings treatments on soil bacterial community diversity was examined using Illumina MiSeq sequencing of 48 rhizosphere soil samples. This analysis assessed α -diversity, which reflects species richness and evenness, and β -diversity, representing differences in community composition. The findings are illustrated in Figure 4a (Chao1 index), Figure 4b (Shannon index), and Figure 4c (PCoA analysis). Compared to the standard full chemical fertilizer application (CK), treatments T1, T2, and T3 all led to increases in both the Chao1 and Shannon diversity indices of the bacterial community. Notably, T1 caused a significant rise in the Shannon index compared to CK. The Chao1 index increased significantly by 104.30% and 177.57% in T2 and T3, respectively, while the Shannon index rose by 25.83% and 37.80% ($p < 0.05$). Additionally, when compared to T2, T3 significantly enhanced the Chao1 and Shannon indices by 35.86% and 9.51%, respectively.

Regarding soil bacterial β -diversity in the maize rhizosphere, principal coordinate analysis (PCoA) based on Bray–Curtis distances was conducted to evaluate similarities and differences in community composition (Figure 4c). The first two principal coordinates, PC1 and PC2, explained 20.65% and 15.29% of the total variance, respectively, together accounting for 35.94%. The use of phosphorus tailings led to distinct clustering of bacterial community structures. Significant differences in community composition were found between the conventional full chemical fertilizer treatment (CK) and each of the tailings

treatments (T1, T2, T3), as well as among T1, T2, and T3 themselves ($p = 0.001$). These results demonstrate that both conventional fertilizer and phosphorus tailings applications substantially modified the rhizosphere bacterial community structure.

