Effects of long-term irrigation on soil phosphorus fractions and microbial communities in *Populus euphratica* plantations

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Abstract

Irrigation has been demonstrated to be effective in managing *Populus euphratica* plantations, but its impacts on phosphorus (P) availability and the soil microbiome have not been fully elucidated. In this study, we compared soil properties, P fractions, phosphatase activities, and microbial communities in the surface soil (0–20 cm) of *P. euphratica* plantations under both drought and irrigation conditions. We found that total P, labile P and moderately labile P all increased significantly under irrigation by 12.3%, 70.1%, and 3.0%, respectively. The increased levels of labile P were primarily driven by higher levels of NaHCO₃-Pi, which increased from 1.9 mg kg⁻¹ to 12.3 mg kg⁻¹. Furthermore, irrigation markedly altered labile P composition and the relative levels of resin P, NaHCO₃-Pi, and NaHCO₃-Po were all impacted. Improved soil moisture increased soil phosphatase activity, suggesting that soil organic P (Po) mineralization was positively affected by irrigation. Moreover, we observed that bacterial diversity, fungal diversity and alkaline phosphatase gene communities, rather than total microbial biomass carbon or total PLFAs, were most significantly impacted by irrigation status. Furthermore, we found positive correlations among inorganic P (Pi) and Bradyrhizobiaceae, Nocardiaceae, and Sphingomonadaceae, whereas negative correlations were found between Burkholderiaceae and Pi, highlighting the diverse functional bacteria involved in P cycling. Our study demonstrates that irrigation can increase soil P availability and supply capacity, with shifts in P composition closely linked to changes in soil microbial characteristics. Water management strategies that target the restoration of soil microbial communities may therefore improve soil quality and enhance soil P cycling.

Introduction

Ecologically vulnerable regions are more susceptible to the impacts of global climate change[1]. Extreme droughts are likely to be more frequent in arid regions[2], and soil moisture is a critical limiting factor for many ecosystems. Soil properties and microbiomes are detrimentally affected by drought, leading to changes in pH levels, organic matter content, microbial diversity, and nutrient dynamics, with the essential macronutrient phosphorus (P) often seriously impacted[3–4]. P enters the soil via weathering of P-bearing primary minerals. Following release into the soil, P undergoes complex geochemical and biological transformations[5], resulting in a diversity of coexisting organic and inorganic forms. The turnover rates and bioavailability of these various forms of P to plants and microbes vary significantly in the soil[6]. Despite studies indicating that increasing frequency of droughts will profoundly impact soil P cycling, few studies fully consider soil P cycling in ongoing drought manipulation experiments in forest ecosystems.

Plants assimilate P from the soil via their root systems. Root exudates, such as organic acids and phosphatase enzymes, facilitate the solubilization and mobilization of soil P[7], and root architecture significantly influences P uptake efficiency[8]. Organic P in the soil is predominantly mineralized by plant roots and soil microorganisms, which produce a variety of enzymes that impact this process, primarily extracellular acid phosphatases (ACPs) and alkaline phosphatases (ALPs)[9].

While plant roots primarily produce ACPs, soil microorganisms, particularly bacteria, are the primary producers of ALPs[10]. Soil ACP and ALP activities have been shown to be directly dependent on soil water availability[11]. Drought can alter the composition and function of microbial communities in the soil, thereby influencing soil nutrient cycling[12]. The relationship between soil P fractions and microbial communities remains unclear and largely depends on which aspects of microbial communities are evaluated. Microbial groups, such as bacteria and fungi, display highly variable capacity to utilize soil P[13]. The abundance, composition, and functional diversity of soil microorganisms can be assessed using microbial biomass (measured through phospholipid fatty acid [PLFA] biomarkers and microbial biomass carbon), composition and diversity of taxonomic communities (taxonomic profiles measured using 16S rRNA or ITS genes and their amplification sequencing or PLFA biomarkers), or potential functions (profiles of functional genes measured using qPCR). Secreted alkaline phosphatase activity is primarily driven by *phoD* and *phoX*[14]. ALP activity and the prevalence of the *phoD* gene have also been shown to be directly correlated[15], and the abundance and diversity of the *phoD* genes are affected by soil pH[16] as well as fertilizer inputs[17]. However, research aimed at explicitly elucidating the association between microbial communities and soil P components is still lacking. Clarifying these relationships will be beneficial to integrating soil microbial processes into soil P cycling models.


