Adding intercropped maize and faba bean root residues increases phosphorus bioavailability in a calcareous soil due to organic phosphorus mineralization

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Abstract

Background and aims Root residues are an important factor influencing soil phosphorus (P) availability for crop uptake, but how the residues from different species combinations in sole cropping or intercropping systems affect soil P pools remains unclear.

Methods Maize and faba bean were planted as either sole crops or intercrops in a P-deficient calcareous soil in cycle 1, and the same cropping systems were added with and without root residues of single or both species in the 2nd and 3rd cycles in a greenhouse experiment. Biomass and P concentration were determined after each cycle, and soil P fractions were measured after final cycle.

Results Addition of a mixture of intercrop root residues increased biomass, total P content, microbial biomass P concentration and soil acid phosphatase activity, compared with addition of root residues of a single crop. Changes in Hedley soil P fractions varied with root residue sources. Sole maize root residue with high C/P ratio caused a considerable depletion of inorganic P (NaHCO₃-Pᵢ, NaOH-Pᵢ and 1 M HCl-Pᵢ), and sole faba bean root residue with lower C/P ratio caused a large depletion in Resin-P and NaHCO₃-Pₒ fractions, and the root residue of intercrops with an intermediate C/P ratio depleted more of the NaHCO₃-Pₒ and conc. HCl-Pₒ fractions. Without adding root residues, sole faba bean depleted more of the Resin-P, NaHCO₃-Pᵢ, NaOH-Pᵢ and NaHCO₃-Pₒ fractions than the other two cropping systems did, because it acquired more P from the soil than other crops did.

Conclusions Adding a root residue mixture of maize and faba bean accelerated soil organic P mineralization (NaHCO₃-Pₒ and conc. HCl-Pₒ) by increasing microbial biomass P concentrations and acid phosphatase activities, and thus enhanced the intercropping advantage in terms of biomass and total P content in a P-deficient soil.
Keywords  Acid phosphatase · Maize/faba bean intercropping · Microbial biomass P · P fractionation · Root residues · Soil properties

Introduction

Phosphorus (P) is an essential macronutrient for plant growth and yield (Cordell and White 2014). The major problem with plant P nutrition is not the P concentration in soil, but its availability to plants, as inorganic P (P\textsubscript{i}) is readily immobilized by oxides of iron and aluminium in acid soils and by calcium in calcareous soils (Hinsinger 2001; Shen et al. 2011). The reliance on large amounts of P-fertilizer input into intensive agricultural systems leads not only to the gradual depletion of finite phosphate rock reserves, but also to environmental problems such as eutrophication of waterways (Fixen and Johnston 2012). Therefore, it is important to identify cropping systems capable of increasing P-acquisition efficiency and enhancing P cycling in agricultural systems, ultimately reducing P-fertilizer input and environmental impact, while securing a sustainable food production (Cong et al. 2020).

Soil P transformation involves complex biochemical processes (Alamgir et al. 2012; Zhang et al. 2004; Zheng et al. 2002), and soil P depletion is primarily caused by plant P uptake. Phosphorus uptake by plants depletes soluble P\textsubscript{i}, which initiates a shift in equilibrium of other soil P pools, mainly by desorption and dissolution, causing changes in soil P dynamics (Kamh et al. 2002). Crews (1996) reported that depletion of labile P\textsubscript{i} pools can trigger the release of P from HCl-P\textsubscript{i} and NaOH-P\textsubscript{i} pools, suggesting P can be desorbed or dissolved from calcium phosphate minerals as well as from non-occluded sites on Fe and Al oxides to replenish the labile P\textsubscript{i} pools. Plants also affect soil P cycling during microbial crop residue decomposition, as P from crop residues can be released to replenish soil solution P\textsubscript{i} or immobilized in microbial biomass (Damon et al. 2014). Bünnemann et al. (2004) showed that less than 20% of P from crop residues enters the Resin-P and microbial biomass P (Microbial-P) pools and Noack et al. (2014) found that more than 40% of crop P residues are converted to Microbial-P, suggesting that the effect of crop residues on soil P availability may be related to the quantity and quality of the residues added (Damon et al. 2014; Soon and Arshad 2002; Stewart et al. 2015). Generally, residues with a P concentration >3 mg P g\textsuperscript{-1} and carbon (C)/P ratio < 200 favor net P mineralization, because these residues contain sufficient P to exceed that taken up by microbial biomass (Alamgir et al. 2012; Damon et al. 2014). Compared with cereal residues, legume residues have higher P concentrations and a lower C/P ratio due to the greater capacity of legumes to acquire soil P (Nuruzzaman et al. 2005). However, immobilization of soil P occurs when residue P content is insufficient to meet the requirement of microbial growth. Hence, the interaction between crop residues and microbial biomass determines the dynamics of P release in soils.

Soil P transformations may also be affected by practices such as sole cropping or intercropping, which is attracting increasingly interests from both scientists and growers (Hinsinger et al. 2011; Li et al. 2007, 2008; Liao et al. 2020). Intercropping, referring to two or more crops grown together on the same field for a certain overlapping period, is a system with high efficiency in P use (Tang et al., 2021; Wang et al. 2020), and the underlying mechanisms have been explored in different studies. Legumes such as faba bean (Vicia faba L.), white lupin (Lupinus albus L.) and chickpea (Cicer arietinum L.) can increase P acquisition by using root exudates (e.g., protons, organic anions, and acid phosphatases), to solubilize P that is largely unavailable to other crops (Cu et al. 2005; Li et al. 2007; Veneklaas et al. 2003). Cereals such as maize (Zea mays L.) and wheat (Triticum aestivum L.) acquire more available soil P through a greater exploration of the soil volume, so it is possible for cereals to benefit from growing with legumes in term of P acquisition (Dissanayaka et al. 2015; Li et al. 2007; Wang et al. 2020; Tang et al., 2021). Thus, complementarity between cereal and legume crops in nutrient-foraging strategies contributes to a P-use advantage of intercropping (Li et al. 1999, 2020; Zhang et al. 2016). There are several studies indicating that mixing cereal and legume residues may enhance the growth and P uptake of crops, because mixed residues provide a greater variety of substrates and ecological niches for microbes (Kaewpradit et al. 2009; Hassan et al. 2013; Yan et al. 2010; Zhou et al. 2020). However, little is known about the response of microbiological properties and
soil P pools to removal or addition of crop residues in maize/faba bean intercropping and whether it affects intercropping advantage.

