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Full Length Article

The sowing ratio in legume intercropping regulates the facilitation of phosphorus uptake in wheat

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ABSTRACT

The intercropping of legumes with wheat is an interesting method to optimize the use of resources such as phosphorus (P) and nitrogen (N). This occurs when the roots of wheat and legumes interact generating positive (facilitation and complementarity) rather than negative interactions (competition). This research aims to assess how the sowing ratio, which affects root interactions, drives the shift from competition to complementarity and facilitation in legume-wheat intercropping, hypothesizing that each legume species exhibits different behaviour. Under climate-controlled conditions, wheat was intercropped with pea, faba bean, and lupin, varying the number of legume plants per pot (legume density). The effects of legume density on competition/complementarity/facilitation were investigated by monitoring root exudation of carboxylates, acid phosphatase, and nutrient acquisition in both wheat and legumes. Legumes enhanced P uptake of wheat, while N was more strongly influenced by the increase in wheat dry matter observed under intercropping. Faba bean and lupin facilitated wheat P uptake at higher density, while pea facilitated P uptake at lower density. The legume species and plant number affected the concentration and composition of carboxylates in the wheat rhizosphere. Shoot P concentration of wheat was positively correlated with oxalate, malate, citrate, and succinate. In the case of lupin intercropping, phosphatase activity contributed more to facilitation than carboxylates. Intercropping with legumes is a valuable strategy for enhancing the use of resources such as P and N when the shift from competition to complementarity and facilitation is triggered by an appropriate sowing ratio, which depends on the legume species.

1. Introduction

The coexistence of multiple species is a prevalent phenomenon in nature. It is a highly efficient strategy through which organisms can optimise the utilisation of resources. Indeed, when a natural ecosystem is converted to an agroecosystem for agricultural purposes, anthropogenic inputs (such as ploughing, fertilisation, weed control and so on) are required to maintain the suitability of the system for a single species. When these external inputs are removed, the system rapidly reconverts to a multispecies ecosystem. In contrast to an agroecosystem, the floristic composition, defined as the ratio of different species within an ecosystem, is achieved in nature through a range of mechanisms, primarily influenced by environmental factors (Bañares-de-Dios et al., 2022).

An agricultural system that more closely resembles the natural settlement of plant communities and is capable of reducing external inputs is intercropping. In intercropping, the cultivation of two or more crops simultaneously results in the utilisation of resources through interactions between the plants, both above and below the ground, which can be positive, negative or neutral. In other words, different species can compete, which is a negative interaction, or they can interact positively by complementary use of resources and, in some cases, facilitate the growth of the surrounding plants. In intercropping, the belowground interactions between roots appear to hold greater significance than the aboveground interactions (Zhang and Li, 2003).

When different functional groups of species are intercropped, significant differences in resource acquisition and utilisation are observed, and complementarity can occur (Reich et al., 2004; Yuan et al., 2018). The intercropping of wheat with legumes results in interspecific complementarity due to the differing strategies employed by these crops in the acquisition and utilisation of resources, thereby limiting interspecific

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competition (Duchene et al., 2017). With regard to N, the natural capacity of legumes to exploit atmospheric N (N_2) avoids interspecific competition between the soil N-dependent wheat and legumes for this element (Hauggaard-Nielsen et al., 2008). Concurrently, the intense uptake of N from the soil by wheat stimulates the growth and activity of nodules in the legume roots, increasing the quantity of N derived from N_2 fixation (Hauggaard-Nielsen et al., 2009; Parsons et al., 1993).

Legumes directly contribute to soil enrichment by significant amounts of N-rich rhizodeposition (also known as N derived from Rhizodeposition-NdfR; Berenstecher et al., 2023; Fustec et al., 2010). Rhizodeposition supplies the rhizosphere with a range of organic and inorganic compounds, including protons (H+), carboxylates and acid phosphatase, which can facilitate P uptake of wheat in intercropping with legumes (Lazali and Drevon, 2021; Lo Presti et al., 2021; Wu et al., 2021). It is challenging to distinguish between complementarity and facilitation in practice (Loreau and Hector, 2001). However, the prevalence of positive interactions over negative ones can vary during the crop cycle and is influenced by the number of plants supported by the environment. In the agroecosystem, the number of plants is determined by the sowing density and, in the case of intercropping, by the sowing proportion between the crops (Li et al., 2016).

The mechanisms regulating plant density through the correction of excessive sowing density, including constant final yield (maximum biomass for a genotype in an environment after a period of growth) and self-thinning (density-dependent mortality at high densities), have been studied in agroecosystems (Deng et al., 2012; Postma et al., 2021; Weiner and Freckleton, 2010; Westoby, 1984). Plant roots can respond to different neighbouring plants and variable environments by modifying their physiological or morphological traits. This ability is known as phenotypic plasticity in functional traits, and is an important mechanism in community assembly and efficient utilisation of resources (Callaway et al., 2003). However, the root response is not uniform; it is species-specific and contingent on: (i) whether the neighbour is a plant of a different or the same species and, in this latter case, whether they are kin or non-kin; (ii) the availability of resources, as well as the combination of the aforementioned factors, which produce contrasting results (El Amrani, 2023; Karlova et al., 2021; Mcnickle and Brown, 2014; Postma et al., 2021; Wang and Callaway, 2021; Yang et al., 2018). In certain instances, elevated plant density has prompted an increase in root proliferation as a means of coping with competition, which can culminate in the phenomenon known as the "tragedy of the commons" (Rehling et al., 2021). In this last case, the below-ground detection of the neighbour plant encourages root growth which is not affected by nutrient status. In other cases, it depends only on the availability of resources following an “ideal free distribution model”. This model was developed to understand why some plants did not respond to below-ground neighbours with root overproliferation (Chen et al., 2015; Mcnickle and Brown, 2014; Zhu et al., 2022). Assuming that the facilitation, complementarity, and competition occur via root interactions when root intermingling is reached, a practical way to modulate intermingling in the field is by acting on the sowing ratio. This research aims to shed light on how the sowing ratio drives the shift from competition to complementarity and facilitation.

For that purpose, it was hypothesised that: (i) in intercropping, the legume-wheat sowing ratio determines the root interactions between the two crops, regulating the shift from positive to negative interactions and vice versa, affecting the use of resources such as N and P and the accumulation of dry matter in wheat; (ii) each legume species, although belonging to the same functional group, can have a different facilitating effect due to differences in root exudation (acid phosphatase and carboxylate release) and competitive ability, so that the same sowing ratio can produce different responses in wheat due to the different intercropped legume species; (iii) acid phosphatase and carboxylates exuded by the legumes facilitate the uptake of sparingly available P by wheat, but this facilitation appears when not masked by interspecific competition.

2. Material and methods

2.1. Plant growth and experimental design: legume species and legume density treatments

In a pot experiment, durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn. cv. Svevo) lupin (*Lupinus albus* L. cv. Multitalia), faba bean (*Vicia faba* var. minor Beck cv. Sikelia) and pea (*Pisum sativum* L. cv. Hardy) were grown either as sole crop or as a wheat/legume intercrop to achieve the cropping treatments (CTR). The cropping treatments were combined with two levels of the legume density treatments (LD) by intercropping one wheat plant with one legume plant (LD1) and one wheat plant with two legume plants (LD2) per pot. CTR and LD were arranged in a two-factorial completely randomised design with four replications. A summary of the cropping treatments (CTR) and legume density treatments (LD) is provided in Table S1 and Fig. 1. To sample at the flowering stage of each legume, three wheat sole crops were established (Wf, Wl and Wp for faba, lupin and pea, respectively) for each legume density level. With regard to the sole cropping of legumes, one legume plant (LD1) and two legume plants (LD2) were grown per pot. In addition, three bare soil (BS) treatments were added and sampled at the flowering stage of faba bean (BSf), lupin (BSl) and pea (BSp) for each legume density level and used as a control for soil variable analysis (Fig. 1).

The plants were grown in a climate chamber until flowering. The climate chamber (KW, Apparecchi Scientifici, Siena, Italy) is a 5-m^2 chamber equipped with temperature, moisture and photoperiod control and provided by high-pressure sodium (HPS) lamps. The climate chamber is located at the agronomy laboratories at the Department AGRARIA, “*Mediterranea*” University of Reggio Calabria, Italy. The growth parameters shown in Table 1 were maintained during the plant growth. The pots used consisted of PVC tubes Ø 14 cm and 30 cm tall filled with soil mixed with perlite (80/20, v/v). The soil used in the experiment was collected from the experimental farm (38° 06′ 37″ N, 15° 39′ 40″ E) of the Department AGRARIA at the “*Mediterranea*” University of Reggio Calabria, Italy and was selected because of its low content of available P (11.33 mg of bicarbonate-extractable P kg^{-1} soil). Main soil properties are listed in Table 2.

2.2. Soil and plant sampling collection and analysis

Soil and plant samples were collected at different flowering times of the legume species, 58, 65 and 86 days after sowing for pea, faba bean and lupin and their respective intercrops, wheat sole crops and bare soils. The soil was carefully removed from the roots and mixed before being collected as bulk soil for chemical and biochemical analysis. The roots were immediately separated from the shoots and gently shaken to remove excess soil leaving only adhering rhizosphere soil. Rhizosphere soil was recovered for carboxylate analyses (quality and quantity of carboxylates), following the modified Pearse et al. (2007) method: a subsample of approximately 10 g of roots with adhering soil was immediately transferred to a 100-mL vial containing 50 mL of 0.2 mM $CaCl_2$. Root samples were gently dunked in the solution for 30 s to remove as much rhizosphere soil as possible. Root damage was minimised; however, it cannot be excluded that some carboxylates did not originate due to cellular damage. For bare soil, 10 g of bulk soil was used instead of roots and adhering rhizosphere soil. After the roots were removed and collected for analysis, a subsample of the extract was filtered through a 0.2-µm syringe filter into a 1-mL HPLC vial. Both rhizosphere extract and bulk soil samples were transferred to a − 20 °C freezer for carboxylate determination and biochemical analyses, respectively. An aliquot of the bulk soil was air dried and 2-mm sieved for measurement of the available P. The recovered root subsample was accurately washed to remove any trace of soil and combined with the remaining root. Roots and shoots were placed in the oven and dried at 60 °C until the weight was stabilised to record the dry weight. Dried roots and shoots were milled and stored for P and N content analyses.

