Plant Soil (2020) 448:587–601
https://doi.org/10.1007/s11104-020-04460-0

REGULAR ARTICLE

Changes in soil phosphorus fractions following sole cropped and intercropped maize and faba bean grown on calcareous soil

**Dan Liao · Chaochun Zhang · Haigang Li ·
Hans Lambers · Fusuo Zhang**

Received: 19 August 2019 / Accepted: 9 February 2020 /

Abstract

Aims This study aimed to investigate the effects of coexistence with faba bean, a phosphorus (P)-efficient crop, on soil-accumulated P use by a maize/faba bean intercropping system on dynamic changes in soil P pool.

Methods Maize and faba bean were grown in P-accumulated soil as either sole cropping or intercropping. After one year (Stage I) or four years (Stage II) of no P application, soil samples were collected respectively and analyzed for soil P pools using sequential fractionation. Aboveground biomass and P content were annually measured from 2013 to 2016 to assess the annual P balance.

Results The intercropped maize/faba bean system showed a P-uptake advantage, with a Land Equivalent Ratio (LER) ranging from 1.2 to 1.5. The average shoot P content over the four years in intercropped maize and faba bean was significantly greater than that of the corresponding sole crops by 29% and 30%, respectively. Over the three-year P depletion period, the three cropping systems primarily depleted the 1 M HCl-P_i fraction, followed by sole maize, which depleted the NaOH-P_i and concentrated HCl-P_o fractions. Sole faba bean depleted the alkali-soluble P_o fraction (extracted by $NaHCO_3$ and NaOH), and the intercropped maize/faba bean system depleted the conc. HCl-P_o fraction, which was similar to the effect of sole maize.

Conclusions Both sole crops and intercrops mainly depleted 1 M HCl-P_i, but differed in P_o depletion. Sole maize and maize/faba bean intercropping depleted the sparingly labile P_o fraction, while sole faba bean depleted the labile and moderately labile P_o fractions.

Keywords Faba bean · Hedley fractionation · Intercropping · Maize · Soil phosphorus fractions

Responsible Editor: Tim S. George

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11104-020-04460-0) contains supplementary material, which is available to authorized users.

D. Liao · C. Zhang (✉) · H. Li · H. Lambers · F. Zhang
College of Resources and Environmental Science/Key Laboratory of Plant-Soil Interactions, Ministry of Education (MOE), China Agricultural University, Beijing, China
e-mail: zhangcc@cau.edu.cn

H. Li
College of Grassland, Resources and Environment, Inner Mongolia Agricultural University, Hohhot, China

H. Lambers
School of Biological Sciences and Institute of Agriculture, The University of Western Australia, Perth, WA, Australia

Introduction

Phosphorus is both an essential and a frequently limiting nutrient for crop growth (Raghothama 1999; Vance 2003). Farmers routinely apply P-containing fertilizers to increase plant-available soil P concentrations and enhance crop yield, but there are increasing concerns regarding its use efficiency (Hinsinger 2001; Vance 2003). Moreover, application of P fertilizers in highly-intensive agriculture can be problematic, because the soils have accumulated substantial amounts of P due to excessive historical P applications (Vance et al. 2003; Sattari et al. 2012). Phosphorus losses from these enriched soil can greatly increase the risk of pollution of aquatic environments (Carpenter et al. 1998). Hence, it is essential to develop P-management strategies to effectively use accumulated soil P, one of which is to increase soil P bioavailability through growing plant species with the ability to scavenge recalcitrant soil P

Richardson et al. (2011) recognize three strategies of plants exploiting soil P resources: (i) “root-foraging,” which is helpful for acquiring more available soil P through a greater exploration of the surface soil; (ii) “P-mining,” which improve the desorption, solubilization, or mineralization of sparingly available inorganic P (P_i) and organic P (P_o) pools by using root exudates such as organic anions and phosphatases; and (iii) “improving internal P-utilization efficiency,” which increases plants yield per unit of P uptake. Although plants can use any of these strategies to acquire soil P, crop species differ in their ability to access P from soil P pools (Simpson et al. 2011). While many crops lack the ability, a limited number of species can acidify the rhizosphere, exude carboxylates, or release phosphatases to acquire sparingly-available P (Gerke 1994; Li et al. 2007; Li et al. 2014; Wang and Shen 2019).

Several legume crops such as white lupin (*Lupinus albus* L.), faba bean (*Vicia faba* L.), and chickpea (*Cicer arietinum* L.), exude large amounts of organic compounds that mobilize sparingly-available soil P (Veneklaas et al. 2003; Cu et al. 2005; Li et al. 2007). For example, faba bean releases protons, malate, and citrate into the rhizosphere to mobilize insoluble Al- and Fe-bound P in acid soils and Ca-bound P in calcareous soil (Li et al. 2007). Chickpea can effectively access P_o through exuding acid phosphatases to hydrolyze P_o into P_i (Li et al. 2003). Additionally, these legumes also potentially promote the growth of intercropped or subsequent cereal crops (Li et al. 2003, 2007; Nuruzzaman et al. 2005). The ability of legumes to secrete P-mobilizing substances to accesses more recalcitrant P sources indicates that they have a potential for enhancing P acquisition of P-inefficient cereal species when they share the rhizosphere in an intercropping system (Dissanayaka et al. 2015).

In several intercropping systems, interspecific facilitation increases P acquisition of cereal species when planted with P-mobilizing legumes. For example, white lupin mobilizes P from insoluble soil P pools to enhance P uptake of intercropped wheat (*Triticum aestivum* L.) (Gardner et al. 1983; Horst and Waschkies 1987; Cu et al. 2005). The same effect has been recorded for groundnut (*Arachis hypogaea* L.) and intercropped maize (Ae et al. 1996; Ae and Otani 1997; El Dessougi et al. 2003). Pigeon pea (*Cajanus cajan* L.) exudes piscidic acid and a *p*-O-methyl derivative that chelates Fe^{3+}, effectively utilizing Fe-bound P and increasing P acquisition when intercropped with sorghum (Ae et al. 1990). In addition to the above interspecific facilitation, complementarity between intercrops also plays an important role in enhancing P uptake of both intercrops. Complementarity can occur in an intercropping system if two intercrops access different soil horizons (Li et al. 2006; Zhang et al. 2014) or different P pools (Cu et al. 2005; Li et al. 2008), and competition for nutrients is consequently mitigated.

The changes in soil P fractions after addition of fertilizer-P have been well documented to predict the response of crops to P fertilizers (Sharpley 1985; Beck and Sanchez 1994). Generally, at low soil P availability, mineralization of P_o pools is the main source of P to crops (Tiessen et al. 1984; Beck and Sanchez 1996). With the addition of fertilizer-P, the amount of P that is not retained in the soil solution or removed by the crop, is transformed into less labile P pools (Schmidt et al. 1996). However, there are few studies on how crops use those less labile P pools. Phosphate rock, the source of P fertilizer, is gradually being depleted and becoming one of the national strategy materials. Hence, utilizing soil-accumulated P and then using less P fertilizer is one of the key issues to achieve sustainable agriculture. The present study used a long-term field experiment located in the North China Plain, where the calcareous soil at the site causes a strong retention of P on to the solid soil phases (Hu et al. 2012). The soil at the site was over-supplied with P fertilizer for four years until the end of the season in 2012. Since then, the soil did not receive nitrogen (N) and P fertilizer for four years. Soil samples were collected, and the P fractions were analyzed after one and four years of P depletion by the following three crop treatments: sole maize, sole faba bean, and intercropping of maize/faba. We hypothesized that (i) over-application of P fertilizer resulted in a greater P accumulation in the legume-only crop than in the cereal-only crop, because these crops differ substantially in their P demand and efficiency in acquiring labile P. We also hypothesized that, over the three-year depletion period, (ii) faba bean would primarily deplete the labile P and less labile P pools by mobilization, and that maize would rely largely on the labile P pools, without exploiting less labile P pools. We hypothesized that in the intercropped system (iii) the depletion of both P pools would be intermediate and expected that intercropped maize/faba bean would increase P utilization compared with that of sole cropping.

Materials and methods

Study area

A long-term field experiment was started in 2009 in Quzhou County (36.93 N, 115.17E; 40 m a.s.l.), Hebei Province, China. The study location has a typical monsoon climate, with an annual mean temperature of 13.1 °C and an annual precipitation of 556.2 mm. At the start of the study in 2009, the soil at the experimental location was a calcareous alluvial soil with a pH of 7.3 (2.5:1 water/soil) and contained 14 g kg^{-1} organic matter, 0.84 g kg^{-1} total N, 12.6 mg kg^{-1} Olsen-P, and 211 mg kg^{-1} exchangeable potassium (K). The methods used for the above analysis follow Bao (2000).

