Carbon and Nutrient Interactions in Cereal-Legume Intercropping Systems: Impacts of Phosphorus Fertilization

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“To the fallen heroes, and to every heart that beats for Bangladesh”

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Declaration of Originality

I hereby declare that, to the best of my knowledge, this thesis is entirely my own work and does not contain any material that has been previously published. All sources of information and any assistance received in the preparation of this thesis have been properly acknowledged. This thesis has not been submitted, in whole or in part, for any degree or for any other purpose.

Md Zillur Rahman

June 2026

Artificial Intelligence Statement

Generative artificial intelligence tools Microsoft Copilot was used in the preparation of this thesis solely for language editing and clarification. All sections in which AI assistance was applied were carefully reviewed and revised by the author. The author takes full responsibility for the content of the submitted thesis and confirms that the work is original and complies with the University of Sydney's guidelines on the use of generative AI.

Md Zillur Rahman

June 2026

Statement of Authorship

This thesis is structured into the following chapters. Chapter 1 provides a general introduction to the topic, including a brief review of the literature and the overall aims and objectives of the study. Chapter 6 presents the main findings of the thesis, along with its limitations and directions for future research. All remaining chapters are research chapters, each containing its own introduction, aims and hypotheses, methodology, results, and discussion. Chapter 2 comprises a comprehensive meta-analysis based on data collected from published literature. Chapters 2, 3, 4, and 5 are written as standalone manuscripts intended for publication, with Chapters 2 and 3 already published as journal articles. I am the first author of the manuscripts presented in all chapters.

Chapter 2 of this thesis is published as "**Rahman, M. Z.,** Akter, S., Keitel, C., & Dijkstra, F. A. (2025). Benefits of phosphorus fertilization in intercropping depend on cropping system: A meta-analysis. Plant and Soil. https://doi.org/10.1007/s11104-025-07695-x."

FAD conceived the research idea and guided to the evaluation of the results. I collected the data, conducted the meta-analysis and meta-regression, performed the interpretation, and drafted the manuscript. All authors contributed to reviewing and editing the manuscript.

Chapter 3 of this thesis is published as "**Rahman, M. Z.,** Akter, S., Bicharanloo, B., Keitel, C., & Dijkstra, F. A. (2025). Yield, nitrogen fixation and carbon allocation to root biomass and respiration in response to phosphorus fertilization in a wheat-chickpea intercropping system. Plant and Soil. https://doi.org/10.1007/s11104-025-08051-9."

I co-designed the study with the co-authors, conducted the experiments, analysed the data, and drafted the manuscript. All authors contributed to reviewing and editing the manuscript.

Chapter 4 of this thesis will be submitted for publication as **Rahman, M. Z.,** Akter, S., Keitel, C., & Dijkstra, F. A. Ammonium-nitrate uptake in cereal-legume intercropping varies with phosphorus availability: A ^{15}N labelling approach.

I co-designed the study with the co-authors, conducted the experiments, analysed the data, and drafted the manuscript. All authors contributed to revising and editing the manuscript.

Chapter 5 of this thesis will be submitted for publication as **Rahman, M. Z.,** Akter, S., Bagheri M., Keitel, C., & Dijkstra, F. A. Linking photosynthetic carbon uptake, belowground allocation, and phosphorus acquisition across species and cropping systems.

I co-designed the study with the co-authors, conducted the experiments, analysed the data, and drafted the manuscript. All authors contributed to revising and editing the manuscript.

I am the corresponding author of all published manuscripts.

Md Zillur Rahman

June 2026

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Professor Feike A. Dijkstra

June 2026

Abstract

Phosphorus (P) deficiency is a pervasive constraint in many agricultural soils, and cereal-legume intercropping is increasingly promoted as a strategy to enhance nutrient use efficiency and yield under low-input conditions. The overall objective of this thesis was to determine how P fertilization regulates productivity, nutrient acquisition, and carbon-nitrogen interactions in cereal-legume intercropping compared with monocropping by evaluating effects on yield, land equivalent ratio (LER), nutrient uptake, biological nitrogen fixation (BNF), nitrogen form preference (NH_4^+ vs NO_3^-), belowground carbon (C) dynamics and species interactions using meta-analysis and isotope-based experiments in a P-limited soil.

A global meta-analysis of 907 observations across 39 studies revealed that P fertilization consistently increased yield and P and N uptake in both monocropping and intercropping systems. Although yield responses were generally greater in monoculture, P fertilization enhanced Land Equivalent Ratio (LER), indicating more efficient land use under intercropping. Non-cereal mixtures showed the largest LER gains, while cereal-based intercrops benefited from P inputs primarily under P-limited conditions. Soil characteristics, climate, and fertilizer duration emerged as major determinants of P responsiveness under intercropping. Although P is known to influence yield and LER in intercropping system, its role in regulating carbon-demanding processes particularly BNF and root respiration along with their interaction with yield formation in component crops remains poorly understood. I therefore conducted a glasshouse experiment and demonstrated that P availability strongly shaped belowground C allocation and biological nitrogen fixation (BNF) in a wheat-chickpea mixture. Chickpea showed substantial increases in biomass, P uptake, and BNF with P fertilization, coupled with reduced C allocation to roots, signalling a strategic reallocation of resources toward symbiotic N fixation that are intensified under intercropping system. Wheat, in contrast, maintained C investment in roots regardless of cropping system, highlighting divergent nutrient acquisition strategies. Despite increasing understanding of P regulation of carbon-demanding processes such as BNF and root respiration along with yield, how P availability influences species-specific NH_4^+ versus NO_3^- uptake and nitrogen competition along with root architecture in cereal-legume intercropping remains unclear. I therefore conducted a ^{15}N pulse-labelling pot experiment showing that wheat was consistently more competitive for nitrate than chickpea, especially with P fertilization, while both species showed similar uptake of $^{15}NH_4^+$. Fine-root traits such as specific root length and root tip density were key predictors of NH_4^+ acquisition under P limitation, underscoring species-specific foraging strategies. In this pot experiment, I further examined how P availability influenced belowground C allocation towards rhizodeposition, root growth, and P uptake. By using a $^{13}CO_2$ pulse-chase method, I demonstrated that chickpea maintained high P uptake with minimal changes in short-term rhizodeposition in response to P fertilization, whereas wheat relied heavily on P fertilization to boost root C investment, rhizodeposition, and P acquisition. Intercropping partly alleviated wheat's P stress by modestly enhancing root C allocation and P uptake.

Together, these studies provide mechanistic insights into how P availability, root traits, and interspecific interactions regulate nutrient uptake and belowground C economy in cereal-legume intercropping, offering pathways for improving P-use efficiency and designing resilient, low-input production systems.

Keywords: Cereal-legume, LER, Belowground C allocation, Respiration, BNF, Rhizodeposition, Nitrate-ammonium uptake, P uptake, Yield, Isotopic tracing

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Publications

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2. **Rahman, M. Z.,** Akter, S., Bicharanloo, B., Keitel, C., & Dijkstra, F. A. (2025). Yield, nitrogen fixation and carbon allocation to root biomass and respiration in response to phosphorus fertilization in a wheat-chickpea intercropping system. Plant and Soil. https://doi.org/10.1007/s11104-025-08051-9.

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2. **Rahman, M. Z**., Akter, S., Bicharanloo, B., Keitel, C., & Dijkstra, F. A. (2024). Enhancing Phosphorus Use Efficiency and Nitrogen Fixation in Wheat-Chickpea Intercropping Systems Under P-Deficient Conditions. Joint SSA & NZSSS Conference, 2-5 December 2024. Rotorua, New Zealand (oral presentation).

Presentation (Domestic)

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2. **Rahman, M. Z**., Akter, S., Keitel, C., & Dijkstra, F. A. (2023). Effects of Phosphorus Fertilization on Plant Growth and Yield in Intercropping and Sole Cropping Systems of Wheat and Chickpea in a Low Phosphorus Soil. Science HDR Conference, the University of Sydney, Camperdown, New South Wales.

3. **Rahman, M. Z**., Akter, S., Keitel, C., & Dijkstra, F. A. (2025). Carbon and Nutrient Interactions in Cereal-Legume Intercropping Systems: Impacts of Phosphorus Fertilization. Higher Degree Research Showcase, School of Life and Environmental Sciences, University of Sydney, Australia

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i) Prime Minister Fellowship, Bangladesh
ii) Francis Henry Loxton Stipend
iii) Francis Henry Loxton Supplementary Scholarship
iv) Sydney International Student Award, University of Sydney
v) Faculty of Science Thesis Completion Scholarship, University of Sydney
vi) Best Presenter (Winner) Award in HDR Showcase, University of Sydney, Australia

Abbreviations

The following is an alphabetised list of singular abbreviations used throughout this thesis. Abbreviations are also defined on their first use in each chapter.

AVP	Available Phosphorus
BNF	Biological Nitrogen Fixation
^{13}C	Carbon-13 Isotope
$^{13}CO_2$	Stable Isotope-labeled Carbon Dioxide
CC	Crop Combination
CI	Confidence Interval
CS	Cropping System
DAS	Days After Sowing
LER	Land Equivalent Ratio
LnRR	Log Response Ratio
NH_4^+	Ammonium
NO_3^-	Nitrate
^{15}N	Nitrogen-15 Isotope
Ndfa	Nitrogen Derived from the Atmosphere
SRL	Specific Root Length
WHC	Water Holding Capacity

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Chapter 1: General Introduction

1.1. Background Information

Crop diversification offers a pathway to reduce agriculture's environmental impact without sacrificing productivity. Intercropping, where two or more species are deliberately grown together on the same land (Vandermeer 1989; Willey 1979), stands out as one of the most promising approaches (Li et al. 2023). Intercropping is a diversification strategy that can reduce input requirements while delivering yields that exceed those expected from the component crops grown as monoculture (Li et al. 2020b; Tamburini et al. 2020). The overyielding often observed in intercrops relative to their monocrops are typically attributed to complementary resource use between species (Lithourgidis et al. 2011; Vandermeer 2009) (14, 15) and may also stem from greater resilience to pests, diseases, and weeds (Gu et al. 2021; Liebman and Dyck 1993; Trenbath 1993). Meta-analyses using extensive datasets show that intercropping can reduce land requirements by approximately 18-23% compared with producing the same crops in monoculture (Li et al. 2020c; Martin-Guay et al. 2018; Xu et al. 2020; Yu et al. 2015). It enables reduced input use such as water and nutrients while achieving higher yields than expected based on the monoculture yields of the component species (Kaci et al. 2022; Stoltz et al. 2013; Wahbi et al., 2016; Wang et al. 2024). Combining species with contrasting resource-acquisition traits for co-growth under intercropping system can enhance resource-use complementarity, thereby promoting intercrop overyielding relative to monocultures (MacLaren et al., 2023). Overyielding in intercropping can be expressed through multiple metrics, but the land equivalent ratio (LER) remains the dominant indicator for quantifying the advantage of intercrops over sole crops (Mead and Willey 1980). Equivalent to the relative yield total (De Wit 1960), LER measures the amount of land a sole crop system would need to produce the same yields produced by the same species when grown together in an intercrop. It is an intensive land use strategy has gained momentum to sustain production while enhancing ecological stability and resource efficiency.

