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Soil microbial functional profiles of P-cycling reveal drought-induced constraints on P-transformation in a hyper-arid desert ecosystem

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Abstract:

Soil water conditions are known to influence soil nutrient availability, but the specific impact of different conditions on soil phosphorus (P) availability through the modulation of P-cycling functional microbial communities in hyper-arid desert ecosystems remains largely unexplored. To address this knowledge gap, we conducted a 3-year pot experiment using a typical desert plant species (*Alhagi sparsifolia* Shap.) subjected to two water supply levels (25 %–35 % and 65 %–75 % of maximum field capacity, MFC) and four P-supply levels (0, 1, 3, and 5 g P m⁻² y⁻¹). Our investigation focused on the soil Hedley-P pool and the four major microbial groups involved in the critical phases of soil microbial P-cycling. The results revealed that the drought (25 %–35 % MFC) and no P-supply treatments reduced soil resin-P and NaHCO₃-Pi concentrations by 87.03 % and 93.22 %, respectively, compared to the well-watered (65 %–75 % MFC) and high P-supply (5 g P m⁻² y⁻¹) treatments. However, the P-supply treatment resulted in a 12 %–22 % decrease in the soil NH₄⁺-N concentration preferred by microbes compared to the no P-supply treatment. Moreover, the abundance of genes engaged in microbial P-cycling (e.g. *gcd* and *phoD*) increased under the drought and no P-supply treatments ($p < 0.05$), suggesting that increased NH₄⁺-N accumulation under these conditions may stimulate P-solubilizing microbes, thereby promoting the microbial community's investment in resources to enhance the P-cycling potential. Furthermore, the communities of *Steroidobacter cummioxidans*, *Mesorhizobium alhagi*, *Devosia geojensis*, and *Ensifer sojae*, associated with the major P-cycling genes, were enriched in drought and no or low-P soils. Overall, the drought and no or low-P treatments stimulated microbial communities and gene abundances involved in P-cycling. However, this increase was insufficient to maintain soil P-bioavailability. These findings shed light on the responses and feedback of microbial-mediated P-cycling behaviors in desert ecosystems under three-year drought and soil P-deficiency.

Keywords: Desert ecosystem, Soil phosphorus fractions, Metagenomics, Soil P cycling, Functional microbiome

Introduction:

Phosphorus (P) is an essential mineral required by all living creatures (Ding et al., 2021; Dodd et al., 2023). While gaseous P compounds are not available in the atmosphere, P-cycling primarily takes place among plants, soil, and microbes (Vitousek et al., 2010; Stutter et al., 2015). Soil microbial P-cycling in drought regions has become a major concern due to altered global precipitation patterns, especially in hyper-arid and P-impooverished desert ecosystems (Hou et al., 2020; Luo et al., 2021). The transformation of P between labile and non-labile fractions occurs depending on environmental conditions (Helfenstein et al., 2018). In dry or semi-arid environments, soil P turnover is known to be exceptionally slow (Niederberger et al., 2019; Gao et al., 2020).

Microbes play a crucial role in the soil microbial P-cycling process, particularly functional microbes associated with P turnover (Wei et al., 2019; Dai et al., 2020). There are four main groups of microbial genes that regulate soil microbial P turnover: P starvation response regulatory genes (*phoU*, *phoR*, *phoB*), P uptake transporter genes (*ugpQ*, *phnD*, *pstB*), organic P (Po) mineralization genes (*appA*, *phoA*, *phoD*), and inorganic P (Pi) solubilization genes (*gcd*, *ppa*, *ppx*) (Liu et al., 2023; Zhang et al., 2023). For instance, the *gcd* gene encodes the membrane-bound quinone protein glucose dehydrogenase (PQQGDH), which plays a role in solubilizing phosphate salts, mainly hydroxyapatite, by enhancing gluconate production (Rodríguez et al., 2006). Other studies have shown that microbes with genes encoding alkaline phosphatase (*phoD*), phytase (*appA*), and C-P lyase (*phn*) have the potential to release available P by mining Po substances in the soil (Grafe et al., 2018). The ability of microbes to efficiently consume and fix P in their biomass depends on the presence of genes encoding high-affinity phosphate-specific transporter proteins (*pst*) and low-affinity inorganic phosphate transporter proteins (*pit*) (Oliverio et al., 2020). Genes involved in regulating P starvation responses help microbes utilize external P sources, and they are also linked to genes involved in P absorption and transport, as well as controlling the expression of genes encoding alkaline phosphatases, especially under limited P conditions (Liang et al., 2022). Some studies in agriculture, forests, and grassland ecosystems have demonstrated that these regulatory genes contribute to microbial P fixation under limited P conditions (Bergkempe et al., 2016; Oliverio et al., 2021). For instance, the genes encoding glycerol-3-phosphate transport proteins (*ugpBCQ*) showed higher abundance in reforested soils with relatively lower available P concentrations (Wu et al., 2022a). On the other hand, other studies in semi-arid natural grassland and beech forest soils suggested that the relative abundance of soil Pi solubilization and Po mineralization genes increased significantly in P-rich soils (Bergkemper et al., 2016; Liu et al., 2023). Moreover, certain phosphate-solubilizing microbes (e.g., *Bacillus*, *Burkholderia*, *Pseudomonas*, and *Rhizobium*) in the plant-soil system have been found to improve rhizospheric function in P-poor soils (Pastore et al., 2020; Shao et al., 2021). However, further investigations are still needed to fully understand the connection between microbes' P acquisition strategies (including Pi dissolution) and

specific soil conditions.

Water, as a major participant in microbial metabolism, plays a crucial role in soil microbial P-cycling (Schimel, 2018). For example, soil water content has been identified as a significant predictor of *phoD* gene community composition in grasslands (Chen et al., 2021). Several studies have suggested that soil drought reduces the metabolic and enzymatic activities of soil microbes (Sardans and Penuelas, 2010; Sun et al., 2017) but increases soil salinity and inorganic N and P concentrations (Zhang and Marschner, 2017). However, conflicting results exist, as some studies have found that drought does not significantly alter soil inorganic N and P concentrations (Jensen et al., 2003) or decrease soil mineralization of nutrients bound to organic matter (Borken and Matzner, 2009). Extreme drought events can even lead to the death of soil microbes, although some may survive under drought conditions by regulating their osmotic potential to enter a dormant state (Schimel, 2018). The inconsistent findings in these studies regarding the relationship between soil nutrient availability and water conditions may be attributed to differences in plant species, soil properties, climate types, and experimental methods employed (Chen et al., 2021; Zhang et al., 2023).

The southern edge of the Taklamakan Desert is a hyper-arid desert ecosystem with high salt concentration, pH value, and depleted soil P (Li et al., 2021a). In recent years, changing global precipitation patterns have resulted in episodic extreme precipitation and drought events during the growing season, posing a threat to vegetation survival and ecosystem structure and function in this region (Tariq et al., 2022). *Alhagi sparsifolia* Shap., a widely distributed leguminous desert species in this area, is severely limited in growth due to P deficiency (Gao et al., 2022). Being more sensitive to soil P-deficiency, *A. sparsifolia* provides an excellent opportunity to study the potential relationship between soil water, P availability, and microbial P-cycling. In this study, we conducted pot experiments under two water conditions and four P-supply levels to investigate the soil Hedley-P pool, functional genes, and functional microbes driving soil microbial P-cycling in the rhizosphere soils of *A. sparsifolia* after three years of treatment. We hypothesized that (1) drought conditions would decrease soil P availability due to impeded P turnover from insoluble P to labile and bio-available P fractions, and (2) the abundance of functional genes associated with soil microbial P-cycling and the community structure of functional microbes would increase under drought compared to well-watered conditions, with a possibly more significant response under low P availability and/or a less pronounced response when P supply is high.

