

## RESEARCH ARTICLE

# Wheat-chickpea and wheat-lupin intercropping systems: Effects on wheat yield and soil properties under contrasting P availability

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## ABSTRACT

Intercropping has been widely studied owing to its potential to increase crop production and improve soil properties. The combination of wheat (*Triticum aestivum* L.) with chickpea (*Cicer arietinum* L.) and lupin (*Lupinus albus* L.), along with P fertilization, has shown promising results in enhancing wheat yield. This study investigated the effects of intercropping wheat with chickpea and lupin, as well as P fertilization, on wheat yield, soil properties, and root morphological characteristics. A field experiment was conducted to evaluate chemical properties, basal soil respiration, and enzymatic activity. The results revealed that intercropping with chickpea and lupin, along with the addition of P, significantly increased the relative wheat yield compared with monoculture. The land equivalent ratio (LER) was also influenced by P fertilization, with wheat/lupin showing higher values than wheat/chickpea. Furthermore, aerial biomass exhibited significant differences between cropping systems and P fertilization, with the P+ treatment (220 kg ha<sup>-1</sup>) resulting in 24% higher aerial biomass during anthesis in the wheat/chickpea intercropping system than in wheat monocrops. The findings of this study indicate that the cropping system did not have a substantial impact on soil chemical properties. However, the interaction between cropping system and P fertilization had a significant effect on soil basal respiration. Although phosphatase activity remained unchanged by the cropping system and P fertilization, urease activity was significantly influenced by both factors, with a notable interaction effect.

**Key words:** Andisol, *Cicer arietinum*, enzymatic activity, interspecific facilitation, LER, *Lupinus albus*, phosphorus, *Triticum aestivum*.

## INTRODUCTION

In Chile, approximately half of the available farmland consists of Andisols, which are known for their strong P-fixation capacity (Mejías et al., 2013). This has led farmers to apply high rates of P fertilizers to meet the nutritional needs of wheat (*Triticum aestivum* L.) This is a significant concern in these soil types because P is a critical macronutrient for plant growth and plays an essential role in various metabolic processes in plants, including photosynthesis, respiration, and protein synthesis (Wang et al., 2023). Hence, root systems have developed both morphological and physiological mechanisms to acquire P when P is scarce in the soil (Wang et al., 2019). Plants employ various morphological strategies to obtain nutrients, including alteration of root distribution, extension of root length, and development of root hairs (Ruiz et al., 2020). Concerning certain physiological root strategies, it is important to emphasize the exudation of extracellular substances such as phosphatases (Lambers et al., 2013). Therefore, prioritizing the cultivation of legume cultivars that release phosphatases to enhance P availability in volcanic soils is recommended. To address this issue, it is necessary to embrace diverse resource-efficient agricultural systems that provide greater resilience to soil and climatic

contingencies. Intercropping systems represent a well-explored option for diversifying crops, involving simultaneous cultivation of two or more species within the same growing season.

Legumes play a key role in N fixation and stimulation of soil microbial communities through active rhizodeposition, leading to enhanced plant biomass and increased yields in companion plants. Latati et al. (2019), in their study on intercropping durum wheat with chickpea, showed a significant increase in the shoot and root biomass of wheat within the intercropped system compared to the monoculture. These results imply that modifications induced by the rhizosphere of the legume in the intercrops promote the uptake of P and N, resulting in increased aboveground biomass, grain yield, and land-use efficiency in the companion crop. However, this improvement has only been observed in cereal crops, suggesting the unidirectional benefit of legumes (Latati et al., 2019; Boudsocq et al., 2022). A similar study by Chaechian et al. (2022) documented an increase in yield components, leading to an enhanced seed yield in the intercropping of wheat and chickpea for both plant species. This growth enhancement was linked to improved water utilization and nutrient availability. The distinct root depths and distributions of wheat and chickpea enable them to absorb water from various soil depths. Thus, this is a clear example of facilitation, an ecological process that occurs when interactions between neighboring plants benefit at least one of them. Conversely, interspecific competition enhances the growth and yield of the dominant crop, whereas competition during the cogrowth phase weakens subordinate crop development (Rodriguez et al., 2020; Zhu et al., 2023).

Lupin improves the absorption of P by inefficient species such as maize through the exchange of rhizosphere functions (Dissanayaka et al., 2015). Furthermore, Betencourt et al. (2012) found that intercropping wheat and chickpea increased the availability of P in the rhizosphere of both plants, especially in soils with limited P content, owing to the rhizosphere alkalization. However, this is recommended as an effective approach to promote wheat growth (Boudsocq et al., 2022). However, there is insufficient evidence to support this in the case of acidic Andisols, which are characterized by low P availability.

Based on earlier research concerning the biological and physiological nutrient acquisition mechanisms in legumes, our hypothesis suggests that in an Andisol, the intercropping system of wheat and Fabaceae will likely exhibit increased soil microbiological activity compared to wheat monoculture. This difference was expected because active rhizodeposition is facilitated by the presence of Fabaceae. Hence, the aims of this study were: i) To assess how the inclusion of two distinct Fabaceae species in an intercropping system affects root morphology, soil chemical properties, and microbial soil activity, such as basal soil respiration, fluorescein diacetate hydrolase (FDAse) activity, and acid phosphatase and urease enzyme activities, in comparison to a wheat monoculture, across three different growth stages (tillering, anthesis, and physiological maturity), and ii) elucidate the relationships among wheat yield, soil properties, and enzyme activity.