Intercropping, by enhancing resource-use efficiency and diversifying crop species, offers a compelling pathway for the sustainable intensification of agriculture. For instance, facilitation in intercropping arises when component species enhance each other's access to water, phosphorus (P), micronutrients like iron (Fe), zinc (Zn), copper (Cu), or nitrogen (N) supplied through biological N fixation, either directly or via rhizosphere-mediated processes (Li et al. 2014; Shen et al. 2013; White et al. 2013a; White et al. 2013b; Zhang et al. 2010). Niche complementarity further contributes to intercrop performance, as species differing in canopy structure or rooting depth can exploit light and soil resources more completely (Hauggaard-

Nielsen et al. 2001; Postma and Lynch 2012; Zhang et al. 2010). Complementarity also occurs when species acquire nutrients in distinct chemical forms (Brooker et al. 2015). Together, these mechanisms generate multiple system-level benefits including improved crop protection, photosynthetic C assimilation, and enhanced uptake of N, P, micronutrients, and water while distributing resource acquisition across time. Collectively, they translate into greater efficiencies in the use of nutrients (P, N, other mineral nutrients), water, light, and assimilates (Brooker et al. 2015). For example, cereal-legume intercropping is well known for improving N availability for both legumes and their companion crops by atmospheric N fixation. Like N, cereal-legume intercropping also serves as a sustainable strategy to enhance P availability to the crops (Li et al. 2007; Tao et al. 2025; Wang et al. 2019).

As the second most key essential nutrient after N, P is both essential for plant growth, but P fertilizers are inherently in limited supply because they mostly originate from non-renewable geological sources (Vance et al. 2000). Most P fertilizers are derived from phosphate rock. However, mineral phosphate reserves are steadily declining, and global stocks could be depleted within the next 50-100 years (Cordell et al. 2009), creating a significant risk for future agricultural productivity. There are no alternatives for P in crop nutrition and global P rock reserves are finite, conditions that may further escalate P fertilizer prices and demand (Dawson and Hilton 2011; Van Vuuren et al. 2010). So, it is vital for improving P fertilizer use efficiency to sustain agricultural productivity and ensuring global food security. However, P-use efficiency can differ substantially across different cropping systems (Yu et al. 2021). These challenges highlight the urgent need for cropping systems that use P more efficiently and sustain productivity under nutrient-limited conditions. Greater P-use efficiency and higher P uptake have been consistently reported in intercropping systems, particularly in legume–cereal combinations (Tang et al. 2021; Tian et al. 2020). The P fertilizer requirement to achieve equivalent yields was 21% lower under intercropping than under sole-cropping systems (Tang et al. 2021). Although P fertilization is known to influence yield, LER, and nutrient uptake in intercropping systems, a comprehensive understanding of how these responses compare with monocropping remains limited. While numerous individual studies have examined P-driven productivity gains across various cropping arrangements, their findings are often inconsistent. This variability arises from differences in climatic zones, species combinations, soil characteristics, management practices, and experimental designs. Consequently, the broader patterns governing crop responses to P fertilization under intercropping remain unclear and insufficiently synthesised.

Phosphorus plays a key role in shaping plant interactions within cereal-legume intercropping systems. The effects of P fertilization in mixed cropping systems can differ markedly from those observed in sole crops because of interspecific interactions, complementary rooting architectures, and rhizosphere-mediated processes. These interactions are tightly regulated by nutrient availability particularly P, which is essential for root growth and underpins the nodule formation and biological N fixation (BNF) in legumes (Mitran et al. 2018; Sulieman et al. 2013; Sulieman and Schulze 2010). Since plants can exploit complementary resource use in intercropping system, legumes may derive greater benefits from P fertilization in intercropping than in monoculture, particularly through enhanced BNF in a P limited soil. BNF is a carbon (C) -intensive process (Minchin and Witty 2005), and the substantial C allocated for BNF can constrain plant growth and yield when other essential nutrients, particularly P, are limiting in the soil. Although the importance of P availability for yield and yield components in intercropping systems is well recognised, its specific contribution to BNF within cereal-legume mixtures remains insufficiently understood. Likewise, the extent to which P shapes yield formation in each component crop-and how it interacts with other C-intensive processes like BNF has received limited attention.

Belowground C allocation reflects the plant's need to balance the C costs of nutrient and water acquisition, as all nutrient-uptake pathways incur a cost of photosynthetically fixed C. For instance, grassland species allocate, on average, roughly one-third of their gross primary productivity to belowground processes, including root biomass production, rhizosphere respiration, and rhizodeposition (Pausch and Kuzyakov 2018a). Plants adjust root biomass and architecture to better exploit nutrient-poor or spatially restricted soil zones when nutrient availability is limited (Giehl and von Wirén 2014). In parallel, plants also expend a large fraction of their C budget on root respiration to support growth and maintenance functions (Pregitzer et al. 2007). Under low P availability, this demand intensifies, as plants frequently allocate more C to root respiration to sustain the metabolic activity required for nutrient foraging (Nielsen et al. 2001; Strock et al. 2018). Consequently, P fertilization can modulate root respiration in cereal-legume intercropping by altering the energy demand for nutrient uptake as well as for BNF. In legumes, for example, BNF substantially elevates root respiration due to the high energetic costs of N fixation in root nodules (Mahon 1977; Ryle et al. 1979). Despite these known physiological demands of P, the extent to which P fertilization shapes BNF, root respiration dynamics, and root biomass formation in cereal-legume mixtures remain insufficiently understood, leaving a critical gap in our understanding of belowground C allocation towards yield formation in intercropping systems.

In intercropping systems, physiological and morphological differences among component species generate complementary patterns of resource use, whereby crops access different forms or layers of a resource, or utilise the same resource at distinct times or depths (Mthembu et al. 2019), ultimately shaping their uptake dynamics and productivity outcomes. These differences are amplified by the inherent variability among species in their capacity to acquire P, which is shaped by contrasting root traits and the degree of mycorrhizal association (Singh Gahoonia and Nielsen, 2004). Rhizosphere processes for belowground C allocation such as root exudation further regulate P mobilisation and uptake by the crops (Dakora and Phillips 2002), with exuded organic compounds enhancing P availability in soils with limited soluble P. Recent work in wheat–chickpea mixtures has shown that mycorrhizal colonisation interacts strongly with P fertilization, affecting not only P uptake and crop yield but also the amount of belowground C allocated to root production and symbiotic partners (Song et al. 2021; Xu and Liu 2024). Together, these mechanisms highlight that P fertilization has cascading effects on nutrient acquisition pathways, particularly the tight coupling between P availability and N uptake in legumes, ultimately shaping growth and productivity. Understanding these interconnected processes is therefore essential for evaluating how intercropping systems coordinate P uptake, N uptake, and the underlying C costs that support both in P-limited soils.

Enhancing N acquisition in cereal-legume intercropping is central to improving nutrient-use efficiency, particularly in P-limited soils where both P and N strongly constrain crop performance. Cereal crops typically face reduced N competition because legumes rely on biological N_2 fixation rather than soil-derived N (Ramirez-Garcia et al. 2014). However, the balance between soil N uptake and BNF in legumes is tightly regulated by P availability, which drives nodulation, nodule function, and shoot biomass production (Almeida et al. 2000; Schulze et al. 2006). Application of P in a P-deficient soil enhances nodulation and BNF rates across legume species (Míguez-Montero et al. 2020; Xia et al. 2013), thereby potentially reducing direct competition with cereals for available soil N. At the same time, cereals with their fibrous, highly exploratory root systems often respond more strongly to P fertilization than tap rooted legumes (Lai and Lawton 1962; Zhao et al. 2025), which may increase their capacity to capture available N under improved P supply.

Competition becomes more complex when considering that ammonium (NH_4^+) and nitrate (NO_3^-) are the two dominant inorganic N forms available to plants (Hobbie and Högberg 2012; Marschner 2008) and plant species frequently exhibit distinct preferences for one form over the other. Such N-form preference can manifest as superior growth, biomass production, or nutrient accumulation when the preferred form is supplied (Britto and Kronzucker 2013). These species-specific differences in N uptake strategies are very common in plants (Qian et al. 2021) which is also relevant in intercropping systems, where cereals and legumes differ fundamentally in root physiology, rhizosphere processes, and the energetic costs associated with N acquisition. Availability of P plays a central role in regulating these processes by influencing root development, exudation patterns, and the efficiency of BNF. As a result, P supply is expected to alter each species' relative reliance on NH_4^+ versus NO_3^-, thereby reshaping competitive dynamics for soil N in intercropping systems.

However, quantifying species-specific uptake of NH_4^+ and NO_3^- in mixed cropping systems remains challenging using conventional approaches, as total N measurements cannot disentangle the relative contributions of different N forms or distinguish between plant species. This limitation constrains our ability to mechanistically understand how P availability mediates N acquisition strategies and interspecific competition. Stable isotope techniques using ^{15}N have been widely applied to trace plant N sources and infer uptake pathways (Gong et al. 2019; Hobbie and Högberg 2012; Kemp et al. 2018). Building on this framework, the use of dual ^{15}N labelling (NH_4^+ and NO_3^-) under controlled P regimes provides a robust approach to directly quantify species-specific N-form uptake. This enables a more precise assessment of N-form preferences and competitive interactions between cereals and legumes in intercropping systems.

While the ^{15}N experiment can help clarify N uptake pathways, understanding nutrient acquisition requires linking these processes with C allocation patterns under intercropping systems. Phosphorus acquisition in cereal-legume systems is strongly shaped by belowground C dynamics, particularly the allocation of photosynthetically derived assimilates to roots and the rhizosphere. A substantial proportion of recently fixed C is rapidly transported belowground and released as rhizodeposits, supporting microbial activity and driving nutrient cycling activity (Bahn et al. 2009; Dilkes et al. 2004; Mencuccini and Hölttä 2010). Living roots typically release 5-20% of their assimilated C into the soil via rhizodeposition (Jones et al. 2009; Pausch and Kuzyakov 2018b), which fuels phosphate-solubilizing microbes capable of mobilizing inorganic and organic P through secretion of organic acids and phosphatases (Avis et al. 2008; Sasse et al. 2018; Tian et al. 2019). This microbial stimulation contributes to the mineralization of soil organic matter and enhances nutrient release (Crow and Wieder 2005; Hasselquist et al. 2016). Intercropping can accentuate these processes: root residues contribute greater C inputs compared with monocultures (Li et al. 2011), and legume-cereal combinations often enhance rhizosphere-mediated P mobilization through biochemical and morphological modifications (Li et al. 2020a; Li et al. 2014; Wang et al. 2020; Zhang et al. 2019).