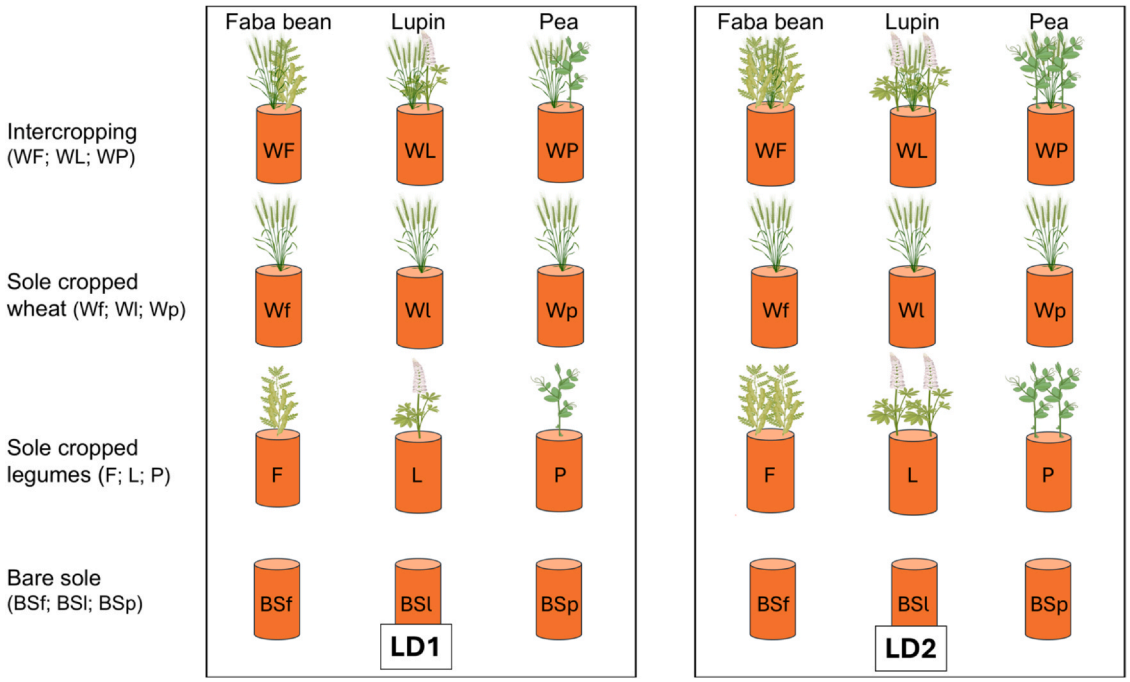

Fig. 1. Graphical representation of the cropping treatments (CTR) combined with the legume density treatments (LD). CTR = WF, WL, WP: wheat intercropped with faba bean, lupin and pea; Wf, Wl, Wp: sole cropped wheat sampled at flowering stage of faba bean, lupin and pea; F, L, P: sole cropped faba bean, lupin and pea; BSf, BSl, BSp: bare soles sampled at flowering stage of faba bean, lupin and pea. LD = LD1 and LD2: pots respectively with one legume plant and two legume plants per pot, grown in intercropping or sole cropping, and the respective BSs and sole cropped wheat.

Table 1
Growth conditions in climate chamber.

Period length (d)	*15*	*20*	*20*	*Until legume flowering*
Day (h)	8	8	10	14
Night (h)	16	16	14	10
Light intensity (μmoles/m^2 /s)	200	255	340	340
Temperature (°C)	8	15	20	25
Relative humidity (%)	60	60	60	60

Table 2
Physical and chemical characteristics of soil used in the experiment.

Soil properties	
Bulk density (g cm^{-3})	1.23
Sand %	36
Silt %	32
Clay %	32
pH $_{CaCl2}$	6.6 ± *0.1*
Total organic carbon (g kg^{-1})	12.55 ± *.4.5*
Total nitrogen (g N kg^{-1})	1.38 ± *0.1*
C/N	9.07 ± *0.1*
$EC_{1:2}$ (dS m^{-1})	0.271 ± *0.021*
CEC ($cmol_{(+)}$ kg^{-1})	20.9 ± *1.3*
$CaCO_3$ (g kg^{-1})	8.4 ± *0.5*
P-Olsen (mg kg^{-1})	11.33 ± *0.2*
NH_4^+ - N (mg kg^{-1})	12.49 ± *0.2*
NO_3^- - N (mg kg^{-1})	26.16 ± *0.2*

Phosphorus concentration in shoots and roots. 100 mg of milled dried roots and shoots were digested with a mixture of nitric and perchloric acid (6:1). The P concentration in the digest was measured by a Lambda Fias UV/VIS Spectrophotometer (Perkin Elmer, Waltham, MA, USA) following the molybdenum blue method (List et al., 1986; Ruzicka and Hansen, 1981).

Nitrogen concentration in shoots and roots. 2.0 g of milled dried roots and shoots were used following the Kjeldahl method (AOAC International, 2016) using a digestor Foss Tecator digest auto (Foss Italia, Padua, Italy) coupled with a Foss Kjeltec 8400 distillation unit (Foss Italia, Padua, Italy).

Ammonium and nitrate content in the soil. Nitrate-nitrogen (NO_3^--N) and ammonium-nitrogen (NH_4^+-N) were extracted using KCl 2 M solution from 5 g of frozen bulk soil (Beemner and Keeney, 1966) and measured by a Lambda Fias UV/VIS Spectrophotometer Perkin Elmer following the Berthelot reaction and Griess-Ilosvay method, respectively.

Soil acid phosphatase activity. Acid phosphatase activity was determined by the method proposed by Tabatabai and Bremner (1969) according to which acid phosphomonoesterase (PME) in soil is expressed as the production of para-nitrophenol (μg PNP h^{-1} g^{-1} of dry soil). Briefly, 1.0 g of the soil stored at − 20 °C was incubated, at 37 °C for 1 h, with para-nitrophenylphosphate in 4 mL of 0.04 M sodium maleate buffer (pH 6.5). After the reaction was stopped with 1 M NaOH, the amount of PNP released by acid phosphomonoesterase activity was measured via a spectrophotometer as absorbance at 400 nm.

Rhizosphere carboxylate determination. The analysis of rhizosphere extracts was performed according to the method suggested by Cawthray (2003) using a HPLC with the PDA detector Altus A-10 (Perkin Elmer, Waltham, MA, USA) and a Kinetex 2.6 μm F5 100 Å column (Phenomenex, CA, USA). The working standards included oxalic, tartaric, malic, malonic, acetic, maleic, citric, cis-aconitic, succinic, fumaric and trans-aconitic acids (Sigma-Aldrich, USA).

P pools in the soil: available and organic P. Available P was extracted with 0.5 M $NaHCO_3$ from 2 g of the air-dried 2-mm sieved soil, according to the Olsen method (Olsen et al., 1954). Organic P was obtained as the difference between 550 °C-ignited and non-ignited soil samples extracted by 0.5 M H_2SO_4 from 2 g of 0.2-mm sieved soil, according to Bowman (1989) and Kuo (1996). P from both the extracts was quantified via spectrophotometry using a Lambda 400 Fias UV/VIS Perkin Elmer (Waltham, Massachusetts, U.S.) according to the molybdenum blue method (List et al., 1986; Ruzicka and Hansen, 1981).

2.3. Statistical analysis

The statistical analyses were performed with R software (R core team, 2022, version 4.2.1) using RStudio (R Studio Team, 2023, version 454). After data normality and homogeneity of its variance were tested by the Shapiro-Wilk test (function "shapiro.test()") and Levene's tests (function "leveneTest()" in car package), experimental data were subjected to a two-way ANOVA (function "aov()") to assess the significance of the fixed effects of CTR, LD, and their interactions (CTR × LD). Tukey's HSD (honestly significant difference) test at *P*-value < 0.05 was used for pairwise multiple comparisons of treatment means. The concentration of the carboxylates was correlated (Pearson r coefficient) to the P concentration in the wheat and legume shoots using the command "cor" in RStudio (version 4.2.1), and the package Corrplot (Wei and Simko, 2021) for graphical displaying.

3. Results

3.1. Dry matter accumulation and partitioning

The total dry matter accumulation was greater in the intercropped wheat than in the sole cropped wheat ($P < 0.001$). However, the results were markedly affected by the interaction between the legume species (CTR) and their density (LD) ($P < 0.001$, Tables 3 and 4). The total dry matter accumulation of wheat was greater when intercropped with lupin and faba bean at LD2, while with pea, it was greater at LD1 (Table 4A). In contrast, among the legumes, intercropping had a significant impact ($P < 0.001$) only on lupin total dry matter (Table 4B), which was greater than that observed in the sole crop. The doubling of the number of legumes at LD2 resulted in a significant reduction in root dry matter in lupin and faba bean ($P < 0.001$) in comparison to LD1. Nevertheless, this phenomenon was not observed in the case of pea (Table S2).

3.2. Phosphorus and nitrogen uptake and their concentrations in shoot

When wheat was intercropped, its P uptake was on average higher than that of the sole crop ($P < 0.001$) and the results varied considerably due to the interaction between legume species (CTR) and the number of legume plants (LD) ($P < 0.001$, Table 3). In intercropping with lupin and faba bean, wheat P uptake was significantly higher than in the sole crop only at LD2 ($P < 0.001$), while at LD1 there was no difference. Conversely, in intercropping with pea, wheat P uptake was always greater than in the sole crop ($P < 0.01$) with negligible differences between LD1 and LD2 (Fig. 2A). Similarly, the concentration of P in the wheat shoot was found to be influenced by the number of legume plants (LD) ($P < 0.01$) and by its interaction with legume species (CTR) ($P < 0.01$, Table 3). This resulted in a significant increase in P concentration in the wheat shoot at LD2 compared to the sole crop when intercropped with pea (Fig. 2B).

