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CULTIVOS INTERCALADOS TRIGO-LEGUMINOSAS Y SU EFECTO EN LA DISPONIBILIDAD DE FÓSFORO

Tesis para optar al grado de Magíster en Ciencias Agronómicas

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RESUMEN

Los andisoles son el orden de suelo menos extenso, cubriendo menos del 1% de la superficie terrestre de la tierra. Sin embargo, en Chile, juegan un papel crucial, constituyendo el 50 % de la producción de cereales del país. A pesar de su importancia, existe una notable escasez de estudios que investiguen el impacto del suelo deficiente en P en la producción de cereales en el sistema de cultivos intercalados. Se realizó un experimento de campo de 2 años con dos niveles de P (con y sin) y sistemas de cultivo (es decir, monocultivo de trigo, cultivo intercalado de trigo/lupino y trigo/garbanzo). Se midieron las propiedades químicas del suelo, la respiración basal del suelo, las actividades enzimáticas, las propiedades morfológicas de las raíces y el rendimiento de los cultivos. El trigo intercalado exhibió un rendimiento relativo de un 60 % superior al del monocultivo de trigo, incluso en los tratamientos sin adición de P. Esto se relacionó con la actividad de la fosfatasa, que aumentó en trigo/lupino, mostrando una actividad 18 % y 30 % superior a la del trigo/garbanzo y el monocultivo de trigo, respectivamente. Además, la proporción de tierra equivalente (LER) de trigo/lupino superó 1 en ambas temporadas, lo que indica una mejor utilización del suelo y los nutrientes. En cambio, el trigo/garbanzo obtuvo un LER inferior a 1 en la segunda temporada. Esto demostró que estas especies exhiben una fuerte competencia, lo que tiene un impacto notable en el rendimiento general. Estos hallazgos sugieren que el cultivo intercalado de trigo/lupino puede mejorar la disponibilidad de nutrientes y el rendimiento, particularmente cuando se depende de bajos niveles de P. Estos resultados apoyan la practicidad del uso de sistemas de cultivos intercalados para mejorar los servicios ecosistémicos y la producción agrícola en un Andisol.

SUMMARY

Andisols are the least extensive soil order, covering less than 1 % of the earth's land surface. However, in Chile, they play a crucial role, constituting 50 % of the country's cereal production. Despite their significance, there is a notable scarcity of studies investigating the impact of P-deficient soil in cereal production in intercropping system. A 2-year field experiment was conducted with two P levels (with and without) and cropping systems (i.e wheat monoculture, wheat/lupin and wheat/chickpea intercropping). Soil chemical properties, basal soil respiration, enzyme activities, root morphological properties and crop yield were measured. The intercropped wheat exhibited a relative yield 60 % higher than that of the wheat monoculture, even in the treatments without P addition. This was related to the activity of phosphatase, which increased in wheat/lupin, showing an activity 18 % and 30 % higher than that in wheat/chickpea and wheat monoculture, respectively. Furthermore, the Land Equivalent Ratio (LER) of wheat/lupin surpassed 1 in both seasons, indicating improved soil and nutrient utilization. In contrast, the wheat/chickpea obtained an LER lower than 1 in the second season. This showed that these species exhibit strong competition, which has a notable impact on the overall yield. These findings suggest that wheat/lupin intercropping can enhance nutrient availability and yield, particularly when relying on low levels of P. These results support the practicality of using intercropping systems to enhance ecosystem services and agricultural production in Andisol.

CAPÍTULO 1

INTRODUCCION GENERAL

Cada vez se están dirigiendo más esfuerzos en la investigación y desarrollo hacia prácticas alternativas a las prácticas agrícolas convencionales que aborden los problemas de la degradación de los recursos naturales (Jensen et al., 2020) y la pérdida de la fertilidad biológica del suelo (Hartman et al., 2018). Una de estas alternativas es muy antigua como el uso de sistemas de cultivos intercalados.

El cultivo intercalado se define como el crecimiento simultáneo de dos o más especies durante una misma estación de crecimiento y pueden estar dispuestos en hileras, franjas o en relevos (Vandermeer, 2012; Wang et al., 2017).

El avance en la comprensión de las interacciones entre los principales nutrientes para los cultivos, como el nitrógeno (N) y el fósforo (P), y la actividad microbiana del suelo proporcionarán nuevas pruebas del valor de los cultivos intercalados de cereales y leguminosas, con el objetivo de reducir el aporte de fertilizantes (Tian et al., 2019). Sin embargo, estos sistemas no han sido demostrados apropiadamente en condiciones edafoclimáticas especiales y limitantes, desde el punto de vista nutricional como la alta fijación de fósforo que ocurre en suelos derivados de cenizas volcánicas en ambientes templados encontrados en el centro-sur de Chile (Mora et al., 2017) que representan entre el 50 y 60% del total de la superficie cultivable de esta zona (Borie and Rubio, 2003).

Las mezclas de cultivos tienen muchas ventajas, comparado con los cultivos únicos, especialmente cuando incluyen legumbres, como las especies de la familia Fabaceae (Verret et al., 2020a). Las leguminosas pueden adaptarse a diferentes patrones de cultivo; fijar N₂; ayudar a mantener una mayor biomasa vegetal y rendimiento del grano de las plantas acompañantes como lo demuestran Latati et al. (2019) en su investigación de cultivo intercalado de trigo duro (*Triticum turgidum durum*) con garbanzo (*Cicer arietinum*) que hubo un aumento significativo de la biomasa de los brotes (44 %) y las raíces del trigo en el sistema intercalado en comparación con el

trigo duro solo. Estos resultados sugieren que cambios inducidos por la rizosfera del garbanzo intercalado facilitó la absorción de P y N, por consiguiente, un aumento en la biomasa aérea, el rendimiento de grano (48 %) y la eficiencia del uso de la tierra para el cultivo de trigo. Sin embargo, este incremento fue solo para el trigo, lo que podría indicar que la ganancia es unidireccional. Este estudio mostró que el garbanzo es una leguminosa que se adapta muy bien a diferentes patrones de cultivo, además de la capacidad de complementarse y facilitar recursos para la planta acompañante. Se han examinado varias especies de leguminosas que exudan aniones orgánicos por medio de los cuales logran acceder al P menos disponible (Mora et al., 2017) y una de las especies de cultivo más estudiada es el lupino blanco (*Lupinus albus*), ya que posee una característica especial que es la formación de raíces de racimo, originalmente llamadas raíces proteoides porque fueron descubiertas en la familia Proteaceae. Estas comprenden muchas raicillas laterales a lo largo de la raíz principal (Ding et al., 2021) y son las responsables de liberar una gran cantidad de exudados radiculares (Lambers et al., 2013) que atraen ciertas cepas bacterianas que desempeñan un importante papel en la salud y nutrición de las plantas (Vora et al., 2021). Este rasgo las ha convertido en plantas ideales para crecer en suelos donde una proporción considerable de P se encuentra en forma no disponible (Dissanayaka and Wasaki, 2021), además destaca su aplicabilidad potencial para mejorar la absorción de especies ineficientes como el trigo, mediante intercambio de funciones de la rizosfera (Dissanayaka et al., 2015). Los estudios de rizobacterias indican que durante el cultivo intercalado, los microbiomas de las dos plantas co-cultivadas podrían tener un conjunto de especies comunes, quizás en diferentes proporciones, que podrían colonizar fácilmente a través de las raíces de ambos tipos de plantas (Vora et al., 2021). Estudios anteriores han demostrado que el uso de lupino blanco intercalado con trigo ha tenido un efecto movilizador de nutrientes en favor del trigo debido a la capacidad del lupino de secretar fosfatasa, donde se han reportado aumentos significativos (152%) en la actividad enzimática, especialmente de fosfatasa ácida (Schoebitz et al., 2020).

Por otro lado, se ha estudiado otros mecanismos por el cual algunas plantas pueden mejorar la absorción de P en condiciones de deficiencia, esto es, con la ayuda de hongos micorrícicos arbusculares (HMA) (Chu et al., 2020), ya que estos contribuyen en la adquisición de P por medio de la colonización de las raíces de las plantas, las micorrizas forman una extensa red de hifas que actúan como una extensión del sistema de raíces ayudando a mejorar la adquisición de nutrientes a través de la exploración de un mayor volumen de suelo (Smith and Read, 2008). Se ha observado que el uso de inoculantes de hongos micorrícicos en plantas intercaladas mejora significativamente la absorción de P, y la tasa de fertilizante de P recomendada puede reducirse hasta un 50 % (Song et al., 2021).