Materials and Methods:

2.1. Experimental Site and Soil Description

The artificial outdoor experimental field used for this study is located near the boundary between the Cele Oasis and the

Taklimakan Desert on the southern edge (37° 00' 57' ' N, 80° 43' 45' ' E, 1353 m elevation). The average annual temperature in this area is 15.85°C, and it experiences a hyper-arid climate with an annual precipitation of 42.62 mm and an annual evaporation of 2700 mm. The soil at the site is classified as aeolian sandy soil, specifically Arenosols. The basic soil properties include a bulk density of 1.34 g cm⁻³, pH of 8.6, organic carbon concentration of 4.66 g kg⁻¹, total nitrogen (TN) concentration of 0.22 g kg⁻¹, total phosphorus (TP) concentration of 0.63 g kg⁻¹, NH₄⁺-N concentration of 1.39 mg kg⁻¹, NO₃⁻-N concentration of 13.07 mg kg⁻¹, Olsen P concentration of 2.31 mg kg⁻¹, and available K concentration of 136.11 mg kg⁻¹.

2.2. Experimental Design

A pot experiment was conducted from 2021 to 2023, with two water treatments: drought (2535% of maximum field capacity) and well-watered (6575% of maximum field capacity). Four soil P-supply treatments were applied, consisting of control (0 g P m⁻² yr⁻¹), low-P (1 g P m⁻² yr⁻¹), medium-P (3 g P m⁻² yr⁻¹), and high-P (5 g P m⁻² yr⁻¹) supplied in the form of NH₄H₂PO₄ (P 27%, N 12% in mass). This resulted in a total of eight treatments: (T1) DP0, drought, and no P-supply; (T2) DP1, drought, and low-P-supply; (T3) DP2, drought, and medium-P-supply; (T4) DP3, drought, and high-P-supply; (T5) WP0, well-watered and no P-supply; (T6) WP1, well-watered and low-P-supply; (T7) WP2, well-watered and medium-P-supply; and (T8) well-watered and high-P-supply. The treatments were replicated four times in a completely randomized design.

Plastic pots with a diameter of 30 cm and height of 100 cm were filled with homogenized topsoil collected from the natural desert habitat (0–30 cm soil layer). After air-drying the soil for two days, it was sieved through a 0.5-cm mesh screen to remove large particles. On April 1st, 2021, the pots were watered to their maximum field capacity (approximately 18%), and five seeds of *A. sparsifolia* were sown in each pot. After one month of growth, the seedlings were trimmed to one healthy and uniform seedling per pot. The P source was administered based on the four P treatments, applying it to each pot with water on May 2nd, 2021, March 24th, 2022, and March 20th, 2023. Soil moisture was monitored daily using a soil moisture rapid detector (Spectrum®, TDR 300, Inc., Illinois, USA) to maintain soil water content at approximately 25–35% for drought treatment and 65–75% for well-watered treatment.

2.3. Soil Sampling

On June 1st, 2023 (the third year) during the vigorous growth stage, rhizosphere soil samples were collected. Fine roots with minimal floating soil were cut using sterilized pruning shears and placed in 50 mL sterile tubes. The samples were immediately transferred to the laboratory in ice boxes. Rhizosphere soil was detached from the surface of the fine roots

using a vortex oscillator. A total of 32 rhizosphere soil samples were obtained (two water treatments, four P-supply treatments, four replicates). After passing through a 2 mm sieve, the soil samples were separated into three subsamples. Subsamples for soil microbial macrogenome analysis were stored in a -80°C freezer, subsamples for soil enzyme activity determination were kept at -4°C (tested within one week), and subsamples used to analyze soil P fraction and other properties were air-dried and kept at room temperature.

2.4. Soil P Fraction and Physicochemical Properties

The soil Hedley P pool was divided into three major categories and nine fractions following the procedure outlined by Hedley et al. (1982) and Tiessen and Moir (2007). Each P fraction was extracted sequentially from the soil using different extractants: 0.5 g ion exchange resin in deionized water (extract resin-P); 0.5 M NaHCO_3 (extract $\text{NaHCO}_3\text{-Pi}$ and $\text{NaHCO}_3\text{-Po}$); 0.1 M NaOH (extract NaOH-Pi and NaOH-Po); hot concentrated HCl (extract conc. HCl-Pi and HCl-Po); 1 M HCl (extract HCl-Pi); and concentrated nitric acid (extract residual-P). Soil Pi fraction and TP were measured using Mo-Sb colorimetry, and soil Po fraction was calculated by subtracting the Pi fraction's TP from each P fraction's TP.

The activities of alkaline phosphatase (ALP), pyrroloquinoline-quinone synthase (PQQS), and phytase were determined using ELISA® Kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., China). Soil microbial biomass P (MBP) concentration was examined using the chloroform fumigation-extraction method after extraction with NaHCO_3 for 24 hours. The exchangeable $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations were determined using an AA3 continuous flow analytical system after extraction with 1 M KCl . Dissolved organic carbon (DOC) concentration was determined using a TOC analyzer after extraction with 0.5 M K_2SO_4 . Soil pH and electrical conductivity (EC) in water were determined using a soil/water ratio of 1:2.5 (w/v) with a PHS-3G digital pH meter (Precision and Scientific Crop., China). Soil Olsen P concentration of basic samples was determined by extracting with 0.5 M NaHCO_3 (pH 8.5). TN concentration was determined using the Kjeldahl method, and available K (AK) concentration was determined by a Flame photometer after extraction with ammonium acetate. Bulk density was calculated using the graduated cylinder technique. The above variables were determined following Bao's (2000) method.

2.5. Soil DNA Extraction and Metagenomic Sequencing

Total microbial genomic DNA samples were extracted using the OMEGA Soil DNA Kit (D5625-01) and stored at -20°C for further assessment. DNA concentration was determined at 260 nm and 280 nm using a fluorescence spectrophotometer (Quantifluor-ST fluorometer, Promega, E6090; Quant-iTPicoGreen dsDNA Assay Kit, Invitrogen, P7589), and DNA quality was confirmed by 1% agarose gel electrophoresis. The extracted total DNA was fragmented into short fragments

using a Whole Genome Shotgun strategy based on the Illumina NovaSeq platform (Illumina, Inc., San Diego, CA, USA). Libraries of inserts of appropriate lengths were constructed, and paired-end sequencing was performed with a sequencing depth of 10 G per sample.

The raw sequencing reads were processed using Cutadapt (v1.2.1) and fastp's sliding-window techniques (Chen et al., 2018). The data were then taxonomically categorized using Kraken2 (Wood et al., 2019) to identify proteins from archaea, bacteria, viruses, fungi, and microbial eukaryotes. Assembly of contigs was performed using Megahit (Liu et al., 2015), and contigs were pooled and clustered using mmseqs2 (Steinegger and Söding, 2017). Non-redundant contigs were aligned to the NCBI-nt database, and genes were predicted using MetaGeneMark (Zhu et al., 2010). CDS sequences were clustered using mmseqs2, and high-quality reads were mapped onto predicted gene sequences using Salmon (Patro, 2015). Abundance values in metagenomes were normalized using the CPM (copy per kilobase per million mapped reads) method. Non-redundant genes were annotated using mmseqs2.

A dataset was searched for 47 genes associated with soil microbial P-transformation and their matching KO numbers (Liang et al., 2022; Wu et al., 2022a, b). These genes were classified into four categories based on their functional roles in soil P-cycling. The predicted gene sequences in the KEGG database were compared with the NCBI non-redundant protein sequence database using Diamond and MEGAN6 to derive the species categorization of particular genes.