We aimed to assess how addition of root residues of single or both species of maize and faba bean alters the dynamics of P pools in a P-deficient calcareous soil and what affects the intercropping effect. We sequentially grew three cycles in pot experiments with the purpose to mimic the cumulated legacy effect generated by crop growth. As one aspect of legacy effects, root residues that remained in the pot after shoots were removed were investigated for their role in achieving an intercropping effect. We tested the following hypotheses: (i) adding root residues increases crop growth and P uptake, consequently leading to intercropping effect; (ii) maize root residues with high C/P induce greater net P immobilization and more depletion of soil P pools than faba bean root residues with lower C/P do; (iii) adding intercrop root residues promotes crop growth more than adding root residues of sole crops in a P-deficient soil, because mixed root residues may be more suitable for microbial growth and therefore accelerate soil organic P mineralization and enhance nutrient availability.

Materials and methods

Experimental set-up

The study included three cycles carried out in a glasshouse at China Agricultural University (40°N, 116.3°E), Beijing, China. The 1st cycle was from December 2017 to March 2018, the 2nd from April to June 2018, and the 3rd from December 2018 to February 2019. During the 1st and 3rd cycles, the glasshouse temperature was maintained at 21–25 °C during the day and 15–18 °C at night, with a photoperiod of 10–12 h throughout the growing period. During the 2nd cycle, the temperature was 24–28 °C during the day and 18–20 °C at night, with a photoperiod of 12–14 h during the growing phase. This study used a glasshouse instead of a growth chamber. The temperature in the glasshouse varied to some extent with the temperature outside, so it would be different between winter and summer. The glasshouse in winter was supplemented with electric light, but the light was not the same intensity as the natural light.

Growth and harvest of the first cycle

We used the soil collected from 0–20 cm topsoil at the Quzhou Experimental Station (36.9°N, 115.2°E; 39.6 m a.s.l.), Hebei Province, China. It is a calcareous alluvial silt loam with the following properties: Olsen-P 5.6 mg kg⁻¹, total C 18.2 g kg⁻¹, total N 1.08 g kg⁻¹, total P 0.7 g kg⁻¹, NH₄OAc-potassium (K) 32.3 mg kg⁻¹ and pH 8.3 (2.5:1 water/soil), all of which were determined before growing plants. The cycle included four treatments (no-plants control, sole maize, sole faba bean and maize/faba bean intercropping), and each treatment had ten replicates. Each pot (top diameter, 20 cm; bottom diameter, 14 cm; height, 14 cm) contained 2.0 kg of the air-dried soil that was beforehand passed through a 2-mm sieve. To ensure sufficient nutrient supply, plants were also fertilized with basal nutrients at the following rates (mg kg⁻¹ soil): 200 N as Ca(NO₃)₂·4H₂O, 100 K as K₂SO₄, 50 Mg as MgSO₄·7H₂O, 2.2 Mn as MnSO₄·H₂O, 2.3 Zn as ZnSO₄·7H₂O, 0.51 Cu as CuSO₄·5H₂O, 0.12 B as H₃BO₃, 0.02 Mo as (NH₄)₆Mo₇O₂₄, and 0.88 Fe as EDTAFe-Na. No P was applied.

The seeds of maize (Zea mays L. cv ZD958) and faba bean (Vicia faba L. cv Lincan5) were first surface-sterilized in 30% v/v H₂O₂ for 10 min, and then rinsed with deionized water. They were soaked in a saturated CaSO₄ solution for 12 h and then grown for 48 h in Petri dishes covered with wet filter papers. After emergence of the radicle, two maize plants or four faba bean plants were kept in each pot for the corresponding sole cropping, in which the plant density was similar to that in the field (Liao et al. 2020). To keep plant density consistent between cropping systems, one maize plant and two faba bean plants were grown in each pot for intercropping. In addition, the study included a no-plant treatment, in which no plant was grown but the soil was treated identically as other treatments.

All pots were arranged in a completely randomized design, and re-randomized weekly during the growing period. The plants were watered every day to maintain a soil pot capacity of 80%. The cycle lasted 91 days, and then plants were harvested. At harvest, after shoots were cut above the soil surface, the plastic pot was cut into halves using scissors and then we removed the soil block containing roots. In the process of separating roots from soil, we sampled roots in two steps with the purpose of collecting as
many roots as possible. First, we carefully removed soil from roots; then, the soil was passed through a 1-mm sieve and all visible roots inside and outside the sieve were collected with tweezers (Wen et al. 2017). Shoots and roots were used for measuring biomass and P concentration. The soils of 10 replications of the same treatment were mixed, homogenized, and stored at 4 °C until the start of cycle 2.

Growth and harvest of the second and third cycles

In the 2nd cycle, we investigated the influence of root residues of the cycle 1 crops on plant growth (Fig. S1). The cycle 2 experiment used the cycle 1 soil, and the soil was added with or without root residues. The stored soil of each treatment in cycle 1 was randomly divided into two even parts; to one part we added corresponding root residues of previous crops (precrops), and to the other part no residues were added. In root residue-added treatments, root samples of precrops were first oven-dried at 45 °C for four days to minimize alteration of plant chemistry, and then cut with scissors into pieces of less than 2 mm before homogeneously mixing them with the soil. The root residues were added at a rate of 2.6 g kg⁻¹, containing a P amount ranging from 1.9 to 2.4 mg kg⁻¹. Detailed information of the root residues is presented in Table 1. All pots received the same amount of nutrients as those in the 1st cycle. The soils were refilled in the same pot, and then used for growing the corresponding crop as in the 1st cycle, i.e. sole maize or sole faba bean-cultured soil, and intercrops planted in both maize and faba bean-cultured soil. The 2nd cycle experiment had seven treatments in total including one no-plant control and six treatments comprising three cropping systems (sole maize, sole faba bean and intercropping) without or with corresponding pre-crop root residues, and each treatment had five replicates. Plants were grown for 56 days, and shoots and roots were harvested separately. Biomass and P content of the shoot samples were determined, and root samples were treated in the same way as in the 1st cycle. Soils of all treatments were collected separately in the same way as that in the 1st cycle, and then stored at 4 °C to be used for the 3rd cycle experiment.

The 3rd cycle had the same experimental setup as the 2nd, but the amount and the nature of the root residues were different from those in the previous run. The root residues used in this cycle had a P content ranging from 1.0 to 1.9 mg kg⁻¹, and a C/P ratio from 257 to 533 (Table 1). Plants were grown for 75 days, and then we sampled shoots, roots, and soil, separately, for different measurements as mentioned below.

