Effect of low-molecular-weight organic acids on phosphorus soil activation: A laboratory study of the soils from Wangbeng section of the Huaihe River Basin, China

Jinxin Zhang[1], Liangmin Gao[1]*, Zhendong Pang[2], Linghan Liu[3], Xiaoqing Chen[1], Shuo Wang[1], Hui Wang[1], Rongrong Tong[1], Chuang Shi[1], Xudong Chen[1]

[1]*School of Earth and Environment, Anhui University of Science and Technology, Huainan, Anhui, P.R. China*
[2]*Environmental Protection Monitoring Station of Huainan city, Huainan, Anhui, P.R. China*
[3]*Zhejiang Wangneng Ecological Technology Co., Ltd., Huzhou, Zhejiang, P.R. China*
*Corresponding author: gaolmin@163.com

Abstract: Farmland soil samples from the northern and southern banks of the Wangbeng section of the Huaihe River Basin, China, were collected and treated with three different low-molecular-weight organic acids (LMWOAs) (malic acid, citric acid, oxalic acid). This study aimed to determine how these acids affect soil phosphorus activation. The results showed that the average activation rate of total phosphorus, inorganic phosphorus, Fe/Al-P and Ca-P in soil samples from the southern bank treated with malic and citric acid was above 162%, except for organic phosphorus, with the highest at 192.04%. The three organic acids displayed significantly greater phosphorus activation in the northern bank soil samples than those of the southern bank. However, the overall average activation rate in the northern bank soils was lower than that of the southern bank. The four factors of phosphorus species, acid species, acid concentration, and treatment time had significant effects on phosphorus activation in the soils from both banks. This study showed that the three organic acids significantly activated inert phosphorus in the soil. Among them, malic acid and citric acid had a stronger effect on activating soil phosphorus and increased the available soil phosphorus utilisation rate.

Keywords: soil property; soil phosphorus mobility; available phosphorus; phosphorus correlation

Phosphorus (P) is an important nutrient in plants. The total phosphorus content in various farmland soils in China is 0.31–1.72 g/kg, with an average of 0.68 g/kg (Wang et al. 2018). The excessive application of phosphate fertilisers caused the accumulation of soil phosphorus and increased the soil total phosphorus content, but generally, the available phosphorus content in soils is often low (Zhang et al. 2019). Wang (2020) found that soil phosphorus fixation mainly occurs within ten days of fertilisation, with 75–90% of the applied phosphate fertiliser

applied retained in the soil (Boschetti et al. 2009, Chakraborty et al. 2011). Most phosphorus in the soil exists in the form of phosphate, although most of the soil phosphorous converts to hydroxyapatite. With only a small amount of phosphorous directly absorbed by plants (Frossard et al. 2000, Cordell and Neset 2014, Augusto et al. 2017), phosphorus deficiency is still one of the main limiting factors in plant growth.

Organic acids play a vital role in soil formation, elemental nutrient availability (Fox and Comerford 1990),

soil phosphorus activation, and reducing heavy metal toxicity in plants (Ash et al. 2017, Wu et al. 2017). There are various low-molecular-weight organic acids (LMWOAs) in soil (Javed et al. 2013), which are the result of the decomposition of animal and plant residues, root secretion (Jones et al. 2003), microorganism synthesis (Strobel 2001, Taghipour and Jalali 2013), the conversion of fertilisers and organic matter, and atmospheric deposition. Citric acid, oxalic acid, tartaric acid, and malic acid are common LMWOAs found in the rhizosphere environment of plants (Moradi et al. 2012). The content of these acids in the soil is relatively high, with the highest cumulative concentration as much as 23 mmol/L (Gerke et al. 2000, Wang et al. 2007). Zhu et al. (2021) studied the regulatory effect of LMWOAs on phosphorus availability in subalpine forest soils in the eastern Qinghai-Tibet Plateau, China. They found that the available phosphorus content in rhizosphere soil was significantly higher than that of ordinary soil, with the related organic acid being primarily citric acid. Pan et al. (2020) found that the available soil nitrogen and the available soil phosphorus were significantly positively correlated with oxalic acid and microbial biomass carbon. Zou et al. (2017) studied the effect of Chinese fir litter-sourced organic acids on soil phosphorus availability. They found that soil organic acids (mainly oxalic acid) in Chinese fir forests of different ages effectively promoted the activation of soil phosphorus. Despite these studies, there are few systematic studies on the effects of organic acid types, concentrations, and culture times on phosphorus activation in different farmland soils.