## MATERIALS AND METHODS

### Site and soil description and experimental design

The present study was conducted at the Instituto de Investigaciones Agropecuarias (INIA) Experimental Station Santa Rosa, located in the Mediterranean region of south-central Chile. This site, which boasts of irrigation facilities, is positioned at 36°3' S, 71°54' W. The study covered one growing season from 31 July 2020 to 17 February 2021. The average temperature recorded during the growing seasons in this area was 14.9 °C, with an annual precipitation of 269 mm. In 2020, the total precipitation measured was 745.8 mm. The soil in the experimental area is classified as a Diguillín series, which is derived from modern volcanic ash of the Andisol order. They were classified as Haploxerands (Soil Survey Staff, 2006). The soil texture was silt loam, and its chemical properties (0-30 cm depth) were as follows: 11.3% organic matter content, pH 5.2, 48.6 mg kg<sup>-1</sup> available N, 7.0 mg kg<sup>-1</sup> Olsen-P content, and 137 mg kg<sup>-1</sup> available K. Before commencing the field experiment, oats were planted in 2019, and lime (CaCO<sub>3</sub>) was applied at a rate of 1000 kg ha<sup>-1</sup> at the beginning of each season.

A two-factor factorial arrangement was used in this study. Three plots were used for the treatment, and in each plot, three plants were sampled (nine plants per treatment). The first factor examined different cropping systems, specifically comparing monocrop and intercropping approaches. Second, we investigated the effects of external addition of P fertilizer, distinguishing between treatments with and without P. We evaluated one monoculture system: Spring wheat (*Triticum aestivum* L.) 'Pantera-INIA'. Additionally, we examined two intercropping systems: Wheat-lupin (*Lupinus albus* L.) 'Alboroto-INIA' and wheat-chickpea (*Cicer arietinum* L.) 'Alfa-INIA'. These treatments were randomly arranged in plots measuring 3.2 m × 4.0 m (12.8 m<sup>2</sup>; 18 plots in total).

For the monoculture setup, the spacing between rows was 20 cm for all crops. The spacing between rows was 20 cm for wheat monoculture. For intercropping systems, row intercropping was 1:1 (a combination of alternate rows of wheat and legumes).

Seed-bed preparation involves two steps. First, a harrow pass was performed, followed by a vibrocultivator. Sowing was performed manually, and before sowing, lupin and chickpea seeds were treated with a gel containing *Rhizobium* sp. at a concentration of  $1.0 \times 10^9$  CFU mL<sup>-1</sup> to ensure effective root nodulation. The seed doses for sowing were 220 kg ha<sup>-1</sup> for wheat and 120 kg ha<sup>-1</sup> for lupin and chickpea.

Fertilization was applied to all cropping systems, including monocultures and intercrops. This treatment involved either the absence of P fertilizer or addition of P fertilizer. For the P fertilization treatment, the soil received triple superphosphate (Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O) at a rate of 220 kg ha<sup>-1</sup>, potassium muriate (KCl) at 220 kg ha<sup>-1</sup>, and urea (CO(NH<sub>2</sub>)<sub>2</sub>) at 103 kg ha<sup>-1</sup> only at sowing. The treatments without P supply were fertilized in the same manner as described above but without the application of triple superphosphate. The treatments were as follows: Wheat monocrop (W) with P (P+) and without P (P-), wheat/lupin (WL) with P (P+) and without P (P-), and wheat/chickpea (WC) with P (P+) and without P (P-).

To control weeds, herbicides were applied exclusively to wheat at the tillering stage. The herbicide products used were 750 g L<sup>-1</sup> MCPA-dimethylammonium (4 kg ha<sup>-1</sup> MCPA; Marks and Company Ltd., Wyke-Bradford, West Yorkshire, England), 600 g kg<sup>-1</sup> metsulfuron-methyl (4 kg ha<sup>-1</sup> Ajax; Hangzhou March Chemicals Co. Ltd., Hangzhou, China), 250 g kg<sup>-1</sup> tritosulfuron (4 kg ha<sup>-1</sup> Arrat; BASF, Ludwigshafen, Germany), and 550 g kg<sup>-1</sup> dicamba-sodium (4 kg ha<sup>-1</sup>; Syngenta, Kwizda Agro GmbH, Leobendorf, Austria). Irrigation was performed during the tillering.

### Soil and plant sampling and harvest

Plant and soil samples were collected from the wheat crops at three phenological stages: Tillering, anthesis, and physiological maturity. Bulk soil was collected from a depth of 0-30 cm depth. Each soil sample was divided into two subsamples. One part was dried and sieved to < 2 mm and used for chemical analyses. The other part was sieved to < 2 mm and stored at -20 °C for determination of soil basal respiration and activities of fluorescein diacetate hydrolase (FDase), acid phosphatase, and urease.

Three plants were collected from the central row of each plot. The plant samples were separated into shoots and roots. The shoots and roots were washed with water after separation. The roots, shoots, and grains were oven-dried in a forced-air oven at 70 °C for 48 h until constant weight was achieved. Biomass (aerial and root) measurements were performed by weighing DM. Below-ground parts of the plants were harvested and dried in craft paper bags. Root parameters, such as length, area, volume, and diameter, were determined using a WinRhizo computerized system (Regent Instruments Inc., Quebec, Canada).

To determine the wheat yield, the grains harvested from one linear meter in the central row of each plot were weighed. An automotive tool (Wintersteiger, Ried im Innkreis, Austria) was used for harvesting. The number of spikes per square meter, seeds per spike, and weight of 1000 seeds were also calculated. The absolute wheat yield was estimated by considering the grain weight and area covered by all plants in each plot. To assess the influence of interspecific competition in each system, relative wheat yield was calculated by dividing the absolute real wheat yield by the plant density in each crop. Lupin and chickpea yields were determined by weighing harvested grains using the same method as that used for wheat. The total production in each intercropped plot was calculated by adding the wheat and legume yields.

