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RESEARCH ARTICLE

Intercropping efficiently utilizes phosphorus resource in soil via different strategies mediated by crop traits and species combination

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Abstract

Background and aims Understanding how long-term intercropping and phosphorus (P)-fertilizer application affect soil P fractions through P-acquisition strategies is critical to maintaining soil P balance in agroecosystems.

Methods We established a long-term field experiment with three P-fertilizer application rates (0, 40, and 80 kg P ha^{-1}) and continuously used four intercropping systems of chickpea/maize, faba bean/maize, oilseed rape/maize, soybean/maize and corresponding five monocultures in 2009. We measured aboveground biomass, shoot P content, soil P fractions, P-related root physiological traits, and soil microbe-related parameters of crop species in 2020. We also calculated the apparent soil P balance (P input into soil minus P harvested from crops) using data from 2009 to 2020.

Results Intercropping enhanced aboveground biomass and shoot P content by 31.2% and 49.4% compared with the weighted means of corresponding monocultures, respectively; intercropping decreased the apparent soil P balance by 37.8% compared with monocultures across three P-fertilizer application rates. Over the 12-year period, chickpea/maize and soybean/maize intercropping systems significantly decreased the soil organic P concentration compared with sole maize; faba bean/maize and oilseed rape/maize intercropping systems significantly decreased soil non-labile P but increased organic P and labile P pool relative to sole maize. Rhizosheath phosphatases and carboxylates (proxied by leaf manganese concentration) might contribute to the depletion of sparingly-available soil P (organic P or non-labile P) in different crop combinations.

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Ran An and Rui-Peng Yu contributed equally to this work.

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Conclusion The higher rhizosheath acid phosphatase activities and carboxylate concentrations may correlate with efficient utilization of sparingly-available soil P resources in intercropping; effective P-fertilizer input enhanced soil P availability and decreased the P surplus in soil which is crucial to enhance crop P uptake.

Keywords Leaf manganese concentration · *phoD* gene abundance · Rhizosheath phosphatase activity · Apparent soil P balance · Soil microbial biomass P · Soil P fractions

Introduction

Phosphorus (P) is a major limiting nutrient for plant productivity in terrestrial environments (Elser et al. 2007; Vitousek et al. 2010). The concentration of available P in soil is quite low, because soil minerals strongly sorb a large proportion of inorganic P or P is associated with organic matter, and hence P availability cannot meet the requirement of plant growth (Hinsinger 2001). Phosphorus-fertilizer input is, therefore, essential for sustainable food production in agriculture. However, excessive P fertilizers result in large amounts of P accumulating in soil and threaten water quality and biodiversity (Vorosmarty et al. 2010). Soil nutrient balance is an important indicator reflecting the losses to the environment. In recent years, a P surplus has accumulated in most farmland in China (Zhang et al. 2019). Moreover, phosphate rock is turned into P fertilizer for crop yield, but this is a non-renewable resource, and the reserves may become exhausted in 93 to 291 years (Cordell and White 2011; Fixen and Johnston 2012; Johnston et al. 2014). China is the major consumer and producer of P fertilizer, and its P reserves will be depleted within a hundred years with increasing P input (Fixen and Johnston 2012; Yu et al. 2021c). Therefore, it is urgent to develop P-management strategies to improve P-use efficiency and decrease P-fertilizer dependency. Combining species with efficient P-acquisition strategies to mine soil P pools in intercropping systems is a promising and economically viable approach to establishing sustainable food-production systems.

Plant species exhibit divergent P-mobilizing capacities (Li et al. 2014). Faba bean is more efficient at mobilizing P by releasing a large amount of carboxylates and protons in the rhizosphere. In contrast, the proton-releasing capacity of maize is too weak to acidify the rhizosphere (Li et al. 2007; Zhang et al. 2016). Plant species have evolved diverse P-acquisition strategies to obtain sufficient P to meet their growth requirement. For example, oilseed rape and maize mainly depend on adjustments of root morphological traits, e.g., an increase in specific root length and lateral root density, to acquire soil P (Calderon-Vazquez et al. 2009). Chickpea and white lupin release root phosphatases and carboxylates that hydrolyze and mobilize organic P; faba bean relies on both root morphological and physiological responses that mobilize P from insoluble inorganic P forms (Isaac and Borden 2019; Lambers 2022; Li et al. 2007; Lyu et al. 2016). When species with diverse P-acquisition strategies grow together, their P-use efficiency may be improved (Li et al. 2014; Yu et al. 2020).

Intercropping attracts increasing attention as a sustainable cultivation method, which can deliver yield advantage and increase soil fertility (Li et al. 2021; Xing et al. 2023). Enhanced P-use efficiency and P uptake have been observed in intercropping, especially in legume/cereal intercropping systems (Tang et al. 2021; Tian et al. 2020a). The advantage of intercropping in P uptake is mainly mediated by complementarity and facilitation. Facilitation is defined as one or more species increasing a function metric (e.g., plant-availability of nutrients) of their neighbors (Brooker et al. 2015; Yu et al. 2021b). For example, white lupin increases the P-acquisition of intercropped maize via mobilization of insoluble P into soluble P in the rhizosphere (Dissanayaka et al. 2015). Complementarity in P uptake suggests P partitioning between species and reduced interspecific competition, leading to greater P acquisition (Hinsinger 2001). For example, in pigeon pea/cereal intercropping systems, pigeon pea can use iron-bound P, while intercropped cereals rely on calcium-bound P (Ae et al. 1990). Thus, intercropping may allow access to different P fractions via various P-acquisition strategies to enhance P uptake in intercropping systems.

Soil microorganisms play an important role in the soil P cycle (Richardson and Simpson 2011). The P held in the biomass of microorganisms is protected from reactions with soil and is potentially available to plant species (Liebisch et al. 2014; Olander and Vitousek 2004). In addition, soil microorganisms may utilize organic P by producing extracellular enzymes, i.e. alkaline phosphatases, which are only produced by microorganisms (Nannipieri et al. 2011). Also, the *phoD* gene has been identified in coding for alkaline phosphatases in bacteria (Gomez and Ingram 1995). Different agronomic managements (e.g., nutrient input and cropping systems) may shift microbial functional profiles of P cycling (Dai et al. 2020; Yu et al. 2021a). A greenhouse experiment also showed that soil microorganisms are associated with intercropping overyielding in faba bean/wheat and faba bean/maize intercropping systems (Wang et al. 2021). Hence, associating soil microbial parameters and P-acquisition strategies with P fractions may provide a novel insight into P utilization in agriculture.

Most previous studies focused on soil P-fraction dynamics and P uptake in a single intercropping system, whereas few linked P-mobilizing capacities with soil P pools in diverse intercropping systems (Liao et al. 2020, 2021; Qu et al. 2022; Tian et al. 2020a). Furthermore, the correlations between P-acquisition strategies and P pools in different crop combinations under field conditions are largely unknown. In this study, we aimed to assess the P pools in soil with three P-application rates (0, 40, and 80 kg P ha^{-1}) and with four intercropping systems with different species combinations and corresponding species monocultures, and also to explore how plant P-acquisition strategies impact soil P pools, using a long-term field experiment. We also measured physiological and microbial parameters in the rhizosheath, namely: pH; acid phosphatase activity; alkaline phosphatase activity; soil microbial biomass P; *phoD* gene abundance. Carboxylates were difficult to measure under field conditions, but leaf manganese concentration ([Mn]) is positively correlated with rhizosheath carboxylate concentrations (Lambers et al. 2021; Yu et al. 2023). Therefore, we measured leaf [Mn] as a proxy for P acquisition via carboxylate release in the rhizosphere. We addressed two key questions: (1) How do P-application rates and intercropping affect productivity, shoot P content, and apparent soil P balance of intercropping systems compared with monocultures? (2) How does intercropping affect soil P pools in different species combinations with different P-acquisition strategies under field conditions?

Material and methods

Study site

The field experiment is located at Baiyun Experimental Station of the Gansu Academy of Agricultural Sciences, Gansu Province (102°40′E, 38°37′N, 1504 m above sea level). This region has a typical arid climate with mean annual temperature and precipitation of 7.7°C and 150 mm, respectively. Total solar radiation is 5988 MJ m^{-2} yr^{-1}, and the average annual frost-free period is 170–180 days. The soil (0–20 cm) of the experimental field is classified as calcareous Aridisol and had the following average composition before establishment of the experiment: pH 8.0, organic matter 19.1 g kg^{-1}, total nitrogen (N) 1.08 g kg^{-1}, Olsen-P 20.3 mg kg^{-1}, and available potassium (K) 233 mg kg^{-1}. In 2020, the Olsen-P concentration in the soil under P0, P40, P80 was 4.8 mg kg^{-1},14.8 mg kg^{-1}, and 29 mg kg^{-1}, respectively.

Experiment design and crop management

The experiment was established in 2009, as a split-plot experimental design with three replicates. The main-plot factor comprised three P-application rates (0, 40, and 80 kg P ha^{-1} y^{-1}). The 40 kg P ha^{-1} fertilizer-application rate is recommended by agronomists to meet the nutrient requirement of maize, and 80 kg P ha^{-1} is the common P-fertilizer application rate for local farmers.

