Maize//soybean intercropping promotes activation of soil phosphorus fractions by secreting more phosphatase in red soil under different phosphorus application rates

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Research Article

Keywords: Maize//soybean intercropping, P application rate, Phosphatase, soil P fractions, Red soil

Posted Date: February 21st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2579986/v1

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Version of Record: A version of this preprint was published at Plant and Soil on September 15th, 2023. See the published version at https://doi.org/10.1007/s11104-023-06252-8.
Abstract

Aims

Rational intercropping plays an important role on improving Phosphorus(P) uptake and utilization. This study aimed to investigate the effects of intercropping on the activation of soil P fractions and available P in acid soil.

Methods

The field experiments were conducted for two consecutive years to investigate the effects of maize intercropping with soybean at different P application rates (0, 60, 90, and 120 kg $P_2O_5$ ha$^{-1}$) on soil P fractions and P turnover.

Results

Compared with the monoculture maize, intercropping significantly increased maize P uptake by 43.6%~74.3% and 45.5%~76.8% in two years, while the intercropping advantage gradually weakened with the increasing of P levels. Intercropping maize promoted the activation of soil P pools, which increased labile P pools by 32.5%~38.4% and 14.4%~82.1%, while reducing non-labile P pools by 7.4%~10.9% and 6.6%~11.6% compared with monoculture maize. Meanwhile, intercropping could deplete NaOH-Po, conc. HCl-Pi, conc. HCl-Po and Residual-P fractions, and increase Resin-P, NaHCO$_3$-Pi, NaHCO$_3$-Po by 4.3%~41.2%, 21.1%~84.6% and 9.7%~98.8%, respectively. In addition, intercropping significantly increased alkaline phosphatase activity (ALP) by 21.2%~42.6% and 19.9%~28.6%, and significantly increased acid phosphatase activity (ACP) by 13.8%~27.1% and 9.5%~13.4% under different P rate. Structural equation model (SEM) showed that both ACP and ALP plays curtail role increased available P directly or indirectly through their effects on organic P turnover.

Conclusions

These result highlight the importance that intercropping maize with soybean increases soil P bioavailability by transforming organic P pools (NaOH-Po and conc. HCl-Po) into soluble phosphate (Resin-P, NaHCO$_3$-Pi and NaOH-Pi) by facilitating the secretion of phosphatase.

Introduction

Phosphorus (P) is one of the most important nutrients for crop growth and plays an important role in maintaining soil fertility and making the agricultural production systems more sustainable (Wang et al. 2015; Ferrol et al. 2019; Liao et al, 2020). However, low P availability is almost universal in most agricultural soils, as P availability is controlled by both the geochemical processes of dissolution, precipitation, and sorption, and the biological processes of mineralization and immobilization (Frossard et al. 2000; Tiessen and Moir. 2007; Hou et al. 2016). Furthermore, the rapid growth of the global food demand relies on a continuous and substantial supply of chemical P fertilizers (Nedelciu et al. 2020; Tian et al. 2021), especially in intensive agricultural systems with P-deficient soils (Chen and Graedel. 2016; Chen et al. 2018; Nesme et al. 2018). Statistics show that global consumption of P fertilizer ($P_2O_5$) consumption increased by 34.5 million tons (Mt) from 1961 to 2019.
The excessive and continuous application of P fertilizer increases soil P accumulation (Liang et al. 2017) and reduces the crop P use efficiency (PUE) to 18–20% (Umar et al. 2020; Yu et al. 2021), which also increasing the risk of soil P losses via runoff and surface water eutrophication (Xu et al. 2020a; Li et al. 2021). In addition, phosphate rock is the source of P fertilizer and a non-renewable resource, which is expected to be severely depleted within 100 years at the current rate of use (Cordell et al. 2009; Penuelas et al. 2013; Herrera et al. 2016). Therefore, improving the use of accumulated soil P and using less P fertilizer is a global challenge to achieve sustainable agriculture.

In soils, P is present in both organic and inorganic forms, and based on the P availability of the different P fractions that soil P forms have been differentiated into several pools with different availability (Tiessen and Moir 2007; Tiessen et al. 1984). Due to the large reactivity of phosphate ions with many soil constituents (e.g. Fe$^{3+}$, Al$^{3+}$, Ca$^{2+}$, etc.) strong retention of phosphorus in the soil solid phases leads to less phosphate being acquired by plants (Hinsinger 2001). To adapt to P deficiency, plants have developed many strategies to improve soil P exploration and utilization, including root foraging, root P mining and improving internal P utilization efficiency (Richardson et al. 2011). For example, improving the desorption, solubilization, or mineralization of sparingly available inorganic P (Pi) and organic P (Po) pools through rhizosphere acidification (Hinsinger 2001; Hinsinger et al. 2003; Tang et al. 2004; Shen et al. 2004; Devau et al. 2011a; Li et al. 2013; Sun et al. 2019), root exudates such as organic anions (Shen et al. 2003; Lambers. 2006; Wang et al. 2007) and phosphatases (Richardson et al. 2000; Li et al. 2004; George et al. 2008; Sun et al. 2020).

Intercropping systems, in which two or more species are grown simultaneously in the same field for at least part of their growth cycle (Trenbath 1976), have benefits for crop yield (Li et al. 2010; Gao et al. 2019), nutrient use (Li et al. 2007; Xu et al. 2020) and disease control (Zhang et al. 2019). A large numbers of evidence has confirmed that intercropped cereals and legumes promote the use of soil P resources (Li et al. 2007; Lahiri et al. 2009; Hinsinger et al. 2011a), and it has been shown that below-ground interactions for P acquisition in intercrops correspond to two main rhizosphere pathways including the direct interspecific facilitation via root-induced exudates and the indirect interspecific facilitation via the action of soil microorganisms (Hinsinger et al. 2011a; Li et al.2014; Li et al.2016). For example, intercropping with maize could increase the rhizosphere acid phosphatase activity of chickpea, and intercropping with wheat could increase rhizosphere malate and citrate concentrations of faba bean, they can hydrolyze or mineralize soil organic P and non-labile inorganic P to significantly improve rhizosphere soil P bioavailability and thus increase P uptake of associated maize and wheat (Li et al. 2004; Li et al. 2016). In addition, white lupin/wheat, groundnut/maize intercropping, and pigeon pea/sorghum intercropping have also been shown to enhance P uptake by mobilizing insoluble soil P pools (Cu et al. 2005; Ae et al. 1996), and faba bean/maize, maize/alfalfa intercropping as well as maize/chickpea intercropping could produce more phosphatase to hydrolyze soluble organic phosphorus to increase P uptake (Zhang et al. 2016; Sun et al. 2020; Li et al. 2004).