The soil samples for chemical and Hedley P-fraction measurement

At the harvest of the 3rd cycle, plants were separated into shoots and roots. Roots were carefully removed from the soil, and shaken gently to remove loosely adhering soil. The soil adhering to roots was defined as rhizosheath soil (Pang et al. 2017). Then roots were transferred to a tube containing 50 mL of 0.2 mM CaCl₂ and gently shaken to dislodge the rhizosheath soil, followed by shaking for 5–10 s to create a homogeneous suspension (Veneklaas et al. 2003).

### Table 1: Amount of total phosphorus (P), carbon (C), nitrogen (N), C:N and C:P ratios in added root residues used in the second and third cycle (n = 5, ± standard deviation)

| Root residue | Biomass (g kg⁻¹) | Total P (mg kg⁻¹) | Total C (mg kg⁻¹) | Total N (mg kg⁻¹) | C:N | C:P |
|--------------|------------------|------------------|------------------|------------------|-----|-----|
| In the second cycle | | | | | | |
| Sole maize | 2.6 | 1.9 ± 0.1b | / | 30 ± 1b | / | / |
| Sole faba bean | 2.6 | 2.4 ± 0.2a | / | 57 ± 6a | / | / |
| Intercropping | 2.6 | 2.3 ± 0.2a | / | 52 ± 11a | / | / |
| In the third cycle | | | | | | |
| Sole maize | 1.2 | 1.0 ± 0.0b | 540 ± 6a | 18 ± 1c | 31 ± 1a | 533 ± 20a |
| Sole faba bean | 1.2 | 1.9 ± 0.1a | 492 ± 12a | 38 ± 1a | 13 ± 0c | 257 ± 16c |
| Intercropping | 1.2 | 1.4 ± 0.3b | 505 ± 119a | 28 ± 9b | 18 ± 2b | 367 ± 29b |
We also sampled moist bulk soil from the no-plant control and placed it in 0.2 mM CaCl₂ (2.5:1 water/soil). A subsample of the extract was filtered into a 1 mL high performance liquid chromatography (HPLC) vial through a 0.22 μm syringe filter for carboxylate analysis in the rhizosheath and bulk soil (Shen et al. 2003). In order to determine the activity of acid phosphatase in the rhizosheath and bulk soil, a 0.5 mL suspension was placed in a 2 mL centrifuge tube with 0.4 mL of 0.2 M sodium acetate buffer (pH 5.2) and 0.1 mL of 0.15 M p-nitrophenyl phosphate (pNP) substrate added, and then incubated at 30 °C for 30 min; p-nitrophenol (pNP) formed after hydrolysis was subsequently extracted with 0.5 mL of 0.5 M NaOH (Alvey et al. 2001). The pNP concentration was measured using a spectrophotometer (UV757T, Shanghai Instrument Co. Ltd., Shanghai, China) at a wavelength of 405 nm. The amount of rhizosheath soil collected differed among treatments. To eliminate effects of a different water/soil ratio on pH determination of rhizosheath extracts, a modified pH (water/soil ratio was adjusted to 2.5:1) was calculated from the measured pH according to Li et al. (2010). The delta pH was defined as the pH difference between rhizosheath and bulk soil, and this calculation was also applied to delta organic acid and delta acid phosphatase activity.

At the harvest of the 3rd cycle, the bulk soil of each treatment was collected, and then Microbial-P, sequential Hedley P fractions, total N and organic C were measured. The air-dried soil samples were used to analyze sequential P fractions as described by Hedley et al. (1982) and modified by Tiessen and Moir (1993). Different extractants were added to 0.5 g of soil in the following sequential order: anion-exchange resin (referred to as Resin-P) and 0.5 M NaHCO₃ (referred to as NaHCO₃-Pi and NaHCO₃-Po), denoting labile inorganic and organic P; 0.1 M NaOH (referred to as NaOH-Pi and NaOH-Po) and 1.0 M HCl (referred to as 1 M HCl-Pi), denoting moderately labile inorganic and organic P; and concentrated HCl (referred to as conc. HCl-Pi and conc. HCl-Po) and concentrated H₂SO₄–H₂O₂ (referred to as Residual-P), denoting non-labile inorganic and organic P. After adding each extractant, we repeated the following steps. The suspension was first stirred for 16 h in a shaker (200 rpm), then centrifuged for 10 min at 25,000 × g at 0 °C; after passing through a 0.45-μm membrane filter, the supernatant was stored prior to colorimetric analysis. Inorganic P was determined according to the method of Murphy and Riley (1962). Like in many previous studies (Li et al. 2008; Hassan et al. 2012; Soltangheisi et al. 2018), we did not directly measure organic P concentration, and it actually was the difference between total P and inorganic P. Organic P concentration in the different extracts (NaHCO₃-P, NaOH-P and conc. HCl-P) was the difference between total P and inorganic P concentration. The total P concentration in the different extracts (NaHCO₃-P, NaOH-P and conc. HCl-P) were determined after digestion of each extract with a 0.2 g ammonium persulfate [(NH₄)₂S₂O₈] solution and 4 mL of 0.9 M H₂SO₄ in an autoclave (103 kPa, 121 °C) for 1 h (Li et al. 2008). To determine the accuracy of the test method, phytate (phytic acid sodium salt hydrate, Sigma, USA) was used as a standard organic P-containing compound to carry out the recovery test, which was 78%. Relatively low recovery of phytate might be because ammonium persulfate incompletely digested phytate-based P (Zhao et al. 2017). The accuracy of the sequential P extraction was assessed by comparing the sum of all P fractions with the concentration of the total P concentration determined according to Ai et al. (2017). The sum of the P fractions was, on average, 110% of the measured total P concentration. The P balance was the difference between P input from root residues and plant seeds and output with the harvest of the plant over three cycles (Table S1). Detailed information of the procedures of soil P extraction and P pool grouping was provided in a previous study (Liao et al. 2020).

Soil microbial biomass P (referred to as Microbial-P) was estimated using the fumigation extraction method (Brookes et al. 1982). The associated processes were as follows: fresh soil was first adjusted to a soil moisture content ranging from 10–15%, and then three individual portions of 2.88 g fresh soil (about 2.5 g dry soil) were weighed and added to jars separately. One portion was fumigated with chloroform, one portion was left unfumigated, and another was spiked with 0.25 mL of 250 mg P as KH₂PO₄ L⁻¹ to assess P recovery. Three soil portions were incubated in a vacuum desiccator for 24 h, followed by extracting with 50 mL of 0.5 M NaHCO₃, respectively. The P concentration was measured using the molybdovanadophosphate method (Olsen et al. 1954). Microbial biomass P was calculated as
chloroform-released P concentration by dividing by 0.4, i.e. assuming that 40% of the P in the biomass is rendered extractable as P by chloroform (Brookes et al. 1982). Chloroform-released Pi concentration was the concentration difference of P between fumigated and non-fumigated soils. Microbial biomass P was corrected by using recovery of added phosphate.