Since 2009, the field had been fertilized with superphosphate at 80 kg P ha^{-1}, urea at 180 kg N ha^{-1}, and potassium sulfate at 50 kg K ha^{-1}. All the P and K fertilizer, as well as half of the total N, were broadcasted and incorporated into the upper 20 cm of the soil prior to planting by using a rotary tiller. The remaining N fertilizer was side-dressed only for maize at V12 stage. The soil Olsen-P reached 16.4 mg P kg^{-1} at the end of the season in 2012. In order to fully exploit the biological potential of crops to unlock the accumulated soil P and increasing the use of recycled P sources, both N and P fertilizer applications were stopped in 2013, while K fertilizer input was maintained.

Experimental design

The field experiment was a completely random design with three replicates. The treatment was cropping systems: sole maize (*Zea mays* L. cv. Zhengdan No. 958), sole faba bean (*Vicia faba* L.cv. Lincan No. 5), or intercropped maize/faba bean. The field was split into 9 plots, each measuring 4.2 m wide and 8 m long, with an area of 33.6 m^2. One cropping system (sole maize, sole faba bean, or intercropped maize/faba bean) was assigned to each plot. Either maize or faba bean was used to maintain a consistent density in-between the plots. For maize, the row distance was 0.4 m, plant spacing was 0.3 m, and the density was 8.33 plants m^{-2}. For faba bean, the row distance and plant spacing were both 0.2 m, and the plant density was 25 plants m^{-2}. Thus, each plot of sole maize or faba bean contained 10 or 21 rows plants, respectively. The maize/faba bean intercropped plots were a replacement design, in which both row distance and plant spacing were identical to those of the corresponding sole crop. The row distance between maize and faba bean was 0.3 m. Each intercropped plot consisted of three strips, each containing two rows of maize grown alternately with three rows of faba bean. Maize strips were 0.8 m wide, and faba bean strips were 0.6 m wide, accounting for 57% and 43% of a whole strip area, respectively (Fig. 1a-c).

All plots were irrigated during the growing season to prevent water stress and weeded manually. Each year from 2013 to 2016, faba bean was sown on 7–11 March and harvested on 3–6 July; maize was sown on 25–28 April and harvested on 7–11 September. The field was fallowed in winter. From 2013 to 2016, the annual mean temperature was 13.5 °C, 15.7 °C, 14.7 °C and 13.4 °C, respectively, and the annual precipitation was 555 mm, 364 mm, 407 mm and 424 mm, respectively.

Collection and measurement of plant and soil samples

At crop maturity each year, the aboveground biomass of four representative faba bean and two maize plants was harvested from each plot, and used for measurements of nutrient concentrations. The middle strip of each plot was used exclusively for grain yield monitoring when the crop was mature. In the yield-monitoring area, all cobs of two rows of maize with adjacent 10 plants in each row or all pods of three rows of faba bean with 20 adjacent plants in each row were collected, and then the grains from the cobs or pods were manually separated after air drying. All plant samples were oven dried at 65 °C for a minimum of 48 h and weighed. After digestion in a mixture of concentrated H_2SO_4 and H_2O_2, P concentrations were determined using the molybdovanadophosphate method by spectrophotometry (UV757T, Shanghai Instrument Co. Ltd., Shanghai, China) (Johnson and Ulrich 1959).

As with most P-related studies, we focused only on the topsoil (0–20 cm), since this layer is recognized to be the most relevant for crop growth (Blake et al. 2003; Wang et al. 2007; Soltangheisi et al. 2018; Sucunza et al.