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Populus euphratica trees play a crucial role in desert ecosystems but are susceptible to the impacts of global climate change. As a dominant tree species in arid areas, P. euphratica can be used for windbreak, sand fixation, and soil water conservation\textsuperscript{19}. It provides multiple ecosystem services as a natural barrier to the expansion of deserts\textsuperscript{20}. Given the projected increase in the frequency and severity of droughts, P. euphratica trees will likely face increasing environmental stresses\textsuperscript{21–23}. The Chinese government has implemented projects aimed at restoring P. euphratica populations, including groundwater irrigation near the Tarim River, which has successfully facilitated the recovery of P. euphratica\textsuperscript{24}. Nevertheless, the impacts of long-term irrigation on soil P cycling processes in P. euphratica plantations, along with the relationships between soil P fractions and soil microorganisms, are currently unknown. In this study, we assessed the impacts of irrigation on soil P pools and the soil microbiome to test three hypotheses: (1) irrigation significantly affects soil P status, particularly labile P fractions; (2) irrigation significantly increases soil phosphatase activity, an outcome that may be correlated with soil properties and microbial changes; (3) the association between P fractions and soil microbes may depend on the metrics used to evaluate microbiome characteristics.

Materials and methods

Field sites
This study was performed on the northwestern border of the Tarim Basin, located in China's Xinjiang Autonomous Region. The climate in the region is characterized by an annual mean air temperature of 10.8°C and a mean annual precipitation of 50 mm. The soil type in the area is classified as calcic xerisol.

A factorial experiment was conducted using two water management conditions, with six replicates each. A total of 12 plots, each measuring 15 m × 10 m, were selected along the upper reaches of the Tarim River (81°17'E, 40°32'N–40°81'N). Populus euphratica trees were planted in 2003 as part of a vegetation restoration project.

For the initial five years, all plots received irrigation. From 2009 to 2021, half of the plots were randomly selected to be irrigated for half a month in March and April (Irrigation treatment), while the other half only received ambient precipitation (drought treatment). Irrigation was maintained for eight hours per day at approximately 50 m\textsuperscript{2} h\textsuperscript{-1}, and the relative soil moisture content was kept at 90% to a depth of 60 cm during the irrigation period\textsuperscript{8}.

Soil sampling
Six 2 × 2 m\textsuperscript{2} sub-plots were randomly selected for each water treatment to ensure representative sampling. In mid-August 2021, three samples were collected from the top 20 cm of soil in each plot and combined to form six composite samples. The composite samples were placed in sterile sealed bags and kept on ice during transportation to the laboratory for processing. Subsequently, the samples were thoroughly mixed and passed through a 2-mm sieve. Portions of each fresh soil sample were stored at 4 °C and −80 °C for further analysis, while the remaining portion was air-dried and divided into an archival sample and a sample used to determine soil properties and P fractions.

Soil properties
Soil pH was determined using a pH meter, with a soil-to-CaCl\textsubscript{2} solution ratio of 1:2.5 (v/v), following standard protocols. Soil organic carbon (SOC) was quantified using the electric sand bath potassium dichromate titration method, as described by Bremner and Jenkinson\textsuperscript{25}. Total nitrogen (TN) content was measured using the micro-Kjeldahl method. NH\textsubscript{4}\textsuperscript{+}-N and NO\textsubscript{3}\textsuperscript{-}N concentrations were extracted from the soil using 1 M KCl at a ratio of 1:5 and analyzed using a Continuous-Flow Auto Analyzer (Bran+Luebbe AA3, Germany). Exchangeable K\textsuperscript{+}, Na\textsuperscript{+}, Ca\textsuperscript{2+}, and Mg\textsuperscript{2+} were extracted using acetamide and analyzed using an inductively coupled plasma emission spectrometer (ICP).