Plant samples for chemical analyses

All shoot and root samples collected across three cycles were first oven-dried for 72 h at 70 °C and then weighed. After grinding, the samples were used to determine P concentrations as detailed above (Johnson and Ulrich 1959). Carbon and nitrogen (N) concentration of plant and soil samples were determined with an Elemental Analyzer (vario MACRO CUBE, Hanau, Germany).

Calculations and statistical analyses

We compared the observed and expected values of shoot biomass, root biomass and total P content (for the whole plant) to assess the intercropping advantage (Loreau and Hector 2001). If the observed value was significantly greater than the expected value, then there was an intercropping advantage. These two values were calculated according to the following formula:

\[ \text{Observed biomass} = M_1 + F_1 \]
\[ \text{Expected biomass} = M_2 + F_2 \]

where \( M_1 \) and \( F_1 \) are the real shoot or root biomass or total P content of maize and faba bean measured for the intercrops; \( M_2 \) and \( F_2 \) are the weighted values for those parameters, and the weight is the corresponding relative density, that is the ratio of number of plants in the intercropping system to that in the sole cropping system (the relative density of each crop was 0.5 in this study).

Three-way analysis of variance (ANOVA), considering crops (maize vs faba bean), cropping systems (sole cropping vs intercropping) and root residue treatments (removed vs added) for soil P fractions, total N and organic C. A t-test was performed for observed and expected values under the same root residue treatment. Significant differences among means were separated by LSD test at a significance of \( P < 0.05 \). All ANOVAs were conducted using the SAS software package (SAS v.8.0).

Results

Intercropping advantage

The maize/faba bean intercropping system showed an advantage in biomass and total P content across three cycles (Fig. 1a-c). Compared with the expected values in the corresponding cycles, the observed shoot biomass was 1.2 times greater in the 2nd cycle when root residues were added, and 1.3 or 1.2 times greater in the 3rd cycle as root residues were added or not, respectively (Fig. 1a). In the 3rd cycle, the observed root biomass was 1.9 or 1.7 times greater than the expected values when root residues were added or not, respectively (Fig. 1b). Compared with the corresponding expected total P content, the observed total P content was 1.2 times greater in the 1st cycle, and also in the 2nd cycle with addition of root residues, and in the 3rd cycle with or without residue addition (Fig. 1c). Addition of root residues had a positive impact on biomass and total P content of intercropping compared with no residue addition, indicating enhanced intercropping advantages. In the 2nd cycle, root residue addition significantly increased the observed shoot biomass and total P content by 17% and 21%, compared with the corresponding expected values (Fig. 1a, c). However, the differences in either biomass or P content between the expected and observed values were negligible in the absence of root residues. In the 3rd cycle, adding root residue increased the observed shoot and root biomass and the observed total P content by 29%, 86%, and 23%, respectively, compared with the corresponding expected values (Fig. 1a-c). Those increases in observed shoot and root biomass and observed total P content without residue addition were 22%, 67%, and 20%, respectively, all lower than the changes under root residue addition.
Plant growth and P uptake affected by intercropping and root residue addition

Regarding individual plant performance, biomass and P content of both shoots and roots of faba bean were greater than those of maize, independent of whether root residues were returned to the soil or not (Tables 2 and S2). Shoot and root biomass and total P content of intercropped faba bean were 28%, 63%, and 37% greater, respectively, as compared with the sole crop. Without root residue addition, compared with the sole crop, intercropped faba bean had a significantly enhanced shoot and root biomass and total P content, 32%, 64% and 77% greater in the 1st cycle, and 40%, 117% and 49% in the 3rd cycle, but those values were rarely altered in the 2nd cycle. Under root residue addition, intercropped faba bean had a significantly greater shoot and root biomass and total P content, 46%, 143% and 46%, respectively, in the 3rd cycles, and an increased total P content of 17% in the 2nd cycle.

In contrast to faba bean, maize in intercropping produced less shoot and root biomass, and had a lower total P content, by 20%, 38% and 31%, respectively compared with the sole maize cropping (Table 2). Without root residue addition, compared with the sole crop, intercropped maize had a lower shoot and root biomass and total P content, by 69%, 72%, and 76% in the 1st cycle, a lower shoot biomass and total P content, by 36% and 51% in the 3rd cycle, respectively, but it had no impact on those values in the 2nd cycle. With root residue addition, intercropped maize increased its shoot biomass and total P content by 59% and 27% in the 2nd cycle, but these values decreased by 25% and 41% in the 3rd cycle, respectively.

The impact of root residue addition on crop performance varied with residue sources and experimental duration. In the 2nd cycle, compared with the treatment without residue added, addition of corresponding sole crop root residues increased shoot biomass and total P content of sole maize by 16% and 24%, but it did not alter those of sole faba bean. However, addition of root residues mixture of intercropped maize and faba bean enhanced shoot and root biomass and total P content of intercropped maize by 52%, 75%, and 49%, and the total P content of intercropped faba bean by 30% (Table 2). In the 3rd cycle, addition of root residues of sole crops or intercrops correspondingly increased shoot and root biomass and total P content of sole maize by 9%, 20%, and 13%, those of intercropped maize by 29%, 25%, and 37%, those of sole faba bean by 11%, 0%, and 39%, and those of intercropped faba bean by 16%, 31%, and 36%, respectively (Table 2). Hence, adding intercrop root

\[ \text{Fig. 1} \text{ Observed and expected shoot biomass (a), root biomass (b), and total phosphorus (P) content (c) of intercrops removed or added root residues in three cycles. The values are means of five replicates + standard deviations. Significant differences between observed values and expected values: no *, } P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001 (t-test) \]
residues resulted in greater biomass and total P content than adding corresponding root residues of a sole crop.