The legume density treatment had a significant impact on both legume P uptake and shoot concentration ($P < 0.01$ and $P < 0.001$, respectively, Table 3). These variables exhibited a broadly similar trend, with the values observed in the pea treatment at LD2 being significantly higher than those of all other treatments. (Fig. 2C and D). Wheat N uptake increased in intercropping with faba bean and lupin at LD2 and in intercropping with pea at both LD2 and LD1, in comparison to sole cropping (Table S3). The CTR and its interaction with LD had a significant impact on legume N uptake ($P < 0.001$, Table 3). The highest N uptake was observed in the sole cropped pea at LD2, which exhibited a statistically significant difference from the lowest value, which was observed in the sole cropped lupin at LD1. Furthermore, the LD had no significant impact on the N concentration in legume shoots (Table S4).

Table 3
Results from the analysis of variance on the effect of legume density treatment (LD), the cropping treatment (CTR) and their interaction (LD × CTR) on measured variables.

	LD	CTR	LD × CTR
Wheat total dry matter (g plant^{-1})	ns	***	***
Legume total dry matter (g plant^{-1})	ns	***	***
Wheat root dry matter (g plant^{-1})	ns	**	ns
Legume root dry matter (g plant^{-1})	***	*	***
Shoot P concentration in wheat (mg g^{-1})	**	ns	**
Shoot P concentration in legumes (mg g^{-1})	***	***	***
P uptake in wheat (mg plant^{-1})	**	***	***
P uptake in legumes (mg plant^{-1})	**	***	***
Shoot N concentration in wheat (mg g^{-1})	ns	***	ns
Shoot N concentration in legumes (mg g^{-1})	ns	***	ns
N uptake in wheat (mg plant^{-1})	*	***	***
N uptake in legumes (mg plant^{-1})	ns	***	***
Soil acid phosphatase activity (μg PNP h^{-1} g^{-1})	***	***	**
Soil organic P (mg kg^{-1})	ns	***	*
Soil available P (Olsen P, mg kg^{-1})	**	***	ns
Soil nitrate (mg kg^{-1})	ns	***	**
Soil ammonium (mg kg^{-1})	ns	***	ns
Total carboxylates in wheat rhizosphere (μmol g^{-1})	**	***	***
Total carboxylates in legume rhizosphere (μmol g^{-1})	ns	***	*
Oxalate concentration in wheat rhizosphere (μmol g^{-1})	***	***	***
Oxalate concentration in legume rhizosphere (μmol g^{-1})	ns	***	***
Malate concentration in wheat rhizosphere (μmol g^{-1})	***	***	***
Malate concentration in legume rhizosphere (μmol g^{-1})	ns	***	**
Citrate concentration in legume rhizosphere (μmol g^{-1})	***	***	**
Succinate concentration in wheat rhizosphere (μmol g^{-1})	***	***	***
Succinate concentration in legume rhizosphere (μmol g^{-1})	***	***	***

ns, not significant; P, phosphorus; N, nitrogen; PNP, para-nitrophenol.
* Significant at $P \leq 0.05$.
** significant at $P \leq 0.01$.
*** significant at $P \leq 0.001$.

3.3. Acid phosphatase activity and pivotal phosphorus pools in the soil

The cropping treatment ($P < 0.001$), legume density ($P < 0.001$) and their interaction ($P < 0.01$) had a significant effect on acid PME activity (Table 3). The lupin-holding soil exhibited the highest acid PME activity among all cropping treatments. Furthermore, acid PME activity was significantly higher at LD2 than at LD1. When lupin was sole cropped or intercropped at LD2, the acid PME activity was significantly greater than that observed in wheat sole crops and bare soils (Table 5). The acid PME activity was higher than that observed in wheat sole crops and bare soils, even in faba bean sole cropping at LD2. In contrast to acid PME activity values, the organic P was not affected by the number of legume plants and exhibited only a slight variation as a result of the crop treatments, with the exception of Wl, where significantly higher values were observed (Table S5A). The doubling of legume plants resulted in a significant depletion of Olsen P ($P < 0.05$). In comparison to the BSs, the Olsen P values were observed to be lower in the treatments with plants, with the exception of P and Wp (Table S5B).

3.4. Pools of nitrogen in the soil: nitrate and ammonia

NO_3^--N in the soil was affected by crop treatment ($P < 0.001$) and by its interaction with legume density ($P < 0.05$, Table 3). NO_3^--N was significantly depleted in all plant-harbouring treatments compared to BSs (Table S6A). NH_4^+-N was only affected by crop treatment

Table 4
Total dry matter accumulation (g plant^{-1}) of wheat (A) and legumes (B) grown in sole crop (W, wheat; F, faba; L, lupin; P, pea) and in intercropping (WF; WL; WP) at two legume densities (LD1 and LD2). f, l, and p indicate the sampling time of wheat (W) corresponding to the different flowering of legumes.

A. Wheat total dry matter (g plant^{-1})

	LD1			LD2			mean		
Wf	21.4	± *1.5*	de	18.6	± *0.7*	ef	*20.0*	± *0.9*	C
Wl	16.9	± *0.4*	ef	16.8	± *0.2*	ef	*16.9*	± *0.2*	CD
Wp	13.7	± *0.3*	f	13.8	± *0.2*	f	*13.7*	± *0.2*	D
WF	24.4	± *1.6*	cd	34.1	± *1.9*	ab	*29.2*	± *2.2*	B
WL	29.3	± *2.1*	bc	36.4	± *0.6*	a	*32.8*	± *1.7*	A
WP	32.7	± *0.5*	ab	20.5	± *0.3*	de	*26.6*	± *2.3*	B
Mean	23.1	± *1.5*		23.4	± *1.8*		23.2	± *1.2*	
LD									*NS*
CTR									*P < 0.001*
LD × CTR									*P < 0.001*

B. Legume total dry matter (g plant^{-1})

	LD1			LD2			mean		
F	30.3	± *3.0*	abc	20.3	± *1.5*	cd	25.3	± *2.4*	BC
L	19.7	± *0.7*	d	21.9	± *1.0*	cd	20.8	± *0.7*	C
P	34.6	± *1.3*	ab	37.8	± *1.7*	a	36.2	± *1.1*	A
WF	34.7	± *2.2*	ab	20.3	± *1.1*	d	27.5	± *2.9*	B
WL	26.0	± *1.5*	bcd	28.3	± *3.8*	abcd	27.2	± *1.9*	B
WP	31.5	± *1.6*	ab	37.5	± *1.9*	a	34.5	± *1.6*	A
Mean	29.5	± *1.3*		27.7	± *1.7*		28.6	± *1.1*	
LD									*NS*
CTR									*P < 0.001*
LD × CTR									*P < 0.001*

The values shown are means ± standard errors. Significance of *P* for legume density (LD), cropping treatments (CTR), and its interactions (LD × CTR) by Anova are reported in italic. On both columns LD1 and LD2 values followed by different lower-case are significant different; mean values followed by different capital letters indicate the significant differences at P ≤ 0.05 (Tukey's HSD test).

($P < 0.001$). The values in F, Wf and WF soil were significantly lower than those in the respective BSf (Table S6B).

3.5. Carboxylates exuded in the rhizosphere

The total amount of carboxylates in the wheat rhizosphere was affected by crop treatment ($P < 0.001$), legume density treatment ($P < 0.01$) and by their interaction ($P < 0.001$, Table 3). The greatest total amount of carboxylates in the wheat rhizosphere was recorded when wheat was grown together with two pea plants (LD2) while no significant differences were found among the other treatments ($P < 0.001$) (Fig. 3A). Significant variation in total carboxylates was observed among the cropping treatments in the rhizosphere of the legumes ($P < 0.001$) with significant interaction between CTR and LD ($P < 0.05$) (Table 3). Significantly higher values were detected in the rhizosphere of intercropped faba bean at LD1 compared to that of the other legumes (Fig. 3B).

The values of total carboxylates reported above were obtained by summing the carboxylates considered for the qualitative composition of the rhizosphere of wheat (Table S7A) and legumes (Table S7B). The concentrations of each carboxylate in the rhizosphere were correlated with the concentrations of P in the shoots of legumes and wheat. A significant correlation ($P < 0.01$) was not identified between carboxylate concentrations and P concentrations in legume shoots. In contrast, a positive correlation was observed between oxalate, malate and citrate and shoot P of wheat, while succinate exhibited a negative correlation (Fig. 4). As a result, these carboxylates were considered to be involved in regulating the P turnover in the rhizosphere and studied in more detail. The concentration of oxalate and malate was significantly higher in the rhizosphere of wheat intercropped with two pea plants (LD2) and two faba bean plants (LD2) than in the respective sole crops (Wp and Wf) (Fig. 5A and C). In contrast, no significant variation was observed in citrate values across different crop treatments in wheat (data not shown). Succinate levels were higher in intercropping with pea and faba bean, although this was only evident at LD1, where it was significantly greater than LD2 (Fig. 6B). In the rhizosphere of the legume, the highest concentration of oxalate was observed in the sole cropped faba bean at LD1, which was significantly different from all other treatments (Fig. 5B). Malate was significantly greater in the faba bean rhizosphere at LD1 than in other legumes (Fig. 5D). Similarly, citrate and succinate were also greater in the faba bean rhizosphere. Notably, citrate release was favoured by LD1 and intercropping, while succinate increased in sole cropping at LD1, followed by intercropping at LD2 (Fig. 6A and C).