The influence of intercropping on crop P uptake and the changes in soil P fractions has been well documented (Liao et al. 2020, 2021; Yang et al. 2022). Liao et al (2020) demonstrated that sole maize and maize/faba bean intercropping depleted the low labile Po fraction, while sole faba bean depleted the labile and moderately labile Po fractions. Li et al (2008) showed that monoculture wheat decreased Resin-P, NaHCO$_3$-P and NaOH-P in its rhizosphere by 24%, 96%, and 10%, whereas NaHCO$_3$-P and NaOH-P were increased by 61% and 10%, respectively, in the rhizosphere of intercropping. In generally, the intercropping could access more available P by releasing more organic anions and phosphatases to transform P fractions into soluble P through solubilization
or mineralization insoluble inorganic P (Pi) and organic P (Po) pools (Wang et al. 2007; Sun et al., 2019; Yang et al. 2022). However, there are few studies on how intercropping changes the soil phosphorus fractions and activates these non-labile P pools in red soil.

The aim of the present study was to investigate the changes in soil phosphorus fractions and P pools in different cropping patterns (sole maize, maize intercropped with soybean) under different P application rates (0, 60, 90 and 120 kg ha\(^{-1}\)) in two consecutive crops and to explore the effect of intercropping on soil phosphorus fractions and bioavailability compared to monoculture. We hypothesized that (i) intercropping maize with soybean would have an advantage in accessing different soil P fractions; (ii) intercropping maize with soybean would alter the transformation of P between P pools to improve P bioavailability; (iii) intercropping maize with soybean would promote organic phosphorus activation by secreting more phosphatase.

Materials And Methods

Experimental design and crop management

The field trial experiment was started in 2017 in Xiaoshao experimental station (24°54′N and 102°41′E 36.93 N, 115.17E), Yunnan Province, China. The study location has a northern subtropical monsoon climate, with an annual mean temperature of 14.4 °C and an annual precipitation of 850 mm. The soil was a typical red soil with a strong capacity for phosphorus fixation. At the beginning of the study in 2017, the basic physicochemical properties of the top 20 cm soil were pH 4.53 (the ratio of water to soil was 2.5:1), organic matter 4.50 g·kg\(^{-1}\), nitrate nitrogen 2.19 mg·kg\(^{-1}\), Olsen-P 4.02 mg·kg\(^{-1}\), bulk density 1.36 mg·cm\(^{-3}\).

The field experiment was conducted with two plant patterns and four P application rates in a randomized complete block design. The main plot was a cropping system, consisting of maize monoculture (MM) and maize intercropping with soybean (IM), and the subplot was P application, including four levels: no P fertilization (P0, 0 kg ha\(^{-1}\)), low P (P60, P reduction by 50% based on conventional P, 60 kg ha\(^{-1}\)), conventional P (P90, 90 kg ha\(^{-1}\)), and high P (P120, P increase by 50% based on conventional P, 120 kg ha\(^{-1}\)). Two plant patterns and four P application rates were combined to make up eight treatments, each treatment was repeated 3 times with the plot area being 26 m\(^2\) (4 m × 6.5 m). Monoculture maize was planted with 50 cm row spacing and 25 cm row spacing, with 25 cm edge distance, the planting density is 75000 plants·ha\(^{-1}\). Intercropping maize/soybean adopted an alternative planting method (maize/soybean 2:2), where two rows of maize were replaced by two rows of soybean with the plant and row spacing of 25 cm×50 cm, respectively.

N fertilizer (46% urea) and potassium fertilizer (50% potassium sulphate) were applied at the local conventional fertilizer rates as 250 kg·ha\(^{-1}\) N and 75 kg·ha\(^{-1}\) K\(_2\)O, respectively, and P fertilizer was added following the experimental design to the corresponding plots. All phosphate fertilizers (12% single superphosphate) and potassium fertilizers (50% potassium sulphate) were applied as basal fertilizer before sowing, while nitrogen fertilizers were applied split into three doses, with 40% of total N as basal fertilizers, 25% and 35% as topdressing at the trumpet stage and top dressing at the big trumpet stage, respectively.

In 2018–2019, maize (Zea mays L, variety of ‘Yunrui 88’) was sown in June and harvested in October, and soybean (Glycine max L, variety of ‘Kaiyu-2’) was sown in March and harvested in August. The experimental plots were then left fallow until the next year’s cropping season. In the sowing, each point sowed 3–4 seeds, and
appropriate irrigation is to ensure the emergence of seedlings, manual thinning when seedling potential uniform to ensure that each empty 2 seedlings, manual tillage weeding in the planting process. After harvesting, the straw was completely removed but root residues remained in the field. During the growing season of maize and soybean, field management of all treatments except plant pattern and P application were identical throughout the growing season.

**Soil and plant sampling and measurements**

The soil samples were collected at the large-bell-mouth stage of maize (30 August in 2018 and 4 September in 2019), when soybean podding drum grain phase. As both crop growth during their peak and the interspecific interactions were strongest at ehe peak, we were able to capture the highest concentrations of compounds in the rhizosphere soil. The soil that remained firmly attached to the root surfaces of underground nodal roots and lateral roots (< 1–4 mm) was sampled as rhizosphere soil.