The land equivalent ratio (LER) served as an indicator of resource use efficiency in intercropping compared with monocultures (Hauggaard-Nielsen et al., 2001). This index quantifies the relative area required in a monoculture to achieve the same yield, considering that both species were present in the intercropping system. The LER value was calculated by summing the proportions of the intercrop and monocrop yields for each species (partial LER for each species) using  $LER = \text{partial LER for wheat} + \text{partial LER for legumes}$ . The partial LER for wheat was obtained by dividing the intercrop wheat yield by the monoculture wheat yield, whereas the partial LER for legumes was determined by dividing the intercrop legume yield by the monoculture legume yield. A LER value greater than one indicates the advantage of intercropping in terms of both yield and land use. However, an LER value of 1 or less suggests that intercropping does not provide any advantages over monoculture. Additionally, when the partial LER for a specific species is greater than 0.5, intercropping is advantageous for that species (Zhu et al., 2023).

### Soil analyses

The soil pH was determined using a 1:5 (w/v) water-to-soil ratio. Available N and P were assessed using the methods described by Jackson (1958) and Watanabe and Olsen (1965), respectively. To analyze basal soil respiration, the closed system soil incubation methodology of Alef and Nannipieri (1995) was utilized, and the results were expressed as micrograms ( $\mu\text{g}$ ) of  $\text{CO}_2$  produced per gram of soil (dry weight) per hour. For the evaluation of fluorescein diacetate hydrolase (FDAse) activity, a modified methodology of Alef and Nannipieri (1995) was employed, and the results were expressed in micrograms ( $\mu\text{g}$ ) of FDAse per gram of soil (dry weight). Acid phosphatase activity was analyzed using the methodology described by Tabatabai and Bremner (1969), and the results were expressed in micromoles ( $\mu\text{mol}$ ) of *p*-nitrophenol (PNP) produced per gram of soil (dry weight) per hour. Urease activity was measured following the procedure outlined by Nannipieri et al. (1980), and the results are expressed in micromoles ( $\mu\text{mol}$ ) of ammonium-N produced per gram of soil (dry weight) per hour.

### Statistical analysis

Data were checked to ensure normal distribution using the Shapiro-Wilk test at  $p < 0.05$ . Data were subjected to two-way repeated measures ANOVA: Cropping system (wheat monoculture and intercropping systems) and fertilization treatment (with and without P). Significant differences were determined using Fisher's least significant difference test ( $p < 0.05$ ). Statistical analyses were performed using SPSS software version 24 (IBM, Armonk, New York, USA).

## RESULTS

### Soil chemical properties

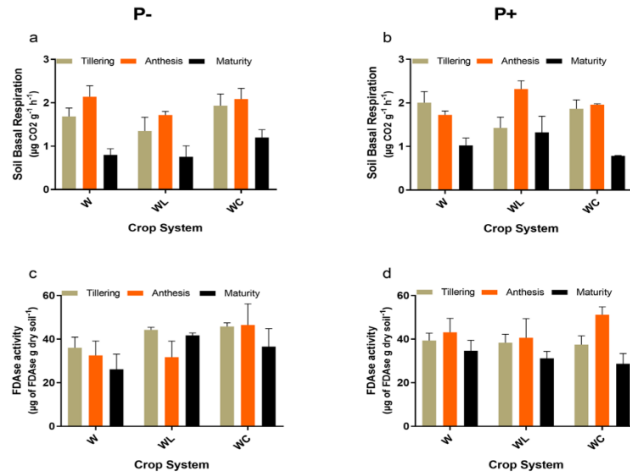
The cropping system did not contribute to modifying soil chemical properties such as pH, nitrates, ammonium, available N, and Olsen-P (Table 1). In addition, we did not observe an increase in the above-mentioned properties when considering P fertilization in soils. The cropping system (CS) did not significantly influence these properties; therefore, intercropping did not significantly affect the nutrient availability. The CS  $\times$  P fertilization interaction was nonsignificant for any property, indicating that the effect of P addition on these soil properties was not affected by the intercropping system.

**Table 1.** Soil pH and available N and P in response to wheat monoculture and wheat-lupin or wheat-chickpea intercropping under two different fertilization doses. Values represent the mean of three replicates. Mean  $\pm$  standard error (Fisher's post hoc test  $p < 0.05$ ). +P: 220  $\text{kg ha}^{-1}$ ; -P: 0  $\text{kg ha}^{-1}$ .

Cropping system	Addition of P	pH	$\text{NO}_3$	$\text{NH}_4$	Available N	Olsen-P
	$\text{kg ha}^{-1}$		$\text{mg kg}^{-1}$	$\text{mg kg}^{-1}$	$\text{mg kg}^{-1}$	$\text{mg kg}^{-1}$
Wheat	+P	5.5 $\pm$ 0.08	4.2 $\pm$ 0.67	3.8 $\pm$ 0.27	8.0 $\pm$ 0.90	7.1 $\pm$ 0.47
	-P	5.4 $\pm$ 0.12	6.3 $\pm$ 1.50	4.8 $\pm$ 0.38	11.0 $\pm$ 1.32	5.2 $\pm$ 0.55
Wheat + lupin	+P	5.4 $\pm$ 0.04	5.1 $\pm$ 1.29	4.0 $\pm$ 0.67	9.2 $\pm$ 1.73	6.4 $\pm$ 1.13
	-P	5.5 $\pm$ 0.02	6.4 $\pm$ 1.07	3.7 $\pm$ 0.34	10.2 $\pm$ 1.37	6.2 $\pm$ 0.20
Wheat + chickpea	+P	5.4 $\pm$ 0.03	5.6 $\pm$ 1.19	4.2 $\pm$ 0.67	9.9 $\pm$ 0.67	5.9 $\pm$ 1.86
	-P	5.5 $\pm$ 0.05	5.0 $\pm$ 1.11	3.7 $\pm$ 0.15	8.7 $\pm$ 1.27	5.4 $\pm$ 0.95
Cropping system (CS)	P-value	0.0996	0.885	0.615	0.958	0.805
P	P-value	0.623	0.352	0.850	0.376	0.306
CS $\times$ P	P-value	0.770	0.514	0.304	0.301	0.691