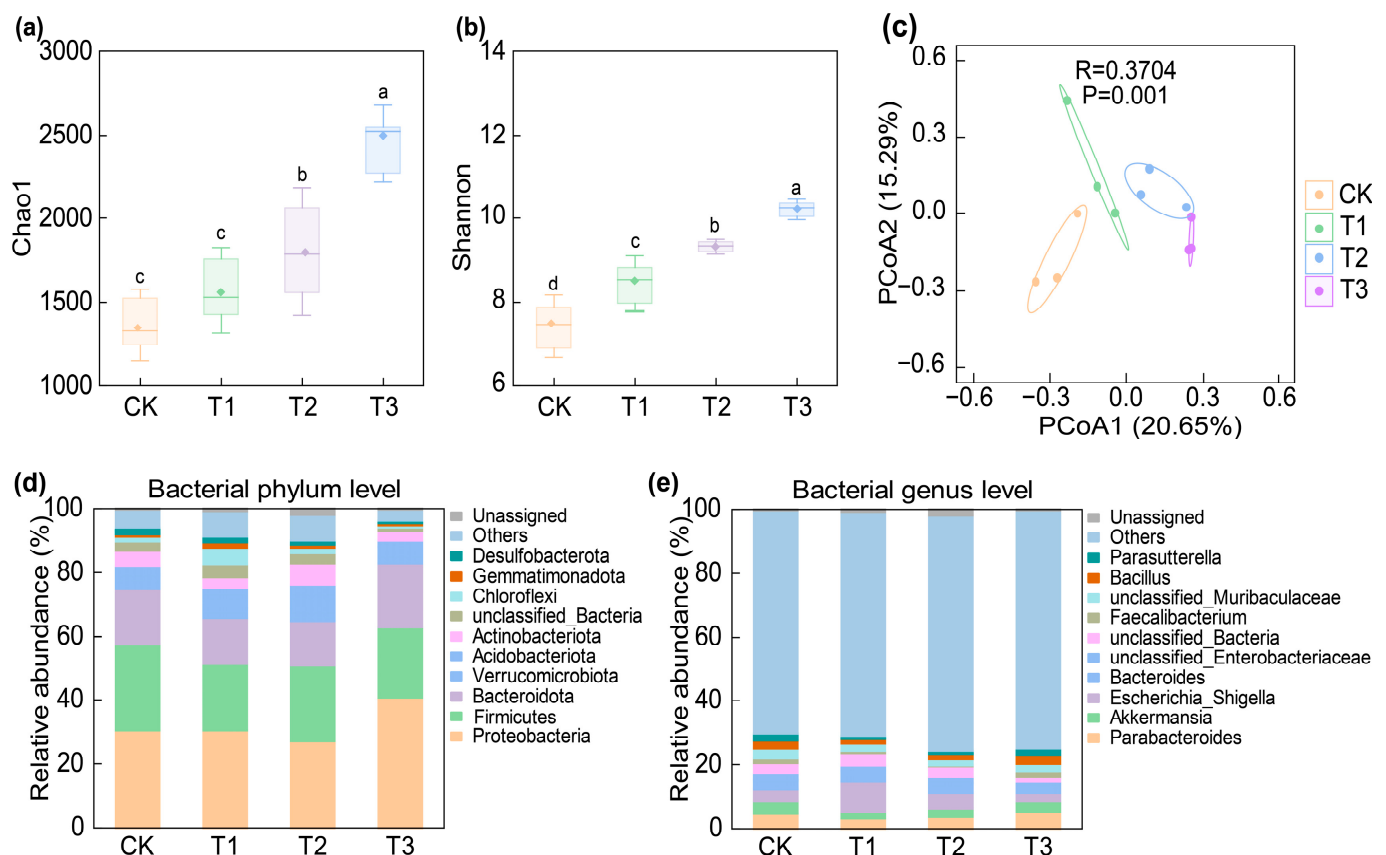


Figure 4. Impact of various phosphorus tailings treatments on soil bacterial α -diversity, β -diversity, and community structure. (a) Richness measured by the Chao1 index; (b) Diversity assessed using the Shannon index; (c) Principal coordinate analysis (PCoA) based on Bray–Curtis distances; (d) Taxonomic composition at the phylum level; (e) Taxonomic composition at the genus level. Error bars represent the mean $\pm$ SD ($n = 3$). In the box plots, the top and bottom edges correspond to the 75th and 25th percentiles, respectively; whiskers indicate one standard deviation above and below the mean; individual data points show replicate samples. Different lowercase letters indicate statistically significant differences between treatments ($p < 0.05$).

3.4.2. Variations in Soil Bacterial Community Composition at Family and Genus Levels

Across all treatments, the five most dominant bacterial phyla in terms of relative abundance were *Proteobacteria*, *Firmicutes*, *Bacteroidota*, *Verrucomicrobiota*, and *Acidobacteriota*. Compared to the control (CK), treatment T3 increased the relative abundance of *Proteobacteria* and *Bacteroidota* by 34.36% and 14.70%, respectively, while decreasing *Firmicutes* by 18.62%. Treatments T1 and T2 led to significant increases in *Acidobacteriota* abundance, rising by 119.58% and 169.01%, respectively.

At the genus level, relative to CK, treatment T3 raised the abundance of *Parabacteroides* by 8.53%. Conversely, treatments T1, T2, and T3 reduced the relative abundance of *Akkermansia* by 47.60%, 32.88%, and 6.72%, respectively, with the reduction under T3 being notably less pronounced than those under T1 and T2 (see Figure 4d,e).

3.4.3. Soil Microbial Co-Occurrence Network Response

Using the original sequence data, we selected outputs with a combined relative abundance greater than 0.001 and identified association pairs with Pearson correlation coeffi-

cients above 0.6 and statistical significance P less than 0.05 to build a microbial co-occurrence network (Figure 5). The findings revealed that the CK treatment included 50 nodes and 107 edges. With the introduction of phosphorus tailings in the T1 and T2 treatments, both the number of nodes and edges gradually declined, indicating a decrease in the complexity of the bacterial network structure. However, under the T3 treatment, the node count increased to 45 and the edge count rose to 95, reflecting a recovery in complexity and strengthened microbial interactions.

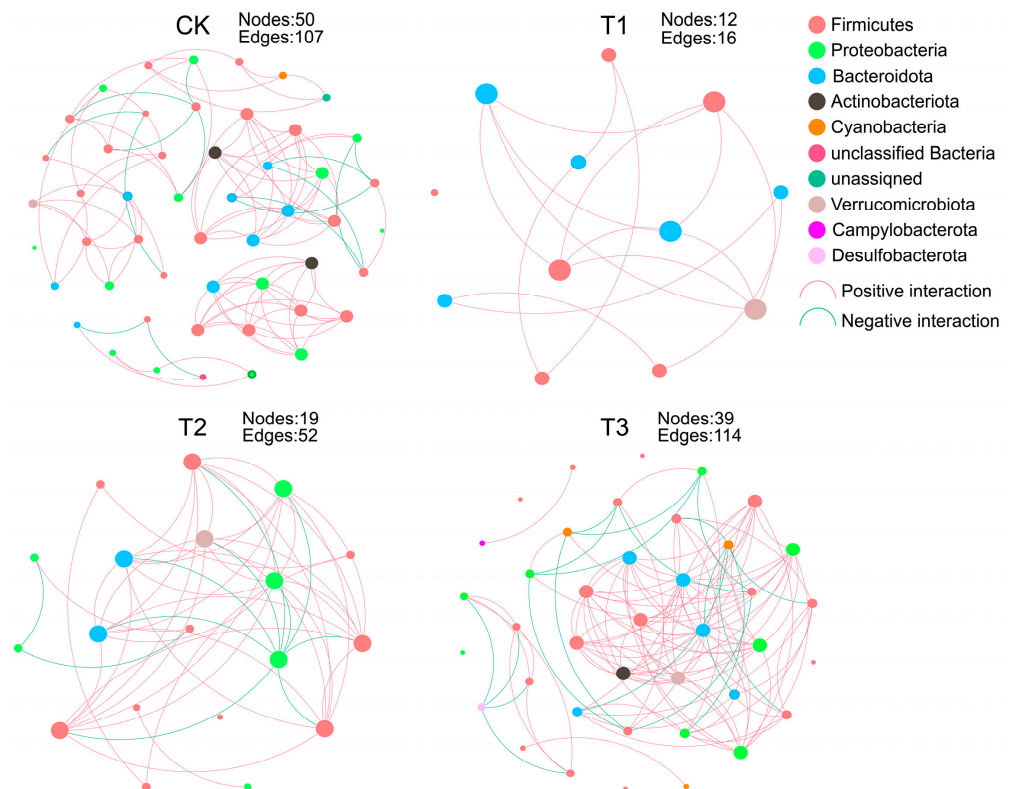


Figure 5. Networks showing bacterial co-occurrence under various phosphorus tailings treatments. Nodes are colored according to bacterial phylum. The size of each node reflects the connectivity of the corresponding operational taxonomic unit (OTU). Edges are colored to indicate correlation type: red for positive correlations and green for negative correlations.