2.6. Statistical Analysis

Analysis of variance (ANOVA) was performed using IBM SPSS 22.0 software to identify the main and interactive effects of water treatment and P-supply treatment on soil Hedley P fractions, physicochemical properties, microbial P-cycling-related functional genes, and functional microbes. Differences between treatment gradients were analyzed using one-way ANOVA followed by Duncan's multiple range tests ($p < 0.05$). Soil microbial beta diversity and major gene community structure were represented by principal coordinate analysis (PCoA) using the Bray-Curtis dissimilarity matrix on the Tutools platform. Redundancy analysis (RDA) was used to analyze relationships between soil P fraction and soil microbial biomass, enzyme activity, and physicochemical properties. Random forest analysis was employed to investigate major soil factors predicting soil microbial alpha and beta diversity on the Tutools platform. Pearson correlation analysis was used to clarify differences in the abundance of 47 soil microbial P-cycling-related functional genes between treatments, combined with random forest analysis on these genes using soil active Pi and Po as the main predictors, resulting in the selection of the four most significantly different functional genes among the four soil microbial P-cycling-related functional genes in terms of their functional roles. Analysis of covariance was utilized to identify differential functional groups at the species level, and random forest analysis and correlation coefficient plots were again used to

identify functional flora associated with soil P fraction and soil biochemical properties. All analyses were performed in R 4.0.4, except for ANOVA, which was conducted using IBM SPSS 22.0 software. The figures were created using GraphPad Prism 9.0 and R 4.0.4, with the association network generated using Gephi 0.9.2.

Results:

3.1 Soil Properties

The drought treatment led to increased soil NO_3^- -N concentration, electrical conductivity (EC), pH, and phytase activity compared to the well-watered treatment (Figs. S1, S2). No P-supply resulted in increased soil alkaline phosphatase (ALP) and pyrroloquinoline-quinone synthase (PQQS) activity and NH_4^+ -N concentration, but decreased soil phytase activity, microbial biomass P (MBP), and dissolved organic carbon (DOC) concentration compared to the high P-supply treatment. Relative to the well-watered and no P-supply treatments, the drought and no P-supply treatment increased PQQS activity, NH_4^+ -N concentration, and EC by 21.43, 14.7, and 33.55%, respectively, but reduced ALP activity by 24.66% (Table 1, $p < 0.05$). Additionally, the drought treatment significantly reduced the concentrations of soil resin-P, NaHCO_3 -Pi, and active Pi by 48.70, 67.96, and 54.40%, respectively, compared to the well-watered treatment ($p < 0.01$) (Table 2 and Fig. S3). Moreover, different levels of P-supply increased the concentrations of various soil P fractions, such as active Pi and Po, with levels 482 and 102% higher under high P-supply compared to no P-supply, respectively. However, the drought and low P-supply treatment decreased resin-P, NaHCO_3 -Pi, and active Pi by 61.11, 61.52, and 59%, respectively, relative to the well-watered and low P-supply treatments ($p < 0.001$). Redundancy analysis (RDA) showed that the variation in soil P fractions was mainly driven by soil MBP, PQQS, DOC, microbial biomass carbon (MBC), EC, phytase, and NH_4^+ -N (Fig. S4).

3.2 Soil microbial alpha and beta-diversity

Both drought and no P-supply treatments significantly increased soil microbial alpha-diversity (Fig. 1a). Although water treatment directly did not affect alpha-diversity variation, it was influenced by P-supply treatment and interactions between water and P-supply treatments ($p < 0.05$). Beta-diversity in treatments with well-watered and no P-supply treatment significantly differed from that in drought and no or low P-supply treatments ($p < 0.001$) along the first component, which explained 48.7% of the variation (Fig. 1b). Additionally, drought and no P-supply were separated from drought and low P-supply along the second component, explaining 15.1% of the variation. Further random forest analysis revealed that in drought and well-watered soils, 54.4 and 42.6% of the variation in microbial beta-diversity was accounted for by soil properties (Figs. 1c, d). Among the soil properties, resin-P and NaHCO_3 -Pi were the primary indicators of beta-

diversity in drought and well-watered soils, respectively. Soil resin-P, $\text{NaHCO}_3\text{-Pi}$, and NaOH-Po were significantly positively correlated with beta-diversity in drought soils, while PQQS and ALP were negatively correlated with beta-diversity (Fig. S5a). In well-watered soils, only soil $\text{NaHCO}_3\text{-Pi}$ and NaOH-Po were strongly positively linked with beta-diversity (Fig. S5b).

3.3 Abundance of soil microbial functional genes associated with P transformation

The majority of the studied genes' abundances decreased with increasing P-supply levels, indicating that soil microbial P-cycling capability was triggered under low P environments (Fig. 2a). The abundance of *gcd*, *phoD*, and *phnD* genes was significantly decreased in high P-supply soil by 17.4, 12.3, and 14.4%, respectively, compared to the no P-supply soil. Additionally, the abundance of *pqqB*, *glpQ*, and *phnMND* under drought conditions was considerably greater than that under well-watered conditions (Fig. 2b). Among them, the abundance of *gcd*, *phoD*, *phnG*, *phnE*, *ugpE*, *pstB*, and *phoR* genes were significantly affected by interactions with water and P-supply treatments (Fig. 3a, $p < 0.05$). Further random forest analysis revealed that *phnDMN*, *glpA*, *pqqE*, *phoD*, and *phoR* were the important predictors for soil active Pi (Fig. 3b). In contrast, *phoD*, *phoR*, *pqqE*, *phnDENI*, and *php* were the important predictors for soil active Po (Fig. 3c). Furthermore, under drought conditions, soil properties had a greater impact on the relative abundance of numerous P transformation-related genes than under well-watered conditions. Under drought conditions, soil $\text{NH}_4\text{-N}$ concentration was strongly positively connected with the abundance of soil P transformation-related genes (Fig. 3d).

3.4 Linkages between the abundance of key genes associated with p cycling and soil properties and their taxonomic compositions

The genes *gcd*, *phoD*, *phnD*, and *phoR* were selected as representative four key genes for further analysis. PCoA indicated that the community structure of these four genes was more strongly affected by drought and low P in soil than well-watered and P-supply conditions (Fig. S6). Compared to well-watered conditions, drought increased the complexity of the functional microbial community ecological network of these four genes (Fig. 4, Table S2). Additionally, soil active P fractions were negatively correlated with the abundance of *gcd*, *phoD*, and *phnD*, but positively correlated with *phoR*, especially in drought soil (Fig. S7). Moreover, the relative abundance of the top 10 species affiliated with these four key genes was significantly influenced by interactions with water and P-supply treatments, except for Proteobacteria bacteria (Fig. 5a, $p < 0.01$). For instance, the relative abundance of *gcd*, *phoD*, *phnD*, and *phoR* genes affiliated with *Steroidobacter cummioxidans*, *Mesorhizobium alhagi*, *Ensifer sojae*, and *Devosia geojensis* was considerably greater in drought and no P-supply conditions than in well-watered and P-supply conditions (Fig. 5b, $p < 0.01$).

3.5 Linkages between the main species in key gene communities associated with p cycling and soil properties

Random forest analysis indicated that soil active P fractions (resin-P, NaHCO_3 -P, and NaOH -P) and DOC were crucial predictors and were significantly positively related to the abundance of *gcd*, *phoD*, *phnD*, and *phoR* gene communities, while soil PQQS activity was significantly negatively correlated with the abundance of these gene communities (Fig. 6). Furthermore, the correlation network demonstrated that the main species in *gcd*-, *phoD*-, *phnD*-, and *phoR*-type communities were closely connected with soil active P fractions and enzyme activities involved in microbial P-cycling. The *gcd* genes affiliated with *Acidobacteria bacterium* and *Devosia geojensis* were significantly positively and negatively correlated with soil active P fractions ($p < 0.05$), respectively (Fig. 6a). Moreover, the *phoD*, *phnD*, and *phoR* genes affiliated with *Acidobacteria bacterium* and *Chloroflexi bacterium* were considerably positively associated with soil active P fractions ($p < 0.05$) (Figs. 6b, c). However, the *phoD*, *phnD*, and *phoR* genes affiliated with *Devosia geojensis* and *Ensifer sojae* were considerably negatively associated with soil active P fractions ($p < 0.05$) (Fig. 6d).