In all plots, three representative maize plants were randomly sampled to obtain rhizosphere soil (including monoculture and intercropping). The three soil samples were mixed then sieved to < 2 mm to remove visible roots, and divided into two parts. One sub-sample was immediately transported to the laboratory and stored at -20°C for the analysis of phosphatase activity (acid phosphatase and alkaline phosphatase), and the other sub-sample was air-dried powder to pass through a 1 mm nylon sieve for measurement of soil Olsen-P and P fractions.

The soil Olsen-P was extracted based on air-dry soil with 0.5 mol L$^{-1}$ NaHCO$_3$ at pH 8.5, and then determined by the ascorbic acid–molybdophosphate blue method (Olsen 1982). P fractions by sequential fractionation as proposed by Hedley et al (Hedley et al. 1982) and modified by Tiessen and Moir (Tissen and Moir. 1993). The sequential extraction steps of soil P fraction and the soil organic P determination method are shown in Table 1. The extractant of P fraction was used for a specific soil P fraction was added to 0.5 g of soil in the following sequential order: anion exchange resin (Resin-P) and 0.5 M NaHCO$_3$ (NaHCO$_3$-Pi and NaHCO$_3$-Po), which represent labile P pools; 0.1 M NaOH (NaOH-Pi and NaOH-Po) and 1.0 M HCl (1 M HCl-Pi), which represent moderately-labile P pools; and concentrated HCl (conc. HCl-Pi and conc. HCl-Po) and concentrated H$_2$SO$_4$–H$_2$O$_2$ (Residual-P), which represent non-labile P pools. At each step, the suspension was stirred on a shaker (200 rpm) for 16 h, centrifuged (10 min at 25,000×g), and the supernatant was then passed through a 0.45-µm membrane filter afterward and stored before colorimetric analysis, whereby the inorganic P was determined according to Murphy and Riley (1962). Ammonium persulfate digestion was used to determine the total P concentration in the different extracts (NaHCO$_3$-P, NaOH-P, and concentrated HCl-P) and organic P concentrations were calculated as the difference between total P and inorganic P (Liao et al. 2020).
Table 1 Sequential P fractions based on the method by Hedley and modified by Tiessen and Moir

<table>
<thead>
<tr>
<th>Step</th>
<th>P Fraction</th>
<th>Extarctant</th>
<th>P pools</th>
<th>Shaking Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Resin-P</td>
<td>A strip of resin + deionized water</td>
<td>Labile P pools</td>
<td>16 h</td>
</tr>
<tr>
<td>2a</td>
<td>NaHCO₃-P(Pi, Po)</td>
<td>0.5 M NaHCO₃ (pH:8.5)</td>
<td>Moderately-labile P pools</td>
<td>16 h</td>
</tr>
<tr>
<td>3a</td>
<td>NaOH-P(Pi, Po)</td>
<td>0.1 M NaOH</td>
<td>Non-labile P pools</td>
<td>16 h</td>
</tr>
<tr>
<td>4</td>
<td>1M HCl-Pi</td>
<td>1M HCl</td>
<td></td>
<td>16 h</td>
</tr>
<tr>
<td>5a</td>
<td>conc. HCl-P(Pi, Po)</td>
<td>concentrated HCl</td>
<td></td>
<td>16 h</td>
</tr>
<tr>
<td>6</td>
<td>Residual-P</td>
<td>concentrated H₂SO₄</td>
<td></td>
<td>16 h</td>
</tr>
</tbody>
</table>

Notes: a: Organic P concentrations were calculated as the difference between total P and inorganic P. The total P concentration in the different extracts (NaHCO₃-P, NaOH-P, and concentrated HCl-P) was determined by ammonium persulfate digestion.

Soil acid and alkaline phosphatase (ACP and ALP) activities were determined by measuring the release of p-nitrophenol by incubating fresh soil (Tabatabai 1994). 1 g fresh soil was added to 0.2 mL toluene, 4 mL buffer, and 1 mL p-nitrophenyl phosphate disodium solution before thoroughly mixing and incubating for 1 h at 37 °C. Subsequently, 0.5 M CaCl₂ 1 mL and 0.5 M NaOH 4 mL were added and the enzyme activity was measured using a colorimetric method (410 nm) as described by Tabatabai (1994). ACP and ALP activities were measured in pH 6.5 and 11.0 buffers, respectively. Enzyme activity was expressed as micrograms of p-nitrophenol produced per gram of dry soil (µg pNPP g⁻¹ fresh soil h⁻¹).

At the crop physiological maturity of the crop each year, the central rows of each experimental block of maize (monoculture and intercropping) were manually harvested and cut at 10 mm above the soil surface, and the sampled plants were separated into maize grain and stalk after drying. All samples were oven-dried at 65°C for 72 h and weighed. The above-ground oven-dried four representative maize plant samples were mixed, ground and passed through a 0.15 mm nylon sieve for plant P analysis. P concentration was measured by the molybdovanadophosphate method (Johnson and Ulrich 1959) after digestion of the plant sample in a mixture of concentrated H₂SO₄ and H₂O₂. Plant P uptake was calculated as the sum of the P uptake by different organs, which was measured as P concentration multiplied by plant dry matter. Soil P balances were calculated as the difference between P inputs and outputs in a natural growing season. P input was estimated annually from P fertilizer application and P out was the P uptake at the harvest of the crop (excluding roots).

Statistics

Statistical analyses were performed using the IBM-SPSS, version 24.0 (SPSS Inc.). One-way ANOVA analysis was performed to identify the significant differences between all treatments, and three-way ANOVA analysis was performed to identify the significant effects of year, plant patterns and P application rates on P fractions. Pearson correlation was employed to simulate the relationship between the soil P fractions and available P and P activation coefficient (PAC). Structural equation modeling (SEM) was run by Amos 24.0 to reveal the causal
relationship between soil phosphatase activities and Olsen-P through the transformation of organic phosphorus (P) fractions (Han et al. 2018).

Results

Effect of intercropping on P uptake and P balance under different P application rates

Two years of field localization experiments showed that intercropping maize with soybean significantly increased crop P uptake and decreased P balance under different P application rates (Fig. 1). Compared with monoculture maize, intercropping enhanced maize P uptake by 43.6%–74.3% and 45.5%–76.8% under different P application treatments in 2018 and 2019, respectively. Regardless of intercropping and monoculture, crop P uptake initially increased and then decreased with the increasing of P rate, with P90 had the highest P uptake.