### Microbiological soil properties

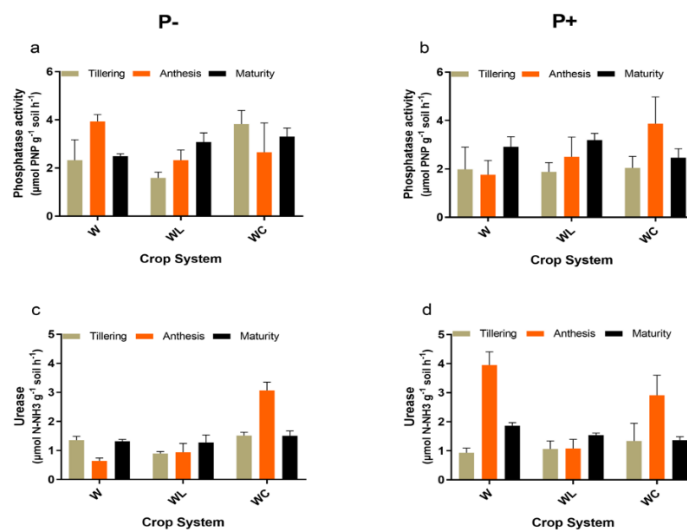
The cropping system and P fertilization did not significantly affect soil basal respiration or FDAse activity. However, the CS  $\times$  P fertilization interaction was significant for basal soil respiration ( $p < 0.05$ ) (Figure 1, Table 2). In addition, acid phosphatase activity was not influenced by cropping system or P fertilization. However, the cropping system and P fertilization ( $p < 0.05$ ) significantly increased the urease activity. The interaction of CS  $\times$  P fertilization regime was significant for urease activity, indicating an effect of P addition in terms of cropping system (Figure 2). Phenological stage (Table 3) was the factor contributing to the highest significant effect on soil basal respiration and FDAse activity ( $p < 0.05$ ).



**Figure 1.** Values of soil basal respiration (a and b) and fluorescein diacetate hydrolase (FDase) activity (c and d). Values represent mean ( $n = 3$ ). Error bars represent standard error (Fisher's post hoc test  $p < 0.05$ ). P-: 0 kg ha<sup>-1</sup> phosphate; P+: 220 kg ha<sup>-1</sup> phosphate; W: wheat monocrop; WL: wheat-lupin; WC: wheat-chickpea.

**Table 2.** ANOVA results (P-value) of the soil microbiological properties, aerial and root biomass, and root characteristics were assessed for monocrops and intercropping systems, as well as for P (P+ and P-). Significant at \*\*\* $p < 0.001$ ; \*\* $p < 0.01$ ; \* $p < 0.05$ ; ns: nonsignificant ( $p > 0.05$ ). Significant differences were determined according to Fisher's LSD. FDase: Fluorescein diacetate hydrolase.

	Soil basal respiration	FDase activity	Phosphatase activity	Urease activity	Aerial biomass	Root biomass	Root length	Root surface area	Root volume	Root diameter
Between subjects										
Cropping system (CS)	0.845	0.289	0.597	0.001**	0.01*	0.294	0.910	0.962	0.522	0.239
P	0.890	0.358	0.703	0.005*	0.07	0.080	0.622	0.872	0.611	0.279
CS × P	0.032*	0.822	0.146	0.001**	0.573	0.425	0.007	0.007	0.007	0.377



**Figure 2.** Values of acid phosphatase (a and b) and urease (c and d). Values represent mean ( $n = 3$ ). Error bars represent standard error (Fisher's post hoc test  $p < 0.05$ ). P-: 0 kg ha<sup>-1</sup> phosphate; P+: 220 kg ha<sup>-1</sup> phosphate; W: wheat monocrop; WL: wheat-lupin; WC: wheat-chickpea; PNP: *p*-nitrophenol.

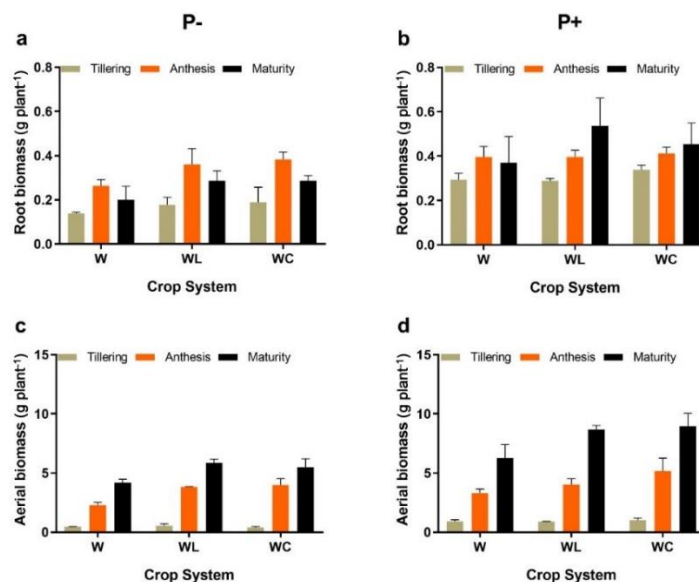
**Table 3.** ANOVA results (P-value) in phenological stage (PS) of the soil microbiological properties, aerial and root biomass, and root characteristics were assessed for monocrops and intercropping systems, as well as for P (P+ and P-). Significant at \*\*\* $p < 0.001$ ; \*\* $p < 0.01$ ; \* $p < 0.05$ ; ns: nonsignificant ( $p > 0.05$ ). Significant differences were determined according to repeated measures. FDAse: Fluorescein diacetate hydrolase.