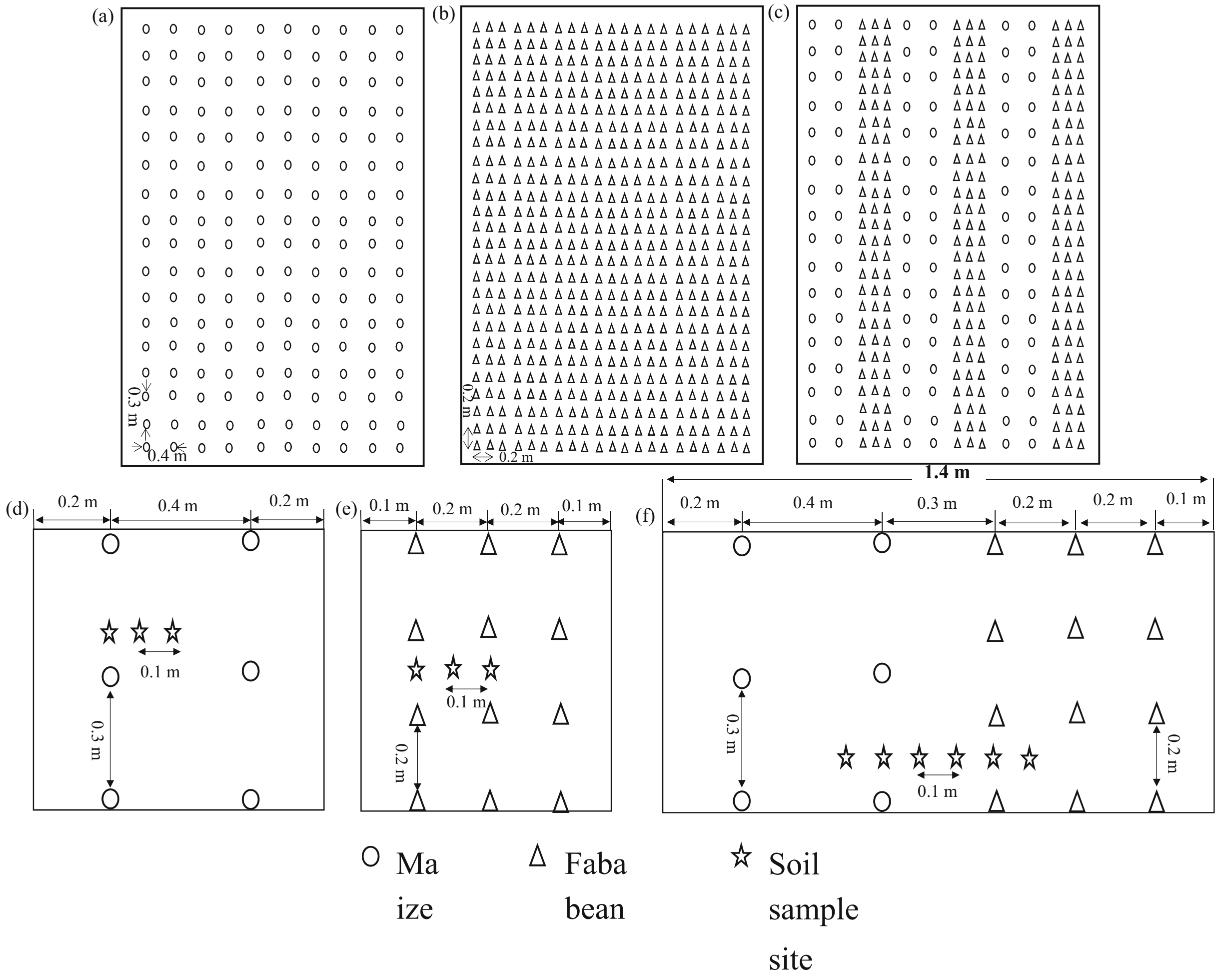


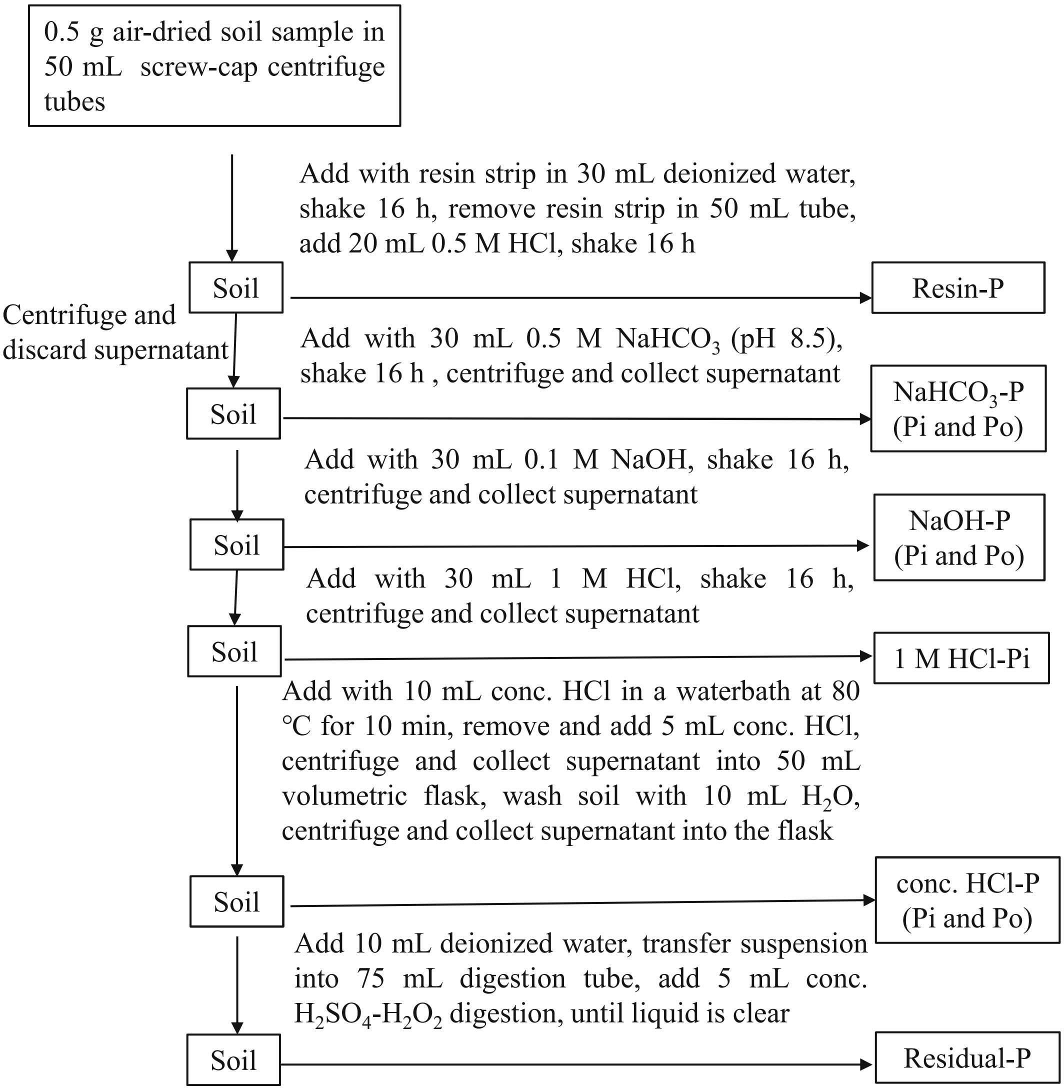
Fig. 1 Layouts of the three cropping systems and soil sampling sites: (a) sole maize; (b) sole faba bean; (c) intercropped maize/faba bean; the soil sampling sites in (d) sole maize, (e) sole faba bean, or (f) in the strips of intercropped maize/faba bean. In a-c, each panel shows an area of 4.2 m width and 8 m length. There were 10 maize rows (0.4 m distance) over this width in panel a (sole maize) and 21 faba bean rows (0.2 m distance) in panel b (sole faba bean). In panel c (intercropped maize/faba bean), there were three strips of two maize rows (at 0.4 m row distance) and three faba bean (at 0.2 m row distance) each, and two paths between the strips, each measuring 0.3 m. There were three placements of soil samples in (d) sole maize from under the center of the maize row at 0.1 m intervals and (e) sole faba bean from under the center of the faba bean row at 0.1 m intervals. There were six placements of soil samples in (f) intercropped maize/faba bean from under the inter-row of maize at 0.1 m intervals

2018; Jimenez et al. 2019). Before the crop was sown in March, 2013, soil samples at each replica plot were collected at depths of 0 to 20 cm to determine the chemical characteristics in the plot for sole maize, sole faba bean, or intercropped maize/faba bean (Table 1). In September, at the end of 2013 (after one year of no P fertilizer, Stage I) and 2016 (after four years of no P fertilizer, Stage II) growing seasons, soil samples at each replica plot from the uppermost 20 cm were collected using a 10 cm diameter soil auger. In order to collect representative soil samples, we took soil cores separately at the upper, middle, and lower site of a plot's diagonal line to form composite samples. Each site included three or six cores at 10 cm intervals for the sole and intercropped system, respectively, as shown in Fig. 1d-f. All soil samples were air-dried and passed through a 2-mm sieve prior to analysis. The samples were used for soil P sequential fractionation (Fig. 2).

Table 1 Chemical characteristics of the top 20 cm soil in the different cropping systems in March 2013 before the crops were sown

Soil characteristics	Cropping system		
	Sole faba bean	Sole maize	Maize/faba bean
pH (1:1 water/soil)	7.6 ± 0.1a	7.7 ± 0.02a	7.6 ± 0.07a
Soil organic matter, g kg^{-1}	13.7 ± 0.4a	13.9 ± 0.3a	13.6 ± 0.2a
Total N, g kg^{-1}	0.83 ± 0.03a	0.86 ± 0a	0.85 ± 0.02a
Olsen-P, mg kg^{-1}	17.1 ± 1.5a	16.2 ± 0.9a	16.0 ± 0.2a
NH_4OAc-extracted K, mg kg^{-1}	75 ± 6a	82 ± 1a	88 ± 7a

Values represent the mean of three replicates ± standard errors(*n* = 3)



0.5 g air-dried soil sample in 50 mL screw-cap centrifuge tubes

Add with resin strip in 30 mL deionized water, shake 16 h, remove resin strip in 50 mL tube, add 20 mL 0.5 M HCl, shake 16 h

Centrifuge and discard supernatant

Add with 30 mL 0.5 M $NaHCO_3$ (pH 8.5), shake 16 h , centrifuge and collect supernatant

Add with 30 mL 0.1 M NaOH, shake 16 h, centrifuge and collect supernatant

Add with 30 mL 1 M HCl, shake 16 h, centrifuge and collect supernatant

Add with 10 mL conc. HCl in a waterbath at 80 °C for 10 min, remove and add 5 mL conc. HCl, centrifuge and collect supernatant into 50 mL volumetric flask, wash soil with 10 mL H_2O, centrifuge and collect supernatant into the flask

Add 10 mL deionized water, transfer suspension into 75 mL digestion tube, add 5 mL conc. H_2SO_4-H_2O_2 digestion, until liquid is clear

Fig. 2 Hedley sequential soil phosphorus (P) fractionation method (Tiessen and Moir 1993). P_i and P_o, represent inorganic and organic P, respectively; conc. HCl indicates concentrated HCl

Soil P fractionation

The method reported by Tiessen and Moir (1993) has been widely used for quantifying various empirically-defined pools of soil P. Briefly, soil (0.5 g) in a 50 mL centrifuge tube was sequentially extracted as follows. (1) To the tube we added a 25 × 62.5 mm anionic resin strip and then 30 mL deionized water, and this was shaken for 16 h at 200 r/s; then the resin trip was placed in a clean 50 mL tube and shaken with 20 mL 0.5 M HCl for 16 h (Resin-P). (2) The water in soil-containing tube was discarded and 30 mL 0.5 M $NaHCO_3$ at pH 8.5 was added, and the suspensions was then shaken for 16 h ($NaHCO_3$-P). (3) 30 mL 0.1 M NaOH was added, and shaken for 16 h (NaOH-P). (4) 30 mL 1 M HCl was added, and shaken for 16 h (1 M HCl-P). (5) The soil residue with 10 mL concentrated HCl was heated at 80 °C in a water bath for 10 min, removed from the bath and then 5 mL of concentrated HCl was added, and the volume adjusted to 50 mL with deionized water (conc. HCl-P). (6) The soil residue was digested with 5 mL concentrated H_2SO_4-H_2O_2 at 350 °C for 3 h (Residual-P). In order to completely recover the soil sample, in between two consecutive steps during processing, the suspensions was centrifuged for 10 min at 25,000×g and 0 °C, and the supernatant passed through a 0.45 μm membrane filter afterwards, and then the filter was rinsed with the next step extractant. Inorganic P (P_i) concentrations for all the extracts were determined within 24 h using the ascorbic acid-molybdenum blue method (Murphy and Riley 1962). The total P (P_t) concentration in the different extracts ($NaHCO_3$-P, NaOH-P and conc. HCl-P) was determined by ammonium persulfate digestion. Organic P (P_o) concentrations were calculated as the difference between P_t and P_i. We duplicated soil testing once every five samples, but we did not test all samples twice, because of the heavy workload of soil P fractionations. In addition, each treatment in the field experiment included three replicates, and soil samples were taken in all plots, so soil sample had three replicates. Hence, we used those two different kinds of replicates to control data quality.

Calculations

(1) The Land Equivalent Ratio (LER) is often considered as an indicator of intercropping benefit (Willey 1979). The LER was calculated according to:

$$\mathrm{LER} = \frac{Y_{if}}{Y_{sf}} \times P_{if} + \frac{Y_{im}}{Y_{sm}} \times P_{im}$$

where Y_{if} and Y_{im} are the P uptake per unit area of intercropped faba bean and maize, respectively. Y_{sf} and Y_{sm} are the P uptake per unit area of sole faba bean and maize, respectively, and P_{if} (43%) and P_{im} (57%) are the relative density of faba bean and maize, respectively, under intercropping relative to sole cropping. If the LER is greater than 1, this indicates the effect of facilitation is greater than that of competition, and there is an intercropping P-uptake advantage.

(2) Soil P balances were calculated for the experimental period as the difference between P inputs and outputs. For the calculation, mean annual values for all replicate plots within each treatment were summed. Phosphorus input was annually estimated from P-fertilizer application. Phosphorus output was P removed with the harvesting of the crop. Phosphorus removal with crops was calculated by multiplying the mean annual shoot biomass of the crop and the shoot P concentration of the crop according to Maltais-Landry et al. (2016). Phosphorus stocks in soil were calculated by multiplying the mean P_t concentrations at the particular soil layer of the treatment, soil bulk density (1.3 g cm^{-3}), and thickness of the soil layer (20 cm).

