

Effects of nitrogen fertilization on soil phosphorus speciation and soil microbial communities in different rotation cropping systems

Zi Yang

Huazhong Agricultural University

Chenyang Cui

Huazhong Agricultural University

Xiaoyang Chao

Huazhong Agricultural University

Di Zheng

Huazhong Agricultural University

Tao Ren

Huazhong Agricultural University

Jianwei Lu

Huazhong Agricultural University

Xiaoming Wang

Huazhong Agricultural University

Wenfeng Tan

Huazhong Agricultural University

Xionghan Feng

fxh73@mail.hzau.edu.cn

Huazhong Agricultural University

Research Article

Keywords: Upland-upland rotation, Paddy-upland rotation, Nitrogen fertilization, Available Phosphorus, Microbial community structure

Posted Date: September 7th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10840035/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Purpose

Crop rotations (upland-upland and paddy-upland) are widely practiced in the Yangtze River Basin, where water and fertilization management strongly influence soil phosphorus (P) dynamics. However, the mechanisms by which nitrogen (N) fertilization strategies affect soil P speciation and microbial communities across these systems remain unclear.

Methods

Based on an 11-year field experiment, this study investigated soil P speciation, microbial communities, and soil physicochemical properties under different N regimes (single-season vs. dual-season) in the paddy-upland (rice-oilseed rape, RO) and upland-upland (corn-oilseed rape, CO) rotations. Using sequential P fractionation and high-throughput sequencing, we examined the responses of soil P speciation and microbial communities to varying N fertilization strategies.

Results

N fertilization significantly enhanced soil P availability. The balanced dual-season fertilization (N150-150) achieved the highest available P (AP) in RO (30.26 mg/kg), nearly double that in CO (14.79 mg/kg). N fertilization decreased soil pH in CO but maintained near-neutral pH in RO. Rotation mode was the primary driver of microbial community structure, with distinct taxa enriched in each system (*Actinobacteria* in CO, *Chloroflexi* in RO). Soil pH drove microbial variation in CO, whereas soil organic matter and AP were key drivers in RO.

Conclusion

Water management serves as the overarching determinant of soil environment, within which N fertilization acts as a key regulatory factor. The balanced dual-season N application (N150-150) was associated with the highest P availability and microbial diversity in the rice-oilseed rape rotation system. N fertilization improves P availability by modulating soil properties and microbial communities, and the balanced dual-season strategy synergistically boosts P supply, promotes soil biodiversity, and supports sustainable cropping intensification in the Yangtze River Basin.

1 Introduction

Paddy-upland rotations (e.g., rice-oilseed rape) and upland-upland rotations (e.g., corn-oilseed rape) are two predominant cropping systems in China's Yangtze River Basin. These systems establish distinct soil microenvironments governed by their contrasting water management regimes (Li et al., 2021). In paddy-

upland rotations, periodic drying and wetting induce frequent fluctuations in soil redox potential, which directly regulates the speciation of phosphorus (P) fractions (Patrick and Khalid, 1974). In contrast, upland-upland rotations maintain a relatively stable aerobic environment that favors the mineralization of organic P (Richardson and Simpson, 2011). These fundamental differences in water regime and redox conditions are expected to create divergent contexts for P dynamics, yet how they interact with long-term N fertilization to shape P fractions and associated microbial communities remains poorly understood.

Soil P availability is a key constraint on global agricultural productivity, through processes including adsorption, precipitation, and biological immobilization, the majority of soil P is rendered unavailable for plant uptake (He et al., 2023), and its regulation differs fundamentally between the two cropping systems due to their contrasting redox environments (Turner and Kim, 2024; Zhou et al., 2016). In paddy-upland rotations, periodic flooding promotes the reductive dissolution of iron-bound P (Fe-P), potentially increasing P availability, while drainage and oxygenation may lead to P re-precipitation (Patrick and Khalid, 1974). A study of sediments in the Chesapeake Bay highlighted the high heterogeneity of P speciation, with iron-bound P and organic P as the dominant forms (Li et al., 2015). In upland-upland rotations, the consistently aerobic environment favors stable P adsorption onto Fe and Al oxides, leading to P fixation and lower availability (Hinsinger, 2001). Consequently, a “phosphorus paradox” prevails in both systems: soils often contain high total P reserves yet supply critically low levels of plant-available P, presenting a fundamental challenge to sustainable agriculture (Richardson et al., 2009). Understanding how N fertilization influences P availability within these two distinct hydrological frameworks is therefore of critical importance.

Nitrogen (N) and P are essential nutrient elements and key factors limiting primary productivity (Zavaleta et al., 2003; LeBauer and Treseder, 2008). The balance between N and P supply is critical for maintaining the stability of terrestrial ecosystems (Li et al., 2020; Yang, 2018). Increased anthropogenic inputs of N and P, driven by human activities and global change, profoundly influence nutrient cycling between soil and plants, thereby altering ecosystem structure and function (Galloway et al., 1996; Bennett et al., 2001; Zhu et al., 2016). Studies have shown that N fertilization generally enhances P availability, with different N fertilizers exert distinct effects. For instance, N application can promote root growth and improve the plant capacity for P acquisition (Grunes, 1959). In the context of contrasting cropping systems, N fertilization may exert system-specific effects on P dynamics due to differences in soil pH regulation, redox conditions, and microbial community composition. For instance, ammonium-based fertilization can lower soil pH and enhance P dissolution in upland systems, while in paddy systems, flooding suppresses nitrification and promotes H^+ -consuming processes that buffer pH changes (Ortas and Rowell, 2004; Kögel-Knabner et al., 2010; Jing et al., 2012; Chialva et al., 2020). In pursuit of high crop yields, excessive application of N and P fertilizers has led to substantial nutrient accumulation in soils, causing severe environmental issues such as eutrophication and harmful algal blooms (Heckrath et al., 1995; Geohring et al., 2001; Powley et al., 2018). Consequently, strategies for rational fertilizer application and controlling nutrient inputs have attracted an increasing attention.

The effects of N fertilization on soil P fractions are likely to differ between paddy-upland and upland-upland rotations due to their contrasting soil chemical environments. N fertilization influences soil P speciation through both direct and indirect pathways. Directly, N fertilization alters soil pH, thereby affecting the dissolution–precipitation equilibria of inorganic P pools; specifically, combined N and P addition lowers pH and ionic strength, activating $\text{Fe}^{3+}/\text{Al}^{3+}$ and increasing Fe-P and Al-P, whereas in calcareous soils, N addition enhances occluded P (O-P) (Zhang et al., 2022). Indirectly, N modulates root exudation and microbial activity, mediating biological P mobilization. In upland systems, acidification resulting from nitrification can promote the dissolution of Fe-P and Al-P, increasing P availability. (Gillespie and Pope, 1991). In paddy systems, the anaerobic environment favors the reductive dissolution of Fe-P, and N inputs may further stimulate this process by promoting iron-reducing microbial activity (Cui et al., 2024; Wang et al., 2021). Despite these advances, most existing studies have focused on single cropping systems or short-term experiments, leaving a critical knowledge gap regarding how long-term N fertilization interacts with different rotation systems, particularly those contrasting in water management, to shape soil P fraction dynamics.

Soil microorganisms are highly sensitive to environmental changes and serve as crucial indicators of soil health, playing fundamental roles in maintaining biodiversity and stabilizing ecosystem functions (Falkowski et al., 2008). In the context of P nutrition, microbial-augmented fertilizers—those amended with functional microorganisms—offers distinct advantages, including being environmentally friendliness, high nutrient use efficiency, and alignment with sustainable agricultural objectives (Feng et al., 2023). Importantly, soil microorganisms are not merely responders to environmental changes but also key mediators of P fractions, linking P fractions to P availability through processes such as mineralization of organic P and solubilization of fixed inorganic P (Richardson and Simpson, 2011). Increasing N fertilization in agriculture has exacerbated nitrogen-phosphorus (N:P) imbalance in soil, drawing research attention to its effects on soil microbial communities (Deng et al., 2012). Different nutrient management strategies, including N fertilization, can induce substantial alterations in the soil environment (Xue et al., 2025). N fertilization alters the living environment of soil microorganisms by directly changing soil chemical conditions (e.g., pH and available N concentration) and indirectly affecting stoichiometric balance (e.g., C:N:P ratios), thereby influencing the overall structure, diversity, and function of soil microbial communities, which in turn impacts key ecosystem processes such as P cycling (Zhu, 2008; Rousk et al., 2010; Ju and Gu, 2017; Oliverio et al., 2020). Given the contrasting soil conditions between paddy-upland and upland-upland rotations, the regulatory mechanisms and ecological effects of N fertilization on soil microbial communities are likely to differ significantly between these two systems, yet this interaction remains underexplored. The application of 16S rRNA gene sequencing has been widely employed to characterize the composition and functional potential of soil microbial communities under such perturbations (Stackebrandt and Goebel, 1994; Wang et al., 2023a; Barberán et al., 2012).

To date, most studies on N fertilization have focused on single-season crops or short-term rotations, limiting our understanding of how long-term rotation practices influence microbial community dynamics (Geisseler and Scow, 2014; Wang et al., 2023a). In particular, the effects of progressive soil N fertilization

concentration gradient on the assembly and succession of microbial communities under long-term rotation practices remain poorly understood (Zeng et al., 2016). Although existing evidence suggests that N fertilization can stimulate microbial-mediated P release (Richardson and Simpson, 2011; Jing et al., 2021), several key knowledge gaps persist. These include: (1) how the coupling between P fractions and microbial communities differs between paddy-upland and upland-upland rotations under long-term fertilization; (2) the successional dynamics of phosphorus-solubilizing microbial communities in environments rich in insoluble P speciation (e.g., Fe-P, Al-P, O-P, and Ca-P); and (3) the extent to which system-specific environmental filters (e.g., redox regime, pH) shape the relationship between P fractions and microbial community assembly. Addressing these gaps is essential for understanding how N fertilization modulates the coupled P-microbial dynamics in diverse cropping systems.