	Soil basal respiration	FDAse activity	Phosphatase activity	Urease activity	Aerial biomass	Root biomass	Root length	Root surface area	Root volume	Root diameter
Within subjects PS	0.0001	0.02	0.08	0.06	0.00003	0.007	0.02	0.64	0.04	0.0003
PS x Cropping system (CS)	0.16	0.81	0.23	0.41	0.03	0.48	0.21	0.72	0.71	0.01
PS x P	0.95	0.95	0.46	0.23	0.003	0.45	0.47	0.75	0.12	0.01
PS x CS x P	0.44	0.75	0.76	0.37	0.72	0.73	0.66	0.12	0.11	0.50

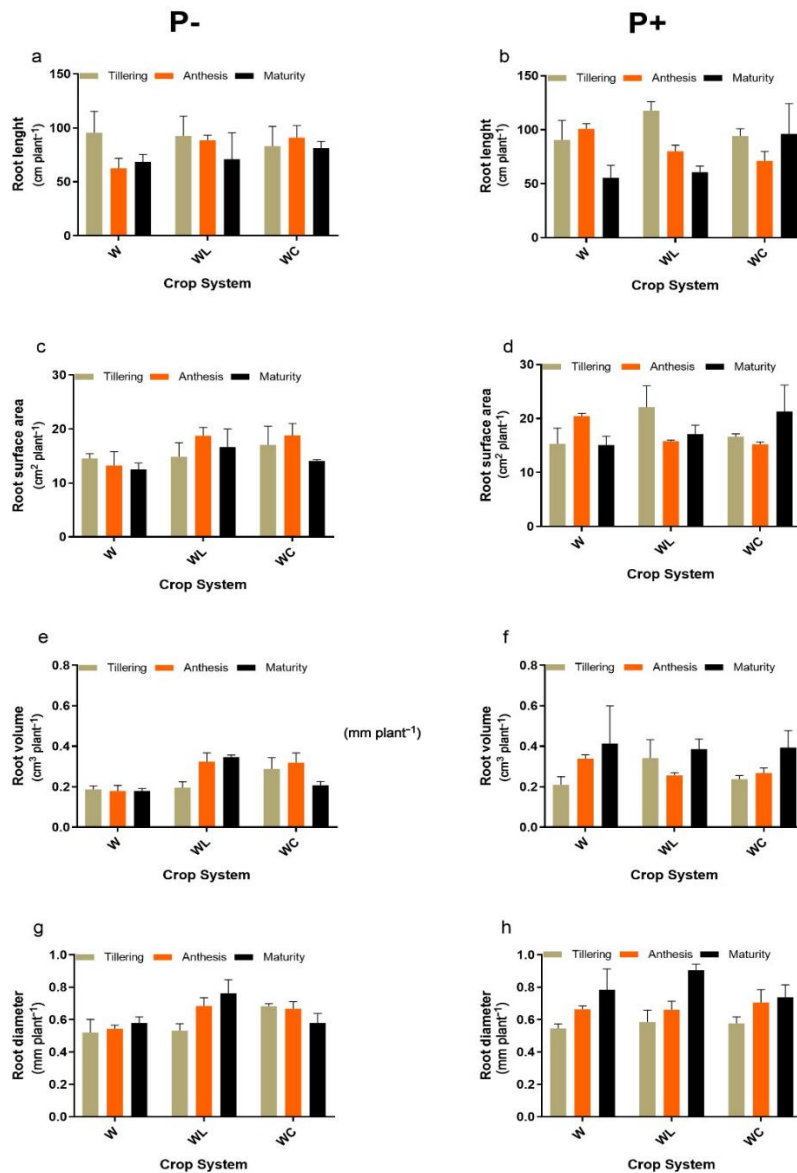
### Roots and aerial wheat plants weight

Concerning root biomass, P fertilization had the highest significance (Figures 3a and 3b). There was nonsignificant effect of cropping system on root biomass. However, the phenological stage had a significant effect on root biomass. Aerial biomass showed significant differences between cropping systems and P fertilization. Aerial biomass in anthesis was 24% higher in the P+ treatment than in the wheat monocrops for wheat/chickpea in the intercropping system (Figures 3c and 3d). Additionally, there was a significant effect on the phenological stage (Table 3).

Root characteristics (length, surface area, and diameter) were not significantly affected by cropping system or P fertilization (Figure 4). In contrast, root volume was significantly affected by P fertilization ( $p < 0.05$ ). However, the only factor that significantly affected the length, root volume, and root diameter was phenological stage (Figure 4 and Table 3).



**Figure 3.** Root biomass (a, b) and aerial biomass (c, d) per plant. Values represent mean (n = 3). Error bars represent standard error (Fisher's post hoc test  $p < 0.05$ ). P-: 0 kg ha<sup>-1</sup> phosphate; P+: 220 kg ha<sup>-1</sup> phosphate; W: wheat monocrop; WL: wheat-lupin; WC: wheat-chickpea.