This study examined typical farmland soils from the Huaihe River Basin, China. Specifically, the soils were separately treated with three LMWOAs (malic acid, citric acid, and oxalic acid). A systematic study was then conducted on the variety, concentration, and cultivation time of the organic acids on the activation of soil phosphorus to explore the effect of LMWOAs on the activation of soil phosphorus. This study aims to provide a scientific basis for the rational application of LMWOAs to improve soil phosphorus availability to reduce the loss of soil phosphorus in agriculture.

MATERIAL AND METHODS

Overview of the study area. There are many tributaries on both sides of the Huaihe River Basin. The river's drainage area is large, making the area prone to flood disasters during flood season and an important agricultural production base in China. In the transitional zone of the north-south climate, the temperature gradually rises from north to south. The average precipitation has gradually decreased from south to north under the influence of topography. This study focused on the Wangbeng section of the Huaihe River Basin in Anhui province, China. The sampling points were concentrated near the Wangjiaba Gate, mainly involving Funan county (115°38'40''N, 32°40'18''E) and Gushi county (115°36'23''N, 32°25'3''E). Funan county has more concentrated agricultural planting with representative farmland soil, while Gushi county and Funan county are symmetrical with respect to the Huaihe River. The sampling points were selected from farmland soils in Funan county on the northern bank and farmland soils in Gushi county on the southern bank. The content of organic carbon and the content of nitrogen was determined using the soil agricultural chemical analysis method. The pH, conductivity and water content of soil were measured by pH meter (PHSJ-4F, Shanghai, China), conductivity meter (DDS-11A, Shanghai, China) and analytical balance (BS224S, Zhejiang, China). And soil granularity was tested by a laser particle analyser (Better-Size 2003).

Experimental materials. The surface soil at the sampling points was collected at a depth of 0–20 cm on November 15, 2020. Table 1 provides the basic properties of the soil samples. The average and maximum soil granularity in the south bank were 10.74 μm and 18.29 μm, respectively. In the north bank, the average soil granularity was 12.90 μm, and the maximum soil granularity was 39.59 μm. Due to the influence of fertilisation, the content of nitrogen and phosphorus in soil was higher. The initial contents of different forms of phosphorus in soil used in the experiment were described as follows: in the soil of the south bank, total phosphorus (TP) content was

Table 1. The physical and chemical properties of the soil samples

Point name	pH	Water content (%)	Conductivity (μs/cm)	Organic carbon (g/kg)	Nitrogen content (mg/kg)	Average soil granularity (μm)
North bank	5.57 ± 0.17	1.80 ± 0.50	89.63 ± 5.23	18.17 ± 0.82	3 103.12 ± 23.86	12.90 ± 0.41
South bank	7.30 ± 0.16	1.80 ± 0.17	35.93 ± 12.18	11.56 ± 0.51	1 154.55 ± 12.58	10.74 ± 0.11

1 042 mg/kg, inorganic phosphorus (IP) was 380 mg/kg, organic phosphorus (OP) was 164 mg/kg, Fe/Al-P was 221 mg/kg, Ca-P was 270 mg/kg. The contents of TP, IP, OP, Fe/Al-P and Ca-P in the soil of the north bank were 2 820, 1 084, 262, 835 and 482 mg/kg, respectively.

Three kinds of LMWOAs (malic acid, citric acid, and oxalic acid) were prepared in the laboratory using pure analytical grade drugs at concentrations of 1, 3, and 5 mmol/L.