The supply of C to these belowground processes is ultimately constrained by photosynthetic capacity, which is strongly linked to leaf N, up to 70% of which is invested in the photosynthetic machinery (Field and Mooney 1986). Higher leaf N supports greater photosynthesis (Evans 1989) and greater C allocation belowground. Legumes, due to their symbiosis with N-fixing bacteria, generally exhibit higher leaf N and photosynthetic capacity than cereals; for example, legume crops contained 40% more leaf N than the cereal crops (Adams et al. 2018). Because cereals are generally more competitive than legumes in acquiring soil N (Chamberlin et al. 1986), legumes in intercrops rely more on atmospheric N fixation to meet their N needs (Rodriguez et al. 2020; Schipanski and Drinkwater 2012). These differences shape their respective belowground C-investment and nutrient-acquisition strategies. Plant strategies to acquire P differ markedly across species. Cereals commonly adopt a morphological strategy, producing highly branched root systems with long root hairs to explore larger soil volumes (Costa et al. 2021; Li et al. 2006; Siegwart et al. 2023; Zhou et al. 2024). Legumes, by contrast, employ a physiological strategy dominated by exudation of protons, phosphatases, and carboxylates that mobilize bound P forms (Hinsinger 2001; Li et al. 2004; Mahajan and Gupta 2009; Zhang et al. 2016). These plant strategies-including root morphological adjustments, secretion of P-mobilizing compounds, and mycorrhizal associations are typically upregulated under P deficiency but downregulated or inhibited when soil P availability is high (Raven et al. 2018), suggesting that P supply modulates interspecific interactions in intercropping systems. These mechanisms have prompted increasing scientific attention because they can reshape P acquisition, BNF efficiency, root respiration, rhizodeposition, and ultimately the nutrient uptake and yield performance of cereal-legume mixtures. To better understand these interacting processes, we investigated how P fertilization influences C allocation and N-P dynamics in a cereal-legume intercropping system using controlled pot experiments and $^{13}CO_2$ and ^{15}N pulse-labelling methods.

1.2. Aims and Objectives

Despite growing interest in cereal-legume intercropping, the linkages between P availability, belowground C allocation, and nutrient acquisition and their consequences for soil biogeochemical processes remain insufficiently understood. These linkages are central to:

- Improving nutrient use efficiency, thereby reducing fertilizer inputs and associated environmental impacts;
- Optimising intercropping system design, particularly species combinations that maximise complementarity rather than competition;
- Guiding genotype selection, including traits related to root architecture, BNF efficiency, and C allocation strategies;
- Enhancing soil fertility and long-term system sustainability, through effects on rhizosphere processes and soil biogeochemical cycling.

To address this gap, I first conducted a meta-analysis to quantify the influence of P fertilization on yield performance, land equivalent ratio (LER), and P and N uptake across diverse intercropping studies. Building on these insights, I then carried out a series of controlled pot experiments to investigate how P availability shapes belowground C investment pathways, including allocation to root and shoot biomass (yield also), root respiration, rhizodeposition, and BNF, alongside associated P and N acquisition in cereal-legume mixtures. Collectively, these approaches provide an integrated assessment of how P fertilization modulates carbon-nutrient interactions and functional complementarity within intercropping systems.

My thesis therefore aims to:

1) quantify how P fertilization influences yield, nutrient uptake (P and N) and LER for both yield and nutrient acquisition in intercropping and monocropping systems, and to determine how these responses are shaped by management practices, soil properties, and climatic conditions (Chapter 2).
2) elucidate how P fertilization affects yield, BNF, and C allocation to root biomass and respiration in legume-cereal intercropping versus monocropping systems in a P-deficient soil (Chapter 3).
3) investigate the preference of NH_4^+ versus NO_3^- uptake in cereal-legume intercropping under low and high P availability using a ^{15}N labelling approach (Chapter 4).

4) investigate how P fertilization and interspecific interactions in cereal-legume intercropping influence above and belowground C allocation and nutrient acquisition (Chapter 5).

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Chapter 2: Benefits of phosphorus fertilization in intercropping depend on cropping system: A meta-analysis

Plant and Soil

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Abstract

Aims: Many agricultural soils suffer from phosphorus (P) deficiency. Intercropping could improve plant P uptake efficiency and yield compared to monocropping systems. How P fertilization affects yield and P uptake in intercropping remains unclear. Methods: In this meta-analysis (including 907 observations from 39 studies) we evaluated how P fertilization influences yield, P and nitrogen (N) uptake in both intercropping and monocropping systems, and their Land Equivalent Ratios (LER) for different crops, soil types, management practices, and climatic conditions. Results: Across all observations, P fertilization increased yields in both cropping systems. However, monocropping exhibited a greater increase in yield. Despite this, the LER increased under P fertilization, indicating that intercropping use land resources more efficiently, likely because certain crops respond more strongly to P fertilization compared to their companion crops. Most pronounced P fertilizer-induced increases in LER were observed in intercropping systems that had no cereals, while cereal yields in intercropping systems increased as much or less compared to monocropping. Positive relationships were observed between increased P and N uptake and yield with P fertilization in both systems. Notably, intercropping demonstrated enhanced efficiency in converting P uptake into yield gains compared to monocropping. Conclusions: P fertilization improves yield, LER, and P uptake in non-cereal intercropping systems while cereal-based intercropping may be more favourable under low P availability conditions. Our findings further emphasize the role of fertilizer duration, soil factors (clay content and pH) and climate in increasing yields and LER with P fertilization, stressing the need for region-specific P management strategies.

Keywords: Competition; Facilitation; Intercropping; Köppen climate zones; Land Equivalent Ratio; Monocropping; Phosphorus uptake

2.1. Introduction

Phosphorus (P) is an essential macronutrient necessary for plant growth, involved in energy transfer, photosynthesis, and nutrient movement within the plant (Schulze et al. 2006; Zhang et al. 2014). Phosphorus fertilization is often required in agricultural systems, but mineral phosphate resources are becoming depleted, and current global reserves may be exhausted in 50–100 years (Cordell et al. 2009) posing a substantial threat to future agricultural productivity. Optimizing P fertilization is thus imperative to ensure sustainable crop yields. However, the efficiency of P use can vary widely between different cropping systems (Yu et al. 2021).

Intercropping systems have been shown a superior nutrient uptake and use efficiency due to complementary interactions between different plant species resulting in greater yields (Li et al. 2014). However, it is unclear how P fertilization effects on yield and nutrient uptake vary between intercropping and monocropping systems among different cropping systems, management practices, soil and climate factors.

Intercropping is an agroecological approach where two or more crop species or genotypes are grown together on the same piece of land for at least part of their life cycle (Vandermeer 1989). It offers potential benefits such as increased overall productivity, improved pest and disease control, and enhanced biodiversity conservation (Lithourgidis et al. 2011; Malézieux et al. 2009). By growing crops together that differ in traits, intercropping can result in more efficient use of radiation, water, space, and nutrients, as well as an extended duration of production cycles (Raza et al. 2022). According to a meta-analysis, intercropping presents a significantly lower risk of damage compared to monocropping, as the likelihood of both component species being adversely affected by biotic and abiotic stress is reduced (Raseduzzaman and Jensen 2017). Given the global challenge of sustainable food production, intercropping emerges as a promising strategy to maximize yield per unit area (Mead and Willey 1980). The Land Equivalent Ratio (LER), a metric used to compare the productivity of intercropping systems to monocropping systems, often reveals the advantages of intercropping in terms of land use efficiency.

Intercropping systems are able to produce more than monocultures due to facilitation, resource sharing, and niche complementarity. For instance, facilitation can occur in cereal-legume intercropping systems where the legume's biologically fixed nitrogen (N) reduces competition with the cereal, allowing cereals to acquire more soil N (Hauggaard-Nielsen and Jensen 2005a; Raseduzzaman and Jensen 2017). Resource sharing under intercropping systems can occur via common mycorrhizal fungal networks (Van Der Heijden and Horton 2009; Walder et al. 2012) or the recycling of nutrients during the process of leaf senescence and root turnover (Li et al. 2014; Zhang et al. 2010). Species with contrasting short and tall shoot architecture, or shallow and deep root structures, exhibit niche complementarity, enabling them to more fully exploit the light and soil resources (Hauggaard-Nielsen et al. 2001; Postma and Lynch 2012; Zhang et al. 2010).

Phosphorus fertilizers play an important role in intercropping systems (An et al., 2023; Hinsinger et al., 2011; Tang et al., 2021), but their effect on yield may depend on many factors, including when, how and how much of the P fertilizer is applied. Crops vary in their P requirements for optimal growth (Li et al. 2011), and when intercropped, a specific amount of P applied could benefit one crop more than the other. The type of P fertilizer, whether it is in inorganic or organic form, will determine when P is released and the efficiency by which P can be taken up by the plant. While most inorganic P fertilizers can be promptly taken up by crops, they may also be removed from plant uptake through fixation in the soil or loss through runoff (Hansen et al. 2002). On the other hand, organic fertilizers, such as compost or manure, release P at a slower rate that are more in synchrony with plant demand, thereby improving the efficiency of P uptake and other nutrients (Shaji et al. 2021). Because crops in intercropping systems do not always have the same phenology, the type and timing of P fertilization could have different effects on P uptake and yield for crops grown in intercropping systems.

The impact of P fertilization on crop production and nutrient uptake is greatly controlled by soil and climate conditions, which can dramatically modify the effectiveness of P utilisation in intercropping systems. The availability of P to plants is determined by soil parameters, including pH, texture, organic matter content, and P sorption capacity (Weil and Brady, 2022), and crops rarely recover more than 30% of the P fertilizer applied (Holford 1997; Syers et al. 2008; Yu et al. 2021). However, because crops vary in their capacity to access P, because of differences in root traits and associations with mycorrhiza (Singh Gahoonia and Nielsen 2004), their responses to P fertilization in intercropping systems may depend on soil characteristics. Likewise, climate factors, including temperature, precipitation, and aridity, not only affect plant growth, but also have the capacity to influence the availability and P dynamics in soil. Precipitation can directly cause soil P leaching and runoff (Sims et al. 1998), whereas high precipitation and temperature accelerate the processes of immobilization, mineralization, and weathering, thereby altering the forms and availability of soil P (Dixon et al. 2016; Siebers et al. 2017; Vitousek et al. 2010). Therefore, it is crucial to comprehend the interplay between soil properties and climate conditions to optimise phosphorus fertilization methods for maximising crop productivity and nutrient uptake in intercropping systems.

By synthesizing data from a wide range of studies conducted worldwide, we aim to elucidate the impacts of P fertilization on yield and nutrient uptake (P and N) in intercropping and monocropping systems, and on the LER for yield and nutrient uptake. We further assessed how these parameters depended on management, soil and climate factors. We hypothesized that yield and nutrient uptake would increase more in intercropping than in monocropping systems in response to P fertilization, so that the LER would increase with P fertilization. We further hypothesized that these responses would be strongest when P is applied in the long-term (> 2 years) in organic form and at high rates (> 50 kg P ha^{-1} yr^{-1}), in soils and climates conducive to high plant P uptake (i.e., soils with neutral pH and low clay content, and temperate and tropical climate conditions), and in cereal-legume intercropping systems.