(3) Changes in soil P concentration over three years were calculated as the difference in P concentration after a four-year period of no P fertilizer application and after one year of no P fertilizer application.

(4) The relative contribution of each P pool to total P reduction was calculated as (Hassan et al. 2012a).

$$\text{Relative contribution } (\%) = \frac{P_a - P_b}{\Delta P} \times 100$$

where P_a and P_b are P concentration of the pool after 1-yr and 4-yr periods of no P fertilizer application, respectively. ΔP is the P_t reduction.

Table 2 The phosphorus (P) balances in the plow layer in the different cropping systems for the period 2009–2016

[a] Treatment and period	Cropping system	P applied (kg ha^{-1})	P removed (kg ha^{-1})	P balances (kg ha^{-1})
Phase I				
2009–2013	Sole maize	320	222	98
	Sole faba bean	320	183	137
	Maize/faba bean	320	228	92
Phase II				
2014–2016	Sole maize	0	87	−87
	Sole faba bean	0	70	−70
	Maize/faba bean	0	104	−104

[a] Since 2009, the field was fertilized with superphosphate at 80 kg P ha^{-1}, and from 2013 onwards, application of P fertilizer was stopped

Statistics

Analysis of variance (ANOVA) was conducted using the SAS software package (SAS v.8.0). Shoot P-uptake data were analyzed using two-way ANOVA, with years (2013, 2014, 2015, and 2016) and cropping systems (sole crop vs intercrop) as the treatment effects. Soil P fractions were analyzed using two-way ANOVA, with years (Stage I and Stage II) and cropping systems (sole maize, sole faba bean and intercropped maize/faba bean) as the treatment effects. The chemical characteristics of soil and changes of P fractions were analyzed using one-way ANOVA (with the three cropping systems as the treatment effect).

Results

Shoot P content and P balance

From 2009 to 2013 (Phase I), the total fertilizer P input was 320 kg P ha^{-1}, and the P output of sole maize, sole faba bean and intercropped maize/faba bean system was 222, 183, and 228 kg P ha^{-1}, respectively, resulting in 98, 137, and 92 kg P ha^{-1} surplus, respectively (Table 2). The P surplus increased soil Olsen-P from the original 12.6 mg P kg^{-1} in 2009 to 16.4 mg P kg^{-1} (the mean of 17.1, 16.2 and 16.0 mg P kg^{-1} in the Table 1) in 2013. From 2014 to 2016 (Phase II), when there was no additional P fertilization, P removal was

Table 3 Shoot phosphorus (P) content (kg ha^{-1}) of faba bean and maize under different cropping systems.

		Year (Y)				
Crop	Cropping system(C)	2013	2014	2015	2016	Average [a]
Maize	Monocropped	28.7 ± 1.1b	27.1 ± 1.5b	30.1 ± 3.0a	29.7 ± 5.4a	28.9 ± 0.8b
	Intercropped	38.5 ± 2.4a	36.4 ± 0.6a	36.2 ± 0.5a	37.6 ± 1.2a	37.2 ± 1.1a
Faba bean	Monocropped	33.8 ± 0.9b	21.0 ± 1.2b	23.7 ± 1.2a	25.6 ± 1.8a	26.0 ± 0.9b
	Intercropped	38.8 ± 0.7a	35.0 ± 1.6a	29.3 ± 2.2a	32.6 ± 2.3a	33.9 ± 0.7a
	Land Equivalent Ratio (LER)	1.3 ± 0.0	1.5 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.0
	Variable	*df*	*F*	*P*		
Maize	Y	3	0.24	0.868		
	C	1	21.7	0.0004		
	Y × C	3	0.22	0.881		
Faba bean	Y	3	13.7	0.0002		
	C	1	45.3	<0.0001		
	Y × C	3	3.13	0.06		

Values are means of three replicates ±standard errors(n = 3). Values followed by the same lower-case letters are not significantly different among different cropping systems in one year at the 5% level by LSD.

[a] Indicates values are averages of four years of sole faba bean or maize or intercropped faba bean or maize

decreased by 61% (sole maize), 62% (sole faba bean), and 54% (intercropping), compared with Phase I, and leading to the P depletion of 87, 70, and 104 kg P ha^{-1} for sole maize, sole faba bean, and intercropped faba/maize, respectively.

From 2013 to 2016, when the LER ranged from 1.2 to 1.5, the maize/faba bean intercropping system had a P-uptake advantage compared with the sole cropping, and average P content of the shoots in the intercropped maize and faba bean plots was significantly increased (29% and 30%, respectively) compared with the corresponding sole crops (Table 3). After one (2013) and two years (2014) of no P fertilizer, the intercropped maize had significantly greater shoot P content (34% in both years), and intercropped faba bean significantly increased shoot P content by 15% and 67% in 2013 and 2014, respectively, compared with the corresponding sole crops. However, after three (2015) and four years (2016) of no P fertilizer, we did not observe a significant intercropping advantage, because the standard error of P content values increased with planting year.

Dynamics of soil P fractions

The dominant P fraction of the soil was 1 M HCl-P_i, which was more than 70% of the total P, followed by conc. HCl-P_o, which was less than 10% of the total P. Resin-P, $NaHCO_3$-P_i, and NaOH-P_i were the smallest fractions, making up 1–2% of the total P (Table 4). The

Table 4 Phosphorus (P) sequential fractionation for soil after one and four years of no P fertilizer application following different cropping systems

P fractions	Sole maize P concentration (conc.)		Sole faba bean P conc.		Maize/faba bean P conc.	
	mg kg^{-1}	[a] %	mg kg^{-1}	%	mg kg^{-1}	%
Stage I (After a one-year of no P fertilizer)						
Resin-P	[b] 11 ± 4a	1	13 ± 3a	1	11 ± 2a	1
$NaHCO_3$-P_i	[c]24±3a*	2	21 ± 1a	2	21 ± 0.2a*	2
NaOH-P_i	15 ± 0.3a*	1	12 ± 1a	1	13 ± 2a	1
1 M HCl-P_i	729 ± 3b	72	689 ± 8c*	71	771 ± 6a**	74
conc. HCl-P_i	39 ± 8a	4	41 ± 2a	4	50 ± 6a	5
Residual-P	62 ± 7a	6	73 ± 0.4a	8	56 ± 6a	5
$NaHCO_3$-P_o	6 ± 2b	1	18 ± 3a*	2	12 ± 1ab	1
NaOH-P_o	21 ± 5a	2	27 ± 4a*	3	20 ± 3a	2
conc. HCl-P_o	106 ± 3a**	10	73 ± 10b	8	86 ± 6ab*	8
Total P	1013 ± 5ab**	100	968 ± 21b	100	1041 ± 8a*	100
Stage II (After a four-year period of no P fertilizer)						
Resin-P	6 ± 0.1a	1	6 ± 1a	1	6 ± 1a	1
$NaHCO_3$-P_i	13 ± 2a	1	16 ± 3a	2	14 ± 1a	1
NaOH-P_i	10 ± 1c	1	14 ± 1b*	1	20 ± 1a**	2
1 M HCl-P_i	695 ± 12ab	70	665 ± 12b	70	731 ± 8a	73
conc. HCl-P_i	65 ± 14a	7	59 ± 3a*	6	87 ± 9a**	9
Residual-P	103 ± 2a*	10	96 ± 3a*	10	63 ± 7b	6
$NaHCO_3$-P_o	16 ± 3a*	2	11 ± 3a	1	18 ± 2a*	2
NaOH-P_o	12 ± 1ab	1	7 ± 0b	1	19 ± 4a	2
conc. HCl-P_o	66 ± 2a	7	72 ± 5a	8	48 ± 6b	5
Total P	987 ± 3a	100	946 ± 12b	100	1008 ± 6a	100

[a] Indicates the percentage of each P fraction to the total P

[b] Values are means of three replicates ± standard errors(n = 3). Values followed by the same lower-case letters are not significantly different among different cropping systems in one year at the 5% level by LSD (horizontal comparison)

[c] Symbols indicate significant differences between Stage I and Stage II: no *, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (t-test)

impact of cropping system on P fractions depended greatly on the depletion duration. After one year of no P fertilizer input (Stage I), the primary P fractions in sole maize, sole faba bean, and intercropped maize/faba bean were 1 M HCl-P_i, followed by conc. HCl-P_o, Residual-P and conc. HCl-P_i (Table 4). Among the three cropping systems, both sole maize and maize/faba bean intercropping had greater 1 M HCl-P_i and conc. HCl-P_o than sole faba bean, but sole faba bean had a greater $NaHCO_3$-P_o.