To address these knowledge gaps, this study investigated the responses of soil physicochemical properties and microbial communities to N fertilization under two contrasting cropping rotation systems over an 11-year long-term experiment. We aimed to (1) determine how varying N fertilization rates and application methods (single-season vs. dual-season) influence soil properties, P fractions, and microbial community structure under different water regimes (paddy-upland vs. upland-upland), and (2) identify the underlying mechanisms and key drivers of the observed responses. Based on the existing knowledge gaps and the design of this long-term experiment, we hypothesized that: (1) water management regime serves as the primary determinant of both soil P fraction distribution and microbial community structure; (2) N fertilization rates and application methods differentially modulate soil P availability and microbial community composition, with effects depending on the specific water regime; and (3) the balanced dual-season N application strategy (N150-150) is associated with higher P availability and microbial diversity, reflecting a closer coupling between P fractions and microbial communities. This research is expected to provide theoretical insights and practical strategies for optimizing farmland nutrient management, enhancing P use efficiency, mitigating P resource waste, and supporting sustainable agricultural development.

2 Materials and methods

2.1 Study site

This study was conducted at the long-term experimental station of Huazhong Agricultural University, located in Wuhan, Hubei Province (30°28'10"N, 114°21'21"E). The station was established in 2012 and is situated in a subtropical monsoon climate zone, characterized by a mean annual temperature of approximately 16°C and mean annual precipitation of around 1200 mm. The soil at the experimental site is classified as a yellow-brown earth according to the Chinese Soil Taxonomy, which corresponds to an Alfisol in the USDA Soil Taxonomy.

Two cropping systems were examined: a paddy-upland rotation (rice-oilseed rape, RO) and an upland-upland rotation (corn-oilseed rape, CO). The experiment was arranged in a randomized complete block design with three replicates. Each plot measured 4 m × 5 m (20 m²), and the layout of the experimental

plots is shown in Tables S1 and S2. The initial soil physicochemical properties at the commencement of the experiment were as follows: under the paddy-upland rotation, soil pH was 6.53, soil organic matter (SOM) 17.99 g kg⁻¹, total nitrogen (TN) 1.09 g kg⁻¹, and available phosphorus (AP) 21.39 mg kg⁻¹; under the upland-upland rotation, soil pH was 6.56, SOM 11.15 g kg⁻¹, TN 0.79 g kg⁻¹, and AP 12.86 mg kg⁻¹.

2.2 Soil sample collection

The experimental field was uniformly divided into two main plots designated for the RO and CO rotation systems, respectively. A 1-meter-wide ridge accompanied by a drainage furrow was constructed between the two rotation areas to mitigate potential waterlogging effects from the paddy field on the corn growth during the rice season.

For each rotation system, five N fertilization treatments were implemented: (1) No N fertilization in both rice/corn and rapeseed seasons (N0-0); (2) 75 kg N hm⁻² applied only in the rice/corn season (N75-0); (3) 150 kg N hm⁻² applied only in the rice/corn season (N150-0); (4) 225 kg N hm⁻² applied only in the rice/corn season (N225-0); and (5) 150 kg N hm⁻² applied in both the rice/corn and rapeseed seasons (N150-150). The experiment was conducted continuously over 11 complete rotation cycles from 2012 to 2023. Across all treatments, P fertilizer (P₂O₅) and potassium fertilizer (K₂O) were uniformly applied at rates of 75 kg hm⁻² and 120 kg hm⁻², respectively, during each crop season (rice, corn, and rapeseed). To support normal crop growth, all treatments also received a uniform application of borax at 15 kg hm⁻².

Soil samples from the 0–20 cm plow layer were collected from each plot using a soil auger following the five-point sampling method. Three replicate samples were randomly taken per treatment. Visible plant roots and gravel were removed by sieving the soil through a 10-mesh sieve. The samples were then sealed, stored in a cooling box, and transported to the laboratory, where they were immediately frozen at -80°C for subsequent microbial analysis. Another portion of each soil sample was air-dried in a well-ventilated shade. The air-dried soil samples were then ground and passed through 10-, 20-, and 100-mesh sieves. The 10-mesh fraction was used for soil pH determination, the 20-mesh fraction for AP analysis, and the 100-mesh fraction for measurements of soil total carbon (TC), TN, soil inorganic P fractions, and other physicochemical properties.

2.3 Soil sample analysis

2.3.1 Soil physicochemical properties and soil phosphorus fractionation

Soil TN and TC contents were determined using an elemental analyzer. Since the soil at the experimental site is classified as an Alfisol in USDA Soil Taxonomy and is non-calcareous (pH ~ 6.5) due to strong leaching under a humid subtropical climate, TC was considered equivalent to soil organic carbon (SOC) (Allison, 1965). Soil organic matter (SOM) content was estimated by multiplying SOC by the Van Bemmelen conversion factor of 1.724, based on the assumption that organic matter contains approximately 58% organic carbon (i.e., $\text{SOC} \times 1.724 = \text{SOM}$) (Bao, 2000; Pribyl, 2010; Minasny et

al.,2020). Total phosphorus (TP) was measured following digestion with a mixture of HClO_4 and H_2SO_4 . AP was extracted with $0.5 \text{ mol}\cdot\text{L}^{-1}$ sodium bicarbonate (NaHCO_3 , pH 8.5) and quantified via the molybdenum blue colorimetry. Soil pH was measured potentiometrically using a water-to-soil ratio of 2.5:1 (Bao, 2000).

Soil inorganic P fractions were analyzed using the sequential fractionation method proposed by Chang and Jackson (1957). Briefly, aluminum-bound phosphorus (Al-P) was first extracted with $0.5 \text{ mol}\cdot\text{L}^{-1}$ NH_4F . After washing the residual soil with saturated NaCl, iron-bound phosphorus (Fe-P) was extracted with $0.1 \text{ mol}\cdot\text{L}^{-1}$ NaOH. Subsequently, occluded phosphorus (O-P) was extracted using $0.3 \text{ mol}\cdot\text{L}^{-1}$ sodium citrate and sodium dithionite, and finally, calcium-bound phosphorus (Ca-P) was extracted with $0.5 \text{ mol}\cdot\text{L}^{-1}$ H_2SO_4 . All extractions were performed under constant shaking conditions at room temperature, and P concentrations in the extracts were determined by the molybdenum blue colorimetric method.

In this study, we focused on soil inorganic P fractions rather than organic P based on the following considerations. First, the sequential fractionation method of Chang and Jackson (1957) is specifically designed for the separation of soil inorganic P pools and is widely used for assessing P availability and speciation in agricultural soils. Second, given that the experimental site is characterized by a humid subtropical climate with strong leaching, soil organic P typically accounts for a relatively small proportion of TP in such highly weathered soils (Cross and Schlesinger, 1995). Third, our preliminary analyses indicated that changes in soil inorganic P fractions were more responsive to long-term N fertilization and rotation practices, thereby providing more direct insights into P availability dynamics. This methodological focus aligns with the primary objectives of this study, which aim to investigate the effects of N fertilization on soil P availability and its underlying mechanisms.

2.3.2 Soil bacterial 16S rRNA gene sequencing and analysis

2.3.2.1 DNA extraction

Total genomic DNA was extracted from soil samples using the E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.) in accordance with the manufacturer's protocols. The quality of the extracted DNA was assessed by 1% agarose gel electrophoresis, and its concentration and purity were measured with a NanoDrop2000 ultra-micro spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.3.2.2 PCR amplification and sequencing library preparation

The hypervariable V3-V4 region of the bacterial 16S rRNA gene was amplified with the barcoded primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Liu et al., 2016). The PCR reaction was performed in a 20 μL mixture containing 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, 0.2 μL of BSA, and 10 ng of template

DNA, with the volume adjusted to 20 μ L using ddH₂O. The thermal cycling conditions comprised an initial denaturation at 95°C for 3 min; 27 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s; followed by a final extension at 72°C for 10 min and a hold at 10°C (Thermocycler: ABI GeneAmp® 9700). The resulting PCR products were visualized and excised from 2% agarose gels, purified with a DNA Gel Extraction Kit (YuHua, China), and quantified using a Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA). Sequencing libraries were constructed from the purified amplicons using the NEXTFLEX Rapid DNA-Seq Kit, following a standard protocol that included adapter ligation, purification of ligated products to remove adapter dimers, library amplification, and final purification.

2.3.2.3 Bioinformatic analysis of sequencing data

Raw paired-end sequencing reads were quality-filtered and trimmed using the fastp software (version 0.19.6) (Chen et al., 2018). The processing step included: (1) trimming 3'-end bases with a quality score below 20; (2) employing a 10 bp sliding window to truncate reads once the average quality score in the window fell below 20; (3) discarding reads shorter than 50 bp or containing ambiguous nucleotides ('N'). Quality-filtered paired-end reads were then merged using FLASH software (version 1.2.11), requiring a minimum overlap length of 10 bp and a maximum mismatch ratio of 0.2 in the overlap region. Demultiplexing (allowing no mismatches in barcodes) and primer sequence correction (allowing up to 2 nucleotide mismatches) were applied to assign sequences to corresponding samples and correct orientation (Liu et al., 2015).

Operational Taxonomic Units (OTUs) were clustered from the high-quality sequences at a 97% similarity threshold using the UPARSE pipeline (v11.0.667), with chimeric sequences detected and removed during the process (Edgar, 2013). To standardize sequencing efforts across samples, all datasets were rarefied to 39,517 sequences per sample prior to downstream alpha and beta diversity analyses. Taxonomic assignment of the OTUs was performed using the RDP classifier (version 2.11) against the SILVA 16S rRNA database (v138) with a confidence threshold of 70% (Wang et al., 2007). The functional potential of the bacterial community was inferred from the 16S rRNA gene data using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, version 2.2.0) (Douglas et al., 2020).

2.4 Statistical analysis

Statistical analyses were performed separately for each rotation system (RO and CO) to evaluate the effects of N fertilization on soil physicochemical properties, P fractions, and microbial community characteristics. One-way analysis of variance (ANOVA) was employed with N fertilization treatment as the fixed factor. Where significant effects were detected, Duncan's multiple range test was applied for post-hoc comparisons, with statistical significance defined at $P < 0.05$. All figures were generated using Origin 2021 (OriginLab Corporation, USA).