Soil pH, organic acid concentrations and acid phosphatase activity in the rhizosheath

The rhizosheath pH was lower than that of the bulk soil (Fig. 2a), while the rhizosheath organic acid concentrations and acid phosphatase activity were higher (Fig. 2b, c). Faba bean had a greater effect on soil pH, organic acid concentrations and acid phosphatase activity than maize did, but intercropping did not significantly alter those effects compared with the corresponding sole crop. Exceptionally, intercropped faba bean increased the rhizosheath organic acid concentration by 6.3 μmol g⁻¹ when root residues of both species were added, compared with sole faba bean. Compared with no root residue addition, adding faba bean root residues reduced pH by 0.14 units, and increased organic acid concentrations by 1.0 μmol g⁻¹ in sole faba bean rhizosheath, and increased acid phosphatase activity by 930 and 1173 μg pNP h⁻¹ g⁻¹, in rhizosheath of sole and intercropped faba

Table 2  Biomass of shoots and roots and total phosphorus (P) content in different cropping systems in treatments involving different root residues in the first, second and third cycle

| Crops        | Cropping systems/ root treatments | Shoot biomass (g plant⁻¹) Removed | Root biomass (g plant⁻¹) Removed | Total P content (mg plant⁻¹) Removed |
|--------------|----------------------------------|-----------------------------------|-----------------------------------|--------------------------------------|
|              |                                  | Removed | Added | Removed | Added | Removed | Added |
| In the first cycle
| Maize        | Sole                             | 4.2 ± 0.4a | / | 1.8 ± 0.1b | / | 5.1 ± 0.3b | / |
|              | Intercrop                        | 1.3 ± 0.1c | / | 0.5 ± 0.1d | / | 1.2 ± 0.1d | / |
| Faba bean    | Sole                             | 3.4 ± 0.2b | / | 1.4 ± 0.1c | / | 4.4 ± 0.3c | / |
|              | Intercrop                        | 4.5 ± 0.6a | / | 2.3 ± 0.5a | / | 7.8 ± 0.8a | / |
| ANOVA        | Factor                           | F | P | F | P | F | P |
| Crops        |                                  | 47.9 | < 0.0001 | 44.3 | < 0.0001 | 212 | < 0.0001 |
| Cropping systems |                                  | 29.6 | < 0.0001 | 3.63 | 0.0747 | 1.42 | 0.2513 |
| In the second cycle
| Maize        | Sole                             | 1.9 ± 0.2c | 2.2 ± 0.2b | 0.5 ± 0.1b | 0.6 ± 0.1b | 3.2 ± 0.5b | 4.1 ± 0.4c |
|              | Intercrop                        | 2.3 ± 0.5bc | 3.5 ± 0.8a | 0.4 ± 0.1b | 0.7 ± 0.1ab | 3.5 ± 0.5b | 5.2 ± 0.8b ** |
| Faba bean    | Sole                             | 2.9 ± 0.1ab | 3.0 ± 0.2a | 0.9 ± 0.1a | 0.7 ± 0.1ab | 5.2 ± 0.5a | 5.4 ± 0.4b |
|              | Intercrop                        | 3.1 ± 0.7a | 3.1 ± 0.5a | 0.9 ± 0.1a | 0.8 ± 0.3a | 5.0 ± 0.9a | 6.5 ± 1.0a * |
| ANOVA        | Factor                           | F | P | F | P | F | P |
| Crops        |                                  | 13.0 | < 0.0001 | 34.7 | < 0.0001 | 54.2 | < 0.0001 |
| Cropping systems |                                  | 10.9 | < 0.0001 | 0.57 | 0.4573 | 7.62 | 0.0095 |
| Root treatments |                                 | 7.26 | < 0.0001 | 1.38 | 0.2484 | 27.3 | < 0.0001 |
| In the third cycle
| Maize        | Sole                             | 2.2 ± 0.1c | 2.4 ± 0.1c | 0.5 ± 0.1bc | 0.6 ± 0.0bc | 3.9 ± 0.1c | 4.4 ± 0.2c |
|              | Intercrop                        | 1.4 ± 0.2d | 1.8 ± 0.3d | 0.4 ± 0.1c | 0.5 ± 0.0c | 1.9 ± 0.4d | 2.6 ± 0.4d |
| Faba bean    | Sole                             | 3.5 ± 0.1b | 3.9 ± 0.3b | 0.6 ± 0.1b | 0.7 ± 0.1b | 4.9 ± 0.2b | 6.8 ± 0.5b *** |
|              | Intercrop                        | 4.9 ± 0.4a | 5.7 ± 0.2a | 1.3 ± 0.1a | 1.7 ± 0.2a | 7.3 ± 0.3a | 9.9 ± 0.7a *** |
| ANOVA        | Factor                           | F | P | F | P | F | P |
| Crops        |                                  | 883 | < 0.0001 | 211 | < 0.0001 | 835 | < 0.0001 |
| Cropping systems |                                  | 30.0 | < 0.0001 | 67.4 | < 0.0001 | 8.12 | 0.0089 |
| Root treatments |                                 | 27.3 | < 0.0001 | 18.5 | 0.0002 | 102 | < 0.0001 |

Values are means ± standard deviations (n=5). Within root residues of pre-crop treatment, values followed by the same lowercase letters are not significantly different (P > 0.05) by LSD test. Significant differences between removed roots and added roots: no *, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001 (t-test). The bold entries indicate P-values < 0.05
bean, respectively (Fig. 2a, c). However, adding maize root residues only significantly increased acid phosphatase activity by 227 μg pNP h⁻¹ g⁻¹ in intercropped maize rhizosheath (Fig. 2c).

Dynamics of soil P fractions and other chemical characteristics

Compared with the unplanted soil, the crop-planted soil had different P fractions depending on cropping systems and root residue addition (Table 3). Irrespective of root residue addition, all three cropping systems greatly depleted NaHCO₃-Pᵢ, NaOH-Pᵢ, 1 M HCl-Pᵢ and NaHCO₃-Pₒ fractions, while accumulating Microbial-P and Residual-P fractions, but they had no significant impact on the NaOH-Pₒ fraction (Fig. 3 and Table 3). All soil P fractions, except NaOH-Pₒ were significantly different between cropping systems (Table 4). Without root residue addition, sole faba bean depleted Resin-P, NaHCO₃-Pᵢ and NaOH-Pᵢ fractions more than sole maize and intercropping did. Under root residue addition, sole maize depleted the NaHCO₃-Pᵢ, NaOH-Pᵢ and 1 M HCl-Pᵢ fractions more than the other two cropping systems did. However, intercropping always caused a greater depletion in NaHCO₃-Pᵢ and conc. HCl-Pᵢ fractions but a greater accumulation of the Microbial-P and conc. HCl-Pᵢ fractions than sole maize and sole faba bean did (Fig. 3 and Table 3). In addition, with or without root residues, the concentration of all P extracted decreased in all three cropping systems. Without root residue addition, the greatest depletion of the sum of all P extracted was recorded for the intercrop, and the lowest for sole maize. Under root residue addition, there were no significant differences in the sum of all P extracted between the three cropping systems.

