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Complementarity and facilitation with respect to P acquisition do not drive overyielding by intercropping

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ABSTRACT

Complementarity in phosphorus (P) acquisition from different sources and facilitation of P uptake have been implicated in yield advantages of intercropping. These beneficial interactions between crop species are expected to be particularly relevant on low-P soils.

Millet and chickpea have previously been found to differ in their ability to access different chemically bound forms of P. Here, we conducted a two-year field experiment on a low-P soil with or without P fertilization to determine whether the resulting potential for complementarity and facilitation with respect to P acquisition is associated with increased P uptake and yield of an intercrop as compared to sole crops.

Alkaline phosphatase activity and carboxylate concentration differed between millet and chickpea, indicating potential complementarity in access to different P sources. Comparison of aboveground P content in the intercrop and the pure stands showed a positive net effect for P uptake ($NE_P > 0$) when no P fertilizer was applied, but this positive net effect for P acquisition was not associated with a yield increase ($NE_Y = 0$). When P fertilizer was applied, there was no significant net increase in P uptake by the intercrop compared to sole crops ($NE_P = 0$), but there was a significant yield gain ($NE_Y > 0$).

Species trait dissimilarities for P acquisition from different sources supported complementarity in, and facilitation of P uptake by millet and chickpea in the field on a low-P soil, but this did not result in yield increase. The finding does not support the notion that complementarity in P acquisition from different sources and facilitation of P uptake are key drivers for overyielding by intercropping on low-P soil.

1. Introduction

Phosphorus (P) is an essential nutrient for plants. Agricultural crops are commonly fertilized with P fertilizer, but a large part of the applied P fertilizer accumulates in the soil (Faucon et al., 2015; George et al., 2016) because the P ions are adsorbed to Al or Fe (hydr)oxides or precipitated as calcium-P (Ca-P) and converted into sparingly soluble forms of P (Hinsinger, 2001; Vance et al., 2003). These sparingly soluble P sources are relatively inaccessible to plants. Plants can access sparingly soluble soil P reserves by the formation of thinner roots, symbioses with mycorrhizal fungi and/or the production of P-mobilizing root exudates (Richardson et al., 2011). Plant species vary widely in their capabilities to mobilize or access sparingly soluble P sources (Pearse et al., 2007). Legumes are in general better able than cereals to mobilize these sparingly soluble P sources, converting these P forms into soluble orthophosphate that can be readily taken up (Li et al., 2014). Companion species of legumes can benefit from this P mobilizing ability of legumes, a phenomenon referred to as facilitation (Li et al., 2014). Cereal/legume intercropping (the cultivation of two or more crop species in the same field (Vandermeer, 1989; Willey, 1990)) is therefore considered an efficient way to optimize the use of poorly available P sources in the soil.

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Improved acquisition of P and increased yield (overyielding) have been observed in intercrops of maize (*Zea mays*) with legumes, e.g., maize/faba bean (*Vicia faba*) (Li et al., 2007), maize/common bean (*Phaseolus vulgaris*) (Latati et al., 2016) and maize/soybean (*Glycine max*) (Wang et al., 2017). These increases in P uptake and yield of intercrops represent an outcome of species interactions in intercropping but the mere observation of greater than expected P uptake by intercropping is not proof that complementarity in or facilitation of P uptake is the underlying mechanism of the increased P uptake because other mechanisms may also be operative. The literature mentions both complementarity and facilitation with respect to P uptake as mechanisms enabling the outcomes of increased P uptake and yield gain of intercrops (Tang et al., 2020).

Complementarity in P uptake is defined as the reduced competition for P between intercropped species as due to their differential accesses to different P sources (Hinsinger et al., 2011), *i.e.*, P partitioning in species mixtures (Turner, 2008). Facilitation is defined as a belowground process where a nutrient-mobilizing species increases the nutrient availability both for itself and for a non-mobilizing neighbour (Brooker et al., 2015; Li et al., 2014).

Most of the studies on P acquisition by intercrops have focused on facilitation of a P-non-mobilizing species by a P-mobilizing species (Faucon et al., 2015; Li et al., 2014; Xue et al., 2016). Studies on facilitation usually concerned intercropped legumes, which, through exudation of P-mobilizing compounds, facilitate P uptake by other crop species, often cereals, especially on P-deficient soils (Latati et al., 2014; Li et al., 2007; Wang et al., 2017). P-mobilizing compounds include carboxylates, phosphatases and protons. Carboxylates can compete with phosphate for the same sorption sites on metal (hydrox)ides and therefore bring P into the soil solution. Phosphatases hydrolyse organic P (Richardson et al., 2009). The release of protons can acidify the rhizosphere and improve the dissolution of Ca-P in alkaline soil (Hinsinger, 2001). Li et al. (2014) hypothesized that mobilization of sparingly soluble P sources plays an important role in overyielding by cereal/legume intercrops. However, Evers et al. (2018) argue the connectedness of acquisition of light, water and nutrients by plants in mixed stands and the difficulty of identifying causes and effects in increased resource capture and overyielding. While it is possible that complementarity or facilitation with respect to P uptake would drive yield increases in agriculture, particularly on P deficient soils, it is likewise possible that complementarity for other factors, e.g., light or water, would drive an increase in biomass increase that – as a consequence – would drive increased P acquisition by intercrops without complementarity in P acquisition from different sources being initial driver of the yield increase. There is therefore uncertainty regarding the importance of complementarity in or facilitation of P uptake as a driver for, or a result of, yield increase of intercrops. This is unlike the situation with respect to N, where ample evidence has been collected demonstrating that complementarity in N acquisition (through N_2-fixation by legumes and mineral N uptake by the companion species) is the main driver for yield increase on soils where N is the yield-limiting element (Hauggaard-Nielsen and Jensen, 2001; Corre-Hellou et al., 2006).

Previous studies have shown that crop species differ in their ability to access various P forms (e.g., Ca-bound P or organic P) (Li et al., 2019; Pearse et al., 2007). If two species with diverging P uptake traits are combined in a mixture, this would enlarge the ways in which the mixture can access P, which could result in a reduced competition for P, and hence allow complementarity and overyielding. Several field studies tested for such complementarity by measuring the depletion of different P pools by species mixtures (Crème et al., 2016; Liao et al., 2020). Lucerne (*Medicago sativa,* a legume species) depleted the available P fraction (extracted by $NaHCO_3$) further than the grass species cocksfoot (*Dactylis glomerata*) and tall fescue (*Festuca arundinacea*) (Crème et al., 2016). Sole maize and maize/faba bean intercropping depleted the sparingly available organic P fraction (extracted by NaOH and concentrated HCl), and sole faba bean depleted the labile and moderately labile organic P fractions (extracted by $NaHCO_3$ and NaOH) (Liao et al., 2020). However, these results of depletion of P fractions by cereals and legumes in intercropping in the field do not support the general notion that legumes are better than cereals able to mobilize sparingly soluble P.

Previously, we tested for complementarity in P uptake between two species with differing abilities to take up different chemical forms of P when grown as pure stands by growing them as mixtures on single or mixed P sources in inert quartz sand in pots (Li et al., 2019). If the mixture took up more P from mixed sources than from a single source, this was interpreted as evidence that the ability to acquire different chemical forms of P resulted in greater P uptake from mixed sources as compared to single P sources. We found in these experiments that millet (*Setaria italica*) was better able to access Ca-bound P than phytate-P, while chickpea (*Cicer arietinum*) could better acquire P from phytate than from Ca-bound P. This trait divergence indeed resulted in increased P uptake from mixed P sources by a millet/chickpea mixture (Li et al., 2019). In follow-up pot experiments with soils containing a natural mixture of different P sources, there was, however, no increased P uptake by millet/chickpea mixture, but we did find that chickpea facilitated P uptake by millet as a result of a greater ability of chickpea to exude carboxylates and acid phosphatase that mobilize sparingly soluble P sources (Li et al., 2019). A pot experiment, however, does not address all possible forms of complementarity or facilitation. For instance, in pot experiments, species are grown simultaneously, while, in the field, there may be a difference in sowing date, as in relay intercropping (Yu et al., 2015). As a C4 species, millet is more adapted to high temperatures than chickpea (C3 species), enabling later sowing and harvesting compared to chickpea. In the field, complementarity may exist if root systems of different species differentially extract P from the soil in space (depth) or time. It is unknown whether complementarity and facilitation with respect to P uptake contribute to the overyielding by intercrops during the whole growing period when there is substantial temporal niche differentiation. This is best studied in the field.