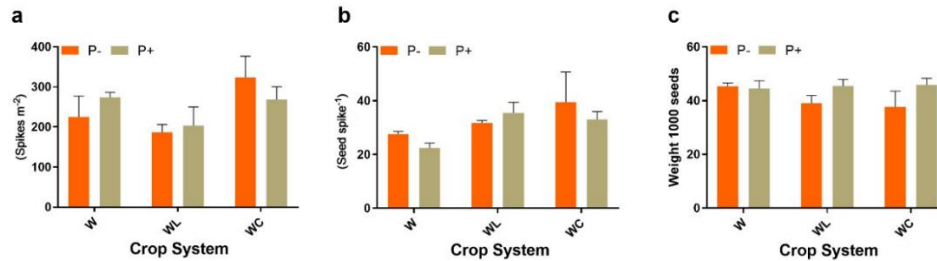


**Figure 4.** Root length (a, b), root surface (c, d), root volume (e, f), and root diameter (g, h) per plant. Values represent mean ( $n = 3$ ). Error bars represent standard error (Fisher's post hoc test  $p < 0.05$ ). P-: 0 kg ha<sup>-1</sup> phosphate; P+: 220 kg ha<sup>-1</sup> phosphate; W: wheat monocrop; WL: wheat-lupin; WC: wheat-chickpea.

### Wheat production and yield components

None of the wheat production and yield components were significantly affected by the cropping system, such as spike m<sup>-2</sup>, seed spike<sup>-1</sup>, and weight of 1000 seeds (Figure 5, Table 4). Cropping system or P fertilization did not significantly affect the absolute yield of wheat (Figure 6a). The results, regardless of cropping system, showed similar yields to wheat monoculture, even though the density of wheat plants was lower in intercropping. The data presented in Figure 6b reveal a substantial impact of intercropping and P incorporation on wheat yield when measured on a plant density basis. The WC and WL systems demonstrated a noticeable improvement in relative wheat yield, which increased by 1.36 and 1.31 t ha<sup>-1</sup>, respectively, compared to the monoculture. These findings were significant and underscored the potential benefits of these intercropping systems. In the absence of P fertilization (P-), the relative yields of wheat were 0.85 and 0.44 t ha<sup>-1</sup> higher than those in the monocrop wheat for W+C and

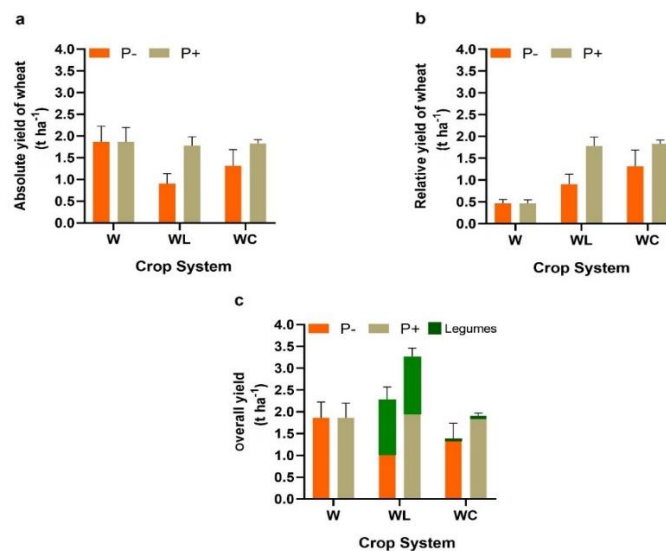
W+L, respectively (Figure 6b). Regarding the overall yield, there had a nonsignificant effect; however, a significant effect was observed in W+L compared with that in W and W+C (Figure 6c). On the other hand, the yield of legumes (Table 5) showed significant differences ( $p < 0.0001$ ) between the species where lupine showed a great yield superiority compared to chickpea, regardless of the cropping system.



**Figure 5.** Number of wheat spikes m<sup>-2</sup> (a), ratio of seed number per spike (b), and kernel weight (c) of wheat. Values represent mean (n = 3). Error bars represent standard error (Fisher's post hoc test  $p < 0.05$ ). P+: 220 kg ha<sup>-1</sup> phosphate; P-: 0 kg ha<sup>-1</sup> phosphate; W: wheat monocrop; WL: wheat-lupin; WC: wheat-chickpea.

**Table 4.** ANOVA results (P values) of wheat yield components such as spikes m<sup>-2</sup>, seed numbers spike<sup>-1</sup> and 1000 seed weight, and wheat grain absolute yield, wheat relative yield, overall yield of plots, land equivalent ratio (LER) and partial LERs. Significant at \*\*\* $p < 0.001$ ; \*\* $p < 0.01$ ; \* $p < 0.05$ ; ns: nonsignificant ( $p > 0.05$ ). Significant differences were determined according to Fisher's LSD.

Variables	Values	Spikes numbers m <sup>-2</sup>	Seeds spike <sup>-1</sup>	1000 seed weight	Wheat absolute yield	Wheat relative yield	Overall yield of plots	LER	Partial LERs
Cropping system (CS)	P-value	0.07	0.11	0.60	0.22	0.0001***	0.005	0.03*	0.01*
P	P-value	0.92	0.54	0.11	0.07	0.017*	0.053	0.02*	0.04*
CS × P	P-value	0.43	0.57	0.37	0.33	0.14	0.26	0.44	0.81



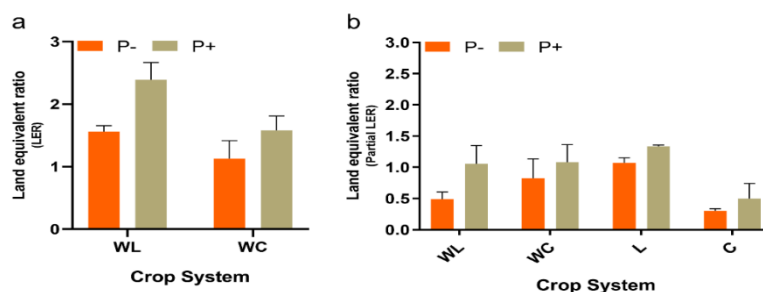
**Figure 6.** Absolute wheat yield (a), relative wheat yield (b), and overall crop yield (c). Values represent mean (n = 3). Error bars represent standard error (Fisher's post hoc test  $p < 0.05$ ). P+: 220 kg ha<sup>-1</sup> phosphate; P-: 0 kg ha<sup>-1</sup> phosphate; W: wheat monocrop; WL: wheat-lupin; WC: wheat-chickpea.



