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RESEARCH ARTICLE

Restoration boosts soil P-cycle multifunctionality in karst ecosystems by modulating soil properties and rare bacterial taxa

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Highlights

- Natural restoration improves karst soil P-cycle multifunctionality compared to artificial restoration and cropland.
- Natural restoration boosts soil organic carbon and bacterial complexity and network tightness.
- Rare *phoD*-harboring bacterial taxa play a more critical role in driving soil P-cycle than dominant taxa.

Abstract

Phosphorus (P) is an essential nutrient element that is critical for plant growth and ecosystem functionality. The soil P cycle plays multiple roles, such as sustaining plant growth and productivity, regulating nutrient balance within ecosystems, and enhancing ecosystem adaptability and resilience. This cycle is influenced by factors such as the restoration approach and microbial community dynamics. However, the extent to which the restoration approach alters the P cycle in karst ecosystems and the underlying microbial mechanisms remain poorly understood. The P-cycle multifunctionality index (P-cycle MFI) serves as a comprehensive indicator for evaluating soil P cycle function, and it provides insights into changes in the P cycle between different restoration approaches. To investigate the shifts in soil P-cycle MFI and microbial mechanisms between different restoration approaches, we analyzed soil available P (AP), total P (TP), microbial biomass P (MBP), and the activities of acid phosphatase (ACP) and alkaline phosphatase (ALP). These data were used to calculate the P-cycle MFI by averaging the Z-scores between two restoration approaches (artificial restoration of forest (AF) and natural restoration of forest (NF)) and a control (cropland, CP) at six subtropical karst ecosystem sites in China. We also determined the soil organic carbon (SOC), exchangeable calcium (Ca) and magnesium (Mg), pH, bulk density (BD), microbial biomass C (MBC), and microbial biomass nitrogen (MBN), as well as the community structure, relative abundance, diversity indices, and co-occurrence networks of *phoD*-harboring bacteria. The results showed that the community structure of *phoD*-harboring bacteria varied significantly among AF, NF, and CP and across different temperature gradients. These bacteria exhibited increasing complexity and tightness in co-occurrence networks from CP to AF and then to NF, along with the ACP and ALP activities, but not the TP and AP contents. The P-cycle MFI values were significantly higher in NF compared to AF and CP, and the variation was significantly explained by restoration approach, temperature, MBC, MBN, SOC, exchangeable Ca, BD, community structure of *phoD*-harboring bacteria, and exchangeable Mg. Furthermore, natural restoration had a more substantial impact on the P-cycle MFI than temperature by enhancing SOC, microbial biomass, the complexity and co-occurrence network tightness of the *phoD*-harboring bacterial community structure, and ACP and ALP activities, but it reduced soil BD. The rare genera of *phoD*-harboring bacteria significantly influenced the variation of soil P-cycle MFI compared to the dominant genera. This study highlights the importance of rare genera of *phoD*-harboring bacteria in driving soil P-cycle multifunctionality in karst ecosystems, with natural restoration being more effective than artificial methods for

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enhancing soil organic matter and microbial community complexity.

Keywords: karst ecosystem, restoration, community, *phoD*-harboring bacteria, P-cycle multifunctionality, rare taxa

1. Introduction

Soil ecosystem multifunctionality refers to the capacity of soil to simultaneously support and maintain multiple ecological functions, such as maintaining soil properties and fertility, carbon sequestration, the nitrogen (N)- and phosphorus (P)-cycles, litter decomposition, and microbial interactions (Deng *et al.* 2024; Wang J Q *et al.* 2024). In terrestrial ecosystems, the P-cycle plays a pivotal role in these functions, as essential ecological processes like plant and microbial growth are intricately linked to P resources (Elser *et al.* 2007). However, more than 43% of global terrestrial ecosystems experience soil P limitation (Du *et al.* 2020; Hou *et al.* 2020). This P limitation can diminish vegetation productivity, biodiversity, and carbon sink capacity (Ford *et al.* 2016; Yang *et al.* 2019; Wei *et al.* 2022), impede ecosystem development and stability, and ultimately impact the provision of ecosystem services (Marklein *et al.* 2016). Furthermore, soil degradation has emerged as a progressively pervasive global concern that is predominantly fueled by irrational land use practices, over-cultivation, soil erosion, and excessive P fertilizer application (Gomiero 2016; Pravalie 2021). These factors result in structural imbalances and reduced soil microbial community diversity, leading to soil acidification and ultimately diminishing the multifunctionality of soil ecosystems (Jia *et al.* 2022). As a result, these issues not only limit the capacity for ecosystem restoration but also compromise the sustainability and efficacy of restoration efforts for ecosystems. Consequently, attaining a comprehensive understanding of the changes and potential influencing factors associated with P-cycle multifunctionality between different restoration approaches, especially in P-limited soils, is essential for achieving sustainable ecosystem restoration and management.

Available P is a fundamental parameter used for characterizing the P-cycle in soils, as it is involved in P transfer between soil (inorganic and organic P), microbes, plants, and litter P pools. However, this highly dynamic indicator undergoes rapid changes due to P uptake by plants and microorganisms, and its measured values are often very low, especially in severely P-limited soils (Pan *et al.* 2016). In some ecosystems, despite high levels of total P and bound P fractions, the P cycle remains minimally active due to the extensive sequestration of P components within stable soil complexes (Chiu *et al.* 2021). Conversely, in other ecosystems characterized by low levels of total and available P, a highly active P cycle can be driven by the high activity of functional microorganisms (Wang S Z *et al.* 2024), such as *phoD*-harboring bacteria. These microorganisms secrete significant amounts of phosphatase into P-limited soils, thereby converting organic P into available inorganic P (Liang *et al.* 2020; Pan *et al.* 2024a, b). Therefore, available P values cannot effectively characterize the functional properties of the soil P-cycle. A more appropriate approach is to assess soil P-cycle multifunctionality, which considers the changes in chemical patterns (including total and available P contents) and the factors influencing P cyclic process (including enzyme activities, and microbial biomass, diversity, and community structure) (Gong *et al.* 2024). Restoration efforts significantly affect soil P-cycle multifunctionality (Sünnemann *et al.* 2023; Yang *et al.* 2023). For example, natural restoration of forests have higher soil moisture content, organic C, phosphatase activity, and microbial biomass and diversity, and altered microbial community structure compared to artificial restoration of forests, thereby enhancing the index of soil P-cycle multifunctionality (Allan *et al.* 2015).

This enhancement may primarily be achieved through greater inputs of plant litter and root exudates into the soils due to vegetation restoration, as well as enhanced microbial diversity and more diverse microbial communities. In recent years, an increasing body of research has demonstrated that the enhanced soil ecosystem multifunctionality index (MFI) is positively correlated with greater microbial diversity (Wagg *et al.* 2014; Liu *et al.* 2022; Xu *et al.* 2023, 2024). This correlation can be attributed to restoration efforts that improve soil water and nutrient conditions, thereby promoting microbial growth (Resch *et al.* 2021; Tian *et al.* 2022). However, some studies suggest that changes in microbial community structure may have a more significant impact on ecosystem multifunctionality than microbial diversity. As restoration progresses, soil microbes exhibit more complex structures and tighter co-occurrence networks, which facilitate nutrient cycling, energy flow, and information transfer (Yang *et al.* 2023; Gong *et al.* 2024). Generally, the dominant taxa characterized by higher relative abundance occupy the largest number of pairwise connections within these co-occurrence network models. Based on these initial observations, researchers have traditionally concluded that dominant taxa play a significant role in soil multifunctionality. However, recent studies indicate that rare microbial taxa (i.e., those with low relative abundance) may be more important for soil nutrient cycling and multifunctionality than the dominant taxa (Soliveres *et al.* 2016; Chen *et al.* 2020; Liu *et al.* 2021; Xu *et al.* 2022; Wang *et al.* 2023). For example, in P-limited soils, rare *phoD*-harboring bacteria drive greater variations in soil phosphatase activity, available P, and ecosystem multifunctionality compared to the dominant taxa (Liu *et al.* 2021; Wan *et al.* 2021; Xu *et al.* 2022; Chen and Wan 2023). Increasing evidence suggests that rare microbial taxa are more sensitive to environmental changes such as land-use changes, vegetation degradation, and vegetation restoration (Liu M J *et al.* 2023). Nevertheless, some studies have shown that dominant taxa can also exhibit sensitivity to environmental changes (Kurm *et al.* 2019; Xing *et al.* 2023). These conflicting results highlight the need for further

research to elucidate the roles of different microbial taxa in the soil P cycle and multifunctionality.