Designing for complementarity in P uptake from different sources by intercrops not only requires an appropriate species choice based on species traits, but it also depends on the soil P condition. According to the stress-gradient hypothesis, competitive interactions between plants dominate in favourable environments, but positive interactions dominate in unfavourable environments (e.g., low nutrient availability) (He et al., 2013; Callaway et al., 2002). We can use P fertilization of a low-P soil as an experimental manipulation to test whether complementarity in P acquisition is a driver for yield increase. If complementarity in or facilitation of P acquisition drives yield increase, we expect that the yield increase is reduced or disappears if sufficient P fertilizer is added, thus effectively removing limitation of growth by P uptake.

Facilitation of P uptake via root exudates requires root proximity (Vengavasi and Pandey, 2018; Hinsinger et al., 2011). In strip intercropping, where one species is sown in strips of several rows alternated with several rows of the other species, facilitative nutrient uptake (e.g., Fe) was only observed for plants in border rows but not for plants in inner rows that were not intermingling extensively with the roots of the other species (Zuo et al., 2000). Complementarity for accessing P sources is likely to be more pronounced for border-row plants than for inner-row plants in intercrops that are grown in multi-row strips, because of the difference between the two in proximity to the companion species. Therefore, comparing performance of outer and inner-row plants is a way to gauge the role of interspecific vs intraspecific interactions. A previous study showed an increase in releases of protons and phosphatases in maize/soybean intercropping compared to sole crops, that could be responsible for the increased soil P concentration in the rhizosphere of intercrops (Wang et al., 2017). Therefore, comparing the root exudates of intercrops and sole crops is useful to determine whether root exudates are responsible for increased P uptake by intercrops compared to sole crops.

In the present study, we conducted a field experiment with millet/

chickpea relay strip intercropping during two growing seasons to test for complementarity and facilitation with respect to P acquisition by intercropping on a low-P soil. We hypothesized that

(1) there is overyielding with respect to P uptake, aboveground biomass and yield of millet/chickpea intercropping: intercrops acquire more P and produce more biomass and yield than expected based on sole crop performance and the mixing ratio of the species in the mixture.
(2) overyielding is caused by complementarity and facilitation with respect to P uptake because (a) millet and chickpea have different root exudates; (b) millet and chickpea deplete different P pools; (c) there is an increase in root exudates in intercrops compared to sole crops; (d) millet and chickpea plants take up more P in border rows than in inner rows.
(3) overyielding with respect to P uptake, biomass and yield of intercrops is more pronounced at a lower P level if complementarity and facilitation with respect to P uptake are drivers of overyielding.

2. Materials and methods

2.1. Site description

The field experiment was conducted in 2017 and 2018 at the Zhangye Experimental Station (38°85′N, 100°38′E) at the Institute of Soils, Fertilizers and Water-Saving Agriculture, Gansu Academy of Agricultural Sciences. The experimental site is located in northwest China, 10 km southwest of Zhangye City, Gansu Province, at an altitude of 1555 m above sea level. The area has an arid climate and the soil type is an Aridisol. The experimental field was used to grow maize without P fertilizer in the previous three years. Soil (depth 0−20 cm) pH was 7.41 (1:2.5 soil: $CaCl_2$), and the soil contained 6.5 mg kg^{-1} Olsen-P, 0.11 g kg^{-1} total N, 0.83 g kg^{-1} total P, 140 mg kg^{-1} exchangeable K and 1.14 g kg^{-1} organic carbon. The monthly total precipitation (mm), mean temperature (℃) and monthly total sunshine duration (h) during the two growing seasons are presented in Table 1.

2.2. Experimental design and crop management

The soil was ploughed before fertilizer application and sowing. The treatments comprised three cropping systems: sole millet (*Setaria italica L.* cv. Longgu 11), sole chickpea (*Cicer arietinum L.* cv. Longying 1), and millet/chickpea intercropping (Fig. 1), and two P fertilizer levels: P0 (without addition of inorganic P) and P100 (with 100 kg P ha^{-1} applied each year as triple superphosphate). The experiments were laid out according to a random block design with two factors and five replicates. The experiment was done in a single field in two subsequent years. Sole crops were grown as rotations (one year millet, the other year chickpea) while intercropped species swapped position within the plot in the second year (small rotation) in accordance with local practice to avoid problems associated with continuous cultivation of a crop species.

Individual plots were 4.75 m long and 6.80 m wide. Each intercrop plot consisted of four strips of 1.70 m width in which four rows of chickpea were alternated with three rows of millet (Fig. 2). Row distance was 20 cm in chickpea and 30 cm in millet, with 25 cm between adjacent chickpea and millet rows in the intercrop. Plant distance in the row was 20 cm in chickpea and 10 cm in millet. Millet and chickpea occupied 53 % and 47 % of the intercropped area, respectively. The design is a replacement design. Crop rows were oriented east-west.

To increase the possibility for intercrops to access different P sources, each plot received an extra 40 kg ha^{-1} P as sodium phytate (< 0.2 % inorganic P, Anhui Huainan Biological Development Co., LTD, China) as a form of sparingly available organic P. N fertilizer was supplied at a rate of 225 kg ha^{-1} pure N as urea. All P fertilizer and 112.5 kg ha^{-1} of the N fertilizer were evenly broadcast and incorporated into the upper 20 cm of the soil before sowing. Another 112.5 kg ha^{-1} of N was applied in the form of urea in early July. No K or organic fertilizer was applied. All plots were irrigated and weeded manually. All plots were irrigated by flooding in accordance with farmer practice. The timing and irrigation amounts in 2017 were: 6 May (90 mm), 15 June (irrigation amount was higher than 120 mm because of a break of the irrigation canal and water was too much for chickpea (at flowering stage) and millet (at the seedling stage)), 15 July (90 mm), 20 August (100 mm). The timing and irrigation amounts in 2018 were: 26 May (90 mm), 25 June (100 mm), 24 July (90 mm), 20 August (100 mm).

In 2017, chickpea was sown on 2 April and harvested on 10 August, and millet was sown on 29 April and harvested on 5 September (Fig. 3). In 2018, chickpea was sown on 23 March and harvested on 2 August, while millet was sown on 27 April and harvested on 21 September. At chickpea sowing, a 25 cm-long PVC pipe of 15 cm diameter was inserted between plant rows in each monoculture plot to prevent root in-growth and to allow collecting reference soil samples at harvest.

2.3. Final harvest and P uptake

At final harvest of each crop species, in 2017, we harvested three adjacent rows of sole millet and four adjacent rows of sole chickpea over a length of 3.15 m, avoiding the outer 80 cm of the rows nearest to the edge of the plot. In 2018, sole millet and chickpea were harvested over an area of 7.2 m^2 per plot. In both years, three rows of millet and four rows of chickpea were harvested in one central strip in each intercropping plot. A sub-sample was randomly taken to determine dry weight (70 °C for 72 h) of straw and grain separately. P concentration of straw and grain was determined using the vanado-molybdate method (Westerman, 1990) after wet digestion with a mixture of concentrated H_2SO_4 and H_2O_2. Total P content was calculated as the sum of the P contents of straw (stems plus leaves) and grain.

2.4. Periodic samples during co-growth to measure biomass and P uptake and collect rhizosphere soil samples

Above- and belowground plant samples were collected from both the sole crops and the intercrop during the co-growth period of millet and chickpea to measure the aboveground biomass and P uptake and determine carboxylate concentration and enzyme activities in the rhizosphere. There were three sampling occasions during the co-growth period in 2017 (Fig. 3). The first sample (I) was on 1 June (the 33rd day of co-growth when chickpea started flowering and millet was at seedling stage), the second sample (II) was on 6 July (the 68th day of co-growth

Table 1
Temperature, precipitation and sunshine during the growing seasons of 2017 and 2018 at Zhangye experimental site.