The sub-plot factors were nine cropping systems comprising five monocultures: faba bean (*Vicia faba* L.cv. Lincan No.5), soybean (*Glycine max* L.cv. Zhonghuang No. 33), chickpea (*Cicer arietimum* L. cv. Longying No. 1), oilseed rape (*Brassica campestris* L. cv. Longyou No. 5) and maize (*Zea mays* L.cv. Xianyu No. 335), and four intercropping systems: faba bean/maize, soybean/maize, chickpea/maize, and oilseed rape /maize. The field was split into 81 plots, with each monocropped plot measuring 4×5.5 m^2 and each intercropped plot measuring 5.6×5.5 m^2. For maize, the inter-row distance was 0.4 m, and the inter-plant distance was 0.25 m. For legumes (i.e. faba bean, chickpea, and soybean), the inter-row distance and inter-plant distance were 0.2 m. For oilseed rape, the inter-row distance was 0.2 m, which was planted by broadcast sowing in each row. Every intercropped plot included four 1.4 m-wide intercropping strips, each of which consisted of two rows of maize and three rows of companion crops (i.e. faba bean, chickpea, soybean, and oilseed rape), with a row distance of 0.3 m between maize and the companion crop. In the intercropped area, maize occupied 57% and the companion crop occupied 43%.

All plots were given a basal application of 60 kg K ha^{-1} as potassium sulfate and corresponding P as superphosphate. Sole maize and intercropping systems were given an identical application of 225 kg N ha^{-1} as urea, one-third of N fertilizer (75 kg N ha^{-1}) was applied as basal fertilizer, and the remaining N fertilizer was divided equally into two portions and applied at the stem elongation stage and the pre-tasselling stage, respectively (Li et al. 2021; Xing et al. 2023). The monoculture companion crops were given a basal application of 75 kg N ha^{-1} as urea, which was half of the total N fertilizer during the entire growth period (Li et al. 2021; Xing et al. 2023). Six irrigations were carried out on 12 May, 2 June, 23 June, 3 July, 29 July, and 15 August with 100 mm for monoculture and intercropping plots in 2020. The amounts of fertilizer and irrigation were recommended by local agronomists.

Faba bean and chickpea were sown on March 22 in 2020. Soybean and oilseed rape were sown on April 17 in 2020; maize was sown on April 26. Faba bean, chickpea, and oilseed rape were harvested in early July; maize and soybean were harvested in late September to early October 2020. At maturity, three continuous rows of companion crops and two rows of maize were harvested per plot and separated into grain and straw. All samples were air-dried for two weeks and weighed.

Soil and plant analyses

Soil and mature leaf samples were collected during flowering stage, with samples of faba bean, chickpea, and oilseed rape collected in June, and the samples of soybean and maize were collected in July. We dug up the roots of the five crops from the 0–30 cm soil layer, then shook the roots to remove loosely adhering soil. The rhizosheath soil was brushed from 5–10 individuals according to the different sizes of the crops. Bulk soil samples of 0–20 cm were collected using an auger (3.5 cm diameter) in all plots. In the monoculture plots, the bulk soil was collected from five cores. In the intercropping plots, the bulk soil was separately collected from each crop strip for five cores. The bulk soil samples were air-dried for measurement of soil P fractions and Olsen-P. All rhizosheath soil samples were mixed through a 2-mm sieve and divided into three parts; one was stored at 4° for measurement of alkaline and acid phosphatase activity, and microbial biomass P; one was air-dried for measurement of soil pH and P fractions; another subsample was stored at -20°C for DNA extraction.

Soil pH was measured in a 10 mM calcium chloride extract (1:2.5) by using an S210 pH meter (Mettler Toledo, Zurich, Switzerland). Soil microbial biomass P was determined after fumigation with $CHCl_3$ for 24 h and then extracted with 0.5 M $NaHCO_3$ (Brookes et al. 1982). Olsen-P was extracted with 0.5 M $NaHCO_3$ and measured by molybdenum blue colorimetry (Bao 2005). Alkaline phosphatase and acid phosphatase activity were measured with *p*-nitrophenol (PNP) as substrate at pH 8.5 and 5.2 using spectrophotometrically at 405 nm, respectively (Tabatabai and Bremner 1969). Shoot P concentration was analyzed using the vanadomolybdate method (Bao 2005). Leaf [Mn] was measured by inductively-coupled plasma optical-emission spectroscopy (ICP-OES; OPTIMA 3300 DV, Perkin-Elmer, Waltham, MA, USA) using mature leaves.

Soil P fractionation

The air-dried bulk and rhizosheath soil samples were sieved through a 0.15-mm mesh and then 0.5 g was used to analyze sequential P fractions. The soil P fractionation was carried out according to a sequential fractionation scheme (Tiessen and Moir 1993) modified from methods described by Hedley et al. (1982). When assessing the soil P fractions, we accept that the biological significance, if any, of some of these fractions is poorly understood (Barrow et al. 2021). In the fractionation scheme, soil P was divided into nine fractions. i.e. resin-extractable P (Resin-P), bicarbonate-extractable P ($NaHCO_3$-Pi and $NaHCO_3$-Po), OH-extractable P (NaOH-Pi and NaOH-Po), dilute HCl-extractable P (1 M HCl-Pi), concentrated HCl-extractable P (conc. HCl-Pi and conc. HCl-Po) and concentrated H_2SO_4-H_2O_2-extractable P (residual-P). After adding each extractant, the suspension was first placed on a shaker (200 rpm) for 16 h, then centrifuged at 25,000 g for 10 min at 0°C; then the corresponding extract was decanted through a 0.45-μm membrane filter into a clean vial for the colorimetric analysis. The inorganic P fraction and residual-P were measured by the method of Murphy and Riley (1962). The total P concentrations ($NaHCO_3$-Pt, NaOH-Pt, and conc. HCl-Pt) were analyzed using the ammonium persulfate digestion method (Li et al. 2008), and the organic P concentrations were obtained by subtracting the inorganic P concentration from the total P concentration (Pt). Total inorganic P was calculated as the sum of Resin-P, $NaHCO_3$-Pi, NaOH-Pi, 1 M HCl-Pi, and conc. HCl-Pi. Total organic P was calculated by adding $NaHCO_3$-Po, NaOH-Po, and conc. HCl-Po. To make comparisons of trends in labile and less-soluble P fractions, we combined the nine P fractions into four P pools according to the activity of the P fraction, including labile P (sum of Resin-P, $NaHCO_3$-Pi, and $NaHCO_3$-Po), moderately-labile P (sum of NaOH-Pi, NaOH–Po, and 1 M HCl-Pi), sparingly-labile P (conc. HCl-Pi and conc. HCl-Po) and non-labile P (residual-P) (Crews and Brookes 2014; Liao et al. 2020).

DNA extraction and sequencing

The DNA was extracted from 0.5 g frozen soil using the FastDNA SPIN kit (MP Bio-medicals, Solon, OH, USA) following the manufacturer's instructions. The eluted DNA was stored at -20 °C for short-term storage. DNA quality was monitored by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE).

Quantitative PCR

Bacterial *phoD* genes were determined by quantitative polymerase chain reaction (qPCR) with the primers ALPS-F730 (5′-CAGTGGGACGACCACGAG GT-3′) and ALPS-R1101 (5′-GAGGCCGA TCG GCATGTCG-3′) (Sakurai et al. 2008) on the C1000 Touch Thermal Cycler real-time PCR system (BIO-RAD, Hercules, California, USA). The PCR mixture (25 μL) contained 13 μL of 2 × TB Green Premix Ex Taq (Takara, Japan), 0.5 μL of each primer, 2 μL of DNA, and 9 μL of sterile ddH_2O. Cycling conditions were as follows: 95 °C for 3 min, 40 cycles of 95 °C for 5 s, and 58 °C for 30 s (Wei et al. 2019). The standard curve was prepared by serial tenfold dilutions of plasmid. The qPCR results were considered acceptable when the R^2 of the standard curve > 0.98.

Calculations

The aboveground biomass produced by intercropping can be compared with the weighted means of two monoculture crops based on their proportions in the intercropping systems. The weighted means of biomass in monocultures were calculated using the following equation:

$$B_{weighted} = B_{monoculture\_a} \times P_a + B_{monoculture\_b} \times P_b$$

where a represents maize, while b represents companion crop (i.e. oilseed rape, chickpea, faba bean, or soybean); $B_{monoculture\_a}$ and $B_{monoculture\_b}$ represent the biomass of crops a and b in monoculture; P_a and P_b refer to the proportions of the area in the intercropping systems, where $P_a = 57\%$ and $P_b = 43\%$. The above formula was also used to calculate the weighted means of shoot P content, soil total inorganic P, soil total organic P and soil residual-P in monocultures.

The soil total inorganic P concentrations in intercropping systems were calculated using the following equation:

$$P_{i\_intercropping} = P_{i\_intercropping\_a} \times P_a + P_{i\_intercropping\_b} \times P_b$$

where $P_{i\_intercropping\_a}$ and $P_{i\_intercropping\_b}$ represent the soil total inorganic P concentration of crops a and b in intercropping. The above formula was also used to calculate the concentrations of soil total organic P, nine soil P fractions, four P pools, aboveground biomass and the shoot P content in intercropping systems.

