

## Effects of *Potamogeton crispus* growth on phosphorus fractions in lake sediments at two nutrient levels

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### Abstract

This study explored the regulatory effects of *Potamogeton crispus* (*P. crispus*) on phosphorus fractions in lake sediments under two nutrient conditions, using a rhizosphere–non-rhizosphere partitioning approach. Sediments from Tangxun Lake (mesotrophic) and Nan Lake (eutrophic), Wuhan, China, were used in a controlled microcosm experiment. The dynamics of seven phosphorus fractions (Ca<sub>2</sub>-P, Al-P, Org-P, Fe-P, O-Al-P, O-Fe-P, and Ca<sub>10</sub>-P) were analyzed across different growth stages. Results showed: (1) In mesotrophic sediments, *P. crispus* significantly reduced labile phosphorus (Ca<sub>2</sub>-P and Fe-P) in the rhizosphere, indicating enhanced uptake and stabilization; (2) In eutrophic sediments, phosphorus fractions including Org-P and Ca<sub>10</sub>-P accumulated in the rhizosphere, reflecting active turnover and long-term retention; (3) O-Fe-P was consistently lower in rhizosphere sediments across both lakes, likely due to rhizosphere-induced redox suppression. These findings highlight the dual role of *P. crispus* in activating and stabilizing phosphorus in sediments and provide theoretical guidance for plant-based lake restoration strategies.

**Keywords:** *Potamogeton crispus*; Phosphorus Fraction; Rhizosphere Effects; Lake Sediment; Eutrophication Control; Submerged Macrophytes; Eutrophic Lake Restoration; Internal Phosphorus Loading

### 1. Introduction

Phosphorus is one of the key nutrients driving eutrophication in freshwater lakes [1]. With increasing control of external nutrient inputs, the internal release of phosphorus from sediments has emerged as a significant bottleneck to further improvements in water quality [2,3,4]. Previous studies have demonstrated that the chemical forms of phosphorus in sediments, their migration mechanisms, and the exchange processes with overlying water collectively determine the potential for internal phosphorus release [5,6].

In the past decade, ecological engineering approaches have gained attention in lake restoration, with submerged macrophytes showing substantial potential due to their unique rhizosphere effects [7,8,9]. Through root-mediated changes in redox conditions, pH, electrical conductivity, and microbial community structure in sediments, submerged macrophytes can influence the stability and bioavailability of different phosphorus fractions. This dual regulatory role allows them to both suppress and promote phosphorus release in lake ecosystems [10,11,12]. Recent findings have

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further highlighted significant differences in how plant species, root activity, and growth stages affect sediment phosphorus dynamics, underscoring the need for deeper investigation into plant–sediment–environment interactions [13,14].

Since 2020, studies on the role of submerged macrophytes in modulating sediment phosphorus fractions have proliferated. For instance, Wang et al. (2021) found that *Vallisneria natans* could stabilize sediment phosphorus by altering Fe-P and Org-P fractions [15]. Similarly, Yuan et al. (2021) reported that *P. crispus* significantly inhibited Fe-P release in eutrophic lakes by promoting the formation of an oxidized rhizosphere layer conducive to phosphorus re-precipitation and fixation [16]. Moreover, Bai et al. (2020) revealed that rhizosphere microenvironmental changes driven by *P. crispus* also influenced the transformation of Ca<sub>2</sub>-P and Ca<sub>10</sub>-P fractions through time-series experiments [17].

However, there remains a lack of systematic comparative studies on the regulatory mechanisms of submerged macrophytes on phosphorus fractions across lakes with differing nutrient levels. In particular, few studies have employed a unified experimental framework to distinguish the roles of rhizosphere and non-rhizosphere zones in phosphorus transformation pathways. Therefore, it is essential to conduct controlled experiments comparing the functions of submerged macrophytes in mesotrophic versus eutrophic lake sediments, in order to clarify their regulatory mechanisms and provide theoretical support for eutrophic lake restoration.

In this study, *P. crispus* was selected as a representative submerged macrophyte. Sediments were collected from Tangxun Lake (mesotrophic) and Nan Lake (eutrophic) in Wuhan, China. Using a partitioned setup to separate rhizosphere and non-rhizosphere zones, the regulatory effects of *P. crispus* at different growth stages on seven phosphorus fractions in sediments were systematically evaluated. The objective was to elucidate the mechanisms by which submerged macrophytes modulate phosphorus speciation in lake sediments and to provide scientific guidance for species selection in the management of lake eutrophication.

## 2. Materials and Methods

### 2.1. Sediment Collection

Sediment samples were collected from the bottom of Tangxun Lake (mesotrophic) and Nan Lake (eutrophic), both located in Wuhan, China. The specific sampling coordinates were as follows:

- Tangxun Lake: N 30°25'09.4", E 114°22'58.3"
- Nan Lake: N 30°28'25.2", E 114°21'57.8"

The basic physicochemical properties of the sediments are summarized in Table 1.