La elección de la combinación adecuada de los cultivos que serán intercalados atiende a las necesidades y condiciones del lugar en el cual serán establecidos.

El trigo es un cultivo relevante para Chile debido a su importancia socioeconómica y el consumo per cápita, es el cultivo más sembrado en el país y permite suplir, en parte, las necesidades de proteínas, minerales y energía de las personas (INE, 2020).

HIPÓTESIS

El sistema de cultivo intercalado trigo/leguminosas en un Andisol tendría una mayor actividad microbiológica del suelo que el monocultivo de trigo debido a la rizodeposición activa en presencia de la leguminosa y como consecuencia, el trigo tendría la capacidad de acceder a formas menos disponibles de P en el sistema de cultivo intercalado, obteniendo una mayor biomasa y rendimiento lo que podría conducir a una disminución en el uso de fertilizantes fosforados.

OBJETIVO GENERAL

Evaluar y comparar la influencia del monocultivo de trigo con dos sistemas de cultivos intercalados: trigo-lupino y trigo-garbanzo, con y sin suministro de fertilizantes P sobre las propiedades químicas y biológicas del suelo, las características radiculares y el efecto de la interacción de estos factores en la disponibilidad y adquisición de P.

OBJETIVOS ESPECIFICOS

- Evaluar los efectos de los dos sistemas de cultivos intercalados sobre la biomasa de trigo, las propiedades de las raíces, el rendimiento y la producción general, en comparación con el monocultivo.
- Evaluar los efectos de los dos sistemas diferentes de cultivos intercalados sobre las propiedades químicas del suelo y la actividad microbiana del suelo, en comparación con el monocultivo de trigo.
- Dilucidar las relaciones entre los rendimientos de los cultivos y las propiedades del suelo, incluida la actividad microbiana.

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CAPÍTULO 2

Intercropping strategies for improving P availability, soil microbiology and wheat yield in P-deficient Andisol

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ABSTRACT

Andisols are the least extensive soil order, covering less than 1 % of the earth's land surface. However, in Chile, they play a crucial role, constituting 50 % of the country's cereal production. Despite their significance, there is a notable scarcity of studies investigating the impact of P-deficient soil in cereal production in intercropping system. A 2-year field experiment was conducted with two P levels (with and without) and cropping systems (i.e wheat monoculture, wheat/lupin and wheat/chickpea intercropping). Soil chemical properties, basal soil respiration, enzyme activities, root morphological properties and crop yield were measured. The intercropped wheat exhibited a relative yield 60 % higher than that of the wheat monoculture, even in the treatments without P addition. This was related to the activity of phosphatase, which increased in wheat/lupin, showing an activity 18 % and 30 % higher than that in wheat/chickpea and wheat monoculture, respectively. Furthermore, the Land Equivalent Ratio (LER) of wheat/lupin surpassed 1 in both seasons, indicating improved soil and nutrient utilization. In contrast, the wheat/chickpea obtained an LER lower than 1 in the second season. This showed that these species exhibit strong competition, which has a notable impact on the overall yield. These findings suggest that wheat/lupin intercropping can enhance nutrient availability and yield, particularly when relying on low levels of P. These results support the practicality of using intercropping systems to enhance ecosystem services and agricultural production in Andisol.

Keywords: Enzyme activities; root characteristics; facilitation; LER

1. INTRODUCTION

The cultivation of wheat (*Triticum aestivum*) is of great relevance at global scale, with a total production of 7.7 M Ton and a cultivated area of 2.2 M ha in 2021 (FAO, 2023). This has led to the development of production models aimed at maximizing yields to ensure food security and maximize economic revenues, with excessive use of agrochemicals (del Pozo et al., 2022). One of the consequences of these intensive production models is the detriment of the balance of agroecosystems, causing losses in soil biodiversity (Hartman et al., 2018), soil and water pollution, and degradation of natural resources (Jensen et al., 2020). In many areas of South America, a large proportion of arable land corresponds to soils derived from volcanic ash (Andisol). In Chile, 50-60 % of the total arable land is located on Andisols (Borie and Rubio, 2003). These soils have a high phosphorus (P) fixation capacity (Mejías et al., 2013), generating limiting conditions for wheat development, since most of the P is not available for plant uptake (Castillo et al., 2022). To address this problem, it is necessary to adopt more diversified and resource-efficient agricultural systems that provide greater resilience to soil and climate contingencies. Intercropping systems are widely studied alternatives for crop diversification, defined as the simultaneous growth of two or more species during the same growing season; intercropping can be mixed or arranged in rows, stripes or relays (Vandermeer, 1989; Z. Wang et al., 2017). Intercropping offers numerous benefits over monocrops, especially when legumes are included (Engbersen et al., 2021; Verret et al., 2020b). Legumes are involved in N₂ fixation and the activation of soil microbial communities by active rhizodeposition, which contributes to increased plant biomass and yields in companion plants (Bacchi et al., 2021). In this sense, Sánchez-Navarro et al. (2019) found that multiple broccoli/cowpea cultivation increased organic carbon, available N, and total N compared to broccoli monoculture, which was attributed to the legume's ability to fix N₂. (Latati et al., 2019b) in their research of intercropping durum wheat with chickpea, showed a significant increase in shoots and roots biomass of wheat in the intercrop compared to the monocrop. These findings suggest that modifications triggered by

the rhizosphere of the intercropped legume facilitate the uptake of P and N, leading to an augmentation in aboveground biomass, grain yield, and land-use efficiency in the associated crop. However, this improvement has only been observed in cereal crops, suggesting a unidirectional benefit provided by legumes (Boudsocq et al., 2022; Latati et al., 2019b). In a similar study, Chaechian et al. (2022) reported an increase in yield components, which in turn improved the seed yield in wheat-chickpea intercropping in both plants. This increase was associated with greater efficiency in water use and availability of nutrients, since the roots of wheat and chickpea, having different depths and distributions, allowed crops to absorb more water from different 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. On the other hand, interspecific competition enhances the growth and yield of the dominant crop, whereas the development of the subordinate crop is weakened due to competition during the joint growth stage (Rodriguez et al., 2020; Yu et al., 2022; Zhu et al., 2023). One of the most limiting macronutrients for plant growth is P because it is a key component of many metabolic processes in plants, including photosynthesis, respiration, and protein synthesis (Wang et al. 2023). Consequently, root systems have developed morphological and physiological mechanisms to access P when its availability in soil is low (Wang et al. 2019; Kumar et al. 2019). Plants develop different morphological strategies to obtain nutrients, such as the development of root hairs (Ruiz et al., 2020) and association with arbuscular mycorrhizal fungi (AMF), that form an extensive hyphal network that improves P acquisition by exploring a larger soil volume (Chu et al., 2020). Inoculating maize/soybean intercropping with AMF can significantly improve P uptake, reducing the recommended P fertilizer rates by 50 % (Song et al., 2021). Similarly, AMF enhance P uptake in chickpeas, with greater root colonization observed under low P levels (Singh et al., 2017). Regarding some of the physiological strategies of the root, it is important to highlight the exudation of organic anions and extracellular substances, such as phosphatases (Lambers et al., 2013). Hence, the selection of legumes that exude organic anions, such as citrate, malate, and succinate (Wang and Lambers, 2020) and enzymes, such as acid phosphatases,

to increase soil P availability, should be prioritized in volcanic soils (Mora et al. 2017). Lupin (*Lupinus albus*) can form cluster roots with many lateral rootlets along the main root (Ding et al., 2021), contributing to the release of large amounts of root exudates like citrate, accounting for 6-23 % of its total dry weight (Lambers et al., 2013). This process generates a mobilizing effect of P from unavailable sources, besides increasing the bioavailability of micronutrients such as Cu, Fe, Zn and Mn (Braum and Helmke, 1995). Intercropping with legumes enhances the diversity and activity of the soil microbiome compared with monocrops, leading to the solubilization of soil nutrients crucial for plant health and nutrition (Vora et al., 2021). Cuartero et al. (2022) observed an increase in the diversity and composition of soil bacteria and fungi in a melon/cowpea intercropping system that promoted N₂ fixation and N assimilation by plants. This trait has made legumes ideal companion plants for intercropping in soils where a high proportion of P is unavailable (Dissanayaka and Wasaki, 2021). In addition, the presence of lupin may improve the absorption of P by inefficient species, such as maize, through an exchange of rhizosphere functions (Dissanayaka et al. 2015). Schoebitz et al. (2020) reported that lupin intercropped with wheat caused a mobilizing effect of nutrients in favor of wheat, owing to the release of phosphatases, with a significant increase in phosphatase activity of 152 % compared to wheat monoculture. Furthermore, Betencourt et al. (2012) demonstrated that intercropping of wheat and chickpea increased the availability of P in the rhizosphere of both species, particularly in soils with low P content, due to rhizosphere alkalization. Although intercropping has been proposed as an efficient system for wheat growth (Boudsocq et al., 2022; Cu et al., 2005; Gardner and Boundy, 1983), it has not been adequately demonstrated for Andisols with limited soil conditions, such as low P availability.