Experimental method. The collected soil samples were spread out on air drying trays until air-dried. After air-drying, the soil samples were crushed with an agate mortar and then ground through a 0.15 mm screen, then 3.0 g of the resulting soil samples were placed into a 50 mL centrifuge tube, and 30 mL of 1, 3, and 5 mmol/L concentrations of each organic acid (malic acid, citric acid, oxalic acid) was added to attain a solid-liquid ratio of 1:10. The mixtures were shaken at 200 r/min at 25 °C for 30, 60, 90, 120, 150, 180, 210, and 240 min so that they mixed uniformly and fully reacted. After each period, the samples were centrifuged at 4 000 r/min for 10 min to separate the soil and water, then the supernatant was removed, and the residue was washed twice with distilled water. The remaining residue was quickly deposited with the help of a centrifuge. Finally, the sediment phosphorus speciation test method SMT (Ruban et al. 1999, Pei et al. 2020) was used to determine the various forms of phosphorus in the sample, including TP, IP, OP, Fe/Al-P and Ca-P. The basic principle of the method for phosphate in the soil is to use different chemical extracts to separate inorganic phosphate in various forms step by step. TP was extracted from the soil by 3.5 mol/L HCl solution after burning at 450 °C. IP was extracted with 1.0 mol/L HCl solution. For OP, the residue obtained by centrifugation in the process of IP determination was cleaned and then burned at 450 °C and extracted with 1.0 mol/L HCl solution. Fe/Al-P was extracted from NaOH solution and 3.5 mol/L HCl solution. The Ca-P was to clean the residue after extraction of NaOH in Fe/Al-P determination with 1 mol/L sodium chloride solution and then extract with 1.0 mol/L HCl solution. Three replicates were completed for each experiment.

Statistical analysis. We used SPSS 26 (Armonk, USA), Origin 2018 (Northampton, USA), and R version 4.0.3 (Auckland, New Zealand) for data processing, statistical analysis, and graph drawing. The soil

phosphorus activation change was calculated per the following formula:

$$A = M_0 - M$$

where: A – amount of phosphorus change; M – phosphorus content the soil under the action of LMWOAs; M_0 – originally measured phosphorus content of the soil. A < 0 indicates that LMWOA has a solidification effect on soil phosphorus, whereas A > 0 represents that LMWOA has an activating effect on soil phosphorus (Chen et al. 2018).

RESULTS AND DISCUSSION

The three LMWOAs selected in this study displayed significant activating abilities on phosphorus in typical soils from the northern and southern banks of the Huaihe River Basin. Malic acid and citric acid-activated soil TP, IP, OP, Fe/Al-P, and Ca-P strongly. Oxalic acid had a certain fixing effect on OP and Ca-P at low concentrations. The order of comprehensive activation capacity was: malic acid ≥ citric acid > oxalic acid. The phosphorus activation ability in the soil from the northern bank was significantly greater than that of the southern bank due to the increased amount of original phosphorus in the northern bank soil.