Microbial data were processed and analyzed on the Majorbio Cloud Platform (<https://cloud.majorbio.com>). Alpha diversity indices (Chao1 and Shannon) were calculated using

mothur software (Schloss et al., 2009), and differences between groups were assessed using the Wilcoxon rank-sum test. Beta diversity was evaluated by principal coordinate analysis (PCoA) based on Bray-Curtis distances. The permutational multivariate analysis of variance (PERMANOVA) was used to test the significance of community structure dissimilarities between groups. To identify differentially abundant bacterial taxa (from phylum to genus level), linear discriminant analysis effect size (LEfSe) was performed (Segata et al., 2011). Distance-based redundancy analysis (db-RDA) was conducted to elucidate the relationships between environmental variables (treatments) and soil bacterial community composition. The influences of key environmental factors, identified by db-RDA, on alpha and beta diversity indices was further assessed via linear regression.

3 Results

3.1 Effects of N fertilization on soil properties

In the present study, the initial soil condition was weakly acidic in both cropping systems. N fertilization exerted contrasting effects on soil pH between the two systems (Fig. 1a, b). In the corn-oilseed rape (CO) rotation, N fertilization significantly reduced soil pH compared with the no-N control, though no further significant decrease was observed with increases N rates. The high N treatment (225-0) decreased soil pH by approximately 0.5 units relative to the N0-0 control. In contrast, under the rice-oilseed rape (RO) rotation, N fertilization increased and stabilized soil pH at a near-neutral level.

N fertilization also significantly influenced the soil C/N ratio and soil organic matter (SOM) content under both cropping systems (Fig. 1c, d). In the CO rotation, the soil C/N ratio increased with N fertilization compared to the control, reaching a maximum (9.1) in the N225-0 treatment. Conversely, in the RO rotation, N fertilization reduced the soil C/N ratio relative to the control, with values remained below the control level (9.2). This divergence may result from N input stimulating microbial mineralization of organic carbon, leading to carbon consumption that potentially exceeded N accumulation. Changes in SOM showed partial correlation with the soil C/N ratio. In the CO system, SOM responded variably to N rates, with the highest value (14.4 g/Kg) occurring under the N75-0 treatment. In the RO system, SOM remained relatively stable across treatments and did not differ significantly, indicating its insensitivity to the applied N fertilization regimes. The maximum SOM content (19.8 g/Kg) in the RO system was recorded under the N150-150 treatment.

3.2 Effects of N fertilization on soil P speciation and availability

N fertilization significantly influenced the distribution of soil P speciation, with distinct patterns observed between the two cropping systems (Fig. 2a). Overall, the RO rotation exhibited higher concentrations of all inorganic P fractions compared to the CO rotation, with Fe-P and O-P being the dominant forms. In the CO rotation, increasing N rates led to significant changes in Fe-P and O-P contents, while Al-P and Ca-P remained relatively stable. Both Fe-P and O-P peaked under the N150-0 treatment. In contrast, Fe-P in the

RO rotation showed no significant response to N fertilization, and both Al-P and Ca-P remained low across all N treatments. Excessive N application (N225-0) reduced the content of all P fractions compared to N150-0, whereas the distribution under N150-150 was similar to that under N150-0.

The soil phosphorus speciation further affected soil P availability (Fig. 2b). Both N fertilization and cropping systems significantly influenced soil AP content, which was generally higher under the RO system. In both systems, the N150-0 treatment resulted in the highest AP content, indicating that moderate N application enhanced P mobilization. However, further increasing the N rate to N225-0 significantly reduced AP in the CO system. Notably, the N150-150 treatment maintained high AP content in both rotations, with values significantly exceeding those of single-season N application treatments. This effect was particularly pronounced in the RO system, where AP under N150-150 was approximately double that in the CO system.

3.3 Key soil microbial taxa associated with soil P fractions

Given the focus of this study, we specifically examined microbial taxa known to be involved in soil P fractions.

P-solubilizing and P-cycling-related taxa: Analysis of soil bacterial community at the phylum level revealed that several taxa with known roles in P cycling differed substantially between the CO and RO systems (Fig. 3). The RO system showed a marked increase in the relative abundance of *Chloroflexi* (Fig. 3), many members of which are iron-reducing bacteria that can mediate the reductive dissolution of Fe-P under anaerobic conditions, thereby enhancing P release. In contrast, the CO system supported significantly higher relative abundances of *Actinobacteria*, *Acidobacteriota*, and *Proteobacteria* (Fig. 3). These taxa are often associated with P solubilization in acidic upland soils: *Actinobacteria* are known to produce organic acids and phosphatases that mobilize fixed P, while *Acidobacteriota* may contribute to P cycling through organic matter decomposition and P mineralization.

Response of P-cycling-related taxa to N fertilization: N fertilization influenced the abundance of these P-related taxa in a system-species manner. In the CO system, as N rate increased, the abundance of *Proteobacteria*—a phylum containing many phosphate-solubilizing bacteria (e.g., *Pseudomonas*, *Bacillus*)—increased significantly, particularly under medium to high N inputs (Fig. 3). This enrichment of copiotrophic *Proteobacteria* aligns with the observed increase in AP under moderate N fertilization (N150-0). In the RO system, the abundance of *Chloroflexi* remained relatively stable across N treatments (Fig. 5), consistent with the stable Fe-P pools observed in this system, and suggesting that iron-reducing P mobilization may be primarily driven by water regime rather than N input.

Unique and shared microbial taxa under different treatments: Venn analysis based on OTUs revealed that the N150-150 treatment harbored a distinct set of unique OTUs compared to its single-season counterpart (N150-0) in both rotations (Fig. 4). This suggests that balanced dual-season N fertilization promotes a unique microbial assemblage that may include P-solubilizing taxa contributing to sustained P mobilization.

Environmental drivers of P-cycling-related microbial communities: Redundancy analysis (RDA) revealed distinct relationships between environmental factors and microbial community structure in the two cropping systems (Fig. 7). In the CO system, soil pH emerged as the dominant driver, while AP and Fe-P also contributed significantly, indicating that P fractions and P availability are key factors shaping the microbial community that in turn mediates P dynamics. In the RO system, AP was identified as a primary driver of microbial community variation, reinforcing the close linkage between P availability and microbial community assembly. Notably, in both systems, the balanced N fertilization (N150-150) was clearly separated from its single-season counterpart (N150-0) in the ordination space (Fig. 7), indicating that balanced N application shapes a distinct microbial niche associated with unique P fraction profiles.

4 Discussion

4.1 Contrasting mechanisms of soil P fraction responses to N fertilization under two crop rotations

By virtue of the 11-year long-term field experiment, this study demonstrates that the rice-oilseed rape (RO) and corn-oilseed rape (CO) rotations constitute two fundamentally distinct soil environment frameworks, under which the effect of N fertilization on soil P speciation and availability differ profoundly. We interpret our key data to elucidate the mechanisms by which N addition differentially influences P fractions across the two cropping systems.

The results show that soil pH revealed opposite responses to N fertilization between the two systems (Fig. 1a, b). In the CO rotation (Fig. 1a), N fertilization significantly reduced soil pH compared with the no-N control, with the high N treatment (N225-0) decreasing soil pH by approximately 0.5 units, consistent with the widespread acidification observed across major agricultural regions in China (Guo et al., 2010). This acidification is mechanistically linked to our P fraction data: in the CO system, the decrease in pH under N fertilization coincided with increases in AP (Gillespie and Pope, 1991). In contrast, under the RO rotation, N fertilization increased and stabilized soil pH at near-neutral levels (Fig. 1b). This pH buffering explains why Fe-P in the RO system showed no significant response to N fertilization, as the neutral pH conditions minimize P solubilization associated with acidification. The evolution of soil carbon-nitrogen stoichiometry (C/N) and SOM content further underscored the systemic differences between the two rotations. In the CO system, N fertilization increased the soil C/N ratio (Fig. 1c), likely due to exogenous N stimulating microbial assimilation of carbon and enhancing N immobilization into microbial biomass (Wang et al., 2023b). By contrast, the decrease in the C/N ratio in the RO system following N fertilization likely reflects accelerated mineralization of organic carbon under anaerobic conditions, where carbon loss outpaces N accumulation (Luo et al., 2017; Geisseler and Scow, 2014). These mechanisms directly influenced SOM dynamics (Fig. 1d). In the CO system, SOM content was sensitive to N fertilizer, peaking at medium rates (e.g., N75-0), whereas high N input (N225-0) likely promoted the mineralization of native organic matter, leading to a decline in SOM. In the RO system, however, SOM remained stable across N levels, highlighting the stronger stabilizing capacity of the paddy environment via organo-mineral

association and physicochemical protection under anaerobic conditions (Zhang et al., 2018). N fertilization also significantly altered soil P availability, consistent with the findings from Jing et al. (2021), who used phosphate oxygen isotope techniques in an Alfisol to demonstrate that N fertilization enhances P bioavailability by stimulating microbial activity and modifying P speciation pathways, with more pronounced effects under medium to high N levels. This isotopic evidence further supports a “priming effect” of N on P cycling and provides mechanistic insights into N-P interactions.

The analyses of soil P fractions provide evidence for P speciation pathways that differ between the two cropping systems. The significantly higher concentrations of inorganic P fractions, particularly Fe-P and O-P, in the RO system (Fig. 2a) are directly attributable to the periodic anaerobic conditions in this system. Intense drying-wetting cycles promote the reductive dissolution of Fe (III)-P to more soluble Fe (II)-forms, facilitating P release (Patrick and Khalid, 1974; Helfenstein et al., 2018). Supporting this interpretation, the enrichment of Fe-P in the RO system coincided with a higher relative abundance of *Chloroflexi* (Fig. 3), iron-reducing bacteria that we detected at significantly higher levels in RO compared to CO. In contrast, the consistently aerobic environment of the CO system favors stable adsorption of P onto Fe and Al oxides, leading to the relative accumulation of Fe-P and Al-P observed in our data (Fig. 2a) (Cui et al., 2022; Zhao et al., 2014).