3.4.4. Community Traits and Environmental Effects of Soil Phosphorus-Depleting Functional Microorganisms (*gcd* and *phoD* Genes)

Bacterial groups containing the key functional genes *gcd* and *phoD*, which play roles in soil phosphorus solubilization, display considerable taxonomic diversity [37]. At the phylum level, several groups such as *Proteobacteria*, *Verrucomicrobia*, *Planctomycetes*, and *Actinobacteria* possess these phosphorus-mobilizing genes. The predominant taxa are mainly found within families like *Rhizobiaceae*, *Pseudomonadaceae*, *Comamonadaceae*, and *Oxalobacteraceae* (see Figure 6b). Redundancy analysis (RDA) results (Figure 6a) show that Axis-1 and Axis-2 account for 34.12% and 29.72% of the variation, respectively, with a combined explanatory power exceeding 60%. These two main axes effectively capture the relationships between bacterial family groups and environmental variables. *Rhizobiaceae* is positively correlated with factors such as electrical conductivity (EC), ammonium nitrogen ($\text{NH}_4^+\text{-N}$), soil organic matter (SOM), and total phosphorus (TP), whereas *Comamonadaceae* and *Caulobacteraceae* show negative correlations with these parameters. The distribution of *Pseudomonadaceae*, *Oxalobacteraceae*, and *Micromonosporaceae* is significantly influenced by pH, available phosphorus (AP), and nitrate nitrogen ($\text{NO}_3^-\text{-N}$).

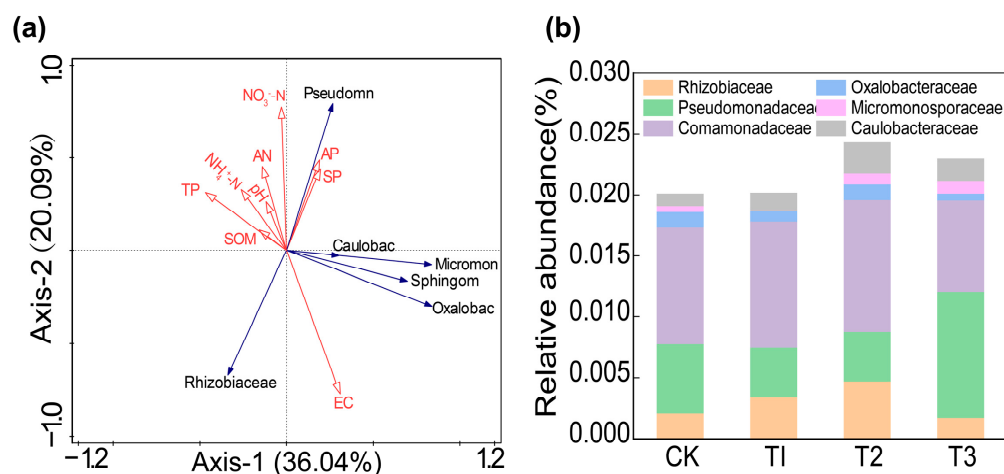


Figure 6. Redundancy analysis (RDA) illustrating the relationships between phosphorus-solubilizing bacteria, soil environmental factors, and the relative abundance of key functional genes. (a) RDA triplot depicting the connections between microbial communities and environmental variables; (b) Relative abundance of the *gcd* and *phoD* genes at the family level. Abbreviations: pH, soil pH; SOM, soil organic matter; EC, electrical conductivity; AN, available nitrogen; $\text{NH}_4^+\text{-N}$, ammonium nitrogen; $\text{NO}_3^-\text{-N}$, nitrate nitrogen; TP, total phosphorus; AP, available phosphorus; SP, soluble phosphorus; Red arrows represent soil environmental factors; while blue arrows indicate bacteria harboring the functional genes *gcd* and *phoD*.

3.4.5. Prediction of Microbial Functions and Their Relationship with Soil Chemical Properties

Analysis of microbial functions revealed clear differences in soil microbial communities across different phosphorus tailings treatments. The CK treatment showed a strong capacity for denitrification, whereas the T3 treatment was characterized by a significant ability to degrade aromatic compounds (Figure 7a). Correlation analysis between microbial functions and soil chemical properties indicated that pH, soil organic matter (SOM), available nitrogen (AN), total phosphorus (TP), available phosphorus (AP), and soluble phosphorus (SP) were positively associated with aromatic compound degradation, while electrical conductivity (EC) was negatively associated. Furthermore, soil pH was positively correlated with chitin decomposition but negatively correlated with denitrification (Figure 7b).