Soil P fractions
Soil P was fractionated using the continuous extraction method originally developed by Hedley et al.\textsuperscript{26} and modified by Tiessen\textsuperscript{27}. The following protocol was used to extract P fractions from 0.5 g of air-dried soil deposited in a 50 mL centrifuge tube: (a) each centrifuge tube received two 9 × 62 mm resin strips along with 30 mL distilled water, followed by stirring at 160 rpm for 16 hours to extract P from the resin strips using 0.5 M HCl (referred to as resin-Pi); (b) following the removal of the aqueous solution, 30 mL of 0.5 M NaHCO\textsubscript{3} at pH 8.5 was added and the tubes were shaken for 16 hours to extract NaHCO\textsubscript{3}-P; (c) NaOH-P was extracted by adding 30 mL of 0.1 M NaOH and rotating the tubes for 16 hours; (d) 30 mL of 1 M HCl was added to each centrifuge tube, and the tubes were shaken for 16 hours to extract 1 M HCl-Pi; (e) 15 mL of concentrated HCl was used to further extract soil residue at 80 °C (conc. HCl-P); (f) P was obtained by boiling the soil residue in 8 mL of concentrated H\textsubscript{2}SO\textsubscript{4} with 10 drops of HClO\textsubscript{4} to obtain residual P.

After extraction, the supernatant was partitioned into two aliquots for Pi and Po determination. The quantification of Pi was carried out using the molybdate-ascorbic acid procedure originally proposed by Murphy and Riley\textsuperscript{28}. Total P (TP) was determined by incubating the supernatant with acidified ammonium persulfate at 121 °C for one hour. TP and Pi were directly measured from the extracts, and Po was calculated by subtracting TP from P\textsuperscript{29}. To determine soil microbial biomass P (MBP), chloroform fumigation and NaHCO\textsubscript{3} extraction were carried out over a 24-hour period, as described by Brookes et al.\textsuperscript{30}. Separate non-fumigated samples were spiked with 25 mg L\textsuperscript{-1} of P to evaluate the efficacy of P recovery during the fumigation process.

Activities of ACP and ALP
ACP and ALP activities in soil samples were determined via the method described by Tabatabai\textsuperscript{31}. Briefly, fresh soil samples (1 g) were incubated at pH 6.5 (for ACP) and pH 11.0 (for ALP) at 37 °C for 1 hour, using p-nitrophenyl phosphate (pNPP) and disodium phenyl phosphate as substrates. ACP and ALP activities were recorded as mg p-nitrophenol and phenol kg\textsuperscript{-1} soil (dry weight) h\textsuperscript{-1}, respectively.

Soil microbial characteristics
We determined microbial biomass through analysis of microbial biomass carbon (MBC) and phospholipid fatty acid (PLFAs). Extraction of MBC was performed by adding 0.5 M K\textsubscript{2}SO\textsubscript{4} to both chloroform-fumigated and unfumigated soil samples, followed by measurement with a computerized total organic carbon analyzer (Analytikjena, Germany). MBC was quantified by calculating the variation in organic carbon extracted between the fumigated and unfumigated soils. PLFAs were determined after freeze-dried soil (5 g) following the proce-
Soil P fractions in *P. euphratica* plantations

dure described by Frostegård et al.[32]. PLFAs were further classified into respective microbial functional groups in accordance with the procedure created by Russ and Chamberlain[33]. Total PLFAs were determined by summing the biomass of all microbial functional groups. The bacterial to fungal ratio (B: F) was calculated by dividing the sum of all bacterial biomarkers by the sum of all fungal biomarkers.

Soil DNA was extracted from freeze-dried soil samples using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, United States), following the manufacturer’s instructions. Amplifications of bacterial 16S (V4-V5) and fungal ITS rRNA genes, as well as quantitative polymerase chain reaction (qPCR) and amplicon sequencing of *phoD* and *phoX*, were performed following the protocols described by Xia et al.[34] and are provided in Supplementary S1.