**Table 5.** ANOVA results of legume yield in monoculture and intercropping. Values represent the mean of three replicates. Mean  $\pm$  standard error (Fisher's post hoc test  $p < 0.05$ ). +P: 220 kg ha<sup>-1</sup>; -P: 0 kg ha<sup>-1</sup>.

Species	Addition of P	Monoculture	Intercropping
		t ha <sup>-1</sup>	
Lupin	+P	1.12 $\pm$ 0.07	1.49 $\pm$ 0.06
	-P	1.27 $\pm$ 0.04	1.37 $\pm$ 0.15
Chickpea	+P	0.17 $\pm$ 0.05	0.07 $\pm$ 0.02
	-P	0.18 $\pm$ 0.03	0.06 $\pm$ 0.01
ANOVA, P values			
Cropping system		0.0001	0.0001
Addition of P		0.11	0.42
CS x P		0.17	0.53

The LER was significantly influenced by P fertilization, with WL showing higher values than WC (Figure 7a). Partial LERs also revealed a significant effect of P fertilization (Figure 7b). The results showed that the partial LERs of lupin P+, WC P+, and WL P+ were 1.33, 1.08, and 1.06, respectively. However, the partial LER for WC P+ was 0.5. Furthermore, it is worth noting that this partial LER of 0.5, which is higher than the LER values obtained for chickpea and wheat grown alone, indicates that the intercropping system may have a positive impact on soil and plant performance compared to the monoculture.



**Figure 7.** Land equivalent ratio (LER) (a) and partial LER for each crop (b). Values represent mean ( $n = 3$ ). Error bars represent standard error (Fisher's post hoc test  $p < 0.05$ ). P+: 220 kg ha<sup>-1</sup> phosphate; P-: 0 kg ha<sup>-1</sup> phosphate; WL: wheat-lupin; WC: wheat-chickpea; L: lupin; C: chickpea.

## DISCUSSION

The pH of bulk soil plays a crucial role in influencing the accessibility of P to plants. Higher pH enhanced the solubility of P in the soil solution. Inorganic P tends to create insoluble compounds when bound to Al and Fe oxides or soil particles, thereby reducing its accessibility to plants (Wang and Lambers, 2020). Nevertheless, as the soil pH increases, these insoluble compounds can dissolve and release inorganic P into the soil solution, thereby enhancing its bioavailability. In this study, different cropping systems showed no notable impact on soil chemical properties. These findings align with the results of Xing et al. (2023), indicating that intercropped legume/maize systems did not influence the total soil N concentration over a 4 yr experimental period. However, noticeable changes were observed after 12 yr, suggesting that changes in total N concentration may be a gradual process.

Intercropping has been shown to enhance plant biomass, grain yield, and overall crop yield by promoting better resource utilization, such as light, soil nutrients, and water. This improved resource capture by intercropped plants can lead to increased productivity despite the potential competition for resources (Mawnai et al., 2021). Intercropping has been shown to have significant implications for soil enzyme activity and nutrient uptake in wheat systems. Studies have indicated that intercropping can enhance soil enzymatic activity, leading to improved decomposition of organic matter and the release of nutrients into the soil (Farooq et al., 2021).

Additionally, intercropping can result in increased acid phosphatase activity and secretion of organic acids in the rhizosphere, facilitating efficient P utilization by wheat through complementary nutrient uptake mechanisms (Li et al., 2016). Furthermore, the positive effect of intercropping on yield can be attributed to factors such as improved microclimate conditions, which can influence crop performance. Intercropping systems can create a more favorable environment for plant growth, potentially enhancing nutrient availability, water retention, and overall plant health. Moreover, resource sharing and potential facilitation between intercropped species can contribute to increased productivity (Chen et al., 2015).

Cropping system and P fertilization affected soil basal respiration. Soil basal respiration, a key indicator of soil microbial activity, is influenced by various factors including soil pH. The relationship between soil pH and basal respiration is complex and can be influenced by other factors, such as C availability, N enrichment, and environmental conditions. Furthermore, soil temperature, moisture content, and organic matter composition also affect basal soil respiration, making it an important factor to consider when assessing and managing soil health. It is important to note that the soil pH can also affect the soil microbial community, as indicated by Zhou et al. (2021). They found that the composition of the soil microbial community was a major factor affecting basal soil respiration, highlighting the intricate relationship between soil pH, microbial communities, and basal respiration (Ontman et al., 2023). The FDAse hydrolytic activity and soil basal respiration peaks during wheat tillering and anthesis were aligned with increased soil microbial activity. The heightened microbial activity during tillering and anthesis was attributed to elevated nutrient uptake by wheat plants (N and P) during these stages. The C exudation by roots serves as a crucial energy source for soil microorganisms, fostering nutrient exchange, with exudate quantity and composition contingent on the plant species and phenological stages (Gargallo-Garriga et al., 2018).

When examining our results, they showed that there was nonsignificant effect of different cropping systems, phenological stages, and P factors on acid phosphatase. Meier et al. (2023) conducted experiments on wheat varieties grown in Andisols with low and high P concentrations. These results highlight the variability in the effect of P dose on acid phosphatase activity, which negatively impacts wheat cultivars and affects growth traits, P uptake, and grain yield. Variations in enzymatic activity were observed, which were primarily influenced by P levels rather than by water availability.