After four years without P fertilizer (Stage II), the dominant P fraction was 1 M HCl-P_i, which made up 70% or more of the total P. This was in accordance with Stage I. However, the second most dominant P fraction differed between crops (Table 4). Both sole maize and sole faba bean contained a Residual-P of up to 10% of the total P, but intercropped maize/faba bean had conc. HCl-P_i of up to 9% of the total P. Among the cropping systems, maize/faba bean intercropping had greater NaOH-P_i, 1 M HCl-P_i, and NaOH-P_o, but a lower conc. HCl-P_o than sole crop. Each cropping system generated larger changes to the soil P fractions at Stage II than in Stage I.

The P_t concentration at Stages I and II ranged from 968 to 1041 mg kg^{-1} and from 946 to 1008 mg kg^{-1}, respectively (Table 4). At Stage I, the total P fractions across the three cropping systems averaged: 82% in P_i forms, 12% in P_o forms, and 6% in Residual-P form. At Stage II, those P fractions averaged: 82% in P_i forms, 9.7% in P_o forms, and 8.7% in Residual-P form. Over the three years of continuous P depletion, the total soil P declined by 26 mg kg^{-1} for sole maize, 22 mg kg^{-1} for sole faba bean, and 33 mg kg^{-1} for maize/faba bean intercropping (Table 4). These values converted, respectively, to 68, 57, and 86 kg P ha^{-1} (due to soil density of 1.3 g cm^{-3} and soil depth of 20 cm), accounting for nearly 78%, 82%, and 83% of the P balance (Table 2).

The changes in P fractions between Stages I and II (over the three-year continuous P depletion) revealed that continuously no P fertilizer input significantly depleted Resin-P, $NaHCO_3$-P_i, 1 M HCl-P_i, NaOH-P_o, conc. HCl-P_o and P_t, but significantly increased conc. HCl-P_i and Residual-P (Table 4, S2 and Fig. 3). The changes in each P fraction mentioned above (except for Residual-P, NaOH-P_o and conc. HCl-P_o) had no significant difference between cropping systems, differing only in the amount (interactions between years (Stage I and Stage II) and different cropping systems: $P > 0.05$; Fig. 3 and Table S2). However, differences in other P fractions (NaOH-P_i, Residual-P, $NaHCO_3$-P_o, NaOH-P_o and conc. HCl-P_o) between Stages I and II depended on cropping system (interactions between years (Stage I and Stage II) and different cropping systems: $P \leq 0.05$; Fig. 3 and Table S2). Sole maize significantly accumulated $NaHCO_3$-P_o and Residual-P, while significantly depleting NaOH-P_i and conc. HCl-P_o, and sole faba bean significantly accumulated NaOH-P_i and Residual-P while significantly depleting $NaHCO_3$-P_o and NaOH-P_o. Maize/faba bean intercropping significantly accumulated NaOH-P_i and $NaHCO_3$-P_o, and significantly depleted conc. HCl-P_o, similar to sole maize (Fig. 3 and Table 4).

Fig. 3 Changes in soil phosphorus (P) fractions after one and four years of no P fertilizer application (after a four-year period of no P fertilizer application minus measurements collected at the end of one year of no P fertilizer application) following different cropping systems

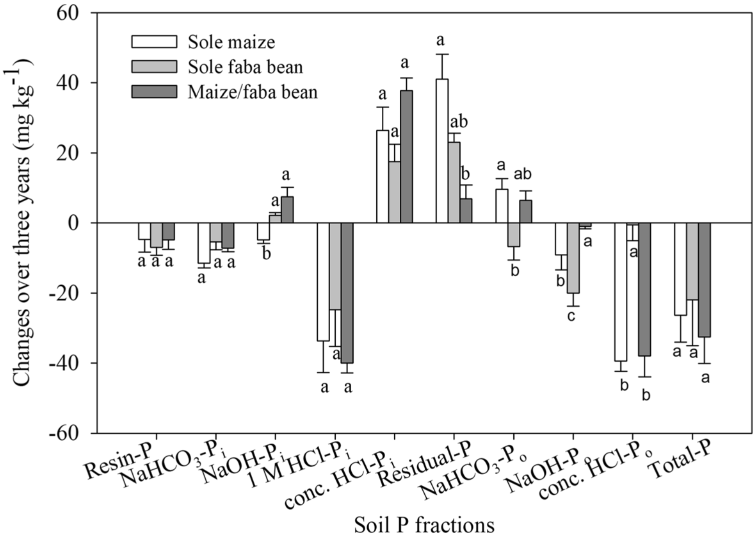


Table 5 The relative contribution of the phosphorus (P) pools as a percentage of the total reduction (%) over three years

Cropping system	[a] Relative contribution of the P pools as percentage of the total reduction												
	Labile P [b]				Moderately labile P [b]				[b] Sparingly labile P			[b] Non-labile P	
	Resin-P	$NaHCO_3$-P_i	$NaHCO_3$-P_o	Sum	NaOH - P_i	NaOH - P_o	1 M HCl -P_i	Sum	conc. HCl -P_i	conc. HCl -P_o	Sum	Residual -P	Sum
Sole maize	5	10		15	5	9	33	47		38	38		
Sole Faba bean	11	8	11	30		31	38	69		1	1		
Maize/faba bean	5	8		13		1	44	45		42	42		

[a] If a P pool increased or was invariant from 2013 to 2016, it is not displayed in the table

[b] Labile P (Resin-P + $NaHCO_3$-P_i + $NaHCO_3$-P_o), moderately labile P (NaOH-P_i + NaOH-P_o + 1 M HCl-P_i), sparingly labile P (conc. HCl-P_i + conc. HCl-P_o) and non-labile P (Residual-P) according to Crews and Brookes (2014) and Ahmed et al. (2019)

The relative contribution of each P fraction's changes to the total reduction differed among cropping systems (Table 5). The moderately labile P pools (sum of NaOH-P_i, 1 M HCl-P_i, and NaOH-P_o) were the main contributors, accounting for 45–69% in the reduction of P_t for the three cropping systems. Second, the labile P pools (sum of Resin-P, $NaHCO_3$-P_i, and $NaHCO_3$-P_o) accounted for 30% in sole faba bean, and the sparingly labile P pools (sum of conc. HCl-P_i and conc. HCl-P_o) accounted for 38% (sole maize) and 42% (maize/faba intercropping).

Discussions

The present study revealed that maize/faba bean intercropping had an advantage in P acquisition from the soil accumulated P, and the advantage existed across the experimental duration. Over three years continuous P depletion, sole faba bean mainly depleted labile and moderately labile P, in accordance with our expectation. Contrary to our hypothesis, sole maize showed a greater depletion of moderately-labile, and sparingly-labile P than sole faba bean. The intercropping system in this case had similar P utilization as sole maize which was distinct from a previous short-term greenhouse study (Li et al. 2008).

In the current study, the Resin-P, $NaHCO_3$-P_i, 1 M HCl-P_i, NaOH-P_o, and conc. HCl-P_o fractions accounted for 95% of the P_t reduction (Table 5), which was close to the value in the literature (Blake et al. 2003). However, the decreases in P_t from Stage I to Stage II in the three cropping systems were less than the P balance of Phase II. As with all field experiments, some of these discrepancies might be attributed to soil sampling and analytical errors and the estimation of the P balances. However, we consider that some of the discrepancy is due to some of P loss from the 0–20 cm topsoil when the estimated P balance was large. Some of the lost P may be retained in the subsoil (Crews and Brookes 2014), but it may also likely be lost with runoff during the three years.

The Resin-P and $NaHCO_3$-P_i are the most available fractions for plant uptake (Bowman and Cole 1978; Tiessen and Moir 1993), so they were significantly depleted with plant uptake, but their contribution to the P_t reduction was little. The HCl-P (the sum of 1 M HCl-P_i, conc. HCl-P_i, and conc. HCl-P_o) that represents moderately labile or stable P compounds is the dominant P fraction in the calcareous soil (Gao et al. 2016), accounting for more than 83% of the P_t in this study (Table 4). The 1 M HCl-P_i was assumed to represent the fraction of calcium phosphate and P associated with Fe or Al oxides (Hedley et al. 1982a, 1983), but we acknowledge that this assumption is an oversimplification (Barrow et al. 2020). Calcium phosphate is the dominant fraction in calcareous soil based on previous studies and the present results. The contribution of 1 M HCl-P_i to the P_t depletion ranged from 33% to 44% (Table 5), and may have resulted from substantial H^+ release by plant roots to dissolve calcium phosphate (Hinsinger 2001). The P_o fractions comprised $NaHCO_3$-P_o, NaOH-P_o, and conc. HCl-P_o, and accounted for nearly 10% of the P_t in our study (Table 4). The moderately-labile and sparingly-labile P_o fractions represented by NaOH-P_o and conc. HCl-P_o had similar contributions to the P_t depletion as 1 M HCl-P_i (Table 5), suggesting that the P_o pools are important in providing plant-available P through mineralization (Stewart and Tiessen 1987; Randhawa et al. 2005). Declines in P_o concentrations with the cessation of fertilizer-P additions have been noted, but are usually only reported with cropping rotations, where the action of tillage stimulates the mineralization of organic matter and associated P_o (Hedley et al. 1982b; Linquist et al. 1997). Reductions in P_o have also been noted in low-fertility grassland soils, where less P_i is available and production is more reliant on mineralization (Sharpley 1985). Thus, the P fractions mentioned above (Resin-P, $NaHCO_3$-P_i, 1 M HCl-P_i, NaOH-P_o and conc. HCl-P_o) were significantly depleted at Stage II compared with Stage I. However, the conc. HCl-P_i and Residual-P proportions significantly increased, which may be the result of P movement from the labile pools into the less labile and even non-labile pools (Walker and Syers 1976; Smeck 1985; Stewart and Tiessen 1987; Negassa and Leinweber 2009). In summary, a negative P balance led to P depletion, consequently reducing labile P and accumulating non-labile P concentrations, indicating that a rational rate of P fertilizer input is necessary to maintain soil fertility while reducing the amount of legacy P and the risk of run-off of P.