Several P fractions including Microbial-P, NaOH-Pₒ, 1 M HCl-Pᵢ and NaHCO₃-Pᵢ, were affected by root residue addition (Table 4). Compared with no root residues, the addition of root residues to sole maize, sole faba bean and the intercrop significantly increased the accumulation of the Microbial-P fraction by 49%, 39%, and 45%, and the depletion of the NaHCO₃-Pₒ fraction by 25%, 22%, and 18%, respectively (Fig. 3 and Table 3). However, this impact of adding root residues on other P fractions differed between cropping systems. Compared with no root residues, sole maize with root residues increased the depletion of NaOH-Pᵢ and 1 M HCl-Pᵢ fractions by 39% and 49%, sole faba bean with root residues decreased the depletion of NaHCO₃-Pᵢ and NaOH-Pᵢ fractions by 39% and 57%, and the intercrop with root residues decreased the depletion of NaOH-Pᵢ fraction.
Table 3  Soil Hedley phosphorus (P) fractions under different cropping systems and unplanted control under different root residues of pre-crop after the third cycle

| P fractions mg kg⁻¹ | Root residues of pre-crop/cropping systems/changes of P fractions | Added roots | Removed roots |
|---------------------|-----------------------------------------------------------------|-------------|---------------|
|                     | No-plant             | Sole maize | Sole faba bean | Intercropping | Sole maize | Sole faba bean | Intercropping | Sole maize | Sole faba bean | Intercropping | Sole maize | Sole faba bean | Intercropping | Sole maize | Sole faba bean | Intercropping |
| Resin-P             |                     | 3.8 ± 0.6Aa | 3.8 ± 1.2a | 0.0           | 2.0 ± 0.5b | -1.8       | 3.0 ± 0.8ab | -0.9 | 3.0 ± 1.2AB | -0.8 | 2.7 ± 0.4B | -1.1 | 3.6 ± 0.9AB | -0.2 |
| NaHCO₃-Pᵢ          |                     | 13.7 ± 0.7Aa | 8.8 ± 0.9c | -4.9          | 9.6 ± 0.4c | -4.1       | 11.4 ± 1.2b | -2.3 | 9.2 ± 0.7C | -4.5 | 11.2 ± 0.5B | **-2.5 | 11.0 ± 1.1B | -2.7 |
| NaOH-Pᵢ            |                     | 11.5 ± 0.6Aa | 8.8 ± 0.8b | -2.8          | 7.3 ± 0.9c | -4.2       | 8.1 ± 0.3bc | -3.3 | 7.5 ± 0.7C | -3.9 | 9.6 ± 1.1B | **-1.8 | 9.6 ± 1.3B | *-1.8 |
| 1 M HCl-Pᵢ         |                     | 534 ± 6.9Aa | 518 ± 5.5b | -16.4         | 523 ± 4.0b | -11.0      | 520 ± 8.2b | -13.7 | 510 ± 4.8C | -24.5 | 519 ± 5.6B | -14.8 | 516 ± 5.2BC | -18.0 |
| conc. HCl-Pᵢ       |                     | 106 ± 2.4Bbc | 102 ± 2.8c | -3.4           | 110 ± 4.6ab | +3.9       | 112 ± 2.0a | +6.1 | 105 ± 3.6B | -0.7 | 109 ± 2.2B | +2.8 | 114 ± 3.9A | +8.5 |
| Residual-P          |                     | 44.8 ± 3.5Cc | 68.7 ± 6.5a | +23.9         | 54.1 ± 4.4b | +9.2       | 52.2 ± 8.7bc | +7.4 | 69.2 ± 7.2A | +24.3 | 49.8 ± 3.3BC | +5.0 | 52.4 ± 1.5B | +7.6 |
| NaHCO₃-P₀          |                     | 17.1 ± 1.0Aa | 8.7 ± 1.3b | -8.4          | 6.7 ± 0.7c**-10.5 | 6.2 ± 0.9c** | -11.0 | 6.6 ± 1.1B | -10.5 | 4.4 ± 1.1C | -12.8 | 4.1 ± 0.8C | -13.0 |
| NaOH-P₀            |                     | 26.2 ± 0.9Aa | 25.3 ± 1.1a | -0.9          | 25.8 ± 2.9a | -0.4       | 26.9 ± 0.9a | +0.7 | 24.4 ± 1.8A | -1.8 | 25.6 ± 2.1A | -0.6 | 26.4 ± 0.8A | +0.2 |
| conc. HCl-P₀       |                     | 27.4 ± 3.1Aa | 25.3 ± 2.8a | -2.1          | 25.3 ± 3.5a | -2.1       | 19.1 ± 2.6b**-8.3 | 25.8 ± 1.3AB | -1.5 | 23.5 ± 1.7B | -3.8 | 15.9 ± 1.5C | -11.5 |
| Sum of all P extracted |                 | 70.7 ± 2.2Aa | 59.3 ± 2.9b | -11.4         | 57.8 ± 2.3b**-12.9 | 52.2 ± 2.5c** | -18.5 | 56.8 ± 3.8B | -13.9 | 53.5 ± 3.0B | -17.2 | 46.4 ± 2.4C | -24.3 |
| Sum of all P extracted b |                | 791 ± 8.1Aa | 780 ± 4.4b | -10.9         | 778 ± 4.8b | -12.8      | 776 ± 5.2b | -14.6 | 773 ± 3.3B | -18.1 | 772 ± 4.2B | -18.4 | 775 ± 8.0B | -15.7 |

a Delta P is the difference in concentration of a given P fraction between the bulk soil of different cropping systems and the unplanted control soil

b Sum of all P extracted is equal to the sum of all P fractions including microbial biomass P

Values are means ± standard deviations (n = 5). The values followed by different lowercase letters indicate significant differences (P ≤ 0.05) between all cropping systems (sole maize, sole faba bean, intercropping and unplanted control) without root residues, and the values followed by different uppercase letters indicate significant differences (P ≤ 0.05) between all cropping systems (sole maize, sole faba bean, intercropping and unplanted control) with root residues. Significant differences between removed roots and added roots: no *, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001 (t-test)
by 45%, but increased the depletion of the conc. HCl-P\textsubscript{o} fraction by 39%.

Compared with the unplanted soil, sole maize without root residue addition significantly decreased soil total N concentration at the harvest of the 3\textsuperscript{rd} cycle (Table 5). Conversely, sole faba bean and the intercrop with added root residues significantly increased the soil total N concentration. Similarly, the soil organic C concentration was significantly increased growing sole faba bean without root residues and in the three cropping systems with root residues, compared with the no-plant control. The addition of root residues significantly increased soil total N and organic C concentrations in the three cropping systems, compared with no added root residues (Table 5).