**Table 1** Basic physicochemical properties of sediments

| Sediment     | pH   | Olsen-P (mg/kg) | Organic Matter (g/kg) | Total P (mg/kg) | Water Content (%) |
|--------------|------|-----------------|-----------------------|-----------------|-------------------|
| Tangxun Lake | 6.14 | 15.33           | 32.86                 | 561             | 59.4              |
| Nan Lake     | 7.58 | 64.52           | 45.9                  | 1570            | 49                |

### 2.2. Plant Materials

*P. crispus* plants used in the experiment were propagated from seeds collected from a waterbody adjacent to Nan Lake (N 30°28'19.72", E 114°21'49.15"), Wuhan, China.

### 2.3. Experimental Design

The experiment was conducted using a pot-culture setup. 1 kg Freshly collected and homogenized sediments (dry weight) was evenly spread into plastic buckets (height: 26 cm; diameter: 23 cm). In each bucket, three nylon mesh bags (300 mesh, 10 cm in diameter, 10 cm in height) were placed, each filled with 30 g homogenized sediments (dry weight) and planted with two uniform *P. crispus* seeds, creating the rhizosphere zone (RS). The surrounding sediment outside the mesh bags served as the non-rhizosphere zone (NRS) (Fig. 1).



**Figure 1** Experimental Design

Buckets were filled with distilled water to a depth of 20 cm using the siphon method and maintained at a constant water level throughout the experiment. For each lake sediment, four replicates were established, along with control treatments without plants (CK). All buckets were cultivated in a rain-sheltered, light-permeable greenhouse.

Sampling was conducted at 30, 90, and 180 days. At each sampling point, the mesh bag with the highest *P. crispus* biomass and the corresponding NRS sediment were collected. The sample codes are listed in Table 2.

**Table 2** Sample codes of sediment treatments

| Lake         | Control (CK) | Rhizosphere (RS) | Non-rhizosphere (NRS) |
|--------------|--------------|------------------|-----------------------|
| Tangxun Lake | CK-T         | RS1-T            | NRS1-T                |
| Nan Lake     | CK-N         | RS1-N            | NRS1-N                |

#### 2.4. Extraction and Determination of Phosphorus Fractions in Sediments

Among the available techniques for analyzing phosphorus fractions in sediments, chemical sequential extraction remains the most mature and widely adopted method [18]. Depending on the type of extractants and the sequence of extraction, various protocols have been developed, each with its own strengths and limitations [18,19]. To comprehensively investigate the mechanisms by which *P. crispus* root growth affects sediment phosphorus speciation, we adopted a modified extraction scheme. This method integrates the classification systems of Chang and Jackson [20] and Jiang and Gu [21,22], along with improvements proposed by Fife [23], Petersen [24], and Zhang [25].

The modified sequential extraction procedure involves seven consecutive steps, each targeting a specific phosphorus fraction in the sediment:

##### 2.4.1. *Ca*<sub>2</sub>-P (loosely bound calcium phosphate)

Weigh 1.00 g (dry weight equivalent) of sediment into a 100 mL polypropylene centrifuge tube. Add 50 mL of 0.25 mol·L<sup>-1</sup> NaHCO<sub>3</sub> (pH 7.5), and shake on a reciprocal shaker for 1 hour at room temperature (25 ± 1°C). Centrifuge at 4000 rpm for 5 minutes and decant the supernatant into a beaker (solution A). Wash the sediment residue once with 50 mL ethanol, centrifuge again, discard the liquid, and retain the residue.

##### 2.4.2. Al-P (aluminum-bound phosphorus)

Add 50 mL of 0.5 mol·L<sup>-1</sup> NH<sub>4</sub>F (pH 8.5) to the residue, shake for 1 hour, then centrifuge. Decant the supernatant into a separate beaker (solution B). Wash the residue once with 50 mL saturated NaCl solution, centrifuge, discard the liquid, and retain the residue.

##### 2.4.3. Org-P (highly active organic phosphorus)

Add 50 mL of 0.7 mol·L<sup>-1</sup> NaClO (pH 8.05) to the residue, mix thoroughly, and incubate in a boiling water bath for 30 minutes. Cool to room temperature, then centrifuge and transfer the supernatant to a 100 mL volumetric flask (solution C). Wash the residue with 50 mL saturated NaCl solution, centrifuge again, and combine the wash with the previous supernatant to reach volume. For Org-P determination, transfer a known volume of solution C to a 100 mL Erlenmeyer

flask, add 10 mL of mixed acid ( $\text{H}_2\text{SO}_4:\text{HClO}_4:\text{HNO}_3 = 1:2:7$  v/v), digest, and measure the inorganic phosphorus concentration.

#### 2.4.4. Fe-P (iron-bound phosphorus)

Add 50 mL of a mixed solution containing  $0.1 \text{ mol}\cdot\text{L}^{-1}$  NaOH and  $0.1 \text{ mol}\cdot\text{L}^{-1}$   $\text{Na}_2\text{CO}_3$  to the residue, stir well, shake for 2 hours, allow to stand for 16 hours, then shake for another 2 hours. Cool and centrifuge. Collect the supernatant in another centrifuge tube (solution D). Wash the residue once with 50 mL saturated NaCl solution, centrifuge, discard the liquid, and retain the residue.

