

Magnesium improves phosphorus availability in the rhizosphere soil of apple by regulating P-cycling microbial communities

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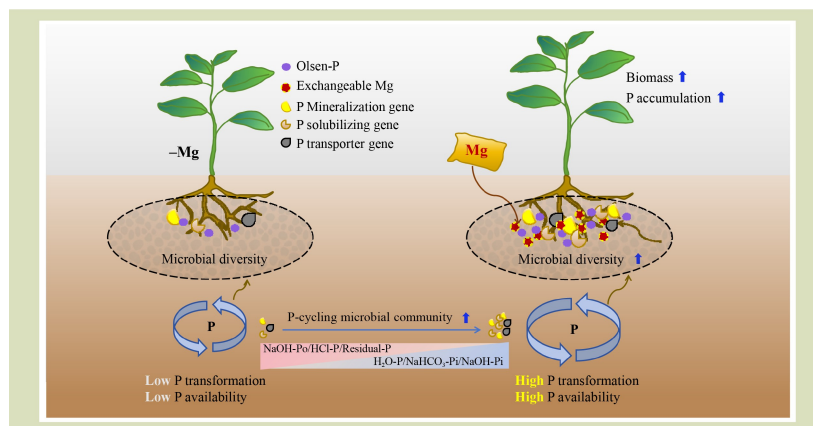
KEYWORDS

Phosphorus availability, microbial community, phosphorus-cycling genes, magnesium, apple

HIGHLIGHTS

- Mg significantly increased soil P availability by elevating labile and moderately labile Pi fractions in the rhizosphere soil.
- Mg increased soil P availability primarily by promoting microbial P mineralization, solubilization, and transport pathways.
- Mg significantly enhanced the abundance of phosphorus-cycling genes, such as *phoD*, *opd*, *gcd*, and *ugpABCE*.

GRAPHICAL ABSTRACT



ABSTRACT

As an essential mineral element for plant growth, Magnesium (Mg) is involved in photosynthesis and influences nutrient availability in soil. However, its impact on Phosphorus (P) availability in apple rhizosphere soil and the underlying microbial regulatory mechanism remain unclear. To investigate whether and how Mg enhances soil P availability, this study integrated metagenomics and bioinformatics to assess the effects of Mg on apple plant growth, P accumulation, P availability, microbial community, and phosphorus-cycling genes (PCGs). The results show that Mg significantly increased soil P availability, particularly the labile and moderately labile Pi fractions. Mg-treated groups showed higher diversity and richness of P-cycling microorganisms and distinct microbial community structures. Metagenomic sequencing revealed a greater enrichment of functional genes involved in organic P mineralization (*phoD* and *opd*), inorganic P solubilization (*gcd*), and P

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transport and regulation (*ugpABCE*) in Mg-treated groups. Significant positive correlations were observed between soil labile P, moderately labile P, and the abundance of PCGs, as well as between soil exchangeable Mg and P-cycling pathways. These findings suggest that Mg enhances P availability by modulating the P-cycling microbial community. Thus, this study proposes that Mg can serve as a rhizosphere prebiotic to reduce the use of phosphate fertilizer in apple production.

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1 Introduction

China is the world's largest apple producer, with a cultivation area of 2.0 million hectares and an output of 49.6 million tons during the 2023 growing season. However, under infertile soil conditions, increased apple production in China has been reliant on high chemical fertilizer input. Currently, the average application rate of phosphate fertilizer in Chinese apple orchards is as high as 570 kg·ha⁻¹, significantly exceeds the recommended dosage (100–150 kg·ha⁻¹)^[1]. P applied to soil is readily fixed by metal cations (Al, Fe, and Ca) or adsorbed onto mineral surfaces, resulting in low P availability. Excessive P fertilizer input increases production costs and leads to environmental issues, such as soil P accumulation, water eutrophication, reduced soil biodiversity, and ecosystem function degradation^[2–5]. Therefore, identifying effective nutrient management strategies to enhance soil P bioavailability and reduce chemical P fertilizer input is of great significance for the green and sustainable development of China's apple industry.

Soil microorganisms play key roles in regulating soil P availability and facilitating plant P acquisition^[6,7]. Microbial processes involved in the soil P cycle primarily include: (1) phosphate starvation response regulation, enabling external P source utilization (encoded by *phoU*, *phoR*, and *phoB*); (2) P uptake and transport systems, utilizing and immobilizing P into microbial biomass; (3) Pi solubilization, mainly through the secretion of organic acids, such as gluconic acid, which is regulated by quinoprotein glucose dehydrogenase (encoded by *gcd*); and (4) Po mineralization, mineralizing Po compounds. Microbially derived acid phosphatases (encoded by *olpA*), alkaline phosphatase (*phoD*), phytase (*appA*), phosphatase (*phnX*), and C-P lyase (*phnJ*) release free orthophosphate from recalcitrant Po forms^[4,8]. The crucial role of soil microorganisms in P activation has been shown through

numerous fertilization experiments. However, these studies have shown the inconsistent responses of soil microbial genes involved in P cycling to fertilization. Specifically, organic amendments or mineral nitrogen (N) and P fertilizers significantly affect the abundance of PCGs. Applying organic matter alone or with inorganic fertilizers increases soil microbial biomass P, soil phosphatase activity, and *phoD* gene abundance. N fertilizer addition suppresses soil microbial biomass P and *phoD* gene abundance but increases *pqqC* gene abundance. P fertilizer application significantly increases microbial biomass P and *phoD* gene diversity^[9]. Combining N and P fertilization enhances acid phosphatase activity, microbial biomass P, *pqqC* gene abundance, and *phoC* gene diversity. The diversity of PCGs increases after partial organic substitution fertilization^[6]. Compost has no significant effect on *phoD* or *pqqC* genes, but biochar and straw increase *pqqC* gene abundance. Compared to straw return, biochar application significantly increases the gene copy number of *phoD*, decreases that of *phoC*, and enhances the abundance of key P functional genes (*phoD*, *gcd*, and *pqqC*)^[10]. Long-term silicon fertilization in rice paddies increased the abundance of Po mineralization functional genes (*phoA*, *phoB*, and *phy*) and Pi solubilization genes (*ppx*, *ppk*, *gcd*), whereas P uptake and transport system genes (*pst*, *pit*) and phosphate starvation response genes (*phoB*) showed no significant changes, which is related to silicon availability, soil pH, plant P uptake, and soil carbon C: P and N stoichiometric ratios^[11]. Partial organic fertilization substitution significantly increased the abundance of Alphaproteobacteria and Gammaproteobacteria, which contain *gcd*. The decrease in soil pH induced by organic substitution negatively affected Pi solubilization and the mineralization capacity, whereas increased soil C:P and N:P ratios had a positive impact^[6].