4 Discussion:

4.1 Effect of drought on soil P availability

The results of this study support the first hypothesis, indicating that drought conditions decreased soil P availability. The reduced P availability can be attributed to drought's impact on the transformation of soil P from recalcitrant to labile fractions and the increased soil movement/migration of P. Water limitation in the soil can alter the bioavailability of elements by affecting their cycling processes. Unlike nitrogen (N), the turnover and migration of P in the soil are influenced by a variety of soil properties. Drought conditions may slow down the turnover rate of P by reducing desorption and diffusion rates of P in the rhizosphere soil of *A. sparsifolia*. Additionally, the increased activity of PQQS and phytase enzymes, as well as the gene abundance of various P-cycling processes, under drought treatment, can be considered an adaptation mechanism to cope with low P conditions and water limitation. However, despite the increase in enzyme activity and microbial P-cycling capacity, it was not sufficient to compensate for the reduction in labile-P produced by soil water reduction under severe drought conditions. This might be due to the fact that water plays a crucial role in various physicochemical reaction processes and its scarcity during severe drought hinders these processes. Furthermore, the increased activity of P-cycling enzymes and microbial genes involved in uptake and transport cannot compensate for the reduced water transport capacity under severe drought conditions. The study also showed that drought significantly increased soil electrical conductivity (EC) and pH values, which might be another important mechanism for drought to reduce soil P availability.

4.2 Effect of drought and P-deficiency on soil P-cycling genes

The study observed that water limitation conditions reduced microbial demand for Pi, alleviating the microbial growth limitation caused by Pi deficiency. The abundance of some P uptake transporter genes increased significantly with soil NH₄⁺-N concentration under drought treatment, indicating that soil NH₄⁺-N accumulation may promote microbial uptake and transport of P. The study also found that drought and low P conditions activated the phoR gene expression in *Bacillus coli*, while the high-affinity pst transporter did not react similarly to the low-affinity pit transporter. This suggests that the linkage between phoR and pst and pit transporters may be partially inhibited under drought and low P conditions. The abundance of genes associated with Po mineralization and Pi solubilization increased under no or low P supply, indicating that these conditions encouraged the synthesis of phosphatases. However, some studies have shown no differences between poor and P-rich forest soils in the functional potential of alkaline phosphatase (ALP), while P-rich forest soils had a higher abundance of gcd genes. This difference might be attributed to the sensitivity of microbes to nutrient availability, especially in soils subjected to drought and high anthropogenic disturbances. Under low P conditions, microbes associated with P-cycling are activated, while in undisturbed soils, microbes may be stabilized under constant long-term conditions.

4.3 Effects of drought and P-deficiency on typical soil pPcycling functional microbes

Pseudomonas, considered a critical microbe involved in soil P-cycling, was found to be enriched in the rhizosphere soil of *A. sparsifolia*, especially under drought and no P-supply conditions. This enrichment implies that when P and water are scarce, the plant-system makes an additional effort to take up P, although it may not be sufficient to maintain similar P uptake levels as in conditions of better P and water availability. Furthermore, *Devosia geojensis* and *Ensifer sojae* showed a significant negative relationship with soil microbial biomass P (MBP), resin-P, and NaHCO₃-Pi concentrations, suggesting that the rhizosphere of leguminous species responds to P-poor soil conditions by enriching *Pseudomonas* to accelerate Po mineralization and Pi solubilization potential. The mechanism by which microbes associated with soil P-cycling drive P-transformation is believed to involve acid production, which dissolves Pi by chelating with metal ions. However, this study found that, under drought and low P conditions, rather than a rise in soil pH, functional genes linked to microbial P-cycling and the dominant microbes of these genes significantly increased. This suggests that lower pH is not necessary for microbial P solubilization, and soil pH remains stable in highly buffered soil. The strategies employed by microbes to mobilize more P under drought and low P conditions include the production of organic acids during metabolism, the secretion of various enzymes for Po mineralization, and the utilization of compounds produced during

metabolism (e.g., $\text{NH}_4^+\text{-N}$) to release additional P. The study confirms the second hypothesis that under drought and low P conditions, microbial P-cycling genes are significantly affected.

5 Conclusions and implications

In conclusion, this study demonstrated that drought and low P conditions had significant impacts on soil P availability and P-cycling processes in the rhizosphere soil of *A. sparsifolia*. Drought conditions led to an increase in soil electrical conductivity, which could accelerate P fixation and consequently reduce the concentrations of labile and moderately labile-P fractions in the soil. However, microbial preference for $\text{NH}_4^+\text{-N}$ concentration was significantly increased under drought and no P-supply treatments compared to well-watered and no P-supply treatments. This observation suggests that under drought conditions, more $\text{NH}_4^+\text{-N}$ accumulation may enhance the growth of P-solubilizing microbes, leading to higher expression of soil microbial P-cycling related functional genes.

The study identified a set of genes associated with P-cycling that were influenced by drought and low P conditions. The abundance of genes encoding *gcd*, *phoD*, *phnG*, *phnE*, *ugpE*, *pstB*, and *phoR* increased under drought and low P conditions, implying that these conditions induced changes in microbial P-cycling functions. The relationship between the abundance of these genes and soil properties was found to be closer under drought than well-watered treatment, particularly with soil $\text{NH}_4^+\text{-N}$ concentration. This indicates that $\text{NH}_4^+\text{-N}$ assimilation by microbes might be a driving factor for the increased expression of P-cycling related functional genes under drought and low P conditions.

Moreover, the study revealed that certain microbial communities affiliated with the major P-cycling genes were enriched under drought and no or low P-supply conditions. *Steroidobacter cummioxidans*, *Mesorhizobium alhagi*, *Devosia geojensis*, and *Ensifer sojae* were among the dominant species in these communities. These findings suggest that the rhizosphere of *A. sparsifolia* responds to drought and low P conditions by promoting specific microbial communities involved in P-cycling.

However, it is crucial to consider that the study area belongs to a hyper-arid desert ecosystem with poor soil structure and limited nutrient availability. Therefore, the role of functional genes and microbes in regulating soil P-cycling under drought conditions may be restricted, and they may not fully compensate for the negative direct effects of drought on P-availability. As a result, the biological ineffectiveness of soil P exacerbated by drought might have a negative feedback on life communities' survival in desert ecosystems. If future drought periods become more severe and prolonged, we can expect a synergistic negative effect on the survival of life communities in these desert ecosystems due to reduced P availability.

In conclusion, this study provides valuable insights into the complex interactions between drought, P-deficiency,

and microbial P-cycling processes in desert ecosystems. Understanding these mechanisms is crucial for predicting the impacts of climate change-induced drought on nutrient availability and ecosystem dynamics in arid regions. Further research is warranted to explore the long-term effects of drought on soil P-cycling and its consequences on desert ecosystem resilience and sustainability.

Declaration of Competing Interest

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.

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520 **Table 1 Soil properties affected by water and P-supply treatments and the overall effects of water and P supplies and their interactions on soil properties**