The karst ecosystem in southwestern China is one of the largest karst landscapes in the world, covering an area of 540,000 km^2 (Yuan 2001). Over the past three centuries, primary forests have been progressively degraded and transformed into agricultural lands or shrublands (Yue *et al.* 2024). Since 1996, extensive ecological restoration projects, such as the grain-for-green and hill closure for afforestation, have been implemented in the karst ecosystems of southwestern China. These projects have substantially enhanced vegetation restoration and effectively mitigated rocky desertification (Wang *et al.* 2019). In karst ecosystems, high levels of calcium (Ca) and pH facilitate the formation of P–Ca complexes in soils, resulting in P limitation for plants and soil microorganisms (Pan *et al.* 2015, 2024a; Zhang *et al.* 2015). To enhance crop productivity, various fertilizers including phosphate, nitrogen, and organic compounds are applied to the croplands in these ecosystems. This practice results in a significantly higher total P contents in the croplands compared to natural lands (including grassland, shrubland, and forest) (Wang *et al.* 2018). Concurrently, other studies have demonstrated that the soil organic matter content, microbial and plant diversity, as well as ecosystem services, are higher in natural restoration of forests than croplands and artificial restoration of forests (Pan *et al.* 2018; Wang *et al.* 2019). These findings suggest a potential enhancement of the soil P-cycle multifunctionality by restoration. However, the specific patterns and variations in soil P-cycle multifunctionality and the underlying microbial mechanisms between different vegetation restoration approaches within P-limited karst ecosystems remain poorly understood.

To gain a deeper understanding of how the patterns and variations in soil P-cycle multifunctionality are regulated by *phoD*-harboring bacteria in karst restoration ecosystems, 90 plots were established at six sites along the latitude of this ecosystem in southwestern China. Soil available P (AP), other soil physicochemical properties, microbial biomass, phosphatase activity, and the P-cycle MFI were analyzed in cropland (CP), artificial restoration of forest (AF), and natural restoration of forest (NF). In addition, the community structure and diversity indices (Chao1 and Shannon–Wiener) of *phoD*-harboring bacteria were also analyzed. The objectives of this study were to: (1) investigate the patterns of soil available P content and P-cycle MFI between two restoration approaches (AF and NF) and cultivated land (CP), and explore their relationships with environmental factors in karst ecosystems; and (2) elucidate the relationships between P-cycle MFI and *phoD*-harboring bacteria between the two restoration approaches. We hypothesized that: (1) Soil available P content will be lower while the P-cycle MFI will be higher in NF than in CP and AF; and (2) some rare taxa of *phoD*-harboring bacteria exert significant and stronger effects on soil P-cycle multifunctionality than the dominant taxa.

2. Materials and methods

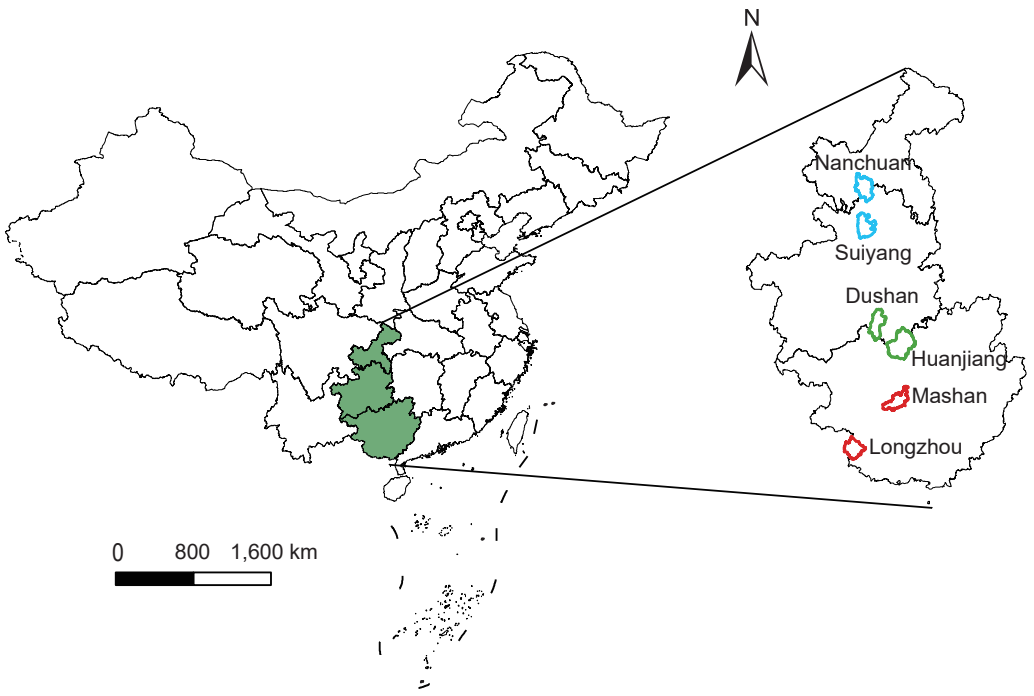
2.1. Study sites

The present study was conducted in the karst regions of southwestern China (106°95′–108°60′E and 22°42′–29°11′N). Climate significantly influences the structure and functionality of soil microbial communities. Consequently, six sampling sites were established along a temperature gradient spanning approximately 1,000 km in this region. These sites are located in Nanchuan County in Chongqing Municipality, Suiyang and Dushan counties in Guizhou Province, as well as Huanjiang, Mashan, and Longzhou counties in Guangxi Zhuang Autonomous Region, China (Fig. 1). These sites all have a subtropical monsoon climate, and they are characterized by mean annual temperatures (MAT) ranging from 12.2 to 21.8°C and average precipitation varying between 1,160 and 1,880 mm across the six locations. Detailed average temperature and precipitation data for each site are summarized in Appendix A.

Two vegetation restoration approaches (AF and NF) and a control group (CP) were established at the six karst region sites. According to the FAOUNESCO Soil Classification System, the soils in this region are classified as lithosols, which is supported by the research conducted by Sun *et al.* (2023). Prior to the 1990s, particularly from 1958 to the mid-1980s, primary karst forests were extensively deforested and degraded into cropland or sparse shrublands due to the increasing demand for food and timber driven by population growth (Pan *et al.* 2015; Xiao *et al.* 2025). To ensure consistency in land use history, all six selected CP sites have maintained continuous maize cultivation for a minimum of 20 years, as confirmed by interviews of local farmers. The AFs were predominantly established following the initiation of ecological restoration projects after 1997 through the planting of *Zenia insignis*, *Populus tomentosa*, and *Cryptomeria fortune* on abandoned cropland and sparse shrublands, with a minimum restoration period of 18 years. The NFs exhibit higher plant diversity and species richness, having undergone restoration primarily from sparse shrublands over a period exceeding six decades. The plant species in the two forests and cropland are listed in Appendix B.

2.2. Plot design and sampling

The experimental plots were established from July to September 2020. At each site, five replicate plots of 30 m×30 m were established for each restoration of forest and cropland. A total of 90 plots (6 sites×3 restoration approaches×5 replicate plots) were set up, with all plots located approximately 10 km apart on low slopes (15°–20°) facing south or southeast. Soil samples were systematically collected in a latitudinal gradient from higher to lower latitudes. Specifically, sampling was conducted in the Nanchuan District of Chongqing Municipality and Suiyang County of Guizhou Province in July 2020; in Dushan County of Guizhou Province and Huanjiang County of the Guangxi Zhuang Autonomous Region in August; and in Mashan County and Longzhou County of the Guangxi Zhuang Autonomous Region in September. This sequential sampling approach maintained uniform temperature conditions of approximately 30°C across all sites. The soil sampling procedure involved excavating 20 soil cores (15 cm depth) of an S-type at each plot using an auger. These cores



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Fig. 1 The six sites located in subtropical karst regions of China.

were then combined into one composite sample, which was subsequently sieved (2 mm) to remove stones, animals, and plant materials. A total of 90 soil samples were collected. All collected samples were immediately stored in a refrigerator and transported every three days to the Huanjiang Observation and Research Station for Karst Ecosystems of Chinese Academy of Sciences in Huanjiang County, Guangxi. Subsequently, the samples were divided into three portions for storage at different temperatures: –80°C for *phoD* gene sequencing, 4°C for measuring enzyme activities and microbial biomass, and air-drying for physical and chemical property analyses.

2.3. Analyses

General soil properties The contents of soil organic carbon (SOC), total phosphorus (TP), exchangeable calcium (Ca) and magnesium (Mg), Olsen-P (AP), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), microbial biomass P (MBP), and the activities of acid phosphatase (ACP) and alkaline phosphatase (ALP), along with soil pH and bulk density (BD), were measured using the methods detailed in Pan *et al.* (2024b).

Soil DNA extraction and sequencing First, soil DNA extraction and quality assessment were carried out. A FastDNA SPIN Kit (MP Biomedicals, Cleveland, OH, USA) was used to extract the DNA from 0.5 g frozen soil samples. The extracted DNA was subjected to qualitative analysis *via* 1% agar gel electrophoresis and quantitative analysis using UV spectrophotometry (Nanodrop, PeqLab, Germany).

Second, the *phoD* gene was amplified using the primers ALPS-F730 (5′-CAGTGGGACGACCACGAGGT-3′) and ALPS-1101 (5′-GAGGCCGATCGGCATGTCG-3′). Each amplification reaction mixture consisted of 25 μL, including 2.5 μL of 10×*Ex Taq* buffer (Mg^{2+} plus), 0.3 μL of *Ex Taq* (TaKaRa, Japan), 1 μL of each of the forward and reverse primers at a concentration of 10 pmol, 1 μL (~30 ng) of DNA template, and ddH_2O to achieve a final volume of 25 μL. The amplification program included an initial denaturation step at 95°C for 3 min; followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s, extension at 72°C for 30 s, and a final elongation at 72°C for 10 min.