Mg, an essential mineral element for plant growth and development, is crucial for activating a large number of

enzymes; thus, it plays an important role in numerous physiological and biochemical processes that affect plant growth, including the synthesis of chlorophyll, nucleic acids, and proteins, the formation and utilization of adenosine triphosphate, and participation in photosynthesis^[12–15]. Mg deficiency alters reactive oxygen species metabolism, photosynthesis, and assimilate distribution in crops, affecting yield and quality^[16]. Research findings indicate that applying MgSO_4 significantly increased the soil microbial invertase and protease activities. Mg altered the soil bacterial community structure; for instance, the relative abundance of *Acidobacterium*, *Mizugakiibacter*, and *Singulisphaera* increased, but that of *Acidiphilium*, *Bradyrhizobium*, and *Gemmatimonas* decreased. Redundancy analysis showed that the soil bacterial community composition was influenced by exchangeable Mg and sulfate concentrations and pH, with exchangeable Mg having the strongest effect on microbial abundance and composition^[17].

The role of Mg in Chinese apple production has received insufficient attention, despite orchard soils often being characterized by high P but low Mg content^[18]. If Mg application enhances soil P availability, reducing P input, it would be highly significant for green and sustainable apple production in China. Previous studies have primarily focused on the role of Mg in photosynthesis, yield and quality formation, abiotic stress, and nitrogen uptake^[19–21]. However, whether Mg improves soil P availability and the underlying microbial community regulation mechanisms remain unclear. We hypothesized that Mg application affects microbial diversity and abundance, alters the composition of the P-cycling microbial community, and enhances P availability in apple soil by promoting microbial P solubilization and inhibiting P fixation. Therefore, the objectives of this study were to (1) confirm the effects of Mg on the soil P fractions, P availability, and soil environmental drivers; (2) elucidate how the abundance, diversity, and composition of P-cycling microorganisms respond to Mg addition; and (3) determine the relationships between P-cycling functional genes and soil P availability, thereby providing insights into the soil microbe-mediated P cycle. This study uncovers a novel role for Mg as a rhizosphere prebiotic, demonstrating its capacity to enhance P availability and presenting a sustainable alternative for reducing phosphate fertilizer inputs in apple production.

2 Materials and methods

2.1 Experimental design and sampling

A pot experiment was conducted in May 2024 at the Science and Technology Innovation Park of Shandong Agricultural University (36°15'N, 117°15'E). The soil used was a clay loam with the following basic physicochemical properties: pH 7.78; OM content, 7.62 $\text{g}\cdot\text{kg}^{-1}$; available N, 60.10 $\text{mg}\cdot\text{kg}^{-1}$; available P, 33.60 $\text{mg}\cdot\text{kg}^{-1}$; and available potassium (K), 148.00 $\text{mg}\cdot\text{kg}^{-1}$.

Four fertilization treatments were established: CK (no MgO), Mg50 (MgO, 50 $\text{mg}\cdot\text{kg}^{-1}$ soil), Mg100 (MgO, 100 $\text{mg}\cdot\text{kg}^{-1}$ soil), and Mg150 (MgO, 150 $\text{mg}\cdot\text{kg}^{-1}$ soil). There were six replicates for each treatment. The Mg fertilizer was Mg sulfate (33.5% MgO). N, P, and K fertilizers were applied uniformly across all treatments at rates of 543 $\text{mg}\cdot\text{kg}^{-1}$ (as urea), 1786 $\text{mg}\cdot\text{kg}^{-1}$ (as superphosphate), and 463 $\text{mg}\cdot\text{kg}^{-1}$ (as potassium sulfate), respectively. Fertilizers were thoroughly mixed with the soil before potting and planting. One-year-old G935 apple plants were planted in the pot in 21 April, 2024. Throughout the experiment, soil moisture was maintained at 60%–80% of field capacity.

After 90 days of treatment, three apple plants from each treatment were randomly selected to determine dry weight and P content. At the same time, rhizosphere soil (soil adhering to 0–2 mm of the root surface) of three apple plants was collected using the shaking method.

2.2 Determination of soil available P, P fractions, exchangeable Mg content and soil enzyme activities

Soil available P was determined using the sodium bicarbonate extraction-molybdenum antimony colorimetric method^[22]. A modified Hedley sequential extraction procedure sequentially using different extractants was used to identify different soil P fractions (Supplementary materials: Text S1). Soil exchangeable Mg was determined by the ammonium acetate exchange-atomic absorption spectrophotometry method^[23]. The soil alkaline phosphatase (ALP) activity was assessed using the disodium phenyl phosphate method^[24]. The phosphodiesterase (PDE) activity was measured using the kit provided by Suzhou Gris Biotechnology Co., Ltd. (microplate assay method).

2.3 Determination of plant P and Mg contents

Take 1 g of dry sample and digest it in a concentrated solution of $\text{H}_2\text{SO}_4\text{-H}_2\text{O}_2$. The P content in apple plants was determined using the phosphomolybdate blue method^[25]. The Mg content was measured by atomic absorption spectrometry method^[25].

2.4 DNA extraction, library construction, microbial sequencing, and bioinformatics analysis

Total DNA was extracted and purified from the samples using a magnetic bead-based soil total DNA extraction kit (DP712-02) obtained from Tiangen Biotech, following the manufacturer's protocol. DNA concentration was quantified using a Qubit 1X dsDNA HS Assay Kit (Invitrogen, Q33230). For each sample, 200 ng of DNA that met quality control standards was fragmented to a target size of 200–500 bp using a Bioruptor™ Pico sonicator (Diagenode). Fragmented DNA was purified using TruSeq library preparation beads. The size distribution of the fragmented DNA was checked using an Agilent 2100 Bioanalyzer with High Sensitivity DNA reagents (Agilent, 5067–4627). Samples that did not meet the desired fragment size range were re-processed. Sequencing libraries were constructed using a TruSeq Nano DNA LT Library Preparation Kit (Illumina, FC-121-4001), with end repair, adapter ligation, index PCR amplification, and purification following the kit's manual. The final libraries were quantified using Qubit 1X dsDNA HS Assay Kits. Paired-end Sequencing (PE150) on an Illumina NovaSeq XP platform (LC Bio Technology Co., Ltd., Hangzhou, China) using the NovaSeq 6000 XP 4-Lane Kit v1.5 (300 cycles) (Illumina, 20043131) (Table S1).