This study shows a great effect of the cropping system on soil P fractions with increasing cultivation duration without additional P fertilizer. At Stage I of P depletion in our study, crop species showed slight differences in 1 M HCl-P_i, $NaHCO_3$-P_o, and conc. HCl-P_o depletion (Table 4). This finding is in accordance with the short-term pot experiment conducted by Dissanayaka et al. (2015). However, at Stage II, when P had not being applied to soil for four years, the difference in P fractions among cropping systems was large (Table 4). It is not supprising that different cropping systems (sole maize, sole faba bean, and maize/faba bean intercropping) all depleted the most labile P (Resin-P and $NaHCO_3$-P_i), and there was no difference between cropping systems (Fig. 3). This finding agrees with most studies (Kamh et al. 1999; Rose et al. 2010; Hassan et al. 2012a, b; Dissanayaka et al. 2015). However, the depletion in other P fractions varied largely with crop biological traits, similar to previous studies (Nuruzzaman et al. 2006; Li et al. 2008; Rose et al. 2010). Faba bean mainly used labile and moderately labile P (Table 5), because of its ability of releasing root exudates to enhance P availability (Lindsay and Moreno 1960; Nuruzzaman et al. 2006; Li et al. 2007, 2016b). Conversely, the ability of maize to modify the rhizosphere is relatively weak, and the crop is reportedly unable to use the acid-soluble P_i pool (Li et al. 2015; Cabeza et al. 2017). However, in the current study, sole maize depleted not only 1 M HCl-P_i and NaOH-P_i, but also P_o, including NaOH-P_o and conc. HCl-P_o (Table 4 and Fig. 3). This might be due to the fact that maize was grown in infertile soil and P acquisition relied greatly on arbuscular mycorrhizas (Miller 2000; Zhu et al. 2005; Liu et al. 2018). Zhang et al. (2018) found that arbuscular mycorrhizal fungi and their hyphae microbiome can promote soil P_o mineralization under field conditions, which was related to the function of the bacterial community on the hyphae surface. The difference in P_o depletion between faba bean and maize might reveal the distinct contributions of released chemical and microbial activity to P_o mineralization, which needs to be tested in future experiments. The NaOH-P_i fraction is readily depleted by wheat and maize (Vu et al. 2008; Cabeza et al. 2017), which was supported by the current results (Fig. 3 and Table 4). Thus, the labile, moderately-labile and sparingly-labile P all contributed to soil P depletion by sole maize (Table 5).Compared with sole maize, soil P fractions of the maize/faba bean intercropping system showed a similar contribution to the P_t reduction and a slightly greater depletion of the moderately-labile P pool (Table 5). In the North China Plains or Northwest China, faba bean is commonly sown in March, and harvested in July, while maize is sown in April and harvested in September (Li et al. 1999; Li et al. 2006; Xia et al. 2013; Li et al. 2016a). After the faba bean harvest, intercropped maize grows alone in the field for more than two months. Soil fractions modified by intercropped faba bean were probably eliminated gradually over the time, leading to a similar contribution of sole maize and the maize/faba bean intercropping system to soil P fraction depletion. The slight difference between the two systems can presumably be attributed to intercropped faba bean residue that changed the C/P ratio in the intercropping soil which is an important factor influencing P_o mineralization (McGill and Cole 1981; Harrison 1982). Hence, the intercropping configuration needs to take into account, when considering P fraction changes in a long-term field experiment which might result in different results between short-term greenhouse experiments and long-term field trials. This area of study needs further investigation.

In this study, the depletion of total P_o (the sum of $NaHCO_3$-P_o, NaOH-P_o and conc. HCl-P_o) in all three cropping systems confirms that plants hydrolyze a small portion of these P_o fractions into P_i forms, before it is taken up or reabsorbed/precipitated. This outcome is in line with results outlined by Li et al. (2015), who found a decrease in the NaOH-P_o and total P_o fractions for both faba bean and maize in the rhizosphere. Crews and Brookes (2014) also found a depletion in the NaOH-P_o and conc. HCl-P_o fractions in continuous unfertilized soil where wheat was grown from 1893 to 2009. Chen et al. (2002) showed that the depletion of NaOH-P_o was related to high concentrations of water-soluble organic carbon, microbial biomass, and high phosphatase activities. Therefore, these results show that the mineralization of soil P_o is related to microorganism and enzyme activities. Furthermore, crop residues may contribute to the P_o pools. Many pot experiments show that the $NaHCO_3$-P_o pool in soil planted with legumes is significantly higher than that of soil planted with cereals, such as common bean and wheat (Li et al. 2008), soybean (*Glycine max* L.), maize (Rubio et al. 2012), and lupine and maize (Dissanayaka et al. 2015). Our findings with faba bean at Stage I also support this assertion, whereas no significant difference between faba bean and maize in this fraction was found at Stage II (Table 4). Over three years of P depletion, maize significantly increases the $NaHCO_3$-P_o fraction, indicating that $NaHCO_3$-P_o was not hydrolyzed or/and mineralized, but rather P_i was converted to P_o by soil microorganisms, possibly due to an increase in the microbial P component of this fraction (Perrott et al. 1989; Guo and Yost 1998).

Besides P fertilizer input and crop physiological trait, soil sample processes, such as drying, storage and re-wetting, can alter the distribution of P among fractions. For example, Turner and Haygarth (2001) showed that drying increased labile forms of P in grassland soils, and especially Resin-P and $NaHCO_3$-P_o. These changes can be attributed to a combination of factors including the release of P from microbial biomass, dissolution of organic colloids and alterations in inorganic P adsorption and diffusion on mineral surfaces (Xu et al. 2011; Bünemann et al. 2013). In the current study, before fractioning P pools, we air-dried soil samples collected in 2016 to keep all collected samples in same condition, because the long-term stored soil samples collected in 2013 samples were air-dried. This might slightly change the value but wouldn't alter the pattern of P fraction depletion. When comparing soil samples in 2013 and 2016, we were most concerned about mineralization of P_o fractions during storage of soil samples in 2013, which would affect analysis of the change in P_o fractions between 2013 and 2016. This concern was somewhat addressed with the findings that virtually all P_o fractions of soil samples in 2013 were higher or equal to those in 2016 (described below in Fig. 3). This coincides with the finding by Blake et al. (2000), who found no evidence of net C or N mineralization in archived samples.

This study indicates that legume/cereal intercropping offers a promising practice to realize agriculture sustainable development, particularly in low-input systems or on nutrient-poor soils, where intercropping is becoming an increasingly attractive cropping system due to its advantages over sole cropping in terms of yield (Li et al. 1999; Li et al. 2010; Gao et al. 2019), nutrient use (Li et al. 2007; Xu et al. 2020), disease control (Zhang et al. 2019), and economic return (Huang et al. 2015). Crop production following the cessation of P-fertilizer additions to P-rich soils can be maintained by unlocking soil P reserves (Gillingham et al. 1990; Dodd et al. 2013; McDowell et al. 2016). Nonetheless, ensuring food security in the long run requires rational P input based on the characteristics of soil and cropping system.

Conclusions

Our study showed that the maize and faba bean intercropping system increased P utilization compared with sole cropping of maize or faba bean. The intercropped maize and faba bean showed a greater average shoot P content over four years than the corresponding sole crops, by 29% and 30%, respectively. Following the cessation of P fertilizer application, maize and faba bean showed different dynamics of various soil P fractions, from the labile fractions to more stable fractions. The largest difference was found for soil P_o pools over three-year cultivation, in which maize depleted the conc. HCl-P_o fraction and faba bean depleted the alkali-soluble P_o fraction (extracted by $NaHCO_3$ and NaOH). These P_o fractions were depleted over the period, under both maize and faba bean, suggesting that it is an important source of plant-available P in non-fertilized conditions.

Changes in soil P_t and P fractions between the two periods we investigated without P fertilizer application suggest that a decline of P_t over the three-year cultivation was mainly related to changes in the moderately labile P pool. Under depletion conditions, all three cropping systems primarily depleted the 1 M HCl-P_i fraction, followed by a monocrop of maize depleting NaOH-P_i and conc. HCl-P_o fractions, and a monocrop of faba bean depleting the alkali-soluble P_o fraction (extracted by $NaHCO_3$ and NaOH). Finally, the intercropped maize/faba bean depleted the conc. HCl-P_o fraction in a similar manner as sole maize did.