4. Discussion

The study was conducted under controlled climatic conditions, in pots filled with soil from an experimental field with known physical and biochemical characteristics. One of the most interesting findings is that the variation in total dry matter accumulation of intercropped wheat as the density of the neighbouring legumes increases.is markedly influenced by the legume species used. Recent studies conducted in both field and controlled environments have linked the benefits of intercropping to the phenotypic plasticity of the facilitated plant. This includes an increase in root biomass and spatial root distribution in response to the facilitator, which may result in enhanced water and nutrient utilisation (Streit et al., 2019; Yu et al., 2020). In our study, the species that was primarily facilitated by intercropping was wheat; however, its root dry matter was not influenced by the neighbouring plant. In contrast, the root dry matter of lupin and faba bean was significantly reduced by growth with a plant of the same species at LD2 compared to the system without competition at LD1. The reduction in

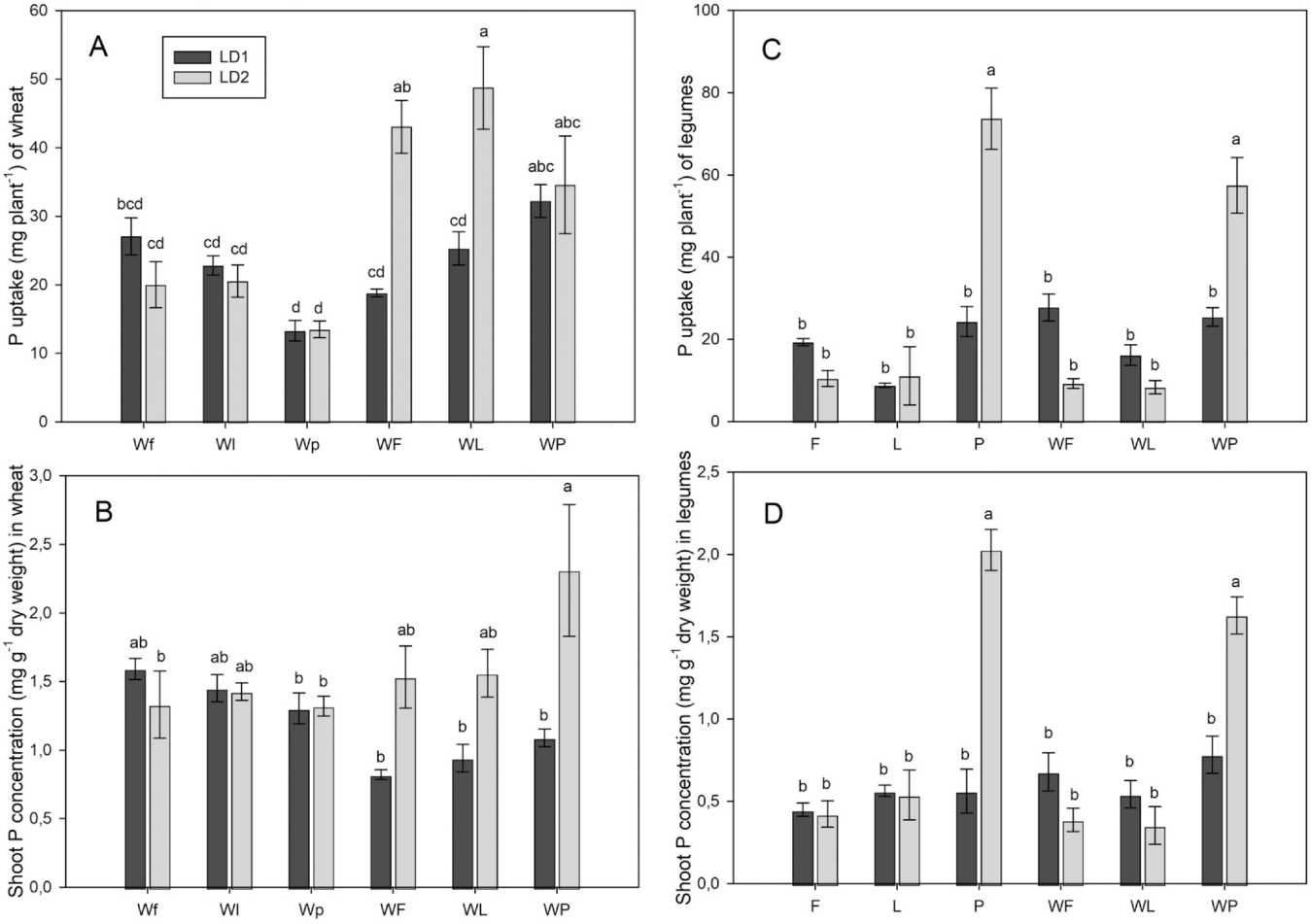

Fig. 2. Phosphorus (P) uptake (mg plant^{-1}, A and C) and P shoot concentration (mg g^{-1} dry weight, B and D) of wheat (A and B) and legumes (C and D) grown in sole cropping (W, wheat; F, faba; L, lupin; P, pea) and in intercropping (WF; WL; WP) at two legume densities (LD1, one legume plant, bars filled with dark grey; LD2, two legume plants, bars filled with light grey). f, l, and p indicate the sampling time of wheat (W) corresponding to the different flowering of legumes. Values are means (n = 4) ± SE. Different letters above the bars indicate significantly different values (P ≤ 0.05; Tukey's HSD test).

root dry matter of lupin and, in particular, faba bean is consistent with the concept of 'constant final yield' (Weiner and Freckleton, 2010). This is evident in the pot environment, where two plants of the same species eventually reach a constant biomass that does not increase further. In other words, the maximum root biomass achievable by two plants of faba bean and lupin was reached, given the limited volume of soil available in the pot. In response to the moderate intraspecific competition, the plants then exhibited a reduction in root growth (Postma

Table 5
Acid phosphatase (PME) activity, expressed as μg of para-nitrophenol (PNP) g^{-1} soil h^{-1} in bare soil (BS), in sole cropping (W, wheat; F, faba; L, lupin; P, pea) and in intercropping (WF; WL; WP) at two legume densities (LD1, one legume plant; LD2, two legume plants). f, l, and p indicate the sampling time of wheat (W) and bare soil (BS) corresponding to the different flowering of legumes.

	LD1			LD2			mean		
BSf	192	± *2.8*	fh	186	± *1.7*	gh	189	± *1.8*	D
BSl	190	± *2.4*	fh	182	± *2.4*	h	186	± *2.1*	D
BSp	175	± *3.9*	h	173	± *4.3*	h	174	± *2.7*	D
F	246	± *23.7*	bf	271	± *9.9*	ad	258	± *12.8*	B
L	285	± *28.6*	ac	305	± *1.1*	ab	295	± *13.8*	A
P	208	± *6.2*	eh	253	± *6.9*	be	230	± *9.5*	BC
Wf	210	± *12.4*	eh	211	± *8.5*	eh	210	± *6.9*	CD
Wl	208	± *11.3*	eh	208	± *9.2*	eh	208	± *6.8*	CD
Wp	207	± *6.6*	eh	206	± *5.0*	eh	207	± *3.9*	CD
WF	225	± *5.6*	dh	247	± *14.3*	bf	236	± *8.3*	BC
WL	244	± *7.5*	cg	329	± *11.2*	a	286	± *17.1*	A
WP	216	± *19.5*	dh	246	± *5.5*	bf	231	± *11.0*	BC
Mean	217	± *5.4*	B	235	± *7.1*	A	**226**	± *4.5*	
LD									*P < 0.001*
CTR									*P < 0.001*
LD × CTR									*P < 0.01*

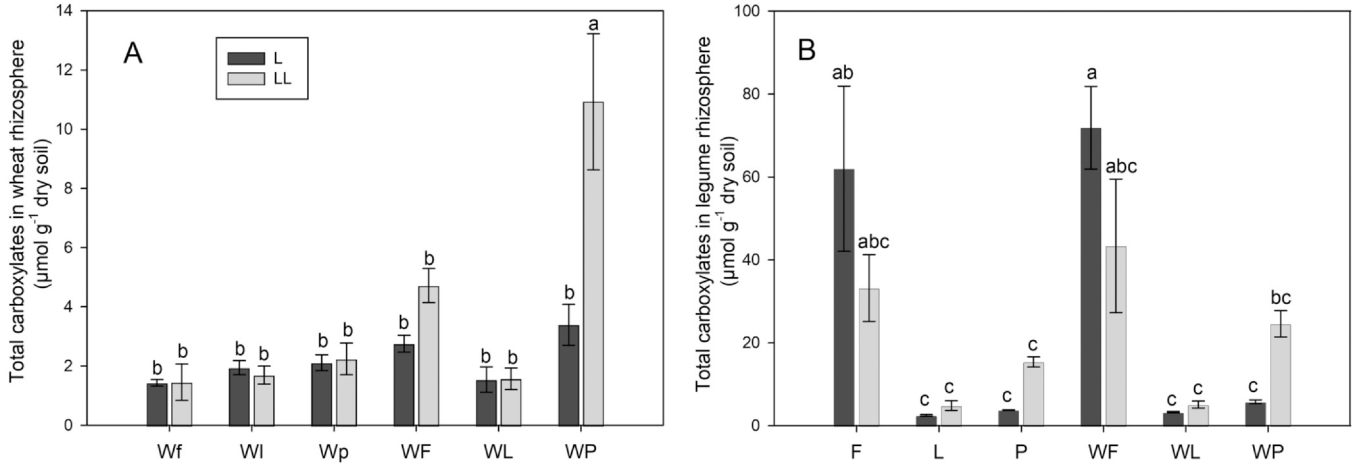

Fig. 3. Total carboxylates in the rhizosphere (µmol g^{-1} dry soil) wheat (A) and legumes (B) grown in sole cropping (W, wheat; F, faba bean; L, lupin; P, pea) and in intercropping (WF; WL; WP) at two legume densities (LD1, one legume plant, bars filled with dark grey; LD2, two legume plants, bars filled with light grey). f, l, and p indicate the sampling time of wheat (W) corresponding to the different flowering of legumes. Values are means (n = 4) ± SE. Different letters above the bars indicate significantly different values (P ≤ 0.05; Tukey's HSD test).