#### 2.4.5. O-Al-P (occluded aluminum-bound phosphorus)

Add 50 mL of  $1 \text{ mol}\cdot\text{L}^{-1}$  NaOH to the residue, stir thoroughly, and incubate in an  $85^\circ\text{C}$  water bath for 1 hour. Cool and centrifuge, then decant the supernatant into a new tube (solution E). Wash the residue with 50 mL saturated NaCl, centrifuge, discard the liquid, and retain the residue.

#### 2.4.6. O-Fe-P (occluded iron-bound phosphorus)

To the residue, add 40 mL of  $0.3 \text{ mol}\cdot\text{L}^{-1}$  sodium citrate solution, then add 0.5 g of sodium dithionite. After equilibrating in a  $75^\circ\text{C}$  water bath, add 10 mL of  $0.5 \text{ mol}\cdot\text{L}^{-1}$  NaOH, and stir for another 10 minutes. Cool and centrifuge. Transfer the supernatant to a 100 mL volumetric flask (solution F). Wash the residue once with 50 mL saturated NaCl solution, centrifuge, and combine the wash with solution F to reach volume. For phosphorus analysis, digest a known volume of F with 10 mL of mixed acid ( $\text{H}_2\text{SO}_4:\text{HClO}_4:\text{HNO}_3 = 1:2:7$ ), then determine the inorganic phosphorus concentration.

#### 2.4.7. $\text{Ca}_{10}$ -P (stable calcium phosphate such as hydroxyapatite)

Add 50 mL of  $0.25 \text{ mol}\cdot\text{L}^{-1}$   $\text{H}_2\text{SO}_4$  to the final residue, shake for 1 hour at room temperature. Centrifuge, decant the supernatant into a separate tube (solution G), and discard the residue.

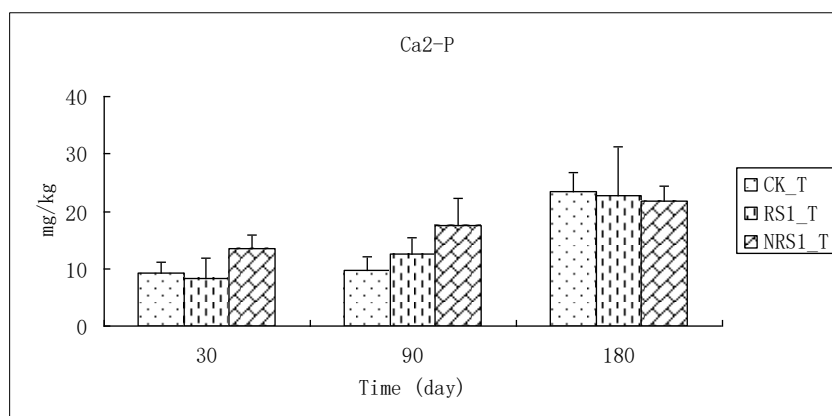
All extracted solutions (A–G) were analyzed for phosphorus content using the molybdenum–antimony anti-colorimetric method. The measurement procedures followed those described in the third edition of Soil and Agricultural Chemistry Analysis by Bao [26].

### 2.5. Statistical Analysis

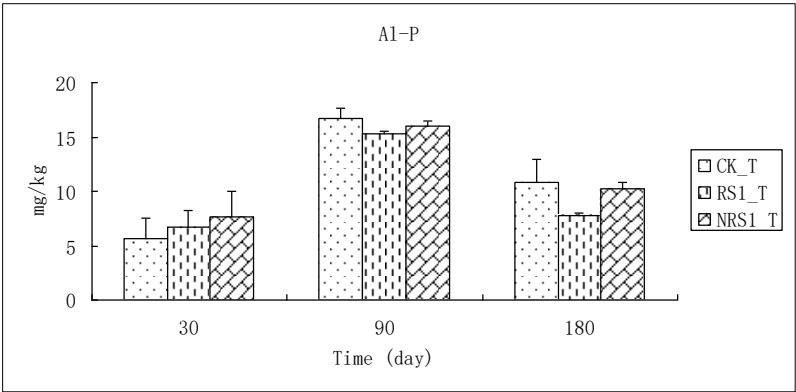
Data were analyzed using SAS 9.4 software. Paired-sample t-tests were conducted to determine significant differences in phosphorus fractions between rhizosphere and non-rhizosphere sediments. Differences were considered statistically significant at  $P < 0.05$ .

## 3. Results

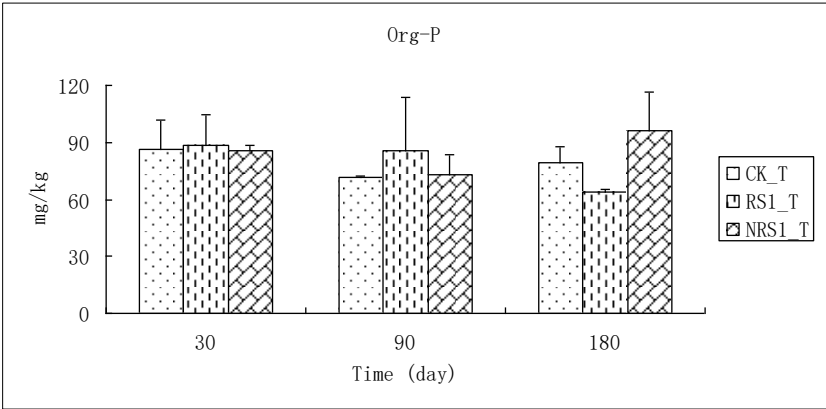
### 3.1. Effects of *P. crispus* on Phosphorus Fractions in Sediments from Tangxun Lake