The result of AP (Fig. 2b) shows that N fertilization enhanced P availability in both systems, but through distinct mechanisms. In the CO system, the increase in AP under moderate N fertilization (N150-0) corresponded directly to the decrease in soil pH (Fig. 1a) and the increase in *Proteobacteria* abundance (Fig. 3), supporting P solubilization and microbial mediation via acidification. In the RO system, the enhancement of AP under N fertilization occurred without significant changes in soil pH or Fe-P pools (Figs. 1b, 2a), suggesting a “N priming effect” on microbial activity as the primary mechanism (Ning et al., 2021). This interpretation is supported by our RDA analysis (Fig. 7b), which identified AP as a primary driver of microbial community variation in the RO system, indicating that P availability and microbial communities are closely coupled.

The results of AP under different N application treatments (Fig. 2b) indicate that the method of N application is as important as the amount. The balanced dual-season fertilization (N150-150) resulted in significantly higher AP compared to its single-season counterpart (N150-0) in both rotations. Specifically, AP under N150-150 was 30.26 mg/kg in RO and 14.79 mg/kg in CO, compared to 28.4 mg/kg and 12.5 mg/kg under N150-0, respectively (Fig. 2b). This demonstrates that synchronized nutrient supply with crop demand throughout the rotation cycle enhances P availability more effectively than concentrating N in a single season.

4.2 Driving factors of soil microbial community structure and their linkages with P fractions

The PCoA, Venn, and RDA analyses provide quantitative evidence for the hierarchical control of soil microbial community assembly, with P fractions serving as key environmental filters.

Water management as the primary determinant: The PCoA analysis (Fig. 6) shows that samples were distinctly separated into two major clusters corresponding to the RO and CO systems, explaining 49.76% and 24.89% of variation respectively on PC1. This quantitative separation provides evidence that water management is the dominant factor shaping microbial community structure (Sun et al., 2018). The phylum-level abundance results (Fig. 3) further show that: the CO system enriched for aerobic groups *Actinobacteria* (average relative abundance 12.8%) and *Acidobacteriota* (10.2%), while the RO system enriched for anaerobic *Chloroflexi* (15.6%). Importantly, at the phylum level, this selective pressure was evident (Fig. 2a): the CO system enriched for typical aerobic groups such as *Actinobacteria* and *Acidobacteriota*, the latter often associated with the slightly acidic conditions of upland soils (Fierer et al., 2012). In contrast, the enrichment of *Chloroflexi* in the RO system corresponded to higher Fe-P and O-P contents in this system, providing indication of a functional linkage between iron-reducing bacteria and Fe-bound P pools (Gui et al., 2016; Zhang et al., 2024).

N fertilization as a secondary driver: The PCoA analysis detected significant separation among N treatments in the CO system (PERMANOVA, $P < 0.01$), but not in the RO system ($P > 0.05$) (Fig. 6). This quantitative difference directly demonstrates that the strength of N fertilization as a driver is constrained by the hydrological context. The RDA analysis (Fig. 7a) quantifies the contribution of environmental factors: in the CO system, soil pH explained 52.09% of community variation (RDA1), while AP and Fe-P contributed 9.01% (RDA2). These findings indicated a direct linkage between microbial community shifts and changes in P fractions. In the RO system (Fig. 7b), SOM (39.06%) and AP (14.94%) were the primary drivers, indicating that even under the dominant influence of water regime, P availability exerts selective pressure on microbial taxa.

4.3 The role and mechanisms of balanced N fertilization in shaping P fractions and microbial communities

The study of P fractions, AP, and microbial community composition provide evidence for the unique role of balanced dual-season N fertilization (150–150). Across both rotations, this treatment resulted in distinct compared to single-season fertilization (150-0) at an equivalent total N rate: In the RO system: AP was 30.26 mg/kg under N150-150 vs. 28.4 mg/kg under N150-0 (Fig. 2b); unique OTUs were 1244 vs. 678 (Fig. 4); *Proteobacteria* abundance increased by approximately 15% (Fig. 3). In the CO system: AP was 14.79 mg/kg under N150-150 vs. 12.5 mg/kg under N150-0 (Fig. 2b); unique OTUs showed distinct separation (Fig. 4); RDA ordination clearly separated C5 from C3 (Fig. 7a).

The RDA analysis (Fig. 7) quantitatively confirms that balanced N fertilization shapes a specific microbial niche associated with observed soil properties. The C5 and P5 treatments were clearly separated from C3 and P3 in the ordination space, with AP and Fe-P identified as key separating variables. This finding supports that balanced N fertilization promotes a distinct microbial assemblage, which may contribute to sustained P mobilization through mechanisms such as organic acid secretion and phosphatase production (Jing et al., 2021).

Therefore, based on our research of P fractions, AP, and microbial community composition, the balanced dual-season N fertilization strategy enhances P availability and shapes microbial community structure, with the effects differing between the two rotation systems. Our data demonstrate that in the CO system, the primary mechanism is P solubilization via acidification (evidenced by pH decrease from 6.56 to 6.06 under N225-0, Fig. 1a), while in the RO system, the primary mechanism is microbial priming (evidenced by stable pH at ~ 6.8 and AP increase from 21.39 to 30.26 mg/kg under N150-150, Figs. 1b, 2b). These findings provide a foundation for developing integrated nutrient management strategies that consider both P fractions and the microbial communities that mediate P dynamics.

5 Conclusion

This study demonstrates that water management serves as the overarching determinant of soil physicochemical properties and microbial community assembly, while N fertilization acts as a critical regulatory lever within this context. By establishing distinct redox environments, water regime governs the evolution of soil pH, P speciation, and SOM dynamics, while simultaneously selecting for system-specific core microbiomes.

The results further reveal that balanced dual-season N fertilization is markedly more effective than optimal single-season application in enhancing P availability and fostering a resilient microbial community. N management exhibited system-specific effects: it acidified CO soils while stabilizing RO soils at near-neutral pH. Overall, the rotation systems characterized by differences in both water regime and crop species, serve as the primary driver of microbial community assembly, selecting for distinct core microbiomes, with N rate further fine-tuning the community.

Based on these findings, we propose the following fertilization management recommendations: (1) For RO rotations, a balanced dual-season N application ($150 \text{ kg N} \cdot \text{hm}^{-2}$ in each season) is recommended to maximize P availability ($30.26 \text{ mg} \cdot \text{kg}^{-1}$) and maintain neutral pH. (2) For CO rotations, moderate single-season N application ($150 \text{ kg N} \cdot \text{hm}^{-2}$) is sufficient to enhance P availability ($12.5 \text{ mg} \cdot \text{kg}^{-1}$), while rates $\geq 225 \text{ kg N} \cdot \text{hm}^{-2}$ should be avoided due to soil acidification. (3) For both systems, excessive N fertilization ($\geq 225 \text{ kg N} \cdot \text{hm}^{-2}$ per season) is discouraged as it reduces P availability and may impair soil microbial diversity.

In conclusion, N fertilization enhances P availability by modifying soil properties and regulating microbial communities. The balanced dual-season N fertilization strategy synergistically improves P availability, promotes soil biodiversity, and supports cropping system sustainability. This approach provides a practical pathway for sustainable nutrient management in intensive cropping systems of the Yangtze River Basin.

Declarations

Competing interests

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

Author Contribution

Author contributions: Data curation, Formal analysis, Methodology, Investigation, Writing – original draft, Writing – review & editing: Zi Yang; Methodology, Writing – review & editing: Chenyang Cui; Investigation, Methodology: Xiaoyang Chao; Writing –review & editing: Di Zheng; Resources, Supervision: Tao Ren; Resources, Supervision: Jianwei Lu; Supervision: Xiaoming Wang; Supervision: Wenfeng Tan; Funding acquisition, Project administration, Resources, Supervision, Methodology, Writing – review & editing: Xionghan Feng. All the authors read and approved the final manuscript.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Nos. 42030709, and W2521004).

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Allison L (1965) Organic carbon. In: Black CA (ed) *Methods of Soil Analysis: Part 2-Chemical and Microbiological Properties*. American Society of Agronomy, pp 1367–1378
2. Bao S (2000) *Soil and Agriculture Chemistry Analysis*, third edn. China Agr. (in Chinese)
3. Barberán A, Bates S, Casamayor E, Fierer N (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J* 6:343–351. <https://doi.org/10.1038/ismej.2011.119>
4. Bennett E, Carpenter S, Caraco N (2001) Human impact on erodible phosphorus and eutrophication: a global perspective. *Bioscience* 51:227–234. [https://doi.org/10.1641/0006-3568\(2001\)051\[0227:HIOEPA\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2001)051[0227:HIOEPA]2.0.CO;2)
5. Chang S, Jackson M (1957) Fractionation of soil phosphorus. *Soil Sci* 84:133–144. <https://doi.org/10.1097/00010694-195708000-00005>
6. Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34:i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>
7. Chialva M, Ghignone S, Cozzi P et al (2020) Water management and phenology influence the root-associated rice field microbiota. *FEMS Microbiol Ecol* 96. <https://doi.org/10.1093/femsec/fiaa146>