According to previous studies, we hypothesized that the wheat/legume intercropping system in an Andisol would have higher soil microbiological activity than the wheat monoculture owing to active rhizodeposition in the presence of the legume. Consequently, wheat would have the ability to access less available forms of P in the intercropped system, with increased biomass and yield. This may lead to a decrease in the use of external P input. To test these hypotheses, a two-year field study was

carried out in an Andisol, comparing a wheat monoculture with two intercropping systems: wheat/lupin and wheat/chickpea, with and without the addition of P fertilizers. The selection of the two legumes species was based on the capacity of the lupin to develop roots in clusters, and the ability of chickpea to associate with AMF. Thus, the objectives of this study were to: i) assess the effects of the two intercropping systems on wheat biomass, root properties, yield, and overall production, compared to the monoculture; ii) evaluate the effects of the two different intercropping systems on soil chemical properties and microbial soil activity, compared to the wheat monoculture; and iii) elucidate the relationships between crop yields and soil properties, including microbial activity.

2. Results

2.1. Soil pH and available nutrients

Cropping season was the factor contributing to the highest significant differences ($p < 0.001$) for pH, nitrates, ammonium, available N and Olsen-P (Table 1). We observed an increase of 6 %, 82 %, 54 %, 77 % and 37 %, respectively, for the abovementioned properties during the second season compared to the first season. The cropping system did not significantly influence these properties, and so intercropping did not significantly affect the availability of nutrients. The P fertilization factor had a significant effect on nitrates, available N and Olsen-P. Nitrates and available N were 29 % and 23 % higher in P-, respectively. However, Olsen-P was 21 % higher under P+ treatment. The interaction cropping system x P fertilization was not significant in any property, indicating that the effect of P addition on these soil properties was not affected by intercropping.

Table 1. Soil pH and available N and P in response to wheat monoculture and wheat-lupin or wheat-chickpea intercropping under different fertilization regimes for two growing seasons. Vales are mean \pm standard error.

Season	Cropping System	Addition of P	pH	NO ₃ (mg kg ⁻¹)	NH ₄ (mg kg ⁻¹)	Available N (mg kg ⁻¹)	Olsen-P (mg kg ⁻¹)
2020-2021	Wheat	P+	5.5 \pm 0.08	4.1 \pm 0.67	3.8 \pm 0.27	8.0 \pm 0.90	7.1 \pm 0.47
		P-	5.4 \pm 0.11	6.2 \pm 1.50	4.7 \pm 0.38	10.1 \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 0.01	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
2021-2022	Wheat	P+	5.7 \pm 0.03	26.3 \pm 4.74	8.2 \pm 1.27	34.5 \pm 3.16	12.1 \pm 0.54
		P-	5.7 \pm 0.01	47.7 \pm 7.36	10.0 \pm 0.45	57.7 \pm 7.08	8.6 \pm 0.52
	Wheat-Lupin	P+	5.7 \pm 0.01	23.9 \pm 4.13	9.8 \pm 0.10	33.8 \pm 4.91	10.8 \pm 0.87
		P-	5.7 \pm 0.003	37.2 \pm 1.65	8.9 \pm 1.20	46.0 \pm 2.64	9.0 \pm 0.57
	Wheat-Chickpea	P+	5.7 \pm 0.04	25.2 \pm 4.06	7.3 \pm 0.61	32.6 \pm 4.18	10.1 \pm 0.44
		P-	5.8 \pm 0.01	27.7 \pm 3.32	9.6 \pm 0.25	37.3 \pm 2.55	7.0 \pm 0.81
Between Subjects							
Cropping System (CS)			0.118 ns	1.511 ns	0.720 ns	2.007 ns	2.362 ns
Phosphorus (P)			0.103 ns	8.594*	2.406 ns	10.029 **	14.916**
CS x P			1.826 ns	1.722 ns	2.829 ns	0.348 ns	1.090 ns
Within Subjects							
Season (S)			122.55***	218.033***	120.591***	407.124***	49.450***
S x CS			0.096 ns	2.393 ns	0.416 ns	3.361 ns	0.588 ns
S x P			0.365 ns	9.209*	1.239 ns	12.752**	3.446 ns
S x CS x P			0.250 ns	1.286 ns	1.173 ns	0.002 ns	0.112 ns

P+: 220 kg ha⁻¹ and P-: 0 kg ha⁻¹. Significant at *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns: not significant ($p > 0.05$).

Fuente: Elaboración Propia

2.2. Microbiological Soil Properties

Intercropping did not significantly affect any of the microbiological properties measured (Fig. 2). However, phosphatase activity tended to be higher under intercropping. The P fertilization factor was only significant for urease activity ($p < 0.01$), with ~35 % higher activity in P- compared to P+ during the second season. The interaction cropping system x fertilization regime was not significant for any of these properties, indicating

no different effect of P addition in terms of cropping system. Growing season was the factor contributing to the highest significant effect on microbiological properties.

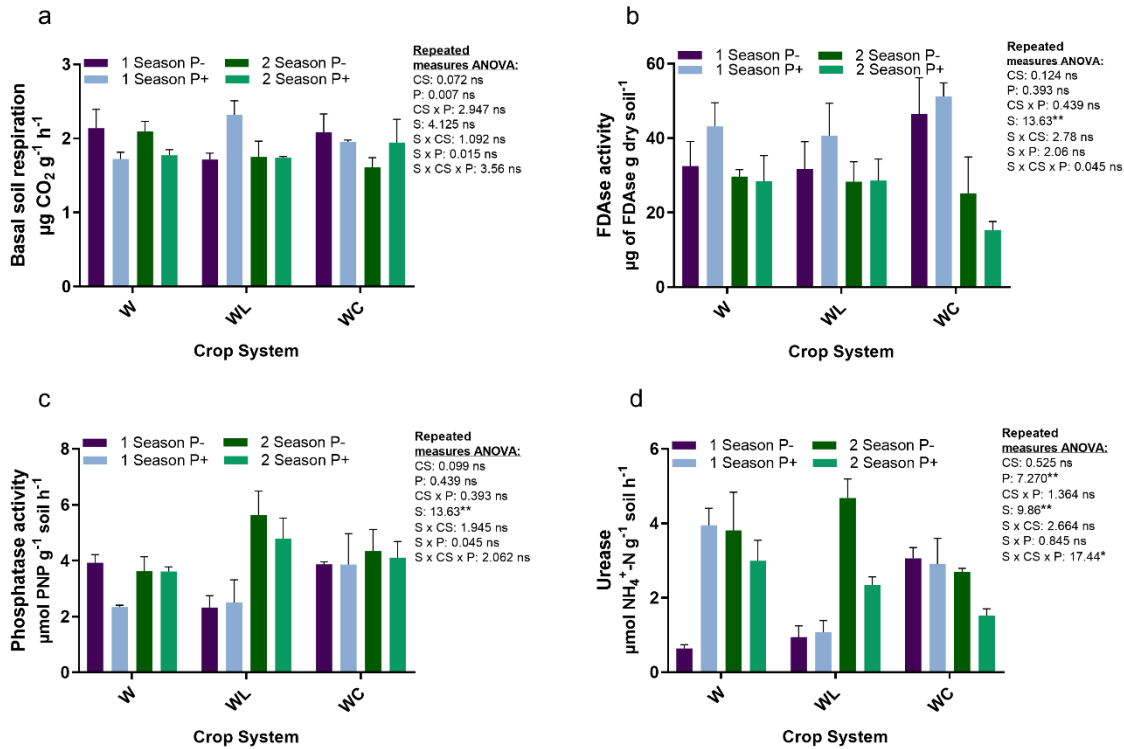


Fig. 1 Values of soil basal respiration (a), FDAse activity (b), acid phosphatase activity (c) and urease activity (d). Values are mean ($n = 3$). Error bars represent the standard error. P+: 220 kg ha^{-1} of phosphate; P-: 0 kg ha^{-1} of phosphate. W (wheat monocrop), WL (wheat-lupin), WC (what-chickpea). 1 season: first growing season; 2 season: second growing season. CS: cropping system; P: fertilization regime; S: growing season. Significant at ** $p < 0.01$; * $p < 0.05$; ns: not significant ($p > 0.05$).