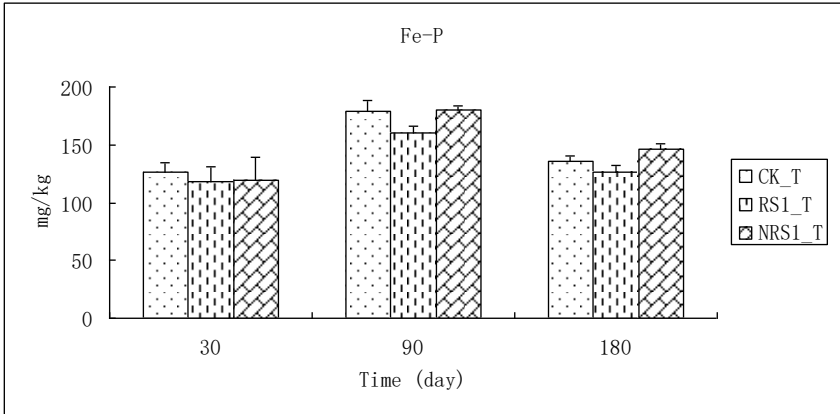
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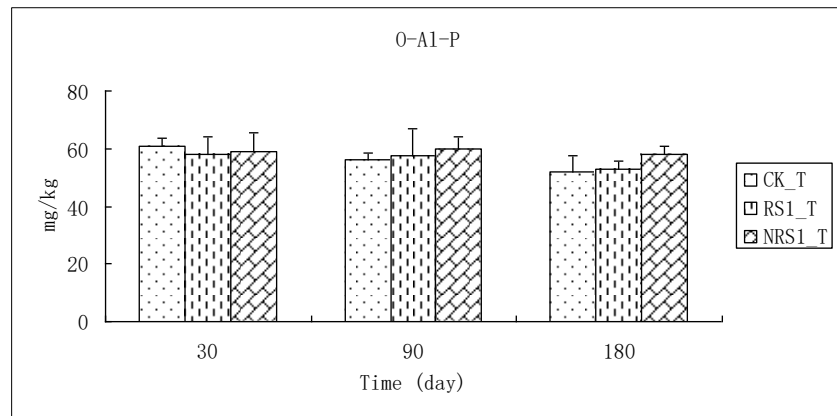
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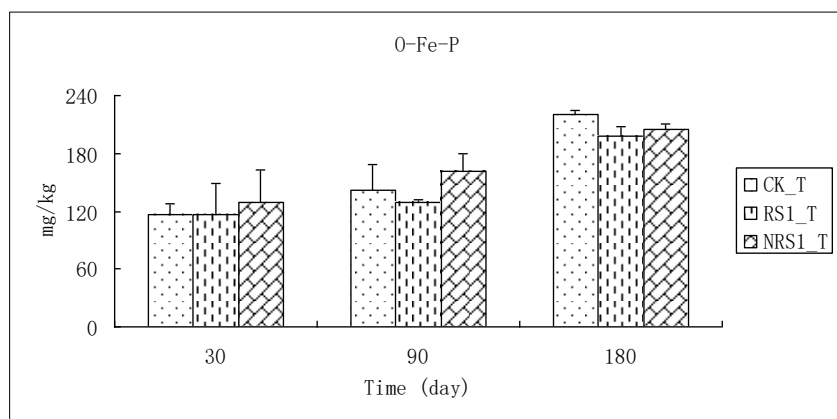
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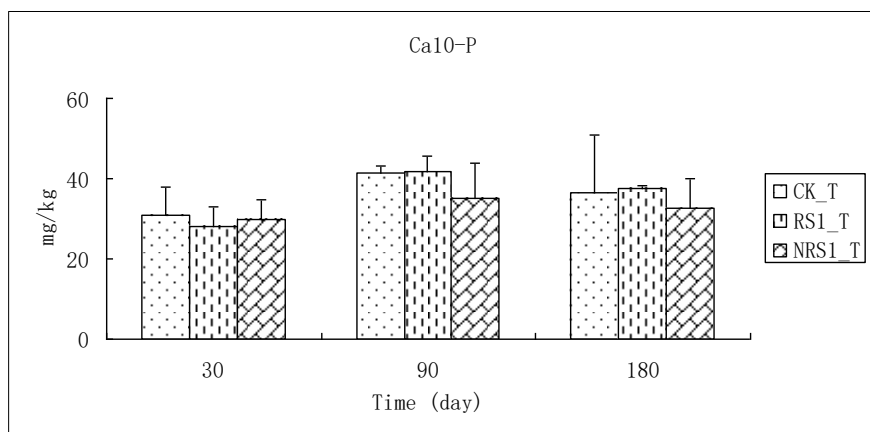
2 d



2 e



2 f



2 g

**Figure 2** Comparisons of phosphorus fractions among control sediments, rhizosphere sediments and non-rhizosphere sediments in Tangxun Lake

In the mesotrophic sediments of Tangxun Lake, *P. crispus* exhibited significant and time-dependent effects on various phosphorus fractions (Fig. 2). The concentrations of  $\text{Ca}_2\text{-P}$  in the early and mid-growth stages (30 days and 90 days), and Fe-P in the mid and later growth stages (180 days) in rhizosphere (RS) sediments were significantly lower than in non-rhizosphere (NRS) sediments ( $P < 0.05$ ), indicating enhanced uptake of labile phosphorus via root absorption. The significant decrease of Fe-P highlights the plant's capacity to regulate mobile phosphorus fractions. This finding aligns

with prior research suggesting that submerged macrophyte roots release oxygen, which oxidizes  $\text{Fe}^{2+}$  to  $\text{Fe}(\text{OH})_3$  colloids capable of binding phosphorus [9,15].