Quality control was performed on raw sequencing data in FASTQ format using fastp (version 0.23.4). Quality-filtered reads from each sample were *de novo* assembled using MEGAHIT (version 1.2.9), generating contigs in FASTA format. Contigs longer than 500 bp were retained. Coding sequences (CDSs) were predicted from these contigs using MetaGeneMark (version 3.26), and CDSs shorter than 100 nt were removed. Redundancy among the predicted CDSs was reduced using MMseqs2 (version 15-6f452) with parameters set at 95% identity and 90% coverage, creating a non-redundant Unigene set in which the longest sequence represented each cluster. Bowtie2 (version 2.2.0) was used to map the quality-filtered reads from each sample to the Unigene set and to calculate read counts (abundance) for each Unigene. Unigenes with total read counts ≤ 2 across all samples were

removed, resulting in the final Unigene set used for downstream analysis. DIAMOND (version 0.9.14) was used to align the Unigene protein sequences against the NR_meta database for taxonomic annotation and against functional databases, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG, version 87.1) for functional annotation (Table S2)^[26]. Abundance profiles for taxonomic and functional classifications were generated based on the Unigene abundance data.

2.5 Statistical analysis

SPSS 20.0 and Excel 2013 were used for data analysis and visualization. Data were subjected to variance (ANOVA), and the differences between means were tested by Tukey's HSD for multiple comparisons and significance analysis. Pearson correlation analysis was performed to identify relationships between soil P fractions and PCGs or P-cycling processes. Principal component analysis (PCA) was conducted using the PCA function of the 'Facto Mine R' package. Correlation network heat maps were generated using the 'psych' package (version 2.4.1). The 'igraph' package was used for network visualization and to calculate the topological parameters. Other graphs and fitting curves were plotted using Origin 2019b software (Origin Lab Corporation, USA).

3 Results

3.1 Apple plant biomass, Mg and P accumulation

Mg significantly affected Mg and P accumulation in apple plants, and the highest P and Mg accumulation were observed in Mg150 treatment (Fig. 1(a,b)). Mg significantly increased the biomass of roots, stems, and leaves, and the biomass increased with the increase of Mg application rate (Fig. 1(c)). Specifically, compared to CK, Mg150 increased the total plant biomass by 65.24% ($p < 0.05$).

3.2 Soil available P and exchangeable Mg content, P fractionation, and phosphorus-transforming enzyme activities

In the rhizosphere soil, soil available Olsen-P and the labile P fractions ($\text{H}_2\text{O-P}$ and $\text{NaHCO}_3\text{-Pi}$), and moderately labile P (NaOH-Pi) increased with the increase in the Mg application rate. In contrast, moderately labile Po (NaOH-Po) consistently

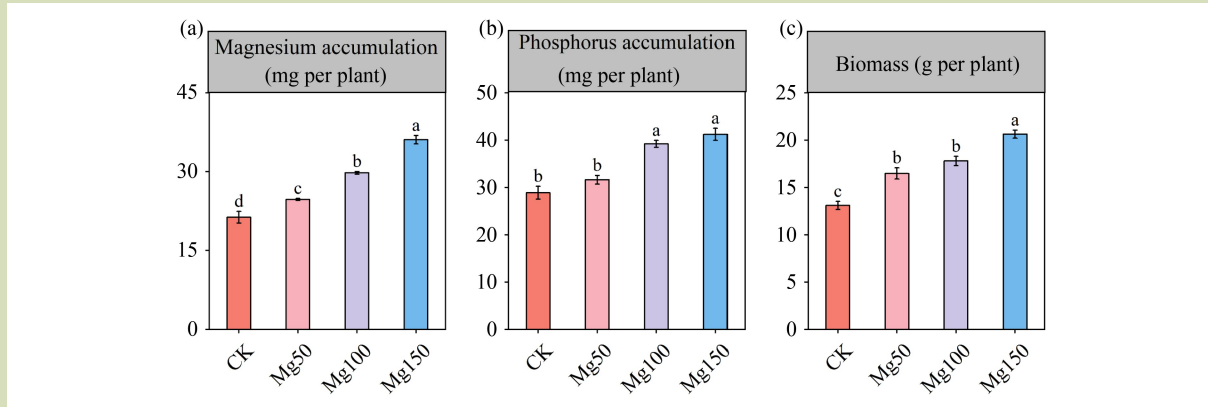


Fig. 1 The effects of different Mg application rates on Mg accumulation (a), P accumulation (b), and apple plant biomass (c). The vertical bar indicates the standard deviation of three replications. Different letters represent significant differences ($p < 0.05$).

showed a decreasing trend. Furthermore, Residual-P, stable P (HCl-P), and moderately labile Po (NaOH-Po) were insensitive to changes in the Mg concentration, showing inconsistent trends (Fig. 2(a)). Soil exchangeable Mg showed an increasing trend, with the largest increase under Mg150 (Fig. 2(c)). ALP activity in the rhizosphere soil increased significantly, with the highest level found under Mg150 (55.45% increase compared to CK) (Fig. 2(d)). The PDE activity in the rhizosphere soil initially increased and then decreased, peaking under Mg100 (Fig. 2(e)).

3.3 Soil microbial community diversity and composition

PCA showed clear spatial separation, indicating a significant effect of Mg application on the soil microbial functional gene community structure (Fig. 3(a)). PCA results showed that the first principal coordinate (PC1) explained 54.36% of the total variance, and the second principal coordinate (PC2) explained 19.92%. PERMANOVA showed a significant difference among treatments ($R^2 = 0.9835$, $p = 0.001$). 19 phosphate-solubilizing microorganisms were identified, and their distributions were compared among samples. The dominant genus among treatments was *Streptomyces*, with an average relative abundance of 34%, followed by *Mycobacterium* and *Bradyrhizobium*, accounting for 16.25 and 15.25%, respectively. The relative abundance of *Streptomyces*, *Bradyrhizobium*, *Pseudomonas*, *Rhizobium*, *Nocardia*, *Bacillus*, *Acinetobacter*, and *Klebsiella* significantly increased in the Mg-treated groups ($p < 0.05$) (Fig. 3(b,c)). α -Diversity analysis revealed that Mg

significantly increased the Shannon and Simpson indices, but without a significant change in the Chao1 index, suggesting that Mg induced a structural reorganization rather than a simple shift in abundance.