The apparent P balance is the difference between P inputs and P removal from aboveground biomass, which was calculated using the following equation (Hua et al. 2016; Liao et al. 2021):

$$\text{ApparentPbalance} = F \times 12 - \sum_{i=2009}^{2020} PC_i$$

where F represents P-application rates (0, 40, and 80 kg ha^{-1}), PC_i represent the shoot P content of cropping systems in i (2009–2020) year.

Statistical analyses

A linear-mixed effect model used the 'nlme' package (Pinheiro et al. 2022). First, aboveground biomass, shoot P content, grain yield, grain P content, inorganic P, organic P, and residual-P were tested using cropping system (i.e. monoculture and intercropping), P-application rate, and crop combination as fixed effects, and block as a random effect. Second, the apparent soil P balance at each P-application rate in different crop combinations was separately analyzed using cropping system (i.e. sole maize, sole companion crop, and intercropping combination) as a fixed effect, and block as a random effect. Third, cropping system (i.e. sole maize, sole companion crop, and intercropping combination) and P-application rate, and their interaction effect on the proportion of four soil P pools, rhizosheath soil properties, and bulk Olsen-P of different crop combinations were tested using cropping system and P-application rate as fixed factors; block was treated as a random factor. Tukey's post-hoc HSD test was conducted at the 5% probability level in linear-mixed effect models.

Principal component analyses (PCA) and PERMANOVA test were conducted using P-related functional traits (rhizosheath acid phosphatase activity, rhizosheath alkaline phosphatase activity, rhizosheath pH, rhizosheath MBP, leaf [Mn], and rhizosheath *phoD* gene abundance), soil P Pools, total organic P, total inorganic P, Olsen-P of different cropping systems (i.e. sole maize, sole companion crop, and intercropping combination), using the 'ggbiplot' package (Vincent 2011). A PERMANOVA test was conducted to calculate the P value between cropping systems using the 'pairwiseAdonis' package (Pedro 2017). Finally, we also examined the relationships between P-related functional traits and shoot P content by correlation analyses in each crop combination. All statistical analyses were performed with R version 4.1.3 (R Development Core Team 2022).

Results

Productivity, plant P content, and apparent soil P balance

Aboveground biomass and grain yield differed among cropping systems (Cs: $P < 0.001$), and P-application rates (P: $P < 0.001$; Fig. 1; Table S1). Intercropping significantly increased aboveground biomass and grain yield compared with the weighted means of the monoculture crops (Fig. 1). Faba bean/maize, soybean/maize, chickpea/maize, and oilseed rape/maize intercropping increased grain yield by 66.7%, 20.8%, 77.4%, and 68.8% without P application, by 33.2%, 26.4%, 38.8%, and 22.5% under P40, and by 36.2%, 16.5%, 43.6%, and 20.8% under P80, compared with the weighted means of corresponding monocultures, respectively (Fig. 1B). The chickpea/maize intercropping combination showed the greatest yield advantage under three P-application rates. There were no significant differences in aboveground biomass and grain yield among crop combinations (Fig. 1A,B). Aboveground biomass and grain yield tended to increase with increasing P-application rates, and the minimum aboveground biomass was observed at the P0 rate (Fig. 1E,F).

Shoot and grain P content differed among cropping systems, crop combinations, and P-application rates (Fig. 1; Table S1). Intercropping significantly increased grain P content and shoot P content compared with the weighted means of the monoculture crops in all crop combinations (Fig. 1). The oilseed rape/maize combination exhibited significantly greater shoot P content than chickpea/maize and faba bean/maize combinations; the soybean/maize combination exhibited significantly greater grain P content than faba bean/maize combinations (Fig. 1C,D). Phosphorus application significantly increased shoot P content and grain P content; the increase of shoot P content and grain P content in intercropping with P40 and P80 was greater than that in P0 (Fig. 1G,H).

Without P input for 12 years, all treatments showed soil P depletion. In contrast, P surplus was shown in all treatments with 80 kg P ha^{-1} application annually. Applying 40 kg P ha^{-1} every year led to a large P surplus for sole companion crops, but a small P surplus for sole maize (33 kg P ha^{-1}). The apparent P balance of intercropping systems under 40 kg P ha^{-1} was in the range of -50 to + 6 kg P ha^{-1}. Intercropping systems and sole maize accumulated less P soil in than sole companion crops (Table 1).

Effect of cropping system on soil total inorganic P, total organic P, and residual P among crop combinations

We calculated total inorganic P, total organic P, and residual-P to assess the utilization of soil P by crop

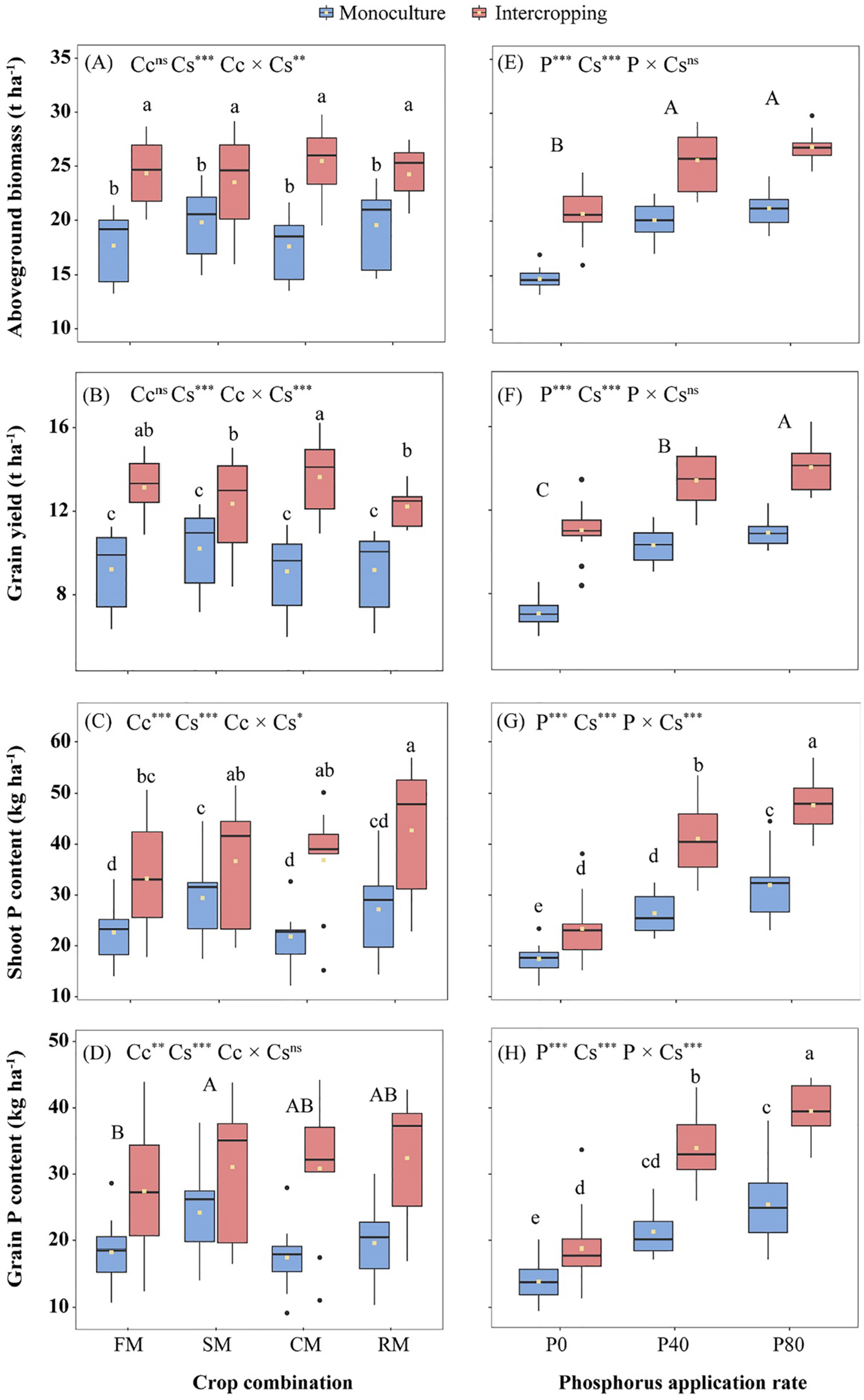

Fig. 1 Effects of crop combination (Cc) and cropping system (i.e. monoculture and intercropping, Cs) on aboveground biomass **A**, grain yield **B**, shoot phosphorus (P) content **C** and grain P content **D**. Effects of P-application rate (factor P) and cropping system (Cs) on aboveground biomass **E**, grain yield **F**, shoot P content **G** and grain P content **H** in 2020. Aboveground biomass, grain yield, shoot P content and grain P content of the monocultures were calculated as the weighted means of corresponding monoculture crops based on their land proportions in intercropping. P0: 0 kg P ha^{-1}; P40: 40 kg P ha^{-1}; P80: 80 kg P ha^{-1}. FM: faba bean/maize combination; SM: soybean/maize combination; CM: chickpea/maize combination; RM: oilseed rape/maize combination. Lowercase letters indicate differences among treatments if the interaction effect is significant. The same letter means there is no significant difference (Tukey HSD). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns, not significant

combinations and cropping systems with different P-application rates. Although there was no significant difference between cropping systems in residual P, total inorganic P, and total organic P of the rhizosheath soil (Fig. S1), intercropping significantly decreased the bulk soil residual-P ($P<0.001$). However, there was no significant difference in the concentrations of total inorganic P and total organic P in