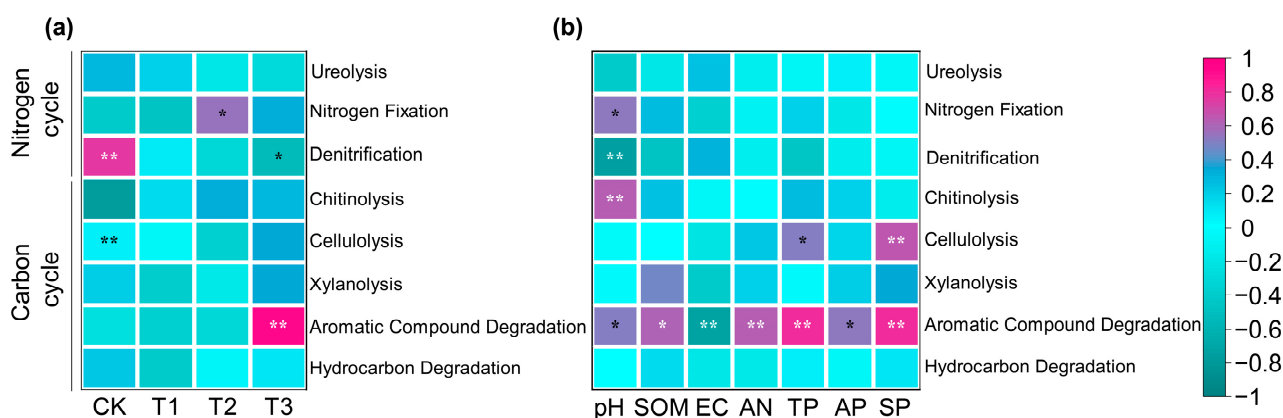


Figure 7. Relationships between microbial functions and soil chemical characteristics under various treatments. (a) Variations in microbial functional profiles among treatments; (b) Correlations between microbial functions and soil chemical properties. The color gradient represents Pearson correlation coefficients. Significance levels: * $p < 0.05$; ** $p < 0.01$. Abbreviations: pH, soil pH; SOM, soil organic matter; EC, cation exchange capacity; AN, available nitrogen; TP, total phosphorus; AP, available phosphorus; SP, soluble phosphorus.

3.5. Correlation Between Soil Chemical Properties and Yield Stratification

This study used Spearman correlation analysis (Figure 8a–d) to explore the relationships between corn yield and soil chemical properties at various soil depths, as well as the interrelationships among these properties. The findings revealed distinct layer-specific patterns: in the 0–10 cm surface layer, corn yield was significantly positively correlated with soil pH and ammonium nitrogen ($\text{NH}_4^+\text{-N}$). At the 10–20 cm depth, yield showed significant positive correlations with soil pH, soil organic matter (SOM), and $\text{NH}_4^+\text{-N}$. In the deeper layers (20–30 cm and 30–40 cm), yield was significantly positively correlated with nitrate nitrogen ($\text{NO}_3^-\text{-N}$) and $\text{NH}_4^+\text{-N}$. Significant correlations among soil properties were mainly observed in the 0–10 cm and 10–20 cm layers. Soil pH was significantly positively correlated with SOM and $\text{NH}_4^+\text{-N}$ at 10–20 cm. Total phosphorus (TP) had significant positive correlations with available nitrogen (AN), $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, but a significant negative correlation with electrical conductivity (EC). Available phosphorus (AP) was significantly positively correlated with $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and TP, while soil phosphorus (SP) showed significant positive correlations with AN, TP, and AP in the 0–10 cm layer.