3.4 Composition and relative abundance of soil PCGs

The total relative abundance of genes involved in purine metabolism and the two-component system showed no significant differences compared to CK. Mg application significantly increased the total relative abundance of genes related to organic phosphoester hydrolysis, transporters, the pentose phosphate pathway, and pyruvate metabolism by 3.17–7.04, 1.28–3.72, and 0.086%–5.59%, respectively. The relative abundance of genes involved in phosphonate and phosphinate metabolism and pyrimidine metabolism was highest under Mg100 (increased by 7.46% and 1.89%, respectively, $p < 0.05$). The abundance of genes related to oxidative phosphorylation was highest under Mg150 ($p < 0.05$) (Fig. 4(a)).

Exogenous Mg application significantly affected the abundance changes of PCGs. The relative abundance of the *phoB* gene was highest among all soil samples; however, its abundance was insensitive to changes in the exogenous Mg content, showing no significant differences among Mg treatment groups. The abundance of genes encoding organic phosphoester hydrolysis (*opd* and *phoD*), transporters (*ugpA*, *ugpB*, *ugpE*, and *ugpC*), and the pentose phosphate pathway (*gcd*) increased with the

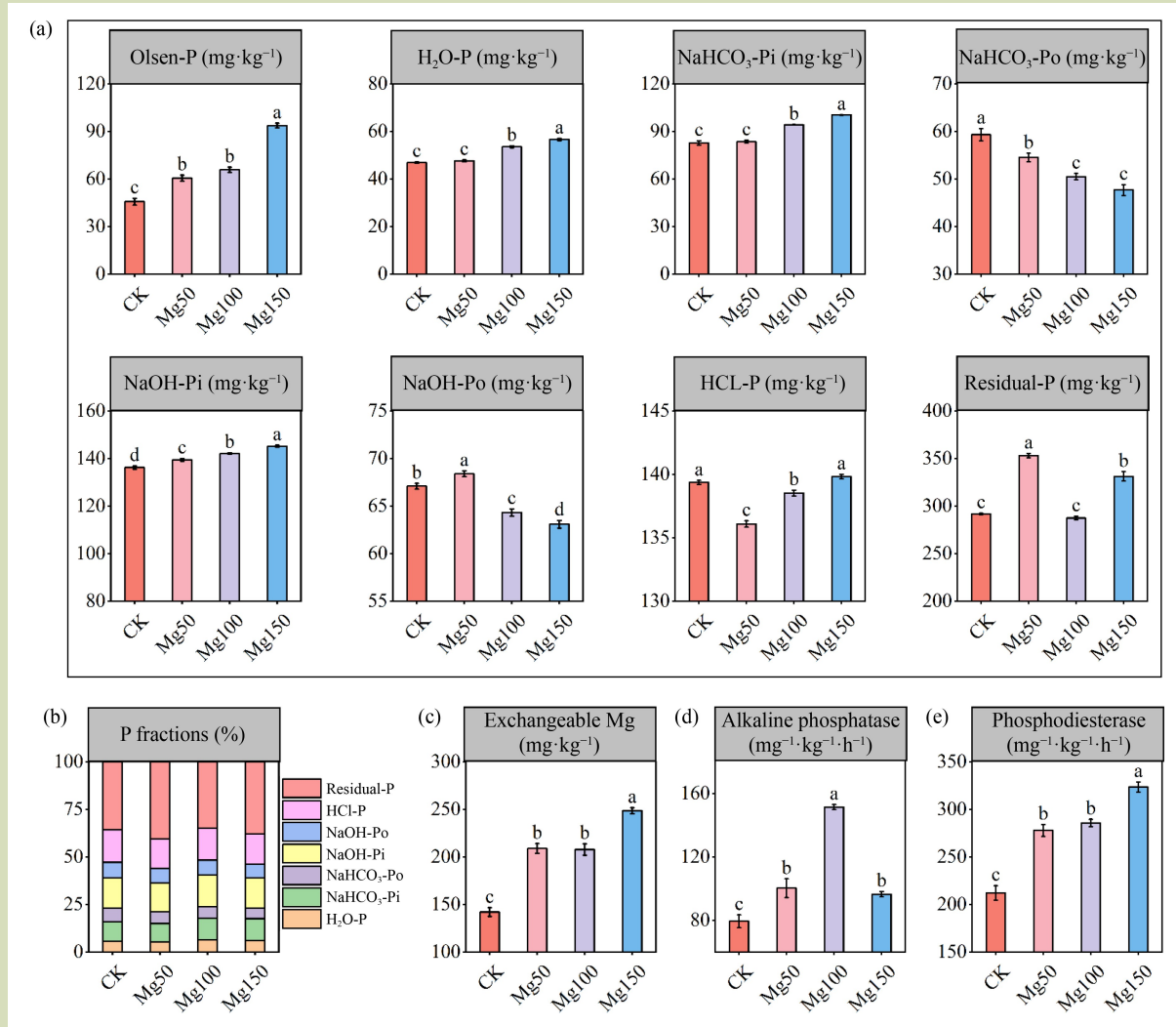


Fig. 2 Changes in the content of each fraction (a), soil P fractions (b), soil Mg content (c), and soil ALP (d) and PDE (e) activities under different treatments. The vertical bar indicates the standard deviation of three replications. Different letters represent significant differences ($p < 0.05$).

increase in the Mg application rate. Exogenous Mg application significantly increased the relative abundance of the *opd*, *phoD*, and *ugpABCE* genes ($p < 0.05$). The abundances of genes encoding organic phosphoester hydrolysis (*phoA*) and transporters (*phnD*, *phnE*) were highest under Mg100 ($p < 0.05$) (Fig. 4(b)).

3.5 Linkages between soil P-cycling functional genes and soil P fractions

Mantel tests were used to determine the associations between

soil P fractions and P-cycling pathways. The soil exchangeable Mg content significantly influenced organic phosphoester hydrolysis, purine metabolism, transporters, the pentose phosphate pathway, pyruvate metabolism, and phosphonate and phosphinate metabolism ($p < 0.05$). Among these, transporters, the pentose phosphate pathway, pyruvate metabolism, and organic phosphoester hydrolysis pathways significantly affected soil P availability and were significantly positively correlated with soil Olsen-P ($p < 0.05$). H₂O-P, NaHCO₃-Pi, and NaOH-Pi were significantly positively correlated with soil Olsen-P ($p < 0.05$) (Fig. 5(a)), suggesting