In the mid and later growth stage,  $\text{Ca}_{10}\text{-P}$  levels increased significantly in the rhizosphere, suggesting that labile phosphorus may have been stabilized into less soluble calcium-bound forms. This trend may be associated with the pH increase and  $\text{Ca}^{2+}$  accumulation near the root zone [27,28].

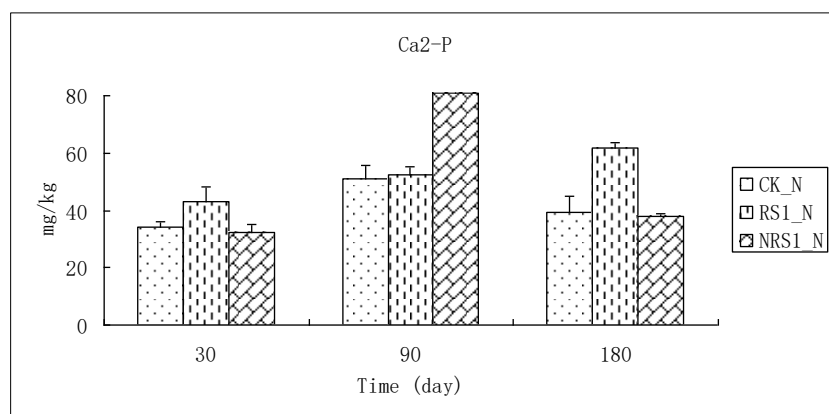
Org-P dynamics were more complex. Although no significant differences were observed in the early stage, Org-P peaked in the rhizosphere during the mid-stage and declined thereafter. This suggests that *P. crispus* might enhance the bioavailability of organic phosphorus through microbial mineralization or enzymatic activity, with time-specific effects.

In contrast, O-Al-P showed minimal differences between RS and NRS sediments across most sampling points, suggesting its stability and limited plant-mediated transformation. In comparison, Al-P concentrations were consistently lower in RS sediments during the mid and late stages, indicating that *P. crispus* may moderately affect the transformation or mobilization of Al-bound phosphorus under mesotrophic conditions.

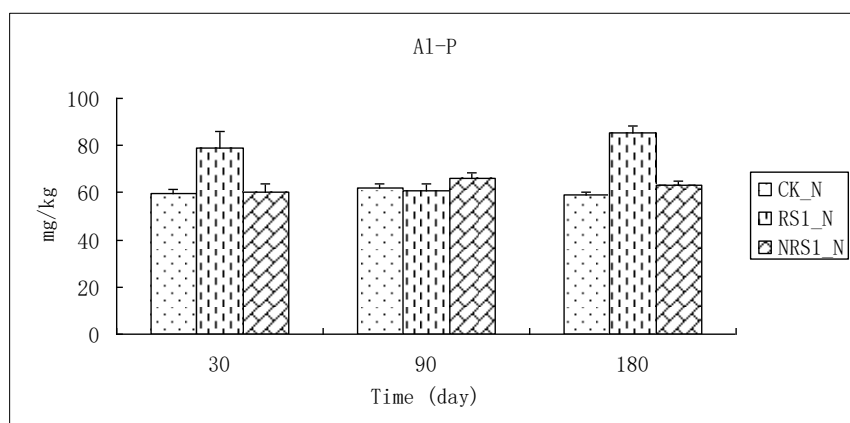
O-Fe-P consistently exhibited lower concentrations in the rhizosphere compared to the non-rhizosphere, albeit without significant differences. This trend suggests a potential inhibitory effect of rhizosphere redox conditions on the formation or release of this fraction [10].

Collectively, these results indicate that *P. crispus* in Tangxun Lake primarily modulates phosphorus transformation between labile ( $\text{Ca}_2\text{-P}$ , Fe-P) and stable ( $\text{Ca}_{10}\text{-P}$ ) fractions, thereby reducing the bioavailability of internal phosphorus under mesotrophic conditions. This finding underscores *P. crispus*' potential in the mesotrophic lake restoration.