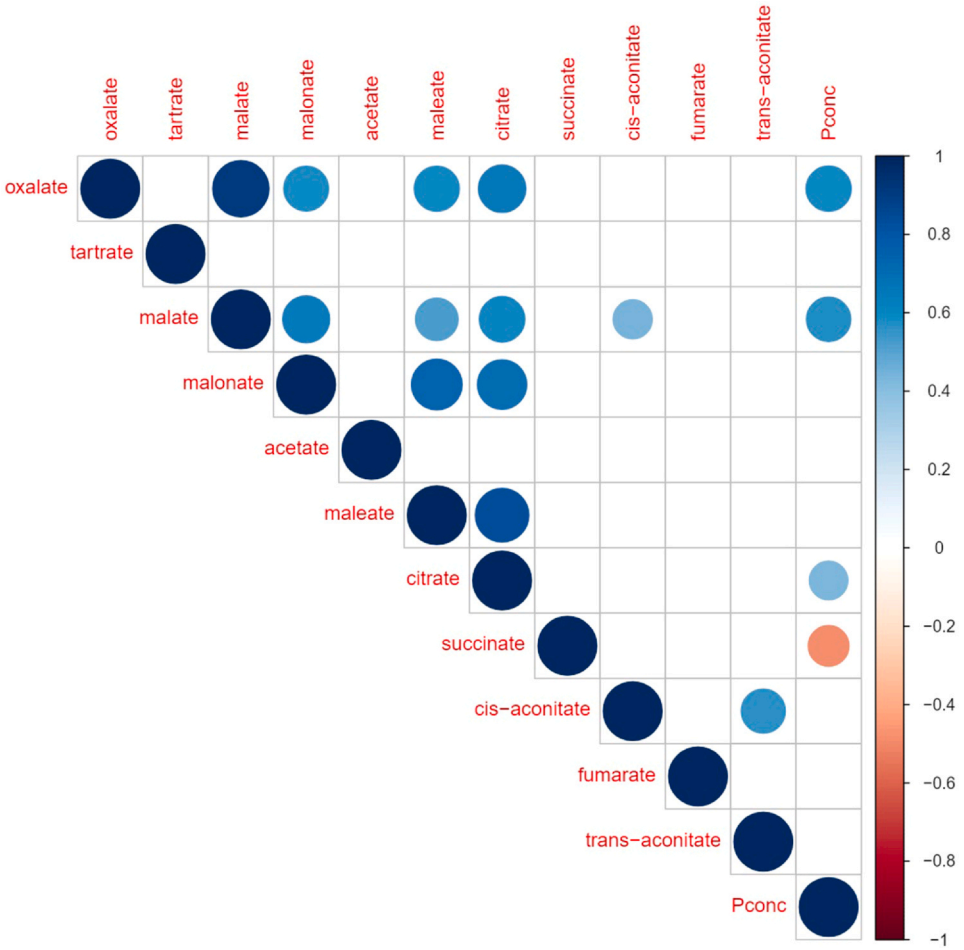

Fig. 4. Pearson correlation matrix between the carboxylates detected in wheat rhizosphere and phosphorus (P) concentration (Pconc) in the wheat shoot. Colour intensity and the size of the circle are proportional to the correlation coefficients (r-value).

et al., 2021; Rehling et al., 2021). It is plausible that this constraint of the legumes may be responsible for enabling intercropped wheat to accumulate a greater quantity of dry matter. In contrast, pea roots were not modified by growing with another pea plant, as previously observed by Meier et al. (2013). This resulted in a doubling of the total legume root biomass and disadvantaged wheat, which benefited from intercropping at LD1 but not at LD2. The dissimilar behaviour of the pea in comparison to the faba bean and lupin serves to corroborate the main role of the root phenotypic response to conspecific co-cultivation (potential intraspecific competition) in either facilitating or competing with the neighbouring crop. The disparity in root plasticity exhibited by the legume species can be attributed to the elevated root dry matter

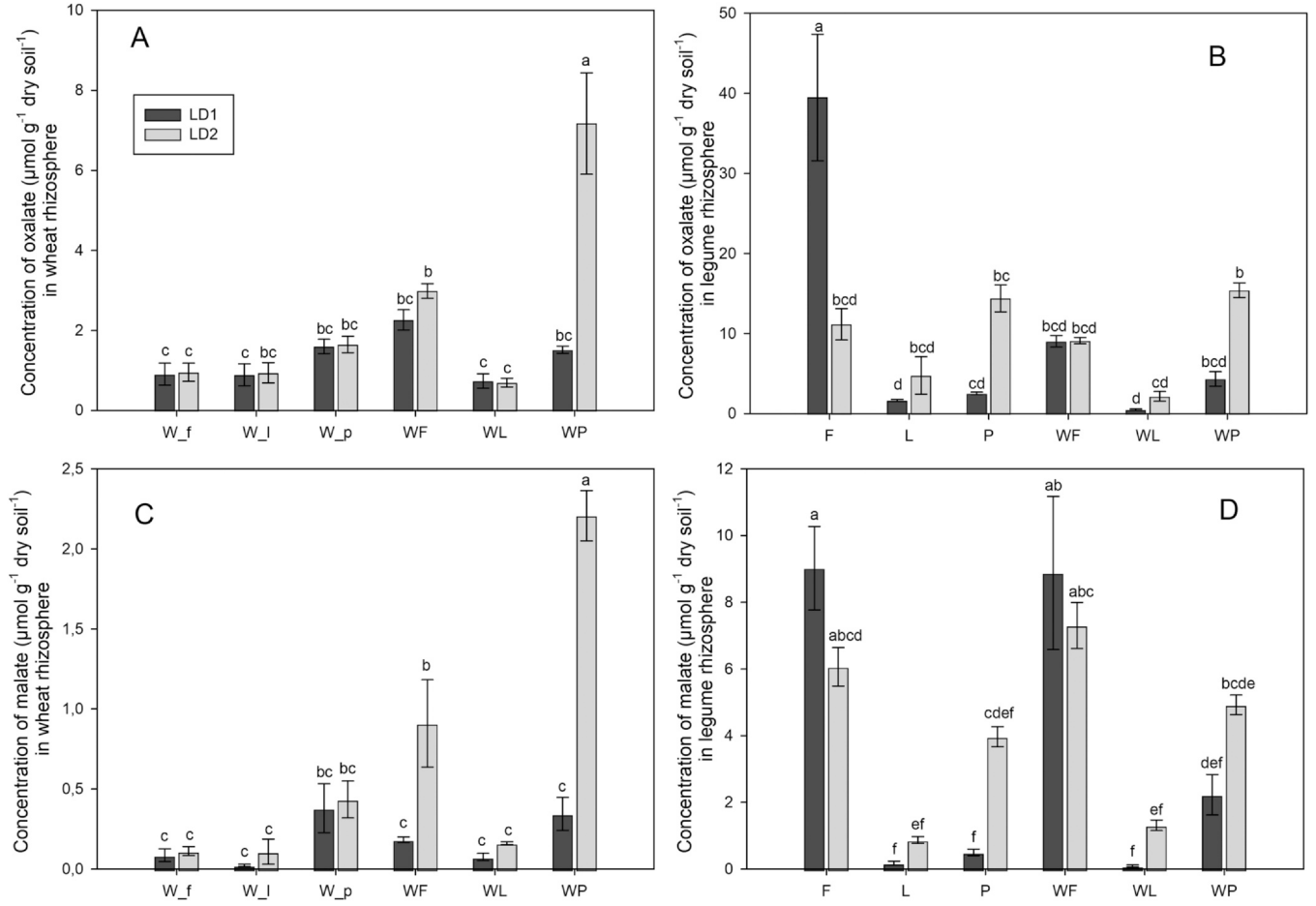

Fig. 5. Concentration (µmol g^{-1} dry $soil^{-1}$) of oxalate (A and B) and malate (C and D) in the rhizosphere of wheat (A and C) and legumes (B and D) grown in sole cropping (W, wheat; F, faba; L, lupin; P, pea) and in intercropping (WF; WL; WP) at two legume densities (LD1, one legume plant, bars filled with dark grey; LD2, two legume plants, bars filled with light grey). f, l, and p indicate the sampling time of wheat (W) corresponding to the different flowering of legumes. Values are means (n = 4) ± SE. Different letters above the bars indicate significantly different values (P ≤ 0.05; Tukey's HSD test).

accumulation by a single plant of faba bean or lupin (Nuruzzaman et al., 2005). The maximum root matter potentially supported by the soil volume was reached by both the faba bean and the lupin at LD1. At LD2, the number of legumes was doubled, and the roots of a single lupin and faba bean plant were subjected to the aforementioned reduction resulting from the competition of another legume plant in the same soil volume. As a result, the greater benefit for wheat dry matter accumulation was achieved at LD2 in intercropping with faba bean and lupin, and at LD1 with pea.

The enhanced yield observed in cereals when intercropped with legumes can be largely attributed to the replacement of interspecific competition with complementary and facilitative relationships, as well as the improvement of resource exploitation, particularly N and P, which are of paramount importance (Duchene et al., 2017). Although the facilitation of both P and N uptake is primarily dependent on direct root contact enabled by the intermingling of the two crops' roots (Hupe et al., 2021; Shao et al., 2021; Xiao et al., 2004; Zhu et al., 2023), the mechanisms of N transfer differ from those of P mobilisation. The variation in N concentration in wheat and legume shoots was minimal and not statistically significant. Therefore, the observed differences in N uptake were largely attributed to variations in dry matter accumulation rather than to the transfer of N. This behaviour may be attributed to the time required for the transfer of N between the two functional groups (from legume to nonlegume crops), which is likely to have exceeded the 86-day duration of the experiment. This result is not unexpected, as previous studies have demonstrated that N transfer is typically lower during the early growing season and increases as the season progresses (Rasmussen et al., 2012) and during the second production year (Chapagain and Riseman, 2014). The transfer of N can occur in young plants through the action of plant-associated mycorrhizae or the exudation of soluble N compounds by legumes (Moyer-Henry et al., 2006; Paynel et al., 2001). However, the primary source of N transfer was the senescence of below-ground legume residues (Høgh-Jensen and Schjoerring, 1997; Moyer-Henry et al., 2006), which occurs in older plants through their degradation and long-term release of N (Høgh-Jensen and Schjoerring, 2001). This assertion is supported by the nitrate- and ammonium-nitrogen values observed in soil with legumes that did not exceed those of bare soil (Table S6). From our results, which referred to the early stages of the crop cycle, legumes did not facilitate wheat N uptake.