Third, DNA sequencing and OTU clustering were conducted. The polymerase chain reaction products were purified using a TIANquick Midi Purification Kit (TIANGEN, China). Sequencing was performed on an Illumina HiSeq2500 platform (Magigene Co., Ltd., Guangzhou, China). Raw sequences containing ambiguous bases shorter than 200 bp, those with average quality scores below 30, and chimeric sequences were excluded using the QIIME 2 platform. High-quality sequences were clustered into operational taxonomic units (OTUs) at a similarity threshold of 97% using UCluster. The sequence data were deposited in the NCBI database under BioProject accession number PRJNA1126443.

Finally, the OTUs of *phoD*-harboring bacteria present in less than 0.1% of the samples were excluded to minimize potential biases during network construction. The Spearman's correlation coefficients among the retained OTUs were calculated by the "cor.test" function from the "psych" package in R version 4.4.0. Correlations with an *r*-value exceeding 0.6 and a *P*-value below 0.05 were employed to construct co-occurrence networks for each restoration forest and the cropland using Gephi version 0.9 (Pan *et al.* 2024b). The Shannon-Wiener and Chao1 diversity indices, as well as

the relative abundance, were then calculated based on the OTUs.

2.4. Statistical analyses

The soil P-cycle MFI for the two restoration approaches of forest and cropland in karst ecosystems were calculated using an averaging approach (Li *et al.* 2022; Yan *et al.* 2023). Specifically, five functional parameters were considered: the contents of AP, TP, and MBP and the activities of ACP and ALP (Huang *et al.* 2021). These parameters were first standardized through Z-score transformation (eq. (1)) and subsequently averaged to derive a soil P-cycle MFI (eq. (2)) for each plot. This index was used to assess the disparities in the P-cycle multifunctionality across different restorations.

$$Zxy=(Fxy-\mu y)/\delta y \quad (1)$$

where Zxy represents the z-score of the yth functional parameter for plot x, and a total of 90 plots were examined in this study; Fxy denotes the value of the yth functional parameter for plot x; μy represents the mean value of the yth functional parameter; and δ represents the standard deviation of the yth functional parameter.

$$\text{P-cycle MFI}=\sum_{y=1}^{5} Zxy/5 \quad (2)$$

The P-cycle MFI was calculated as the composite average of the z-scores derived from five distinct functional parameters at each plot.

Principal coordinate analysis (PCoA) was used to investigate the dissimilarities in soil *phoD*-harboring bacterial structures among CP, AF, and NF using the "vegan" package in R version 4.3.1 (R Core Team 2024). In addition, significant differences were observed in the community structures of *phoD*-harboring bacteria across three temperature gradients: the low temperature regions (MAT<13°C) in Nanchuan and Suiyang, the middle temperature regions (13°C<MAT<19°C) in Dushan and Huanjiang, and the high temperature regions (MAT>19°C) in Mashan and Longzhou.

An initial assessment of the normality of distribution and homogeneity of variance was conducted for all data. Subsequently, differences in soil available and total P contents, microbial biomass C, N, and P contents, acid and alkaline phosphatase activities, and the P-cycle MFI among the different forests or among the three temperature regions were assessed using two-way analysis of variance (Two-way ANOVA; P<0.05), as well as the *phoD*-harboring bacterial Chao1 and Shannon-Wiener diversity indices.

Random forest model is an ensemble learning algorithm that effectively manages complex ecological data, including nonlinear relationships and interactions between variables. This model can be used to evaluate the importance of individual environmental variables in shaping ecosystem structure, function, and processes by evaluating the relative significance of various features. Consequently, it facilitates the selection of optimal variables (Rodriguez-Galiano *et al.* 2012). Therefore, the random forest model has become an invaluable tool for ecologists in understanding and identifying key drivers, leading to its widespread application in ecological research in recent years (Breiman 2001; Shu *et al.* 2023). To determine the relative importance of parameters influencing the P-cycle MFI, such as microbial biomass, nutrient contents, diversity indices and communities of *phoD*-harboring bacteria, and MAT, a random forest model was implemented using the "randomForest" package in R version 4.4.0 to calculate the mean squared error (MSE, %). In addition, a generalized additive model (GAM) implemented with the "mgcv" package in R was used to establish the relationships between P-cycle MFI and each of the parameters. Furthermore, the random forest model was also applied to assess the relative importance of different *phoD*-harboring bacterial genera on the P-cycle MFI.

Structural equation model (SEM) is an advanced statistical methodology for investigating causal relationships among multiple variables. It synthesizes elements of factor analysis and multiple regression, and facilitates the construction of graphical path models that elucidate the complex networks of causal relationships between independent and dependent variables. SEM allows for the quantification of direct, indirect, and total effects of dependent variables on independent variables, thereby revealing the underlying mechanisms within ecosystems (Sutton-Grier *et al.* 2010). In this study, we used SEM *via* IBM SPSS AMOS 28 to evaluate the significant relationships among restoration efforts, MAT, SOC, microbial biomass, the *phoD*-harboring bacterial community, ACP and ALP activities, and the soil P-cycle MFI.

3. Results

3.1. Bacterial community structures, diversity, co-occurrence networks, and relative abundance

The *phoD*-harboring bacterial community structure differed among the three lands (Fig. 2-A). In each type of land, the community structure was different between the low, middle, and high temperature gradients of the different sites (Fig. 2-B–D). The Chao1 and Shannon–Wiener indices exhibited significantly higher values in the NF than in the CP and AF, while no significant differences were observed between the CP and AF (Fig. 2-E and I). The Chao1 indices were the highest in middle temperature region in CP and AF (Fig. 2-G and H); and the Shannon-Wiener indices decreased and increased with increasing temperatures in CP and AF, respectively (Fig. 2-J and K). These two diversity indices increased with the increasing temperature in NF (Fig. 2-H and L).

The genus level co-occurrence networks of soil *phoD*-harboring bacteria in CP, AF, and NF had 857, 846, and 949 nodes, respectively; and included 57,454, 63,280, and 104,542 edges, respectively (Appendix C). The complexity, tightness, and stability of the co-occurrence networks increased from CP to AF and then to NF, with average path lengths ranging from 2.22 to 2.17 and 1.98 and densities ranging from 0.16 to 0.18 and 0.23 (Fig. 3-A–C; Appendix C). Furthermore, the Zi-Pi sub-figures showed that the connectors of the co-occurrence networks in soil *phoD*-harboring bacteria increased from CP to AF and then to NF (Fig. 3-D–F). In addition, the nodes, edges, and densities at the genus level of soil *phoD*-harboring bacteria were the highest in the middle temperature gradient in CP and NF, but the average path length was the opposite (Appendices D