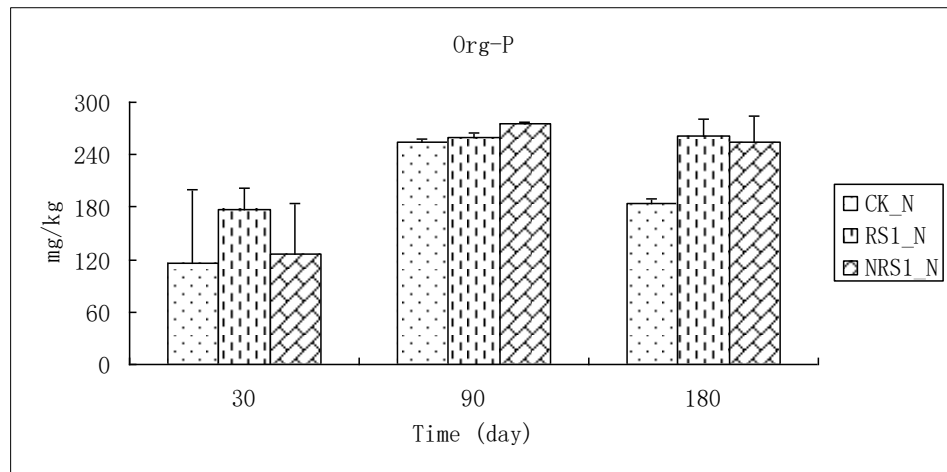
### 3.2. Effects of *Potamogeton crispus* on Phosphorus Fractions in Sediments from Nan Lake