Table 1 The apparent phosphorus (P) balance (kg ha^{-1}) in the topsoil layer (0–20 cm) from 2009 to 2020

Crop combination	Cropping system	Apparent P balance (kg ha^{-1})		
		P0	P40	P80
Faba bean/maize	Sole maize	-379b	33b	435b
	Sole faba bean	-141a	307a	749a
	Faba bean/maize	-369b	-26c	417b
Soybean/maize	Sole maize	-379b	33b	435b
	Sole soybean	-214a	241a	681a
	Soybean/maize	-356b	6b	434b
Chickpea/maize	Sole maize	-379b	33b	435b
	Sole chickpea	-152a	324a	757a
	Chickpea/maize	-395b	6b	442b
Oilseed rape/maize	Sole maize	-379ab	33ab	435ab
	Sole oilseed rape	-118a	274a	707a
	Oilseed rape/maize	-382b	-50b	393b

Note: Shown are the means. Lowercase letters indicate differences among cropping systems for a given P-application rate and crop combination. The same letter means there is no significant difference (Tukey HSD)

the bulk soil compared with the weighted means of the monoculture crops (Fig. 2; Table S2). The chickpea/maize combination had the greatest bulk soil total inorganic P concentration, and that of the faba bean/maize combination was the lowest (Fig. 2A). The oilseed rape/maize combination had the greatest bulk soil total organic P concentration, and that of the soybean/maize combination was the lowest. The soybean/maize combination exhibited the greatest bulk soil residual-P concentration among the four crop combinations, and oilseed rape/maize had the lowest soil residual-P concentration (Fig. 2B,C). The interaction effect between crop combination and cropping system was significant in bulk soil inorganic-P and residual-P concentration, in which the faba bean/maize intercropping system showed significantly lower inorganic-P and oilseed rape/maize intercropping system showed significantly lower residual-P concentrations than those of the weighted means of the monoculture crops (Fig. 2A,C). In the rhizosheath soil, we also found a significant interaction effect between crop combination and cropping system in faba bean/maize combination, in which faba bean/maize intercropping system also exhibited a lower inorganic-P concentration than sole faba bean/maize did (Fig. S1). The concentration of soil total inorganic P, total organic P, and residual-P in the bulk soil and rhizosheath soil increased with increasing P-application rates (Fig. 2D,E,F,S1).

Impacts of P application and cropping system on soil P

In the bulk soil, the moderately-labile soil P pool (the sum of NaOH-Pi, NaOH-Po, and 1 M HCl-Pi) represented the largest proportion of total P in all treatments, accounting for 67% to 74% of total P in all treatments, followed by sparing-labile P, ranging from 11 to 17% (Fig. 3). The proportions of bulk soil P pools were affected by cropping system and P-application rates in different intercropping systems (Fig. 3; Table S3). Phosphorus-application rate significantly increased the labile soil P proportion under the four intercropping systems, but significantly decreased the sparingly-labile P proportion and non-labile P proportion in different intercropping systems (except the sparingly-labile P proportion in the oilseed rape/maize system). The results about the proportions of the four P pools in the rhizosheath soil were similar as those in the bulk soil (Fig. S2).

In the faba bean/maize combination, the total bulk soil P concentration comprised 82.3% inorganic P, 8.8% Po and 8.9% residual P (Table S4). Among the three systems, sole faba bean exhibited a greater labile-P pool than the faba bean/maize intercropped system and sole maize system ($P < 0.01$). The faba bean/maize intercropped system and sole faba bean system had less Pi, moderately-labile P and non-labile P than the sole maize (Fig. 4). In the oilseed rape/maize combination, the total bulk soil P concentration was composed of 82.7% Pi, 9.2% Po and 8.1% residual-P (Table S5). Labile-P and sparingly-labile P concentrations among cropping systems were dependent on P-application rate (Fig. 5). Sole maize exhibited a greater non-labile P pool but less soil Po than sole oilseed rape and oilseed rape/maize intercropping systems did. The faba bean/maize intercropping system, oilseed rape/maize intercropping system, and corresponding sole companion crop systems exhibited more soil organic P but less non-labile P than the sole maize system did.

In the chickpea/maize combination, the total soil bulk P concentration comprised 84.6% Pi, 7.2% Po,

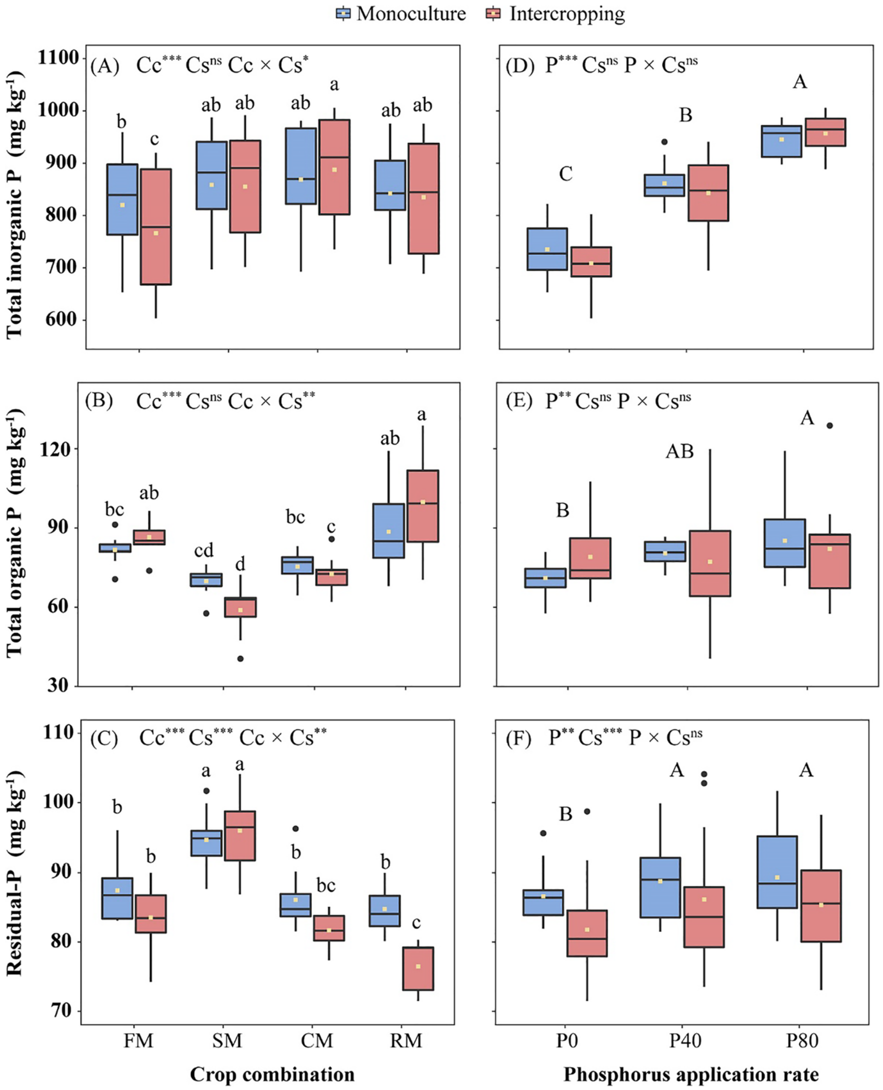

Fig. 2 Effects of crop combination (Cc) and cropping system (i.e. monoculture and intercropping, Cs) on total soil inorganic phosphorus (P) **A**, total soil organic P **B**, and soil residual-P **C** in the bulk soil. Effects of P-application rate (factor P) and cropping system (Cs) on total soil inorganic phosphorus **D**, total soil organic phosphorus **E**, and soil residual-P **F** in the bulk soil. Total soil inorganic P, total organic P, and residual-P of the monocultures were calculated as the weighted means of corresponding monoculture crops based on their land proportions in intercropping. P0: 0 kg P ha^{-1}; P40: 40 kg P ha^{-1}; P80: 80 kg P ha^{-1}. FM: faba bean/maize combination; SM: soybean/maize combination; CM: chickpea/maize combination; RM: oilseed rape/maize combination. Uppercase letters refer to differences among P-application rates. Lowercase letters indicate differences among treatments if the interaction effect is significant. The same letter means there is no significant difference (Tukey HSD). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant

and 8.2% residual P (Table S6). The sole chickpea and/or chickpea/maize intercropping system showed a greater labile-P pool and Pi but less non-labile P and Po than sole maize did. There were no significant differences among cropping systems in the soil moderately-labile P and sparingly-labile P pools (Fig. 6).