8. Cross A, Schlesinger W (1995) A literature-review and evaluation of the Hedley fractionation- Applications to the biogeochemical cycle of soil-phosphorus in natural ecosystems. *Geoderma* 64:197–214. [https://doi.org/10.1016/0016-7061\(94\)00023-4](https://doi.org/10.1016/0016-7061(94)00023-4)
9. Cui C, Li J, Yang Z et al (2024) Formation, transformation and utilization of soil occluded phosphorus. *J Plant Nutr Fert* 30:1413–1421. <https://doi.org/10.11674/zwyf.2024220>
10. Cui E, Lu R, Xu X et al (2022) Soil phosphorus drives plant trait variations in a mature subtropical forest. *Global Change Biol* 28:3310–3320. <https://doi.org/10.1111/gcb.16148>
11. Deng Y, Jiang Y, Yang Y et al (2012) Molecular ecological network analyses. *BMC Bioinformatics* 13:113. <http://www.biomedcentral.com/1471-2105/13/113>
12. Douglas G, Maffei V, Zaneveld J et al (2020) PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* 38:685–688. <https://doi.org/10.1038/s41587-020-0548-6>
13. Edgar R (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 10:996–998. <https://doi.org/10.1038/nmeth.2604>
14. Falkowski P, Fenchel T, Delong E (2008) The microbial engines that drive Earth's biogeochemical cycles. *Sci* 320:1034–1039. <https://doi.org/10.1126/science.1153213>
15. Feng J, Chen L, Xia T et al (2023) Microbial435 fertilizer regulates C: N: P stoichiometry and alleviates phosphorus limitation in flue-cured tobacco436 planting soil. *Sci Rep* 13:10276. <https://doi.org/10.1038/s41598-023-37438-w>
16. Fierer N, Lauber C, Ramirez K et al (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J* 6:1007–1017. <https://doi.org/10.1038/ismej.2011.159>
17. Galloway J, Howarth R, Michaels A, Nixon S, Prospero J, Dentener F (1996) Nitrogen and phosphorus budgets of the North Atlantic Ocean and its watershed. *Biogeochemistry* 35:3–25. <https://doi.org/10.1007/BF02179823>
18. Geisseler D, Scow K (2014) Long-term effects of mineral fertilizers on soil microorganisms - A review. *Soil Biology Biochem* 75:54–63. <https://doi.org/10.1016/j.soilbio.2014.03.023>
19. Geohring L, McHugh O, Walter M, Steenhuis T, Akhtar M, Walter M (2001) Phosphorus transport into subsurface drains by macropores after manure applications: implications for best manure management practices. *Soil Sci* 166:896–909. <https://doi.org/10.1097/00010694-200112000-00002>
20. Gillespie A, Pope P (1991) Consequences of rhizosphere acidification on delivery and uptake kinetics of soil phosphorus. *Tree Physiol* 8:195–204. <https://doi.org/10.1093/treephys/8.2.195>
21. Grunes D (1959) Effect of Nitrogen on the Availability of Soil and Fertilizer Phosphorus to Plants. *Adv Agron* 11:369–396. [https://doi.org/10.1016/S0065-2113\(08\)60127-3](https://doi.org/10.1016/S0065-2113(08)60127-3)
22. Gui J, Chen X, Liu M, Zhuang X, Sun Z, Hu F (2016) Influences of water-saved and nitrogen-reduced practice on soil microbial and microfauna assemblage in paddy field. *CJAE* 27:107–116 PMID: 27228599

23. Guo J, Liu X, Zhang Y et al (2010) Significant Acidification in Major Chinese Croplands. *Sci* 327:1008–1010. <https://doi.org/10.1126/science.1182570>
24. He X, Augusto L, Goll D et al (2023) Global patterns and drivers of phosphorus fractions in natural soils. *Biogeosciences* 20:4147–4163. <https://doi.org/10.5194/bg-20-4147-2023>
25. Heckrath G, Brookes P, Poulton P, Goulding K (1995) Phosphorus leaching from soils containing different phosphorus concentrations in the Broadbalk experiment. *J Environ Qua* 24:904–910. <https://doi.org/10.2134/jeq1995.00472425002400050018x>
26. Helfenstein J, Tamburini F, Von S et al (2018) Combining spectroscopic and isotopic techniques gives a dynamic view of phosphorus cycling in soil. *Nat Commun* 9:3226. <https://doi.org/10.1038/s41467-018-05731-2>
27. Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237:173–195. <https://doi.org/10.1023/A:1013351617532>
28. Jing J, Zhang F, Rengel Z, Shen J (2012) Localized fertilization with P plus N elicits an ammonium-dependent enhancement of maize root growth and nutrient uptake. *Field Crop Res* 133:176–185. <https://doi.org/10.1016/j.fcr.2012.04.009>Get rights and content
29. Jing D, Yan Y, Ren T et al (2021) Effects of nitrogen application rate on phosphorus transformation in an Alfisol: Results from phosphate-oxygen isotope ratios. *Appl Geochem* 134:105094. <https://doi.org/10.1016/j.apgeochem.2021.105094>
30. Ju X, Gu B (2017) Indexes of nitrogen management. *Acta petrol sin* 54:281–296 (in Chinese)
31. Kögel-Knabner I, Amelung W, Cao Z et al (2010) Biogeochemistry of paddy soils. *Geoderma* 157:1–14. <https://doi.org/10.1016/j.geoderma.2010.03.009>
32. LeBauer D, Treseder K (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecol* 89:371–379. <https://doi.org/10.1890/06-2057.1>
33. Li W, Joshi S, Hou G, Burdige D, Sparks D, Jaisi D (2015) Characterizing Phosphorus Speciation of Chesapeake Bay Sediments Using Chemical Extraction, ^{31}P NMR, and X-ray Absorption Fine Structure Spectroscopy. *Environ Sci Technol* 49:203–211. <https://doi.org/10.1021/es504648d>
34. Li L, Li X, Liu B, Lei J, Yue Z, Li C (2020) Imbalanced stoichiometric patterns in foliar nutrient resorption response to N and P addition in grazing alpine grassland. *ACTA OECOL* 102:103505. <https://doi.org/10.1016/j.actao.2019.103505>
35. Li X, Ren T, Lu J (2021) Integrated regulation of nitrogen fertilizer for increasing yield and efficiency of rice-oilseed rape rotation system in the Yangtze River Basin. *J Huazhong Agricultural Univ* 40:13–20 (in Chinese)
36. Liu J, Hu Y, Yang J, Abdi D, Cade-Menun B (2015) Investigation of Soil Legacy Phosphorus Transformation in Long-Term Agricultural Fields Using Sequential Fractionation, P K-edge XANES and Solution P NMR Spectroscopy. *Environ Sci Technol* 49:168–176. <https://doi.org/10.1021/es504420n>
37. Liu C, Zhao D, Ma W et al (2016) Denitrifying sulfide removal process on high-salinity wastewaters in the presence of *Halomonas* sp. *Appl Microbiol Biotechnol* 100:1421–1426.

<https://doi.org/10.1007/s00253-015-7039-6>

38. Luo G, Ling N, Nannipieri P et al (2017) Long-term fertilisation regimes affect the composition of the alkaline phosphomonoesterase encoding microbial community of a vertisol and its derivative soil fractions. *Biol Fert Soils* 53:375–388. <https://doi.org/10.1007/s00253-015-7039-6>
39. Minasny B, McBratney A, Wadoux A, Akoeb E, Sabrina T (2020) Precocious 19th century soil carbon science. *Geoderma Reg* 22. <https://doi.org/10.1016/j.geodrs.2020.e00306>
40. Ning J, Arenberg M, Arai Y (2021) Phosphorus mineralization affected by urea in an intensively managed agricultural soil. *Soil Sci Soc Am J* 85:2067–2076. <https://doi.org/10.1002/saj2.20324>
41. Oliverio A, Geisen S, Delgado-Baquerizo M et al (2020) The global-scale distributions of soil protists and their contributions to belowground systems. *Sci Adv* 6:eaax8787. <https://doi.org/10.1126/sciadv.aax8787>
42. Ortas I, Rowell D (2004) Effect of ammonium and nitrate on indigenous mycorrhizal infection, rhizosphere pH change, and phosphorus uptake by sorghum. *Commun Soil Sci Plan* 35:1923–1944. <https://doi.org/10.1081/LCSS-200026820>
43. Patrick W, Khalid R (1974) Phosphate release and sorption by soils and sediments-effect of aerobic and anaerobic conditions. *Sci* 186:53–55. <https://doi.org/10.1134/S1064229325600514>
44. Powley H, Krom M, Van C (2018) Phosphorus and nitrogen trajectories in the Mediterranean Sea (1950–2030): Diagnosing basin-wide anthropogenic nutrient enrichment. *Prog Oceanogr* 162:257–270. <https://doi.org/10.1016/j.pocean.2018.03.003>
45. Pribyl D (2010) A critical review of the conventional SOC to SOM conversion factor. *Geoderma* 156:75–83. <https://doi.org/10.1016/j.geoderma.2010.02.003>
46. Richardson A, Barea J, McNeill A, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* 321:305–339. <https://doi.org/10.1007/s11104-009-9895-2>
47. Richardson A, Simpson R (2011) Soil microorganisms mediating phosphorus availability. *Plant Physiol* 156:989–996. <https://doi.org/10.1104/pp.111.175448>
48. Rousk J, Bååth E, Brookes P, Lauber C et al (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* 4:1340–1351. <https://doi.org/10.1038/ismej.2010.58>
49. Schloss P, Westcott S, Ryabin T et al (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl Environ Microb* 75:7537. <https://doi.org/10.1128/AEM.01541-09>
50. Segata N, Izard J, Waldron L et al (2011) Metagenomic biomarker discovery and explanation. *Genome Biol* 12. <https://doi.org/10.1186/gb-2011-12-6-r60>
51. Stackebrandt E, Goebel B (1994) A place for DNA-DNA reassociation and 16S ribosomal-RNA sequence-analysis in the present species definition in bacteriology. *INT J SYST BACTERIOL* 44:846–849. <https://doi.org/10.1099/00207713-44-4-846>