Year	Month	April	May	June	July	August	September
2017	Average temperature (°C)	13	16	23	24	22	18
	Total precipitation (mm)	8	13	4	14	57	3
	Total sunshine duration (h)	301	280	330	299	206	282
2018	Average temperature (°C)	12	18	23	24	22	15
	Total precipitation (mm)	12	11	10	44	31	31
	Total sunshine duration (h)	280	295	269	277	240	263

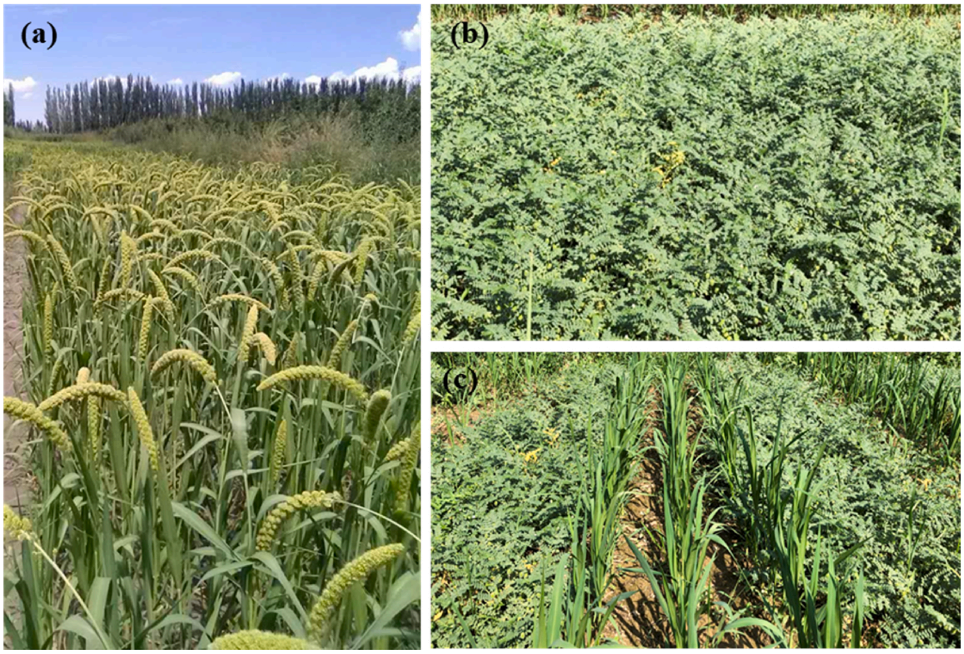

Fig. 1. The three cropping systems in this study. (a) sole millet (at grain filling stage), (b) sole chickpea (at podding stage), (c) millet/chickpea intercropping with alternating strips of three rows of millet and four rows of chickpea (at podding stage of chickpea).

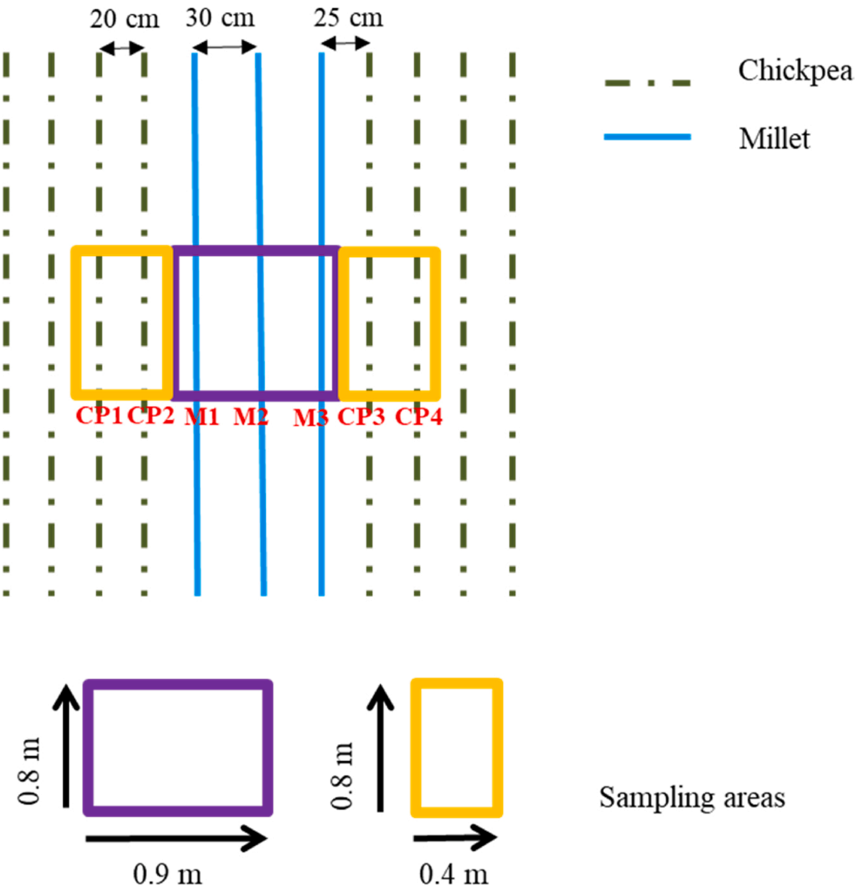

Fig. 2. Diagrammatic representation of the millet/chickpea intercropping strips. CP1-4 represent intercropped chickpea (border rows CP2 and CP3, inner rows CP1 and CP4). M1-3 represent intercropped millet (border rows M1 and M3, inner row M2) in the strip.

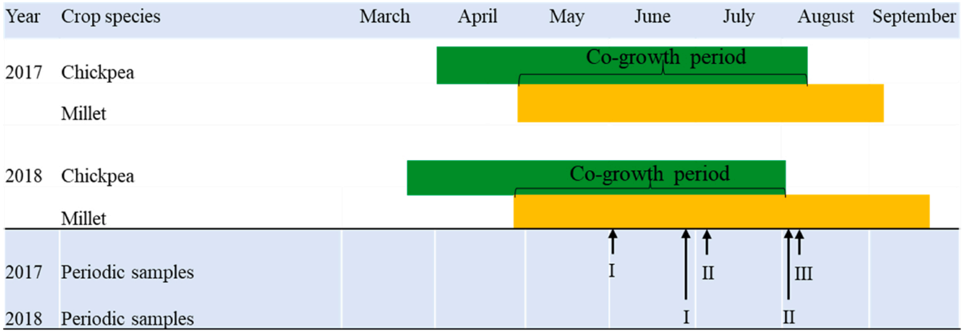

Fig. 3. Diagrammatic representation of the time of sampling during the co-growth period of millet and chickpea in two years; The green and yellow bars represent the period that the chickpea and millet are growing in the field respectively. The short arrows represent the three periodic samples in 2017, and the long arrows represent the two periodic samples in 2018. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

when chickpea was at podding stage and millet was at stem elongation), and the third sample (III) was on 9 August (the 102nd day of co-growth when chickpea was mature and millet was at grain filling stage). Samples were taken twice during the co-growth period in 2018. The first sample (I) was on 28 June (the 62nd day of co-growth when chickpea was at podding stage and millet was at stem elongation), and the second sample (II) was on 2 August (the 97th day of co-growth when chickpea was mature and millet was at grain filling stage).

At each sampling, the aboveground biomass was collected in 0.80 m row length of three millet rows (M1, M2, M3 in Fig. 2), and 0.80 m row length of four chickpea rows (CP1, CP2, CP3, CP4) in intercropping plots. The samples were processed separately for each row to determine differences between inner and outer rows of the species strips. In pure stands, the aboveground biomass was collected in 0.80 m row length of millet or chickpea. All sampled shoots were oven-dried at 70 °C for 72 h to estimate above-ground biomass.

Roots of sole millet, sole chickpea, intercropped millet (M1, M2, M3) and intercropped chickpea (CP1, CP2) were excavated at each sampling occasion (0−20 cm depth). Roots of two sampled plants were shaken to remove the loosely adhering soil, then the roots with tightly adhering rhizosphere soil were immersed in 50 mL of 0.2 mM $CaCl_2$ solution and shaken carefully to collect the rhizosphere soil solution. A subsample of the rhizosphere soil solution was stored at −20 °C prior and six types of carboxylates were identified (tartrate, malate, citrate, succinate, fumarate and trans-aconitate) and their concentrations were determined using HPLC. The sediment in the rhizosphere soil solution was dried to determine dry weight of rhizosphere soil. After shaking the roots of the other two sampled plants, the rhizosphere soil was collected by brushing the roots carefully. The collected rhizosphere soils were stored at 4 °C for the determination of enzyme activities. Subsamples of the rhizosphere soils were dried to determine P fractions.

The solution : rhizosphere soil ratios differed depending on root size and the amounts of rhizosphere soil. We cannot exclude that the carboxylate concentration in the solution is buffered by the soil, hence we calculated exudate ratios (of the same species when grown in mixture compared to when grown alone) under two extreme assumptions: 1) The soil solid phase is inert and does not buffer the carboxylate concentration, and 2) The soil solid phase completely buffers the carboxylate concentration. Under the first assumption, the carboxylate concentration in the extract is a dilution of the rhizosphere solution concentration, and the carboxylate concentration can be expressed in μmol g^{-1} soil dw, based on the measured dry weight of the rhizosphere soil. Under the second assumption, the carboxylate concentration in the extract is the same as the concentration in the rhizosphere soil solution (expressed in μmol L^{-1}). We calculated the ratio of (mixed plant):(sole plant) under both assumptions to see if there was a significant increase (both ratios $\pm 2 \times SE > 1$) or decrease (both ratios $\pm 2 \times SE < 1$) of exudation in response to a heterospecific neighbour (Table S1). In other cases, we refer to the outcome as no increase/no decrease. A similar procedure was followed for the comparison of carboxylate concentration between two species, the ratios were calculated as the ratio of (chickpea) : (millet) under these two extreme assumptions (Table S2).