**Discussion**

Adding root residues enhanced the intercropping advantage in terms of biomass and total P content and also altered soil P fractions. Addition of root residues increased the soil Microbial-P pool, and depleted almost all soil P fractions (NaHCO\textsubscript{3}-P\textsubscript{i}, NaOH-P\textsubscript{i}, 1 M HCl-P\textsubscript{i} and NaHCO\textsubscript{3}-P\textsubscript{o}), but the depletion varied with the source of root residues. Root residues of faba bean (low C/P ratio) caused a greater increase in Microbial-P and depletion in NaHCO\textsubscript{3}-P\textsubscript{o}, and root residues of maize (high C/P ratio) caused a larger depletion of soil inorganic Hedley P fractions (NaHCO\textsubscript{3}-P\textsubscript{i}, NaOH-P\textsubscript{i}, and 1 M HCl-P\textsubscript{i}). However, a root residue mixture of those two crops had a medium C/P ratio and resulted in a more pronounced impact than faba bean root residues alone.

Maize and faba bean show a different growth response to P-poor soils, because of their differences in seed P content (Cerino Badone et al. 2012; Vasic et al. 2012) and rhizosphere processes (Li et al. 2007, 2013; Zhang et al. 2012; Zhou et al. 2009). Those traits allow faba bean to grow better on P-impoverished soils than maize. The maize/faba bean intercropping system in this study had advantages (observed values > expected values) over sole cropping in terms of biomass and total P content, but both advantages were less than those in a the field (Li et al. 1999, 2003; Liao et al. 2020). In the field, intercropped maize relative to a sole crop recovers or even overshoots the depressed growth after intercropped
Faba bean is harvested (Li et al. 1999). In the glasshouse experiment, intercropped maize and faba bean were sown and harvested at the same time and faba bean accumulated more biomass than maize, because of its adaptations to low-P soil (Zhang et al. 2012). Thus, the intercropped maize was negatively affected by competition with faba bean, particularly in winter (the 1st and 3rd cycles) when the light intensity and temperature were suboptimal and aboveground interactions become stronger (Liu et al. 2011). Hence, intercropping advantages in this study mainly contributed to enhanced growth and P uptake of faba bean. Adding root residues to the P-poor soil boosted the growth and P content of both maize and faba bean. Those positive effects likely resulted from the release of P during residue decomposition (Hasbullah and McNeill, 2011; Hassan et al. 2013; Jiang et al. 2021). Previous studies have shown that orthophosphate, which is the dominant P form in crop residues, will be partially returned to the soil (Ebuele et al. 2016; Noack et al. 2012; White and Ayoub 1983). Crop residue input may also enhance the abundance of soil microbes, which transform other forms of soil P to plant-available P (Alori et al. 2017; Turmel et al. 2015). Our study demonstrated that input of root residues significantly changed soil P fractions after the third cycle, including Microbial-P, NaOH-P, 1 M HCl-P and NaHCO₃-Po fractions; however, those effects varied with the source of residues. The decrease in the sum of all soil P extracted was more than the P balance of the identical cropping system over three cycles. This might be due to soil sampling and analytical errors and the estimation of the P balances. However, we consider that some of the discrepancy is due to low measurement values of Microbial-P (Brookes et al. 1982), because P from plant seeds and/or root residues stimulates the P uptake of soil microbial biomass. Phosphorus availability to plant is generally considered to decrease with increasing strength of the extractant in sequential fractionation (Hedley et al. 1982). The labile P fractions (Resin-P, NaHCO₃-Pi and NaHCO₃-Po) are considered the most available for plant growth (Bowman and Cole 1978; Tiessen and Moir 1993), and these were depleted by all three cropping systems, relative to the unplanted soil. Sole faba bean increased depletion of the labile P pools, such as the Resin-P fraction, associated with the other two cropping systems, possibly due to a greater P content of sole faba bean (Hassan et al. 2012; Nuruzzaman et al. 2005). Both NaOH-P and 1 M HCl-P are moderately labile P fractions, and they declined in all three cropping systems. The NaOH-P decrease caused by organic acids in root exudates and/or root residues was probably due to lower P sorption of clay minerals by competing organic anions with orthophosphate for the same sorption sites (Al- and Fe-oxide binding sites) (Gerke 1994; Guppy et al. 2005). Root-induced chemical changes (proton release into the rhizosphere) result in dissolution of the acid-extractable inorganic P.

### Table 5  Soil chemical characteristics after the third cycle

| Soil chemical properties | Root residues of pre-crop/cropping system | Added roots |
|-------------------------|------------------------------------------|-------------|
|                         | No-plant | Removed roots | Sole maize | Sole faba bean | Intercropping | Sole maize | Sole faba bean | Intercropping |
| Total N (mg g⁻¹)        | 1.08±0.07Ba 0.98±0.06b 1.10±0.06a 1.09±0.06a | 1.05±0.01BA 1.20±0.05A* 1.16±0.07A |
| Organic C (mg g⁻¹)      | 8.34±0.15Dbc 8.14±0.09e 8.63±0.24a 8.45±0.28ab | 8.61±0.11C*** 9.75±0.18A*** 9.23±0.14B*** |
| ANOVA                   | Total N | P                  | Organic C | F         | 0.0001 | P                  | 52.4 | <0.0001 |
| Factor                  | Cropping systems (C) | 9.86 | <0.0001 | 52.4 | <0.0001 |
| Root treatments (R)     | 10.4 | **0.0029** |
| C×R                     | 1.29 | 0.2950 |

Values are means± standard deviations (n = 5). Within root residues of pre-crop treatment, values followed by the same letters are not significantly different (P > 0.05) by LSD test. Significant differences between removed roots and added roots: no *, P > 0.05; *, P<0.05; **, P < 0.01; *** P < 0.001 (t-test). The bold entries indicate P-values < 0.05.
fractions, first and most likely calcium phosphate in calcareous soil (Li et al. 2008). In the study, faba bean decreased its rhizosheath pH more than maize did due to proton release associated with biological N₂ fixation. However, intercropped maize did not promote proton release of intercropped faba bean, possibly owing to the abundant supply of fertilizer N in this study (Fan et al. 2006; Table S3). In this study, we aimed to examine different characteristics of the rhizosphere, but the amount of rhizosheath soil that was brushed from the root surface was too small, roughly 2 g, to measure all characteristics. Hence, we used an approach that is commonly adopted in rhizosphere studies for assessing multiple traits (Pearse et al. 2007; Li et al. 2010; Lyu et al. 2016; Wen et al. 2017; Wen et al. 2019). In addition, this approach uses living roots during extraction in 50 mL CaCl₂ solution; so, it is better than the traditional way to test for organic acids and acid phosphatase activity that are closely related with root vitality. The measured rhizosheath pH values will be rectified to the ones examined in routine ratio of water to soil which might cause some errors. If the correction curve used for rectifying the measured values is prepared carefully, the errors should be small.