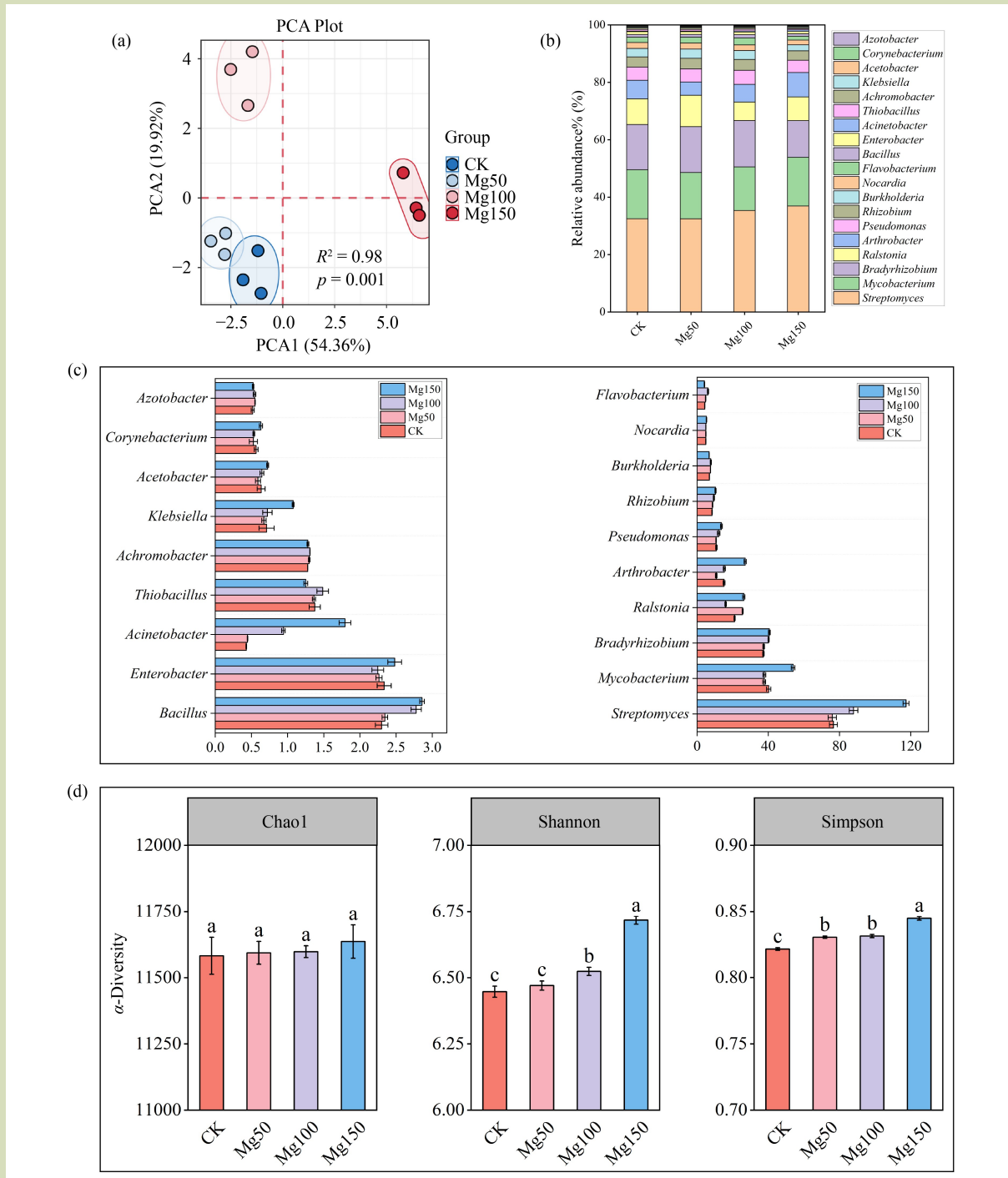


Fig. 3 Differential characteristics of P-cycling microbial functional gene abundances based on PCA using the ‘Facto Mine R’ package (a). Relative abundance of microbial taxa (genus level) containing PCGs under different fertilization treatments (b,c). α-Diversity of the P-Cycling Microbial Community under Different Fertilization Treatments(d). Bar chart show the distribution of α-diversity (Shannon、Simpson and Chao1 index) in the four groups and intergroup statistics. Bar charts show the relative abundance of dominant classes under the four treatments. Data are presented as the mean ± standard error (n = 3).