Fuente: Elaboración Propia

2.3. Wheat aerial and root biomass and root characteristics

Aerial biomass did not show significant differences between the growing seasons (Fig. 3a). However, there was a significant effect of the cropping system and the addition of P, showing that plants growing in intercropping and under P+ had the highest aerial biomass. In fact, aerial biomass was 20 % higher in the P+ treatment than in the P -

treatment. For root biomass, the growing season was the factor with the highest significance, with root biomass approximately 30 % higher in the second growing season (Fig. 3b). There was no significant effect of cropping system on root biomass. However, the addition of P (P+) significantly increased root biomass by 20 % ($p < 0.05$), regardless of the cropping system.

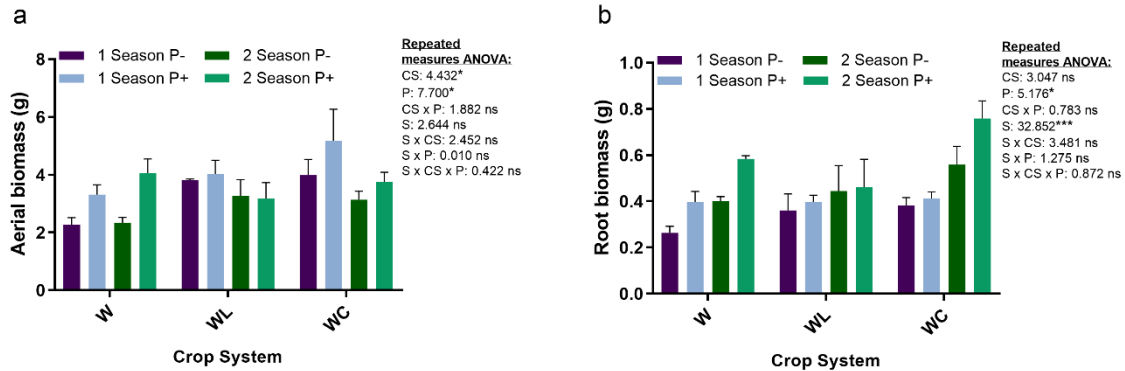


Fig. 2 Aerial biomass (a) and root biomass (b) of wheat. Values are mean ($n = 3$). Error bars represent the standard error. P+: 220 kg ha⁻¹ of phosphate; P-: 0 kg ha⁻¹ of phosphate. W (wheat monocrop), WL (wheat-lupin), WC (wheat-chickpea). 1 season: first growing season; 2 season: second growing season. CS: cropping system; P: fertilization regime; S: growing season. Significant at ** $p < 0.01$; * $p < 0.05$; ns: not significant ($p > 0.05$).

Fuente: Elaboración Propia

Root characteristics (length, surface area, volume, and diameter) were not significantly affected by the cropping system or the addition of P (Fig. 4). The only factor significantly affecting these properties was the growing season, with root length (Fig. 4a), surface area (Fig. 4b), volume (Fig. 4c), and average diameter (Fig. 4d) significantly higher in the second growing season by 33 %, 41 %, 50 %, and 17 %, respectively.

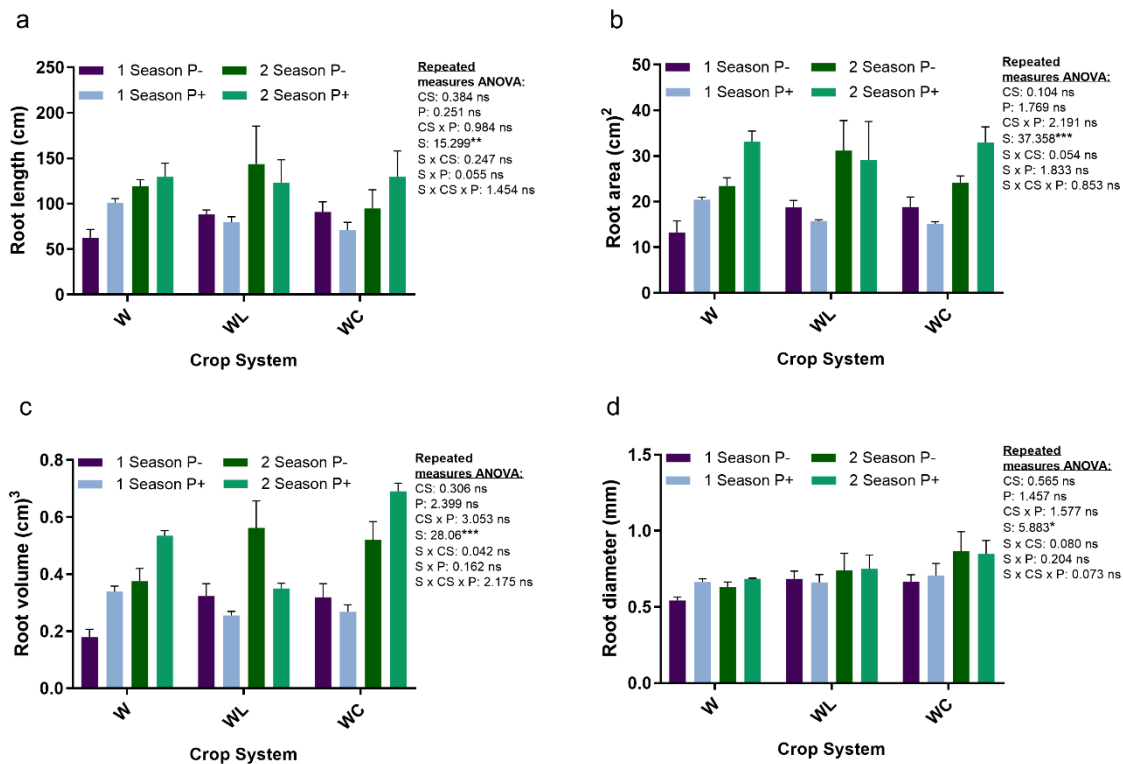


Fig. 3 Root length (a); root surface area (b); root volume (c) and root average diameter (d) of wheat. Values are mean (n = 3). Error bars represent the standard error. P+: 220 kg ha⁻¹ of phosphate; P-: 0 kg ha⁻¹ of phosphate. W (wheat monocrop), WL (wheat-lupin), WC (wheat-chickpea). 1 season: first growing season; 2 season: second growing season. CS: cropping system; P: fertilization regime; S: growing season. Significant at ** $p < 0.01$; * $p < 0.05$; ns: not significant ($p > 0.05$).

Fuente: *Elaboración Propia*

2.4. Wheat production and quality parameters

The absolute yield of wheat showed a significant effect of the growing season, with wheat yield increasing by 62 % in the second season (Fig. 5a). Absolute wheat yield was significantly affected by the cropping system and P fertilization, with the highest average values under monocrop, owing to higher plant density, and addition of P. Nonetheless, in the first growing season, regardless of P fertilization, intercropped wheat showed similar yields to wheat monoculture ($p > 0.05$), even though the density

of wheat plants was lower in intercropping. In the second season, the monoculture of wheat showed higher yields than the wheat intercropped with both legumes under the same fertilization conditions, exceeding approximately 2 ton ha⁻¹. However, when analyzing the absolute yield of wheat without P supply, the difference decreased to 0.7 ton ha⁻¹. Wheat yield expressed on a plant density basis (relative yield) (Fig. 5b) showed an opposite trend to the absolute yield. There was a strong influence of intercropping and the addition of P on relative yield, with the W+C and W+L systems providing a relative wheat yield 69 % and 66 % higher than that of the monoculture, respectively. In the absence of P fertilization (P-), the relative yields of wheat were 60 % and 55 % higher than those in the monocrop wheat for W+C and W+L, respectively (Fig. 5b). Regarding the overall yield, there was a significant effect of the growing season, cropping system and fertilization regime (Fig. 5c). On average, the total yield in the second season increased by 56 % compared with in that in the first season. When analyzing the behavior of the cropping systems in the first season in detail, the overall production of W + L under P- was significantly higher than that of the monoculture of wheat with P+. In the second season, the yield of wheat monoculture was not significantly different to the overall production of the W+L system under both P+ and P - regimes. The overall yield of the W+C system was significantly lower than those of the wheat monoculture and W+L systems. The addition of P significantly increased the yield by approximately 20 %.