The effect of organic acids on the activation of soil phosphorus. Figure 1 show the average activation amount of the tested forms of phosphorus in the soil samples. The LMWOAs displayed significant differences in the activation of the various forms of phosphorus in the same soil. Malic acid had a greater ability to activate soil phosphorus than citric acid, while oxalic acid had the weakest ability. This result is mainly related to the chemical properties of each organic acid. The first dissociation constants of malic acid, citric acid, and oxalic acid are 3.4, 3.14, and 1.2. The larger the first dissociation constant, the stronger the soil activation ability. The activation rate of phosphorus in the southern bank soil samples under malic acid and citric acid was 162%, except for OP. The highest activation rate was for Ca-P in the southern bank soil under 1 mmol/L citric acid, with an activation rate of 192.04%. The activation amount in the northern bank soil was greater than that of the southern bank, but the average activation rate was lower. Lu et al. (1999) studied the activation of four LMWOAs on different types of soils. They found that oxalic acid was the strongest in calcareous soil, while tartaric acid was the weakest, but also found that there were significant differences in acidic soils, where citric acid had the strongest activation ability. Zhang et al.
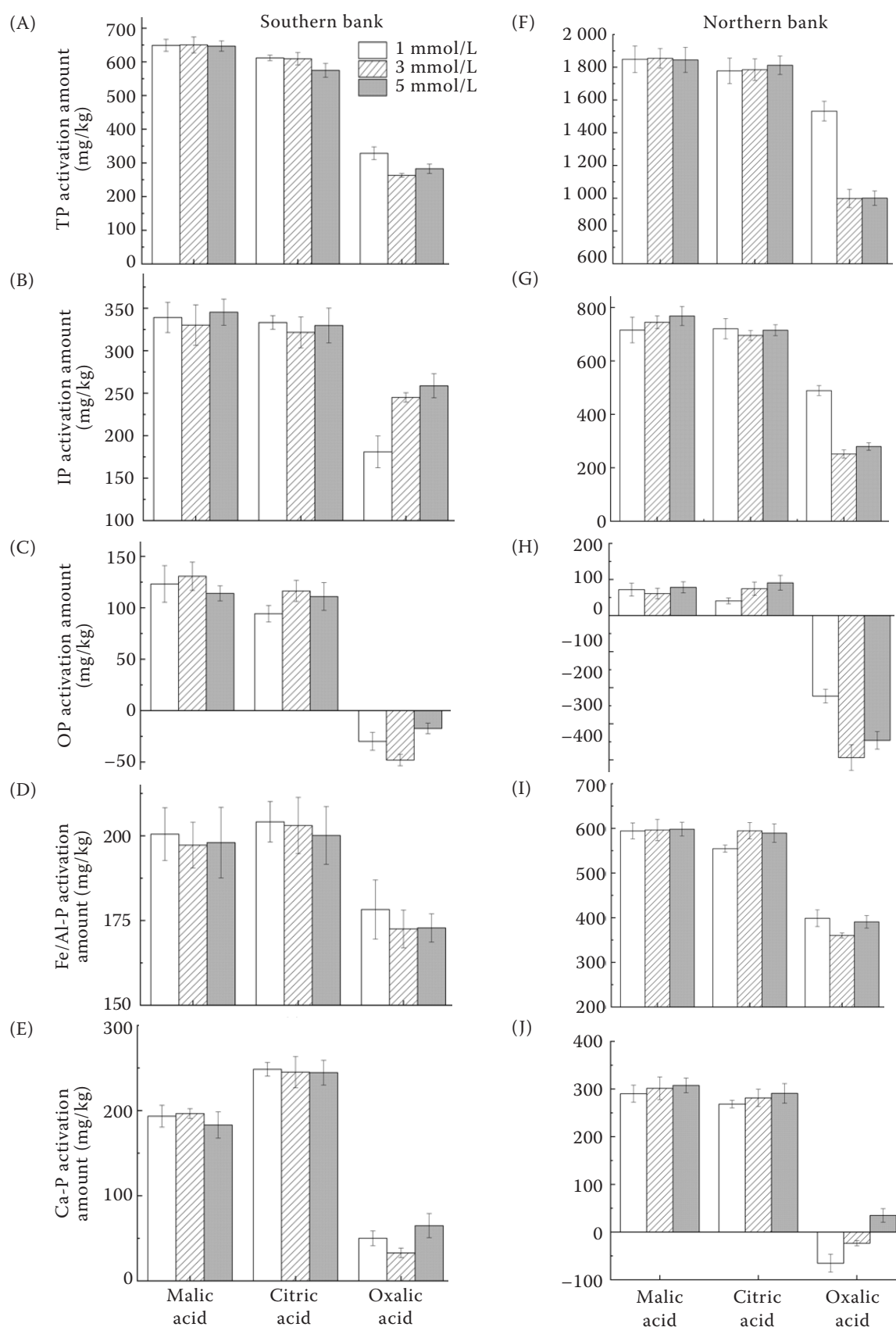
Figure 1. Effect of three organic acids on the activation of various forms of phosphorus in the southern bank and in the northern bank soil. TP – total phosphorus; IP – inorganic phosphorus; OP – organic phosphorus

(2018) found that the phosphorus release capacity of four LMWOAs to iron phosphate is oxalic acid > mixed acid > formic acid ≈ acetic acid. The contents of OP and Ca-P in the soils on the northern and southern banks increased, indicating that oxalic acid had a fixed effect on OP and Ca-P in the soil, a finding which contrasts with the effects of the other acids (Figure 1C, H, J).