The effect of P fertilization on urease activity in soils has been a subject of interest. Several studies have provided insights into the relationship between P fertilization and urease activity, and found that long-term application of N fertilizer significantly decreased urease activity (Dabin et al., 2019). This suggests that N fertilization may affect urease activity, indicating a potential interaction between N and P fertilization in influencing soil enzyme activity. Further research is needed to determine the specific mechanisms by which N fertilization affects urease activity, and to explore the potential implications of this interaction for soil enzyme activity in different agricultural systems. Future studies should investigate the impact of various N fertilization strategies on urease activity as well as the long-term effects of these strategies on soil enzyme activity in agricultural systems to gain a deeper understanding of the complex relationship between N nutrition and soil enzyme function.

The relationship between changes in soil enzyme activities and nutrient uptake by plants is complex and may not always show a direct correlation because of various influencing factors within the soil-plant system. While alterations in soil enzyme activities can affect nutrient cycling and substrate availability, the efficiency of nutrient uptake by plants is also influenced by soil microbial communities, plant-microbe interactions, and the overall soil nutrient status (Zhou and Staver, 2019). The interaction between cropping systems and P fertilization, for instance, can have a significant effect on soil basal respiration, whereas urease activity is notably influenced by both factors, indicating the complexity of the relationship between soil enzymes and nutrient uptake (Waratadar et al., 2022). Therefore, the impact of changes in soil enzyme activities on nutrient uptake is multifaceted and dependent on a combination of factors within the soil environment and plant-microbe interactions.

The root and plant biomass of wheat increased significantly with the addition of P. These results are consistent with those of previous studies (Wang et al., 2023), which reported that the availability of P improved root characteristics such as root volume. This is partly explained by the fact that P is necessary for the formation of new cells and for cell division, which are essential for root growth and development. Therefore, the availability of P can limit or favor root growth, and hence, the absorption of other nutrients (Wang et al., 2023).

However, the development of aerial biomass in wheat intercropped with the addition of P increased compared to other wheat monocrops, which shows the great capacity of Fabaceae to mobilize nutrients, thus favoring wheat.

The relative yield of wheat increased significantly with intercropping and P application. This increase was related to seeds spike<sup>-1</sup>. Similar results were reported by Yang et al. (2022), who investigated the effect of different intercropping systems (maize/legumes and non-legumes) on maize yield and efficiency in the use of P, and their results showed that the intercropping of peanut/maize and soybean/maize yielded the highest yield of maize compared to the monoculture. It is worth mentioning that adequate P nutrition enhances wheat growth and development, particularly yield. Adequate P nutrition increases the number of seeds per spike in wheat. This is attributed to the fact that a significant portion of the P absorbed by wheat is deposited in the grain, contributing to improved performance and higher yields. The P application has been linked to positive effects on yield components, such as grain number per spike, 1000-grain weight, and yield per plant (Wu et al., 2022). Hence, the presence of Fabaceae in an intercropping system generates positive interactions, such as complementarity of resource use through interspecific facilitation and decreases intraspecific competition between plants of the same species, consequently improving production per plant (Rodriguez et al., 2020).

The findings from this study revealed that wheat quality parameters and absolute yield were not significantly influenced by the cropping system or P fertilization. However, the results demonstrated a significant effect of intercropping and the addition of P on relative yield, with the intercropping systems providing higher relative yields than the monoculture, particularly in the absence of P fertilization. Additionally, this study showed that LER was significantly influenced by P fertilization, with certain intercropping systems exhibiting higher values. Furthermore, the partial LERs of specific crop combinations were notably affected by P fertilization, indicating their impact on the productivity of the intercropping systems. The results of van der Werf et al. (2020) align with recent research emphasizing the importance of nutrient management and intercropping strategies in enhancing crop productivity and resource use efficiency. These findings also support the potential benefits of intercropping systems, particularly in terms of the relative yield and LER, which could contribute to sustainable agricultural intensification. Moreover, this study underscores the need for further investigation of the mechanisms underlying the observed interactions between cropping systems, P fertilization, and soil and crop properties, as well as their implications for sustainable agricultural practices (van der Werf et al., 2020). Overall, the results provide valuable insights into the complex dynamics of crop production and highlight the significance of integrated nutrient management and intercropping strategies for optimizing agricultural productivity and sustainability.

## CONCLUSIONS

The results showed that the cropping system did not significantly affect the soil chemical properties. Additionally, soil basal respiration and fluorescein diacetate hydrolase activity were not significantly influenced by the cropping system or P fertilization, although the interaction between cropping system and P fertilization had a significant effect on soil basal respiration. Furthermore, while phosphatase activity remained unaffected by the cropping system and P fertilization, urease activity was significantly influenced by both factors, with a notable interactive effect. The P fertilization had the most significant effect on root biomass, whereas aerial biomass exhibited notable differences between cropping systems and P fertilization. Although wheat quality parameters and absolute yield were not significantly affected by the cropping system or P fertilization, relative yield, expressed on a plant density basis, was significantly affected by intercropping and P addition, with intercropping systems providing higher relative yields than monocultures, particularly in the absence of P fertilization. This study also revealed the significant effects of P fertilization on the land equivalent ratio and partial land equivalent ratio, indicating the influence of P fertilization on the productivity of intercropping systems. These findings underscore the potential benefits of intercropping systems in enhancing resource-use efficiency and overall productivity, emphasizing the need for further research on the mechanisms underlying the observed interactions and their implications for sustainable agricultural intensification.

### Author contribution

Conceptualization: M.S., D.C-R. Methodology: M.S., D.C-R., I.M-T. Validation: N.A. Formal analysis: N.A. Resources: M.S., D.C-R., I.M-T. Data curation: N.A. Writing the original draft: N.A., M.S. Writing-review and editing: M.S. Project administration: M.S. Funding acquisition: M.S., D.C-R., I.M-T. All coauthors reviewed the final version and approved the manuscript before submission.

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