2.2. Materials and Methods

The studies were acquired using the Google Scholar and Scopus databases. We conducted a search using the terms "intercrop*" AND "phosphorus fertili*" and "intercrop" AND "mixture" AND "phosphorus fertil" to specifically look for publications published between 1987 and 2024. A total of 437 publications were identified in Scopus using these keywords, whereas 287 publications were retrieved in Google Scholar. We screened all publications and only included studies on intercropping systems of two crops and where both crops were also grown as monocultures. Furthermore, studies were required to have at least two different rates of P fertilization. For studies lacking a control treatment without P fertilization, we used the P treatment with the lowest P application as the control. We only included publications where the yield, P uptake, or N uptake were provided for crops grown in monoculture and intercropping, or where their respective LER was reported (see below). In a few studies where yield data were missing but plant biomass data were available, biomass data were used instead. A total of 39 studies were selected based on these criteria, including six publications acquired from the China National Knowledge Infrastructure (www.cnki.net), and that were translated from Chinese using Google Translate and DeepL Pro (see Supplementary Information for a full list of publications). Out of the 39 studies, six were pot studies, while the remaining were field studies. The selected studies encompassed research conducted across 13 countries spanning five continents. Because many studies included different treatments (e.g., different crop combinations, fertilizer type and amount, planting density), different treatments were included as separate observations so that we ended up with a total of 907 observations (see Supplementary Information for raw data and references used).

The LER is calculated by adding up the relative yields of two species in intercropping compared to the yields of their respective monocrops (Vandermeer 1989):

$$LER = (Y_{a,i}/Y_{a,s} + Y_{b,i}/Y_{b,s}) \quad (1)$$

where, Y_a and Y_b are the yields of the two crops a and b in intercropping (i) and mono cropping (s). The LER measures the amount of land needed to produce the same yield of a monocrop as a unit area of an intercrop, under the same management conditions (Neamatollahi et al. 2013). An LER greater than one signifies that a bigger land area is required to achieve the same output quantities of species a and b when using monocrops compared to using an intercrop. Similarly, LER values can be calculated for plant P and N uptake. We calculated the LER for yield, plant P uptake and N uptake for studies where these values were not directly reported. We then used six response variables in our meta-analysis. These include yield, P uptake and N uptake in monocultures and mixtures (intercropping), and their respective LERs (LER-Yield, LER-P Uptake and LER-N Uptake).

We collected data on various management parameters including experimental duration, P fertilizer type and amount. The P fertilizer was commonly applied once during the growing season in the majority of studies; however, in a few studies where P fertilizer was applied more than once during the growing season, the cumulative amount was calculated as a single dose. Due to the limited number of observations for studies with more than one application, we were unable to assess P fertilizer frequency effects. The P fertilizer was applied in inorganic form (superphosphate, triple superphosphate, rock phosphate, monocalcium phosphate, monopotassium phosphate, diammonium phosphate, dipotassium phosphate, inositol hexaphosphate, ferric phosphate, aluminum phosphate, or as ash,) organic form (manure or compost) or a mixture of inorganic and organic P (manure+triple superphosphate, manure+ash). We collected soil attributes such as clay content and pH. When soil texture was provided instead of clay content, we estimated the clay content using the US Department of Agriculture soil texture triangle. We further recorded initial soil P availability when reported where different methods were used, including Olsen-P, Bray-1, Bray-2, resin extraction, and double lactate-extractable P. Using the geographical coordinates of the field trial sites, we applied the Köppen climate classification system to determine the Köppen climate zones using the Köppen-Geiger climate map (Kottek et al. 2006).

To assess the management, soil, and climate parameters, we combined observations into specific categories. Experimental duration was grouped into two categories: Short (observations made within 2 years since the experiment started), and Long (observations made beyond 2 years). The P fertilizer application rates were divided into two categories: High P (exceeding 50 kg P ha^{-1} yr^{-1}) and Low P (equal to or less than 50 kg P ha^{-1} yr^{-1}) whereas P fertilizer types were separated into Inorganic P, Organic P, or Mixed P. For soil parameters, observations were classified according to clay content, with the category Low Clay defined as less than or equal to 30% and the category High Clay defined as over 30%. The soil pH categories were grouped into Acidic (pH < 5.5), Neutral (pH > 5.5 and ≤ 7.5), and Basic (pH > 7.5). Finally, for the Köppen climate zones we used the following four categories: Arid, Temperate, Tropical, and Cold (continental).

Crops were grouped into the following crop type categories: Cereal (maize *Zea mays*, wheat *Triticum auestivum*, rice *Oryza sativa*, barley *Hordeum vulgare*, sorghum *Sorghum bicolor*), Legume (chickpea *Cicer arietinum*, soybean *Glycine max*, faba bean *Vicia faba*, gliricidia *Gliricidia sepium*, pigeon pea *Cajanus cajan*, peanut *Arachis hypogaea*, common bean *Phaseolus vulgaris*, lupine *Lupinus perennis*, cowpea *Vigna unguiculata*, mungbean *Vigna radiata*, and alfalfa *Medicago sativa*), and Other (clover *Trifolium sp.,* guinea grass *Megathyrsus maximus*, Java grass (*Cyperus sp.*), rapeseed *Brassica napus*, cassava *Manihot esculenta*, sweet potato *Ipomoea batatas*, tomato *Solanum lycopersicum*, potato *Solanum tuberosum*, turnip *Brassica rapa*). We then assessed the impact of P fertilization on the yield, plant P and N uptake of a particular crop types, referred to as the target crop, grown either alone or in combination with another crop (the companion crop). This resulted in 12 separate categories for "Target and Companion Crops": Cereal, Legume, Other, Cereal-Cereal, Legume-Legume, Cereal-Legume, Cereal-Other, Legume-Cereal, Other-Cereal, Legume-Other, Other-Legume and Other-Other, with the target crop mentioned first. Specifically, when a single crop is mentioned, it indicates the crop was grown in monoculture. By contrast, to assess the role of P fertilization on LER-Yield, LER-P Uptake and LER-N Uptake in different crop combinations, the mixtures were categorised into six distinct crop combinations: Cereal-Cereal, Legume-Legume, Cereal-Legume, Cereal-Other, Legume-Other, and Other-Other. Data from each study were directly extracted from tables and text, while we used GetData 2.24 software to extract data from figures.

2.2.1. Data analysis

The natural logarithm of the response ratio (lnRR) between the mean of each response variable for the treatment group ($\overline{X}_{Treatment}$) and the mean of the corresponding response variable for the control group ($\overline{X}_{Control}$) (Hedges et al. 1999) was calculated to quantify the effect size caused by the P fertilization:

$$\ln RR = \ln (\bar{X}_{Treatment}/\bar{X}_{Control}) \quad (2)$$

Due to the absence of reported standard deviation and/or standard errors in the majority of studies, it was not possible to calculate the variance needed to weight effect sizes across all observations. Instead, we implemented a weighting (W) based on replication (Van Groenigen et al. 2011; Wu et al. 2011):

$$W = (n_{Treatment} \times n_{Control})/(n_{Treatment} + n_{Control}) \quad (3)$$

where $n_{Treatment}$ and $n_{Control}$ denote the number of replicates for the P treatment and control (no or low P) plots, respectively. We employed random-effects models in the MetaWin software 2.1 (Rosenberg et al. 1997) and derived mean effect sizes alongside 95% bootstrapped confidence intervals (CIs) for yield, P Uptake and N uptake in monocultures and mixtures, and for LER-Yield, LER-P Uptake and LER-N Uptake across the entirety set of observations. The 95% bootstrapped CIs were generated after 4999 iterations. Significant P fertilization effects were ascribed when the 95% CIs did not intersect with zero. To assess differences among categorical variables, $Q_{between}$ statistics were employed, with significance set at a threshold of $P_{random} < 0.05$.

Given that the majority of studies encompassed several observations, we aimed to ascertain the independence of observations within each study. We did this by randomly taking a pair of observations from each study (one for monocultures and one for mixtures) and then compared the analysis of the random set with the entire dataset. Results obtained from the random subset with those derived from the entire dataset revealed that mean effect sizes were consistent (see Table S2.1), and we are therefore confident that observations within a study can be regarded as independent (Vilà et al. 2011). We used the Egger's test and Rosenthal's Fail-Safe Analysis to evaluate the possibility of publication bias in our analyses (Han and Zhu 2020; Koricheva et al. 2013; Rosenberg 2005). For the Egger’s test, p values ≥ 0.05 indicate the absence of publication bias, while a fail-safe number exceeding $5n + 10$ (n is the number of observations) indicates robustness (Rosenberg 2005). Based on the Egger’s test there may have been some publication bias for P uptake, but according to Rosenthal’s Fail Safe Analysis, our results are robust (Table S2.2). The statistical analyses were performed using MetaWin software 2.1 (Rosenberg et al. 1997).

2.3. Results

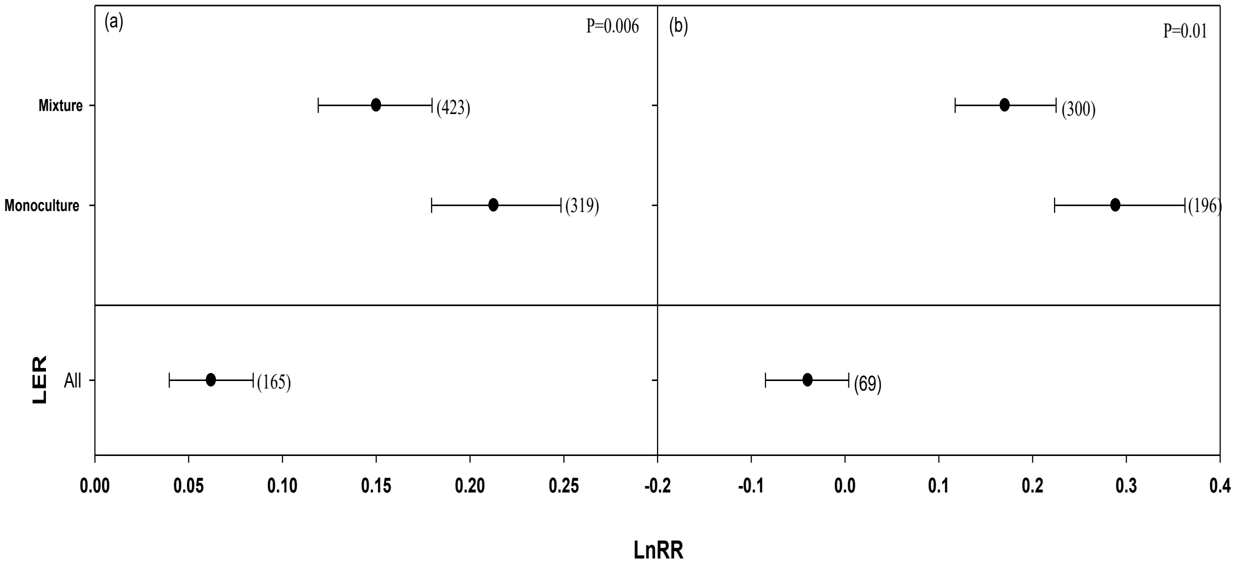

Figure 2. 1. Effect of phosphorus (P) fertilization (LnRR) on yield (a) and plant P uptake (b) of monocultures and mixtures and on their respective Land Equivalent Ratio (LER). Error bars represent 95% bootstrapped confidence intervals (CIs), and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences between monocultures and mixtures ($P<0.05$).