Although intercropping could reduce the P balance by 17.1%–33.4% and 19.9%–32.4% for all P application treatments compared to monoculture maize and P120 treatment had the highest P balance in 2018 and 2019, respectively, but the P balance was gradually increased with the increase of P application rate and the intercropping advantage was gradually weakened with the increase of P application rate (Fig. 1).

Effect of intercropping on soil P fractions under different P application rates

Intercropping significantly affected the content and proportion of inorganic and organic P (Fig. 2). The inorganic P was the main P source (sum of Resin-P, NaHCO$_3$-Pi, NaOH-Pi, 1 M HCl-P, conc. HCl-Pi and Residual-P) with the greater proportion of total P, ranging from 57.7–75.3% and 59.4–76.6%, the proportion of organic P (NaHCO$_3$-Po, NaOH-Po, and conc. HCl-Po) ranging from 24.7–42.3% and 23.4–40.6%, respectively.

Regardless of intercropping or monoculture, the proportion of inorganic P had an increasing trend while the proportion of organic P had a reducing trend with the increased of P application rate for two consecutive seasons. P application rate significantly increased the content of inorganic P by 23.6–65.8% and 58.2–97.9% in 2018 and 2019, respectively, while reduced the content of organic P by 4.4–28.8% and 8.9–13.6% (except P60 treatment in 2019).

Overall, intercropping reduced the content of inorganic P by 1.8% and 2.8%, and reduced the content of organic P by 2.9% and 3.9% compared with monoculture in 2018 and 2019, respectively. Compared to monoculture, intercropping could significantly reduce the proportion of organic P and increase the proportion of inorganic P in P60 (Fig. 2), and intercropping significantly decreased the content of organic P by 7.4% compared with monoculture at P60 in 2019.

According to the difference in available P activity in the all phosphorus fractions, we divided all 9 phosphorus fractions into 3 phosphorus pools including labile P pools, moderately-labile P pools and non-labile P pools. In two consecutive growing seasons, non-labile P pools (sum of conc. HCl-Pi, conc. HCl-Po, and Residual-P) represented the largest proportion of total P in all treatments, ranging from 60.2–69.8% and 56.7–68.9% (in 2018
and 2019), followed by the moderately-labile P pools (sum of NaOH-Pi, NaOH-Po and 1 M HCl-P), which changed slightly from 26.2–26.9% and 26.1–30.0%, and the labile-P pools (sum of Resin-P, NaHCO$_3$-Pi, and NaHCO$_3$-Po), which represented only 3.6–10.9% and 3.6–15.2% (Fig. 3).

Intercropping greatly affected the content of P pools and the proportion of these pools to the total P under different P application rates (Fig. 3). For all P application rates, intercropping increased labile P pools by 35.7% and 37.5% on average, and moderately-labile P pools by 4.4% and 2.9%, while reducing non-labile P pools by 8.6% and 10.0% in 2018 and 2019, respectively. Compared with monoculture maize, intercropping maize significantly increased labile P by 32.5%–38.4% and 14.4%–82.1%, while significantly reduced non-labile P by 7.4%–10.9% and 6.6%–11.6% under different P application rates in 2018 and 2019, respectively.

With the increased P application rate, the proportion of labile P pools was significantly increased while the proportion of non-labile P pools was significantly reduced and no obvious effect to moderately-labile P pools. Intercropping on a whole reduced the proportion of non-labile P pools while increasing the proportion of labile P pools and moderately-labile P pools (Fig. 3).

Over two consecutive growing seasons, intercropping significantly affected soil P fractions under different P application rates (Fig. 4). P application caused a larger Resin-P, NaHCO$_3$-Pi, NaHCO$_3$-Po, NaOH-Pi, 1 M HCl-Pi, conc. HCl-Pi and Residual-P fractions, while smaller NaOH-Po and conc. HCl-Po fractions regardless of monoculture or intercropping.

Intercropping maize increases the content of Resin-P, NaHCO$_3$-Pi, NaHCO$_3$-Po, NaOH-Pi, and 1 M HCl-Pi fractions by 19.6%, 55.7%, 31.1%, 13.8% and 94.3% on average, while it reduces the content of NaOH-Po, conc. HCl-Pi, conc. HCl-Po and residual-P fractions by 2.5%, 9.5%, 4.3% and 10.1%, respectively, compared to monoculture maize under different P application rates.

In the P0 treatment, intercropping maize significantly increased NaOH-Pi by 8.6 mg kg$^{-1}$ while decrease NaOH-Po, conc. HCl-Pi, conc. HCl-Po and Residual-P by 6.36 mg kg$^{-1}$, 7.77 mg kg$^{-1}$, 12.62 mg kg$^{-1}$, and 18.24 mg kg$^{-1}$ in 2018, and increased Resin-P, NaHCO$_3$-Po, NaOH-Pi, 1M HCl-Pi by 0.23 mg kg$^{-1}$, 11.10 mg kg$^{-1}$, 11.07 mg kg$^{-1}$, and 2.04 mg kg$^{-1}$ and decreased NaOH-Po, conc. HCl-Po, Residual-P by 6.88 mg kg$^{-1}$, 11.84 mg kg$^{-1}$, and 20.12 mg kg$^{-1}$ in 2019, respectively, compared to monoculture maize.

In the P60 treatment, increase maize increased NaHCO$_3$-Pi, NaOH-Pi, and 1M HCl-Pi by 9.04 mg kg$^{-1}$, 10.76 mg kg$^{-1}$, and 6.38 mg kg$^{-1}$ while decreased NaOH-Po, conc. HCl-Pi and Residual-P by 11.97 mg kg$^{-1}$, 10.52 mg kg$^{-1}$, and 14.43 mg kg$^{-1}$ in 2018, and increased NaHCO$_3$-Pi, NaHCO$_3$-Po, NaOH-Pi, and 1M HCl-Pi by 6.17 mg kg$^{-1}$, 6.67 mg kg$^{-1}$, 10.37 mg kg$^{-1}$, and 7.91 mg kg$^{-1}$ while decreased conc. HCl-Pi and conc. HCl-Po by 29.68 mg kg$^{-1}$ and 11.96 mg kg$^{-1}$ in 2019, compared with monoculture, respectively.