The enzyme activities were determined within one week after sampling. Soil solution for the determinations of enzyme activities was obtained by gently shaking 2 g moist rhizosphere soils with 8 mL of deionized water for 1 min. After settling, the suspension was collected for the determinations of enzyme activities and the sediment was dried at 90 °C for 24 h to determine dry weight as a reference base.

Phytase activity was assessed according to Richardson et al. (2000): 0.5 mL of soil solution was mixed with 2 mL of 30 mM MES [2-(N-morpholino) ethanesulfonic acid] buffer (pH 5.5), 0.5 mL of 2 mM EDTA (ethylenediaminetetraacetic acid) and 0.5 mL of 20 mM Na-phytate (Sigma, St. Louis, MO, USA). The mixture was incubated for 1 h at 37 °C and the reaction was terminated by addition of 1 mL of 25 % trichloroacetic acid (TCA). Solutions were subsequently centrifuged at 12,000 × *g* for 10 min to remove soil particles. A control was determined in parallel for each soil sample and TCA was added prior to incubation. The orthophosphate concentration in the supernatant was determined by measuring absorbance at 882 nm using the molybdenum-blue reaction (Murphy and Riley, 1962). Phytase activity was expressed as µg released P per hour per gram soil.

Alkaline phosphatase activity was assayed according to (Neumann, 2006): 0.5 mL of soil solution was transferred into 2 mL Eppendorf reaction vials, then 0.4 mL of 100 mM Trizma buffer (pH 7.4) and 0.1 mL of 150 mM substrate [*p*NPP (*p*-nitrophenyl phosphate); Sigma St. Louis, MO, USA] was added. The mixture was incubated for 30 min at 30 °C, after that the reaction was terminated by addition of 0.5 mL of 0.5 M NaOH and centrifuged for 10 min at 12,000 × *g* to remove soil particles. A control was determined in parallel for each soil sample to correct for background coloration. The supernatant was measured spectrophotometrically at 405 nm to determine the absorbance. Alkaline phosphatase activity was measured from the release of *p*-nitrophenol (PNP) and expressed as µ mol PNP per hour per gram soil.

2.5. *Soil P fractionation*

At final harvest of each species, the soil in the PVC columns was collected as reference soil. P fractions of rhizosphere soils in monoculture plots of P0 treatments and P fractions of reference soils were determined using the method described by Tiessen and Moir (1993). The detailed procedure is described in Method S1.

2.6. *Data analysis*

Observed grain yield (or aboveground biomass, P content) is the sum of the grain yields (aboveground biomass, or P content) of millet ($Y_{I,M}$)

and chickpea ($Y_{I,CP}$) in intercropping:

$$\text{Observed yield} = Y_{I,\,M} + Y_{I,\,CP} \tag{2}$$

In strip intercropping systems, the expected yield is calculated from the land shares (LS_M and LS_{CP}) and crop yields ($Y_{M,M}$ and $Y_{M,CP}$) of each species in monoculture.

$$\text{Expected yield} = LS_M \times Y_{M,M} + LS_{CP} \times Y_{M,CP} \tag{3}$$

Where $LS_M = 0.53$, $LS_{CP} = 0.47$.

The net effect is the difference between observed yield and expected yield (Loreau and Hector, 2001):

$$\text{Net effect (NE)} = \text{Observed yield} - \text{Expected yield} \tag{4}$$

In a two-species mixture, the NE is equal to the sum of two components, the complementarity effect (CE) and the selection effect (SE) (Loreau and Hector, 2001):

$$NE = CE + SE = 2{*}\overline{\Delta RY} * \overline{M} + 2 * \text{cov}(\Delta RY, M) \tag{5}$$

The CE is calculated by multiplying $\overline{\Delta RY}$, the average relative yield gain of the two species, and $\overline{M}$, the average sole crop yield of the two species. The SE is equal to twice the covariance of relative yield gain and monoculture yield, $\text{cov}(\Delta RY, M)$.

Relative yield gain is defined as the difference between actual and expected relative yield:

$$\Delta RY_i = RY_i - RY_i^0 \tag{6}$$

where RY_i is the actual relative yield of a species and RY_i^0 is the expected relative yield. Actual relative yield is the yield in the intercrop (per unit area of the whole crop) divided by the yield in the sole crop (De Wit, 1960):

$$RY_i = Y_I / Y_M \tag{7}$$

Expected relative yield is equal to the land share of a species in the intercrop (LS) (Li et al., 2020).

We applied two-way ANOVA with cropping system and P level as fixed factors and block as a random effect to analyse data of biomass, yield and P content for millet and chickpea separately for each year. We used three-way ANOVA with crop species, cropping system and P level as fixed factors and block as a random effect to analyse data of phytase activity and alkaline phosphatase activity of millet and chickpea at each sampling date (R package *nlme*, (R C Team, 2014)). T-test was used to analyse the effect of P level on the harvest index of millet or chickpea and also the difference of NE, CE and SE from 0.

Table 3
P values of two-way ANOVA (P input level × cropping system) to analyse data on aboveground biomass, grain yield and P content (straw P + grain P) of millet and chickpea in sole crops and intercropping.

Year	Dependent variable	Species	Significance level (*P* value)		
			Phosphorus input (P)	Cropping system (C)	C × P
2017	Biomass	Millet	0.10	0.21	0.19
		Chickpea	0.20	0.84	0.95
	Yield	Millet	0.48	0.48	0.08
		Chickpea	0.14	0.76	0.51
	P content	Millet	0.001**	0.67	0.13
		Chickpea	0.04*	0.52	0.57
2018	Biomass	Millet	0.06	0.11	0.69
		Chickpea	0.07	0.08	0.70
	Yield	Millet	0.14	0.04*	0.56
		Chickpea	0.11	0.15	0.60
	P content	Millet	<0.003**	0.31	0.81
		Chickpea	0.005**	0.34	0.84

Note: The two-way ANOVA was carried out for 2017 and 2018 separately, and for millet and chickpea separately, with biomass, grain yield or P content as the dependent variables, with the P input levels and cropping systems as fixed factors and block as a random effect. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

3. Results

3.1. Aboveground biomass, grain yield and aboveground P content

P uptake responded positively to fertilization with P in both years (Tables 2 and 3). However, the biomass and yield of both species in the pure stands and intercrop did not respond to application of P fertilizer in 2017, while the biomass but not the yield of both species responded positively to the application of P fertilizer in 2018 (Tables 2 and 3). Significant net effects of intercropping were found for P uptake ($NE_P > 0$) at P0 in 2018 and for grain yield ($NE_Y > 0$) in the P100 treatment in 2018, but no significant net effects were recorded in other cases. The average harvest indices (HI) of both millet and chickpea were 0.20 and 0.33 in 2017 respectively, which were significantly lower than that in 2018 (HI of millet = 0.45, HI of chickpea = 0.54) ($P < 0.0001$, Fig. S1).

The observed aboveground biomass in intercropping was similar to the expected biomass based on monocultures at both P levels in both growing seasons (Fig. 4a). There was, however, significant overyielding of grain yield at the high P level and overyielding of P content at low P level in 2018: the observed grain yield of intercrop was 0.6 ± 0.2 Mg ha^{-1} higher than expected from sole crops at the high P level in 2018 ($P < 0.05$, Fig. 4b). This net effect was entirely due to the complementarity effect (0.6 ± 0.2 Mg ha^{-1}) (Table 2). The observed aboveground P content in the intercrop was 2.4 ± 0.8 kg ha^{-1} higher than expected at

Table 2
Aboveground biomass, grain yield and P content (straw and grain P) of millet and chickpea and observed, expected biomass, grain yield and P content, net effect, complementarity effect and selection effect (means ± standard error (n = 5)) of millet/chickpea intercropping in different treatments and years.