In the case of P, both its shoot concentration and uptake in wheat were found to be significantly affected by the cropping treatment and legume density treatment. However, the rates varied significantly due to the species of legume intercropped. Indeed, when wheat was grown with one plant of lupin or one plant of faba bean, the absorption of P was not facilitated by the intercropping of these species. In such circumstances, any facilitations that might have occurred could have been masked by interspecific competition (Li et al., 2016). Therefore, the positive effect of intercropping on wheat was observed solely in terms of dry matter accumulation. The doubling of lupin and faba bean in the intercropping system resulted in a significant enhancement in wheat P uptake and growth. It was hypothesised that in these circumstances, the intraspecific competition between the two legume plants limited their competitive pressure on the wheat. Concurrently, the intraspecific

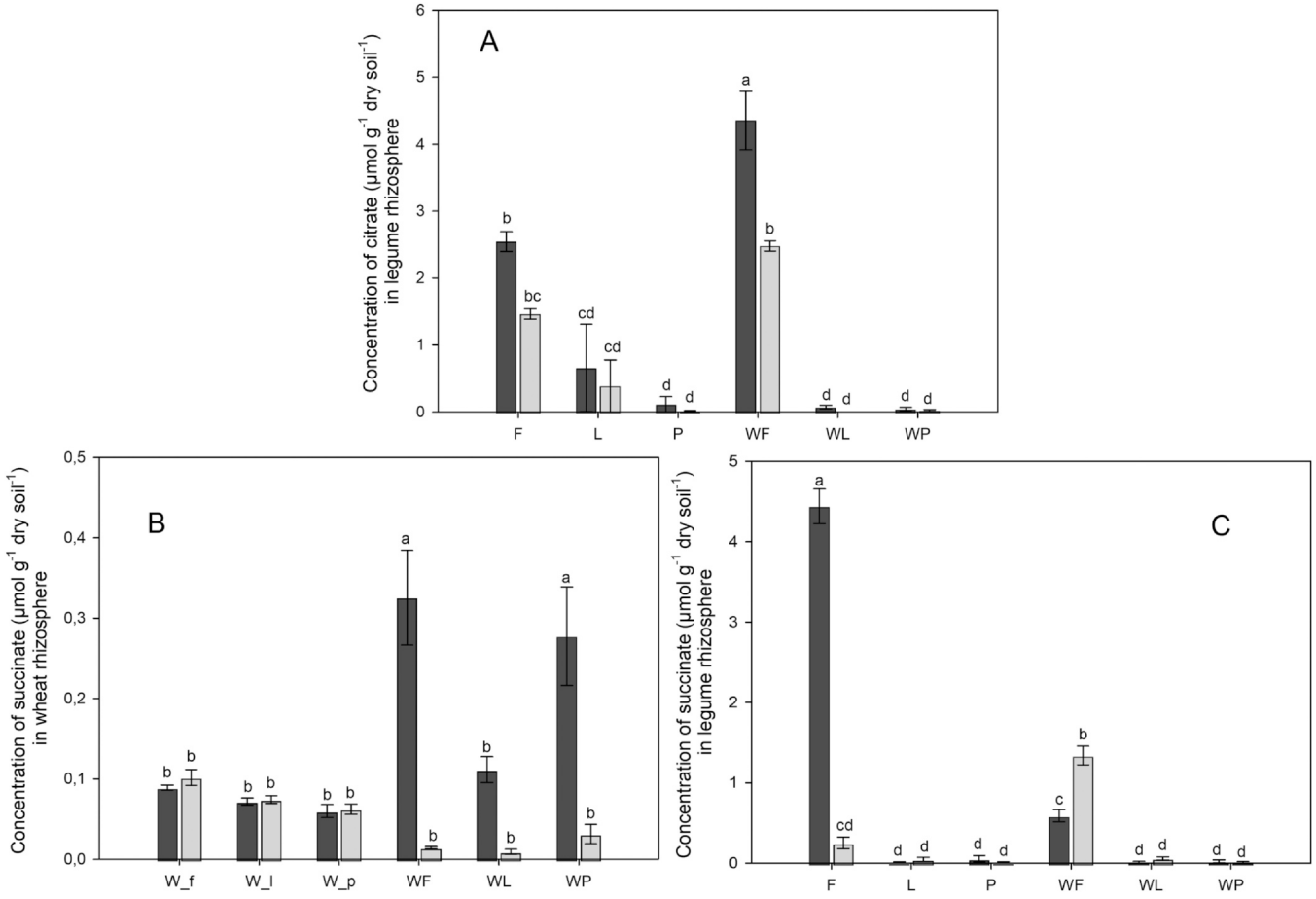

Fig. 6. Concentration (µmol g^{-1} dry $soil^{-1}$) of citrate (A) and succinate (B and C) in the rhizosphere of wheat (B) and legumes (A and C) grown in sole cropping (W, wheat; F, faba; L, lupin; P, pea) and intercropping (WF; WL; WP) at two legume densities (LD1, one legume plant, bars filled with dark grey; LD2, two legume plants, bars filled with light grey). f, l, and p indicate the sampling time of wheat (W) corresponding to the different flowering of legumes. Values are means (n = 4) ± SE. Different letters above the bars indicate significantly different values ($P \leq 0.05$; Tukey's HSD test).

competition prompted the growth of the legume roots in a direction away from the conspecific neighbour. This, in combination with the increase in the overall root biomass resulting from the doubling of the plants, favoured intermingling with wheat roots and, subsequently, facilitative interactions (Zhang et al., 2020; Zhu et al., 2023). In the case of pea, at LD2 there was no limitation of the legume growth caused by negative intraspecific interactions. It appeared that pea growth was favoured by co-cultivation with a conspecific plant. Consequently, in intercropping at LD2, wheat growth was negatively affected by interspecific competition, while its shoot P concentration was significantly high, indicating that P absorption was still facilitated. Indeed, the elevated P concentration in the wheat shoot was accompanied by the highest root proliferation observed in the legumes (pea), which could enable greater intermingling of the roots, thereby fostering more interactions. Despite the highest shoot P concentration being recorded in intercropping with pea at LD2, wheat P uptake was greater in lupin and faba bean intercropping due to higher dry matter accumulation. However, pea at LD2 facilitated P availability not only for the intercropped wheat but also for itself, exhibiting the highest values of P shoot content and P uptake. This contrasts with the other legumes, which were not favoured by conspecific co-cultivation. The high P shoot concentration observed in both pea and wheat in intercropping implies considerable potential for pea in facilitating P mobilisation, provided that its strong interspecific competition is effectively managed during intercropping.

The enhanced P uptake observed in intercropping with legumes is largely attributed to the carboxylates, phosphatases and protons exuded by the roots of the P-mobilising legume, which are shared with the non-P-mobilising crop through root intermingling (Cu et al., 2005; Duchene et al., 2017; Li et al., 2013; Tang et al., 2021). In this study, the highest shoot P concentration in wheat was associated with the highest concentration of carboxylates in its rhizosphere, which were detected in intercropping with two pea plants. It seems reasonable to posit that the majority of the carboxylates present in the wheat rhizosphere originated from the pea roots in close proximity, given that a higher concentration was detected in the proximal pea rhizosphere than in that of wheat cropped alone. In contrast, the concentration of carboxylates in wheat was lower in intercropping with faba bean, which was less effective than pea in transferring carboxylates to the wheat rhizosphere despite its high rate of carboxylate exudation (An et al., 2023) (Fig. 3A and B). This can be explained by the wide differences in root morphology, with pea exhibiting a greater number of fine roots compared to faba bean. Fine roots enabled pea to explore a larger volume of soil with the same root dry matter, resulting in the delivery of root exudates to numerous localised soil volumes, making up for the slow diffusion of carboxylates and enzymes in the soil (Hinsinger et al., 2009). Nevertheless, the contribution of faba bean to wheat growth is supported by the improved P uptake at LD2. The low carboxylate concentration in the lupin rhizosphere contrasts with the findings of other studies (Lyu et al., 2016; Wang et al., 2024). On the other hand, the low exudation of carboxylates in lupin was compensated by the rates of acid PME activity in intercropping, which were the highest detected. This indicates that the exudation of acid PME plays a pivotal role in the facilitation exerted by lupin.