**Statistical analysis**

T-tests were employed to determine variations in soil properties, P fractions, ACP and ALP, total PLFAs, MBC, MBP, bacterial and fungal Shannon-diversity, copy numbers of *phoD* and *phoX* genes, and alpha diversity (richness and Shannon-diversity) of alkaline phosphatase gene communities between the drought and irrigation treatment groups. The Wilcoxon test was used to detect variations in the relative abundance of *phoD* and *phoX* bacteria at the family and genus levels between the two treatments. The ‘ggvegan’ R package was used to conduct a covariance analysis for soil properties and P fractions. To investigate the associations between soil properties and soil P fractions, we employed redundancy analysis (RDA). Additionally, the ‘vegan’ R package was employed to conduct principal coordinates analysis (PCoA) for *phoD* and *phoX* community composition and procrustean analysis among the *phoD* and *phoX* community with soil P fractions. The correlations between soil P fractions with the *phoD* and *phoX* community, as well as MBP, ACP, and ALP, were performed using the ‘psych’ and ‘pheatmap’ R packages. All box plots were generated utilizing the ‘ggplot2’ package in R.[35]

**Results**

**Soil properties, P fractions, and their associations**

Compared to the drought treatment, irrigation significantly increased several soil physical and chemical attributes, including WC, pH, TN, SOC, NH₄⁺-N, TP, and TPi (Table 1). The labile P fraction represented approximately 1-3% of TP, while the moderately labile P fraction accounted for 84-89% and decreased under irrigation (Fig. 1a). The relative content of resin-Pi decreased slightly, whereas the amount of NaHCO₃-Pi in the labile P fraction increased significantly with irrigation (Fig. 1b). The NaHCO₃-Pi fraction accounted for approximately 56% and 28% of labile P under irrigation and drought treatments, respectively (Fig. 1b). The components comprising moderately labile P were minimally altered by irrigation (Fig. 1a). The ratio of NaOH-extracted P in the moderately labile P fraction increased by 2% comparing drought to irrigation treatment, while the fraction of 1 M HCl-Pi was reduced by 2% (Fig.

![Fig. 1](image-url) Percentage of each phosphorus (P) fraction under different water management treatments. (a) Total P. (b) Labile P. (c) Moderately labile P. (d) Sparingly labile P.

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Soil phosphorus (P) sequential fractionation under different water management treatments

<table>
<thead>
<tr>
<th>P fraction (mg kg⁻¹)</th>
<th>Irrigation</th>
<th>Drought</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labile P</td>
<td>1.15 ± 0.27</td>
<td>0.50 ± 0.06</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Resin-Pi</td>
<td>12.31 ± 2.40</td>
<td>1.88 ± 0.51</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>NaHCO₃-Pi</td>
<td>8.69 ± 0.82</td>
<td>4.25 ± 0.49</td>
<td>0.03</td>
</tr>
<tr>
<td>ΣLabile P</td>
<td>22.15 ± 1.67</td>
<td>6.62 ± 0.54</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Moderately labile P</td>
<td>7.02 ± 0.79</td>
<td>2.07 ± 0.12</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>NaOH-Pi</td>
<td>16.41 ± 1.83</td>
<td>6.98 ± 0.17</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>1 M HCl-Pi</td>
<td>521.10 ± 12.37</td>
<td>505.52 ± 1.56</td>
<td>0.03</td>
</tr>
<tr>
<td>ΣModerately labile P</td>
<td>544.53 ± 13.51</td>
<td>511.58 ± 1.54</td>
<td>0.02</td>
</tr>
<tr>
<td>Slightly labile P</td>
<td>27.50 ± 1.84</td>
<td>21.35 ± 1.73</td>
<td>0.01</td>
</tr>
<tr>
<td>Conc. HCl-Po</td>
<td>9.79 ± 1.38</td>
<td>6.68 ± 1.50</td>
<td>0.02</td>
</tr>
<tr>
<td>ΣSlightly labile P</td>
<td>37.29 ± 2.75</td>
<td>28.04 ± 1.95</td>
<td>0.17</td>
</tr>
<tr>
<td>Nonlabile P</td>
<td>41.76 ± 5.11</td>
<td>26.22 ± 0.82</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Bold numbers indicate significant differences (p < 0.05) between treatments.