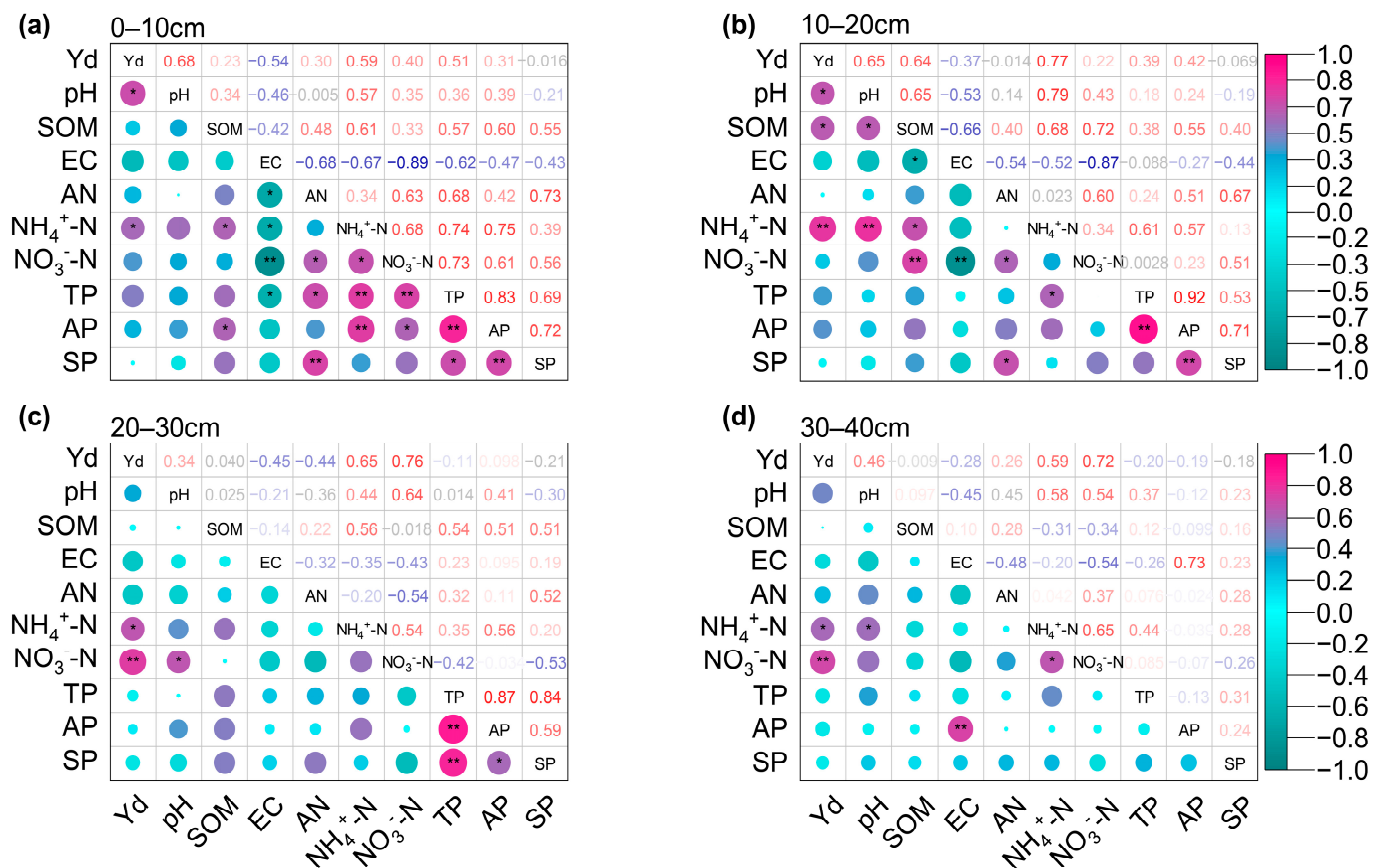


Figure 8. Correlation between soil chemical characteristics and corn yield. (a) 0–10 cm soil layer; (b) 10–20 cm soil layer; (c) 20–30 cm soil layer; (d) 30–40 cm soil layer; Yd: Corn yield; pH: Soil pH; SOM: Soil organic matter; EC: Soil cation exchange capacity; AN: Available nitrogen; $\text{NH}_4^+\text{-N}$: Ammonium nitrogen; $\text{NO}_3^-\text{-N}$: Nitrate nitrogen; TP: Total phosphorus; AP: Available phosphorus; SP: Soluble phosphorus. The color gradient represents the correlation coefficient values (red indicates positive correlations, blue indicates negative), and the size of the dots corresponds to the strength of the correlation. Asterisks * and ** indicate significance levels of $p < 0.05$ and $p < 0.01$, respectively.

3.6. Key Pathways Through Which Phosphorus Tailings Regulate Corn Yield and Soil Bacterial Functions

The surface soil layer showed the most significant changes in chemical properties, such as pH and available phosphorus, as well as in the abundance of phosphorus-solubilizing bacteria containing the *gcd* and *phoD* genes after treatment with phosphorus tailings. The interactions between phosphorus tailings, soil, and microorganisms in this layer played a crucial regulatory role in influencing maize productivity. To quantify the direct and indirect effects of soil properties, pH, and the *gcd*/*phoD*-bearing bacterial community on maize yield, we used structural equation modeling (SEM), which helped clarify the main pathways by which phosphorus tailings enhance yield (Figure 9). The SEM results indicated a good model fit (goodness-of-fit index = 0.647), confirming the robustness of the findings.