Acknowledgments This work received financial support from the National Key R & D Program (Grant Number 2017YFD0200200/2017YFD0200207), the '973' project of Chinese Ministry of Science and Techonology (Grant Number 2015CB150400) and the National Natural Science Foundation Project (Grant Number 31210103906). The authors acknowledge support through the European Union's Horizon 2020 Program for Research & Innovation under Grant Agreement Number 727217 (ReMIX: Redesigning European cropping systems based on species MIXtures).

References

Ae N, Otani T (1997) The role of cell wall components from groundnut roots in solubilizing sparingly soluble phosphorus in low fertility soils. Plant Soil 196:265–270

Ae N, Arihara J, Okada K, Yoshihara T, Johansen C (1990) Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science 248:477–480

Ae N, Otani T, Makino T, Tazawa J (1996) Role of cell wall of groundnut roots in solubilizing sparingly soluble phosphorus in soil. Plant Soil 186:197–204

Ahmed W, Huang J, Kaillou L, Qaswar M, Khan MN, Chen J, Sun G, Huang QH, Liu YR, Liu GR, Sun M, Li C, Li DC, Ali S, Normatov Y, Mehmood S, Zhang HM (2019) Changes in phosphorus fractions associated with soil chemical properties under long-term organic and inorganic fertilization in paddy soils of southern China PloS One 14

Bao S (2000) Soil chemical analysis. China Agriculture Press, Beijing [in Chinese]

Barrow N J, Sen A, Roy N, Debnath A (2020) The soil phosphate fractionation fallacy. Plant Soil [in perss]

Beck MA, Sanchez PA (1994) Soil-phosphorus fraction dynamics during 18 years of cultivation on a typic paleudult. Soil Sci Soc Am J 58:1424–1431

Beck MA, Sanchez PA (1996) Soil phosphorus movement and budget after 13 years of fertilized cultivation in the Amazon basin. Plant Soil 184:23–31

Blake L, Goulding KWT, Mott CJB, Poulton PR (2000) Temporal changes in chemical properties of air-dried stored soils and their interpretation for long-term experiments. Eur J Soil Sci 51:345–353

Blake L, Johnston AE, Poulton PR, Goulding KWT (2003) Changes in soil phosphorus fractions following positive and negative phosphorus balances for long periods. Plant Soil 254:245–261

Bowman RA, Cole CV (1978) Transformations of organic phosphorus substrates in soil as evaluated by NaHCO3 extractions. Soil Sci 125:49–54

Bünemann EK, Keller B, Hoop D, Jud K, Boivin P, Frossard E (2013) Increased availability of phosphorus after drying and rewetting of a grassland soil: processes and plant use. Plant Soil 370:511–526

Cabeza RA, Myint K, Steingrobe B, Stritsis C, Schulze J, Claassen N (2017) Phosphorus fractions depletion in the rhizosphere of young and adult maize and oilseed rape plants-P. J Soil Sci Plant Nutr 17:824–838

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol Appl 8:559–568

Chen CR, Condron LM, Davis MR, Sherlock RR (2002) Phosphorus dynamics in the rhizosphere of perennial ryegrass (*Lolium perenne* L.) and radiata pine (*Pinus radiata* D. Don.). Soil Biol Biochem 34:487–499

Crews TE, Brookes PC (2014) Changes in soil phosphorus forms through time in perennial versus annual agroecosystems. Agric Ecosyst Environ 184:168–181

Cu STT, Hutson J, Schuller KA (2005) Mixed culture of wheat (*Triticum aestivum* L.) with white lupin (*Lupinus albus* L.) improves the growth and phosphorus nutrition of the wheat. Plant Soil 272:143–151

Dissanayaka D, Maruyama H, Masuda G, Wasaki J (2015) Interspecific facilitation of P acquisition in intercropping of maize with white lupin in two contrasting soils as influenced by different rates and forms of P supply. Plant Soil 390:223–236

Dodd RJ, McDowell RW, Condron LM (2013) Changes in soil phosphorus availability and potential phosphorus loss following cessation of phosphorus fertiliser inputs. Soil Res 51:427–436

El Dessougi H, Dreele AZ, Claassen N (2003) Growth and phosphorus uptake of maize cultivated alone, in mixed culture with other crops or after incorporation of their residues. J Plant Nutr Soil Sci 166:254–261

Gao X, Shi D, Lv A, Wang S, Yuan S, Zhou P, An Y (2016) Increase phosphorus availability from the use of alfalfa (*Medicago sativa* L.) green manure in rice (*Oryza sativa* L.) agroecosystem. Sci Rep 6

Gao HX, Meng WW, Zhang CC, van der Werf W, Zhang Z, Wan SB, Zhang FS (2019) Yield and nitrogen uptake of sole and intercropped maize and peanut in response to N fertilizer input Food Energy Secur e187

Gardner WK, Barber DA, Parbery DG (1983) The acquisition of phosphorus by Lupinus-Albus L. 3. The probable mechanism by which phosphorus movement in the soil root interface is enhanced. Plant Soil 70:107–124

Gerke J (1994) Kinetics of soil phosphate desorption as affected by citric-acid. Z Pflanzenernähr Bodenkd 157:17–22

Gillingham A, Richardson S, Power I, Riley J (1990) Long term effects of withholding phosphate application on North Island hill country: Whatawhata research Centre. Proc N Z Grassl Assoc 51:11–16

Guo F, Yost RS (1998) Partitioning soil phosphorus into three discrete pools of differing availability. Soil Sci 163:822–833

Harrison AF (1982) Labile organic phosphorus mineralization in relationship to soil properties. Soil Biol Biochem 14:343–351

Hassan HM, Marschner P, McNeill A, Tang C (2012a) Growth, P uptake in grain legumes and changes in rhizosphere soil P pools. Biol Fertil Soils 48:151–159

Hassan HM, Marschner P, McNeill A, Tang CX (2012b) Grain legume pre-crops and their residues affect the growth, P uptake and size of P pools in the rhizosphere of the following wheat. Biol Fertil Soils 48:775–785

Hedley MJ, White RE, Nye PH (1982a) Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. emerald) seedlings. New Phytol 91:45–56

Hedley MJ, Stewart JWB, Chauhan BS (1982b) Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci Soc Am J 46:970–976

Hedley MJ, Nye PH, White RE (1983) Plant-induced changes in the rhizosphere of rape (*Brassica napus* var. emerald) seedlings. IV. The effect of rhizosphere phosphorus status on the pH, phosphatase activity and depletion of soil phosphorus fractions in the rhizosphere and on the cation-anion balance in the plants. New Phytol 95:69–82

Hinsinger P (2001) Bioavailability of inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil 237:173–195

Horst WJ, Waschkies C (1987) Phosphatversorgung von sommerweizen (*Triticum aestivum* L.) in mischkultur mit weiBer lupine (*Lupinus albus* L.). Z Pflanzenernähr Bodenkd 150:1–8

Hu B, Jia Y, Zhao ZH, Li FM, Siddique KHM (2012) soil P availability, inorganic P fractions and yield effect in a calcareous soil with plastic-film-mulched spring wheat. Field Crop Res 137:221–229

Huang CD, Liu QQ, Heerink N, Stomph T, Li BS, Liu RL, Zhang HY, Wang C, Li XL, Zhang CC, van der Werf W, Zhang FS (2015) Economic performance and sustainability of a novel intercropping system on the North China plain. PLoS One 10

Jimenez JLG, Healy MG, Daly K (2019) Effects of fertiliser on phosphorus pools in soils with contrasting organic matter content: a fractionation and path analysis study. Geoderma 338:128–135

Johnson CM, Ulrich A (1959) Analytical methods for use in plant analysis. Calif Agric Exp Sta Bull 766:25–78

Kamh M, Horst WJ, Amer F, Mostafa H, Maier P (1999) Mobilization of soil and fertilizer phosphate by cover crops. Plant Soil 211:19–27

Li L, Yang S, Li X, Zhang F, Christie P (1999) Interspecific complementary and competitive interactions between intercropped maize and faba bean. Plant Soil 212:105–114

Li L, Tang CX, Rengel Z, Zhang FS (2003) Chickpea facilitates phosphorus uptake by intercropped wheat from an organic phosphorus source. Plant Soil 248:297–303

Li L, Sun J, Zhang F, Guo T, Bao X, Smith FA, Smith SE (2006) Root distribution and interactions between intercropped species. Oecologia 147:280–290

Li L, Li SM, Sun JH, Zhou LL, Bao XG, Zhang HG, Zhang FS (2007) Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. Proc Natl Acad Sci U S A 104:11192–11196

Li H, Shen J, Zhang F, Clairotte M, Drevon JJ, Le Cadre E, Hinsinger P (2008) Dynamics of phosphorus fractions in the rhizosphere of common bean (*Phaseolus vulgaris* L.) and durum wheat (*Triticum turgidum durum* L.) grown in monocropping and intercropping systems. Plant Soil 312: 139–150