We also found that the same LMWOA had a significantly different effect on the activation amount of phosphorus in the different soil samples. Under the same acid concentrations and OP and Ca-P, the activation amount of phosphorus in the northern bank soil was about three times that of the soil from the southern bank, with an activation amount ordered as malic acid ≥ citric > oxalic acid. This may be related to soil type, the distribution of various forms of phosphorus, and the nature of the soil vegetation. In addition, there are certain differences in the form and quantity of IP in the soils from each bank. The availability of phosphorus in the soil from the northern bank was higher than that from the southern bank. The organic acids were easy to resolve, and the original phosphorus content in the soil from the northern bank was greater than that of the southern bank. Thus, the organic acids could activate more phosphorus in the northern bank soil. In addition, citrate, malate, and oxalate are conducive to the formation of stable 5-bond or 6-bond ring structures with trivalent cations (such as Al^{3+} and Fe^{3+}). This explains why activated Fe/Al-P is moderately unstable phosphorus that is more firmly held on the surface of Fe and Al oxides by chemical adsorption. However, citric acid has a stronger affinity for trivalent

metal ions that dominate in acidic soils, such as Al^{3+} and Fe^{3+}, which explains why it may show a stronger activation ability than oxalic acid.

The change in acid concentration affected the overall activation of phosphorus in the soil on both banks. Increased citric acid concentrations within a certain range gradually increased the activation amount of various forms of phosphorus in the soil, with a similar change trend for all three concentrations. This is similar to the experimental results of Fang et al. (2007). They found that the activation ability of organic acids to soil phosphorus at a certain concentration increases with increasing concentrations. In contrast, oxalic acid was more conducive to the activation of soil phosphorus at low concentrations. As shown in Figure 1C, H, the fixation effect of oxalic acid on soil OP first increased and then decreased, and the conducive effect was enhanced as the acid concentration increased, but more research is needed to determine the most conducive concentration level. Soil organophosphorus is a very complex problem, many components and structures are not clear, most of the organophosphorus exist in the form of polymer, the effectiveness is not high. The activation effect of oxalic acid on acidic soil with different phosphorus fertility was weak. The inhibition of LMWOAs on P was more obvious in low and medium fertility soils with low available P content. The soil phosphorus level, especially the available phosphorus content, also affected the soil phosphorus adsorption capacity. Although different organic acids could reduce the soil phosphorus adsorption capacity, the decrease of
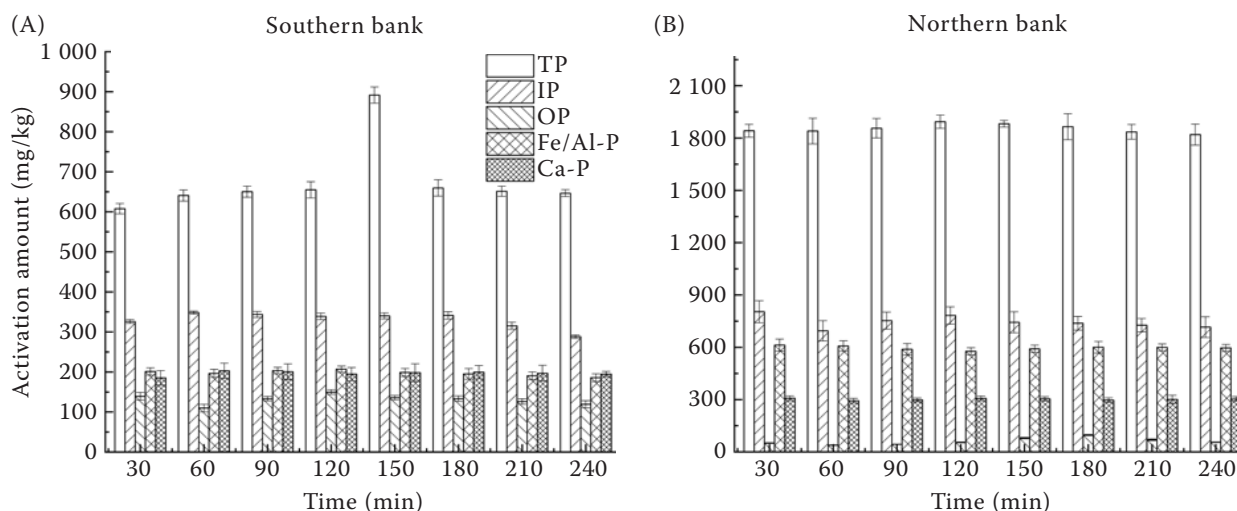