A significant correlation was observed between the concentration of the carboxylates in the rhizosphere and the P concentration in the shoot, specifically in wheat plants. However, no such correlation was

evident in legumes. This is an expected result since faba bean, pea and lupin are species that respond to P-deficiency with increased carboxylate release (Wiche et al., 2023). In this experiment, the differences in P-deficiency among the treatments were insufficient to allow differentiation of root exudation (Table S5). Consequently, the quantitative and qualitative variations of carboxylates can be more readily and accurately identified as the underlying cause of P shoot variations in wheat, rather than being attributed to the consequence of plant P status in legumes. Therefore, oxalate, malate, citrate, and succinate, which correlated with P of the wheat shoot, were considered to be the carboxylates most involved in P mobilising. The concentration of oxalate and malate in the rhizosphere of wheat was significantly higher in intercropping with two plants of pea and faba bean than in the respective sole crops (Fig. 5A and C). The observed increase can be attributed to the exudation of the nearby legume plants, which were significantly higher and more effectively diffused at LD2. This is due to the greater root proliferation, which favoured root intermingling between the two crops. Indeed, due to the poor mobility of carboxylates and enzymes, which are strongly adsorbed to soil particles, they diffuse only a few millimetres in the surrounding root area (Hinsinger et al., 2009; Nuruzzaman et al., 2006). Consequently, the concentration of oxalate and malate in the rhizosphere of wheat was considerable and positively correlated with the concentration of P in the wheat shoot, indicating that these carboxylates may facilitate the uptake of P by wheat. (Figs. 4 and 5). In the case of citrate, although a positive correlation was observed between citrate levels and shoot P of wheat, the low concentration of citrate in the wheat rhizosphere, which did not differ between sole cropping and intercropping (data not shown), indicates that citrate was not as effective as other carboxylates in facilitating wheat P uptake by legumes. It is noteworthy that in this study the exudation of tricarboxylic acid citrate by legumes was generally low in comparison to that of the dicarboxylic acids malate and oxalate. This finding is in contrast with other studies where citrate was efficiently secreted by legumes to cope with P deficiency (Li et al., 2016; Schwerdtner et al., 2022). However, the relatively low exudation rate of citrate by legumes is the reason why its values were low in the wheat rhizosphere. Succinate was the only carboxylate that exhibited a negative correlation with the P concentration of wheat shoots, and was also the only carboxylate that, with the exception of faba bean, was present in greater quantities in wheat than in legume rhizospheres. It is hypothesised that succinate in the wheat rhizosphere originated mainly from the wheat roots rather than from the legume. Despite the relatively moderate concentration, the release of succinate can be interpreted as one of the responses to the near legume, as evidenced by the significant variation in its release when wheat was intercropped with one or two legume plants. This is analogous to the findings of a previous study on the interaction of maize with lupin, which also observed a notable variation in the release of succinate in maize intercropped with lupin (Schwerdtner et al., 2022). In contrast to malate and oxalate, succinate accumulation in the wheat rhizosphere was significantly higher at LD1 than LD2. Indeed, in this latter case, the profuse exudation of carboxylates, such as oxalate and malate, from the expanded legume roots may inhibit the release of succinate by the wheat roots.

The release of carboxylates generally played a primary role in facilitating wheat P uptake when intercropped with pea and faba bean. However, in the case of lupin, the carboxylate content retrieved in the rhizosphere was too low and not significantly different from that of wheat cropped alone, to explain the detected facilitation. In contrast, the soil harbouring lupin exhibited significantly elevated acid PME activity compared to that of the other treatments, a finding that has been corroborated by prior research (Nuruzzaman et al., 2006; Wu et al., 2021). It is hypothesised that, in intercropping with faba bean, acid PME activity acted in combination with carboxylates to facilitate wheat P uptake. However, in intercropping with lupin, it was hypothesised that acid PME activity was the primary factor. This hypothesis is also supported by two assumptions: i) the highest P uptake of wheat, which was recorded in wheat-lupin intercropping at LD2, was accompanied by the highest acid PME activity recorded in the experiment; ii) in wheat-lupin intercropping, at LD2 acid PME activity was 30 % greater than at LD1 which resulted in a 90 % increase in P uptake by wheat at LD2. As previously stated, the observed increase in acid PME activity cannot be attributed to an enhanced root proliferation, as only pea exhibited a notable increase in total root dry matter at LD2, while the values for lupin and faba bean remained comparable to those observed at LD1. Thus, when lupin was intercropped, despite the modest root proliferation associated with LD2, acid PME activity increased more markedly compared to other legumes. Lupin showed more plasticity in acid PME activity than pea and faba bean (Ma et al., 2021; Wu et al., 2021). This plasticity can be interpreted as a compensatory response to P depletion induced by the neighbouring wheat.

5. Conclusions

Intercropping is an interesting agroecological tool for improving the use of resources and reducing anthropogenic inputs when positive interactions overcome negative interactions between crops. This study hypothesised that a way to modulate the shift from negative to positive interactions and favour facilitation is by acting on the sowing ratio between the two crops composing the intercrop. This first hypothesis was confirmed, since the wheat P uptake and dry matter accumulation were significantly affected by the legume density with significant variations due to legume species. When lupin and faba bean were grown using two plants per pot, their root growth was limited, and wheat dry matter and nutrient uptake were favoured. Pea, on the contrary, was improved by the neighbouring of another plant of the same species, increasing its growth and nutrient uptake and limiting wheat growth. In this last case, the facilitation exerted by the legumes was masked by competition. This finding confirms the second hypothesis that each legume species can exhibit a different facilitative effect due to the differences in root exudation and competitive pressure. When intercropped with two pea plants, wheat benefited from the increase of root exudates from the pea roots which heightened its P concentration in the shoot but, its growth, was contextually limited by competition. In this case, the competition overcame the facilitation, confirming the third hypothesis according to which facilitation exerted by root exudates is evident when not masked by interspecific competition. The differences observed among the legumes, besides the exudation profile of the single species, could also reflect the differences in root morphology that drive root intermingling; however, this subject was not investigated in the present study and is an interesting topic for future research. This study does not comply with the sowing ratio commonly adopted in arable cropping systems, but aims to clarify the mechanisms that regulate the root interactions by stressing them. Nevertheless, our results can be useful for innovative cropping systems such as temporary intercropping. In this last case, the legume can be sown at high density and its termination can be planned, before the competition with wheat occurs, to maximise the facilitation. Further trials in the field will enable the evaluation of disproportionate legume density in combination with different timing and methodologies of legume termination.

CRediT authorship contribution statement

Emilio Lo Presti: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Michele Monti:** Writing – review & editing, Supervision, Resources, Data curation, Conceptualization. **Maurizio Romeo:** Investigation, Formal analysis, Data curation. **Monica Bacchi:** Supervision, Conceptualization. **Carmelo Santonoceto:** Supervision, Conceptualization. **Giovanni Preiti:** Supervision, Conceptualization. **Irene Maria Grazia Custureri:** Investigation, Formal analysis, Data curation. **Giuseppe Badagliacca:** Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ijagro.2024.100024.