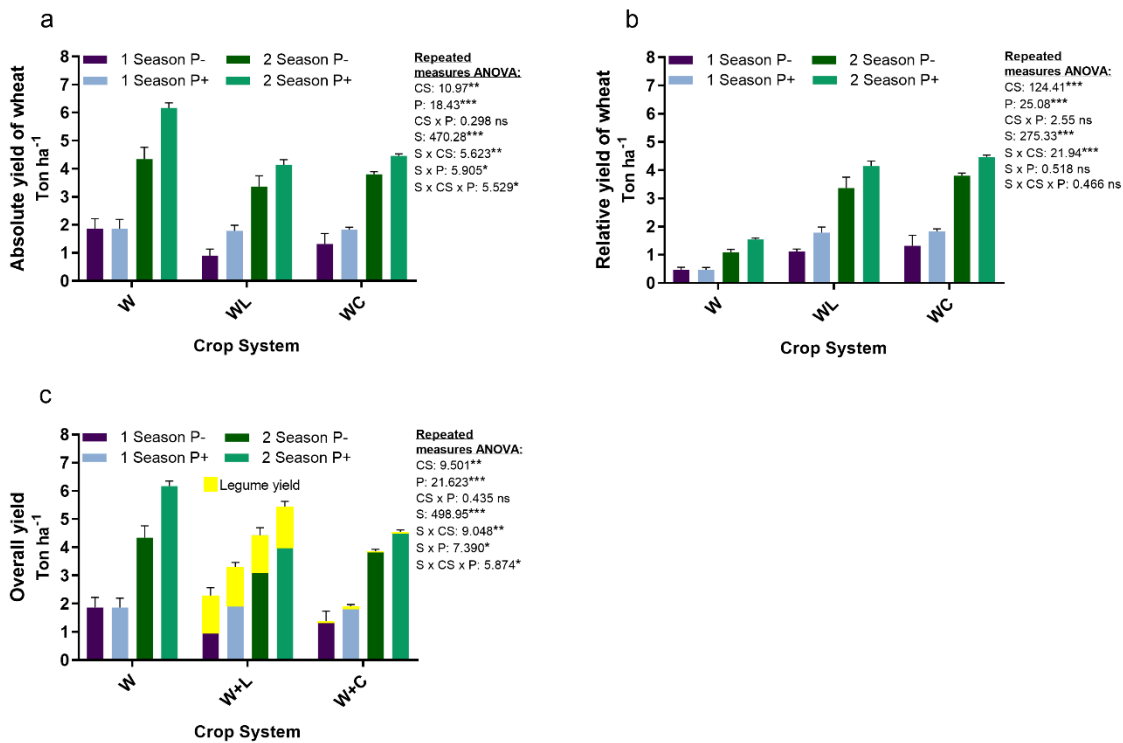


Fig. 4 Absolute wheat yield (a), relative wheat yield (b) and overall crop yield (c). Values are mean ($n = 3$). Error bars represent the standard error. P+: 220 kg ha⁻¹ of phosphate; P-: 0 kg ha⁻¹ of phosphate. W (wheat monocrop), WL (wheat-lupin), WC (wheat-chickpea). 1 season: first growing season; 2 season: second growing season. CS: cropping system; P: fertilization regime; S: growing season. Significant at ** $p < 0.01$; * $p < 0.05$; ns: not significant ($p > 0.05$).

Fuente: Elaboración Propia

All wheat quality parameters were significantly affected by the growing season, with highest values during the second season (Fig. 6). The effect of P fertilization was not significant in any of the quality parameters. The cropping system only significantly affected the ratio seeds/spike, with highest values under intercropping, by 23 % and 21 % in W+C and W+L compared to the monoculture, respectively.

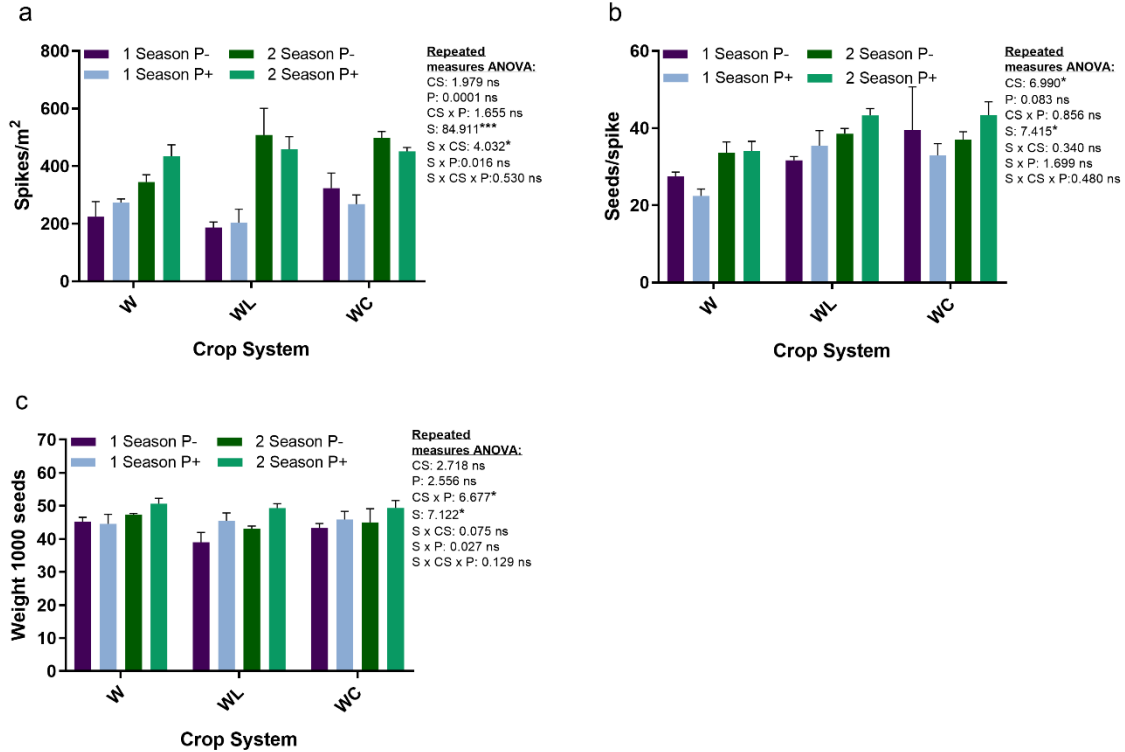


Fig. 5 Number of wheat spikes per m² (a); the ratio seed number per spike (b) and the kernel weight (c) of wheat. Values are mean (n = 3). Error bars represent the standard error. P+: 220 kg ha⁻¹ of phosphate; P-: 0 kg ha⁻¹ of phosphate. W (wheat monocrop), WL (wheat-lupin), WC (wheat-chickpea). 1 season: first growing season, 2 season: second growing season. CS: cropping system; P: fertilization regime; S: growing season. Significant at *** $p < 0.001$; * $p < 0.05$; ns: not significant ($p > 0.05$).

Fuente: Elaboración Propia

The LER was significantly influenced by the cropping system, with W+L showing higher values than W+C (Figure 7a). LER was >1 in all cases except for W+C P-, that showed unsustainable values < 1. LER values showed this descending order during the first season: (WL P+) 2.3 > (WC P+) 1.58 > (WL P-) 1.56 > (WC P-) 1.1, and the following descending order for the second season: (WL P+) 1.9 > (WL P-) 1.7 > (WC P-) 1.0 > (WC P+) 0.8 (Fig. 7a). Partial LERs also revealed a significant effect of cropping system (Fig. 7b). The results showed that the partial LERs of lupin,

wheat/chickpea, and wheat/lupin were 1.1, 0.8 and 0.7, respectively. However, the partial LER of chickpea when intercropped with wheat was 0.2.