In the P90 treatment, intercropping maize significantly increased NaHCO$_3$-Pi, NaOH-Pi, and 1 M HCl-Pi by 9.66 mg kg$^{-1}$, 6.81 mg kg$^{-1}$ and 6.46 mg kg$^{-1}$ while decreased NaOH-Po and conc. HCl-Pi by 8.29 mg kg$^{-1}$ and 13.05 mg kg$^{-1}$ in 2018, and increased NaHCO$_3$-Pi and 1M HCl-Pi by 6.50 mg kg$^{-1}$ and 3.36 mg kg$^{-1}$ while decreased conc. HCl-Pi and Residual-P by 30.38 mg kg$^{-1}$ and 7.20 mg kg$^{-1}$ in 2019, compared with monoculture maize, respectively.
In the P120 treatment, intercropping maize significantly increased NaHCO$_3$-Pi, NaHCO$_3$-Po and NaOH-Pi by 7.64 mg kg$^{-1}$, 11.30 mg kg$^{-1}$ and 23.13 mg kg$^{-1}$ while reduced 1M HCl-Pi, conc. HCl-Pi and Residual-P by 1.56 mg kg$^{-1}$, 21.38 mg kg$^{-1}$ and 14.13 mg kg$^{-1}$ in 2018, and increased NaHCO$_3$-Pi, NaHCO$_3$-Po and NaOH-Pi by 12.24 mg kg$^{-1}$, 9.82 mg kg$^{-1}$ and 8.29 mg kg$^{-1}$ while reduced NaOH-Po, conc. HCl-Pi by 10.79 mg kg$^{-1}$ and 25.71 mg kg$^{-1}$ in 2019, compared with monoculture, respectively.

Year significantly affected all P fractions except NaOH-Po and Residual-P, P rate and plant pattern both significantly affected all P fractions, and the interaction of the plant pattern and P rate were affected all fractions except Resin-P, NaCO$_3$-Po, and NaOH-Po, and the interaction of year and P rate affected all P fractions except conc.HCl-Po, while the interaction of year and plant pattern affected only 1 M HCl-Pi and conc.HCl-Pi, and the interaction of year, cropping system, and P rate affected only NaOH-Pi (Table 2).

<table>
<thead>
<tr>
<th>Factors</th>
<th>Df</th>
<th>Resin-P</th>
<th>NaHCO$_3$-Pi</th>
<th>NaHCO$_3$-Po</th>
<th>NaOH-Pi</th>
<th>NaOH-Po</th>
<th>1M HCl-Pi</th>
<th>conc. HCl-Pi</th>
<th>conc. HCl-Po</th>
<th>Residual-P</th>
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<td>Plant pattern(PP)</td>
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<td>P rate(P)</td>
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<td>Y×PP</td>
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<td>PP×P</td>
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<td>Y×PP×P</td>
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</table>

Note: Df, degrees of freedom; ns, no significant difference * $P<0.05$, ** $P<0.01$, *** $P<0.001$

The changes in P fractions between intercropping and monoculture (including 4 P rates) revealed that intercropping could deplete more non-labile P pool (including NaOH-Po) to increase crop P uptake and reduce P balance (Fig. 1, Fig. 6). Covering all P application treatments in two consecutive years, intercropping depleted NaOH-Po (3.39 ~ 11.97 mg kg$^{-1}$), conc. HCl-Pi (3.72 ~ 30.38 mg kg$^{-1}$), conc. HCl-Po (4.89 ~ 12.62 mg kg$^{-1}$ in P0, P60 and P90) and Residual-P (2.13 ~ 20.12 mg kg$^{-1}$), but obviously increased Resin-P (0.11 ~ 0.33 mg kg$^{-1}$), NaHCO$_3$-Pi (0.98 ~ 12.24 mg kg$^{-1}$), NaHCO$_3$-Po (2.80 ~ 11.30 mg kg$^{-1}$), NaOH-Pi (5.37 ~ 23.13 mg kg$^{-1}$) and 1 M HCl-Pi (1.22 ~ 7.91 mg kg$^{-1}$) compared with monoculture. The changes in each P fraction mentioned above were significantly affected by the P rate and plant pattern independently, and the interactions between the P rate and plant pattern were significantly affected NaHCO$_3$-Pi, NaOH-Pi, 1 M HCl-P, conc.HCl-Pi, conc.HCl-Po and Residual-P (Table 2).
Effect of intercropping on soil available P content and P activation coefficient under different P application rates

Soil available P (including Olsen-P, Resin-P and NaHCO$_3$-Pi) and P activation coefficient (PAC) in the rhizosphere soil were significantly affected by P application rate and/or plant pattern (Fig. 6). P application (P60, P90, and P120) significantly increased Olsen-P by 1.8 ~ 3.0 times and 1.5 ~ 2.5 times, respectively, in 2018 and 2019, compared with P0 treatment, and P application also increased Resin-P by 1.7 ~ 4.4 and 1.3 ~ 3.2 times related to no P application, as well as increased NaHCO$_3$-Pi by 5.4 ~ 15.8 and 3.1 ~ 7.3 times, respectively. In addition, the P application increased the PAC by 1.4 ~ 2.3 times and 0.8 ~ 1.3 times in 2018 and 2019, respectively.

Intercropping had an advantage for increasing soil available P and P activation coefficient, increasing Olsen-P by 12.5% and 15.4%, increasing Resin-P by 19.6% and 16.9%, increasing NaHCO$_3$-Pi by 55.7% and 33.7%, and increasing PAC by 11.3% and 15.3% at all P application rates relate to monoculture in 2018 and 2019, respectively. Compared with monoculture, intercropping maize significantly increased Olsen-P and Resin-P by 25.9% and 41.2% at P60 in 2019. Otherwise, intercropping significantly increased soil NaHCO$_3$-Pi by 84.6%, 52.4%, 27.3% and 35.8%, 21.1%, 35.4% in 2018 and 2019, respectively, compared with monoculture maize (at P60, P90, and P120 treatments), respectively (Fig. 6). The intercropping advantage was gradually weakened with the increasing of P application rate.