Year	Variables	P input levels	Millet		Chickpea		Net effect	Complementarity effect	Selection effect
			Mono	Inter	Mono	Inter			
2017	Biomass (Mg ha^{-1})	P0	13.4 ± 1.1	12.0 ± 1.0	2.7 ± 0.7	3.7 ± 1.3	−0.3 ± 0.8	1.2 ± 1.8	−1.5 ± 1.1
		P100	13.3 ± 2.0	14.0 ± 1.3	4.7 ± 1.2	4.8 ± 2.2	0.4 ± 0.9	0.0 ± 1.2	0.4 ± 0.5
	Yield (Mg ha^{-1})	P0	2.7 ± 0.3	2.4 ± 0.3	0.9 ± 0.3	1.1 ± 0.4	0.0 ± 0.2	0.2 ± 0.4	−0.2 ± 0.2
		P100	2.4 ± 0.5	2.9 ± 0.3	1.3 ± 0.4	1.7 ± 0.3	0.5 ± 0.3	0.5 ± 0.3	0.0 ± 0.1
	P content (kg ha^{-1})	P0	14.3 ± 2.8	12.7 ± 3.0	5.6 ± 1.6	6.5 ± 2.3	−0.4 ± 1.1	0.2 ± 2.1	−0.5 ± 1.1
		P100	19.8 ± 1.2	28.5 ± 5.5	11.1 ± 3.0	11.2 ± 5.5	4.7 ± 2.4	3.1 ± 2.7	1.5 ± 0.7
2018	Biomass (Mg ha^{-1})	P0	11.4 ± 0.5	10.3 ± 0.7	4.3 ± 0.4	5.1 ± 0.5	−0.2 ± 0.3	0.5 ± 0.4	−0.6 ± 0.4
		P100	12.3 ± 0.7	11.6 ± 0.4	5.8 ± 0.7	6.2 ± 1.0	−0.2 ± 0.6	0.3 ± 0.9	−0.5 ± 0.5
	Yield (Mg ha^{-1})	P0	4.5 ± 0.4	5.1 ± 0.6	2.1 ± 0.3	2.9 ± 0.2	0.5 ± 0.3	0.7 ± 0.3	−0.1 ± 0.2
		P100	4.9 ± 0.4	5.8 ± 0.5	2.9 ± 0.3	3.2 ± 0.4	0.6 ± 0.2*	0.6 ± 0.2*	0.0 ± 0.1
	P content (kg ha^{-1})	P0	16.6 ± 1.3	19.3 ± 1.5	9.5 ± 1.0	11.7 ± 1.1	2.4 ± 0.8*	2.7 ± 0.8*	−0.3 ± 0.3
		P100	26.9 ± 3.0	28.8 ± 0.8	15.9 ± 1.8	17.2 ± 2.6	1.6 ± 1.1	2.8 ± 1.6	−1.2 ± 1.1

Note: Biomass, yield and P content of intercrops were calculated based on the land areas occupied by each crop only. Asterisks represent significant difference compared to 0. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

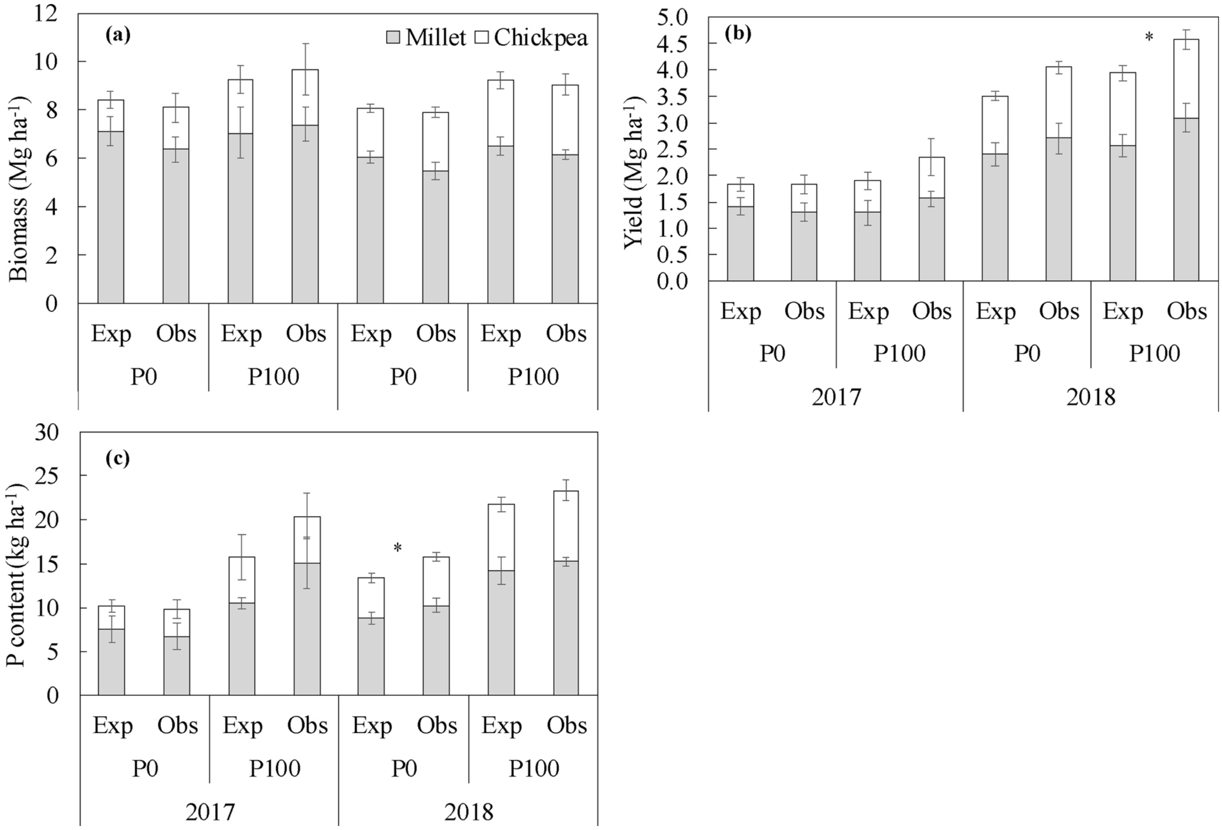

Fig. 4. Expected (Exp) and observed (Obs) total and species-specific aboveground biomass (a), grain yield (b), and aboveground P content (c) of millet/chickpea intercropping in two growing seasons and at two levels of P input (P0 and P100). Error bars represent standard error of the mean (n = 5). Asterisks indicate a significant difference between observed and expected, * $P < 0.05$.

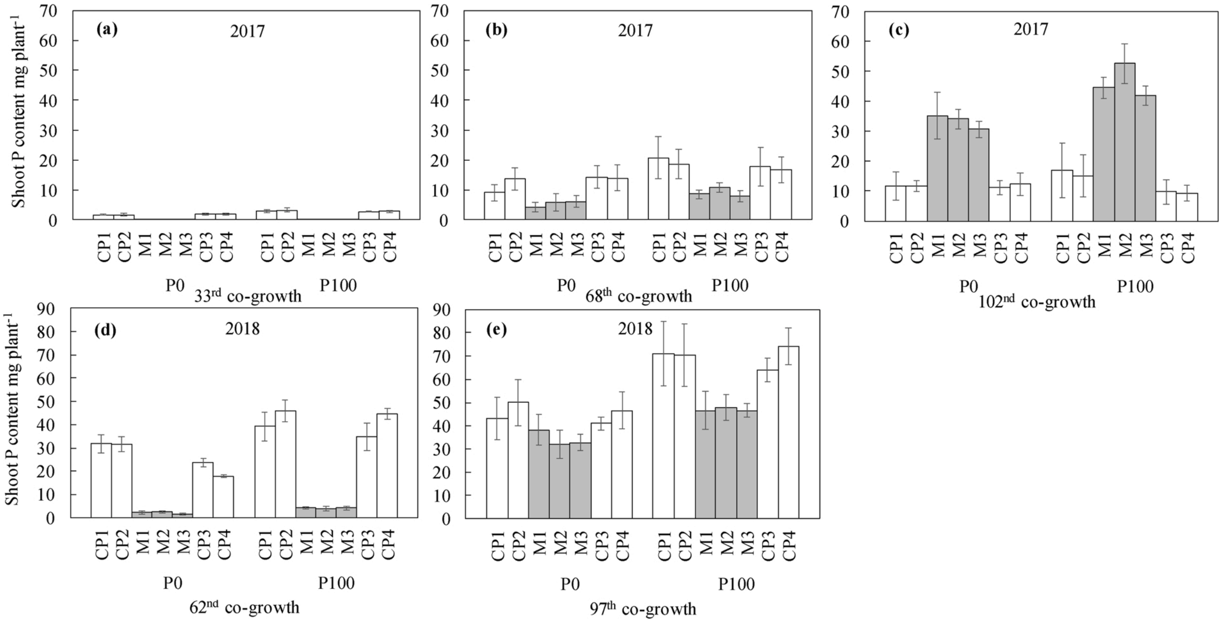

Fig. 5. Shoot P content of millet and chickpea in border rows and inner rows of the intercrop in two seasons. CP1, CP4 and M2 represent inner rows of chickpea and millet, respectively, and CP2, CP3, M1 and M3 represent border rows of chickpea and millet. Error bars represent standard errors (n = 5).

the low P level in 2018 ($P < 0.05$, Fig. 4c). This net effect was entirely due to the complementarity effect (2.7 ± 0.8 Mg ha^{-1}, Table 2) while the selection effect was not significantly different from zero (-0.3 ± 0.3 Mg ha^{-1}, Table 2). In 2017, the observed yield and P content of intercrops were similar to expected (Fig. 4b, c).