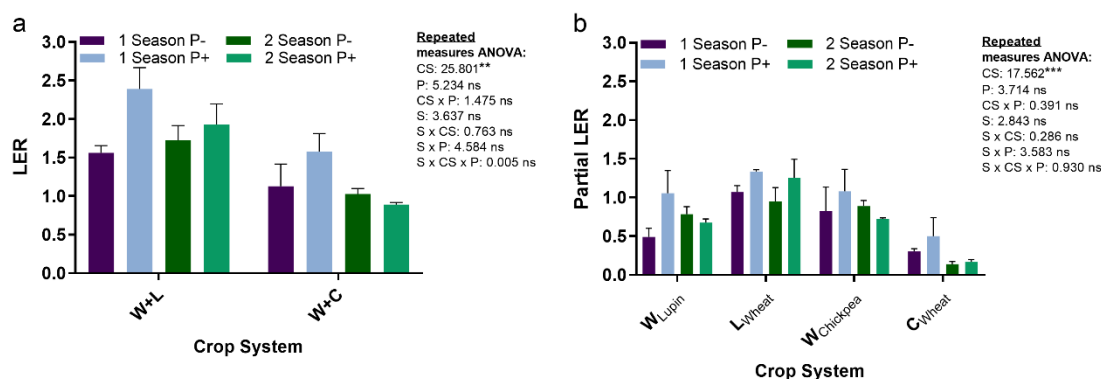


Fig. 6 Land equivalent ratio (LER) (a) and partial LER for each crop (b). Values are mean (n = 3). Error bars represent the standard error. P+: 220 kg ha⁻¹ of phosphate; P-: 0 kg ha⁻¹ of phosphate. W (wheat monocrop), WL (wheat-lupin), WC (wheat-chickpea). 1 season (First growing season), 2 season (Second growing season). CS: cropping system; P: fertilization regime; S: growing season. Significant at *** $p < 0.001$; ns: not significant ($p > 0.05$).

Fuente: *Elaboración Propia*

Table 2. Multiple linear regression model for absolute, relative, global yield and root biomass in intercrops and monocrops

Y	X	m	Partial correlation	β	R ²	R ² Adj	F value
Absolute yield wheat	Constant (b)	-15.22			0.82	0.78	22.21***
	Ammonium	0.08	0.18	0.14			
	pH	2.27	0.26	0.25			
	Olsen-P	0.17	0.39	0.27			
	Root biomass	0.98	0.17	0.09			
	Kernel weight	0.04	0.33	0.17			
	Spikes/m ²	0.003	0.31	0.21			
Relative yield wheat	Constant (b)	-2.75			0.72	0.68	20.06***
	Phosphatase	0.10	0.17	0.11			
	Root weight	1.68	0.27	0.18			
	Spikes/m ²	0.005	0.60	0.48			
	Seeds/spike	0.05	0.42	0.30			
Overall yield	Constant (b)	-17.28			0.80	0.77	31.31***

	Ammonium	0.06	0.11	0.10			
	pH	3.18	0.39	0.36			
	Nitrates	0.008	0.09	0.07			
	Olsen-P	0.29	0.63	0.47			
Root biomass	Constant (b)	-2.008			0.58	0.50	6.85***
	Available N	-0.002	-0.23	-0.28			
	Olsen-P	0.016	0.29	0.26			
	pH	0.36	0.33	0.42			
	Phosphatase	0.015	0.20	0.15			
	Aerial biomass	0.052	0.45	0.35			
	Root length	0.001	0.36	0.30			

m: unstandardized coefficients; β : standardized coefficients. Significant at *** $P < 0.001$.

Fuente: Elaboración Propia

Multiple linear regression analysis (Table 2) showed that absolute wheat yield was positively related to ammonium, pH, Olsen-P, root weight, weight 1000 seeds and spikes/m². The relative yield of wheat was related to acid phosphatase activity, root weight, spikes/m², and seeds/spike. The overall yield was related to pH, ammonium, nitrates and Olsen-P. On the other hand, the root biomass was positively related to Olsen-P, pH, phosphatase, aerial biomass and root length and negatively related to available N.

3. Discussion

3.1 Chemical and biological properties of soil

Soil pH is a critical factor that affects P availability of P for plants. The pH shifted from strongly acidic during the first season to moderately acidic during the second. This increase was related to the consecutive application of lime to the soil before the establishment of the crop in both seasons. This pH change can increase the availability of P in the soil solution, which explains the increase in Olsen-P in the second growing season. This is because in acidic soils, inorganic P tends to form insoluble compounds when retained by oxides of Al and Fe or by soil particles, which makes it less available to plants (Y. Wang & Lambers, 2020). However, as the soil pH increases, these insoluble compounds can dissolve and release inorganic P into the soil solution, increasing its bioavailability (Hinsinger, 2001). In contrast, Olsen-P showed a tendency to decrease in intercropping compared to monoculture. This decrease may be related to the

greatest uptake of P by intercropped plants than by the single species, as previously reported by Wang et al. (2014) and Schoebitz et al. (2020). Regarding the available N content, the highest concentration under no P fertilization may be explained by the fact that P fertilization can increase the uptake of N by plant roots, resulting in a decrease in N compounds in the soil solution (Vázquez et al., 2020; Wu et al., 2022). Thus, P fertilization may have a depleting effect on soil N reserves. In this study, cropping systems had no significant effect on soil chemical properties; these results are similar to those obtained by Xing et al. (2023), who reported that intercropped legume/maize systems did not affect the concentration of total N in the soil after 4 years of the experiment, but after 12 years, suggesting that changes in total N concentration may be a slow process.

Microbial activity is a biological indicator of soil quality (Meena, 2018; Sharma et al., 2020). However, intercropping had no effect on basal soil respiration or enzyme activity. Acid phosphatase is an enzyme that hydrolyzes organic P compounds and releases the organic P available to the plant (O'Sullivan et al., 2020). This enzyme plays a significant role in the volcanic soils, which are characterized by a low availability of P (Borie and Rubio, 2003). When examining our results, they showed that although there was no significant effect of different cropping systems and the P factor on the activity of this enzyme, there was a trend of increasing acid phosphatase activity in wheat intercropped with lupin in the absence of P compared to monoculture. White lupin can solubilize P in soil by forming cluster roots that release large amounts of carboxylates and acid phosphatases. These mechanisms allow white lupin to access both inorganic and organic forms of P in the soil (Lambers et al. 2013; Dissanayaka and Wasaki 2021). In addition to exudation by plants, soil microorganisms produce acid phosphatase in response to P deficiency (O'Sullivan et al. 2020). These results are consistent with those of previous studies (Boudsocq et al., 2022; Dissanayaka and Wasaki, 2021), in which white lupin stands out for its highly efficient mechanisms in the acquisition of P, such as exudation of high amounts of acid phosphatase. Schoebitz et al. (2020) observed that lupine increased nutrient availability and uptake by wheat in wheat/lupine

intercropping in acidic soils, which was associated with physiological lupin traits such as high phosphatase activity of the rhizosphere and the release of carboxylates, which could increase the availability of P for both crops (Honvault et al., 2021)-

The activity of urease increased under conditions of P deficiency in all cropping systems. Our results are contrary to those obtained by Wang et al. (2008), who observed that the low availability of N promoted a greater activity of urease, and that fertilization with P caused an increase in the absorption of N by the plants depleting the available N, which increased the production of urease to supply the deficiency of N. However, our results are aligned with Wang et al. (2018), who found that urease activity responded differently depending on soil type: in Luvisols with a pH of 5.45, very similar to the pH of the soil in our study, fertilization with N and N + P had a negative effect on urease activity. In contrast, in Lixisols (pH 6.5), the addition of P caused a significant increase in the urease activity. These findings show that pH is a factor that conditions the response of the soil to P fertilization, and that has a modulating effect on urease production and release.