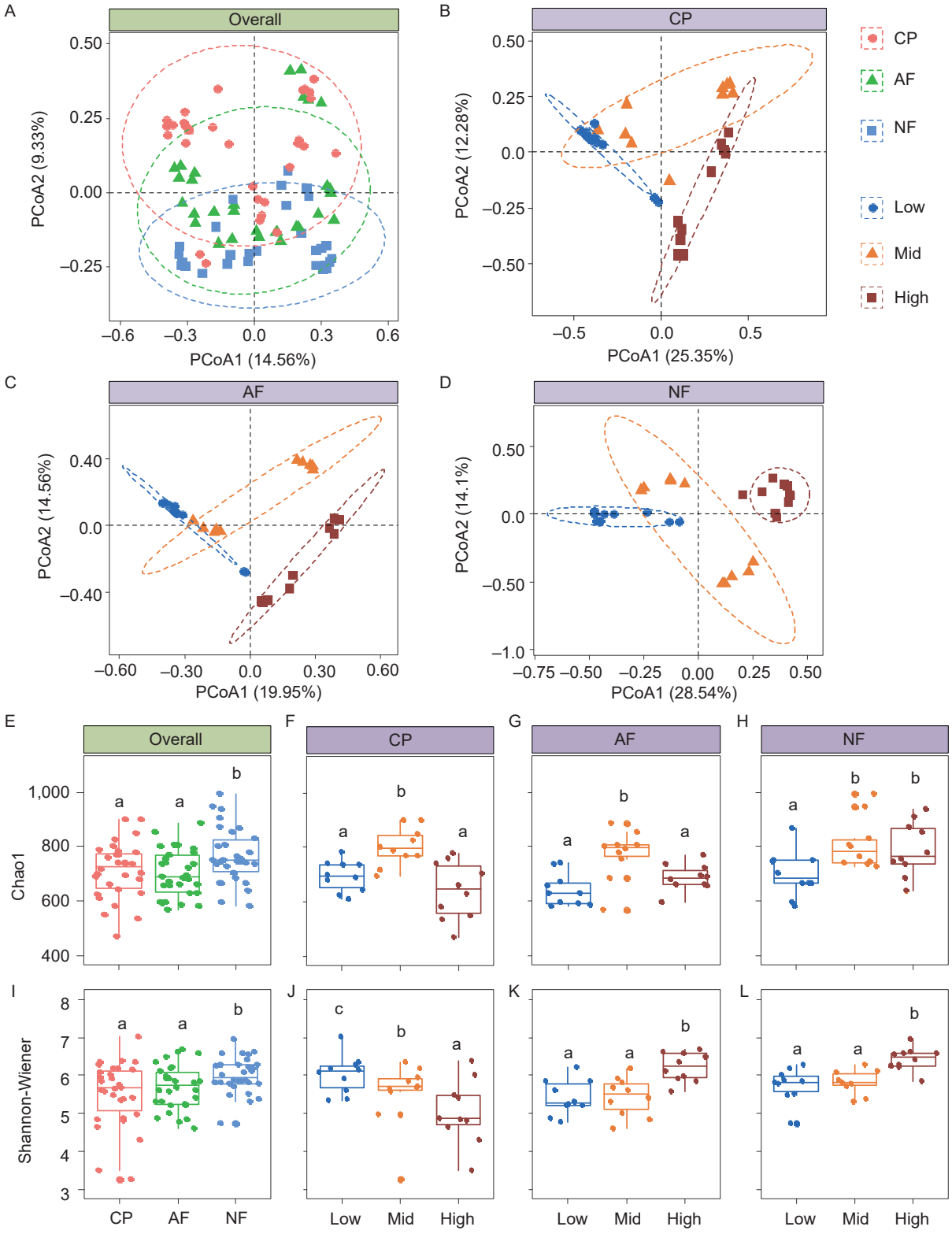


Fig. 2 The community structure and diversity indices of soil *phoD*-harboring bacteria differed between the restoration forests and between the temperature gradients. CP, cropland; AF, artificial restoration of forest; NF, natural restoration of forest. Low, low temperature gradients; Mid, middle temperature gradients; High, high temperature gradients.

and E). However, the nodes, edges, and densities increased with increasing temperature, while the average path lengths decreased (Appendices D and E).

At the genus level (>5%) of *phoD*-harboring bacteria in CP (Appendix F-a), *Pseudomonas* (14.82%) was the most dominant genus, followed by *Ralstonia* (10.95%), *Pseudolabrys* (9.95%), *Chelatococcus* (9.01%), *Methylobacterium* (7.71%), and *Bradyrhizobium* (6.89%). In low temperature regions of CP, *Pseudomonas* (17.78%) was the most dominant genus, followed by *Pseudolabrys* (14.23%), *Bradyrhizobium*

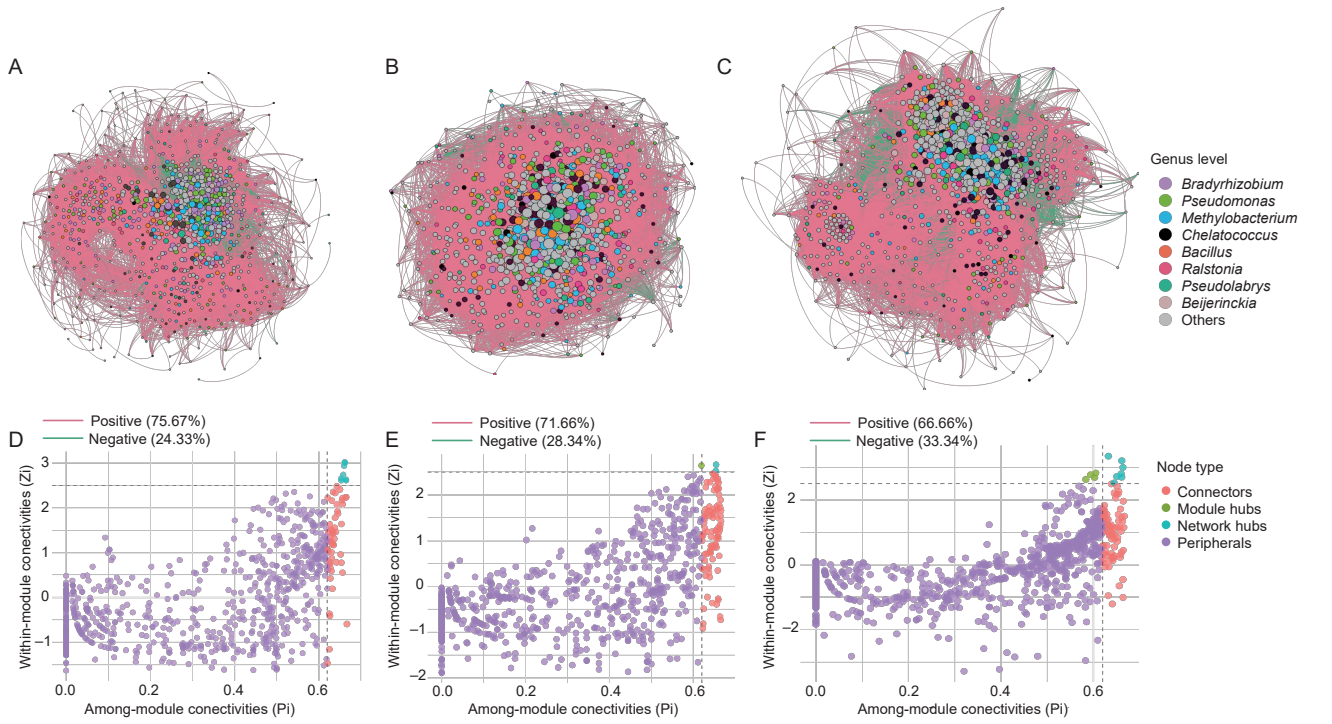


Fig. 3 Co-occurrence networks at the genus level in cropland (CP; A), artificial restoration of forest (AF; B), and natural restoration of forest (NF; C) in karst areas. The red and green edges represent positive and negative relationships, respectively. Zi-Pi plots show the distribution of *phoD*-harboring bacterial OTUs in CP (D), AF (E), and NF (F). The threshold value of Zi is 2.5, and that of Pi is 0.62.

(10.55%), *Methylobacterium* (10.49%), *Chelatococcus* (7.99%), and *Beijerinckia* (5.10%). In middle temperature regions of CP, *Ralstonia* (16.51%) was the most dominant genus, followed by *Pseudolabrys* (13.86%), *Pseudomonas* (13.21%), *Chelatococcus* (9.50%), *Bradyrhizobium* (6.00%), *Methylobacterium* (5.72%), and *Bacillus* (5.16%). In high temperature regions of CP, *Ralstonia* (13.89%) was the most dominant genus, followed by *Pseudomonas* (13.48%), *Collimonas* (10.00%), *Chelatococcus* (9.53%), *Methylobacterium* (6.92%), and *Bacillus* (6.64%).

At the genus level (>5%) of *phoD*-harboring bacteria in AF (Appendix F-b), *Chelatococcus* (10.14%) was the most dominant genus, followed by *Ralstonia* (9.55%), *Pseudolabrys* (9.04%), *Methylobacterium* (8.46%), *Pseudomonas* (6.14%), and *Ramlibacter* (5.29%). In low temperature regions of AF, *Pseudolabrys* (17.98%) was the most dominant genus, followed by *Beijerinckia* (9.53%), *Methylobacterium* (9.42%), *Chelatococcus* (6.72%), *Ramlibacter* (5.73%), *Bradyrhizobium* (5.46%), and *Methylomonas* (5.39%). In middle temperature regions of AF, *Ralstonia* (16.02%) was the most dominant genus, followed by *Methylobacterium* (11.16%), *Chelatococcus* (10.31%), *Ramlibacter* (8.61%), *Collimonas* (7.80%), *Pseudolabrys* (6.92%), and *Pseudomonas* (6.37%). In high temperature regions of AF, *Chelatococcus* (13.38%) was the most dominant genus, followed by *Ralstonia* (9.64%), *Pseudomonas* (9.27%), *Bacillus* (6.71%), and *Brasilonema bromeliae* (5.77%).

At the genus level (>5%) of *phoD*-harboring bacteria in NF (Appendix F-c), *Chelatococcus* (12.54%) was the most dominant genus, followed by *Pseudomonas* (11.74%), *Ramlibacter* (8.89%), *Methylobacterium* (8.13%), *Pseudolabrys* (6.09%), and *Beijerinckia* (6.51%). In low temperature regions of NF, *Chelatococcus* (15.32%) was the most dominant genus, followed by *Methylobacterium* (13.47%), *Pseudolabrys* (9.23%), *Beijerinckia* (9.11%), and *Collimonas* (8.52%). In middle temperature regions of NF, *Pseudomonas* (17.55%) was the most dominant genus, followed by *Chelatococcus* (14.12%), *Ramlibacter* (8.76%), *Methylobacterium* (7.95%), *Pseudolabrys* (7.73%), and *Mesorhizobium* (5.70%). In high temperature regions of NF, *Ramlibacter* (15.33%) was the most dominant genus, followed by *Pseudomonas* (14.08%), *Chelatococcus* (8.20%), *Beijerinckia* (7.23%), and *Arenibacterium* (5.04%).

3.2. Soil general properties, P fractions, phosphatase activities, and P-cycle MFI

The SOC, MBC, MBN, and exchangeable Ca and Mg contents progressively increased from CP to AF and then to NF, whereas soil BD exhibited the opposite trend (Table 1). In CP, SOC and MBC decreased with increasing temperature, while exchangeable Ca and BD were the opposite. In both AF and NF, SOC, MBN, exchangeable Ca and Mg, and pH increased with rising temperatures.