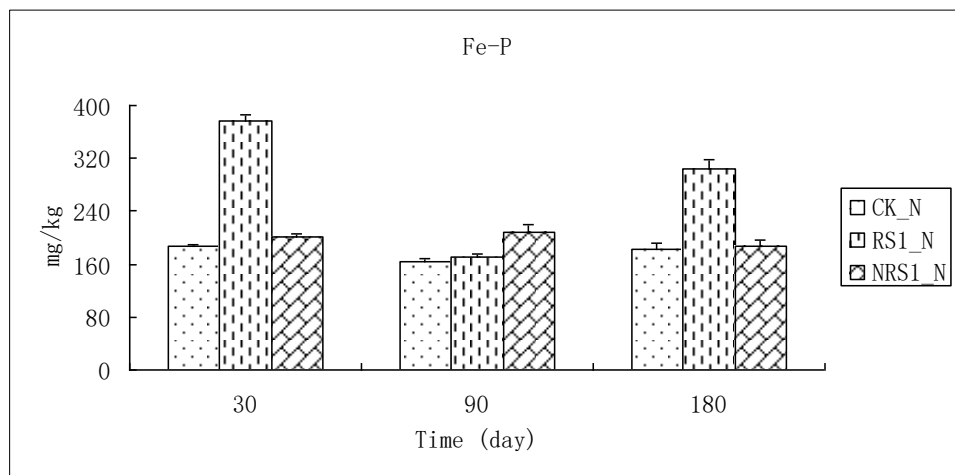
3 a



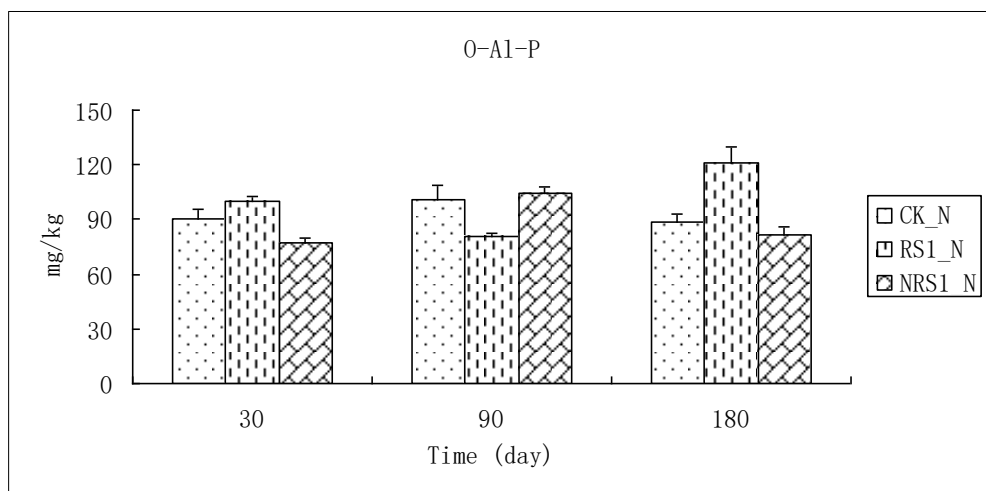
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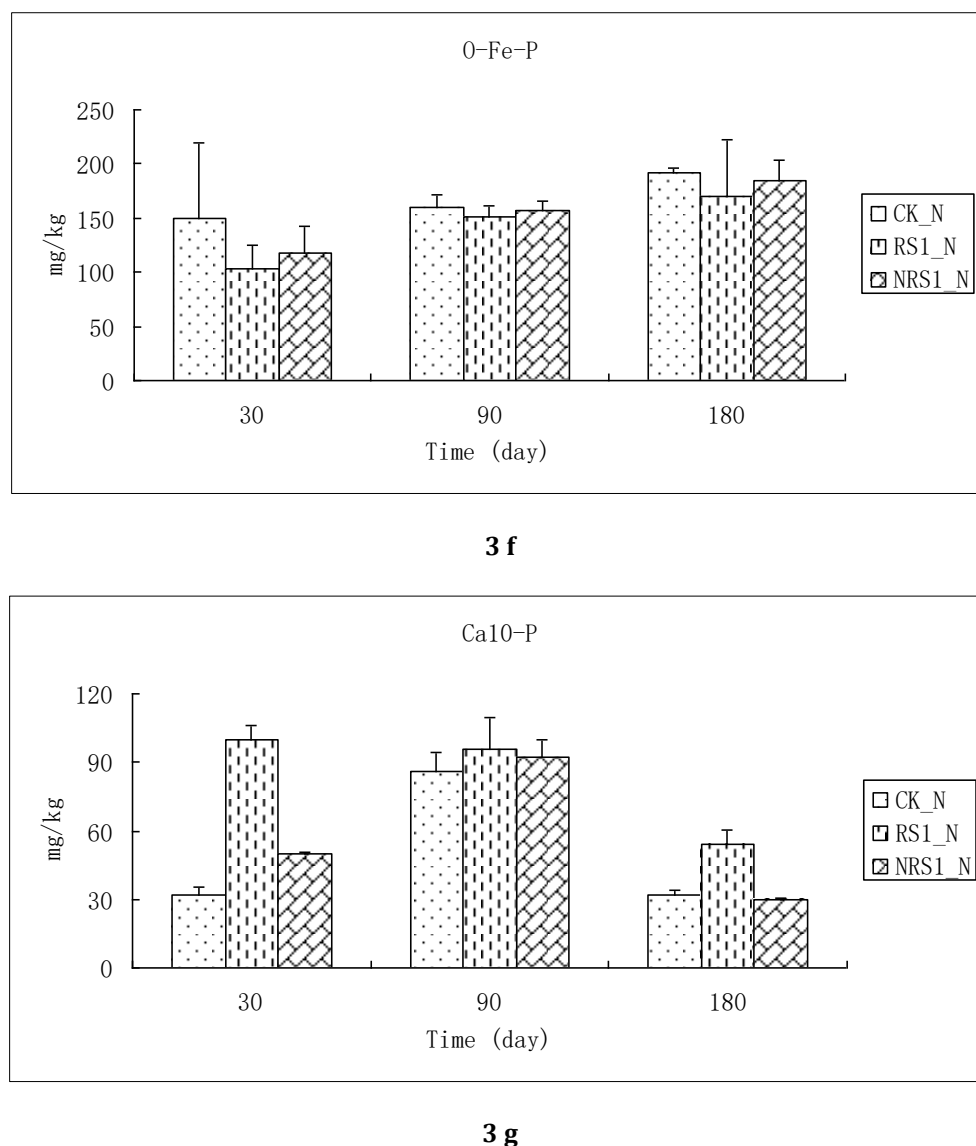


**3 d**



**3 e**





**Figure 3** Comparisons of phosphorus fractions among control sediments, rhizosphere sediments and non-rhizosphere sediments in Nan Lake

In the eutrophic sediments of Nan Lake, *P. crispus* had a more systematic and pronounced impact on phosphorus fractions (Fig. 3). During the early and later growth stages, concentrations of  $\text{Ca}_2\text{-P}$ ,  $\text{Al-P}$ ,  $\text{Org-P}$ ,  $\text{Fe-P}$ ,  $\text{O-Al-P}$ , and  $\text{Ca}_{10}\text{-P}$  in the rhizosphere were generally higher than in non-rhizosphere sediments, with most differences reaching significant or highly significant levels ( $P < 0.01$ ). These results suggest that under eutrophic conditions, *P. crispus* enhances the mobilization and retention of multiple phosphorus fractions in the rhizosphere, leading to their significant accumulation in rhizospheric sediments.

$\text{Fe-P}$  and  $\text{O-Al-P}$  were particularly noteworthy. The significant increase in  $\text{Fe-P}$  in the rhizosphere may be attributed to the release of organic acids or low-molecular-weight ligands by the roots, promoting  $\text{Fe}^{3+}$  complexation and phosphorus solubilization [16]. The increase of  $\text{O-Al-P}$  implies that even aluminum-bound phosphorus may be mobilized under eutrophic conditions due to rhizosphere disturbances.

$\text{Ca}_{10}\text{-P}$  remained consistently higher in the rhizosphere throughout the experimental period, indicating a possible stabilization of phosphorus into more recalcitrant forms. This conversion could mitigate internal phosphorus release and serve as a long-term sedimentary sink.

The dynamic changes in  $\text{Org-P}$  also reflected rhizosphere effects. The observed fluctuations of  $\text{Org-P}$  in Nan Lake sediments indicate a high degree of phosphorus turnover. The rhizosphere showed elevated  $\text{Org-P}$  levels in the early

and late stages, with a mid-stage dip relative to the non-rhizosphere. This pattern suggests that Org-P is actively involved in biogeochemical cycling in the rhizosphere, potentially undergoing mineralization or conversion into other phosphorus forms [15].

O-Fe-P levels were consistently lower in the rhizosphere, indicating that reductive rhizosphere conditions may limit the formation of this oxidized phosphorus form. This trend parallels observations from Tangxun Lake and reinforces the notion that the rhizosphere modulates redox-sensitive phosphorus dynamics.