Figure 2. Changes in the activation amount of various forms of phosphorus in the soil on the northern and southern banks over time. TP – total phosphorus; IP – inorganic phosphorus; OP – organic phosphorus

the maximum phosphorus adsorption capacity was significantly affected by the soil available phosphorus content. In the oxalic acid activation experiment on soil Ca-P, it was observed that the higher the oxalic acid concentration, the stronger the activation ability. This could be due to the release of phosphorus from various calcium phosphate minerals through the formation and precipitation of calcium oxalate. The reaction between oxalates is rapid, so the release of oxalate-mediated phosphorus from the soil is also rapid. In contrast, our results showed that citric acid and malic acid were much more effective than oxalic acid at activating phosphorus. This difference is due to the effect of the phosphorus-containing minerals and metals in the soil on the reaction time rate of organic acids was unknown for our samples and may have differed. Thus, further work is needed to determine whether the determined reaction time is related to the activation of soil phosphorus.

Comparison of the activation amount of different forms of phosphorus in soils on the north and south banks. The different organic acids tested in this study had different effects on the various forms of soil phosphorus since they each had different properties. These organic acids can interact with iron, aluminium, and other oxides to reduce the adsorption of organic matter in the soil. In addition, LMWOAs have a pH of about 3, which can dissolve insoluble phosphorus-containing compounds. There are also interactions between the anions in the organic acids and metal ions, such as Fe, Al, and Ca, which activate phosphorus in the soil.

Since malic acid activated the most soil phosphorus on both banks, it was selected for further analysis. Figure 2 compares changes in phosphorus activation in soil samples from both banks, which was under 3 mmol/L malic acid at different incubation times. It shows that the phosphorus activation amount in the soil from the northern bank is greater than that of the southern bank, which may be due to a large difference in the ortginal phosphorus contents on the two banks. This finding is consistent with the analysis in the previous two sections. The three LMWOAs used in the experiment had a weak ability to activate OP in the soil, which was even fixed. The OP value in Figure 2 was still positive, which may be due to malic acid dissolving insoluble phosphorus-containing compounds and activating part of the soil phosphorus. Similarly, the three LMWOAs studied by Chen et al. (2018) solidified soil OP in Korean pine plantations and larch plantations. Except for OP, the activation amounts of TP, IP, Fe/Al-P, and Ca-P all roughly doubled compared to the original soil, indicating the significant effect LMWOAs can have on soil phosphorus. The activation of soil phosphorus by organic acids is a slow and continuous, comprehensive dynamic process, further in-depth and detailed research is needed regarding experiment duration.

Table 2. Multi-factor variance analysis of soil phosphorus species, acid species, acid concentration and treatment time on the north and south banks

Source of variation	North bank		South bank	
	type III sum of squares	P	type III sum of squares	P
Phosphorus species (i)	279 516 311.3	0.000	16 664 625.04	0.000
Acid type (ii)	24 046 683.65	0.000	3 652 113.954	0.000
Acid concentration (iii)	394 116.137	0.000	55.396	0.373
Processing time (iv)	26 731.045	0.000	6 647.017	0.000
$i \times ii$	3 442 276.531	0.000	1 940 034.280	0.000
$i \times iii$	821 661.625	0.000	98 369.658	0.000
$i \times iv$	64 816.937	0.000	41 891.245	0.000
$ii \times iii$	1 244 315.170	0.000	10 918.222	0.000
$ii \times iv$	43 686.501	0.000	38 653.499	0.000
$iii \times iv$	13 676.335	0.000	51 936.516	0.000
$i \times ii \times iii$	1 525 604.168	0.000	239 102.637	0.000
$i \times ii \times iv$	178 771.104	0.000	102 340.616	0.000
$i \times iii \times iv$	220 141.215	0.000	177 268.169	0.000
$ii \times iii \times iv$	44 969.855	0.000	86 390.438	0.000
$i \times ii \times iii \times iv$	345 814.367	0.000	254 404.294	0.000

Multivariate analysis of variance. SPSS was used to test the data for normality and homogeneity of variance. According to the Kolmogorov-Smirnov test (K-S test), the *P*-value of the significance test was greater than 0.05, indicating that the data met the normal distribution. According to the one-way ANOVA test, the median-based homogeneity of variance test showed $P > 0.05$, indicating that the data satisfied the hypothesis of homogeneity of variance.