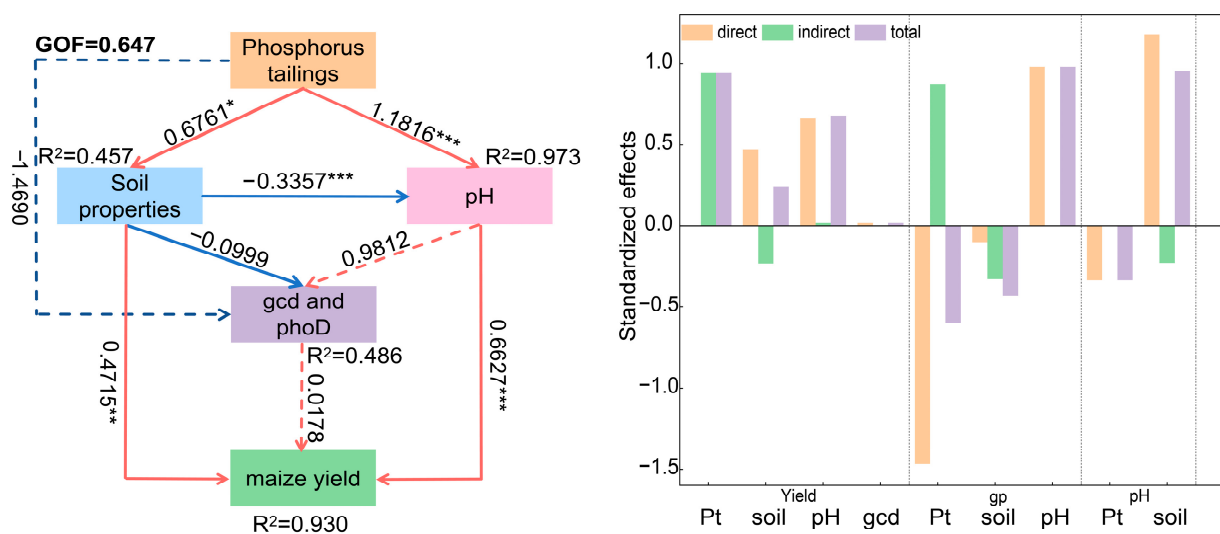


Figure 9. Displays an analysis conducted with partial least squares path modeling (PLS-PM) to investigate the impact of soil characteristics on seasonal corn yield. Solid arrows represent statistically significant positive path coefficients, while dashed arrows indicate non-significant coefficients, based on a p -value cutoff of 0.05. The numbers next to the arrows show standardized path coefficients. The box plot depicts the loadings between latent variables—phosphorus tailings, soil properties, soil pH, and phosphorus-accumulating bacterial communities carrying *gcd* and *phoD* genes—and the observed variable, yield. Here, R^2 denotes the proportion of variance in crop yield explained by the model. GOF stands for goodness of fit. The panel on the right illustrates the direct, indirect, and total effects. Abbreviations: Pt for phosphorus tailings; soil for soil properties; gp for phosphorus-accumulating bacterial communities linked to the *phoD* gene; Yield for corn yield. Significance levels are indicated as * for $p < 0.05$; ** for $p < 0.01$; and *** for $p < 0.001$; Orange box: Phosphorus tailings; Blue box: Soil properties; Pink box: Soil pH; Purple box: Bacteria carrying *gcd* and *phoD* functional genes; Green box: corn yield.

The path coefficients from the SEM showed that phosphorus tailings had a significant positive impact on soil properties, particularly a strong positive effect on soil pH. Soil properties also had a highly significant positive influence on pH. Both soil properties and pH significantly and positively affected corn yield, with soil pH exerting a notably strong effect. In contrast, the abundance of phosphorus-solubilizing bacteria carrying the *gcd* and *phoD* genes had only a slight positive effect on yield, which was not statistically significant ($p > 0.05$). According to the model, the total effect sizes of each factor on corn yield were phosphorus tailings, 0.9408; soil pH, 0.6801; soil properties, 0.2414; and the *gcd*/*phoD* bacterial community, 0.0178. Therefore, the factors' relative importance in descending order was phosphorus tailings > soil pH > soil properties > *gcd*/*phoD* phosphorus-solubilizing bacterial community.

4. Discussion

4.1. Impact of Phosphorus Tailings on Corn Yield and Quality

Phosphate tailings, a calcium-magnesium byproduct generated from phosphate mining, represent a resource that can be utilized effectively. This study found that, compared to the control group (CK), various treatments involving phosphate tailings significantly increased corn yields. The most effective treatment (T3: 80% conventional fertilizer combined with phosphate tailings and organic fertilizer) resulted in a 13.72% increase in corn yield and a 6.02% increase in 100-kernel weight relative to CK ($p < 0.05$). These findings are consistent with Mao et al. [38], who reported that converting dolomite phosphate tailings (DPR) into controlled-release phosphorus fertilizer notably boosted biomass production in corn and sorghum. The yield enhancement is attributed to the slow-release phosphorus properties of phosphate tailings. When used alongside organic fertilizers, the organic acids produced during decomposition help dissolve the insoluble phosphate in the tailings and reduce phosphorus fixation and leaching. For example, Shao et al. [39] showed that low-grade phosphate rock (APR) activated with sodium lignosulfonate (SL) and humic acid (HA) reduced phosphorus accumulation loss by 65.2% and 65.3%, respectively, compared to calcium superphosphate treatment. Additionally, Dai et al. [40] developed an acid-activated phosphorus tailings-based controlled-release soil agent (SLPs) that effectively controlled phosphorus release, increasing soil available phosphorus from 0.23 mg/g to 2.53 mg/g and soil organic matter (SOM) from 8.6 g/kg to 40.19 g/kg. This significantly enhanced soil phosphorus and nitrogen availability, thereby promoting corn growth [41]. Beyond yield improvements, the T3 treatment also significantly enhanced corn quality. Compared to CK, T3 increased protein content by 18.04% and crude fiber content by 9.00% in corn kernels ($p < 0.05$). This improvement is likely due to the comprehensive nutrient supply provided by organic fertilizers, which not only contain abundant organic matter but also trace elements such as calcium, magnesium, and sodium that help balance soil nutrients and improve nutrient uptake by corn. For instance, Fang et al. [42] found in sweet corn studies that reducing chemical fertilizer application while using organic fertilizers significantly increased vitamin C, soluble protein, soluble sugar, and starch content—trends that align with the quality enhancements observed here. Furthermore, Paramesh et al. [43] demonstrated that combining inorganic amendments (like phosphorus tailings with reduced chemical fertilizer) and organic fertilizers synergistically improved soil nutrient cycling, creating favorable conditions for both increased corn yield and quality through the accumulation of metabolic products.