In summary, *P. crispus* in Nan Lake established a dynamic phosphorus transformation interface, exhibiting a three-phase regulatory process: activation, uptake, and stabilization. These results emphasize the dual role of submerged macrophytes in enhancing both the bioavailability and long-term retention of phosphorus in eutrophic sediments.

#### 4. Discussion

This study demonstrates that the regulatory effects of *P. crispus* on sediment phosphorus fractions vary significantly across lakes with different trophic statuses. These variations are closely related to sediment physicochemical properties, root physiological activity, and overall nutrient conditions in the water body.

In the mesotrophic environment of Tangxun Lake, labile phosphorus forms such as Ca<sub>2</sub>-P and Fe-P were significantly reduced in rhizosphere sediments, particularly during the mid-growth stage. This suggests that *P. crispus* promotes phosphorus uptake through its root system and alters the rhizosphere environment to reduce the availability of mobile phosphorus forms [6,7,15]. The observed decrease in Fe-P content likely results from oxygen release by roots, which promotes Fe<sup>2+</sup> oxidation and subsequent formation of Fe(OH)<sub>3</sub> colloids, effectively immobilizing phosphorus [9,16].

O-Fe-P remained at lower levels in rhizosphere sediments throughout the growth period, albeit without statistical significance. This suggests that *P. crispus* may inhibit the formation of this fraction by modulating redox conditions. Given that O-Fe-P formation typically requires strong oxidation, it is less likely to accumulate under mesotrophic conditions.

Increased Ca<sub>10</sub>-P levels in the later stages may indicate stabilization of phosphorus via conversion to calcium-bound forms, potentially driven by rhizosphere pH shifts or Ca<sup>2+</sup> accumulation. This aligns with Yang (2010) [27] and Lu et al. (1999) [28], who proposed that rhizosphere activity promotes phosphorus transformation from exchangeable to recalcitrant forms.

In contrast, the eutrophic sediments of Nan Lake showed stronger and more comprehensive responses to *P. crispus* activity. Rhizosphere concentrations of Ca<sub>2</sub>-P, Fe-P, O-Al-P, and Ca<sub>10</sub>-P were significantly higher in early and later growth stages, suggesting that the plant's physiological activity—and its impact on phosphorus cycling—is intensified under nutrient-rich conditions [16,17,28].

Elevated Fe-P in the rhizosphere may result from the release of organic acids or chelators by roots, facilitating Fe-P dissolution and redistribution [16,27]. The increase in O-Al-P suggests that even relatively stable aluminum-bound phosphorus forms may be affected by root-induced biological disturbances under eutrophic conditions.

Temporal fluctuations in Org-P levels further support the role of the rhizosphere in phosphorus cycling. The decline observed during the mid-growth stage, followed by a late-stage increase, may reflect a phase of active mineralization and subsequent phosphorus re-accumulation in the rhizosphere. This pattern suggests a coupling between microbial degradation and root uptake, resulting in dynamic phosphorus turnover [15, 29].

Notably, O-Fe-P remained consistently lower in rhizosphere sediments, with a clear decreasing trend throughout the growth period. Although the differences were not statistically significant, this persistent pattern supports the idea that the rhizosphere's redox state inhibits O-Fe-P formation, limiting its potential as a long-term phosphorus sink [10,15].

#### 5. Conclusion

Through a comparative study of phosphorus fractions in rhizosphere and non-rhizosphere sediments from lakes with different nutrient statuses, this study elucidates the ecological function of *P. crispus* in regulating internal phosphorus cycling. The main conclusions are as follows:

- *P. crispus* altered sediment phosphorus dynamics in a nutrient-dependent manner. In mesotrophic Tangxun Lake, it reduced labile phosphorus (Ca<sub>2</sub>-P and Fe-P) in the rhizosphere, suggesting uptake-driven depletion and stabilization. In eutrophic Nan Lake, it promoted the accumulation of Org-P, Ca<sub>10</sub>-P, and other fractions in the rhizosphere, indicating a more active turnover and transformation process.
- Fe-P showed the most pronounced and consistent response to rhizosphere processes, followed by Ca<sub>10</sub>-P. In contrast, O-Fe-P exhibited consistently lower levels in the rhizosphere without significant differences, likely reflecting suppressed oxidation rather than active regulation.
- Org-P displayed dynamic fluctuations in eutrophic sediments, highlighting its dual role as a bioavailable phosphorus source and an intermediate in phosphorus transformation.
- Rhizosphere effects play a pivotal role in phosphorus transformation. Root-mediated processes such as oxygen release, pH modulation, and the secretion of organic acids and enzymes significantly alter the rhizosphere microenvironment, impacting phosphorus availability, mineralization, and stabilization. The rhizosphere acts as a "hotspot" for phosphorus transformation and should be a focal point in ecological restoration strategies.

These findings emphasize the potential of submerged macrophytes as bioregulators of internal phosphorus loading. Their differential responses across nutrient gradients reinforce the importance of site-specific and mechanism-driven restoration approaches targeting the sediment–plant interface. Future research should combine in situ monitoring and multi-omics approaches to explore microbial contributions to phosphorus regulation in the rhizosphere and develop integrated plant–microbe remediation frameworks.

## Compliance with ethical standards

### Acknowledgement

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### Disclosure of conflict of interest

No conflict of interest to be disclosed.

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