3.2. Root and aboveground biomass and morphological characteristics of wheat roots

The root biomass of wheat showed a significant increase in the second season of cultivation, influenced by the addition of P, which was prominent in the wheat/chickpea treatment. Our results showed a positive relationship between root biomass, root morphological characteristics such as length, and chemical properties of soil, such as pH and Olsen-P, and a negative relationship with available N. The most important factor that could explain the increase in root biomass in the second year is the change in pH, which allowed a greater bioavailability of P (Wang and Lambers, 2020) and the consequent absorption by the roots (Hinsinger et al., 2011). These results are consistent with those of previous studies (Loudari et al., 2022; Wang et al., 2023; Wang et al., 2008) reporting that the availability of P improved root characteristics such as length. This is partly explained by the fact that P is necessary for the formation of new cells and 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 (Liu et al., 2023; Wang et al., 2023). However, in this study, we observed that a higher root biomass did not contribute to greater growth and development of the aerial biomass, contrary to the results obtained by Liu et al. (2022). This may be because the plant requires carbon investment to develop longer roots or a higher root biomass (Honvault et al., 2021; McGrail et al., 2023). This implies an expenditure of energy destined for root growth, which can limit the resources available for the growth of the aerial part of the plant (Viana et al., 2022). On the other hand, the development of length, area and volume in the roots of wheat intercropped with lupine without the addition of P increased with respect to the other two systems, which shows the great capacity of lupine to mobilize nutrients, thus favoring wheat.

3.3. Crop production

The relative yield of wheat significantly increased under intercropping, even in treatments without P supply. This increase was related to the spikes/m², seeds/spike, acid phosphatase activity and root biomass. Similar results have been reported in the literature. For example, Yang et al. (2022) 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 obtained the highest yield of maize compared to monoculture. This increase was positively correlated with acid phosphatase and P availability. A system intercropped with legumes can increase the availability of nutrients through different mechanisms that come from root interactions and their closest environment (Lambers et al., 2018). For example, when legumes are intercropped with a highly N-competitive cereal such as barley or wheat (Hauggaard-Nielsen et al., 2001; Zhang et al., 2017), there is an increase in the fixation of N₂ in response to the high absorption of mineral N by cereals (Bedoussac and Justes, 2010). Thus, there is an increase in the N availability for cereals (Yu et al., 2022). An adequate availability of P can increase the number of seeds per spike, thus obtaining a higher yield (Peng and Li, 2005). Hence, the presence of legumes in an intercropping system generates positive interactions such as complementarity of resource use through interspecific facilitation, and also

decreases intraspecific competition between plants of the same species, consequently improving production per plant (Duchene et al., 2017; Fan et al., 2020; Rodriguez et al., 2020; Yu et al., 2022).

The monoculture of wheat obtained the highest total production owing to the highest density of plants, but the relative yield values indicate that when accompanied by legumes, facilitation processes occur and intraspecific competition is reduced. This led to a higher number of seeds per plant. In contrast, the intercropping system of wheat and chickpea resulted in the lowest total production. This may be related to the competitive advantage of wheat in nutrient acquisition (Bedoussac and Justes, 2010; Hauggaard-Nielsen et al., 2001). These results are consistent with those obtained by Latati et al. (2019) in an experiment with durum wheat intercropped with chickpea, concluding that wheat benefited at the expense of chickpeas from strong competition for resources. This same behavior was observed by Li et al. (2001), where wheat benefited from soybeans in terms of nutrient acquisition, which was attributed to wheat's greater competitive capacity compared to soybeans, where wheat eventually dominated (Homulle et al. 2022). Consequently, chickpea yield was very low (75 % decrease in P+ and 81 % decrease in P- compared to their respective monoculture), and therefore, the total yield of the two species was lower than that of the wheat monoculture. In contrast, the wheat and lupin intercropping system showed high production, regardless of P fertilization, showing the most stable total yield of the three systems. These results suggest that the mechanisms responsible for the increase in total yield in the wheat/lupin system are the processes of resource facilitation by lupin that improve wheat yield, with a benefit of both species. These findings have been widely reported by intercropping studies (Cu et al., 2005; Gardner and Boundy, 1983; Schoebitz et al., 2020). Thus, lupin can be suggested as a species that can improve the acquisition of resources (especially P) as companion plant through different mechanisms to mobilize P (O'Sullivan et al., 2020) and indirectly increase the availability of other nutrients, leading to increased yields (Dissanayaka et al., 2015; Dissanayaka and Wasaki, 2021).

It is important to highlight that the absolute wheat yield responded in a very variable way to the supply of P in each season. In the first season, the monoculture and intercropping systems with supply of P showed similar yields of approximately 1.8 Ton ha⁻¹, despite the lowest density of plants in the intercrops. This suggests that legumes are involved in increasing wheat yields under unfavorable soil conditions, such as low pH. However, when P was not applied, the wheat monoculture produced higher yield than the intercropped systems. Thus, it can be inferred that the lack of P and low pH increased P deficiency, which affected the mechanisms of legumes to access nutrients. In contrast, in the second season, improvements in soil chemical conditions, mostly by increases in pH and P availability (owing to increased pH), favored the development and activity of the legumes, increasing wheat production in the absence of P addition. These findings suggest that intercropping systems can maintain high wheat yields under low plant densities.

LER resulted in values >1 for the lupin/wheat intercropping for the two crop seasons, demonstrating an advantage in the productivity of both crops intercropped over wheat monoculture. These results could be explained by functional facilitation; that is, greater availability and better use of resources provided mainly by legumes (Homulle et al., 2022; Mahmoud et al., 2022). In this line, partial LERs were only valid for wheat (LER 0.8 and 0.7) and lupin (LER = 1.1). Chickpeas obtained a partial LER below that considered beneficial for the crop (LER = 0.2). These LER values are aligned with those of previous studies (Latati et al. 2019; Zhu et al. 2023), which showed that the partial LERs of legumes such as chickpea and grass pea are generally lower than those of cereals. This indicates that legumes behave as altruist companions, ceding the most available nutrients to companion plants, which normally grow more in detriment to legume development (Marcos-Pérez et al., 2023). Thus, it seems that chickpea would have suffered from strong competition by wheat, resulting in low yield when intercropped with wheat, which is not an advantageous cropping system. In contrast, lupin can improve wheat development and production when intercropped, leading to high yields for both crops.

4. Materials and Methods

4.1. Site and soil description and experimental design

This study was conducted at the INIA Experimental Station Santa Rosa (36°3' S, 71°54'W) in the Mediterranean climate region of south-central Chile, under irrigated conditions. The mean annual temperature is 13 °C and the mean annual precipitation is 980 mm. The total precipitation was 745.8 mm in 2020 and 649 mm in 2021 (Agromet 2023). The details of precipitation and air temperatures in both growing seasons are shown in Fig. 1. The soil belongs to the Diguillín series, coming from modern volcanic ashes of the Andisol order, classified as Typic Haploxerands (Soil Survey Staff., 2022). The texture is silt loam, and the chemical soil properties (0-30 cm) are: organic matter, 11.3 %; pH 5.2, available N, 48.6 mg kg⁻¹; Olsen-P, 7.0 mg kg⁻¹ and available K, 137 mg kg⁻¹. Before initiating this field experiment, oat was planted in 2019, and lime (CaCO₃) was applied at 1000 kg ha⁻¹ at the beginning of each season. A factorial arrangement was used, with two factors and three replicates per treatment. The first factor was the cropping system (monocrop vs. intercropping), whereas the second factor was the external addition of P fertilizer (with and without P). Thus, we compared a spring wheat monoculture (*T. aestivum* cultivar Pantera-INIA), lupin monoculture (*L. albus* cultivar Alboroto-INIA), and chickpea monoculture (*C. arietinum* cultivar Alfa-INIA) with wheat-lupin and wheat-chickpea intercropping systems during two growing seasons (2020-2021 and 2021-2022) (cropping system factor). Each growing season lasted from July/August to February. The spacing between rows was 20 cm for the three monocultures. For intercropping systems, we implemented row intercropping 1:1 (a combination of alternate rows of wheat and legume), with 40 cm between rows. Thus, the density of wheat plants in the monoculture was 62.5 plants m⁻², while it was 15.6 plants m⁻² in the intercropping systems.