Effect of intercropping on rhizosphere soil ALP and ACP under different P application rates

This study also found that intercropping significantly affected the ACP and ALP under different P application rate (Fig. 7). Compared with monoculture, intercropping maize increased ALP by 21.2%~42.6% and 19.9%~28.6%, respectively, and the differences were significant for all P application rates except P0 over two years. Meanwhile, intercropping also significantly increased the ACP by 13.8%~27.1% and 9.5%~13.4% than monoculture under different P application rates. Regardless of monoculture or intercropping, the ALP gradually decreases while the ACP was first to increase then decreased with the increase of P application, as well as the intercropping advantage gradually weakened.

Relationship between soil P fractions with available P and PAC

The correlations between the P fractions and available P and PAC were demonstrated through a heat map analysis (Fig. 8). Irrespective of monoculture or intercropping maize, available P and PAC were all significantly positively related to NaHCO$_3$-Po, NaOH-Pi, 1M HCl-P, conc.HCl-Pi, and Residual-P fractions, and Labile P, Moderately-labile P, Non-labile P, and inorganic P pools ($p < 0.05$, $p < 0.01$), while significantly negatively related to NaOH-Po and conc. HCl-Pi fractions and organic P pools (except PAC, which was not significantly related to conc.HCl-Po in intercropping maize). Thus, we think that organic P pools (including NaOH-Po and conc. HCl-Po) could be transformed into inorganic P pools to become available P.

Regulation of intercropping on soil phosphorus pools
We constructed a structural equation model (SEM) to explore the influence of intercropping on the increase in available P via regular acid phosphatase activity (ACP), and alkaline phosphatase activity (ALP) (Fig. 9). The most parsimonious model explained 91% (monoculture maize) and 85% (intercropping maize) of the variance in available P. Both ACP and ALP affected Olsen-P directly or indirectly through their effects on organic P turnover. In detail, regardless of intercropping or monoculture maize, ACP showed a significant positive impact on NaHCO$_3$-Po to increase Olsen-P, and a significant negative impact on conc.HCl-Po transformation NaOH-Po or NaHCO$_3$-Po into Olsen-P, while ALP mainly through significantly negative effect on NaOH-Po or NaHCO$_3$-Po transformation into available P.

Although ACP had a greater influence on NaHCO$_3$-Po in monoculture maize ($p = 0.58^{***}$) than intercropping maize ($p = 0.36^{**}$), the influence was sample NaHCO$_3$-Po turnover into Olsen-P in monoculture and intercropping. While ACP had a greater effect on conc.HCl-Po in intercropping ($p=-0.63^{***}$) than in monoculture ($p=-0.53^{**}$). Furthermore, we found a significant negative relationship between ALP and NaHCO$_3$-Po only in intercropping maize.

**Discussions**

**Effect of intercropping on P uptake and P balance**

Crop P uptake and farmland soil P balance could be used as an important basis for the evaluation of fertilization technical measures after P application (Yan et al. 2020). Under the condition of no phosphorus application, soil phosphorus is in a long-term deficit state, while when the P uptake by crops is lower than P fertilizer application, there is P surplus and accumulation in the soil or loss (Bai et al. 2013; Liao et al. 2021). Phosphorus carried out by crops is the main expenditure of soil phosphorus, and the more phosphorus is uptake, the less phosphorus accumulation. Our study showed that maize intercropping with soybean had an advantage in crop P uptake, and could decrease soil apparent P balance (Fig. 1). Similar results were found that intercropping maize with faba bean could increase crop P uptake and reduce P balance (Liao et al. 2020, 2021). Furthermore, early research has confirmed that intercropping maize with soybean could increase maize P uptake and soil P availability as well as phosphatase activity (Wang et al. 2017; Yang et al. 2022). However, with the increase of P application rate, the crop P uptake first increased and then decreased, and the soil apparent P balance was always gradually increased, the advantages of intercropping were gradually weakened. This suggests that maize intercropping with soybean could get a greater advantage in increasing P uptake and reducing P balance when the reasonable application of phosphorus fertilizer.

**Effect of intercropping on P bioavailability and P fractions**

Soil P is mainly present in insoluble forms that are poorly available for crop uptake, thus P bioavailability is especially critical in the red soil due to low P bioavailability (Duchene et al. 2017). It is well documented that different soil P fractions and bioavailability can be affected by different soil mechanisms, such as fertilization and intercropping (Maharjan et al. 2018; Liao et al. 2020; Mahmood et al. 2020, 2021). Different P fractions and P pools in soils exist in complex equilibria and their concentration in soil solution depends on the balance between P mobilization and P uptake by plants (Ye et al. 2015; Yan et al. 2020). In this study, P application
significantly increased soil bioavailable P (including soil Olsen-P, Resin-P, NaHCO$_3$-Pi), and intercropping maize with soybean could enhance soil available P by increasing Olsen-P by 12.5% and 15.4%, increasing Resin-P by 19.6% and 16.9%, increasing NaHCO$_3$-Pi by 55.7% and 33.7% for all P application rate over two consecutive growing seasons than the monoculture maize, especially the P60 treatment showed a significant difference (Fig. 6). Meanwhile, the P activation coefficient (PAC) as a crucial indicator of soil P availability related to the ratio of available P to total P (Wu et al., 2017) that intercropping also showed a higher value than monoculture. Intercropping resulted in higher available P and achieved greater P uptake, indicating that the intercropping was able to efficiently access more P as compared to monoculture (Liao et al. 2021). This may be because intercropping with soybean could promote phosphorus activation from insoluble P pools to enhance maize P uptake (Mayakaduwage et al. 2020; Wang et al. 2017).