	ALP ($\mu\text{mol min}^{-1} \text{L}^{-1}$)	PQQS ($\mu\text{mol min}^{-1} \text{L}^{-1}$)	Phytase ($\mu\text{mol min}^{-1} \text{L}^{-1}$)	MBP (mg kg^{-1})	$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	$\text{NO}_3^-\text{-N}$ (mg kg^{-1})	DOC (mg kg^{-1})	EC ($\mu\text{s cm}^{-1}$)	pH
Water treatment (W)									
Drought	70.43 $\pm$ 9.13	84.11 $\pm$ 17.00	110.12 $\pm$ 13.48	2.90 $\pm$ 1.41	1.21 $\pm$ 0.52	13.62 $\pm$ 2.38	26.22 $\pm$ 4.13	319.60 $\pm$ 57.30	9.21 $\pm$ 0.09
Well-watered	87.73 $\pm$ 14.71	75.13 $\pm$ 11.97	99.49 $\pm$ 14.56	4.02 $\pm$ 1.86	1.87 $\pm$ 0.55	10.08 $\pm$ 1.79	33.86 $\pm$ 6.97	266.61 $\pm$ 79.05	9.15 $\pm$ 0.05
P-supply treatment (P)									
No P	93.48 $\pm$ 15.20a	97.14 $\pm$ 11.62a	91.67 $\pm$ 11.15c	1.88 $\pm$ 0.19c	1.77 $\pm$ 0.68a	11.37 $\pm$ 1.90a	24.95 $\pm$ 3.61b	324.44 $\pm$ 68.54a	9.17 $\pm$ 0.11a
Low P	83.87 $\pm$ 10.63ab	85.58 $\pm$ 4.25b	97.51 $\pm$ 11.84c	2.10 $\pm$ 0.83c	1.46 $\pm$ 0.62b	12.19 $\pm$ 2.95a	29.82 $\pm$ 4.82ab	301.55 $\pm$ 65.71a	9.20 $\pm$ 0.09a
Media P	74.34 $\pm$ 7.77bc	74.28 $\pm$ 5.73c	108.54 $\pm$ 8.30b	3.09 $\pm$ 1.38b	1.55 $\pm$ 0.67b	11.95 $\pm$ 3.53a	30.25 $\pm$ 5.76ab	274.16 $\pm$ 72.55a	9.17 $\pm$ 0.05a
High P	64.63 $\pm$ 7.24c	61.49 $\pm$ 5.28d	121.51 $\pm$ 6.38a	4.67 $\pm$ 1.60a	1.38 $\pm$ 0.59b	11.89 $\pm$ 2.85a	35.14 $\pm$ 8.87a	259.26 $\pm$ 77.40a	9.17 $\pm$ 0.05a
ANOVA <i>p</i> value									
W	< 0.001	0.096	0.039	0.066	0.002	< 0.001	0.001	0.038	0.021
P	< 0.001	< 0.001	< 0.001	< 0.001	0.043	0.950	0.022	0.223	0.915
W*P	0.014	0.028	0.881	0.058	0.040	0.373	0.464	< 0.001	0.370

521 **Note:** Different lower-case letters are significantly different ($p < 0.05$). Values are means $\pm$ standard deviation, n=16 for water treatment, n=8 for P-supply treatment.

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527 **Table 2 Soil P fractions affected by water and P-supply treatments and the overall effects of water and P supplies and their interactions on soil P fractions**

	Labile P (mg kg ⁻¹)			Moderately labile P (mg kg ⁻¹)		Recalcitrant P (mg kg ⁻¹)				Active Pi (mg kg ⁻¹)	Active Po (mg kg ⁻¹)
	Resin-P	NaHCO ₃ -Pi	NaHCO ₃ -Po	NaOH-Pi	NaOH-Po	1 M HCl-Pi	conc. HCl-Pi	conc. HCl-Po	Residual-P		
Water treatment (W)											
Drought	5.74±2.85	5.20±2.86	4.12±2.93	4.43±2.81	5.63±1.45	428.99±8.10	30.56±1.61	18.80±1.98	27.95±1.60	15.38±7.45	9.75±3.95
Well-watered	11.19±6.12	16.23±11.43	5.99±2.75	6.30±3.72	5.72±1.07	428.72±5.80	29.46±1.40	19.75±1.94	27.52±1.47	33.73±20.80	11.71±3.33
P-supply treatment (P)											
No P	2.21±0.19d	1.82±0.22c	3.25±1.46b	3.02±0.67c	4.10±0.46b	425.55±6.14b	29.67±1.85a	19.13±1.36a	28.36±1.23ab	7.05±1.046c	7.34±1.57c
Low P	7.49±3.64c	7.33±3.93b	3.79±1.46b	3.65±1.29bc	5.78±0.63a	426.80±6.18ab	29.82±1.34a	18.63±1.20a	27.05±1.10bc	18.47±8.75bc	9.57±1.94bc
Media P	10.47±3.69b	15.79±8.55a	4.94±2.20b	5.37±1.44b	6.24±1.22a	428.95±4.61ab	29.54±1.24a	19.10±0.71a	26.72±1.35c	31.63±13.55ab	11.18±2.09b
High P	13.70±4.80a	17.94±12.45a	8.23±3.51a	9.41±4.25a	6.58±0.80a	434.11±8.17a	31.01±1.68a	20.24±3.48a	28.80±1.53a	41.06±20.22a	14.82±4.14a
ANOVA <i>p</i> value											
W	0.003	0.001	0.073	0.119	0.847	0.913	0.047	0.180	0.431	0.002	0.141
P	<0.001	0.001	0.001	<0.001	<0.001	0.059	0.227	0.434	0.009	< 0.001	< 0.001
W*P	< 0.001	< 0.001	0.879	0.131	0.829	0.948	0.700	0.997	0.620	< 0.001	0.860

528 **Note:** Different lower-case letters are significantly different ($p < 0.05$). Values are means ± standard deviation, n=16 for water treatment, n=8 for P-supply treatment. Active Pi

529 = resin-P + NaHCO₃-Pi + NaOH-Pi; Active Po = NaHCO₃-Po + NaOH-P.

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Figure captions

Fig. 1 Microbial diversity and the effects of soil parameters on the microbial diversity in drought and well-watered soils. The analyses are based on microbial profiles at species annotation L7. AVONA performed the individual and interactive effects of water and P-supply treatment on the soil microbial alpha- and beta-diversity. W: water treatment; P: P-supply treatment; W*P: the interactions with water and P-supply treatment. ***, $p < 0.001$. The importance of each predictor was determined by the increase in MSE. The asterisk symbol indicates significant differences between microbial diversity and soil properties (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). ALP, alkaline phosphatase; PQQS, pyrroloquinoline-quinone synthase; DOC, dissolved organic carbon.

Fig. 2 The effects of water and phosphorus status on the relative abundances of genes responsible for soil microbial P-cycling potential. (a) displays the relative abundance of 47 genes, the asterisk symbol indicates significant differences between the four P treatments under drought or well-watered treatments ($p < 0.05$, LSD). (b) displays the 12 genes with statistically significant variations in the relative abundance of all 47 genes under the two water treatments ($p < 0.01$, LSD), the asterisk symbol indicates significant differences between the two water treatments ($p < 0.05$, LSD).

Fig. 3 Abundance of phosphorus transformation-related genes in soil under different water and phosphorus levels. (a) displays the abundance of phosphorus transformation-related genes. The asterisk symbol indicates significant differences in the interaction between water treatments and phosphorus treatments. (b) displays the gene predictors of soil active Pi. The importance of each predictor was evaluated by the increase in MSE. Green denotes a negative correlation, whereas blue denotes a positive correlation. (c) displays the association between the abundance of phosphorus transformation-related genes and soil basic properties. Screening criteria: R-value > 0.6 ; correlation algorithm is spearman. Shown only at $p < 0.05$. Asterisks indicate that the difference is significant (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). DOC, dissolved organic carbon, EC, electrical conductivity.

Fig. 4 Association network analysis of species of soil phosphorus cycling functional genes *gcd*, *phoD*, *phnD* and *phoR* in drought and well-watered treatments. Nodes represent the abundance of species. Their colors distinguish the module they belong to, and their size represents the degree.

Fig. 5 Taxonomic assignments of the *gcd*, *phoD*, *phnD* and *phoR* genes at the species level. (a) displays the relative abundance (%) of species (top 10). The asterisk symbol indicates significant differences in the interaction between water and phosphorus treatments. (b) displays the response of different species relative abundance (top 6) to water and phosphorus treatments and analysis of variance. Where the asterisk indicates that this corresponding species relative abundance reached a significant difference level under the water and phosphorus treatments (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Fig. 6 Correlations between indicator abundance and soil properties and random forest analysis to identify the main predictors of composition for the *gcd*, *phoD*, *phnD* and *phoR* gene communities. Only significant correlations are included ($p < 0.05$). The red line denotes positive correlation, and the green line denotes negative correlation. DOC, dissolved organic carbon; ALP, alkaline phosphatase; PQQS, pyrroloquinoline-quinone synthase. The importance of each predictor was evaluated by the increase in MSE. Asterisks indicate that the relationship between the relative abundance of the *gcd*, *phoD*, *phnD* and *phoR* gene communities and soil properties was significant (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Blue and green represent positive and negative correlations, respectively.