The contents of soil TP and AP in CP were significantly higher than those in both AF and NF (Fig. 4-A and B). The MBP content and P-cycle MFI were greater in NF than in AF and CP, with the lowest values observed in AF (Fig. 4-C and F). Conversely, the activities of ACP and ALP increased from CP to AF and then to NF (Fig. 4-D and E). In CP, the TP, AP, and P-cycle MFI were the highest in the high temperature regions but the lowest in the middle temperature regions (Appendix G-a, d and p).

Table 1 Soil properties in the three temperature gradients of three restoration approaches in karst areas[1)]

Vegetation[2)]	Temperature gradient	SOC (g kg^{-1})	MBC (mg kg^{-1})	MBN (mg kg^{-1})	ExCa (mg kg^{-1})	ExMg (mg kg^{-1})	pH	BD (g cm^{-3})
CP	Low	27.06±1.79 b	364.10±29.44 b	39.60±2.98 a	6.87±0.83 a	1.36±0.22 a	6.30±0.16 a	1.10±0.02 a
	Middle	21.12±2.13 ab	207.42±33.7 a	35.02±3.15 a	12.80±0.90 ab	2.69±0.54 a	6.58±0.08 a	1.26±0.03 b
	High	17.96±1.23 a	130.98±15.48 a	30.53±2.15 a	22.37±9.73 b	1.18±0.15 a	6.35±0.24 a	1.28±0.01 b
	Total	22.05±1.20 A	234.17±23.59 A	35.05±1.70 A	14.01±3.37 A	1.74±0.23 A	6.41±0.1 A	1.21±0.02 B
AF	Low	27.05±2.15 a	430.23±20.99 a	98.90±5.14 a	4.23±0.40 a	1.99±0.53 ab	5.91±0.31 a	1.21±0.04 a
	Middle	33.25±1.61 a	445.18±40.88 a	107.4±5.37 ab	16.12±1.61 b	1.10±0.11 a	6.30±0.19 a	1.14±0.02 a
	High	43.23±2.13 b	574.75±47.34 a	122.34±8.47 b	51.99±5.22 c	3.79±0.63 b	7.34±0.12 b	1.12±0.05 a
	Total	34.51±1.66 B	483.39±24.40 B	109.55±4.05 B	24.11±4.16 B	2.29±0.34 A	6.52±0.17 A	1.16±0.02 B
NF	Low	52.30±3.07 a	1060.96±39.31 a	133.75±7.40 a	22.32±3.05 a	3.38±0.79 a	5.87±0.16 a	0.93±0.04 a
	Middle	65.01±4.06 b	1025.72±121.53 a	182.84±10.67 b	33.15±2.81 a	8.23±1.26 b	6.74±0.07 b	0.95±0.04 a
	High	56.46±2.94 b	1170.13±68.87 a	190.63±10.87 b	50.27±1.58 b	8.01±1.45 b	7.07±0.06 b	1.02±0.05 a
	Total	57.92±2.13 C	1085.60±48.05 C	169.07±7.18 C	35.25±2.57 C	6.54±0.79 B	6.56±0.11 A	0.97±0.02 A

[1)] SOC, soil organic carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; ExCa, exchangeable Ca; ExMg, exchangeable Mg; BD, bulk density.
[2)] CP, cropland; AF, artificial restoration of forest; NF, natural restoration of forest.
Different capital letters represent differences in the restoration forests and cropland; different lowercase letters represent differences in the temperature gradients for the same land type (P<0.05).

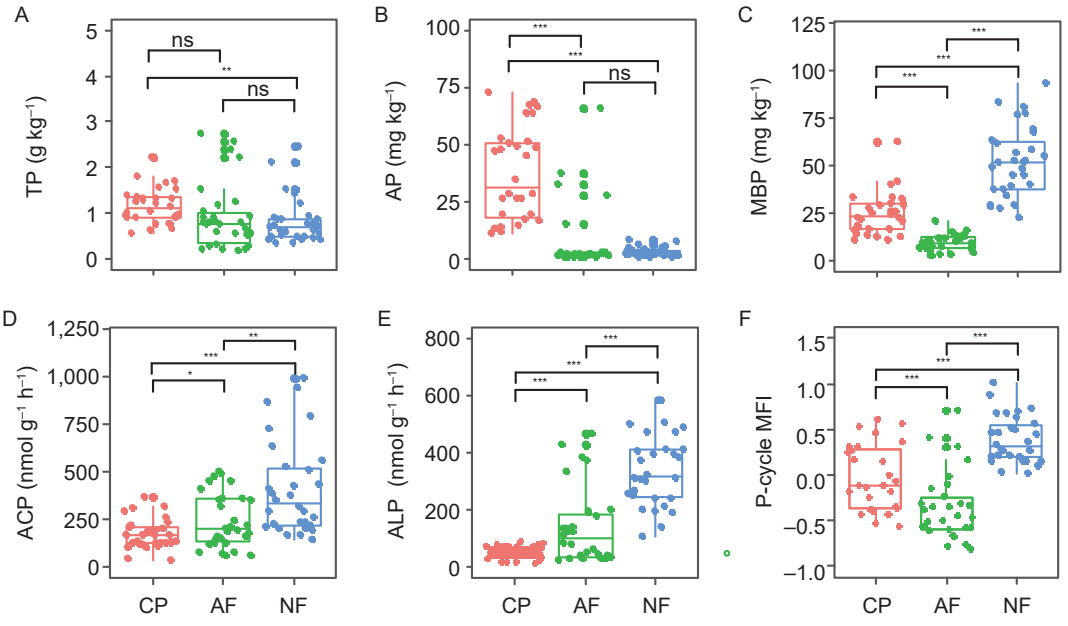


Fig. 4 Soil P contents, phosphatase activities, and P-cycle multifunctionality index (MFI) differed between different restoration forests and cropland. CP, cropland; AF, artificial restoration of forest; NF, natural restoration of forest. TP, total phosphorus; AP, available P; MBP, microbial biomass P; ACP, acid phosphatase; ALP, alkaline phosphatase. *, P<0.05; **, P<0.01; ***, P<0.001; ns, no significant difference.

The ACP activities in CP decreased with increasing temperature (Appendix G-j). In AF, the TP and AP contents, ALP activity, and P-cycle MFI increased with rising temperatures (Appendix G-b, e, n and q), while ACP activity showed the opposite trend (Appendix G-k). In NF, the TP and MBP contents were the highest at the middle temperature (Appendix G-c and i); ALP activities increased with increasing temperature (Appendix G-o), whereas ACP activity and P-cycle MFI exhibited the opposite patterns (Appendix G-l and r).

3.3. Factors influencing soil available P and P-cycle MFI

The soil P-cycle MFI was significantly and primarily explained by restoration, followed by MAT, MBC, MBN, SOC, exchangeable Ca, BD, *phoD*-harboring bacterial community structure, and exchangeable Mg (Fig. 5-A). In addition, the P-cycle MFI showed significant and positive correlations with restoration approach, MBC, MBN, SOC, and exchangeable Ca and Mg, but a significant and negative correlation with BD (Fig. 5-B). These results show that restoration has a greater influence on the P-cycle MFI than temperature. The results of the SEM showed that restoration significantly modified the physical and chemical properties of soil, such as decreasing BD, altering the *phoD*-harboring bacterial community structure, increasing microbial biomass, and enhancing phosphatase activity, thereby promoting the

multifunctionality of the soil P cycle (Fig. 6). In addition, the temperature variations also influenced the *phoD*-harboring bacterial community structure, increased microbial biomass and phosphatase activity, and ultimately enhanced the multifunctionality of the soil P cycle (Fig. 6).

3.4. Genera related to the variation of multifunctionality

In CP (Fig. 7-A), the variation in soil P-cycle MFI was significantly explained by several non-dominant genera with relative abundances of <1%, including *Spiribacter* (0.373%), *Allocoleopsis franciscana* (0.107%), *Psychrobacter* (0.101%), *Nostoc piscinale* (0.0578%), *Salinicola* (0.579%), *Tistlia* (0.116%), *Aestuariivirga* (0.698%), *Methyloferula* (0.0801%), *Rubrivivax* sp. (0.0707%), *Mastigocladopsis repens* (0.197%), and *Verminephrobacter* (0.71%). In addition, some dominant genera (relative abundances of >1%) such as *Ramlibacter* (2.15%), *Bradyrhizobium* (6.89%), and *Pseudolabrys* (9.95%) also played a significant role.