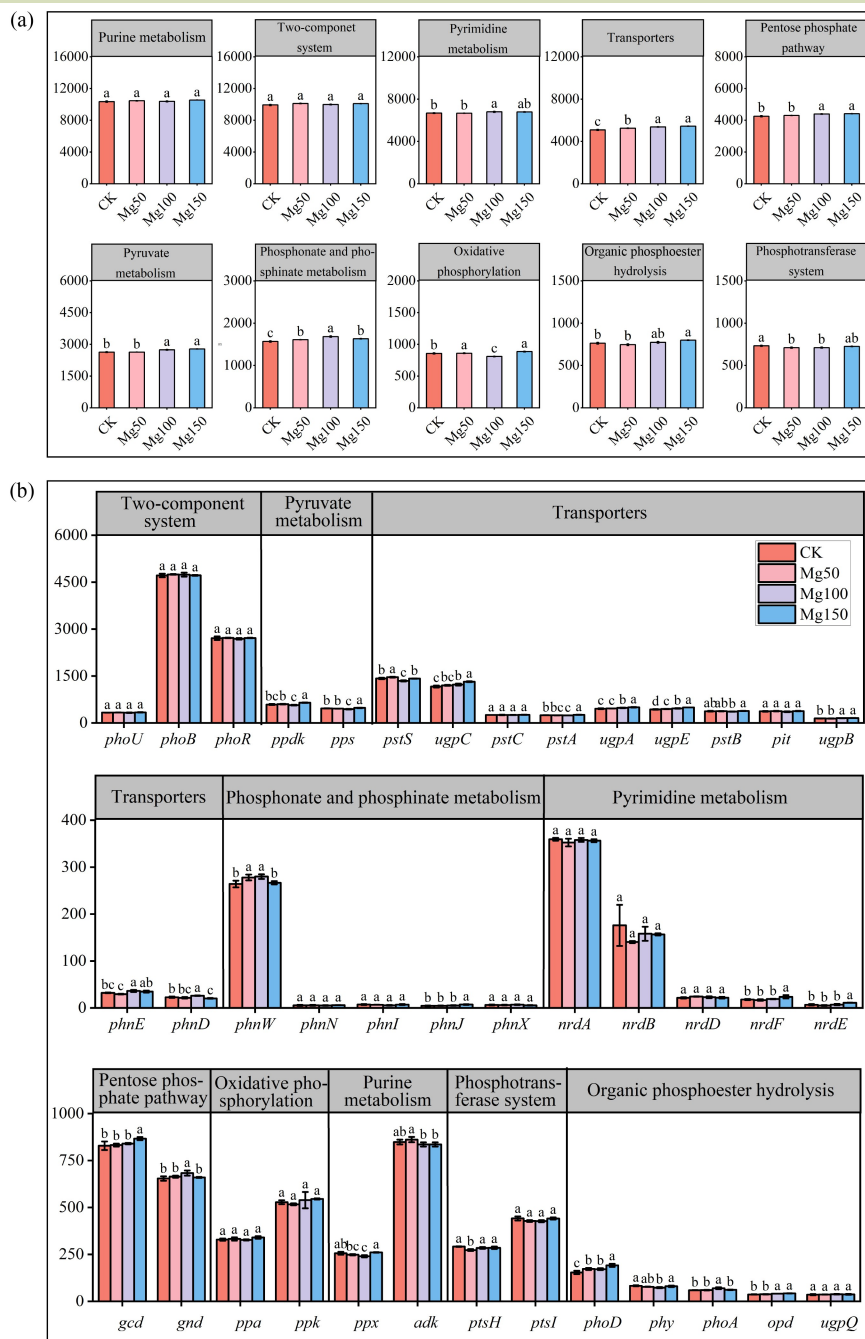


Fig. 4 Changes in the relative abundance of genes responsible for 10 soil P-cycling pathways (a) and individual genes (b) under different treatments. Data are presented as the mean \pm standard error ($n = 3$). Different letters indicate significant differences among treatments ($p < 0.05$).

that these P fractions are key factors influencing soil P availability.

The Spearman correlation matrix between soil PCGs and soil P fractions showed that genes encoding organic phosphoester

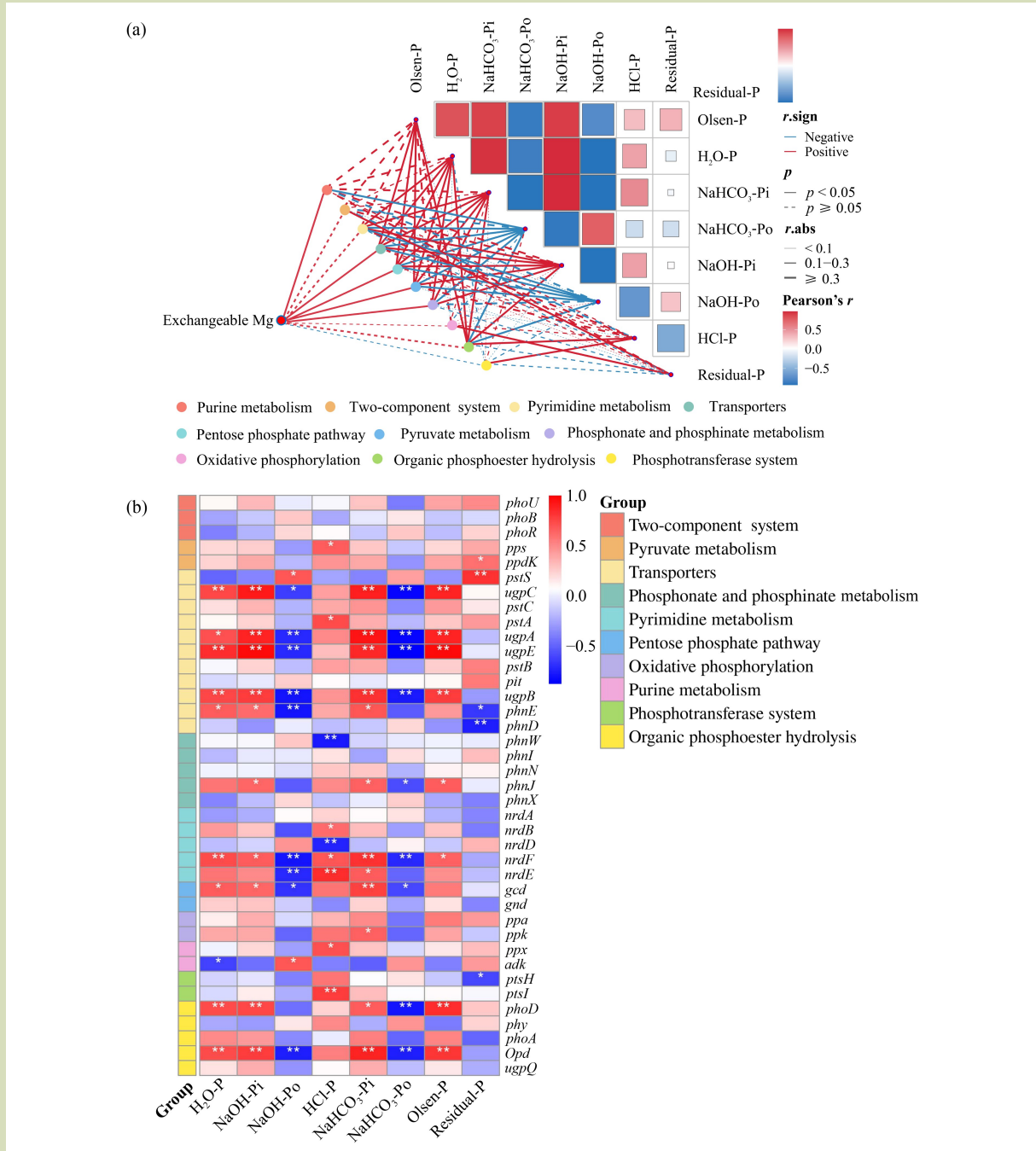


Fig. 5 Network heat map analysis of soil P fractions, P-cycling pathways, and soil exchangeable Mg (a), and the correlation matrix between PCGs and soil P fractions (b). Lines represent correlations between P-cycling pathways and soil environmental factors; the heat map represents correlations among environmental factors. The line thickness corresponds to the strength of the correlation (absolute value of Mantel's r) between P-cycling pathways and environmental factors. The line color indicates positive or negative correlations. Different colors in the heat map represent positive or negative correlations, with color depth indicating the correlation strength. Colors indicate correlation coefficients (r values), with red representing positive correlations and blue representing negative correlations. Only significant correlations ($p < 0.05$) are displayed. Asterisks indicate significance levels: * $p \leq 0.05$, ** $p \leq 0.01$.

hydrolysis (*opd* and *phoD*) and transporters (*ugpABCE*) were significantly positively correlated with Olsen-P, H₂O-P, NaHCO₃-Pi, and NaOH-Pi. Genes involved in phosphonate and phosphinate metabolism (*phn*) and pyrimidine metabolism (*pndE*) showed significant positive correlations ($p < 0.05$) but were significantly negatively correlated with NaOH-Po and NaHCO₃-Po (Fig. 5(b)). This indicates that these functional genes significantly activate the soil P pool and promote P availability.