Table 1. Effects of irrigation on soil properties (mean ± standard error) in *P. euphratica* plantations

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Irrigation</th>
<th>Drought</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WC</td>
<td>25.26 ± 0.54</td>
<td>6.93 ± 0.95</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>pH</td>
<td>8.41 ± 0.05</td>
<td>8.72 ± 0.02</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TN (g kg⁻¹)</td>
<td>1.18 ± 0.05</td>
<td>0.76 ± 0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>SOC (g kg⁻¹)</td>
<td>40.13 ± 0.22</td>
<td>32.12 ± 0.29</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>NO₃⁻-N (mg kg⁻¹)</td>
<td>6.35 ± 1.82</td>
<td>4.55 ± 0.90</td>
<td>0.16</td>
</tr>
<tr>
<td>NH₄⁺-N (mg kg⁻¹)</td>
<td>2.15 ± 0.23</td>
<td>1.25 ± 0.11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TP (g kg⁻¹)</td>
<td>0.65 ± 0.01</td>
<td>0.57 ± 0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>DON (mg kg⁻¹)</td>
<td>7.96 ± 0.47</td>
<td>9.65 ± 0.27</td>
<td>0.05</td>
</tr>
<tr>
<td>AK (g kg⁻¹)</td>
<td>0.41 ± 0.09</td>
<td>0.32 ± 0.05</td>
<td>0.12</td>
</tr>
<tr>
<td>DOC (g kg⁻¹)</td>
<td>0.26 ± 0.03</td>
<td>0.39 ± 0.07</td>
<td>0.17</td>
</tr>
<tr>
<td>Na⁺ (g kg⁻¹)</td>
<td>1.85 ± 0.06</td>
<td>1.82 ± 0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>Ca²⁺ (g kg⁻¹)</td>
<td>15.75 ± 0.51</td>
<td>13.95 ± 0.34</td>
<td>0.86</td>
</tr>
<tr>
<td>Mg²⁺ (g kg⁻¹)</td>
<td>0.83 ± 0.08</td>
<td>0.61 ± 0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>TPI (g kg⁻¹)</td>
<td>0.57 ± 0.01</td>
<td>0.53 ± 0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>P/Pt (%)</td>
<td>88.15 ± 0.91</td>
<td>92.29 ± 0.30</td>
<td>0.68</td>
</tr>
<tr>
<td>Po/Pt (%)</td>
<td>11.85 ± 0.91</td>
<td>7.71 ± 0.30</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Bold numbers indicate significant differences (p < 0.05) between treatments.

Irrigation led to a slight variation in the relative concentration of conc. HCl-Pi, which varied between 74−76% under the two water treatments (Fig. 1d).

The concentrations of labile and moderately labile P increased markedly under irrigation (Table 2). The Pi and Po fractions in the labile P and moderately labile P fractions also increased under irrigation. Although sparingly labile P values were not significantly different between irrigation and drought treatments, irrigation had significantly higher conc. HCl-Pi and conc. HCl-Po. The soil phosphatase enzymes and microbial characteristics

Under irrigation, there was a significant increase in MBP, ACP, and ALP relative to the irrigation treatment (Fig. 2a−c). Notably, irrigation increased ACP more strongly than ALP (Fig. 2a, b). Significant and positive relationships were identified between labile P and moderately labile P, as well as between residual P and MBP, ACP, and ALP. However, HCl-P did not exhibit noteworthy associations with MBP, ACP, or ALP, with the exception of MBP and conc. HCl Po (Fig. 3). To assess the impact of water management on soil microbial characteristics, microbial analyses were conducted using three metrics: biomass changes, taxonomic profiles, and functional changes (Fig. 4a−g). This analysis showed that soil microbial biomass was greater under irrigation than under drought, regardless of whether it was measured using MBC or total PLFAs (Fig. 4a and b). While there were no substantial differences between the two treatments in the relative proportions of bacteria and fungi based on PLFA classification, the diversity of bacteria and fungi under irrigation was higher than under drought when it was assessed using bacterial 16S and fungal ITS rRNA gene amplifications (Fig. 4c−e). Additionally, phoD and phoX copy numbers were higher under irrigation than under drought (Fig. 4f and g).