In AF (Fig. 7-B), the variation in soil P-cycle MFI was significantly explained by the non-dominant genera

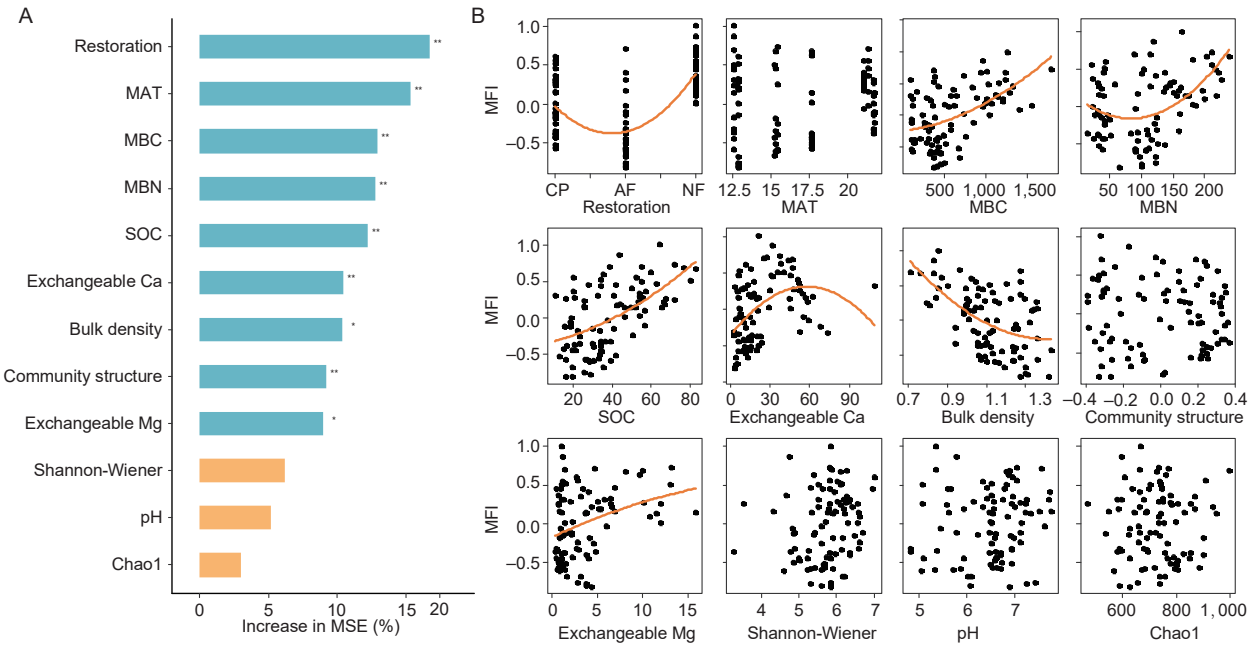


Fig. 5 Relative importance for explaining the variations of the soil P-cycle multifunctionality index (MFI; A) using the random forest model, and their relationships calculated by the generalized additive models (B). SOC, soil organic carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MAT, mean annual temperature; MSE, mean square error, expressed as MSE (%), was used to calculate the relative importance of the measured variables.

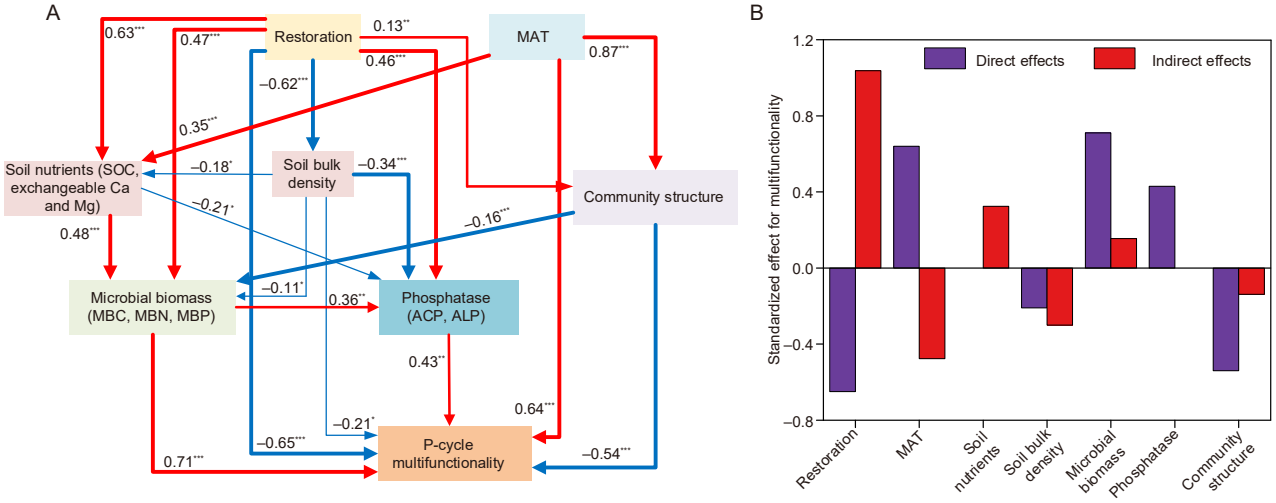


Fig. 6 The influence path of soil P-cycle multifunctionality (A) and the direct and indirect effects of factors on this index (B) using the structural equation model (SEM). The parameters of this model are X_2=6.846, *df*=8, *n*=90, CFI=0.981, AGFI=0.916, *P*=0.553, and RMSEA=0.000. Soil nutrients include soil organic carbon (SOC), and exchangeable Ca and Mg; microbial biomass includes microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP); phosphatase activities include acid phosphatase (ACP) and alkaline phosphatase (ALP) activities; MAT, mean annual temperature.

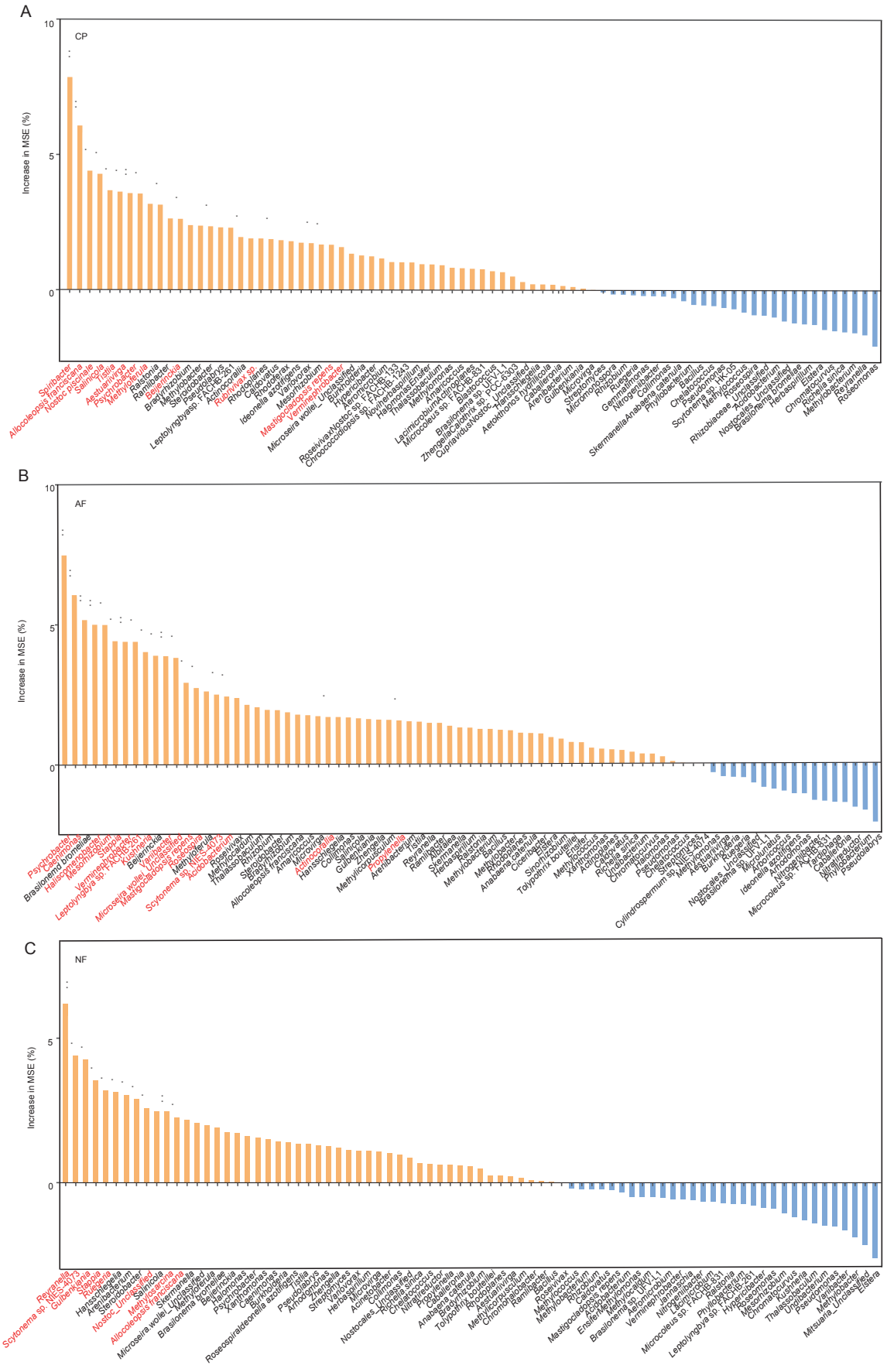


Fig. 7 Relative importance of *phoD*-harboring bacterial genus for explaining the variations in the soil P-cycle multifunctionality index in cropland (CP; A), artificial restoration of forest (AF; B), and natural restoration of forest (NF; C) using the random forest model. MSE, mean square error. The red text in the figure indicates the non-dominant genera.