Both monocultures and mixtures showed increased yield responses to P fertilization (Fig. 2. 1a). Yield responses in monocultures were significantly higher than in mixtures ($P = 0.006$). Furthermore, P fertilization consistently raised the LER of yield across all observations. Similar results were observed for plant P uptake responses in monocultures and mixtures, and the LER for plant P uptake (Fig. 2.1b).

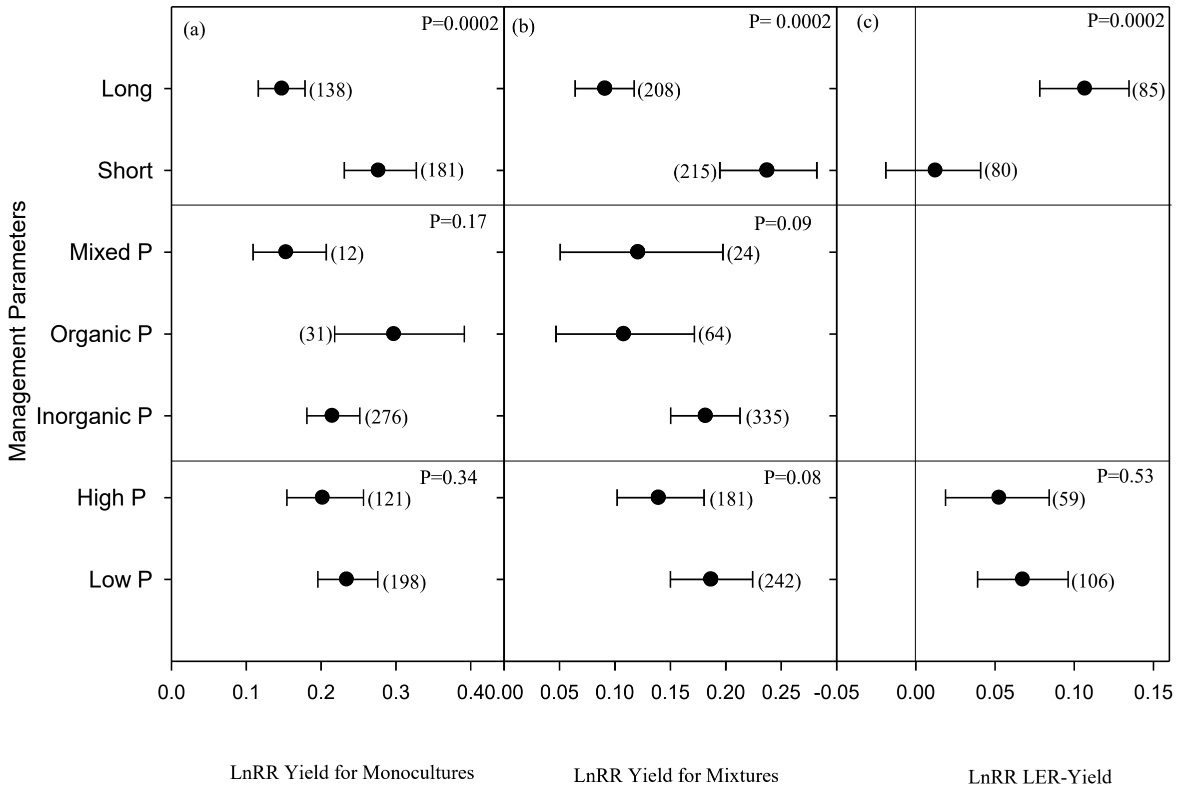

Figure 2. 2. Effect of phosphorus (P) fertilization (LnRR) on yield of monocultures (a) and mixtures (b), and on the Land Equivalent Ratio (LER-Yield) (c) separated by management parameters (year based experimental timeframe, P fertilizer class and P application levels). Error bars represent 95% confidence intervals (Cls) and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences among categories ($P<0.05$).

We observed that variations in experimental timeframe, P fertilizer types, and P application levels resulted in varied yields and LER values for both monocultures and mixtures. In experiments exceeding a two-year duration (Long), the impact of P fertilization on both monoculture and mixture yields were smaller compared to experiments lasting two years or less (Short, $p = 0.002$ and $p = 0.002$, Fig. 2. 2a, b, respectively). Additionally, the application of P increased the LER-Yield only in experiments lasting longer than two years ($p = 0.002$, Figure 2. 2c). We also assessed experimental duration effects for low (equal to or less than 50 kg P $ha^{-1}yr^{-1}$) and high application rates (exceeding 50 kg P ha^{-1} yr^{-1}) separately, where we found similar results (but with larger confidence intervals and lower significance, data not shown), suggesting that the experimental duration effects were not biased by fertilizer application rates. We observed no significant differences among the different types of P fertilizer application on yield, both in monocultures and mixtures (Fig. 2. 2a, b), while there were not enough observations to assess fertilizer type effects on LER-Yield. Similarly, there were insufficient observations to evaluate the effects of fertilizer type on LER-P uptake (Fig. S2. 1). Moreover, we observed no significant differences between low (equal to or less than 50 kg P ha^{-1} yr^{-1}) and high rates (exceeding 50 kg P ha^{-1} yr^{-1}) of P fertilization in yield of monocultures and mixtures (Fig. 2. 2a, b), and in LER-Yield (Fig. 2. 2c).

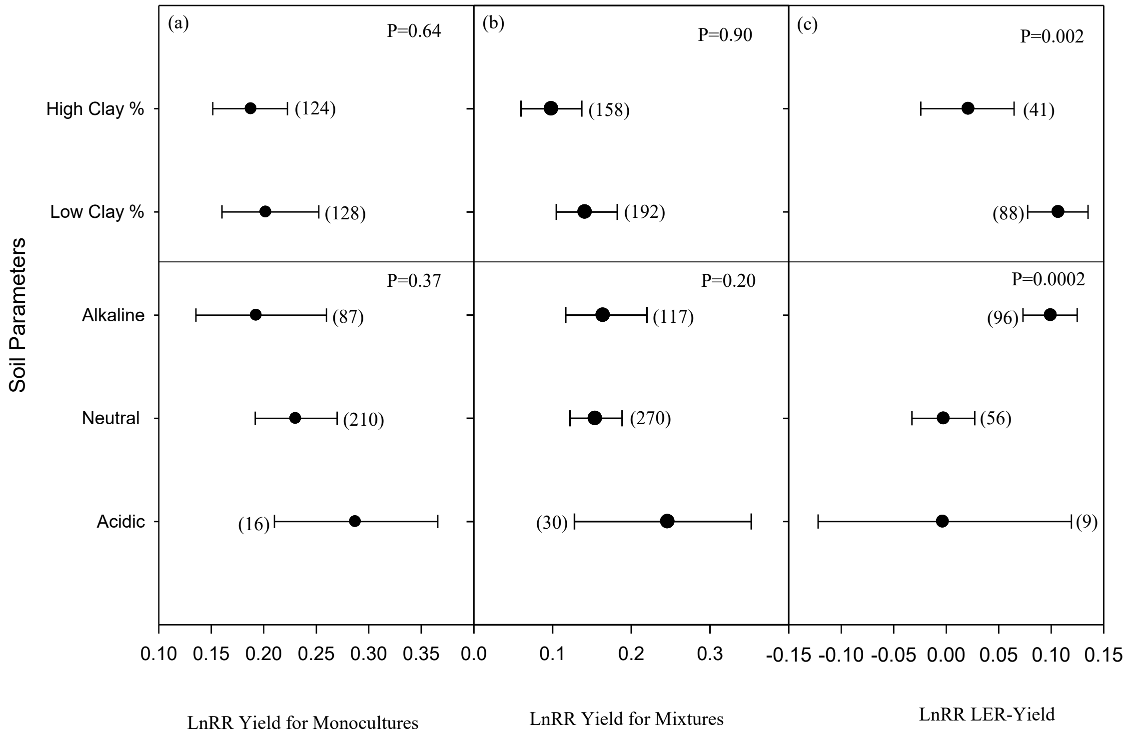

Figure 2. 3. Effect of phosphorus (P) fertilization (LnRR) on yield of monocultures (a) and mixtures (b), and on the Land Equivalent Ratio (LER-Yield) (c), separated by soil parameters (clay% and soil pH). Error bars represent 95% bootstrapped confidence intervals (CIs), and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences among categories (P<0.05).

Effect of phosphorus (P) fertilization (LnRR) on yield of monocultures (a) and mixtures (b), and on the Land Equivalent Ratio (LER-Yield) (c), separated by soil parameters (clay% and soil pH). Error bars represent 95% bootstrapped confidence intervals (CIs), and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences among categories (P<0.05).

The influence of P fertilization on monoculture and mixture yield, as well as LER-Yield, was consistently positive across all soil parameters (Fig.2. 3). We observed no significant difference in yield responses between low clay percentage (30% or less) and high clay percentage (more than 30%), for both monocultures and mixtures. However, the LER-Yield increased more with P fertilization in low clay than in high clay soils (P = 0.002, Fig. 2. 3c). We also observed no significant differences in the effect of P fertilization on yield among different soil pH classes in monocultures and mixtures (Fig. 2. 3a, b), but the LER-Yield response was highest in basic soils (pH > 7.5, P = 0.0002, Fig. 2. 3c).