52. Sun W, Xiao E, Pu Z et al (2018) Paddy soil microbial communities driven by environment- and microbe-microbe interactions: A case study of elevation-resolved microbial communities in a rice terrace. *Sci Total Environ* 612:884–893. <https://doi.org/10.1016/j.scitotenv.2017.08.275>
53. Turner B, Kim P (2024) Terminology for residual and legacy phosphorus. *Plant Soil* 501:237–239. <https://doi.org/10.1007/s11104-024-06538-5>
54. Wang Q, Garrity G, Tiedje J, Cole J (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73:5261–5267. <https://doi.org/10.1128/AEM.00062-07>
55. Wang R, Liu H, Sardans J et al (2021) Interacting effects of urea and water addition on soil mineral-bound phosphorus dynamics in semi-arid grasslands with different land-use history. *Eur J Soil Sci* 72:946–962. <https://doi.org/10.1111/ejss.13046>
56. Wang K, Ren T, Yan J et al (2023a) Soil phosphorus availability alters the effects of straw carbon on microbial mediated phosphorus conversion. *Plant Soil* 491:575–590. <https://doi.org/10.1007/s11104-023-06134-z>
57. Wang Y, Huang Y, Song L et al (2023b) Reduced phosphorus availability in paddy soils under atmospheric CO₂ enrichment. *Nat Geosci* 16:162–168. <https://doi.org/10.1038/s41561-022-01105-y>
58. Xue X, Ren C, Zhao C et al (2025) Litter removal and nitrogen deposition alter soil microbiological community composition and diversity in a typical rubber (*Hevea brasiliensis*) plantation of Hainan, China. *Appl Soil Ecol* 208:105969. <https://doi.org/10.1016/j.apsoil.2025.105969>
59. Yang H (2018) Effects of nitrogen and phosphorus addition on leaf nutrient characteristics in a subtropical forest. *Trees* 32:383–391. <https://doi.org/10.1007/s00468-017-1636-1>
60. Zavaleta E, Shaw M, Chiariello N et al (2003) Grassland responses to three years of elevated temperature, CO₂, precipitation, and N deposition. *Ecol Monogr* 73:585–604. <https://doi.org/10.1890/02-4053>
61. Zeng J, Liu X, Song L et al (2016) Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biol Biochem* 92:41–49. <https://doi.org/10.1016/j.soilbio.2015.09.018>
62. Zhang X, Xin X, Zhu A et al (2018) Linking macro aggregation to soil microbial community and organic carbon accumulation under different tillage and residue managements. *Soil Tillage Res* 178:99–107. <https://doi.org/10.1016/j.still.2017.12.020>
63. Zhang Y, Li X, Liu H et al (2022) Effects of nutrient and water addition on soil inorganic phosphorus fractions in an old-field grassland. *Chin J Appl Ecol* 33:369–377. <https://doi.org/10.13287/j.1001-9332.202202.010>
64. Zhang X, Wu N, Ke Z et al (2024) Anaerobic Degradation of Dicamba via a Novel Catabolic Pathway by a Consortium Enriched from Deep Paddy Soil. *J Agric Food Chem* 72:1035–1043. <https://doi.org/10.1021/acs.jafc.3c07903>
65. Zhao J, Ni T, Li Y et al (2014) Responses of Bacterial Communities in Arable Soils in a Rice-Wheat Cropping System to Different Fertilizer Regimes and Sampling Times. *PLoS ONE* 9.

<https://doi.org/10.1371/journal.pone.0085301>

66. Zhou J, Wu Y, Bing H et al (2016) Variations in soil phosphorus biogeochemistry across six vegetation types along an altitudinal gradient in SW China. CATENA 142:102–111.
<https://doi.org/10.1016/j.catena.2016.03.004>

67. Zhu Z (2008) Research on soil nitrogen in China. Acta Petrol Sin 45:778–783 (in Chinese)

68. Zhu J, Wang Q, He N et al (2016) Imbalanced atmospheric nitrogen and phosphorus depositions in China: implications for nutrient limitation. JGR-B 121:1605–1616.
<https://doi.org/10.1002/2016JG003393>

Figures

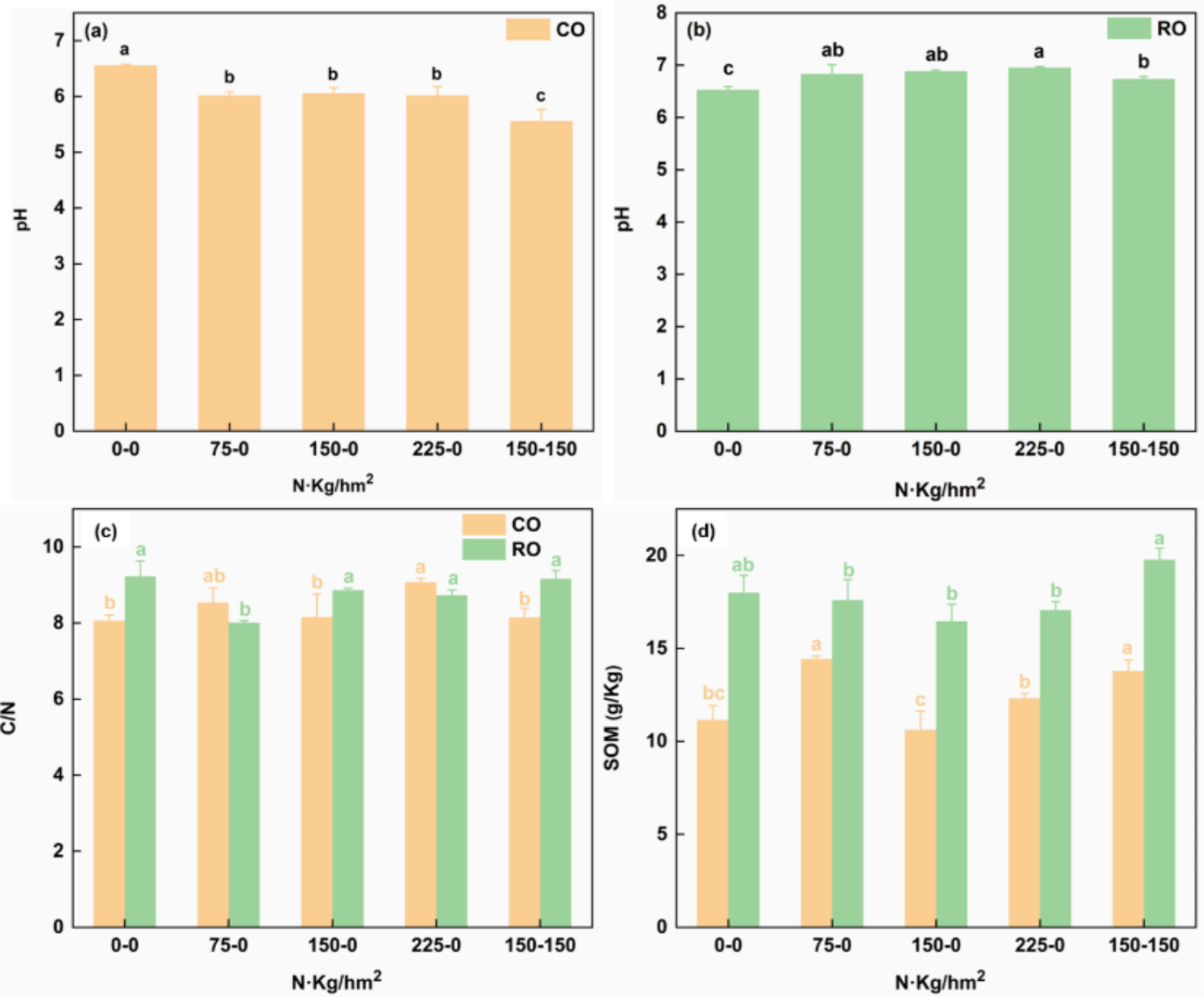


Figure 1

Soil properties under different nitrogen fertilization treatments in the corn-oilseed rape (CO) and rice-oilseed rape (RO) rotations. (a) soil pH in CO, (b) soil pH in RO, (c) soil C/N ratio, and (d) soil organic matter (SOM) content. Different lowercase letters indicate significant differences among treatments within each rotation system at $P < 0.05$. Treatment abbreviations: 0-0: no N application in either season; 75-0: 75 kg N hm^{-2} in corn/rice season and 0 kg N hm^{-2} in oilseed rape season; 150-0: 150 kg N hm^{-2} in corn/rice season and 0 kg N hm^{-2} in oilseed rape season; 225-0: 225 kg N hm^{-2} in corn/rice season and 0 kg N hm^{-2} in oilseed rape season; 150-150: 150 kg N hm^{-2} in corn/rice season and 150 kg N hm^{-2} in oilseed rape season.

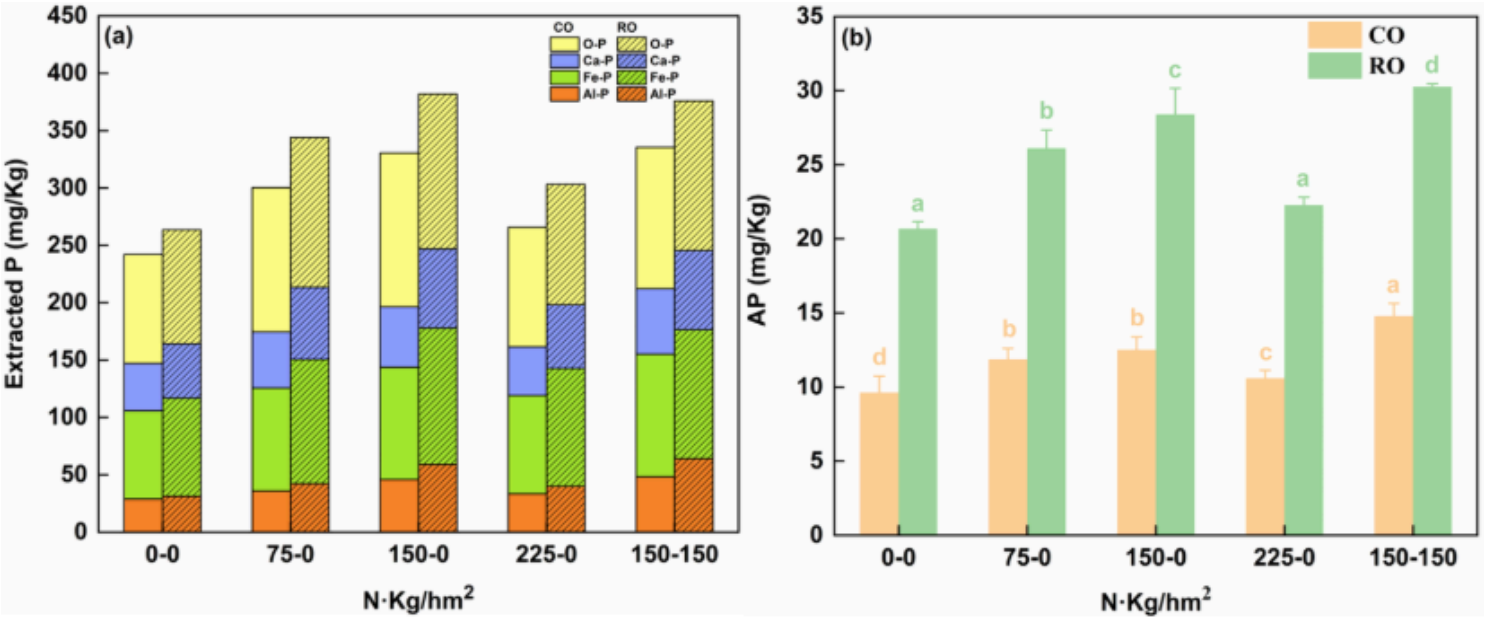


Figure 2

Effects of different nitrogen fertilization treatments on (a) soil phosphorus fractions and (b) available phosphorus under the corn-oilseed rape (CO) and rice-oilseed rape (RO) rotations. Different lowercase letters indicate significant differences among treatments at $p < 0.05$. Note: P fractions are defined as: O-P: occluded P; Fe-P: iron-bound P; Ca-P: calcium-bound P; Al-P: aluminum-bound P. Treatment abbreviation: 0-0, 75-0, 150-0, 225-0, and 150-150 denote seasonal nitrogen application rates (kg N hm^{-2}) for the corn/rice and oilseed rape seasons, respectively.