4.2. Impact of Phosphorus Tailings on Soil Bacterial Community Structure and Function

Soil microbial communities are fundamental drivers of soil ecosystem functions, with their diversity and structural variations directly indicating soil health and nutrient transformation efficiency [44]. Although phosphorus tailings are industrial waste products, their effects on soil microorganisms in acidic red soils have not been thoroughly studied. This research utilized 16S Illumina sequencing technology, specifically high-throughput Illumina MiSeq sequencing of 16S rRNA genes, to assess how different phosphorus tailings treatments influence soil bacterial communities. The results showed that bacterial α -diversity, measured by the Chao1 and Shannon indices, increased by 177.57% and 37.80%, respectively, under the T3 treatment compared to the control (CK). Importantly, T3 significantly outperformed T2 in enhancing microbial diversity, with the Chao1 index rising by 35.86% and the Shannon index by 9.51%. This demonstrates that combining phosphorus tailings with organic fertilizer is more effective at boosting bacterial diversity and richness than using phosphorus tailings alone. Additionally, β -diversity analysis (Figure 4c) revealed that different phosphorus tailings treatments substantially altered

the soil microbial community structure, distinctly separating them from the CK treatment. This observation is consistent with previous studies showing that phosphorus tailings application can modify soil microbial community composition [45]. Under T3 treatment, the relative abundance of *Proteobacteria* and *Bacteroidota* increased significantly by 34.36% and 14.70%, respectively, compared to CK, while Firmicutes decreased by 18.62%. This shift likely results from the combined effect of phosphorus tailings and organic fertilizer, which not only supply abundant carbon sources for microbes but also improve soil aeration and nutrient balance, selectively enriching carbon-utilizing groups such as *Proteobacteria* and *Bacteroidota*. Functional predictions (Figure 7) indicate that T3 treatment notably enhances the degradation of aromatic compounds. This corresponds with the increased abundance of *Proteobacteria* and *Bacteroidota*, dominant phyla involved in efficient organic matter breakdown, which drive the decomposition of complex organic compounds like aromatics, thereby accelerating soil carbon and nitrogen cycling [46,47]. Tang et al. [48] also showed that organic fertilizer application modifies soil properties, influencing microbial community structure.

The study further examined functional microorganisms involved in phosphorus transformation, focusing on carriers of the *gcd* and *phoD* genes, based on Zhao et al.'s [49] work on microbial communities linked to organic phosphorus mineralization (*phoD*) and inorganic phosphorus solubilization (*gcd*). Screening identified dominant phosphorus-cycling bacteria from the *Rhizobiaceae*, *Pseudomonadaceae*, *Comamonadaceae*, *Oxalobacteraceae*, *Micromonosporaceae*, and *Caulobacteraceae* families. Redundancy analysis (Figure 6a) highlighted soil alkalinity (AN), ammonium nitrogen ($\text{NH}_4^+\text{-N}$), available phosphorus (AP), and ecological capacity (EC) as key factors regulating the structure of phosphorus-cycling microbial communities. Notably, treatments combining reduced chemical fertilizer with phosphorus tailings (T2 and T3) significantly increased the relative abundance of *gcd*- and *phoD*-harboring phosphorus-cycling bacteria compared to the control, with T3 showing the greatest effect (Figure 6a). This aligns with findings by Yu [37] and Zhou [50], who reported that optimized phosphorus inputs—such as sustained-release phosphorus from tailings—combined with organic amendments can synergistically promote the enrichment of phosphorus-solubilizing microbes. The elevated abundance of these functional bacteria under T3 further enhanced soil phosphorus cycling capacity, providing a biological foundation for sustained phosphorus availability to crops.

Importantly, this study covers only a two-year observation period, while soil microbial succession is naturally a long-term ecological process. The observed increases in bacterial diversity and changes in community composition under the T3 treatment probably reflect the microbial community's initial adaptation to enhanced soil conditions, such as higher pH, greater phosphorus availability, and added organic carbon. However, it is still uncertain whether these changes will lead to a stable, directional succession or if they will experience temporary fluctuations. Therefore, extended monitoring over a longer period is necessary to determine the progression of microbial succession and the consistency of their functional roles.

4.3. Mechanism Linking Soil Properties, Microbial Functions, and Corn Yield

Using Spearman correlation analysis, this study explored the relationships between soil microbial community functions and environmental factors under various phosphorus tailings treatments, aiming to clarify how environmental factors regulate these functions and to reveal the intrinsic connections between microbial activity and maize yield. The results showed that in the top root zones (0–10 cm and 10–20 cm soil layers), soil pH and ammonium nitrogen ($\text{NH}_4^+\text{-N}$) were significantly positively correlated with yield. Additionally, in the 10–20 cm layer, soil organic matter (SOM) also exhibited a significant