There was no difference in shoot P content (Fig. 5), shoot biomass (Fig. S2) and shoot P concentration (Fig. S3) of millet and chickpea between inner rows and border rows in intercropping at any sampling date in any of the two seasons. The lack of border row effects indicates absence of relevant interspecific interactions between millet and

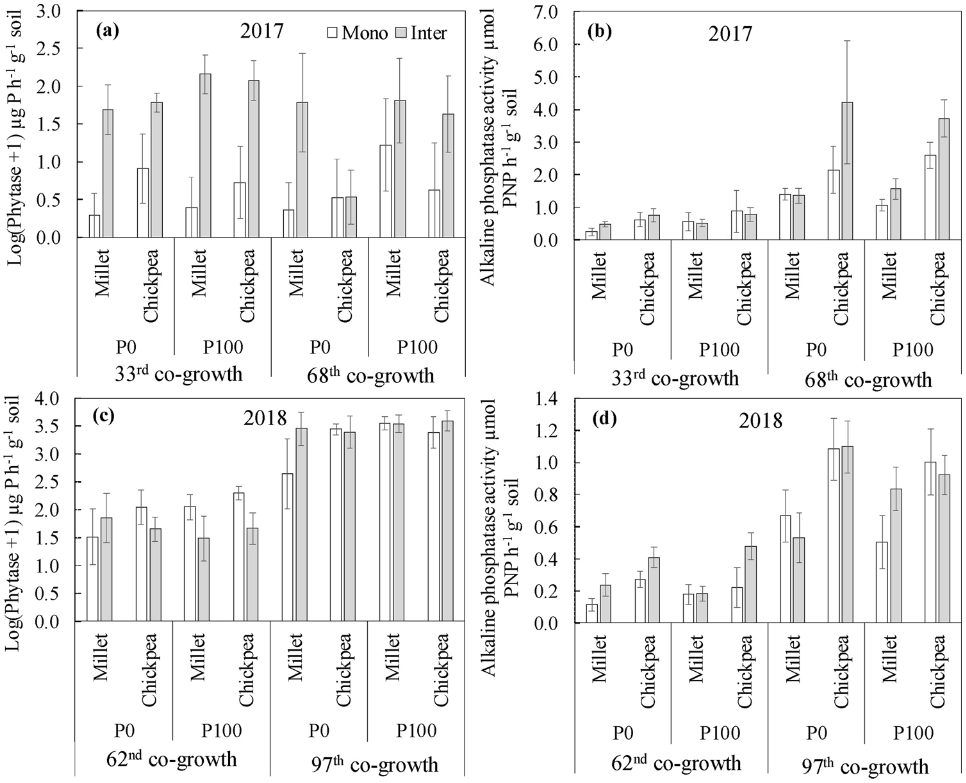

Fig. 6. Enzyme activities in the rhizospheres of millet and chickpea grown in monoculture or intercropping. (a, c) Phytase activity and (b, d) alkaline phosphatase activity: at the 33rd day and 68th day of co-growth in 2017 (a, b) and the 62nd and 97th day of co-growth in 2018 (c, d). Error bars represent standard errors (n = 5).

chickpea.

3.2. *Root exudates*

On the 33rd day of the co-growth period in 2017, phytase activity was on average four times higher in intercrops than in pure stands, irrespective of species, P level or their interactions (Table S3; Fig. 6). At the 68th day of co-growth in 2017, phytase activity was 2.2 times higher at the high P level than at the low P level, independent of species or cropping system or their interactions (Table S3) (Fig. 6a). In 2018, on the contrary, there was no difference in phytase activity between millet and chickpea, or between intercrops and monoculture, or between high P and low P (Table S3).

Alkaline phosphatase activity of chickpea was higher than that of millet at each sampling date in both years (Table S3, Fig. 6b, d). At the 62nd day of co-growth in 2018, the alkaline phosphatase activity was 1.7 times higher in intercrops than in monocultures regardless of species and P levels. There was no difference in both phytase and alkaline phosphatase activity between millet or chickpea plants in different rows (Fig. S4).

The main carboxylate components of millet and chickpea were malate, succinate and citrate, and the fractional contribution of these carboxylates varied over time (Fig. S5). The difference in carboxylate concentration between millet and chickpea also varied over time. The carboxylate concentration in the rhizosphere was higher in sole millet than in sole chickpea at the low P level on the 62nd day of the co-growth period in 2018 but not at any other sampling moment (Table S2). The rhizosphere carboxylate concentration was higher in intercropped millet than in sole millet at the 33rd day of co-growth period at high P level in 2017 (Table S1), while intercropped chickpea had a lower rhizosphere carboxylate concentration than sole chickpea at the 97th day of co-growth at the high P level in 2018.

4. Discussion

This study addressed three questions: (1) is there a positive net effect of millet/chickpea relay strip intercropping on P uptake, biomass and yield; (2) is there evidence for complementarity and facilitation with respect to P uptake; (3) do complementarity and facilitation with respect to P uptake drive a positive net effect of intercropping. The first question did not receive a straightforward “yes or no” answer: We observed a positive net effect of intercropping on aboveground P content ($NE_P > 0$) at zero P input, and there was a positive net effect for grain yield ($NE_Y > 0$) with P fertilizer input in 2018. However, neither effect was consistent across the two years, and the positive net effect on yield at high P in 2018 occurred without a positive net effect on P uptake, whereas the positive net effect on P uptake at low P in 2018 occurred without a positive net effect on biomass or yield.

The requirement for a proper test for complementarity and facilitation with respect to P uptake is that P is an important growth-limiting factor under the conditions of the study. The biomass and shoot P content of both species responded positively to the application of P fertilizer (Table 3) in 2018, so this condition was fulfilled in 2018. However, because of a flooding event in 2017, the grain yields of both millet and chickpea were low (Table 2), representing half of grain yield of the same cultivars reported in previous studies (Deng et al., 2013; Xia et al., 2013). As similar biomass was produced in both years while grain yield was lower in 2017, we found lower harvest indices in 2017 than in 2018 (Fig. S1). Consequently, the results in the first season did not allow for a strong test for complementarity and facilitation with respect to P uptake and grain yield by intercropping. The results in 2018 are considered representative for the potential for complementarity in this intercropping system. The results of 2017 are still relevant as they do still test complementarity, albeit under conditions where another factor than P shortage may have affected plant performance, and because of the very low P content of the native soil and previously demonstrated potential for complementarity between millet and chickpea in their access to different forms of sparingly soluble P (Li et al., 2019).

We found overyielding by millet/chickpea intercropping compared to their sole crops in 2018: there was a positive net effect of intercropping on aboveground P content at low P level, and on yield at high P level (Fig. 4b, c). The former was not associated with extra biomass and yield (Table 2). Yield of millet was higher in intercropping than in the sole cropping regardless of P level (Tables 2 and 3). This indicates reduced competition for resources in intercropping compared to sole cropping, as reflected by the positive complementarity effect of yield of the intercrop at a high P level, and a positive complementarity effect of P content of the intercrop at a low P level (Table 2).

The possible occurrences of P partitioning and facilitation of P uptake by intercrops were determined by the measurements of root exudates of crops in intercropping and monoculture. At each sampling date in both years, alkaline phosphatase activity of chickpea was higher than that of millet (Fig. 6). On the 62nd day of the co-growth period in 2018, the carboxylate concentrations in the rhizosphere at low P level were higher for millet than for chickpea (Table S2). We furthermore observed increased alkaline phosphatase activity in intercrops compared to sole crops in both species at both P levels, but decreased carboxylate concentration in intercropped chickpea compared to sole chickpea. Rhizosphere parameters hinted at a potential for complementarity and facilitation. Nevertheless, P uptake was similar in different rows of intercrop strips, contradicting a potentially positive effect of interspecific plant-plant interactions on P uptake.