Fig. 7 A summary diagram showing the responses of the soil P availability, and soil properties, genes associated with P-cycling to drought and well-watered conditions

Fig. 1

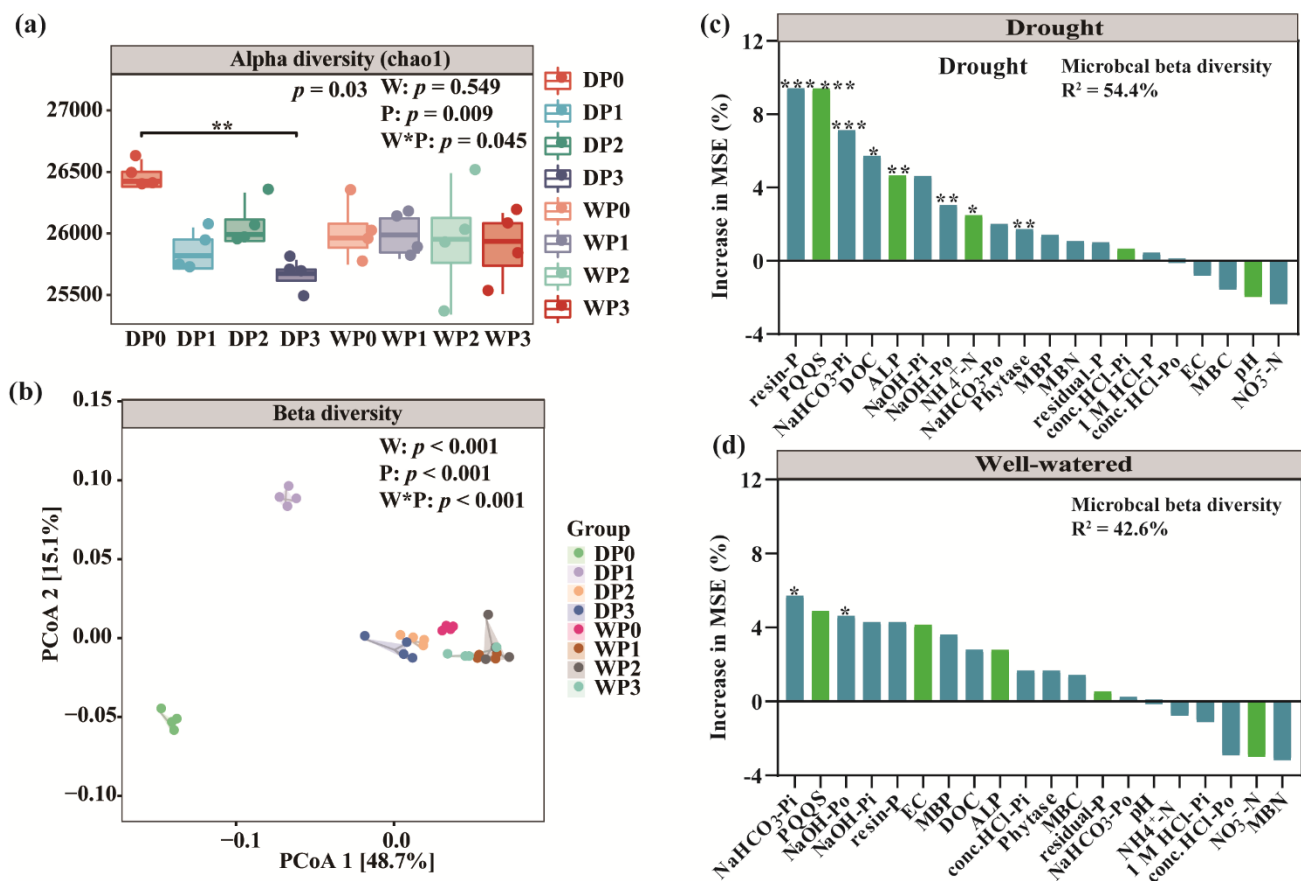


Fig. 2

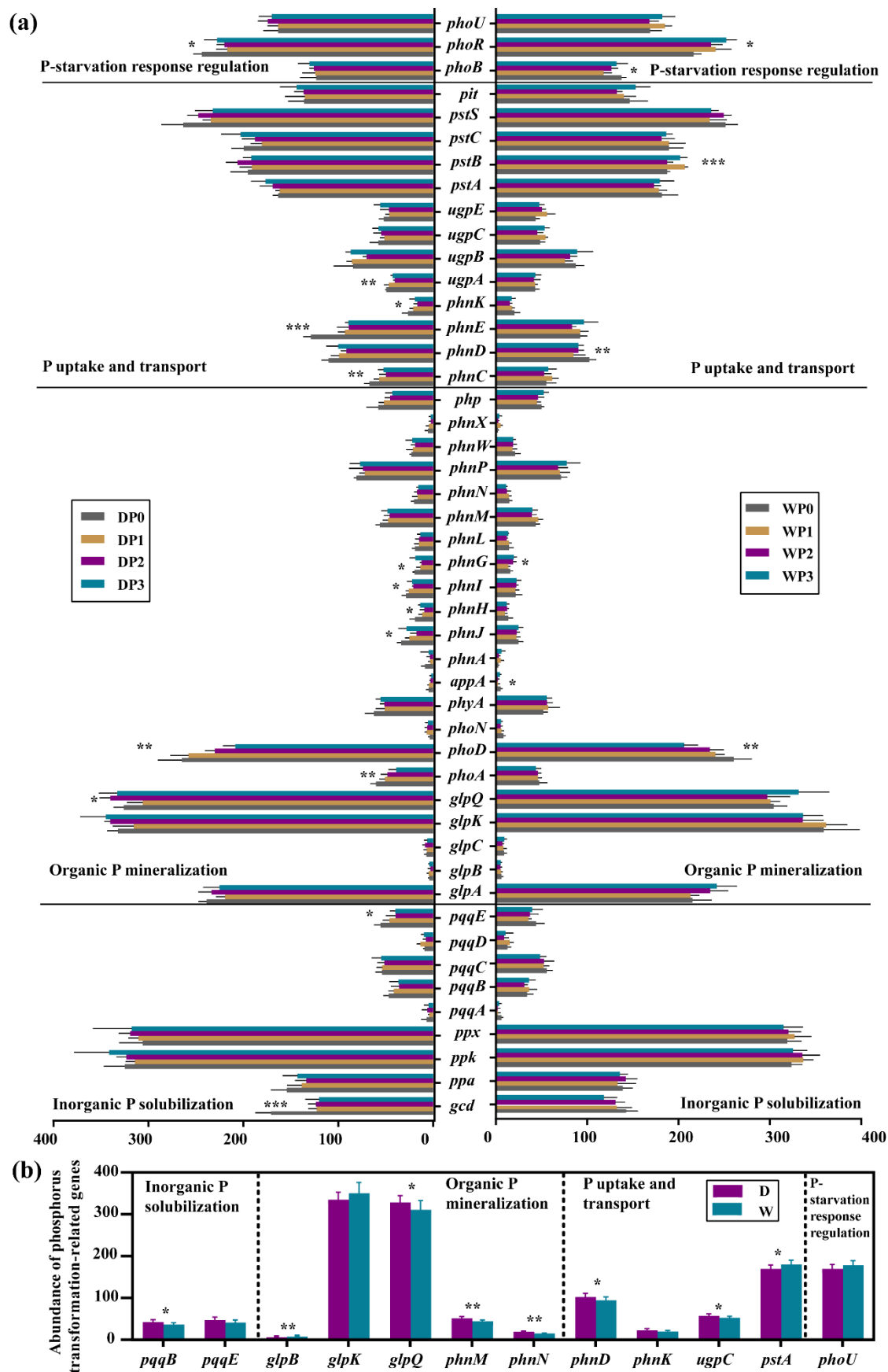


Fig. 3

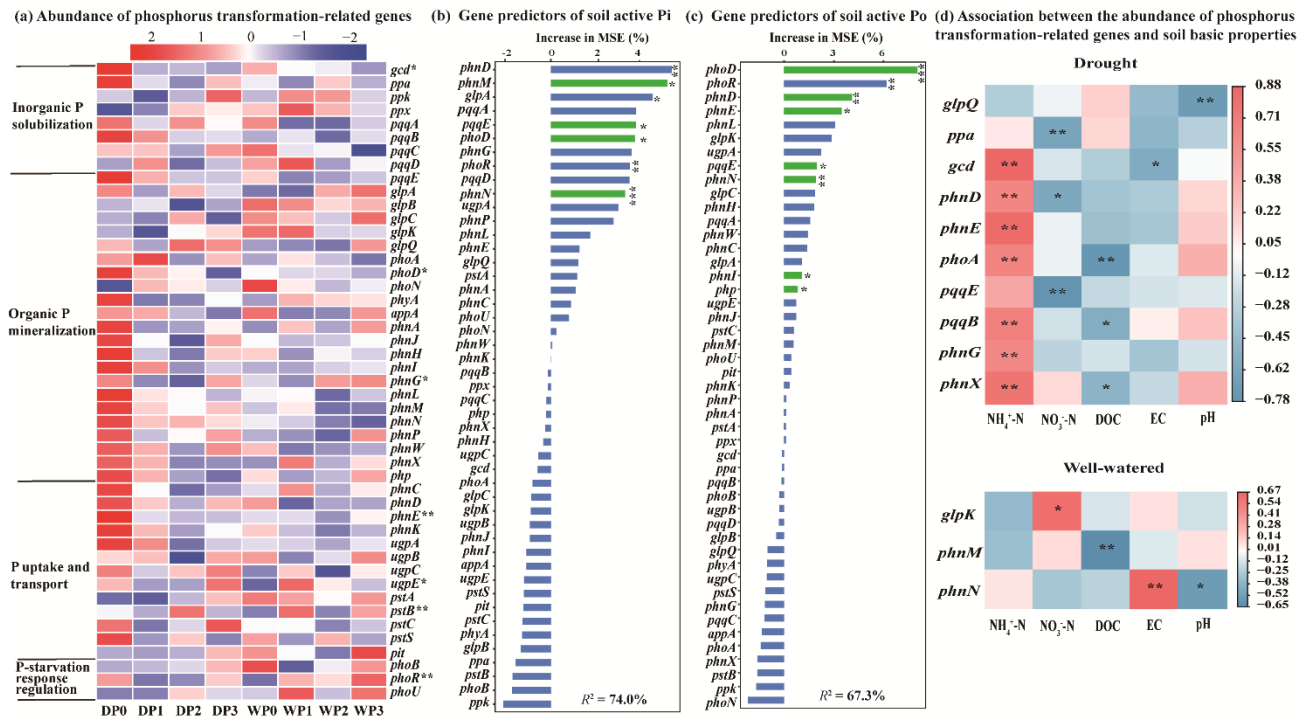


Fig. 4

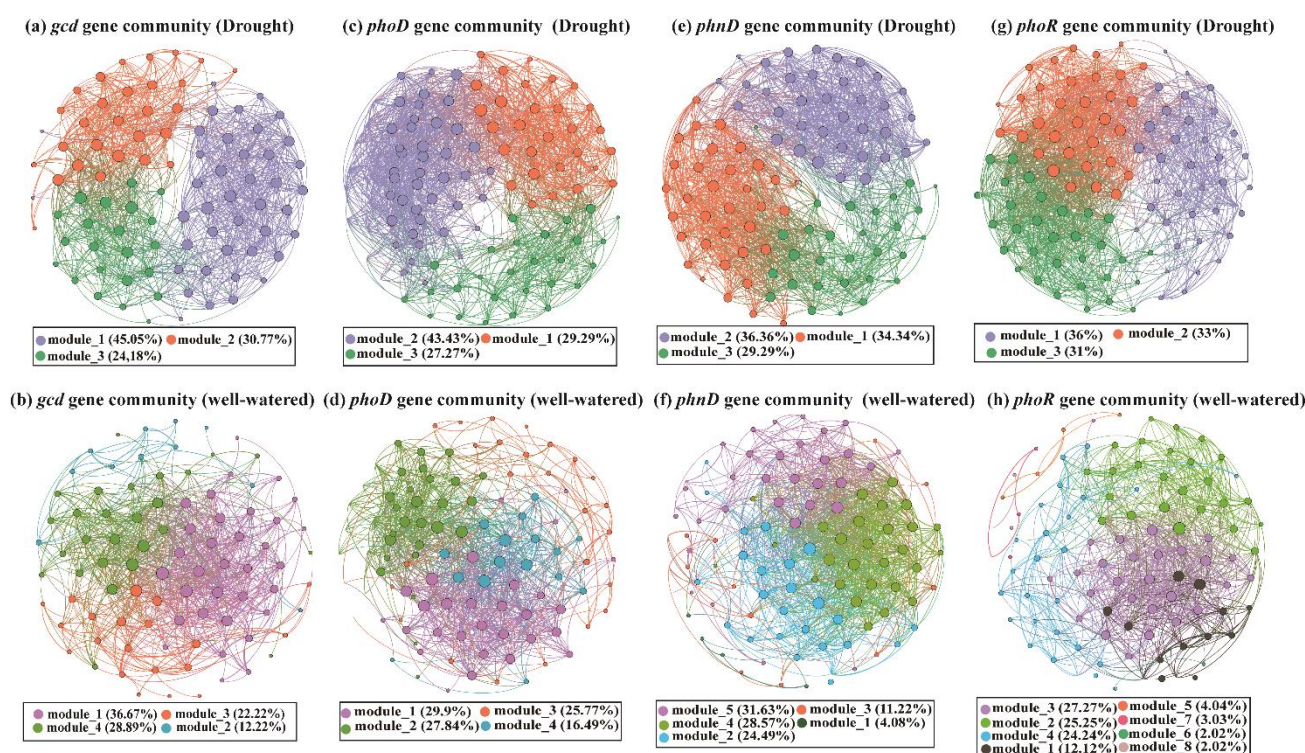


Fig. 5