The continuous P fertilization applied to the soil was distributed into different pools and caused a large accumulation of Resin-P, NaHCO$_3$-Pi, NaHCO$_3$-Po, NaOH-Pi, 1 M HCl-Pi, conc. HCl-Pi and Residual-P fractions, except NaOH-Po and conc. HCl-Po fractions (Yan et al. 2020; Mahmood et al. 2021). The maize intercropping with soybean showed a higher content of Resin-P, NaHCO$_3$-Pi, NaHCO$_3$-Po, NaOH-Pi, and 1 M HCl-Pi fractions, while a lower content of NaOH-Po, conc. HCl-Pi, conc. HCl-Po and Residual-P fractions compared with monoculture maize in two years (Fig. 4 and Fig. 5). From the above, it is clear that intercropping increased the available P fractions and decreased the insoluble P fractions (conc. HCl-Pi) and mainly organic P fractions (NaOH-Po and conc. HCl-Po). This may be that in maize/soybean system facilitates the P turnover between different P fractions, and interspecific interactions facilitate maize and soybean crop to raise the effective P concentration by secreting organic acids or phosphatase to mobilize and hydroponic insoluble forms of P (Li et al. 2007; Wang et al. 2017). And the increase of available P in the rhizosphere soil of intercropping maize may be due to that root exudate secretion and soil P mobilization co-occur with the depletion of P in the rhizosphere, and can counteract the uptake-driven depletion (Sun et al. 2019; Yang et al. 2022).

The soil P pool was an important indicator to divide the different characteristics or activity P fractions into different P pools. In this study, the non-labile P pools (sum of conc. HCl-Pi, conc. HCl-Po, and Residual-P) had the highest content and proportion of total P; followed by moderately-labile P pools (sum of NaOH-Pi, NaOH-Po and 1 M HCl-IP), while the labile-P pools (sum of Resin-P, NaHCO$_3$-Pi, and NaHCO$_3$-Po) were the lowest (Fig. 3). Continuous application of P fertilizer not only increases the labile-P content (plant-available P forms) and mod-labile P content (sparingly soluble P forms) but also increases the non-labile P content (insoluble P forms) (Soltangheisi et al. 2018, 2020). The maize intercropping with soybean showed a higher content of soil labile-P pools and moderately-labile P pools, while a slightly lower non-labile P pools (Fig. 3). This was likely the major reason for intercropping acquire more P uptake through transformation more non-labile P pools. Large numbers of research have demonstrated intercropping could promote P turnover by mobilizing P from the absorbed mineral or organic fractions in the rhizosphere soil through root exudate induced rhizosphere changes in pH, organic anions, and phosphatase activity (Hinsinger et al. 2011a; Shen et al. 2011; Li et al. 2014; Sun et al., 2019).

Furthermore, we divided all P fractions into organic P (NaHCO$_3$-Po, NaOH-Po, and conc. HCl-Po) and inorganic P (sum of resin-P, NaHCO$_3$-Pi, NaOH-Pi, 1 M HCl-P, conc. HCl-Pi and Residual-P). The result showed that intercropping maize could reduce both organic P and inorganic P, especially in low P treatments (P0 and P60 treatments) compared to monoculture. And we found that intercropping had a greater effect on organic P
activation than inorganic P activation (Fig. 2), therefore, we presumed that organic P turnover could be a major mechanism for facilitating crop P uptake in maize and soybean intercropping system. Indeed, regardless of monoculture or intercropping maize that the available P were all significantly positively related to inorganic P pools \( (p < 0.05, p < 0.01) \), while significantly negatively related to NaOH-Po and conc. HCl-Po fractions and organic P pools (Fig. 7). This indicated that organic P pools (including NaOH-Po and conc. HCl-Po) could convert into inorganic P to become available P.

**Intercropping promotes phosphorus activation by secreting more phosphatase**

Plants and soil microorganisms play an important role in soil phosphorus transformation through the secretion of organic anions and extracellular enzymes to mobilize P from insoluble to soluble soil P pools and enhance P uptake (Ren et al. 2019; Zhang et al. 2020). Phosphatase as a critical extracellular enzyme involved in the conversion of organic phosphorus to inorganic P is mainly secreted by the higher plants and microorganisms, including acid phosphatase (ACP) and alkaline phosphatase (ALP) (Nannipieri et al. 2011). Some studies have shown that ACP is mainly secreted by microorganisms, while ALP is mainly derived from the plant root, which is more sensitive to fertilization than microorganisms (Richardson et al. 2009; Zhou et al. 2011; Huang et al. 2016; Wang et al. 2017). Soil microorganisms may utilize sugars and amino acids as a C source from plant rhizosphere and mineralize organic P by trading off the production of extracellular enzymes (Heuck et al. 2015).

Enhanced phosphatase activity has been reported in numerous plant species, especially under P-deficient conditions (Li et al., 2004; George et al. 2008; Liang et al. 2017; Wang et al. 2017). Previous research showed organic P fractions decreased associated with an increase in phosphatase activity to meet the increased P demand (Fan et al. 2018). This was consistent with the P dynamics (decreased non-labile P, and increased moderately labile P and labile P) in our study. In this study, a structural equation model (SEM) was conducted to confirm that both ACP and ALP affected available P directly or indirectly through their effects on organic P turnover (Fig. 8). Meanwhile, we also observed phosphatase activity (ALP and ACP) were higher in maize rhizosphere of intercropping with soybean than monoculture maize (Fig. 9), this could reveal that intercropping facilitates the organic P turnover through regular acid phosphatase activity (ACP), alkaline phosphatase activity (ALP). And the NaOH-Po and conc. HCl-Po may be the key P fractions to deplete turnover into available P (NaOH-Pi) to support plant P uptake. A similar result was confirmed by Liao et al. (2021) that maize intercropping with faba bean accumulated NaOH-Pi while depleting NaOH-Po. Additionally, a large number of studies had indicated that the legume crop is more capable of utilizing soil organic P and inorganic P than maize in cereal/legume intercropping systems because the root system of the legumes secretes a greater amount of acid phosphatase or organic acid to mobilize more soil P, and maize can also utilize more P from legumes through positive interspecific facilitation (Stoddard et al. 2010; Zhang et al. 2016; Chen et al. 2018; Wang et al. 2017).