3.6 Co-occurrence of microbial genes and microorganisms involved in soil microbial P cycling

Correlation networks primarily reflect correlations between genes and microorganisms. Different nodes represent different genes/microbes, and the line thickness indicates the Pearson correlation coefficient. To assess network complexity and stability, key topological metrics were calculated (Table S3). In the gene network, *opd*, *phoD*, *gcd*, *ugpABCE*, *pstA*, *nrdE*, *phnJ*, and *ppdK* had strong correlations with other genes, indicating their key roles in the P cycle (Fig. 6(a)). In the microbial network, *Klebsiella*, *Acetobacter*, *Corynebacterium*, *Escherichia*, *Brevibacterium*, *Pseudomonas*, *Arthrobacter*, *Mycobacterium*, and *Streptomyces* had strong correlations with other microbes, suggesting that they play crucial roles in the microbial community (Fig. 6(b)).

4 Discussion

4.1 Effects of Mg on microbial community structure and enzyme activity

Microorganisms are a vital component of soil, playing crucial roles in regulating soil fertility, plant growth, and nutrient cycling. The soil microbial biomass and activity are influenced by Mg nutrient inputs^[17]. In this experiment, the relative abundance of bacterial genera, such as *Streptomyces*, *Bradyrhizobium*, *Pseudomonas*, *Rhizobium*, and *Bacillus*, significantly increased under Mg treatment. Studies have shown that *Pseudomonas* is a phosphate-solubilizing bacterium that plays an important role in the P cycle^[27]. *Bacillus* promotes plant growth and has been demonstrated to play significant roles in C and P cycling^[28]. *Streptomyces*, which belongs to the Actinobacteria phylum, has been shown to possess the capability to promote plant growth and inhibit pathogens^[29]. *Bradyrhizobium* enhances urea metabolism and increases peanut yield^[30], and *Rhizobium* fixes atmospheric nitrogen (N₂)^[31]. Furthermore, our results showed that in the microbial co-occurrence network, key taxa, such as *Klebsiella*, *Acetobacter*, *Corynebacterium*, *Escherichia*, *Brevibacterium*, *Pseudomonas*, *Arthrobacter*, *Mycobacterium*, and *Streptomyces*, had high centrality values, potentially playing pivotal roles in the microbial community in response to soil exchangeable Mg.

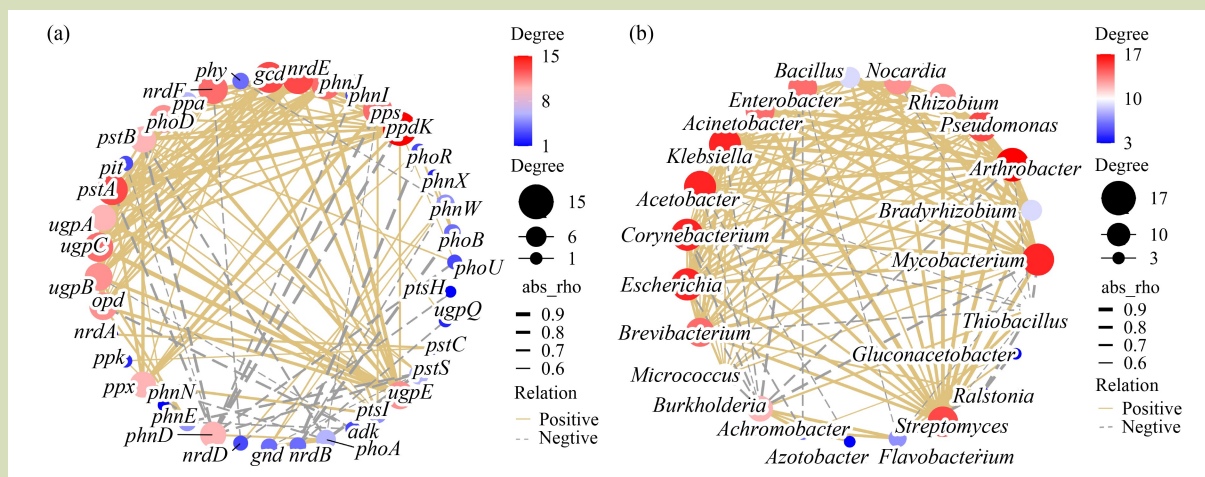


Fig. 6 Correlation network analysis of genes (a) and microorganisms (b). In the network, nodes represent different genes or microbial genera, colored and sized according to their centrality value. Edges represent significant correlations ($p < 0.05$), with thickness indicating the strength of the correlation ($|r|$ value) and color indicating positive (yellow) or negative (gray) correlations. Only strong correlations with $|r| > 0.6$ are displayed.

The elevated centrality of *Klebsiella* and *Escherichia* in the co-occurrence network, which diverges from patterns observed in typical orchard fields, is likely attributable to pot-specific conditions. The enriched water and nutrient availability in pots promotes copiotrophic populations, resulting in a community composition that contrasts with field soils^[32]. The enrichment of these beneficial microorganisms in the rhizosphere increases the abundance of soil P cycle-related genes and enhances the activity of enzymes related to Po mineralization and Pi solubilization, improving soil P availability and suppressing plant soil-borne pathogens.

Enzyme activity reflects soil microbial function. ALP is a key enzyme, as it hydrolyzes phosphate monoesters (the main form of Po), converting soil Po into Pi for plant uptake^[33,34]. ALP plays a dominant role in Po mineralization, aiding in the conversion of insoluble P and Po into available P, enhancing soil fertility by enriching available P. Measuring the ALP activity in the soil determines the overall nutrient availability controlled by the soil microbiome and soil health status. Both ALP and PDE activities serve as good indicators for regulating P turnover (i.e., Po mineralization). The addition of mineral and organic fertilizers in the P cycle system promotes soil available P^[35,36]. In this study, the activity of the P-cycle enzyme ALP significantly increased under Mg application compared to CK. This may be related to the important role played by Mg in the enzyme structure and metabolic biochemistry, as Mg application enhances the dehydrogenase, urease, and alkaline and acid phosphatase activities^[17].