In the soybean/maize combination, the total bulk soil P fractions averaged: 84.1% in Pi forms, 6.5% in Po forms, and 9.4% in residual P (Table S7). There were no significant differences among cropping systems in labile-P, moderately-labile P pool and Pi. Sole soybean and soybean/maize intercropping systems

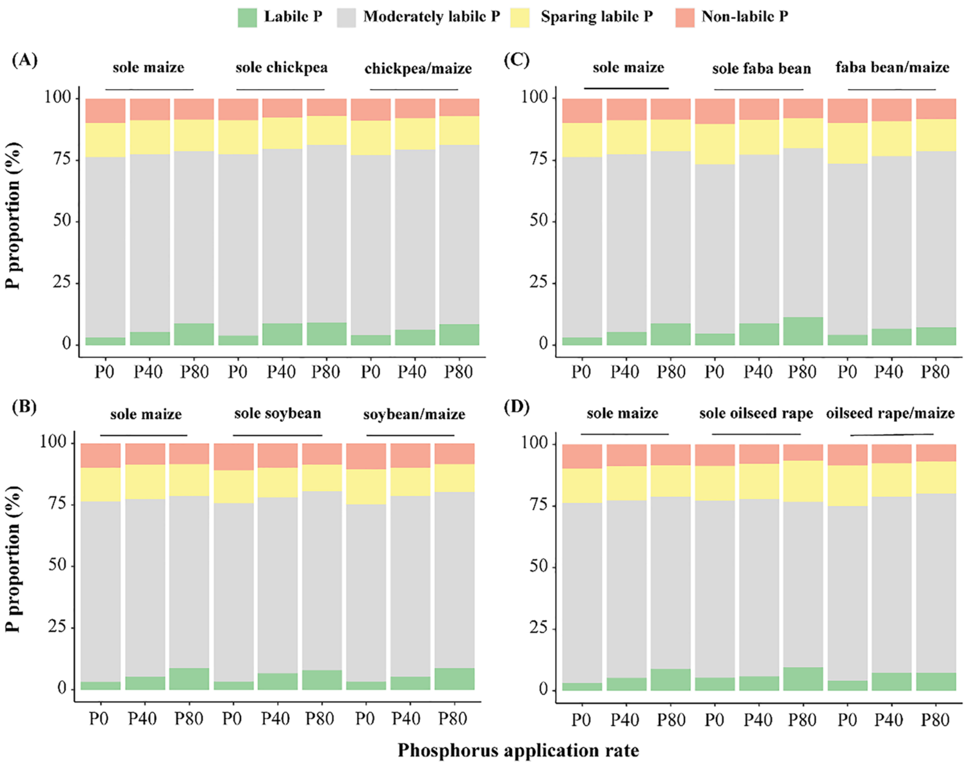

Fig. 3 Proportions of different soil bulk phosphorus (P) pools in cropping systems (i.e. sole maize, sole companion crop, intercropping combination, Cs) with three P-application rates. Shown are chickpea/maize **A**, soybean/maize **B**, faba bean/maize **C**, and oilseed rape/maize **D** combinations. Labile P pool includes Resin-P, $NaHCO_3$-Pi, and $NaHCO_3$-Po; moderately-labile P pool includes NaOH-Pi, NaOH-Po, and 1 M HCl-Pi; sparingly-labile P pool includes conc. HCl-Pi and conc. HCl-Po; non-labile P pool is residual-P; P0: 0 kg P ha^{-1}; P40: 40 kg P ha^{-1}; P80: 80 kg P ha^{-1}

exhibited less sparingly-labile soil P and Po than sole maize, in which the decrease of sparingly-labile P pool and Po under P40 and P80 was larger than those without P application (Fig. 7). The concentration of soil Po in the chickpea/maize intercropping system, soybean/maize intercropping system, and corresponding sole companion crop systems was lower than that of sole maize system. Our results also show that the chickpea/maize and soybean/maize systems had a lower percentage of total organic P, but a greater percentage of total inorganic P than the faba bean/maize and oilseed rape/maize systems (Tables S4-S7).

Correlations between soil P pools and root physiological traits

The crops showed significant differences in physiological traits in each crop combination. In the oilseed rape/maize combination, sole oilseed rape and oilseed rape/maize intercropping system exhibited greater acid phosphatase activity, greater leaf [Mn], lower pH, and lower *phoD* gene abundance in rhizosheath than sole maize (Table S4). In the faba bean/maize combination, sole faba bean showed greater rhizosheath microbial biomass P, leaf [Mn] and bulk Olsen P, but a lower *phoD* gene abundance than sole maize did (Table S4). In the soybean/maize combination, the soybean maize intercropping system showed greater microbial biomass P and leaf [Mn], but lower *phoD* gene abundance than sole maize did (Table S4). In the chickpea/maize combination, the chickpea/maize intercropping system exhibited lower *phoD* gene abundance than sole maize did (Table S4).

Principal component analysis (PCA) was used to explore how P-related functional traits and soil P pools were associated with intercropping under different crop combinations. In the chickpea/maize system, PCA axis 1 explained 32.8% of the

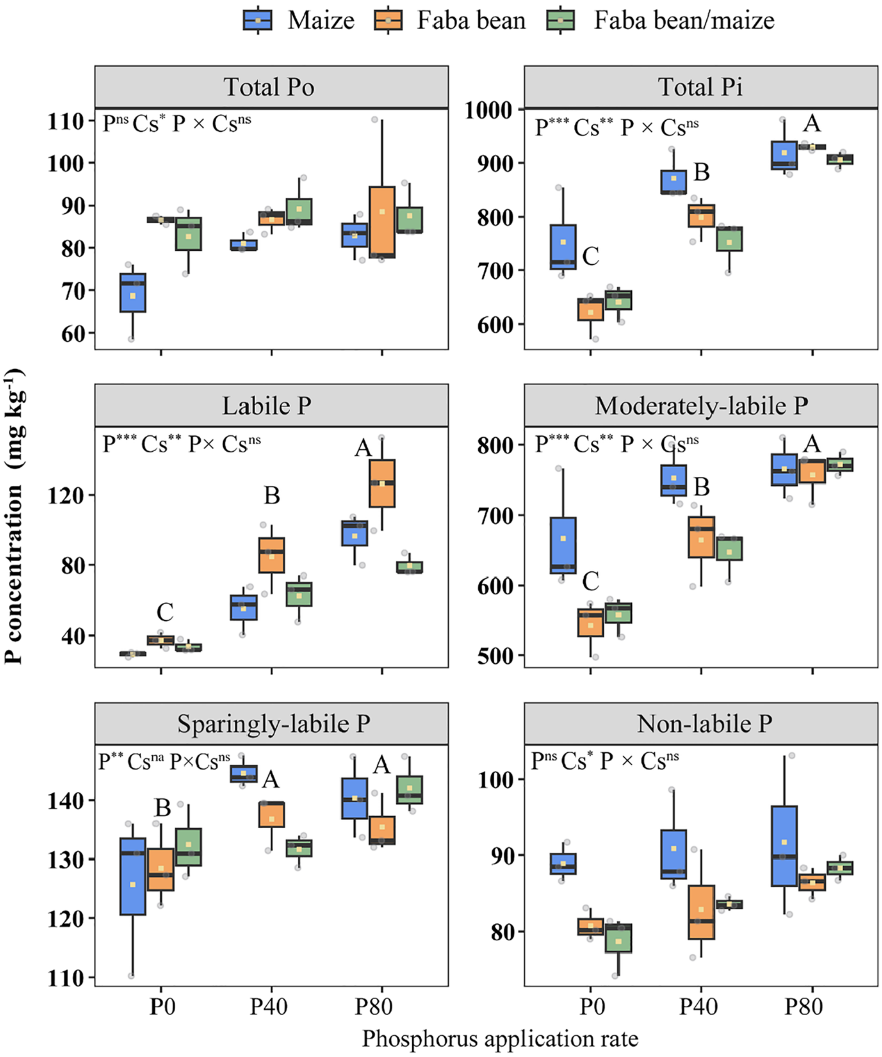

Fig. 4 Effects of phosphorus (P)-application rate (factor P) and cropping system (i.e. sole maize, sole faba bean, and intercropping combination, Cs) on bulk soil P in faba bean/maize combination. P0: 0 kg P ha^{-1}; P40: 40 kg P ha^{-1}; P80: 80 kg P ha^{-1}. Uppercase letters refer to differences among P-application rates. The same letter means there is no significant difference (Tukey HSD). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant

variance, and PCA axis 2 explained 15.7%. The sole chickpea and chickpea/maize intercropping systems showed less total soil organic P but more inorganic P than sole maize did, while the total soil organic P concentration was negatively correlated with rhizosheath phosphatase activity; pH was negatively correlated with total inorganic P and Olsen P (Figs. 6 and 8A; Table S6). In the soybean/maize system, the two principal components explained 53.1% of the variance. The sole maize system was separated from the sole soybean and soybean/maize intercropping system. The sole soybean and soybean/maize intercropping system showed a lower total soil organic P concentration, which was negatively correlated with rhizosheath acid phosphatase activity, rhizosheath alkaline phosphatase activity, rhizosheath microbial biomass P and leaf [Mn] (Fig. 8B). The two PCA axes in the faba bean/maize and oilseed rape/maize systems accounted for 54.2% and 72.9%, respectively. Sole faba bean, sole oilseed rape, and corresponding intercropping systems showed greater soil total organic P concentrations, but lower residual-P concentrations, and the soil residual-P concentrations were negatively correlated with rhizosheath acid phosphatase activity and alkaline phosphatase activity (Figs.4, 5 and 8C,D). In addition, the intercropping systems and companion crops showed a greater labile P pool, but a lower non-labile P pool concentration than sole maize, except for soybean/maize combinations (Fig. 8E-H). All the companion