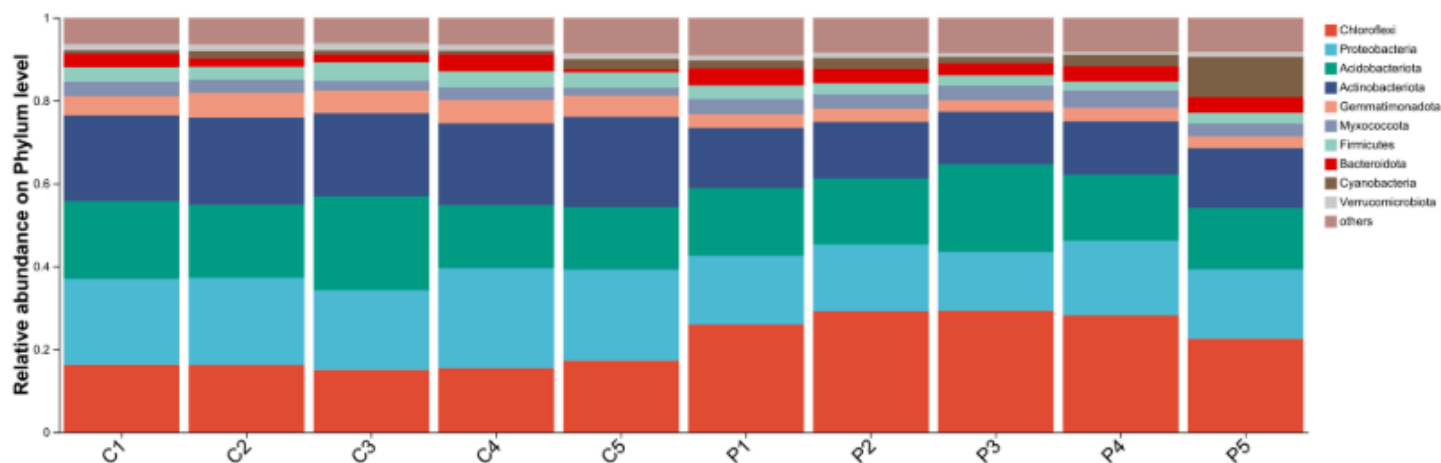


Figure 3

Relative abundance of soil bacterial communities at the phylum level under different nitrogen fertilization treatments in the corn-oilseed rape (CO) and rice-oilseed rape (RO) rotations. Note: Treatments for the CO rotation (denoted as C) are: C1 (0-0), C2 (75-0), C3 (150-0), C4 (225-0), and C5 (150-150). Treatments for the RO rotation (denoted as P) are: P1 (0-0), P2 (75-0), P3 (150-0), P4 (225-0), and P5 (150-150). Treatment codes (e.g., 150-150) represent nitrogen application rates (kg N hm^{-2}) in the corn/rice and oilseed rape seasons, respectively.

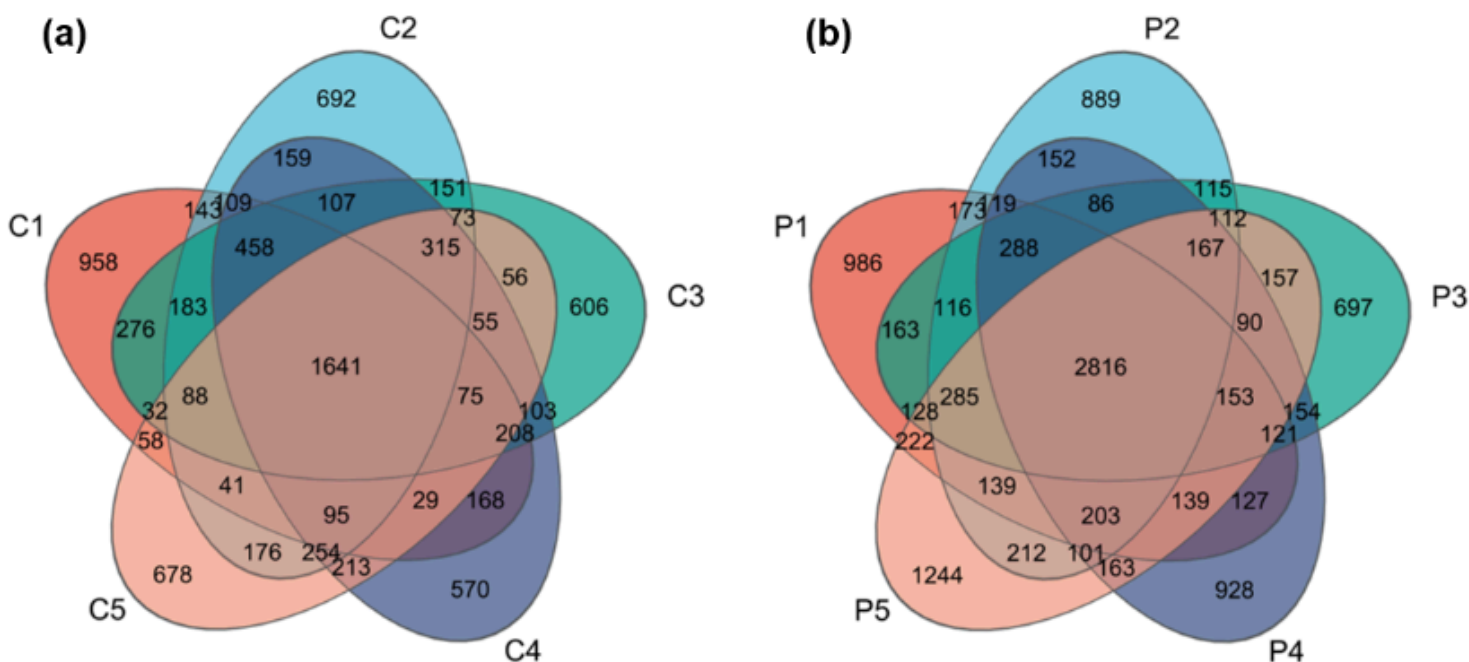


Figure 4

Soil bacterial count at the OTU level under different nitrogen treatments in (a) corn-oilseed rape (CO) and (b) rice-oilseed rape (RO) rotations. Note: For the CO rotation (denoted C), treatments are: C1 (0-0), C2 (75-0), C3 (150-0), C4 (225-0), C5 (150-150). For the RO rotation (denoted P), treatments are: P1 (0-0), P2 (75-0), P3 (150-0), P4 (225-0), P5 (150-150).

(75-0), P3 (150-0), P4 (225-0), P5 (150-150). Codes (e.g., 150-150) represent nitrogen application rates (kg N hm⁻²) in the corn/rape and oilseed rape seasons, respectively.