Notably, all soil microbial parameters except the bacteria-to-fungi ratio were positively correlated with elevated levels of soil P (Fig. 5a). Among the evaluated soil microbial characteristics, microorganism composition and functional levels exhibited better explanatory power for the variations in soil P fractions (Fig. 5b).

The richness and diversity of the bacterial phoD genes were significantly higher under irrigation than under drought (Supplemental Fig. S2a and b). In contrast, no major variations were observed between treatments in the richness and diversity of bacterial phoX genes (Supplemental Fig. S2b). Principal coordinate analysis (PCoA), conducted using the Bray-Curtis distance matrix, indicated significant variations in phoD and phoX gene communities between treatments (Supplemental Fig. S2c and d).

The taxonomic composition of phoD and phoX gene bacteriocommunities was assessed at the family and genus levels, where the relative abundances exceeded 0.01% (Supplemental Fig. S3a−d). Specifically, phoD gene reads were primarily classified into 14 families and 14 genera, while phoX gene reads were classified into 14 families and 13 genera. Analysis of phoD gene community composition at the family level revealed that irrigation had a considerable impact on the relative abundance of Bradyrhizobiaceae, Nocardiaceae, Sphingomonadaceae, and Burkholderiaceae (Supplemental Fig. S3a). Furthermore, at the genus level, irrigation increased the relative abundance of Bradyrhizobium and Rhodococcus (Supplemental Fig. S3b). Similarly, phoX gene community composition analysis demonstrated that irrigation significantly affected the relative abundance of Phyllobacteriaceae and Xanthomonadaceae at the family level. In contrast, at the genus level, the relative abundance of Halomonas and Rhodopirellula reduced and increased during irrigation, respectively (Supplemental Fig. S3c and d).

Procrustean analysis confirmed a strong relationship...
between the structure of the phoD gene community at Operational Taxonomic Unit (OTU) level and soil P fractions across treatment type (Fig. 6a). However, soil P fractions were not affected by the composition of the phoX gene community (Fig. 6b).

We next conducted a correlation analysis between the phoD and phoX gene communities and soil P fractions. At the family level of the phoD gene community, relative abundances of Bradyrhizobiaceae, Nocardiaceae, and Sphingomonadaceae were positively correlated with resin-Pi, NaHCO3-Pi, NaHCO3-Po, NaOH-Pi, and NaOH-Po. Meanwhile, Burkholderiaceae was negatively correlated with all P fractions except NaHCO3-Po (Fig. 6c). Spearman’s correlation analysis revealed a significant positive correlation between NaHCO3-Po and Myelobacteriaceae, conc. HCl-Pi with Oxalobacteraceae, Acetobacteraceae, and Myelobacteriaceae, and residual-P with Bradyrhizobiaceae, Sphingomonadaceae, and Myelobacteriaceae (Fig. 6c).

At the genus level of the phoD community, the relative abundance of Bradyrhizobium and Rhodococcus was positively correlated with resin-Pi, NaHCO3-Pi, NaHCO3-Po, NaOH-Pi, and NaOH-Po. Bradyrhizobium was also positively correlated with residual-P (Fig. 6e). Further, Methylobacterium was positively correlated with resin-Pi, NaHCO3-Po, conc. HCl-Pi, and residual-P (Fig. 6e). In contrast, the relative abundance of Mesorhizobium was negatively correlated with NaHCO3-Po and conc. HCl-Pi (Fig. 6e).