Addition of root residues significantly decreased the depletion of the NaOH-P₁ fraction in both sole faba bean and the intercropping system, but it increased the depletion of NaOH-P₁ and 1 M HCl-P₁ fractions in sole maize, compared with no addition of root residues. Damon et al. (2014) found that most P released from crop residues is recovered as chemically-sorbed inorganic P (NaOH-extractable) for all soils they studied. Our results about the effects of root residues of sole faba bean and intercrops are consistent with their studies, indicating that addition of root residues to soil in low-P systems can compensate for the reduction of these inorganic pools, but the effect of sole maize root residues is different, enhancing NaOH-P₁ depletion. Maize root residues have a high C/P ratio; so, adding those induced net P immobilization and more depletion of soil P pools than happened without root residues (Alamgir and Marschner 2013). This may explain why growth and P content of sole maize were less than those of sole faba bean and intercrops, even though the former caused a large depletion of soil inorganic P pools (NaHCO₃-P₁, NaOH-P₁ and 1 M HCl-P₁). The non-labile P fractions (conc. HCl-P₁ and Residual-P) increased in three cropping systems relative to the unplanted soil which may be the result of P transformation from the labile and the less labile P pools to non-labile P pools (Liao et al. 2020; Negassa and Leinweber 2009).

Although Microbial-P is a small pool (about 2% of the sum of all P extracted), it turns over quickly and therefore becomes a continuous source of P for plants (Capek et al. 2021; Damon et al. 2014; Forsmark et al. 2021). Compared with the unplanted soils, the Microbial-P concentration significantly increased in all three cropping systems, and more so in the maize/faba bean intercropping system. Thus, greater plant diversity may stimulate soil microbial activity by providing a greater quantity and variety of plant-derived resources for microbes (Chen and Chen 2019). Sekaran et al. (2020) indicated that kura clover/prairie cordgrass intercropping increased Microbial-C and Microbial-N due to the supply of readily available C substrate through root exudates and soil organic matter as well as availability of mineral N through N-fixation. Our study supports this, since we found a higher concentration of soil organic acids and organic C in the intercropping system. Thus, intercropped maize had a lower total P content than sole maize due to the increased competition of the microbial pool for P in intercropping. The Microbial-P concentration was enhanced by the addition of root residues, with the most pronounced impact generated by the root residue mixture of maize and faba bean. The reason is that the mixed root residues had different residue qualities, which may provide a greater variety of substrates and niches for microbes (Chapman and Newman et al. 2010; Chen and Chen 2019; Wang et al. 2019). This might increase microbial biomass, diversity and activity, and therefore accelerate soil organic P (such as NaHCO₃-P₀ and conc. HCl-P₀) mineralization, and thus enhance nutrient availability. However, that needs to be further tested.

Soil microorganisms play an important role in the dynamics of crop residues by converting organically-bound P to plant-available P (Damon et al. 2014). The depletion of organic P in three cropping systems, especially the NaHCO₃-P₀ fraction, was greater with root residues than without which may be explained by faster microbial mineralization and secretion of acid phosphatases. The roots can release acid phosphatases and also release more carbon into the soil to stimulate phosphatase production by microorganisms to hydrolyze organic P (Marschner et al. 2005).
This can be understood as ‘carbon priming effects’ when adding a small amount of root residues to the soil stimulates microbial biomass to mineralize soil organic P (Kuzyakov 2010; Pascault et al. 2013; Lu et al. 2021). Thus, stimulation of the microbial biomass and secretion of acid phosphatases to mineralize soil organic P are potential strategies for matching soil inorganic P supply with crop demands. This knowledge is essential to enhance farmers’ ability to efficiently manage nutrients in farming systems. However, in this short-term study, the root residues were finely cut and thoroughly incorporated into soil which was different to that in the field, where the root residues are often of greater size and presented in varying amounts in the soil. This may increase the release of P into the soil solution and accelerate microbial turnover. The plants grown in pots will be smaller than those in the field, due to the limiting rooting area and available nutrients. However, this study focused on the impacts of root residues on soil P fractions and intercropping advantages; plant size may not alter the pattern of the impact, but it might change the extent of the impact. The best way for this kind of studies is to use 32P-labeled residues to trace the fates of P from residues in plant-soil-microbe interactions, to provide guidelines for crop nutrient management (Nachtimuthu et al. 2009; Noack et al. 2014).

Conclusions

Compared with sole cropping, intercropping increased shoot and root biomass of faba bean by 28% and 63%, total P content by 37%, averaged over three cycle cycles, while decreasing those values in maize by 20%, 38%, and 31%, respectively. Adding intercrop root residues increased the biomass and total P content more than adding residues of a sole crop did, consequently enhancing the intercropping advantage in terms of biomass and total P content. Root residues increased soil acid phosphatase activity and Microbial-P, but depleted almost all soil P fractions (NaHCO$_3$-P$_r$, NaOH-P$_r$, 1 M HCl-P$_r$, and NaHCO$_3$-P$_o$), while the depletion varied with root residue sources. Adding sole maize root residues with a high root C/P ratio depleted the NaHCO$_3$-P$_r$, NaOH-P$_r$, and 1 M HCl-P$_r$ fractions more, whereas sole faba bean root residues with a low root C/P ratio depleted the Resin-P and NaHCO$_3$-P$_o$ fractions more.

Root residue mixtures with an intermediate C/P ratio depleted the NaHCO$_3$-P$_r$ and conc. HCl-P$_o$ fractions more, but increased the Microbial-P fraction more. Thus, mixtures of root residues of maize and faba bean accelerated soil organic P mineralization (NaHCO$_3$-P$_o$ and conc. HCl-P$_o$) by increasing microbial biomass and acid phosphatase activity. In addition, without root residues, sole faba bean depleted the Resin-P, NaHCO$_3$-P$_r$, NaOH-P$_r$ and NaHCO$_3$-P$_o$ fractions more than the other two cropping system did.

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Data Availability Available on request.

Code availability Not applicable.

To be used for all articles, including articles with biological applications.

Not applicable.

Declarations

Conflicts of interest/Competing interests The authors declare that they have no competing interests.

Humans and/or animals Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals.

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