positive correlation with crop production. These findings confirm that applying phosphorus tailings and organic fertilizers boosts crop yields by alleviating soil acidification (raising pH) and enhancing nutrient availability (increasing $\text{NH}_4^+\text{-N}$ and SOM). However, in the deeper 20–40 cm soil layer, only nitrate nitrogen ($\text{NO}_3^-\text{-N}$) and $\text{NH}_4^+\text{-N}$ showed significant positive correlations with yield, suggesting that nutrients in deeper soil are less influenced by treatments and play a secondary role in yield formation. Previous research has identified soil pH, SOM, available nitrogen (AN), total phosphorus (TP), available phosphorus (AP), soil phosphorus (SP), and electrical conductivity (EC) as significant factors affecting carbon cycling functions in soil microbial communities, with soil pH being the primary regulator of nitrogen cycling. This aligns with the meta-analysis by Zhong et al. [51], which found the strongest link between soil pH changes and the abundance of microbial nitrogen cycling genes. In contrast, Wang et al. [52] reported that total organic carbon (TOC), total nitrogen (TN), and heavy metals (Zn/Cd) were dominant in controlling carbon-phosphorus cycling in abandoned lead-zinc mining soils. The discrepancy arises from differences in soil disturbance types: Wang's study focused on heavy metal-contaminated soils, whereas this study examined acidic soils remediated with phosphorus tailings. The carbon-nitrogen cycling functions of soil microbes act as a crucial link between soil health and crop productivity. Li et al. [53] demonstrated that microbial carbon-nitrogen cycling efficiency directly influences crop growth and yield. Nonetheless, soil phosphorus content also affects these functions. Notably, Wang et al. [54] found that soil physicochemical properties, rather than microbial communities, primarily regulate carbon and nitrogen cycling.

To further elucidate causal relationships following phosphorus tailings application, partial least squares path modeling (PLS-PM) was used (Figure 9). Structural equation modeling (SEM) is a robust method for testing hypothesized causal links by assessing how well a proposed model fits observed data [55,56]. The analysis revealed that phosphorus tailings indirectly enhance maize yield through two main pathways: first, by significantly increasing soil pH to reduce acidification in red soils; second, by substantially improving soil physical and chemical properties, thereby boosting nutrient availability. Soil properties also showed a strong positive influence on soil pH, indicating that phosphorus tailings can synergistically optimize pH by enhancing overall soil nutrient status. Among factors directly driving yield, soil pH had the strongest positive effect, followed by soil properties, while phosphoregulative bacteria harboring *gcd* and *phoD* genes had only marginal positive effects. This causal pathway—phosphorus tailings → soil pH/soil properties → maize yield—constitutes the core regulatory mechanism in treatment T3. The limited impact of *gcd/phoD*-containing phosphoregulative bacteria may reflect short-term experimental limitations, but their potential role in long-term soil phosphorus cycling deserves further attention [57].

4.4. Research Limitations

It is important to note that the results presented here come from a relatively short-term (two-year) field experiment. Although notable changes in soil microbial community structure, diversity, and specific functional potentials were observed in response to the combined phosphorus tailings and organic fertilizer treatment (T3), the long-term development and stability of these microbial successions have yet to be confirmed. The limited direct influence of *gcd/phoD*-containing phosphorus-solubilizing bacteria on yield in our structural equation model (Figure 9), despite their increased abundance under T3, may be due to the relatively brief duration of the study. A two-year timeframe might not be sufficient to fully capture delayed or cumulative microbial functional responses to changes in the soil environment (such as pH increase and nutrient availability), especially regarding

the formation of stable, functionally effective microbial communities involved in complex processes like phosphorus cycling and their subsequent effects on plant growth. Future research with longer monitoring periods is necessary to confirm the persistence of these microbial community changes and their lasting impact on soil health and crop productivity in acidic red soils.

Additionally, this study was carried out in a specific experimental field with controlled plot sizes. Soil properties, microbial communities, and crop responses can exhibit much greater spatial variability in larger, more heterogeneous agricultural landscapes or under different management histories. The magnitude of the observed effects may differ in such settings. Therefore, caution should be exercised when applying these findings to broader regions, and validation across diverse field environments with varying inherent spatial variability is recommended.

5. Conclusions

This study investigated an acidic red soil corn system and assessed the combined effects of phosphorus tailings and organic fertilizers over a two-year field experiment. The findings revealed that phosphorus tailings had a significant impact on the diversity, composition, and structure of soil microbial communities. When fertilizer use was reduced by 20% and phosphorus tailings were applied together with organic fertilizers, soil pH and phosphorus availability improved notably. These improved soil conditions promoted the gradual restoration of bacterial diversity, network stability, and aromatic compound degradation, with a specific increase in phosphorus-degrading bacterial groups containing phosphoredoxin (*gcd*) and *phoD* genes. Phosphorus tailings indirectly boosted corn yield by synergistically regulating soil properties and pH, contributing a total effect value of 0.9408. Even with a 20% reduction in fertilizer, treatment T3 significantly outperformed the control (CK), achieving a 13.72% increase in corn yield, a 6.02% higher hundred-grain weight, an 18.04% greater protein content, and a 9.00% decrease in crude fiber content, thereby fulfilling the goals of “reducing fertilizer use, utilizing phosphorus tailings resources, and improving crop quality through synergistic enhancement.” Since this study only covered two years, the microbial responses observed provide just an initial glimpse. Therefore, extended long-term research is essential. Future studies should assess the environmental effects of applying phosphorus tailings, such as possible heavy metal buildup, the stability of microbial functions, and the ongoing availability of phosphorus. Additionally, long-term research is necessary to fully understand any delayed microbial functional changes and their lasting impact on soil fertility and crop productivity. Overall, these results offer a practical approach and scientific basis for the combined use of phosphorus tailings and organic fertilizers in farmland, supporting sustainable management of acidic soils.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

SOM	Soil organic matter
AN	Available nitrogen
TP	Total phosphorus
AP	Available phosphorus
SP	Soil soluble phosphorus
EC	Cation exchange capacity
SEM	Structural equation model

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