**Discussion**

Compared to drought, irrigation increased TP and TPI due to significant increases in labile and moderately labile P. Labile P, which serves as the primary source of P for plant growth, increased substantially by 2.34 fold (Table 2) [36,37]. However, labile P only accounted for 3% of TP under the irrigation treatment (Fig. 1a), indicating that available P deficiency was still an important limiting factor in this ecosystem’s productivity. Nevertheless, improving soil moisture increased litter production and soil coverage, thereby reducing P leaching from the soil surface [38]. Additionally, higher litter input and the proliferation of roots have been shown to increase Po, and the partial decomposition of Po by plants and microorganisms can increase the availability of resin P [39,40]. NaHCO3-P increased significantly under irrigation and altered labile P composition, with NaHCO3-Pi gradually coming to dominate this fraction (Fig. 1b). Activated soil phosphatase promoted the release of inorganic P from NaHCO3-Pi, which represents a labile form of P that can rapidly dissolve and mineralize. These characteristics act to supplement P in deficient soils, mitigating declines in resin-Pi and NaHCO3-Pi.

NaOH-P is a component of moderately labile P pool in soils and requires long-term mineralization before it is available to plants [41]. Its presence therefore reflects the soil’s future potential to supply P. Irrigation can increase NaOH-P content, likely as a result of the influx of external sources of carbon [39,42]. P represented by HCl-P is associated with calcium and is likely derived from primary minerals [43,44]. We found that 1 M HCl-P was the predominant form at our study location, accounting for approximately 70% of TP (Fig. 1c). Recent studies have shown that the mean residence time of HCl-P can range from years to millennia and that the average turnover rate of Ca-phosphate is
indicating that it is highly stable. However, it should be noted that the stability of HCl-P is significantly influenced by the soil pH. In alkaline soils, the HCl-P pool is typically comprised of highly stable calcium-P minerals. Long-term irrigation can lead to increased soil moisture and decreased pH (Supplemental Fig. S1) and may facilitate the weathering of primary minerals and the desorption of Ca-associated P. Residual P, bound by secondary minerals, represents...
the most stable P fraction in soils. Although it is typically unavailable to plants and soil microorganisms, desorption and weathering can eventually mobilize it for plant uptake\cite{47}.

The mineralization of Po to Pi is believed to be heavily influenced by phosphatases\cite{48,49}. A substantial increase in both ACP and ALP activities in response to irrigation was observed in our study (Fig. 2a and b) and may be linked to increased litter input and higher soil water content. Irrigation stimulates enzyme
activity because water increases the connectivity between soil pores and the availability of nutrient resources to meet the physiological needs of microorganisms\cite{9}. Soil microbial biomass is increasingly recognized as a critical driver of soil P dynamics and we found a positive correlation between soil microbial biomass P (MBP) and most soil P fractions (Fig. 3)\cite{30,51}. Furthermore, irrigation increased MBP (Fig. 2c), while drought has been shown to inhibit microbial growth and cause the release of significant quantities of P, resulting in a reduction of MBP\cite{52}.

Irrigation affected several soil microbiome characteristics, including microbial biomass, taxonomy, and functional profiles. Irrigation has been shown to increase both total or active microbial biomass and active microbial biomass in soil, as well as bacterial and fungal diversity (Fig. 4a and b). This suggests that adequate water availability facilitates the metabolic activities of soil microorganisms, promoting their growth and propagation. Moreover, water availability can influence soil microbial function\cite{53,54}. Irrigation increased the number of phoD and phoX bacteria (Fig. 4c) and the diversity of phoD (Supplemental Fig. S2a and b), a gene encoding an important phosphatase enzyme involved in P cycling. This indicates that water availability enhances the ability of soil microbes to perform essential functions, such as nutrient cycling. Therefore, irrigation can significantly impact soil microbial function, ultimately affecting the overall health of soil ecosystems.

We also found that the composition and function of microorganisms, rather than total microbial biomass, significantly influenced soil P fractions, as demonstrated by variations in the diversity of bacteria and fungi and the copy numbers of phoX genes. However, it will be important to understand how variations in soil moisture and pH and to changes in the composition and functional profiles of soil microorganisms, mainly bacteria possessing phoD genes. However, it will be necessary to fully characterize the allocation of foliar-P fractions of *P. euphratica* and its relationship with soil-P fractions in the future. These findings underscore the potential impacts of water management on soil P dynamics.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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