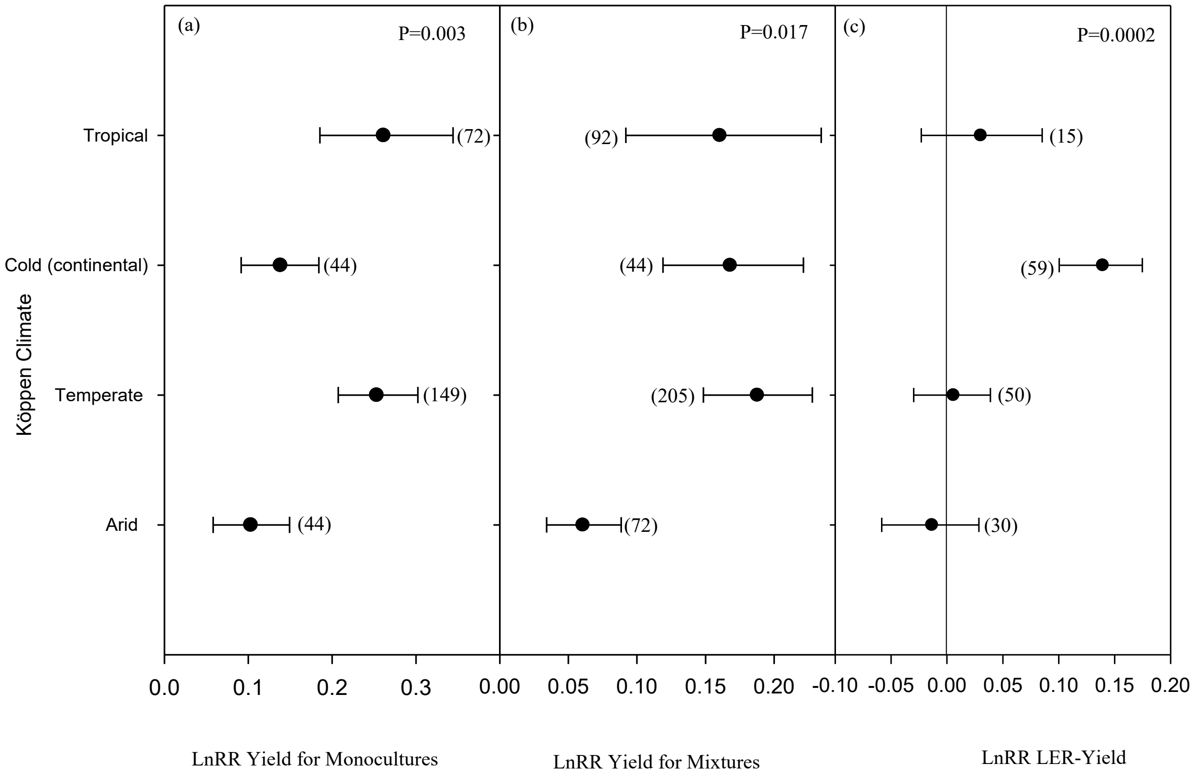

Figure 2. 4. Effect of phosphorus (P) fertilization (LnRR) on yield of monocultures (a) and mixtures (b), and on the Land Equivalent Ratio (LER-Yield) (c), separated by different Köppen climate zones, (Tropical, Cold (continental), Temperate, and Arid). Error bars represent 95% bootstrapped confidence intervals (CIs), and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences among categories (P<0.05).

Across various Köppen climate zones, we observed significant differences in yield in monocultures (P = 0.003) and mixtures (P = 0.02), and in LER-Yield (P = 0.0002) in response to P fertilization (Fig. 2. 4). Yield responses for monocultures and mixtures were lowest in the arid climate zone, and were highest in the tropical zone for monocultures, and highest in the temperate zone for mixtures (Fig. 2. 4a, b). In contrast, the LER-Yield only significantly increased in response to P fertilization in the cold (continental) climate zone (Fig. 2. 4c).

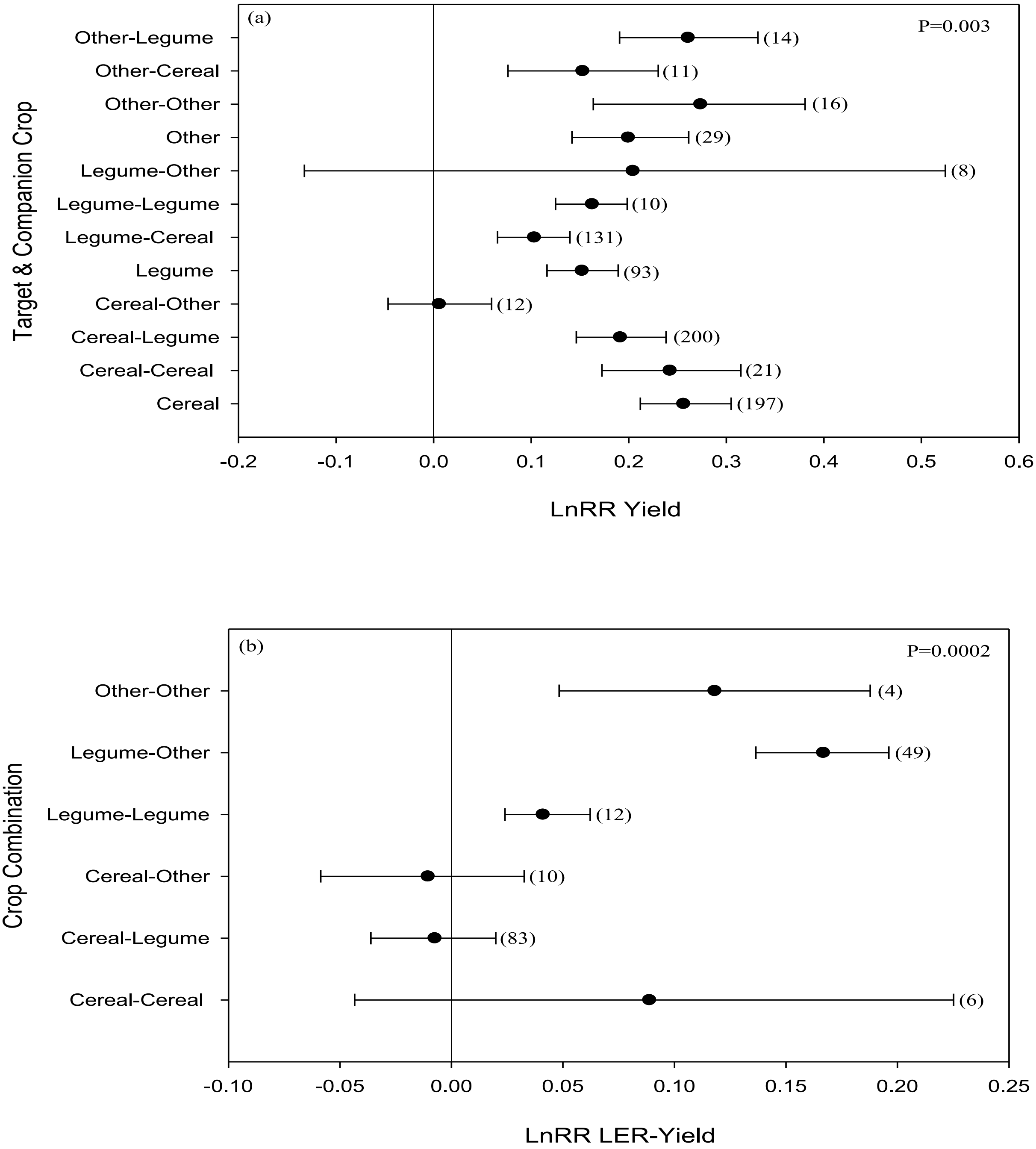

Figure 2. 5. Effect of phosphorus (P) fertilization (LnRR) on the yield of a designated target crop type grown either as a monoculture or in combination with a specific companion crop (a), and on the Land Equivalent Ratio (LER-Yield) across different crop combinations (b). Error bars represent 95% bootstrapped confidence intervals (CIs), with the number of observations shown in brackets next to the error bars. The effect of P fertilization is considered significant

when the 95% CIs do not overlap with zero. P-values indicate significant differences among the categories ($P < 0.05$).

The effect of P fertilization on yield varied significantly among target crop types, either cultivated independently or in association with a designated companion crop type ($P=0.003$, Fig. 2. 5a). The most pronounced P fertilizer-induced responses were observed in non-cereal and non-legume crops (other crops) when cultivated either in monoculture or in association with cereal crops, legumes, or other crops. The yield of cereal crops did not increase with P fertilization when grown with other non-cereal and non-legume crops, but yield did increase when grown in monoculture and when grown with legumes and other cereal crops. In general, the smallest yield increases were observed for legumes (except for cereal crops grown with non-cereal and non-legume crops), but we note large variation in the yield response of legumes when grown with non-cereal and non-legume crops, most likely because of the low number of observations.

The influence of P fertilization on Land Equivalent Ratio (LnRR-LER) significantly varied among diverse crop combination types ($P = 0.0002$, Fig. 2. 5b). Application of P positively impacted LER-Yield in intercropping systems that included legumes, except for cereal-legume intercropping systems. The LER-Yield in response to P fertilization also significantly increased in intercropping systems without cereals and legumes (Other-Other), but we note the low number of observations. We conducted similar analyses for plant P uptake, plant N uptake, and their LER (LER-P Uptake and LER-N Uptake) and found similar patterns as for yield and LER-Yield (Figs.2.S1-S9).

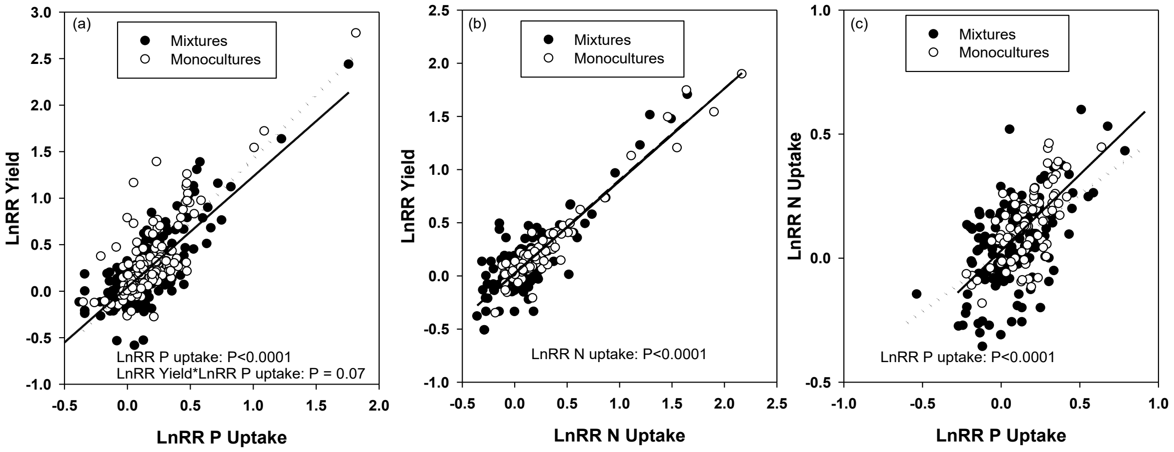

Figure 2. 6. Relationship between (a) the effect size of yield (LnRR Yield) and phosphorus uptake (LnRR P Uptake), (b) between LnRR Yield and the effect size of nitrogen uptake (LnRR N Uptake), and (c) between LnRR N Uptake and LnRR P Uptake under monocultures and mixtures (dotted line represents the mixtures whereas the straight line represents monocultures).

We observed strong positive relationships between the increase in P uptake (LnRR P Uptake) and the increase in crop yield (LnRR Yield) in response to P fertilization, both for monocultures and mixtures ($P < 0.0001$, Fig. 2. 6a). Specifically, an equivalent rise in P uptake resulted in a more pronounced increase in yield in intercropping systems compared to monoculture. The slopes of the regression lines differed between intercropping and sole cropping systems, although the LnRR Yield × LnRR P Uptake interaction was marginally significant ($P = 0.07$). There was also a strong positive relationship between the increase in N uptake (LnRR N Uptake) and yield and between LnRR P uptake and LnRR N uptake ($P< 0.0001$, Fig. 2. 6b, c). However, there were no significant differences in the slope between monocultures and mixtures.