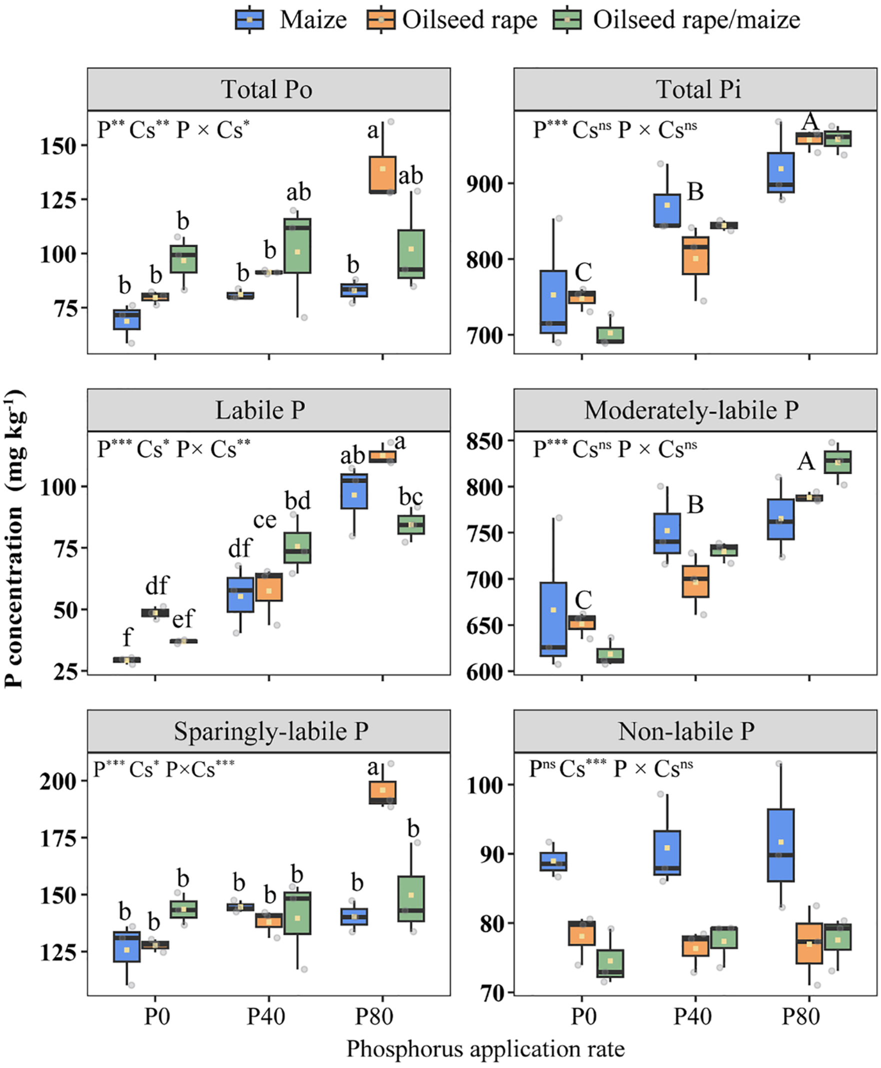

Fig. 5 Effects of phosphorus (P)-application rate (factor P) and cropping system (i.e. sole maize, sole oilseed rape, and intercropping combination, Cs) on bulk soil P in oilseed rape/maize combination. P0: 0 kg P ha^{-1}; P40: 40 kg P ha^{-1}; P80: 80 kg P ha^{-1}. Uppercase letters refer to differences among P-application rates. Lowercase letters indicate differences among treatments if the interaction effect is significant, the middle letters are omitted if there are more than two letters (e.g., the letters 'bd' are concisely expressed as 'bcd'). The same letter means there is no significant difference (Tukey HSD). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ns, not significant

crops and corresponding intercropping systems exhibited higher rhizosheath acid phosphatase activity, rhizosheath alkaline phosphatase activity, rhizosheath microbial biomass P and leaf [Mn] than sole maize which partly reflected the stronger rhizosheath P-mobilizing capacity (Fig. 8).

Discussion

Intercropping with effective P-application rates reduced the soil P surplus

Overall, our results show that intercropping systems were associated with significantly greater biomass, grain yield and P content than the weighted means of corresponding monocultures, irrespective of P-application rate (Fig. 1). Several experiments and meta-analyses have also shown positive effects of biodiversity on productivity and shoot P content (Mudare et al. 2022; Tang et al. 2021; Yu et al. 2020). After 12 years, oilseed rape/maize intercropping reduced the soil residual-P concentration compared with corresponding monocultures (Fig. 2), while achieving greater shoot P content, indicating intercropping was more efficient at accessing soil P compared with monocultures. Long-term P-fertilizer application has caused a P surplus and increased soil P accumulation (Table 1). Excessive P in soil is highly susceptible to eroding into waterways, resulting in eutrophication of

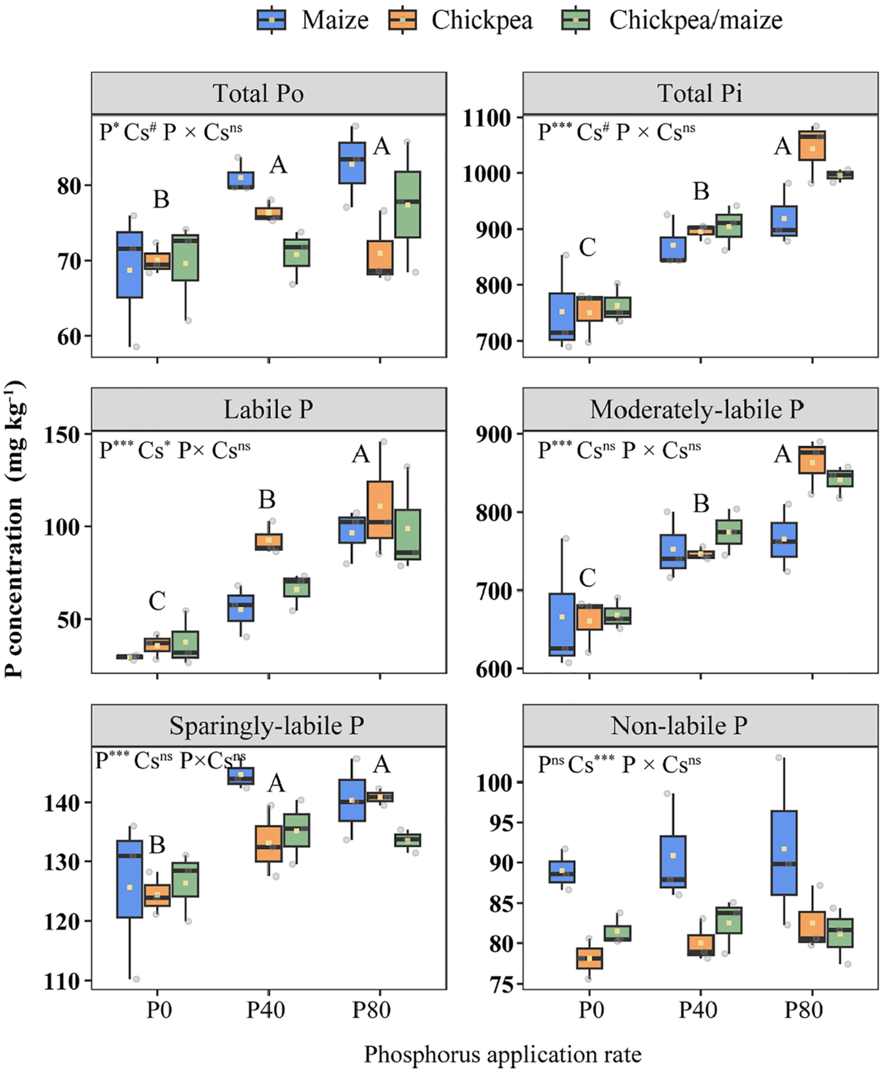

Fig. 6 Effects of phosphorus (P)-application rate (factor P) and cropping system (i.e. sole maize, sole chickpea, and intercropping combination, Cs) on bulk soil P in chickpea/maize combination. P0: 0 kg P ha^{-1}; P40: 40 kg P ha^{-1}; P80: 80 kg P ha^{-1}. Uppercase letters refer to differences among P-application rates. The same letter means there is no significant difference (Tukey HSD). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant

rivers and lakes (Qiu 2010; Zhang et al. 2019). We found that intercropping can reduce the P surplus in soil compared with companion crop monocultures, especially when applying 40 kg P ha^{-1} fertilizer, as the P surplus was close to zero (Table 1). Our findings corroborate previous studies, which showed a negative effect of intercropping on P balance (Liao et al. 2021, 2020). Our evidence suggests that intercropping with appropriate P-fertilizer application can increase productivity, meet plant P requirements, and better maintain soil P balance than monocultures do.