The data satisfied all the premises and assumptions of the analysis of variance, so the analysis of variance could be performed. A multi-factor analysis of variance was performed on typical soil types of phosphorus, acid types, acid concentrations, and treatment time for both banks. Table 2 shows the results. The original hypothesis of the multi-factor analysis of variance test for typical soils on both banks was that each factor had no significant effect on activating each form of phosphorus. The chosen significance level of the test was $P < 0.05$.

It can be seen from Table 2 that the P-value of the soil acid concentration in the south bank is greater than 0.05, indicating that the acid concentration has no significant effect on the changes of phosphorus activation in various forms of typical soils in the south bank, while the *P*-value of phosphorus species, acid species and treatment time are less than 0.05, indicating that these factors have a significant impact on the changes of phosphorus activation in typical soils on the north and south banks. In addition, the *P*-value of the interaction of various factors in typical soils on the north and south banks were all less than

0.05, indicating that the interaction of these factors on the changes in the activation of various forms of phosphorus was significant. Chen et al. (2018) found that changes in the content of inorganic phosphorus components in the soil are related to factors such as inorganic phosphorus forms, organic acid types, cultivation time, acid concentration changes, and forest types. From the point of view of the sum of squared deviations of the single factor contribution, excluding the difference in the content of various phosphorus species, the sum of squared deviations of acid species is greater than the sum of squared deviations contributed by other factors, so the acid species have the most significant influence on the changes in the activation of various forms of phosphorus in typical soils on the north and south banks. When implementing measures to increase the availability of soil phosphorus, it is possible to consider adding a certain concentration of malic acid and citric acid to the soil. Changes in the amount of phosphorus activation in the soil were affected by the interaction of the different phosphorus forms, types of LMWOA, organic acid concentrations, treatment time, and soil types. In this study, the most significant factor affecting the phosphorus activation in soils from both banks was the type of organic acid, followed by the organic acid concentration and treatment time.

There is a certain correlation among various forms of phosphorus in soil, as shown in Figure 3. The correlation coefficients among TP, IP and Fe/Al-P all reach 0.97, showing a significant positive correlation. The diversity of phosphorus in the soil, the complexity of the reaction with various substances, and the self-regulation of the soil system, etc., make various forms of phosphorus have a certain degree of mutual influence and restriction. The activation of soil phosphorus by LMWOAs is a slow and continuous comprehensive dynamic process, which needs to be further studied in order to better understand the biogeochemical cycle and mechanism of phosphorus in the soil system.
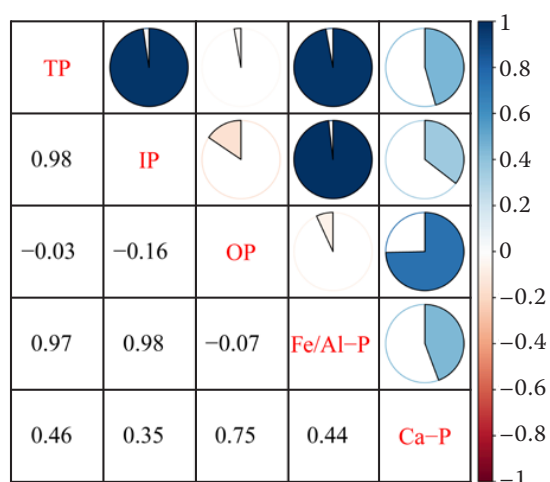
Figure 3. Correlation among various forms of phosphorus in soil. TP – total phosphorus; IP – inorganic phosphorus; OP – organic phosphorus

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