2.4. Discussion

Phosphorus fertilization plays a crucial role in agriculture, greatly impacting crop yields in monoculture and intercropping systems. In this meta-analysis we assessed whether P fertilization would significantly enhance yield and the uptake of P and N in intercropping systems more than in monocropping systems, resulting in greater LER values. We further assessed to what degree these responses depended on management (duration, type and amount), soil parameters (pH, texture), climate, and crop combination. The results offer important insights into the efficacy of P fertilization across different cropping systems and underscore the potential of intercropping to improve agricultural sustainability.

In contrast to what we hypothesized, monocultures showed a significantly higher yield response to P fertilization compared to mixtures. A lower yield response in mixtures may be due to factors such as interspecific competition for nutrients and light, which can limit the performance of individual crops. Despite a lower yield response, P fertilization consistently elevated the LER-Yield across all observations. This implies that even though individual crop yields in mixtures might not increase as dramatically as in monocultures, the overall efficiency of land use is enhanced. The improved LER-Yield indicates that P fertilization can substantially improve resource use efficiency in intercropping systems, leading to higher total productivity per unit area compared to sole cropping (An et al. 2023; Hinsinger et al. 2011; Tang et al. 2021).

2.4.1. Impact of experiment duration, fertilizer type and application rate

In contrast to what we hypothesized, studies lasting more than two years showed a reduced effect of P fertilization on yields in both monocultures and mixtures compared to those of shorter duration (Fig. 2. 2a, b), suggesting that the benefits of P fertilization may plateau or decline over time. We are unclear why this is, but continuous fertilization tends to result in a build-up of organic and inorganic P pools in the soil (Gupta et al. 2007; Reddy et al. 2000). If there were P fertilization legacy effects in the control plots in the studies used in our meta-analysis (e.g., because control plots were not always in the same location), this could then have resulted in smaller P fertilization effects in the long term. Interestingly, only studies exceeding two years reported increased LER for yield (Fig. 2. 2c). These results are in agreement with findings that long-term P fertilization enhances soil P availability and plant uptake efficiency in intercropping systems compared to monocultures (An et al. 2023). While yield responses to P fertilization may diminish over time, intercropping systems tend to offer greater stability year-to-year compared to monocultures (Li et al. 2021), potentially improving long-term LER-Yield.

No significant differences in crop yield were observed when comparing the application of various types of P fertilizers, while there were not enough observations to evaluate the impact of different P fertilizer types on LER-yield (Fig. 2. 2). Others have observed that combining organic fertilizer with inorganic fertilizer can result in higher grain yields in both intercropping and monocropping (Muyayabantu et al. 2012), possibly because of the enhanced availability of N and other (micro-)nutrients that are added with the organic fertilizer, along with improvements in other soil parameters (such as pH). However, we found no support for this in our meta-analysis, but we note the relatively low number of observations for mixed fertilizers. Surprisingly, no significant differences in crop yield or LER-yield were observed when comparing low (≤50 kg P ha^{-1} yr^{-1}) and high (>50 kg P ha^{-1} yr^{-1}) P application rates, suggesting that the P fertilizer use efficiency in mono- and intercropping were independent of fertilizer rate. In a global meta-analysis, Yu et al. (2021) found that the P fertilizer use efficiency in cereal crops varied strongly for rates below 200 kg P ha^{-1} yr^{-1}, and where the P fertilizer use efficiency depended more strongly on soil factors such as pH. Also, in our meta-analysis, variation in P fertilizer use efficiency among crops, soil type and factors such as climate, may have obscured any effects of P application rate on yield and LER-Yield. Furthermore, we observed that initial soil P availability varied substantially among studies (from 2.35 to 42.2 mg P kg^{-1} soil), which further could have obscured P application type and rate effects on yield and LER-Yield. We note however, that initial soil P availability was measured using different methods (Olsen-P, Bray-1, Bray-2, resin extraction, double lactate extraction) making it difficult to compare these values among studies.

2.4.2. Impact of soil properties and climate zone

The increased yield responses to P fertilization in both monoculture and intercropping systems were independent of clay content and soil pH. Conversely, and in support of what we hypothesized, the LER-Yield increased more with P fertilization in soils with low clay content compared to those with high clay content (Fig. 2. 3c). While clay-rich soils tend to have a greater P sorption capacity (Gérard 2016) and availability (Hou et al. 2018), P mobility tends to be higher in lighter and coarse textured soils, where P diffusion faces fewer restrictions (Lambers et al. 2008). This could then possibly have reduced competition for P between crops and enhanced the synergistic effects of intercropping. The LER-Yield further increased the most in alkaline soils (Fig. 2. 3c), suggesting that intercropping systems could particularly benefit from P fertilization under alkaline conditions. We expected greatest benefits under neutral conditions when soil P availability and plant uptake tend to be highest (Marschner et al. 2005). However, in intercropping systems with legumes, rhizosphere acidification by the legume could improve P availability and uptake by the companion crop in alkaline soils (Latati et al. 2014). This improved P use efficiency and inter-specific facilitation may then have resulted in greater LER-Yield responses to P fertilization in alkaline soils.

The lowest yield responses for both monocultures and mixtures were found in the arid climate zone (Fig. 2. 4a, b), likely due to limited water availability so that any responses to P fertilization will be small. For instance, plant P uptake by wheat plants almost tripled as soil moisture content rose from 30% to 75% of water holding capacity, even in the absence of P supply to the crop (He et al. 2002). Conversely, the temperate and tropical zones showed the highest yield responses for monocultures and mixtures, likely because water availability was not constraining P uptake in those regions. Therefore, the pronounced yield responses in tropical and temperate zones may reflect higher baseline precipitation supporting greater nutrient uptake. Furthermore, soils in tropical climates are often highly weathered that are low in P (Vitousek et al. 2010). As such, it is not too surprising that some of the greatest effects of P fertilization are observed in tropical climates. In contrast to yield responses, P fertilization increased the most LER-yield in the cold (continental) climate zone (Fig. 2. 4c). Phosphorus availability is often limited due to slower mineralization rates at lower temperatures (Grierson et al. 1999). Therefore, in cold (continental) climates that also tend to have low precipitation, P fertilization may benefit growth of both crops in intercropping systems. Additionally, the higher LER-Yield in the cold climate zone may be due to a longer growing season, providing more time for both crops in intercropping systems to respond to P fertilization.

2.4.3. Impact of target crop and companion crop

Cereal crops did not show yield increases when intercropped with “Other” crops (crops that are not a cereal or a legume) after P fertilization (Cereal-Other, Fig. 2. 5a). We are unclear why this would be, but perhaps these other crops competed strongly for P so that the P fertilization did not result in any yield gain for the cereal. We note however, the low number of observations for Cereal-Other crop combination in our meta-analysis, and more research on these crop combinations is warranted. On the other hand, significant yield increases were observed when cereals were intercropped with legumes or other cereals. However, those increases were as large as when cereals were grown in monoculture. Similar results were observed for P uptake (Fig. S2.5). This suggests that P fertilization did not change competition for P when cereals are grown with legumes or other cereals. Yield increases in intercropping systems have been attributed to facilitation and complementarity mechanisms that enhance nutrient release and use. For instance, legumes can increase the activity of arbuscular mycorrhizal fungi (AMF) enhancing nutrient acquisition for legumes, but which may indirectly promote P uptake in cereals (Qiao et al. 2015). Intercropping cereals with other cereals or legumes can augment P uptake due to variations in root growth dynamics and the ability of some crops to mobilize P for neighbouring species through the production phosphatases, protons and carboxylates (Li et al. 2014). As discussed above, when grown with legumes, cereals can gain more P and increase yield due to rhizosphere acidification caused by the legume, thereby enhancing P availability (Hauggaard-Nielsen et al. 2009; Jensen 1996). Additionally, P fertilization can boost N fixation in legumes, thereby providing more N to the cereal and further promoting soil acidification and P acquisition (Hinsinger et al. 2011; Stern 1993). Our results indicate that P fertilization did not result in additional yield gains for the cereal when intercropped with a legume, suggesting that the legume competed as strongly as the cereal for the applied P. Interestingly, legume and other non-cereal crops intercropped with cereals showed somewhat lower yield increases with P fertilization compared to their monocultures. This suggests that cereals were strong competitors for P thereby suppressing yield responses of the legumes or other non-cereal crops. Additionally, the yield responses of legumes grown with other non-cereal crops, and yield responses of other non-cereal crops in all intercropping systems varied significantly, likely due to the limited number of observations.

In contrast to what we hypothesized, cereal-legume intercropping systems did not exhibit a significant increase in LER-Yield with P fertilization (Fig. 2. 5b). We expected that the addition of P could boost biologically fixed N by the legume thereby allowing cereals to acquire more soil N (Hauggaard-Nielsen and Jensen 2005b; Raseduzzaman and Jensen 2017) and overall, more efficient resource use. However, the LER-N uptake actually decreased with P fertilization in the cereal-legume combination (Fig. S2. 10), but with limited number of observations. Our results are consistent with the observations made by Xiao et al. (2021), who found that he LER-Yield for cereal-legume mixtures were not influenced by increased rates of P fertilization. As discussed above, this could be attributed to cereals acting as strong competitors for P, which may have limited the yield response of the legume component (Fig. S2. 5a), thereby resulting in no effect on LER-yield. Similarly, cereals might exhibit competitive dominance for P when grown alongside other crops (Fig. 2. 5a), leading to a similar lack of impact on LER-Yield (Fig. 5b). The fibrous roots of cereal crops can also be more plastic compared to the tap root systems of legumes that was shown for maize grown with soybean (Yang et al. 2022), and which may play a crucial role in these competitive dynamics. Thus, the contrast between increased yield responses of cereals and legumes or other crops to P fertilization in intercropping systems (Fig. 2. 5a) and lack of an LER-Yield response when cererals are included (Fig. 2. 5b) demonstrates that aggressive P uptake by cereals may remove overall yield benefits and could limit complementary interactions in these systems.

The largest increase in LER-Yield in response to P fertilization was observed in intercropping systems with legumes and other non-cereal crops (Fig. 2. 5b). This suggests that facilitation complementarity effects of legumes intercropped with these other crops can be enhanced with P fertilization, improving efficient use of P to boost overall productivity. Possibly, the root structures of these other non-cereal crops minimize competition for resources like P and other resources, resulting in more efficient resource use and improved productivity under intercropping system. We are not aware of any studies that have assessed the role of root structures for yield in intercropping systems of legumes and other non-cereal crops, and we believe this is an area requiring further research.

2.4.4. Relationships among the effect size of yield, P uptake, and N uptake

The positive relationships between the effect size of yield and P uptake (Fig. 2. 6a) highlights the essential role of P in enhancing crop productivity. Intercropping systems in particular, showed a more pronounced yield increase per unit of P uptake increase compared to monoculture systems, although the difference in slope was only marginally significant. This suggests that when intercropped, crops can be more efficient in converting the applied P into yield. We are unclear why this would be, but others have suggested that interspecific competition and facilitation in intercropping systems can increase nutrient use efficiency and thereby increased crop productivity (Zhang and Li 2003). Our analysis also revealed a significant relationship between the effect size of yield and N uptake, and between the effect size of P uptake and N uptake (Fig. 2. 6b), supporting the well-documented effect of P fertilization on enhancing both plant growth and N uptake (Jiang et al. 2019). This also indicates that the interaction between P and N availability is interconnected and crucial for growth-related processes in plant production systems. This reciprocal interaction suggests that, despite their distinct roles, N and P facilitate overall plant growth and metabolism. Despite significant relationships between the effect sizes of yield and N uptake, and between P uptake and N uptake, there were no substantial differences in the slopes between monocultures and intercropping systems, indicating that at least for N the basic relationship between nutrient uptake and yield increase is similar between the two cropping systems. However, the overall yield improvements in intercropping systems with P fertilization highlight their potential benefits in optimizing P use efficiency and enhancing productivity (Toker et al. 2024).