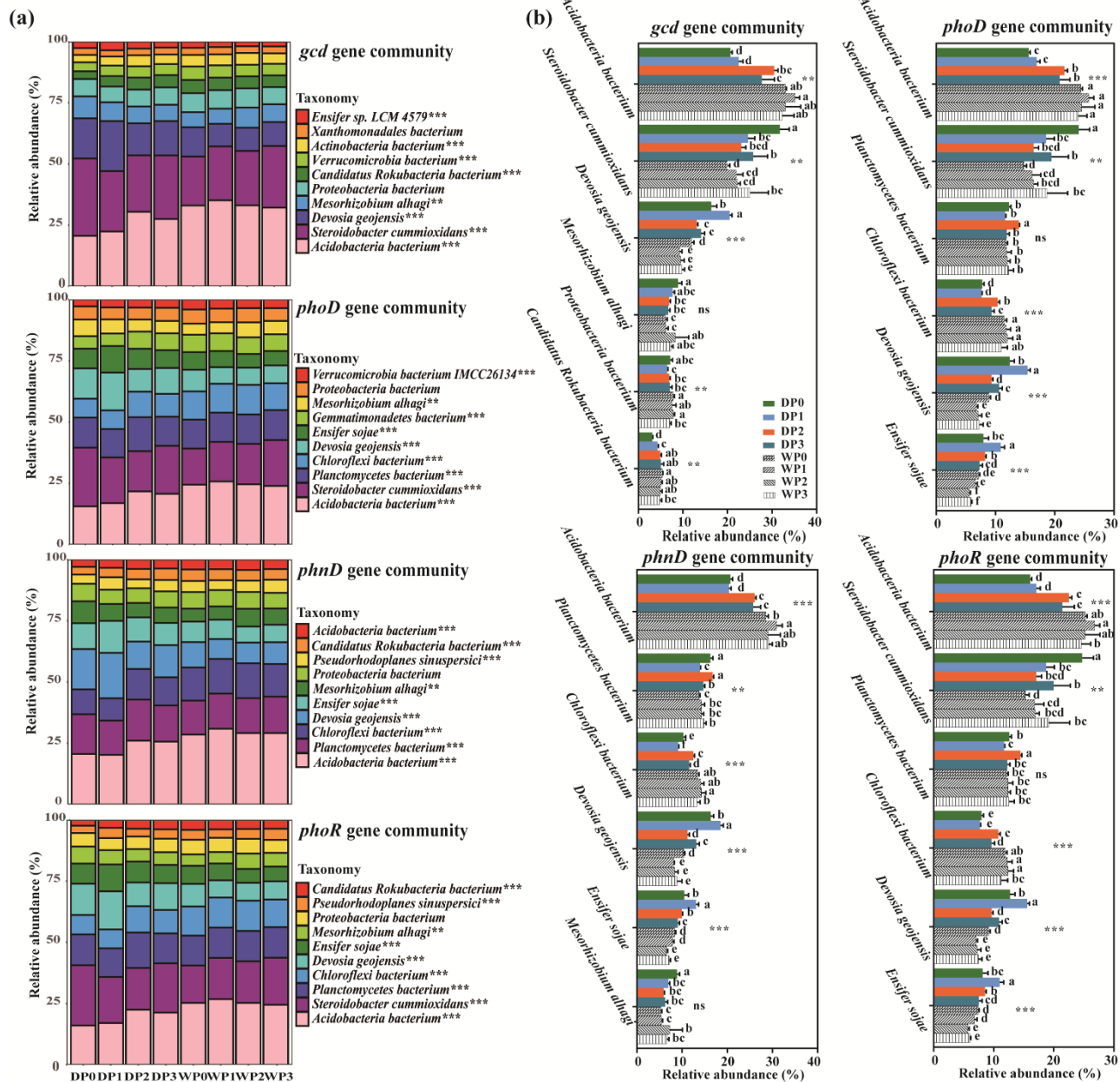


Fig. 6

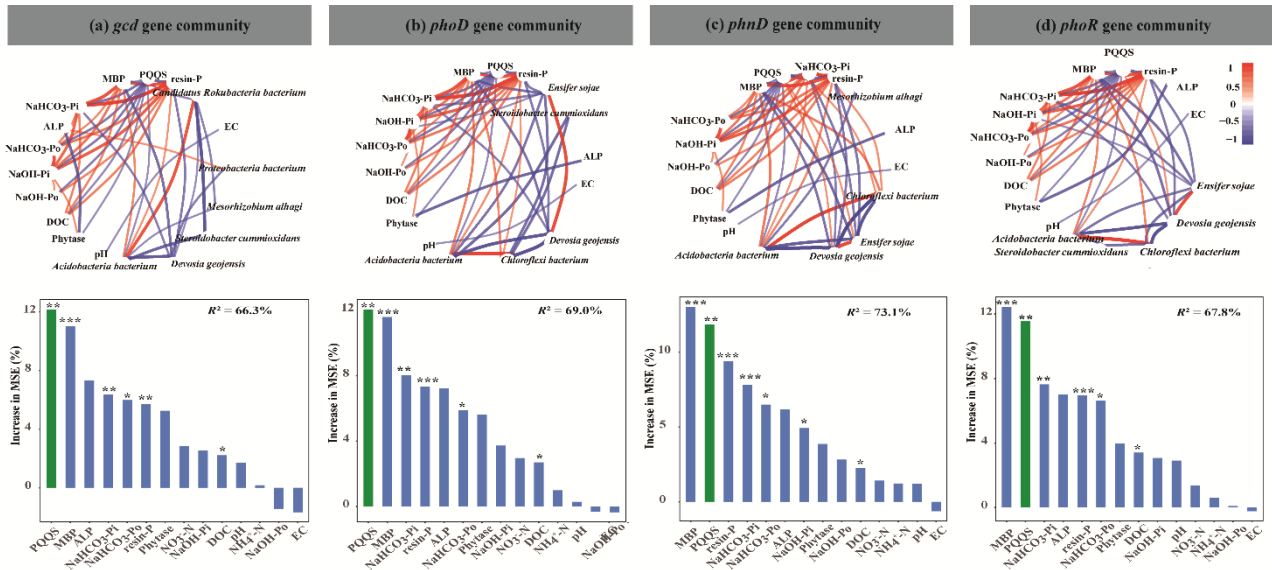
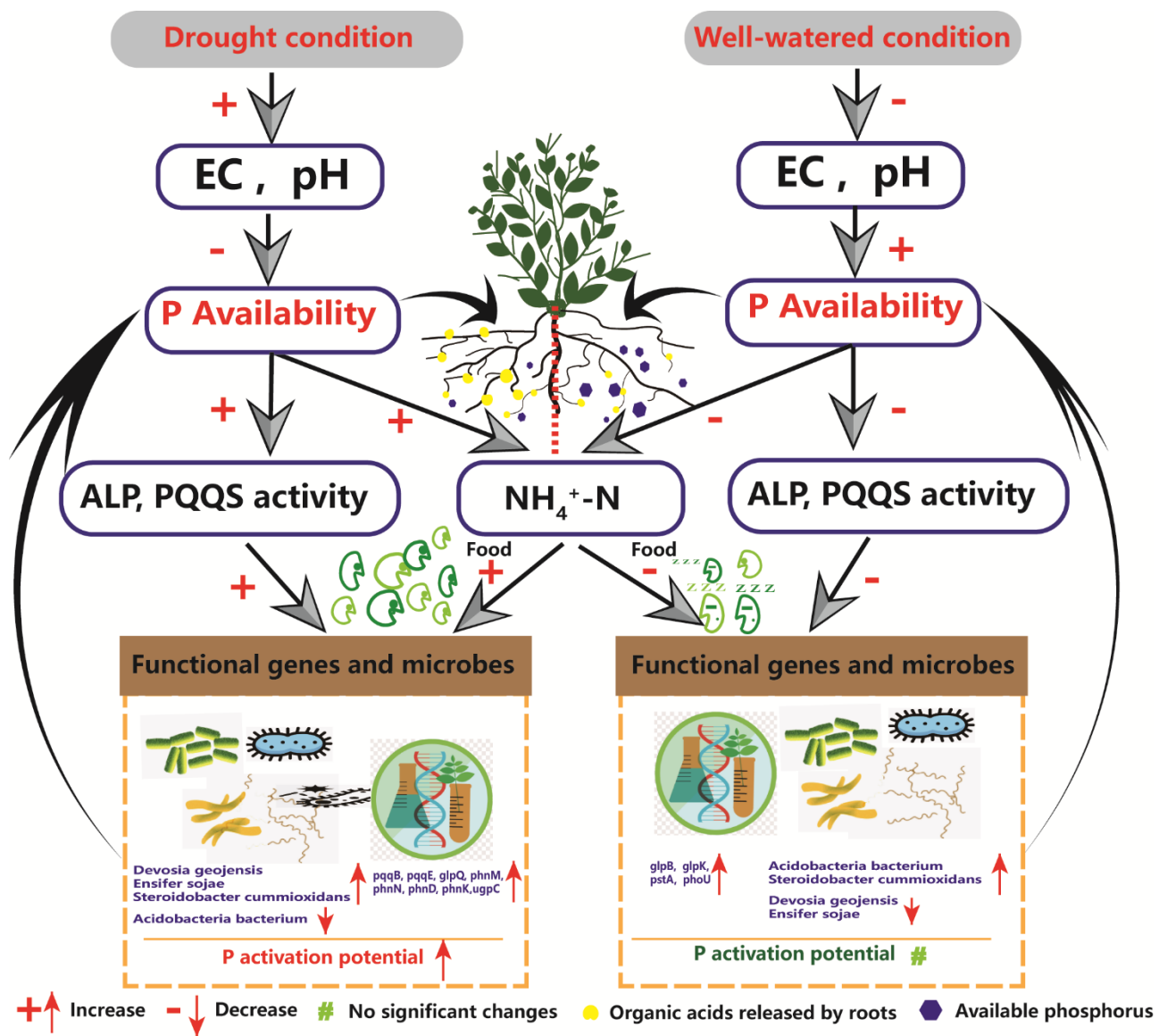


Fig. 7



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