Phosphorus-fertilizer application rates directly affected soil P. Specifically, P application increased soil inorganic P, labile P pool and moderately-labile P pool in all crop combinations, resulting in a greater labile P proportion, a lower sparingly-labile P proportion, and a lower non-labile P proportion than plots without P fertilizer (Figs. 3, 4, 5, 6 and 7). Similar responses were also found in a faba bean/maize intercropping system with two P-fertilizer rates on calcareous soil (Liao et al. 2021) and in an oilseed rape-rice cropping system with five P-fertilizer rates on acidic soil (Yan et al. 2022). The concentration of the labile soil P pool increased with P-application rates which indicates that P fertilizer might accumulate in the labile P fractions and be available to crops (Tian et al. 2020b). This suggests that supplying P fertilizer is crucial to maintain P bioavailability for long-term cultivation.

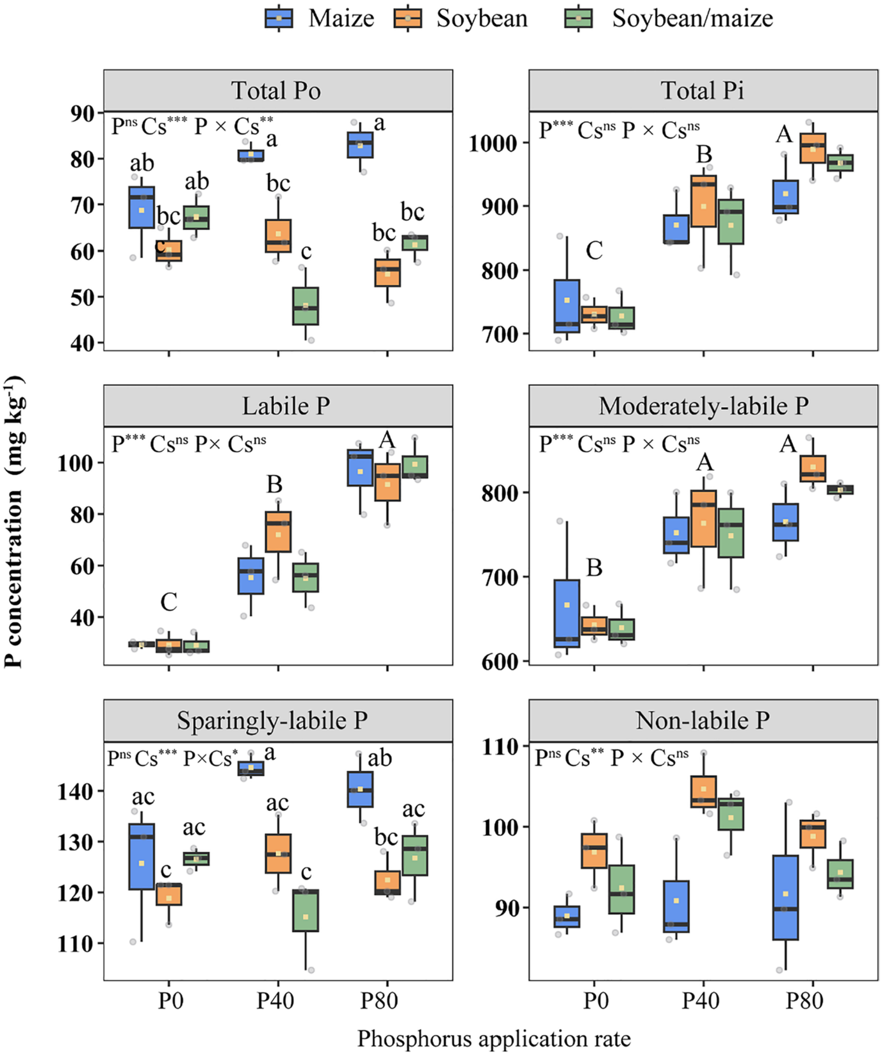

Fig. 7 Effects of phosphorus (P)-application rate (factor P) and cropping system (i.e. sole maize, sole soybean, and intercropping combination, Cs) on bulk soil P in soybean/maize combination. P0: 0 kg P ha^{-1}; P40: 40 kg P ha^{-1}; P80: 80 kg P ha^{-1}. Uppercase letters refer to differences among P-application rates. Lowercase letters indicate differences among treatments if the interaction effect is significant, the middle letters are omitted if there are more than two letters (e.g., the letters ‘ac’ are concisely expressed as ‘abc’). The same letter means there is no significant difference (Tukey HSD). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant

The correlations between P-acquisition strategies and soil P pools in different crop combinations

The results of rhizosheath soil P pools may reflect P-mobilization abilities of crop species, while the results exhibited great variation in the field (Fig. S1). We focused on P-mobilization abilities of selected crops and its impact on companion crop in our previous article (An et al. 2023). Here, we pay more attention to the changes in soil P pools which can show the impact of cropping combinations/systems on P pool dynamics over the experimental period. In the present experiment, chickpea/maize and soybean/maize intercropping systems decreased the total soil Po concentration compared with sole maize; faba bean/maize and oilseed rape/maize intercropping systems showed a greater concentration of total organic P, but a lower non-labile soil P concentration than sole maize (Figs. 4, 5, 6, 7, and 8). The difference in soil P pools was related to diverse P-acquisition strategies of crops (Dissanayaka et al. 2015; Li et al. 2004; Liao et al. 2020; Wen et al. 2020). In chickpea/maize and soybean/maize combinations, we found a negative correlation

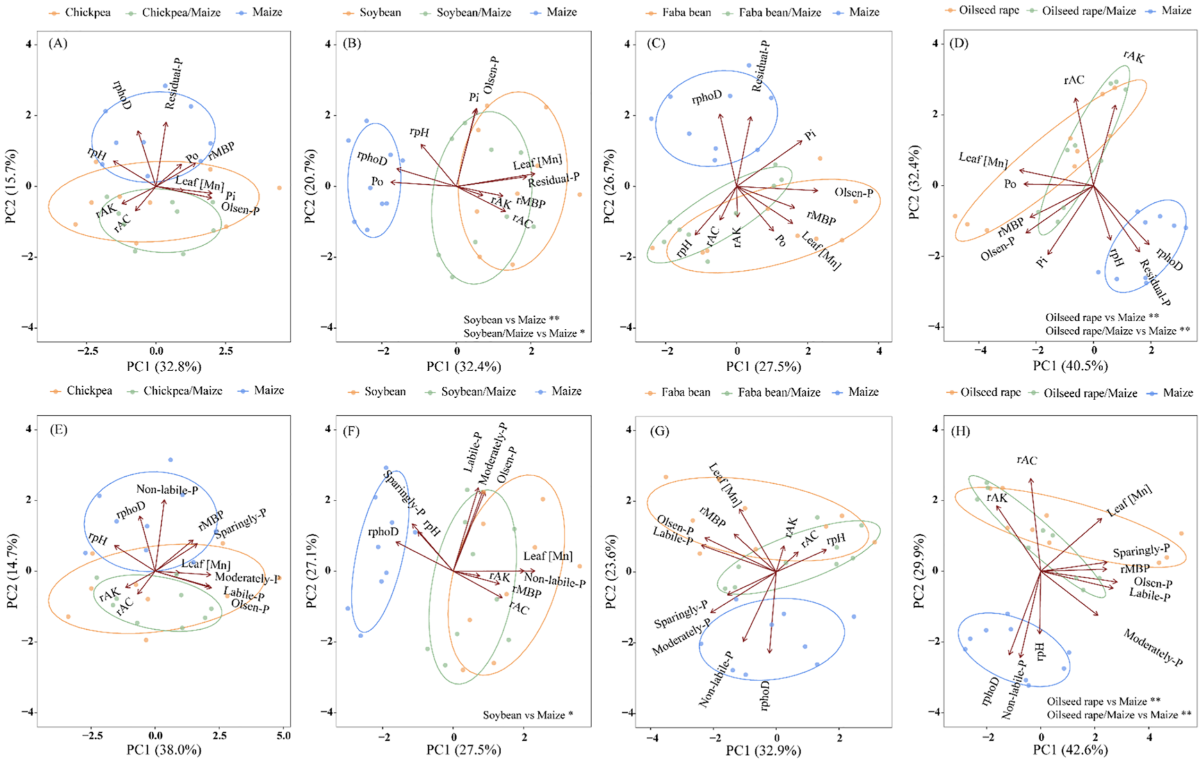

Fig. 8 Principal component analysis (PCA) among plant phosphorus (P)-related traits and soil total bulk soil inorganic P, total bulk soil organic P, bulk soil residual-P and bulk Olsen P of different cropping systems in the four crop combinations in 2020 (A-D).Principal component analysis (PCA) among plant P-related traits and bulk soil P pools and bulk Olsen P of different cropping systems (i.e. sole maize, sole companion crop, and intercropping combination) in the four crop combinations in 2020 (E–H). PC1 represents the first axis, PC2 represents the second axis, and the percentage number represents the proportion of variation the axis could explain. rAC, rhizosheath acid phosphatase activity; rAK, rhizosheath alkaline phosphatase activity; rpH, rhizosheath pH; rMBP, rhizosheath microbial biomass P; leaf [Mn]: leaf manganese concentration; rphoD, rhizosheath *phoD* gene abundance; Pi, total soil inorganic P; Po, total soil organic P; Moderately-P, moderately-labile P;Sparingly-P, sparingly-labile P. * means the differences between cropping systems by PERMANOVA test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant

between total soil organic P concentration and rhizosheath acid phosphatase activity (Fig. 8A,B), consistent with previous reports, indicating that chickpea and soybean can hydrolyze organic P by releasing acid phosphatase, leading to belowground facilitation in organic P utilization in intercropping (Belinque et al. 2015; Kong et al. 2014, 2018; Li et al. 2004). In addition, leaf [Mn] (a proxy for carboxylate release) showed a negative correlation with total soil organic P concentration in the soybean/maize combination (Fig. 8). Rhizosheath pH was negative correlated with the pools of Olsen P, labile P and moderately-labile P in chickpea/maize and faba bean/maize combinations (Fig. 8E,G). The exudate release in the rhizosphere caused acidification in the soil, which is critical to mobilize sorbed soil P into available P (Hinsinger et al. 2015; Lambers et al. 2015).