**Conclusions**

Our study results showed that intercropping maize with soybean increased plant P uptake and mitigated the soil apparent P balance, and the intercropping advantage is gradually weakened with increasing P application. Intercropping maize with soybean could deplete NaOH-Po, conc. HCl-Pi, conc. HCl-Po and residual-P fractions, and increase Resin-P, NaHCO₃-Pi, NaHCO₃-Po, NaOH-Pi, and 1 M HCl-Pi fractions compared to monoculture. In
addition, intercropping increased the content of labile P pools (35.7% and 37.5%) and moderately-labile P pools (4.4% and 2.9%), while reducing non-labile P pools (8.6% and 10.0%). Furthermore, intercropping also significantly increase the activity of ACP and ALP, and structural equation model (SEM) showed that both ACP and ALP play curtail role increased available P directly or indirectly through their effects on organic P turnover. These results confirm that intercropping promotes the turnover of organic P pools (NaOH-Po and conc. HCl-Po) into soluble phosphate (Resin-P, NaHCO$_3$-Pi and NaOH-Pi) through regulation phosphatase activation. While suitable P application may be more conducive to the intercropping promote P activation and crop P uptake.

**Declarations**

**Funding** This study was supported by the National Natural Science Foundation Project (Grant Number 32260805, 31760615) the National Key R & D Program (Grant Number 2022YFD1901503), and the Major Science and Technology Special Project of Yunnan Province (202102AE090030).

**Competing Interests** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Author Contributions** Long Zhou, Lizheng Su, Hongmin Zhao, Shirui Wang contributed to the study conception and design. Material preparation, data collection and analysis were performed by Long Zhou, Lizheng Su and Shirui Wang. The first draft of the manuscript was written by Long Zhou and all authors commented on previous versions of the manuscript. Yi Zheng and Li Tang contributed to Writing—reviewing and editing, project administration, supervision, funding acquisition. All authors read and approved the final manuscript.

**Data Availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures
Figure 1

Effect of intercropping (sole maize and intercropping maize represent MM and IM) on Phosphorus (P) uptake and P balance under different P application rates (0, 60, 90, and 120 kg ha\(^{-1}\) annually, P0, P60, P90, and P120, respectively) in 2018 and 2019. Values are means of three replicates ± standard errors (n=3), and values followed by the same lower-case letters are not significantly different among different cropping systems and the P application rates at the 5% level by the LSD.

Figure 2

Effect of intercropping (sole maize and intercropping maize represent MM and IM) on the proportions of inorganic and organic P fractions under different P application rates (0, 60, 90, and 120 kg ha\(^{-1}\) annually, P0, P60, P90, and P120, respectively) in 2018 and 2019. Values are means of three replicates ± standard errors (n=3), and
values followed by the same lower-case letters are not significantly different among different cropping systems and the P application rates at the 5% level by the LSD.

**Figure 3**

Effect of intercropping (sole maize and intercropping maize represent MM and IM) on the proportions of labile, moderately-labile and non-labile P fractions under different P application rates (0, 60, 90, and 120 kg ha\(^{-1}\) annually, P0, P60, P90, and P120, respectively) in 2018 and 2019. Values are means of three replicates ± standard errors (n=3), and values followed by the same lower-case letters are not significantly different among different cropping systems and the P application rates at the 5% level by the LSD.

**Figure 4**
Effect of intercropping (sole maize and intercropping maize represent MM and IM) on the sequential soil P fractions under different P application rates (0, 60, 90, and 120 kg ha\(^{-1}\) annually, P0, P60, P90, and P120, respectively) in 2018 and 2019. Values are means of three replicates ± standard errors (n = 3), and values followed by the same lower-case letters are not significantly different among different cropping systems and the P application rates at the 5% level by the LSD.

**Figure 5**

Change in soil P fractions for intercropping maize and monoculture (soil P fractions of intercropping maize minus the corresponding P fractions of monoculture maize represent MM and IM) under four P application rates (0, 60, 90, and 120 kg ha\(^{-1}\) annually, P0, P60, P90, and P120, respectively) in 2018 and 2019. Values are means of three replicates ± standard errors (n = 3), means with the same letters are not significantly different (P < 0.05) by the LSD test.
Effect of intercropping (sole maize and intercropping maize represent MM and IM) on Olsen-P, P activation coefficient (PAC), Resin-P and NaHCO$_3$-Pi under different P application rates (0, 60, 90, and 120 kg ha$^{-1}$ annually, P0, P60, P90, and P120, respectively) in 2018 and 2019. Values are means of three replicates ± standard errors (n=3), and values followed by the same lower-case letters are not significantly different among different cropping systems and the P application rates at the 5% level by the LSD.

Figure 6
Effect of intercropping (sole maize and intercropping maize represent MM and IM) on acid phosphatase activity (ACP), alkaline phosphatase activity (ALP) under different P application rates (0, 60, 90, and 120 kg ha$^{-1}$ annually, P0, P60, P90, and P120, respectively) in 2018 and 2019. Values are means of three replicates ± standard errors (n=3), and values followed by the same lower-case letters are not significantly different among different cropping systems and the P application rates at the 5% level by the LSD.
Figure 8

Person relationships between P fractions with available P and PAC in rhizosphere soil in monoculture maize (MM) and intercropping maize (IM)

Figure 9

Structural equation model (SEM) related to soil phosphatase mobilization organic phosphorus (P) fractions into available P in monoculture and intercropping maize. Numbers in $R^2$ indicate the variance explained by the model. Numbers on arrows are standardized path coefficients, arrow thickness represents the magnitude of the path coefficient. Significance levels are as follows: *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Red arrows indicate positive effects, blue arrows indicate negative effects, and dashed arrows indicate non-significant paths, which were removed in the final model. ACP: acid phosphatase activity, ALP: alkaline phosphatase activity.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- data.xlsx