References

An, R., Yu, R.P., Xing, Y., Zhang, J.D., Bao, X.G., Lambers, H., Li, L., 2023. Intercropping efficiently utilizes phosphorus resource in soil via different strategies mediated by crop traits and species combination. Plant Soil 1–21.
AOAC International, 2016. Official Methods of Analysis, fifteenth ed. AOAC International,, Arlington, VA, USA.
Bañares-de-Dios, G., Macía, M.J., de Carvalho, G.M., Arellano, G., Cayuela, L., 2022. Soil and climate drive floristic composition in tropical forests: a literature review. Front Ecol. Evol. 10, 866905.
Beemner, J.M., Keeney, D.R., 1966. Determination and isotope-ratio analysis of different forms of nitrogen in soils: 3. Exchangeable ammonium, nitrate, and nitrite by extraction-distillation methods. Soil Sci. Soc. Am. J. 1, 577–582.
Berenstecher, P., Conti, G., Faigón, A., Piñeiro, G., 2023. Tracing service crops' net carbon and nitrogen rhizodeposition into soil organic matter fractions using dual isotopic brush-labeling. Soil Biol. Biochem 184, 109096.
Bowman, R.A., 1989. A sequential extraction procedure with concentrated sulfuric acid and dilute base for soil organic phosphorus. Soil Sci. Soc. Am. J. 53, 362–366.
Callaway, R.M., Pennings, S.C., Richards, C.L., 2003. Phenotypic plasticity and interactions among plants. Spec. Feature Ecol. 84, 1115–1128.
Cawthray, G.R., 2003. An improved reversed-phase liquid chromatographic method for the analysis of low-molecular mass organic acids in plant root exudates. J. Chromatogr. A 1011, 233–240.
Chapagain, T., Riseman, A., 2014. Barley-pea intercropping: effects on land productivity, carbon and nitrogen transformations. Field Crops Res 166, 18–25.
Chen, B.J.W., During, H.J., Vermeulen, P.J., de Kroon, H., Poorter, H., Anten, N.P.R., 2015. Corrections for rooting volume and plant size reveal negative effects of neighbour presence on root allocation in pea. Funct. Ecol. 29, 1383–1391.
Cu, S.T.T., Hutson, J., Schuller, K.A., 2005. Mixed culture of wheat (*Triticum aestivum* L.) with white lupin (*Lupinus albus* L.) improves the growth and phosphorus nutrition of the wheat. Plant Soil 272, 143–151.
Deng, J., Zuo, W., Wang, Z., Fan, Z., Ji, M., Wang, G., Ran, J., Zhao, C., Liu, J., Niklas, K.J., Hammond, S.T., Brown, J.H., 2012. Insights into plant size-density relationships from models and agricultural crops. Proc. Natl. Acad. Sci. USA 109, 8600–8605.
Duchene, O., Vian, J.F., Celette, F., 2017. Intercropping with legume for agroecological cropping systems: complementarity and facilitation processes and the importance of soil microorganisms. A review. Agric. Ecosyst. Environ. 240, 148–161.
El Amrani, B., 2023. Exploring the importance of root architecture plasticity in plant adaptation to environmental constraints. Plant Species Biol. 38, 234–244.
Fustec, J., Lesuffleur, F., Mahieu, S., Cliquet, J.B., 2010. Nitrogen rhizodeposition of legumes. A review. Agron. Sustain. Dev. 30 (1), 57–66 2010 30.
Hauggaard-Nielsen, H., Jørnsgaard, B., Kinane, J., Jensen, E.S., 2008. Grain legume–-cereal intercropping: the practical application of diversity, competition and facilitation in arable and organic cropping systems. Renew. Agric. Food Syst. 23, 3–12.
Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M., Jensen, E.S., 2009. Pea–barley intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. Field Crops Res 113, 64–71.
Hinsinger, P., Bengough, A.G., Vetterlein, D., Young, I.M., 2009. Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321 (1), 117–152 2009 321.
Høgh-Jensen, H., Schjoerring, J.K., 1997. Interactions between white clover and ryegrass under contrasting nitrogen availability: N 2 fixation, N fertilizer recovery, N transfer and water use efficiency. Plant Soil 197, 187–199.
Høgh-Jensen, H., Schjoerring, J.K., 2001. Rhizodeposition of nitrogen by red clover, white clover and ryegrass leys. Soil Biol. Biochem 33, 439–448.
Hupe, A., Naether, F., Haase, T., Bruns, C., Heß, J., Dyckmans, J., Joergensen, R.G., Wichern, F., 2021. Evidence of considerable C and N transfer from peas to cereals via direct root contact but not via mycorrhiza. Sci. Rep. 11, 11424.
Karlova, R., Boer, D., Hayes, S., Testerink, C., 2021. Root plasticity under abiotic stress. Plant Physiol. 187, 1057–1070.
Kuo, S., 1996. Phosphorus. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Agronomy 9. ASASSSA, Madison, WI.
Lazali, M., Drevon, J.J., 2021. Mechanisms and adaptation strategies of tolerance to phosphorus deficiency in legumes. Commun. Soil Sci. Plant Anal. 52, 1469–1483.
Li, C., Dong, Y., Li, H., Shen, J., Zhang, F., 2016. Shift from complementarity to facilitation on P uptake by intercropped wheat neighboring with faba bean when available soil P is depleted. Sci. Rep. 6 (1), 1–8 2016 6.
Li, H., Zhang, F., Rengel, Z., Shen, J., 2013. Rhizosphere properties in monocropping and intercropping systems between faba bean (Vicia faba L.) and maize (Zea mays L.) grown in a calcareous soil. Crop Pasture Sci. 64, 976–984.
List, D., Ruwisch, I., Langhans, P., 1986. Potential applications of flow injection analysis in fruit juice analysis. Fleuss. Obst 53 (10).
Lo Presti, E., Badagliacca, G., Romeo, M., Monti, M., 2021. Does legume root exudation facilitate itself P uptake in intercropped wheat? J. Soil Sci. Plant Nutr. 21, 3269–3283.
Loreau, M., Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412 (6842), 72–76 2001 412.
Lyu, Y., Tang, H., Li, H., Zhang, F., Rengel, Z., Whalley, W.R., Shen, J., 2016. Major crop species show differential balance between root morphological and physiological responses to variable phosphorus supply. Front Plant Sci. 7, 235088.
Ma, X., Liu, Y., Shen, W., Kuzyakov, Y., 2021. Phosphatase activity and acidification in lupine and maize rhizosphere depend on phosphorus availability and root properties: coupling zymography with planar optodes. Appl. Soil Ecol. 167, 104029.
Mcnickle, G.G., Brown, J.S., 2014. An ideal free distribution explains the root production of plants that do not engage in a tragedy of the commons game. J. Ecol. 102, 963–971.
Meier, I.C., Angert, A., Falik, O., Shelef, O., Rachmilevitch, S., 2013. Increased root oxygen uptake in pea plants responding to non-self neighbors. Planta 238, 577–586.
Moyer-Henry, K.A., Burton, J.W., Israel, D.W., Rufty, T.W., 2006. Nitrogen transfer between plants: a 15N natural abundance study with crop and weed species. Plant Soil 282, 7–20.
Nuruzzaman, M., Lambers, H., Bolland, M.D.A., Veneklaas, E.J., 2005. Phosphorus benefits of different legume crops to subsequent wheat grown in different soils of Western Australia. Plant Soil 271, 175–187.
Nuruzzaman, M., Lambers, H., Bolland, M.D.A., Veneklaas, E.J., 2006. Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. Plant Soil 281, 109–120.
Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U. S. Dep. Agric Circular No. 939.
Parsons, R., Stanforth, A., Raven, J.A., Sprent, J.I., 1993. Nodule growth and activity may be regulated by a feedback mechanism involving phloem nitrogen. Plant Cell Environ. 16, 125–136.
Paynel, F., Murray, P.J., Bernard Cliquet, J., 2001. Root exudates: a pathway for short-term N transfer from clover and ryegrass. Plant Soil 229, 235–243.
Pearse, S.J., Veneklaas, E.J., Cawthray, G., Bolland, M.D.A., Lambers, H., 2007. Carboxylate composition of root exudates does not relate consistently to a crop species' ability to use phosphorus from aluminium, iron or calcium phosphate sources. N. Phytol. 173, 181–190.
Postma, J.A., Hecht, V.L., Hikosaka, K., Nord, E.A., Pons, T.L., Poorter, H., 2021. Dividing the pie: a quantitative review on plant density responses. Plant Cell Environ. 44, 1072–1094.
Rasmussen, J., Gylfadóttir, T., Loges, R., Eriksen, J., Helgadóttir, Á., 2012. Spatial and temporal variation in N transfer in grass-white clover mixtures at three Northern European field sites.
Rehling, F., Sandner, T.M., Matthies, D., 2021. Biomass partitioning in response to intraspecific competition depends on nutrients and species characteristics: a study of 43 plant species. J. Ecol. 109, 2219–2233.
Reich, P.B., Tilman, D., Naeem, S., Ellsworth, D.S., Knops, J., Craine, J., Wedin, D., Trost, J., 2004. Species and functional group diversity independently influence biomass accumulation and its response to CO_2 and N. Proc. Natl. Acad. Sci. USA 101, 10101–10106.
Ruzicka, J., Hansen, E.H., 1981. Flow Injection Analysis. John Wiley & Sons,, New York.
Schwerdtner, U., Lacher, U., Spohn, M., 2022. Lupin causes maize to increase organic acid exudation and phosphorus concentration in intercropping. J. Sustain. Agric. Environ. 1, 191–202.
Shao, Z.Q., Zheng, C.C., Postma, J.A., LU, W.L., Gao, Q., Gao, Y.Z., Zhang, J.J., 2021. Nitrogen acquisition, fixation and transfer in maize/alfalfa intercrops are increased through root contact and morphological responses to interspecies competition. J. Integr. Agric. 20, 2240–2254.
Streit, J., Meinen, C., Rauber, R., 2019. Intercropping effects on root distribution of eight novel winter faba bean genotypes mixed with winter wheat. Field Crops Res 235, 1–10.
Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem 1, 301–307.
Tang, X., Zhang, C., Yu, Y., Shen, J., van der Werf, W., Zhang, F., 2021. Intercropping legumes and cereals increases phosphorus use efficiency; a meta-analysis. Plant Soil 460, 89–104.
Wang, R., Funayama-Noguchi, S., Xiong, Z., Staudinger, C., Wasaki, J., 2024. Phosphorus absorption kinetics and exudation strategies of roots developed by three lupin species to tackle P deficiency. Planta 259, 1–14.
Wang, S., Callaway, R.M., 2021. Plasticity in response to plant–plant interactions and water availability. Ecology 102, e03361.
Wei, T., and Simko, V., 2021. R package 'corrplot': Visualization of a Correlation Matrix (Version 0.92). Available from ⟨https://github.com/taiyun/corrplot⟩.
Weiner, J., Freckleton, R.P., 2010. Constant final yield. Annu Rev. Ecol. Evol. Syst. 41, 173–192.

Westoby, M., 1984. The self-thinning rule. Adv. Ecol. Res 14, 167–225.
Wiche, O., Dittrich, C., Pourret, O., Monei, N., Heim, J., Lambers, H., 2023. Relationships between carboxylate-based nutrient-acquisition strategies, phosphorus-nutritional status and rare earth element accumulation in plants. Plant Soil 489, 645–666.
Wu, A., Fang, Y., Liu, S., Wang, H., Xu, B., Zhang, S., Deng, X., Palta, J.A., Siddique, K.H., Chen, Y., 2021. Root morphology and rhizosheath acid phosphatase activity in legume and graminoid species respond differently to low phosphorus supply. Rhizosphere 19, 100391.
Xiao, Y., Li, L., Zhang, F., 2004. Effect of root contact on interspecific competition and N transfer between wheat and faba bean using direct and indirect 15 N techniques. Plant Soil 262, 45–54.
Yang, X.F., Li, L.L., Xu, Y., Kong, C.H., 2018. Kin recognition in rice (*Oryza sativa*) lines. N. Phytol. 220, 567–578.
Yu, R.P., Li, X.X., Xiao, Z.H., Lambers, H., Li, L., 2020. Phosphorus facilitation and covariation of root traits in steppe species. N. Phytol. 226, 1285–1298.
Yuan, Z., Ali, A., Wang, S., Gazol, A., Freckleton, R., Wang, X., Lin, F., Ye, J., Zhou, L., Hao, Z., Loreau, M., 2018. Abiotic and biotic determinants of coarse woody productivity in temperate mixed forests. Sci. Total Environ. 630, 422–431.
Zhang, D., Lyu, Y., Li, H., Tang, X., Hu, R., Rengel, Z., Zhang, F., Whalley, W.R., Davies, W.J., Cahill, J.F., Shen, J., 2020. Neighbouring plants modify maize root foraging for phosphorus: coupling nutrients and neighbours for improved nutrient-use efficiency. N. Phytol. 226, 244–253.
Zhang, F., Li, L., 2003. Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient-use efficiency. Plant Soil 248, 305–312.
Zhu, S.G., Cheng, Z.G., Wang, J., Gong, D.S., Ullah, F., Tao, H.Y., Zhu, H., Duan, H.X., Yang, Y.M., Xiong, Y.C., 2023. Soil phosphorus availability and utilization are mediated by plant facilitation via rhizosphere interactions in an intercropping system. Eur. J. Agron. 142, 126679.
Zhu, Y.H., Weiner, J., Jin, Y., Yu, M.X., Li, F.M., 2022. Biomass allocation responses to root interactions in wheat cultivars support predictions of crop evolutionary ecology theory. Front Plant Sci. 13, 858636.