Root exudates differed between species and cropping systems. The higher alkaline phosphatase activity of chickpea than millet suggests higher ability of chickpea to access organic P. Carboxylate concentration of millet was mostly similar to that of chickpea except at the last sampling in 2018 when carboxylate concentration of millet was higher than chickpea (Table S2). This means that both millet and chickpea exuded carboxylates, which mobilize the Ca-bound P (Hoffland et al., 1989; Lambers et al., 2012) and promote the desorption of organic P in the soil for hydrolysis by phosphatase (Gerke, 2015; George et al., 2005; Tinker and Nye, 2000). In our previous pot experiment (Li et al., 2021), both the carboxylate concentration and acid phosphatase activities were higher in chickpea than in millet. Temporal fluctuations of root exudates at different plant growth stages could be related to this discrepancy (Mimmo et al., 2011; Li et al., 2016). At the first sampling in 2018, the higher alkaline phosphatase activity in the rhizosphere of intercrops compared to the sole crops, regardless of species and P levels, indicates potential P mobilization by intercrops. However, during the co-growth period in 2018, the rhizosphere carboxylate concentrations of intercrops were mostly similar to, or lower than in sole crops. Lower carboxylate concentrations could limit the efficiency of higher phosphatase activity in intercropping. Similar P content (Fig. 5) and biomass (Fig. S2) of plants in different rows of intercropping strips suggest no increased P uptake in response to a heterospecific neighbour in intercropping, although there were differences in root exudates between millet and chickpea.

The third question was answered negatively: while some mechanisms for complementary P uptake were found, and intercropping was characterized by some positive net effects compared to expectations based on sole crop performance, no evidence was found that the net effect of intercrop was driven by complementarity and facilitation with respect to P uptake. Yield and P uptake were uncoupled. The increased P uptake by intercrops at a low P level did not result in a yield increase of intercrops, and the yield increase of intercrops at high P level was not associated with an increased P uptake by intercrops compared to sole cropping.

We did not find evidence that the two species tap on different P fractions (Fig. S6) or that one species facilitates P acquisition by the other species. Complementarity (or facilitation) is often used as both a cause of enhanced ecosystem functioning in diverse communities and a consequence of some community processes (Barry et al., 2019). The measurements at the plant level (biomass, yield and P uptake) represent the outcomes but not the underlying mechanisms. The differences in rhizosphere enzyme concentrations provided the potential to cause complementarity or facilitation, but they did not result in increased yield or P uptake. These results were similar to those obtained in a pot experiment by Phoenix et al. (2020) who provided some evidence for P partitioning without any impact on P uptake by, and growth of, mixtures compared to monocultures. This means that the mechanism of complementarity in or facilitation of P uptake was not the only cause of the positive net effect that we found; the net effect was independent of P level. Previous studies likewise reported no change in overyielding by intercropping in response to a P fertilizer gradient on a low P soil (Tang et al., 2016; Li et al., 2018). This indicates that overyielding can be achieved at both high and low P levels, and that P partitioning in intercropping is a phenomenon that is not highly related to overyielding, and cannot be regarded as the main driver of overyielding.

In the present study, the identified positive complementarity effects at high and low P levels may have been caused by other factors than complementarity in P uptake or facilitation of P uptake. Chickpea, a C3 species was sown and harvested earlier than millet (C4 species), resulting in temporal complementarity between two species. A previous meta-analysis showed that intercrops of C3/C4 combination and temporal niche differentiation allow temporal and spatial complementarity in acquiring light or soil resources (water, N or P) between intercropped species (Li et al., 2020). The later sown species may additionally benefit from N (and P) mineralization from decomposing roots of the earlier harvested species (Cong et al., 2015). Moreover, fertilizer input increases the net effect of relay strip intercropping (Li et al., 2020) because sufficient nutrient availability promotes the recovery growth of the later-sown species (millet in the present study) after harvest of the early-sown species (chickpea).

5. Conclusions

Millet and chickpea are species with complementary traits for acquisition of sparingly soluble P. We selected these species to test in the field whether complementary traits for P acquisition and resulting P partitioning can drive agronomically relevant levels of overyielding. The two species differed in carboxylate concentrations and alkaline phosphatase activity in the rhizosphere. Consistent with this difference in P acquisition traits, we found an increase in P uptake by the intercrop in the low-P treatment in one of the two experimental years. This increase in P uptake was, however, not associated with overyielding. On the other hand, in the same year, overyielding occurred in the high-P treatment in which P fertilizer was added to supplement the low-P soil at the site. This overyielding by intercropping was not associated with increased P uptake by intercropping. In the first year, a flooding event affected all experimental treatments, potentially affecting the potential for complementarity and facilitation. Results in the second year provide evidence for complementary traits for P acquisition from different sources, but no evidence for agronomically relevant overyielding as a result of related P partitioning. On the other hand, complementarity for other factors associated with differences in other species traits, e.g., growing period, resulted in positive overyielding in the high-P treatment. Results clearly show that complementary traits (e.g., differences in root exudates) do not guarantee overyielding at the crop level.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fcr.2021.108127.

References

Barry, K.E., Mommer, L., van Ruijven, J., Wirth, C., Wright, A.J., Bai, Y., Connolly, J., De Deyn, G.B., de Kroon, H., Isbell, F., Milcu, A., Roscher, C., Scherer-Lorenzen, M., Schmid, B., Weigelt, A., 2019. The future of complementarity: disentangling causes from consequences. Trends Ecol. Evol. 34 (2), 167–180.

Brooker, R.W., Bennett, A.E., Cong, W.-F., Daniell, T.J., George, T.S., Hallett, P.D., Hawes, C., Iannetta, P.P.M., Jones, H.G., Karley, A.J., Li, L., McKenzie, B.M., Pakeman, R.J., Paterson, E., Schöb, C., Shen, J., Squire, G., Watson, C.A., Zhang, C., Zhang, F., Zhang, J., White, P.J., 2015. Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. New Phytol. 206 (1), 106–117.

Callaway, R.M., Brooker, R.W., Choler, P., Kikvidze, Z., Lortie, C.J., Michalet, R., Paolini, L., Pugnaire, F.I., Newingham, B., Aschehoug, E.T., Armas, C., Kikodze, D., Cook, B.J., 2002. Positive interactions among alpine plants increase with stress. Nature 417 (6891), 844–848.

Cong, W.F., Hoffland, E., Li, L., Six, J., Sun, J.H., Bao, X.G., Zhang, F.S., van der Werf, W., 2015. Intercropping enhances soil carbon and nitrogen. Glob. Change Biol. 21 (4), 1715–1726.

Corre-Hellou, G., Fustec, J., Crozat, Y., 2006. Interspecific competition for soil N and its interaction with N-2 fixation, leaf expansion and crop growth in pea-barley intercrops. Plant Soil 282 (1-2), 195–208.

Crème, A., Rumpel, C., Gastal, F., de la Luz Mora Gil, M., Chabbi, A., 2016. Effects of grasses and a legume grown in monoculture or mixture on soil organic matter and phosphorus forms. Plant Soil 402 (1-2), 117–128.

De Wit, C.T., 1960. On competition. Verslagen landbouwkundige onderzoekingen 66.8 Pudoc. Centrum voor landbouwpublikaties en landbouwdocumentatie, Wageningen, The Netherlands.

Deng, C., Feng, Z., Jiang, H., 2013. A variety contrast test of double ridge whole-mulching furrow sowing millet. Ningxia J. Agric. For. Sci. Technol. 54, 9–10 (in Chinese).

Evers, J.B., van der Werf, W., Stomph, T.J., Bastiaans, L., Anten, N.P.R., 2018. Understanding and optimizing species mixtures using functional–structural plant modelling. J. Exp. Bot. 70 (9), 2381–2388.

Faucon, M.P., Houben, D., Reynoird, J.P., Mercadal-Dulaurent, A.M., Armand, R., Lambers, H., 2015. Advances and perspectives to improve the phosphorus availability in cropping systems for agroecological phosphorus management. In: Sparks, D.L. (Ed.), Advances in Agronomy, vol 134, pp. 51–79.

George, T.S., Richardson, A.E., Simpson, R.J., 2005. Behaviour of plant-derived extracellular phytase upon addition to soil. Soil Biol. Biochem. 37 (5), 977–988.

George, T.S., Hinsinger, P., Turner, B.L., 2016. Phosphorus in soils and plants - facing phosphorus scarcity. Plant Soil 401 (1-2), 1–6.

Gerke, J., 2015. The acquisition of phosphate by higher plants: effect of carboxylate release by the roots. A critical review. J. Plant Nutr. Soil Sci. 178 (3), 351–364.