4.2 Regulatory mechanisms of Mg on the soil P cycle

Soil microbial P metabolism functional genes play a central role in regulating the soil P cycle. Their functions broadly include transport systems, organic phosphoester hydrolysis, pyruvate metabolism, two-component system, pentose phosphate pathway, phosphotransferase system, oxidative phosphorylation, phosphonate and phosphinate metabolism, and purine and pyrimidine metabolism^[37]. These functional genes influence Pi solubilization and Po mineralization. This study found that Mg application significantly increased the abundance of genes related to such pathways as organic phosphoester hydrolysis, transport systems, and the pentose phosphate pathway. *phoD* is a key gene in the organic phosphoester hydrolysis pathway that encodes a monomeric enzyme hydrolyzing phosphate monoesters and diesters,

driving the conversion of Po to Pi, and acting as an important biomarker for soil P biotransformation^[8,9,38]. In this study, the increase in the relative *phoD* abundance and ALP activity under Mg treatment and the increase in the labile and moderately labile Pi fractions indicate the key role of Mg in Po mineralization.

One important mechanism for microbial solubilization of immobilized Pi is organic acid excretion, with gluconic acid being the most common organic acid produced by phosphate-solubilizing bacteria. The *gcd* gene, encoding quinoprotein glucose dehydrogenase, catalyzes the oxidation of glucose to gluconic acid, which acidifies the periplasmic space, significantly enhancing the dissolution of mineral phosphates and thus playing a core role in microbial P metabolism^[37,39]. Consistent with the findings in vegetables soil^[6], the abundance of the *gcd* gene in this study increased significantly with Mg application and showed a strong positive correlation with Olsen-P and other parameters, indicating that *gcd* plays an important role in microbial P solubilization.

Mg treatment significantly increased the abundance of the *ugpABCE* genes encoding glycerol-3-phosphate transporters and showed a positive correlation with Olsen-P, suggesting its important role in promoting microbial P uptake. Research has shown that the *ugpABCE*-mediated transport pathway links the synergistic mechanism between microbial P metabolism and plant P absorption^[37,40]. To identify key genes in the soil P cycle responsive to Mg, we constructed a correlation network for related genes. *opd*, *phoD*, *gcd*, *ugpABCE*, *pstA*, *nrde*, *phnJ*, and *ppdK* had high centrality values, indicating their key roles in promoting Po mineralization and Pi solubilization, which warrants further in-depth study. It is worth noting that the abundance of *phoA*, *phnD*, and *phnE* exhibits a nonlinear response to increasing magnesium concentration. The effects of nitrogen on soil phosphorus cycling genes (*aphA*, *appA*, *phnGHIJLM*, *phnN*, *phnP*, *phoN*, and *upgQ*) exhibited the same trend^[41]. This may be attributed to the osmotic stress induced by elevated Mg concentrations, which restricts microbial cell growth and protein translation, resulting in reduced abundance of related functional genes^[42].

4.3 Impact of Mg on soil P transformation, availability, and utilization efficiency via microbial P cycling functions

Our results indicated that soil exchangeable Mg promoted ALP

activity and was significantly positively correlated with soil P transformation processes (transport systems, pentose phosphate, pyruvate metabolism, and the organic phosphate ester hydrolysis). The increased ALP activity and enhanced soil phosphorus transformation processes together contributed to the rise in soil available P content^[34,35,43]. Due to the multiple interactions between P and soil, a large portion of applied P is adsorbed and fixed in the soil and converted into insoluble forms, leading to a gradual decrease in soil P availability^[5]. Mg has the potential to act as a regulator of nutrient cycling^[17]. This study demonstrated that Mg significantly altered the P characteristics of apple orchard soil. Mg treatment increased the soil Olsen-P content, particularly the forms readily absorbed and utilized by plants, such as H₂O-P, NaHCO₃-Pi, and NaOH-Pi, while it reduced the content and proportion of the NaHCO₃-Po form. Although studies directly investigating the effects of Mg on soil P fractions are still scarce, research has revealed that different exogenous interventions can achieve the targeted regulation of specific P fractions through common mechanisms, such as altering soil physicochemical properties or microbial activity. For instance, a study on the transformation of heavy metals and P fractions during sludge composting under functional membrane coverage found that coverage significantly increased the H₂O-P content of the soil^[44]. The interaction between warming and P addition has been shown to directly affect soil P availability, increasing the percentage of NaHCO₃-Pi in the Pi pool of a desert grassland soil^[45]. Biochar treatment has been shown to enhance the mobility of traditional P fractions and reduce the NaHCO₃-extractable Po component in long-term fertilized arid soil^[46]. A recent study found that ammonium polyphosphate treatment significantly increased the NaOH-Pi concentration in the rhizosphere soil of wheat^[47].

Path analysis of 10 correlation pathways between soil exchangeable Mg and the soil P cycle indicated that Mg significantly influenced transport systems, the pentose phosphate pathway, pyruvate metabolism, and the organic phosphate ester hydrolysis pathway. These pathways were significantly positively correlated with Olsen-P and identified as key biological pathways for enhancing soil P availability.

Previous research has confirmed that organic phosphate ester hydrolysis is crucial for P cycle transformation, and the increase in soil P availability and transformation is associated with an increased abundance of genes related to organic phosphate ester hydrolysis (e.g., *phoX* and *phoD*)^[43]. Concurrently, microbial regulation, transport, and the uptake and assimilation of P sources from the environment primarily rely on transport proteins and pyruvate and pentose phosphate metabolism^[37]. These key pathways are derived from a recently compiled genomic database for the P cycle; however, evidence of their functions and regulation in the plant kingdom, particularly in fruit tree systems, remains limited.

5 Conclusions

This study systematically revealed the regulatory mechanism of Mg input on the soil P cycle and its promotion of P availability. Mg influences the structural composition and abundance of soil microorganisms by regulating the diversity and composition of functional genes involved in the P cycle, which significantly enriches key functional genera, such as *Streptomyces*, *Pseudomonas*, and *Bacillus*. Mg significantly enhanced the abundance of functional genes, such as *phoD*, *opd*, *gcd*, and *ugpABCE*. Soil exchangeable Mg was consistently positively correlated with the P cycle-related metabolic pathways of these functional genes, indicating that Mg-mediated microbial P mineralization, solubilization, and transport are the primary pathways for soil P cycling and transformation. Mg significantly promoted soil P transformation and availability by reshaping the microbial community structure, providing a theoretical basis and technical pathway for alleviating soil P fixation and enhancing P nutrient cycling efficiency in agricultural ecosystems. These findings broaden our understanding of how microbial P cycle functional genes respond to Mg fertilizer and enable us to design nutrient management strategies based on Mg-P synergy, achieving efficient P fertilizer utilization and green agricultural production through the regulation of microbially driven P transformation. Follow-up research is needed to determine how Mg affects P cycle-related microbial processes across a wider range of crop species and soil types.

Supplementary materials

The online version of this article at <https://doi.org/10.15302/J-FASE-2027709> contains supplementary materials (Text S1; Tables S1–S3).

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Compliance with ethics guidelines

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