Li QZ, Sun JH, Wei XJ, Christie P, Zhang FS, Li L (2010) Overyielding and interspecific interactions mediated by nitrogen fertilization in strip intercropping of maize with faba bean, wheat and barley. Plant Soil 339:147–161

Li L, Tilman D, Lambers H, Zhang FS (2014) Plant diversity and overyielding: insights from belowground facilitation of intercropping in agriculture. New Phytol 203:63

Li G, Li H, Leffelaar PA, Shen J, Zhang F (2015) Dynamics of phosphorus fractions in the rhizosphere of faba bean (*Vicia faba* L.) and maize (*Zea mays* L.) grown in calcareous and acid soils. Crop Pasture Sci 66:1151–1160

Li B, Li YY, Wu HM, Zhang FF, Li CJ, Li XX, Lambers H, Li L (2016a) Root exudates drive interspecific facilitation by enhancing nodulation and N_2 fixation. Proc Natl Acad Sci U S A 113:6496–6501

Li C, Dong Y, Li H, Shen J, Zhang F (2016b) Shift from complementarity to facilitation on P uptake by intercropped wheat neighboring with faba bean when available soil P is depleted Sci Rep 6

Lindsay WL, Moreno EC (1960) Phosphate phase equilibria in soils. Soil Sci Soc Am J 24:177–182

Linquist BA, Singleton PW, Cassman KG (1997) inorganic and organic phosphorus dynamics during a build-up and decline of available phosphorus in an ultisol. Soil Sci 162:254–264

Liu F, Xu YJ, Han GM, Wang W, Li XY, Cheng BJ (2018) Identification and functional characterization of a maize phosphate transporter induced by mycorrhiza formation. Plant Cell Physiol 59:1683–1694

Maltais-Landry G, Scow K, Brennan E, Torbert E, Vitousek P (2016) Higher flexibility in input N:P ratios results in more balanced phosphorus budgets in two long-term experimental agroecosystems. Agric Ecosyst Environ 223:197–210

McDowell RW, Condron LM, Stewart I (2016) Variation in environmentally- and agronomically-significant soil phosphorus concentrations with time since stopping the application of phosphorus fertilisers. Geoderma 280:67–72

McGill WB, Cole CV (1981) Comparative aspects of cycling of organic C, N, S and P through soil organic-matter. Geoderma 26:267–286

Miller MH (2000) Arbuscular mycorrhizae and the phosphorus nutrition of maize: a review of Guelph studies. Can J Plant Sci 80:47–52

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Negassa W, Leinweber P (2009) How does the Hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: a review. J Plant Nutr Soil Sci 172:305–325

Nuruzzaman M, Lambers H, Bolland MDA, Veneklaas EJ (2005) Phosphorus uptake by grain legumes and subsequently grown wheat at different levels of residual phosphorus fertiliser. Aust J Agric Res 56:1041–1047

Nuruzzaman M, Lambers H, Bolland MDA, Veneklaas EJ (2006) Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. Plant Soil 281:109–120

Perrott KW, Maher FM, Thorrold BS (1989) Accumulation of phosphorus fractions in yellow-brown pumice soils with development. N Z J Agric Res 32:53–62

Raghothama KG (1999) Phosphate acquisition. Annu Rev Plant Phys 50:665–693

Randhawa PS, Condron LM, Di HJ, Sinaj S, McLenaghen RD (2005) Effect of green manure addition on soil organic phosphorus mineralisation. Nutr Cycl Agroecosyst 73:181–189

Richardson AE, Lynch JP, Ryan PR, Delhaize E, Smith FA, Smith SE, Harvey PR, Ryan MH, Veneklaas EJ, Lambers H, Oberson A, Culvenor RA, Simpson RJ (2011) Plant and microbial strategies to improve the phosphorus efficiency of agriculture. Plant Soil 349:121–156

Rose TJ, Hardiputra B, Rengel Z (2010) Wheat, canola and grain legume access to soil phosphorus fractions differs in soils with contrasting phosphorus dynamics. Plant Soil 326:159–170

Rubio G, Faggioli V, Scheiner JD, Gutierrez-Boem FH (2012) Rhizosphere phosphorus depletion by three crops differing in their phosphorus critical levels. J Plant Nutr Soil Sci 175

Sattari SZ, Bouwman AF, Giller KE, van Ittersum MK (2012) Residual soil phosphorus as the missing piece in the global phosphorus crisis puzzle. Proc Natl Acad Sci U S A 109:6348–6353

Schmidt JP, Buol SW, Kamprath EJ (1996) Soil phosphorus dynamics during seventeen years of continuous cultivation: fractionation analyses. Soil Sci Soc Am J 60:1168–1172

Sharpley AN (1985) Phosphorus cycling in unfertilized and fertilized agricultural soils. Soil Sci Soc Am J 49:905–911

Simpson RJ, Oberson A, Culvenor RA, Ryan MH, Veneklaas EJ, Lambers H, Lynch JP, Ryan PR, Delhaize E, Smith FA, Smith SE, Harvey PR, Richardson AE (2011) Strategies and agronomic interventions to improve the phosphorus-use efficiency of farming systems. Plant Soil 349:89–120

Smeck NE (1985) Phosphorus dynamics in soils and landscapes. Geoderma 36:185–199

Soltangheisi A, Rodrigues M, Arruda Coelho MJ, Gasperini AM, Sartor LR, Pavinato PS (2018) Changes in soil phosphorus lability promoted by phosphate sources and cover crops. Soil Tillage Res 179:20–28

Stewart JWB, Tiessen H (1987) Dynamics of soil organic phosphorus. Biogeochemistry 4:41–60

Sucunza FA, Gutierrez Boem FH, Garcia FO, Boxler M, Rubio G (2018) Long-term phosphorus fertilization of wheat, soybean and maize on Mollisols: soil test trends, critical levels and balances. Eur J Agron 96:87–95

Tiessen H, Moir JO (1993) Characterization of available P by sequential extraction. In: Carter MR (ed) Soil sampling and methods of analysis. Lewis, Ann Arbor, pp 75–86

Tiessen H, Stewart JWB, Cole CV (1984) Pathways of phosphorus transformations in soils of differing pedogenesis. Soil Sci Soc Am J 48:853–858

Turner BL, Haygarth PM (2001) Biogeochemistry-phosphorus solubilization in rewetted soils. Nature 411:258–258

Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol 157:423–447

Veneklaas EJ, Stevens J, Cawthray GR, Turner S, Grigg AM, Lambers H (2003) Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. Plant Soil 248:187–197

Vu DT, Tang C, Armstrong RD (2008) Changes and availability of P fractions following 65 years of P application to a calcareous soil in a Mediterranean climate. Plant Soil 304:21–33

Walker TW, Syers JK (1976) Fate of phosphorus during pedogenesis. Geoderma 15:1–19

Wang L, Shen J (2019) Root/rhizosphere management for improving phosphorus use efficiency and crop productivity. Better Crops 103:36–39

Wang X, Lester DW, Guppy CN, Lockwood PV, Tang C (2007) Changes in phosphorus fractions at various soil depths following long-term P fertiliser application on a black Vertosol from South-Eastern Queensland. Soil Res 45:524–532

Willey RW (1979) Intercropping-its importance and research needs: part 1. Competition and yield advantages Field Crops Abs 32

Xia HY, Zhao JH, Sun JH, Bao XG, Christie P, Zhang FS, Li L (2013) Dynamics of root length and distribution and shoot biomass of maize as affected by intercropping with different companion crops and phosphorus application rates. Field Crop Res 150:52–62

Xu G, Sun JN, Xu RF, Lv YC, Shao HB, Yan K, Zhang LH, Blackwell MSA (2011) Effects of air-drying and freezing on phosphorus fractions in soils with different organic matter contents. Plant Soil Environ 57:228–234

Xu Z, Li CJ, Zhang CC, Yu Y, van der Werf W, Zhang FS (2020) Intercropping maize and soybean increases efficiency of land and fertilizer nitrogen use-a meta-analysis. Field Crop Res 246:107661

Zhang CC, Postma JA, York LM, Lynch JP (2014) Root foraging elicits niche complementarity-dependent yield advantage in the ancient 'three sisters' (maize/bean/squash) polyculture. Ann Bot 114:1719–1733

Zhang L, Shi N, Fan J, Wang F, George TS, Feng G (2018) Arbuscular mycorrhizal fungi stimulate organic phosphate mobilization associated with changing bacterial community structure under field conditions. Environ Microbiol 20:2639–2651

Zhang CC, Dong Y, Tang L, Zheng Y, Makowski D, Yu Y, Zhang FS, van der Werf W (2019) Intercropping cereals with faba bean reduces plant disease incidence regardless of fertilizer input- a meta-analysis. Eur J Plant Pathol 154:931–942

Zhu JM, Kaeppler SM, Lynch JP (2005) Topsoil foraging and phosphorus acquisition efficiency in maize (*Zea mays*). Funct Plant Biol 32:749–762