Psychrobacter (0.193%), *Caenimonas* (0.408%), *Haliscomenobacter* (0.0869%), *Verminephrobacter* (0.161%), *Variibacter* (0.064%), *Mesorhizobium* (0.308%), *Stappia* (0.296%), *Leptolyngbya* sp. FACHB-261 (0.25%), *Kushneria* (0.0993%), *Microseira wollei*_Unclassified (0.843%), *Mastigocladopsis repens* (0.248%), *Roseospira* (0.308%), *Scytonema* sp. NIES-4073 (0.573%), *Acidobacterium* (0.202%), *Actinocorallia* (0.186%), and *Propylenella* (0.0857%). Dominant genera such as *Brasilonema bromeliae* (1.97%) and *Beijerinckia* (4.42%) also contributed significantly.

In NF (Fig. 7-C), the variation in soil P-cycle MFI was significantly influenced by the non-dominant genera *Reyranella* (0.352%) and *Methylosarcina* (0.269%), followed by *Scytonema* sp. NIES-4073 (0.0754%), *Gulbenkiania* (0.32%), *Stappia* (0.0477%), *Ruegeria* (0.148%), *Nostoc_* Unclassified (0.208%), and *Allocoleopsis franciscana* (0.0446%). Dominant genera such as *Hansschlegelia* (1.23%), *Arenibacterium* (2.54%), and *Steroidobacter* (1.29%) also played a crucial role.

Notably, a wide variety of rare genera were classified within the phyla Proteobacteria and Acidobacteria. The Proteobacteria included rare genera *Spiribacter*, *Psychrobacter*, *Salinicola*, *Aestuariivirga*, *Methyloferula*, *Rubrivivax* spp., *Verminephrobacter*, *Caenimonas*, *Haliscomenobacter*, *Variibacter*, *Mesorhizobium*, *Stappia*, *Roseospira*, *Gulbenkiania*, *Ruegeria*, *Hansschlegelia*, *Arenibacterium*, and *Steroidobacter*. The genus *Acidobacterium* was identified in the phylum Acidobacteria.

4. Discussion

4.1. Restoration type and temperature impact soil P-cycle multifunctionality

In the present study, the indices of soil P-cycle multifunctionality were the highest in natural restoration of forest, followed by cropland, and then artificial restoration of forest. This suggests that natural restoration is more effective than artificial restoration for enhancing soil P-cycle multifunctionality, which essentially supports the first hypothesis. Previous studies have demonstrated that the soil environment and microbial community structure and diversity significantly influence soil ecosystem multifunctionality (Wagg *et al.* 2014; Guo *et al.* 2021; Wan *et al.* 2021; Wang N *et al.* 2024). The findings of this study align with those earlier reports. Notably, karst ecosystems in southwestern China are characterized by severe P limitation, pronounced human-land conflicts, and ecological fragility. Consequently, soil P-cycle multifunctionality serves as a critical indicator for evaluating the effectiveness and sustainability of ecological restoration efforts in this region. Further research is needed to elucidate the specific factors and underlying mechanisms influencing soil P-cycle multifunctionality during ecological restoration.

Restoration efforts enhance the multifunctionality of the soil P-cycle by increasing soil organic matter and reducing bulk density, and by modifying the microbial community structure and activity (Stürck and Verburg 2017; Zhang *et al.* 2024). First, vegetation restoration typically leads to greater soil organic carbon (Ying *et al.* 2023; Cui *et al.* 2024), as observed from cropland to artificial and natural forests. Soil organic carbon is crucial for microbial activity, which impacts the P cycle, especially in P-limited soils (Chen X T *et al.* 2024), since soil organic carbon is the primary source of carbon and energy for soil microorganisms. The higher exchangeable Ca and Mg levels in natural forests, along with their positive correlations with P-cycle multifunctionality, support this mechanism. This occurs because these factors indicate greater rates of Ca-bound P dissolution and organic matter decomposition (Pan *et al.* 2024a). Despite lower total and available P levels, natural forests showed the highest P-cycle multifunctionality index, indicating more active microbial P transformation. Conversely, croplands had higher total and available P contents but a lower P-cycle multifunctionality index, likely due to fertilizer use. Second, soil bulk density is another critical parameter influencing the P cycle (Mashagiro *et al.* 2024). Lower bulk density can enhance soil structure, aeration, and water permeability, thereby promoting microbial growth and reproduction and extracellular enzyme activities (van Leeuwen *et al.* 2017; Zhu *et al.* 2021; Azene *et al.* 2023). The results of this study showed reduced soil bulk density with advancing vegetation restoration. Thus, restoration efforts enhance soil properties and microbial activities, thus boosting the multifunctionality of the soil P cycle (Fig. 8).

Temperature is another critical factor influencing the multifunctionality of the soil P cycle, which results from the interactive feedback between vegetation, soil, and microorganisms. There are two possible reasons for this phenomenon. (1) An elevated temperature can enhance microbial growth and increase the abundance and expression of genes involved in the P cycle. This effect is particularly pronounced in the karst regions of southwestern China, where water and heat coincide seasonally (Zhang *et al.* 2023). Note that the abundance and expression of functional genes involved in the microbial P cycle may be closely related to soil P fractions across the different temperature regions. Previous research has shown that in karst areas, the content of soil-bound inorganic P increases with rising temperature, while the available P content decreases, especially in forests (Pan *et al.* 2024a). This trend leads to a greater degree of P limitation as temperatures rise. To compensate for the reduced available P, microorganisms upregulate the expression of phosphatase genes, thereby enhancing phosphatase secretion into the soils. This study also found that the alkaline phosphatase activities in both artificial and natural restoration of forests increased with rising temperatures, whereas acid phosphatase activity exhibited the opposite trend. These findings suggest that *phoD*-harboring bacteria, which carry alkaline phosphatase genes, play a crucial role in improving the soil P cycle (Liang *et al.* 2020; Wei *et al.* 2021). However, in cropland, the alkaline phosphatase activity did not increase with rising temperatures due to the higher available P content at this land, which could meet the P requirements of plants and microorganisms under conditions of lower microbial activity. (2) The elevated nutrient demand of vegetation stimulates the activity of

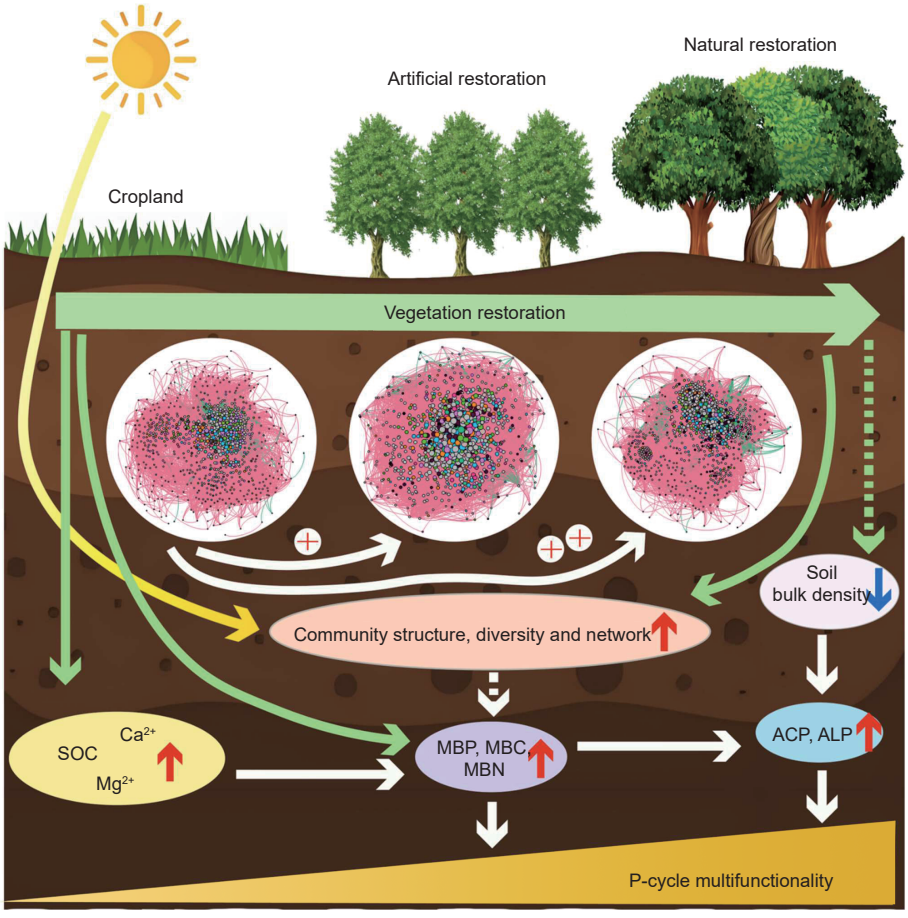


Fig. 8 Effects of restoration on soil properties and *phoD*-harboring bacteria on P-cycle multifunctionality. SOC, soil organic carbon; Ca^{2+}, exchangeable Ca; Mg^{2+}, exchangeable Mg; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus; ACP, acid phosphatase; ALP, alkaline phosphatase.

associated microorganisms. Generally, plant productivity is higher in warmer regions, leading to greater P requirements (Ziegler *et al.* 2024). In nutrient-limited regions or during the progress of restoration, the mycorrhizal uptake strategy is more prevalent (Wu *et al.* 2025). This approach emphasizes the collaboration between symbiotic fungi and free-living microorganisms to enhance nutrient acquisition. Specifically, free-living microorganisms obtain soluble organic matter from mycorrhizae to support their survival and reproduction, while simultaneously converting and activating soil P. In turn, the fungi facilitate the absorption of P by plant roots. Many bacteria, such as *phoD*-harboring bacteria, are examples of free-living microorganisms that enhance phosphatase activity by upregulating *phoD* gene expression (Fig. 7). Elevated temperatures increased the metabolic activity of these bacteria.