The seed bed preparation consisted of a rastra pass followed by a vibrocultivator. Sowing was performed manually. Lupin and chickpea seeds were sprayed with a gel with *Rhizobium* sp. at a rate of 1.0 x 10⁹ colony forming units mL⁻¹ just before sowing

to ensure effective root nodulation. The seed dose for sowing was 220 kg ha⁻¹ for wheat and 120 kg ha⁻¹ for lupin and chickpea. The fertilization treatment was also applied in all cropping systems, monocultures, and intercrops, consisting of the absence of P fertilizer or the addition of P fertilizer. For the P fertilization treatment, the soil received triple superphosphate at 220 kg ha⁻¹, potassium muriate at 220 kg ha⁻¹, and urea at 103 kg ha⁻¹ only at sowing. Treatments without a supply of P were fertilized in the same manner as described above, but without triple superphosphate application. Thus, the treatments were: i) wheat monocrop (W) with P (P+) and without P (P-); ii) wheat/lupin (WL) with P (P+) and without P (P-); and iii) wheat/chickpea (WC) with P (P+) and without P (P-). Treatments were randomly set up in plots of 3.2 × 4.0 m² established in triplicate. For weed control, herbicide treatment was performed exclusively on wheat during the tillering stage. The products consisted of MCPA 4 kg ha⁻¹ of 750 g L⁻¹ MCPA-dimethylammonium, Ajax 4 kg ha⁻¹ of 600 g kg⁻¹ metsulfuron-methyl, Arrat 4 kg ha⁻¹ of 250 g kg⁻¹ tritosulfuron, and 550 g kg⁻¹ dicamba-sodium. Irrigation was applied only during the first growing season of the tillering stage.

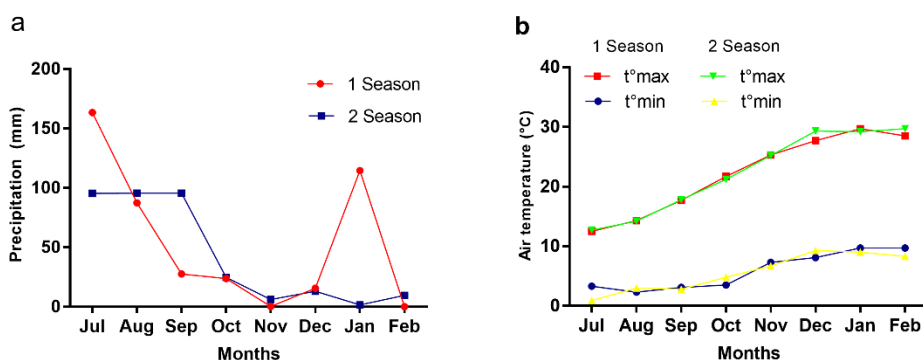


Fig. 7 Climatological data of precipitations (a) and temperatures (b) in the growing period of the crops.

Fuente: Elaboración Propia

4.2. Soil and plant sampling and harvest

Plants and soils (0-30 cm) were collected exclusively from the wheat crop in the anthesis state (November) from each plot. Three plants placed in the central row of

each plot, and adhering soil to the roots was collected. Each soil sample was then separated into two portions. One portion of soil was sieved to < 2 mm, and then used for chemical analyses. The other portion was sieved to < 2 mm and stored at -20 °C for microbiological analyses.

The plant samples were separated into shoots and roots and dried in an oven at 70 °C for 48 h until a constant weight was reached. The aerial and root biomass were determined by weighing the dry matter. The belowground parts of the plants were harvested and dried in Kraft paper bags. Root parameters such as length, area, volume, and diameter were determined using a WinRhizo computerized system (Regent Instruments Inc., Quebec, Canada) (Q. Wang et al., 2021).

Wheat yield was determined by weighing the harvested grains from one linear meter in the central row of each plot. Harvest was carried out using an automotive (Winterseiger) for trials. Spikes/m², seeds/spike, and a weight of 1000 seeds were also calculated. The absolute wheat yield was estimated by considering the grain weight and area of all the plants in each plot. The relative wheat yield was calculated by dividing the absolute real wheat yield by the density of the plants in each crop to assess the influence of interspecific competition in each system. Lupin and chickpea yields were determined in the same way as wheat by weighing the harvested grains. The total production was calculated by the addition of wheat and legume yields in each intercropped plot.

Land equivalence ratio (LER) was used for grain yield as an indicator of resource use efficiency in intercropping over monocultures (Hauggaard-Nielsen et al., 2001). This index gives the relative area required of monoculture to obtain the same yield (of both species) as in intercropping. The LER value is calculated as the sum of the proportions of the intercrop yield and the monocrop yield of each species (partial LER for each species)

(Equation 1):

$$\text{LER} = \text{partial LER for wheat} + \text{partial LER for legume} \quad \text{Equation 1}$$

Where partial LER for wheat is the ratio wheat yield in intercrop to wheat yield in monocrop, and partial LER for legume is the ratio legume yield in intercrop to legume yield in monocrop. If the LER > 1, there is an advantage of intercropping in terms of

yield and land use; if $LER \leq 1$, there is no advantage of intercropping for monocrop (Jensen et al., 2015). When partial $LER > 0.5$ indicates the advantage of intercropping for that species (Zhu et al. 2023).

4.3. Soil analyses

Soil pH was measured in water 1:5 (w/v). The available N and P were measured using the methods described by Jackson (1958) and Watanabe and Olsen (1965), respectively. Basal soil respiration was analyzed using the closed system soil incubation methodology (Alef and Nannipieri 1995). The results obtained were expressed as μg of CO_2 produced per gram of soil (dry weight) per hour. The activity of fluorescein diacetate hydrolase (FDAse) was analyzed using the modified methodology by Alef and Nannipieri (1995) and was expressed in μg of FDAse per gram of soil (dry weight). For the analysis of acidic phosphatase activity, we used Tabatabai and Bremner (1969) methodology. The results obtained were expressed in the μmol of PNP per gram of soil (dry weight) per hour. Urease activity was measured according to (Nannipieri et al., 1980) was expressed in μmol of ammonium-N produced per gram of soil (dry weight) per hour.

4.4. Statistical analysis

Data were checked to ensure normal distribution using the Shapiro-Wilk test at $p < 0.05$. Data were subjected to three-way repeated measures ANOVA, with season (2020-2021 and 2021-2022) as the within-subject factor, and cropping system (wheat monoculture and intercropping systems) and fertilization treatment (with and without P) as between-subject factors. The relationships between these properties were studied using multiple regression and Pearson's correlations. Multiple linear regression analysis ($Y = m_1X_1 + m_2X_2 + \dots + m_nX_n + b$) was carried out with all data from the two growing seasons using backward methods, with absolute wheat yield, relative wheat yield, or overall production as independent variables and soil properties, plant biomass, crop quality parameters, and root characteristics as dependent variables. Standardized coefficient (β) and partial correlation values were used for analysis. The β coefficient is

the estimated value resulting from the analysis performed on variables that have been standardized to have a variance of 1 to determine which of the independent variables has a greater effect on the dependent variable. Therefore, the variables with larger β coefficients contribute more to the model. A partial correlation indicates the correlation between the dependent variable and one independent variable when the linear effects of the remaining variables are eliminated. Unstandardized coefficients (m) were used to fit the values of yield versus those calculated using the regression model. Statistical analyses were performed using the IBM SPSS software version 24.

5. Conclusions

This field study highlights the benefits of introducing lupin as a companion species in a wheat-intercropped system in a P-deficient acidic Andisol. Higher yield per plant was observed in intercropped wheat compared to monoculture, related to a higher number of spike development, root biomass, and phosphatase activity, which contributed to increased availability of soil P for wheat uptake. However, the absolute wheat yield was higher under monoculture because of the highest density of plants compared with intercropping. Nonetheless, the overall production in the wheat+lupin intercropping system reached the values of the wheat monocrop, highlighting the efficiency of the system to produce two commodities with the same quantity of inputs. However, the interactions of wheat with chickpea and lupin were very different. The wheat/chickpea combination revealed intense competition, where wheat was the dominant component in the system affecting chickpea development and yield. In contrast, wheat/lupine exhibited a facilitating relationship that improved the productivity of both species. The wheat/lupin system demonstrated that it was possible to reduce the use of P fertilizers without greatly compromising wheat yield, as evidenced by LER values, which were > 1 , indicating an advantage in the efficient use of soil and nutrient resources compared with wheat monoculture. These findings highlight the importance of considering the effects of facilitation and competition among species to maintain stable production, reduce dependence on external inputs, such as P, and promote agroecosystem sustainability.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Natalie Aravena, Dalma Castillo-Rosales, Iván Matus, Felipe Noriega, Raúl Zornoza and Mauricio Schoebitz. The first draft of the manuscript was written by Natalie Aravena and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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