In faba bean/maize and oilseed rape/maize combinations, we did not find a negative correlation between rhizosheath phosphatase activity and soil total organic P concentration, in which rhizosheath phosphatase activity was negatively correlated with the residual soil P concentration (Fig. 8). In the present experiment, faba bean and oilseed rape exhibited greater phosphatase activity than other species did (An et al. 2023); these probably mobilized a non-labile P pool into available P pools, resulting in a greater labile P pool, but a lower non-labile P concentration in intercropping systems than in sole maize

(Figs. 4, 5 and 8). Leaf [Mn] was negatively correlated with residual-P concentration in oilseed rape/maize combination (Fig. 8). The combination of carboxylates and enzymes may partly explain the depletion of P in soil (Clarholm et al. 2015; Giles et al. 2017). Therefore, combining species with desirable P-acquisition strategies to fully mine soil P pools in the diverse intercropping systems is crucial for sustainable P management.

The correlations between soil microorganisms and soil P in different crop combinations

Soil microorganisms are integral to soil P cycling in agroecosystems (Dai et al. 2020; Wei et al. 2019). The P held within soil microorganisms accounts for approximately 2% to 10% of the total P concentration, which generally exceeds that in plant biomass (Richardson and Simpson 2011). Microbial biomass P is potentially available to plant species and modulates soil P availability (Liebisch et al. 2014). The immobilized P within the biomass is maintained in labile forms and is protected from reactions with soil (Olander and Vitousek 2004). In this study, sole companion crops and intercropping systems had greater rhizosheath microbial biomass P than sole maize which indicates a potentially stronger competition of soil microorganisms to hold soil P (Fig. 8; Table S8). We also found a positive correlation between rhizosheath microbial biomass P and soil Olsen-P, except in the soybean/maize combination (Fig. 8). This partly supports that microbial biomass P can reflect soil P fertility (Peng et al. 2021). The changes in microbial biomass P are related to P concentration in the soil which depends on P-fertilizer application and agronomic management (Hallama et al. 2021; Liao et al. 2022; Peng et al. 2021).

The *phoD* gene, encoding an alkaline phosphatase that mediates organic P hydrolysis, is negatively correlated with soil organic P concentration (Fraser et al. 2015). Consistent with these findings, we found negative correlations between soil organic P and rhizosheath *phoD* gene abundance in oilseed rape/maize combinations (Fig. 8), indicating the contribution of microorganisms in P utilization. However, *phoD* gene abundance did not show a positive correlation with alkaline phosphatase activity in our study (Fig. 8). Similar results were also found in a soybean pot trial with three P-input rates (Tian et al. 2021).

Sole maize showed significantly higher rhizosheath *phoD* gene abundance than companion species and intercropping systems (Fig. 8; Table S8) which might be related to the microorganisms associated with arbuscular mycorrhizal fungal hyphae (Lang et al. 2022; Liu et al. 2018). Microorganisms associated with arbuscular mycorrhizal fungal hyphae can hydrolyze organic P and promote organic P turnover (Wipf et al. 2019). Other microbial functional genes of P cycling and microbial compositions need to be tested in future research. Thus, the results of microorganisms may be a consequence of intercropping, species characteristics, and P-fertilizer rate, which is related to P uptake and soil nutrient dynamics (Dai et al. 2020; Roohi et al. 2020; Yang et al. 2022).

The species differed in their P-acquisition strategies as well as associations with soil microorganisms, which are integral to the soil P cycle and mediate P availability, which is a crucial mechanism underpinning belowground facilitation in intercropping systems (Yu et al. 2021a). Intercropping significantly increased productivity and plant P content, compared with monocultures. Among the intercropping systems, chickpea/maize intercropping showed the greatest grain yield advantage, followed by faba bean/maize (Fig. 1). The chickpea/maize intercropping system had less total Po and non-labile P but more labile P than sole maize which correlated with leaf [Mn] and rhizosheath phosphatase activity (Figs. 6 and 8A,E); faba bean/maize intercropping system had less non-labile P but more labile P than sole maize, and the depletion of non-labile P was correlated with pH and rhizosheath phosphatase activity (Figs. 4 and 8C,G). The greater release of carboxylates and phosphatase in the rhizosheath would mobilize sorbed P and organic P into available P pools which promotes P uptake (Clarholm et al. 2015; Li et al. 2014; Giles et al. 2017). Thus, soil P pools and bioavailability, pH, and rhizosheath phosphatase activity played an important role in enhancing plant P uptake, which further contributes to crop productivity of intercropping (Yang et al. 2022).

Species in intercropping systems showed greater P-mobilizing capacities while changing soil P pools and enhancing shoot P content and aboveground biomass in the four crop combinations. Thus, it is understandable that plant physiological traits were correlated with different soil P fractions. Previous studies showed that intercropping enhances nutrient-use efficiency, increases nutrient uptake, and enhances crop productivity, while not all crop combinations have an intercropping advantage (Li et al. 2021; Tang et al. 2021). Efficient P-mobilizing species intercropped with inefficient species enhanced the availability of soil P by mobilizing sorbed soil P which increases the P acquisition of inefficient neighbors (Li et al. 2014). Crop species showing greater morphological plasticity in intercropping may acclimate to the heterogeneous environment, leading to P-uptake advantages via interspecific facilitation (Schneider and Lynch 2020; Yu et al. 2020). Furthermore, when P-efficient genotypes are combined with diverse agronomic strategies (i.e. intercropping) with complementary P-acquisition strategies under effective P-fertilizer management, the P-use efficiency may increase (Cong et al. 2020). Thus, establishing desirable species combinations is vital to enhance productivity and shoot P uptake, and maintain the soil P balance under high chemical fertilizer inputs (Cheriere et al. 2020).

Long-term P-fertilizer application results in a large amount of legacy P accumulating in soil or lost to the environment. Soil legacy P represents a secondary P resource, which can substitute for P fertilizer and become a source for crop use (Rowe et al. 2016). Species exhibit different P-acquisition strategies to obtain enough available P for their growth (Li et al. 2007; Zhang et al. 2016) which can help mine legacy P in different soils. Overall, optimizing a rational intercropping system involving complementary P-acquisition strategies is crucial for sustainable P management. The P-fractionation procedures modified by Hedley have limitations, and further analyses should be carried out in conjunction with the methods such as Energy Dispersive X-ray Spectra (EDS) analysis (Barrow et al. 2021).

Conclusion

Our study shows that intercropping significantly increased aboveground biomass, grain yield and plant P content, significantly mitigating soil P balance compared with companion crop monocultures. Under 40 kg ha^{-1}, intercropping can exhibit a relatively high P content and prevent a P surplus in soil. Furthermore, we explored the correlations between P-acquisition strategies and soil P pools in different crop combinations under field conditions. Chickpea/maize and soybean/maize intercropping systems significantly decreased the soil organic P pool compared with sole maize under P-application conditions. Faba bean/maize and oilseed rape/maize intercropping systems significantly decreased the non-labile soil P pool but increased that organic P pool more than the sole maize system did. Furthermore, rhizosheath acid phosphatases and carboxylates may contribute to depleting sparingly-available soil P (organic P or non-labile P pools) in different crop combinations; rhizosheath microbial biomass P and *phoD* genes may also be related to the increase of soil P availability through mobilizing organic P. These results show that P-acquisition traits of species in intercropping systems change soil P pools to enhance the system's P uptake while reducing P-fertilizer requirements. Our findings highlight the importance of P-acquisition strategies in designing intercropping systems and in decreasing P accumulation in soil for sustainable P management.

Authors' contributions Long Li, Ran An, Rui-Peng Yu, Yi Xing, Xing-Guo Bao, and Jiu-Dong Zhang designed and managed the experiment. Ran An, Rui-Peng Yu, and Yi Xing collected data and performed analyses. Ran An drafted the paper, and Rui-Peng Yu, Hans Lambers and Long Li contributed substantially to the revisions.

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

Declarations

Competing interest The author declare to have no competing interests.

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