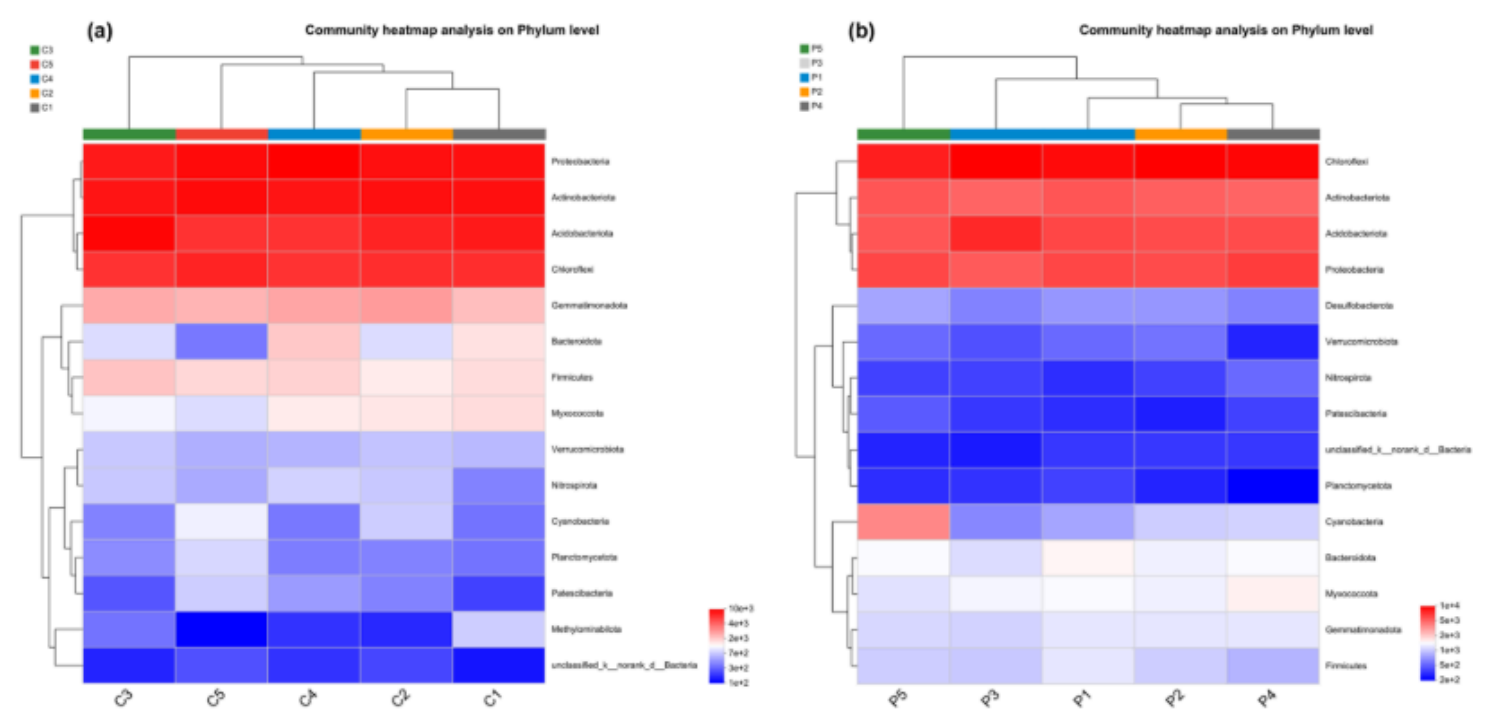


Figure 5

Clustering heatmap of soil bacterial communities at the phylum level under different nitrogen fertilization treatments in (a) corn-oilseed rape (CO) and (b) rice-oilseed rape (RO) rotations. Note: Treatment codes for the CO rotation (C): C1 (0-0), C2 (75-0), C3 (150-0), C4 (225-0), C5 (150-150). For the RO rotation (P): P1 (0-0), P2 (75-0), P3 (150-0), P4 (225-0), P5 (150-150). Codes (e.g., 150-150) denote nitrogen application rates (kg N hm⁻²) in the corn/rape and oilseed rape seasons, respectively.

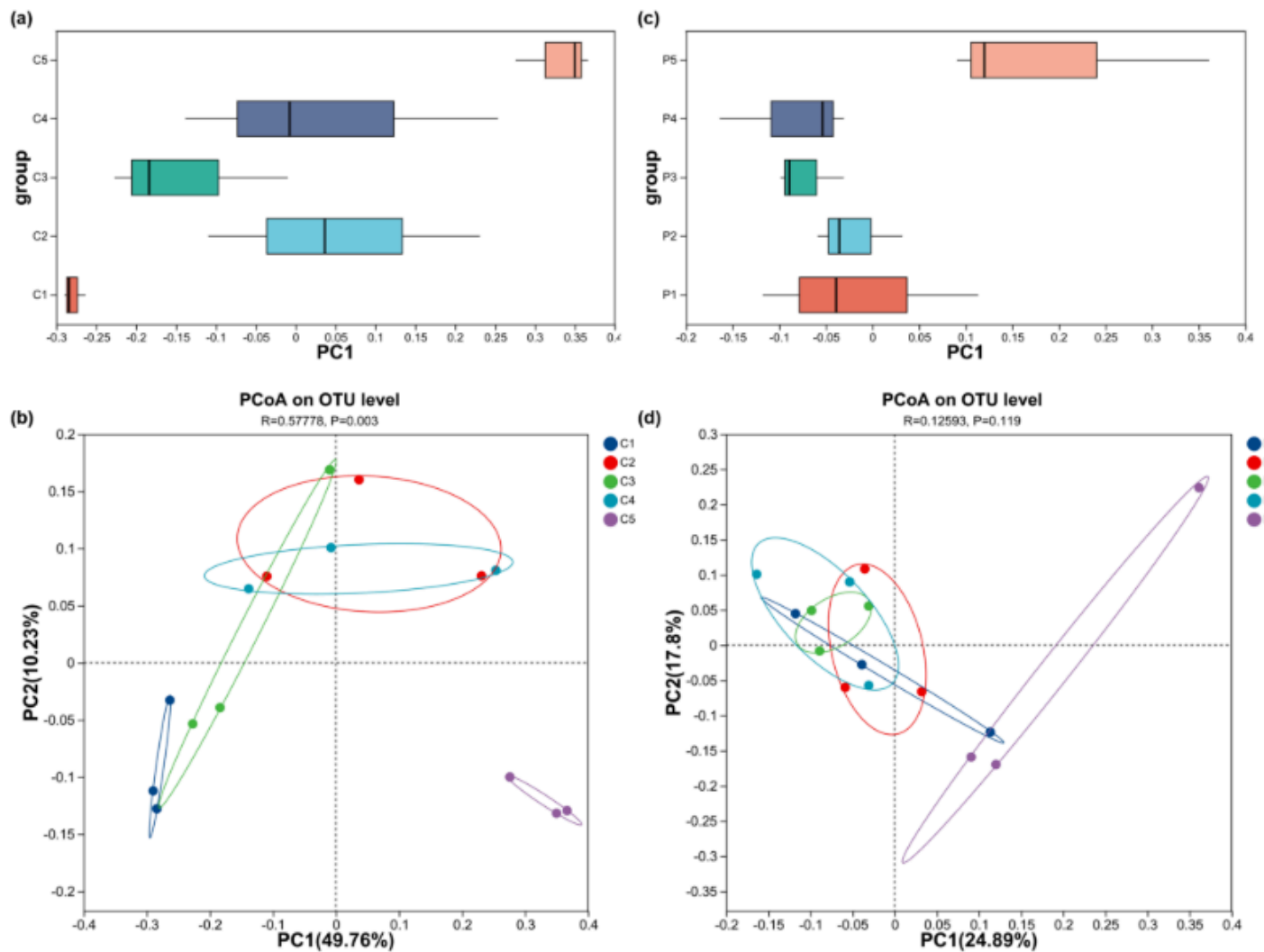


Figure 6

Principal coordinate analysis (PCoA) of soil bacterial communities at the OTU level under different nitrogen fertilization treatments in the corn-oilseed rape (CO) (a, b) and rice-oilseed rape (RO) (c, d) rotations. Note: Treatment codes for the CO rotation (C): C1 (0-0), C2 (75-0), C3 (150-0), C4 (225-0), C5 (150-150). For the RO rotation (P): P1 (0-0), P2 (75-0), P3 (150-0), P4 (225-0), P5 (150-150). Codes (e.g., 150-150) denote nitrogen application rates (kg N hm^{-2}) in the corn/rice and oilseed rape seasons, respectively.

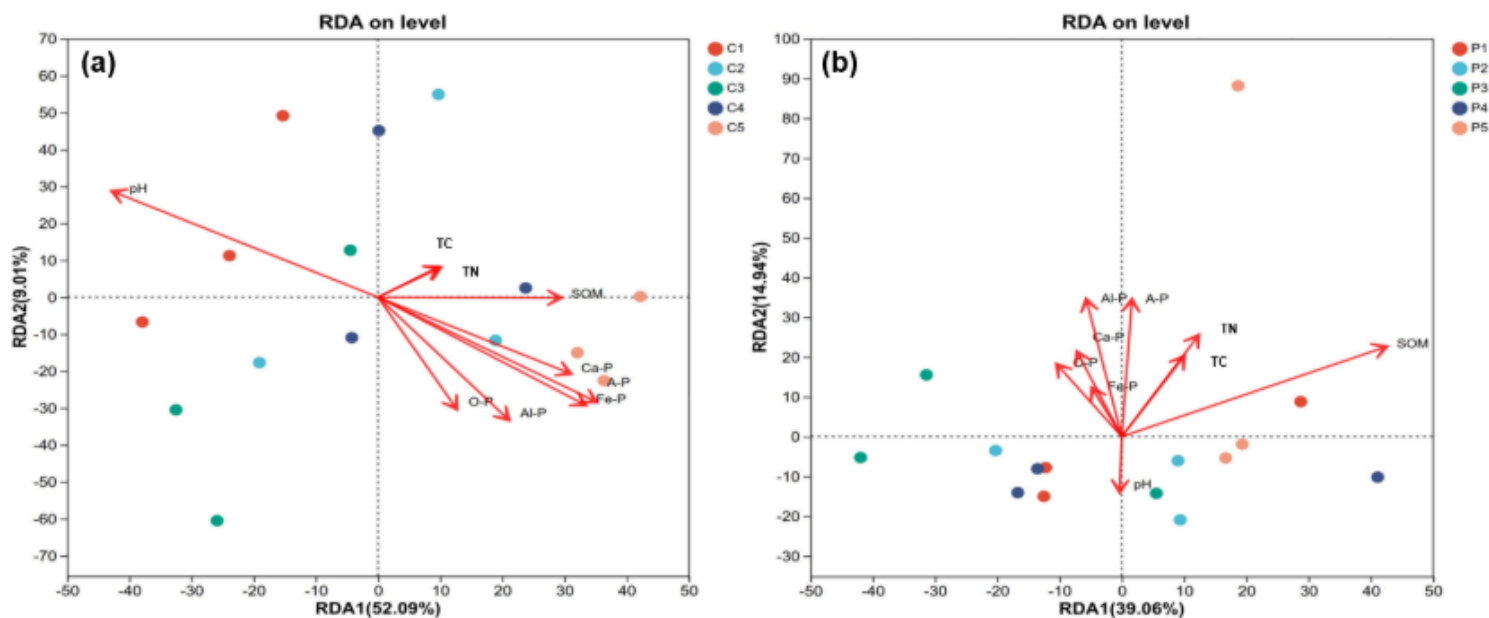


Figure 7

Redundancy Analysis (RDA) of soil bacterial communities (genus level) with soil chemical properties in (a) corn-oilseed rape (CO) and (b) rice-oilseed rape (RO) rotations. Note: Soil properties are: SOM, soil organic matter; TC, total carbon; TN, total nitrogen; A-P, available phosphorus; O-P, Occluded P; Fe-P, Iron-bound P; Al-P, aluminum-bound; Ca-P, calcium-bound P. Treatment codes for the CO rotation (C) are: C1 (0-0), C2 (75-0), C3 (150-0), C4 (225-0), C5 (150-150). For the RO rotation (P): P1 (0-0), P2 (75-0), P3 (150-0), P4 (225-0), P5 (150-150). Codes (e.g., 150-150) denote nitrogen application rates (kg N hm^{-2}) in the corn/rice and oilseed rape seasons, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplementarytables.docx](#)