Hauggaard-Nielsen, H., Jensen, E.S., 2001. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N availability. Field Crops Res. 72 (3), 185–196.

He, Q., Bertness, M.D., Altieri, A.H., 2013. Global shifts towards positive species interactions with increasing environmental stress. Ecol. Lett. 16 (5), 695–706.

Hinsinger, P., 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil 237 (2), 173–195.

Hinsinger, P., Betencourt, E., Bernard, L., Brauman, A., Plassard, C., Shen, J.B., Tang, X. Y., Zhang, F.S., 2011. P for two, sharing a scarce resource: soil phosphorus acquisition in the rhizosphere of intercropped species. Plant Physiol. 156 (3), 1078–1086.

Hoffland, E., Findenegg, G.R., Nelemans, J.A., 1989. Solubilization of rock phosphate by rape. 2. Local root exudation of organic-acids as a response to P-starvation. Plant Soil 113 (2), 161–165.

Lambers, H., Bishop, J.G., Hopper, S.D., Laliberte, E., Zuniga-Feest, A., 2012. Phosphorus-mobilization ecosystem engineering: the roles of cluster roots and carboxylate exudation in young P-limited ecosystems. Ann. Bot.-Lond. 110 (2), 329–348.

Latati, M., Blavet, D., Alkama, N., Laoufi, H., Drevon, J.J., Gerard, F., Pansu, M., Ounane, S.M., 2014. The intercropping cowpea-maize improves soil phosphorus availability and maize yields in an alkaline soil. Plant Soil 385 (1-2), 181–191.

Latati, M., Bargaz, A., Belarbi, B., Lazali, M., Benlahrech, S., Tellah, S., Kaci, G., Drevon, J.J., Ounane, S.M., 2016. The intercropping common bean with maize improves the rhizobial efficiency, resource use and grain yield under low phosphorus availability. Eur. J. Agron. 72, 80–90.

Li, L., Li, S.M., Sun, J.H., Zhou, L.L., Bao, X.G., Zhang, H.G., Zhang, F.S., 2007. Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. Proc. Natl. Acad. Sci. U. S. A. 104 (27), 11192–11196.

Li, L., Tilman, D., Lambers, H., Zhang, F.S., 2014. Plant diversity and overyielding: insights from belowground facilitation of intercropping in agriculture. New Phytol. 203 (1), 63–69.

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

Li, X.F., Wang, C.B., Zhang, W.P., Wang, L.H., Tian, X.L., Yang, S.C., Jiang, W.L., van Ruijven, J., Li, L., 2018. The role of complementarity and selection effects in P acquisition of intercropping systems. Plant Soil 422 (1–2), 479–493.

Li, C., Kuyper, T.W., van der Werf, W., Zhang, J., Li, H., Zhang, F., Hoffland, E., 2019. Testing for complementarity in phosphorus resource use by mixtures of crop species. Plant Soil 439 (1-2), 163–177.

Li, C., Hoffland, E., Kuyper, T.W., Yu, Y., Li, H., Zhang, C., Zhang, F., van der Werf, W., 2020. Yield gain, complementarity and competitive dominance in intercropping in China: a meta-analysis of drivers of yield gain using additive partitioning. Eur. J. Agron. 113, 125987.

Li, C., Kuyper, T.W., van der Werf, W., Zhang, J., Li, H., Zhang, F., Hoffland, E., 2021. A conceptual framework and an empirical test of complementarity and facilitation with respect to P uptake by species mixtures. Pedosphere. In press.

Liao, D., Zhang, C., Li, H., Lambers, H., Zhang, F., 2020. Changes in soil phosphorus fractions following sole cropped and intercropped maize and faba bean grown on calcareous soil. Plant Soil.

Loreau, M., Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412 (6842), 72–76.

Mimmo, T., Hann, S., Jaitz, L., Cesco, S., Gessa, C.E., Puschenreiter, M., 2011. Time and substrate dependent exudation of carboxylates by *Lupinus albus* L. and *Brassica napus* L. Plant Physiol. Biochem. 49 (11), 1272–1278.

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

Neumann, G., 2006. Quantitative Determination of Acid Phosphatase Activity in the Rhizosphere and on the Root Surface. Handbook of Methods used in Rhizosphere Research, p. 536.

Pearse, S.J., Veneklaas, E.J., Cawthray, G., Bolland, M.D.A., Lambers, H., 2007. Carboxylate composition of root exudates does not relate consistently to a crop species' ability to use phosphorus from aluminium, iron or calcium phosphate sources. New Phytol. 173 (1), 181–190.

Phoenix, G.K., Johnson, D.A., Muddimer, S.P., Leake, J.R., Cameron, D.D., 2020. Niche differentiation and plasticity in soil phosphorus acquisition among co-occurring plants. Nat. Plants 1–6.

R C Team, 2014. R: A Language and Environment for Statistical Computing. R foundation for statistical computing, Vienna, Austria.

Richardson, A.E., Hadobas, P.A., Hayes, J.E., 2000. Acid phosphomonoesterase and phytase activities of wheat (*Triticum aestivum* L.) roots and utilization of organic phosphorus substrates by seedlings grown in sterile culture. Plant Cell Environ. 23 (4), 397–405.

Richardson, A.E., Hocking, P.J., Simpson, R.J., George, T.S., 2009. Plant mechanisms to optimise access to soil phosphorus. Crop Pasture Sci. 60 (2), 124–143.

Richardson, A.E., Lynch, J.P., Ryan, P.R., Delhaize, E., Smith, F.A., Smith, S.E., Harvey, P.R., Ryan, M.H., Veneklaas, E.J., Lambers, H., Oberson, A., Culvenor, R.A., Simpson, R.J., 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. Plant Soil 349 (1-2), 121–156.

Tang, X., Placella, S.A., Daydé, F., Bernard, L., Robin, A., Journet, E.P., Justes, E., Hinsinger, P., 2016. Phosphorus availability and microbial community in the rhizosphere of intercropped cereal and legume along a P-fertilizer gradient. Plant Soil 1–16.

Tang, X., Zhang, C., Yu, Y., Shen, J., van der Werf, W., Zhang, F., 2020. Intercropping legumes and cereals increases phosphorus use efficiency; a meta-analysis. Plant Soil.

Tiessen, H., Moir, J.O., 1993. Characterization of available P by sequential extraction. In: Carter, M.R. (Ed.), Soil Sampling and Methods of Analysis, pp. 75–86.

Tinker, P.B., Nye, P.H., 2000. Solute Movement in the Rhizosphere. Oxford University Press.

Turner, B.L., 2008. Resource partitioning for soil phosphorus: a hypothesis. J. Ecol. 96 (4), 698–702.

Vance, C.P., Uhde-Stone, C., Allan, D.L., 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol. 157 (3), 423–447.

Vandermeer, J.H., 1989. The Ecology of Intercropping. Cambridge University Press.

Vengavasi, K., Pandey, R., 2018. Root exudation potential in contrasting soybean genotypes in response to low soil phosphorus availability is determined by photo-biochemical processes. Plant Physiol. Biochem. 124, 1–9.

Wang, X., Deng, X., Pu, T., Song, C., Yong, T., Yang, F., Sun, X., Liu, W., Yan, Y., Du, J., 2017. Contribution of interspecific interactions and phosphorus application to increasing soil phosphorus availability in relay intercropping systems. Field Crops Res. 204, 12–22.

Westerman, R.L., 1990. Soil Testing and Plant Analysis. Soil Science Society of America, Inc.

Willey, R.W., 1990. Resource use in intercropping systems. Agric. Water Manage. 17 (1–3), 215–231.

Xia, H.Y., Wang, Z.G., Zhao, J.H., Sun, J.H., Bao, X.G., Christie, P., Zhang, F.S., Li, L., 2013. Contribution of interspecific interactions and phosphorus application to sustainable and productive intercropping systems. Field Crops Res. 154, 53–64.

Xue, Y., Xia, H., Christie, P., Zhang, Z., Li, L., Tang, C., 2016. Crop acquisition of phosphorus, iron and zinc from soil in cereal/legume intercropping systems: a critical review. Ann. Bot.-Lond. 117 (3), 363–377.

Yu, Y., Stomph, T.-J., Makowski, D., van der Werf, W., 2015. Temporal niche differentiation increases the land equivalent ratio of annual intercrops: a meta-analysis. Field Crops Res. 184, 133–144.

Zuo, Y., Zhang, F., Li, X., Cao, Y., 2000. Studies on the improvement in iron nutrition of peanut by intercropping with maize on a calcareous soil. Plant Soil 220 (1), 13–25.