4.2. Rare taxa influence soil P-cycle multifunctionality

Vegetation restoration has a significant impact on the structure and diversity of soil microbial communities, ultimately enhancing the role of certain rare taxa in the P cycle. In the cropland in this study, non-dominant genera comprising 86.67% (13 out of 15, $P<0.05$) of all significant genera influenced the multifunctionality indices of the soil P-cycle. In the artificial restoration of forest, this proportion was 88.89% (16 out of 18). In natural restoration of forest, non-dominant genera accounted for 72.72% (8 out of 11) of all significant genera that affected this index. These findings were similar to those of previous studies which showed that rare genera of *phoD*-harboring bacteria have a greater role in P transformation than the dominant genera (Wei *et al.* 2019; Liu *et al.* 2021), which supports our second hypothesis. This phenomenon may be attributed to several factors.

First, functional redundancy of the dominant taxa. One study has observed that the abundances of certain *phoD*-harboring bacterial taxa, notably the dominant genera *Pseudomonas* and *Bradyrhizobium*, significantly increase under low P conditions (Chen M *et al.* 2024). However, these taxa did not substantially account for the changes in P-cycle multifunctionality observed in this study (Fig. 7). For instance, *Bradyrhizobium*, the predominant genus in this investigation, can fix atmospheric nitrogen. This nitrogen fixation may supply essential nutrients to other *phoD*-harboring bacteria, thereby enhancing their vitality and ultimately promoting soil P transformation (Kurth *et al.* 2021). Despite numerous pairwise correlations involving the dominant genera within co-occurrence network models across all three restoration stages (Fig. 3), these genera might exhibit functional

redundancy or act as cooperative species by providing necessary nutrients (Eaton *et al.* 2020).

Second, niche differentiation of non-dominant microbial taxa and their sensitivity to environmental changes. Following vegetation restoration, soil bulk density was found to decline (Table 1), resulting in an improvement in spatial niches, primarily due to an increase in large soil aggregates (Hu and Lan 2020). Numerous studies have demonstrated that rare taxa in soil microbial communities exhibit more unique predicted functional traits (Li *et al.* 2019, 2025; Yang *et al.* 2022; Mohapatra *et al.* 2023; Liu *et al.* 2025), such as P transformation. This is largely due to heightened competition among microorganisms for limited space and nutrient resources. To ensure survival, rare microbial groups are compelled to colonize within soil aggregates or in areas with scarce resources (Zheng *et al.* 2021; Simon *et al.* 2024), thereby promoting the transformation and utilization of sequestered P (Wan *et al.* 2025). This process contributes to both spatial and resource niche differentiation. In addition, a diverse array of rare genera within the phyla Proteobacteria and Acidobacteria were identified in this study. As members of the eutrophic bacterial group, these taxa play a critical role in the degradation of organic matter (Wu *et al.* 2009), thereby facilitating the transformation of organic P derived from organic matter and the soil P-cycle. The observed positive relationships between the P-cycle multifunctionality index and the soil organic carbon, total and available P, exchangeable Ca, acid and alkaline phosphatase activities, and microbial biomass support this mechanism (Appendix H and Fig. 5). Consequently, the rare taxa of *phoD*-harboring bacteria contribute to the degradation of organic matter for P transformation while occupying more specialized ecological niches, ultimately enhancing the multifunctionality of the soil P cycle in karst ecosystems.

Third, non-dominant taxa significantly contribute to enhancing community stability and complexity. The *phoD*-harboring bacteria promote soil P transformation and activation by increasing community complexity and strengthening co-occurrence network connectivity in P-limited environments (Pan *et al.* 2024a, b). In this process, a greater number of non-dominant *phoD*-harboring bacterial taxa are integrated into the co-occurrence network. This study also found that the tightness and complexity of the *phoD*-harboring bacterial co-occurrence network progressively increased from cropland to artificial restoration of forest, and then to natural restoration of forest (Fig. 3). Consequently, the non-dominant *phoD*-harboring bacteria exhibited a higher explanatory power in driving P-cycle multifunctionality, thereby supporting the hypothesis that non-dominant groups enhance both community stability and complexity.

4.3. Implications of soil P-cycle multifunctionality for karst ecological restoration

Based on the findings of this study, natural restoration of forest exhibited higher multifunctionality of soil P-cycle than artificial restoration of forest, making it a more suitable approach for the ecological restoration of fragile karst ecosystems. This is attributed to its greater soil organic matter content and improved soil texture, which enhances soil microbial biomass and activity and modulates the microbial community structure. However, our previous studies indicated that after more than three decades of ecological restoration in karst ecosystems, many forests remain in the shrub stage (Pan *et al.* 2015, 2024b). The implementation of artificial replanting by introducing specific tree species into these shrublands may serve as an effective approach to facilitate the transition from shrubs to arbor forests. In artificial restoration of forest, the contents of soil total and available P, as well as phosphatase activities and organic carbon contents, were found to be lower than in natural restoration of forest. This led to a reduced P-cycle multifunctionality index. To enhance the multifunctionality of soil P-cycle in artificial restoration of forest, strategies for increasing the total P supply and organic matter content must be considered, and two recommendations can be proposed. (1) Introduce deep-rooted plants to enhance the organic matter and P contents. In karst regions, the landscape is characterized by exposed bedrock and shallow soil layers, leading to the widespread distribution of shallow-rooted plants (Ding *et al.* 2021). Deep-rooted species, such as *Quercus glauca* Thunb., can extract P from deep soil or rock surfaces through fissures in karst critical zones. This P will be subsequently transferred to the topsoil *via* litterfall, thereby enhancing the P content of the topsoil (Pan *et al.* 2018). (2) Optimize the structure of vegetation by introducing leguminous plants to increase the organic matter content. Leguminous plants not only enhance the soil nitrogen content through biological nitrogen fixation but also augment the input of soil organic carbon and organic P *via* their litter decomposition. In addition, legumes can exploit P resources by stimulating arbuscular mycorrhizal colonization in roots (Liang *et al.* 2022), indicating that legumes play a crucial role in promoting the P cycle.

In karst regions, large amounts of cropland must be preserved rather than converted to forests to ensure the food security of local populations. However, our findings indicate that despite substantial fertilizer application in croplands, the P-cycle multifunctionality index remains low. This phenomenon is likely due to the low soil organic matter and microbial biomass contents. To address this issue, enhancing soil organic matter content through the following measures is imperative. (1) Promote straw incorporation into the soil rather than chemical fertilizers. In karst regions, maize straw is frequently harvested for use as livestock fodder for cattle and sheep. However, a greater proportion of straw should be retained in the field (Li R *et al.* 2020). Furthermore, using organic materials such as livestock manure, silkworm droppings, and treated sludge represents another effective strategy to enhance the soil organic matter content (Xie *et al.* 2001; Li F Y *et al.* 2020). For instance, a long-term field study spanning 25 years in a karst ecosystem demonstrated that the application of organic manure significantly increased soil organic carbon by 27.4–53.8% compared to the use of chemical fertilizers (Liu Y L *et al.* 2023). In addition, incorporating biochar into croplands can also enhance the soil organic carbon content (Zhou *et al.* 2019). (2) Incorporate legumes, such as soybeans, into the agricultural system to increase the soil organic matter content

and enhance phosphorus availability. Both intercropping and rotation of soybean and maize can enhance organic matter content by increasing the inputs of root exudations and litter (Zhang *et al.* 2017). Furthermore, the introduction of P-solubilizing functional microorganisms in intercropping or rotation systems can further promote soil P solubilization.

5. Conclusion

This study offers novel insights into the effects of restoration on soil physicochemical properties and *phoD*-harboring bacterial taxa, thereby promoting soil P-cycle multifunctionality in karst ecosystems. The contents of soil total and available P declined from cropland to artificial and natural restoration of forests. In contrast, this study revealed increases in soil organic carbon, exchangeable calcium, exchangeable magnesium, microbial biomass carbon and nitrogen, as well as the activities of acid and alkaline phosphatases and the indices of soil P-cycle multifunctionality. More rare genera were found to have significant impacts on the variation of soil P-cycle multifunctionality compared to the dominant genera. In addition, restoration had a more substantial impact on soil P-cycle multifunctionality than temperature. Natural restoration was found to reduce soil bulk density while enhancing soil organic carbon, microbial biomass, complexity and the co-occurrence network tightness of *phoD*-harboring bacterial community structure, along with greater activities of acid and alkaline phosphatase, ultimately boosting soil P-cycle multifunctionality. The findings of this study highlight the significant impact of a natural restoration strategy and soil organic carbon, bulk density, and rare taxa of *phoD*-harboring bacteria on enhancing soil P-cycle multifunctionality.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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