2.5. Conclusion

We explored the effects of P fertilization on grain yield, P uptake, and N uptake and their LER values in a variety of intercropping combinations. Using a meta-analysis of 39 studies encompassing different crops, soil types, management practices, and climatic conditions, the analysis revealed that across all observations, yield responses were more pronounced in monocultures than in mixtures, but that P fertilization consistently enhanced LER-Yield. The results highlight the critical role of duration, soil factors (clay content and pH) and Köppen climate zones in increasing LER-Yield with P fertilization, stressing the need for tailored P management strategies for different regions. Additionally, LER-Yield showed no responses to P fertilization in intercropping systems with cereals, possibly because of the strong competitive ability of cereals to take up P. On the other hand, the strongest positive effects of P fertilization on LER-Yield occurred in intercropping systems that had no cereals and suggest that P fertilization may be most beneficial in those intercropping systems. These findings are vital for improving agricultural efficiency, especially in areas with limited arable land and high demand for food production, contributing to the broader goal of sustainable resource management and reduced input reliance.

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Data availability

All data is provided in the Supplementary Information

Competing Interests

Authors declare no competing interests

2.6. References

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Supplementary for Chapter 2

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Table S2.1. Comparison of results from the meta-analyses using all observations and one random observation per study.

Response	**Category**	**n**	**LnRR**	**95% CI**	
Variable				**min**	**max**
Yield	All observations monocultures	423	0.2214	0.1916	0.2537
	All observations mixtures	319	0.1651	0.1372	0.1918
	1 Random observation per study monocultures	39	0.2926	0.1998	0.3940
	1 Random observation per study mixtures	39	0.2689	0.1868	0.3581
P Uptake	All observations monocultures	196	0.3071	0.2528	0.3687
	All observations mixtures	300	0.2087	0.1645	0.2542
	1 Random observation per study monocultures	39	0.5721	0.341	0.8199
	1 Random observation per study mixtures	39	0.5731	0.3542	0.797

Table S2.2. Results for publication bias of the yield, phosphorus (P) uptake, and nitrogen (N) uptake response ratios to phosphorus fertilization (Egger's test Z and P-values, and fail-safe numbers). If the fail-safe number is larger than 5n + 10 (*n* is the number of observations), then the results are robust regardless of any potential publication bias.

Response variable	**n**	**Z**	**P value**	**Fail-Safe number**
Yield	907	-1.880	0.06	11423
P Uptake	496	-2.103	0.04	8448

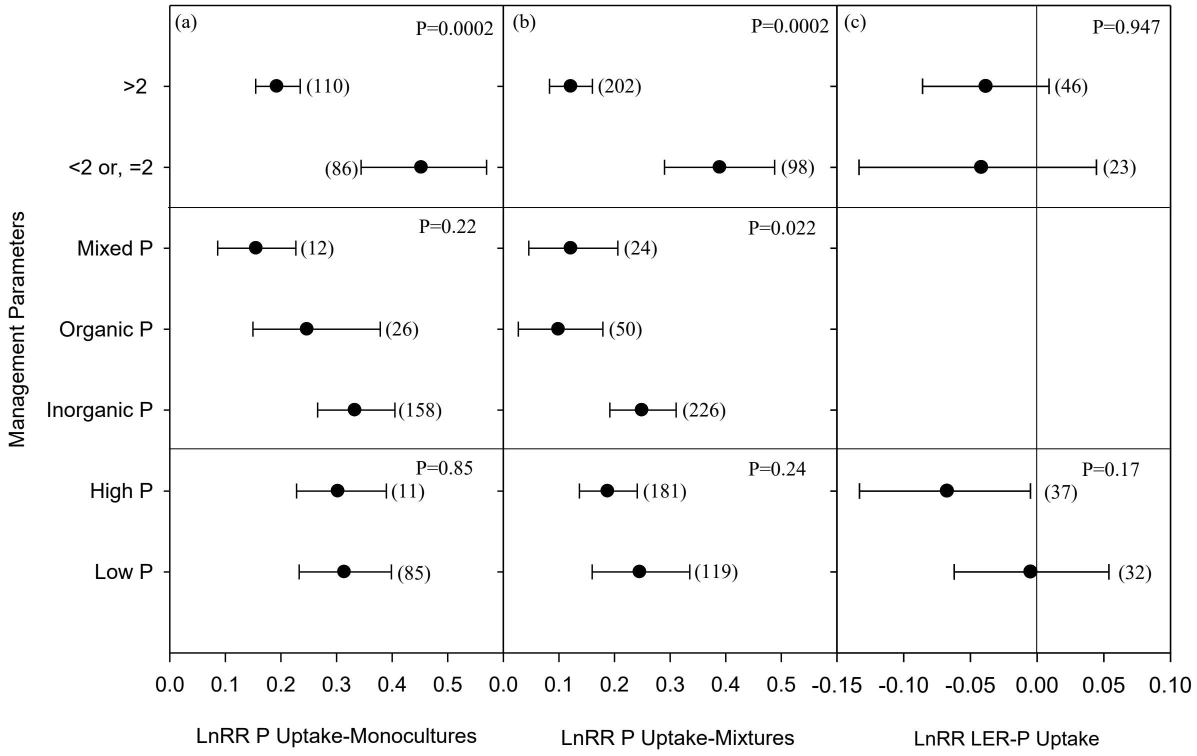


Figure S2. 1 Effect of phosphorus (P) fertilization (LnRR) on P Uptake by monocultures (a) and mixtures (b), and on the Land Equivalent Ratio (LER-P Uptake) (c) separated by management parameters (year based experimental timeframe, phosphorus fertilizer class and phosphorus application levels). Error bars represent 95% confidence intervals (Cls) and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences among categories (P<0.05).

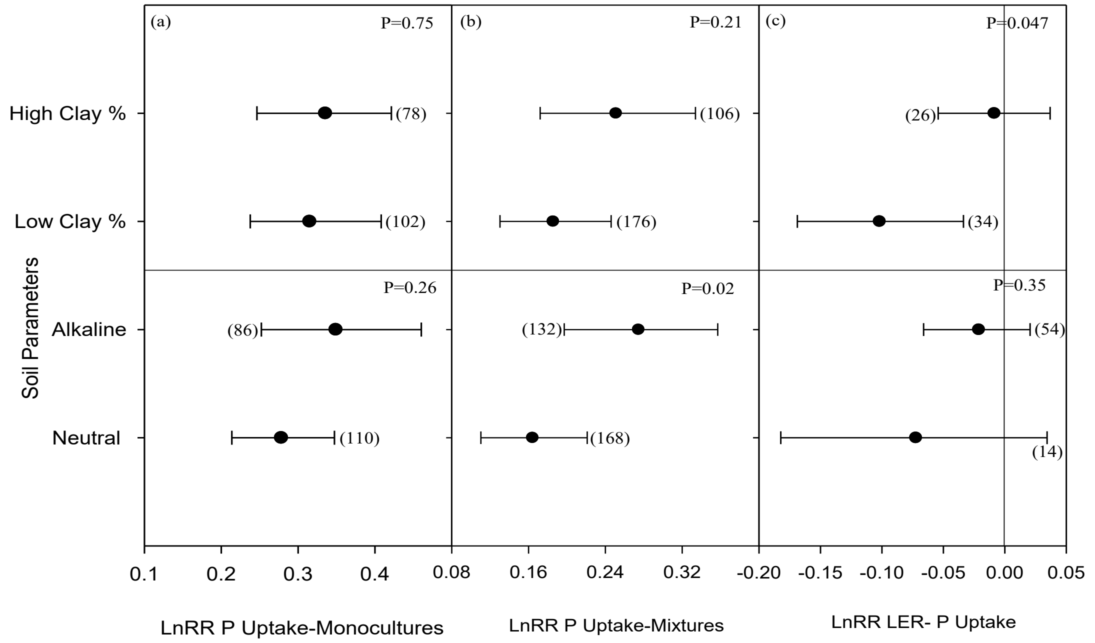


Figure S2. 2. Effect of phosphorus (P) fertilization (LnRR) on P Uptake by monocultures (a) and mixtures (b), and on the Land Equivalent Ratio (LER-P Uptake) (c), separated by soil parameters (clay% and soil pH). Error bars represent 95% bootstrapped confidence intervals (CIs), and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences among categories (P<0.05).

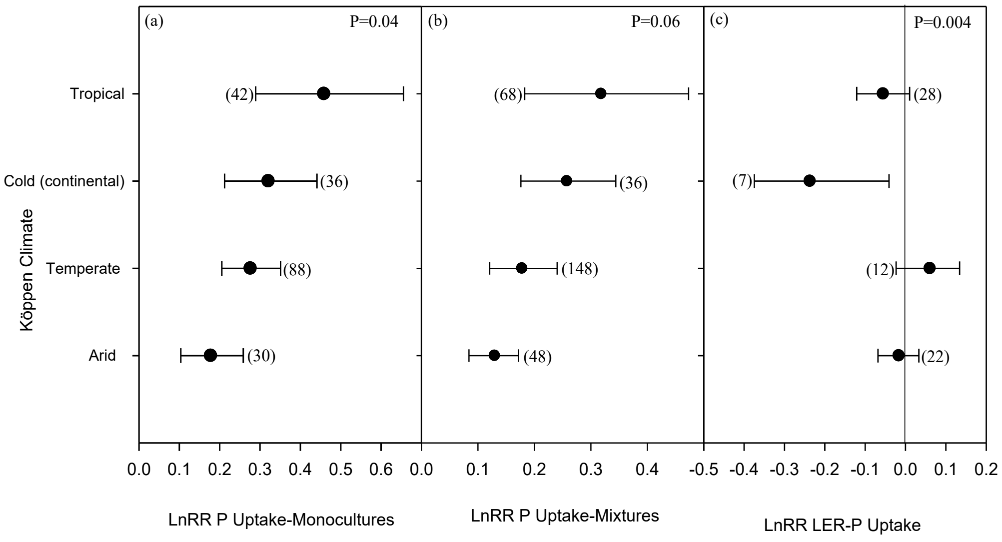


Figure S2. 3. Effect of phosphorus (P) fertilization (LnRR) on P Uptake by monocultures (a) and mixtures (b), and on the Land Equivalent Ratio (LER-P Uptake) (c), separated by different Köppen climate zones, (Tropical, Cold (continental), Temperate, and Arid). Error bars represent 95% bootstrapped confidence intervals (CIs), and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences among categories (P<0.05).

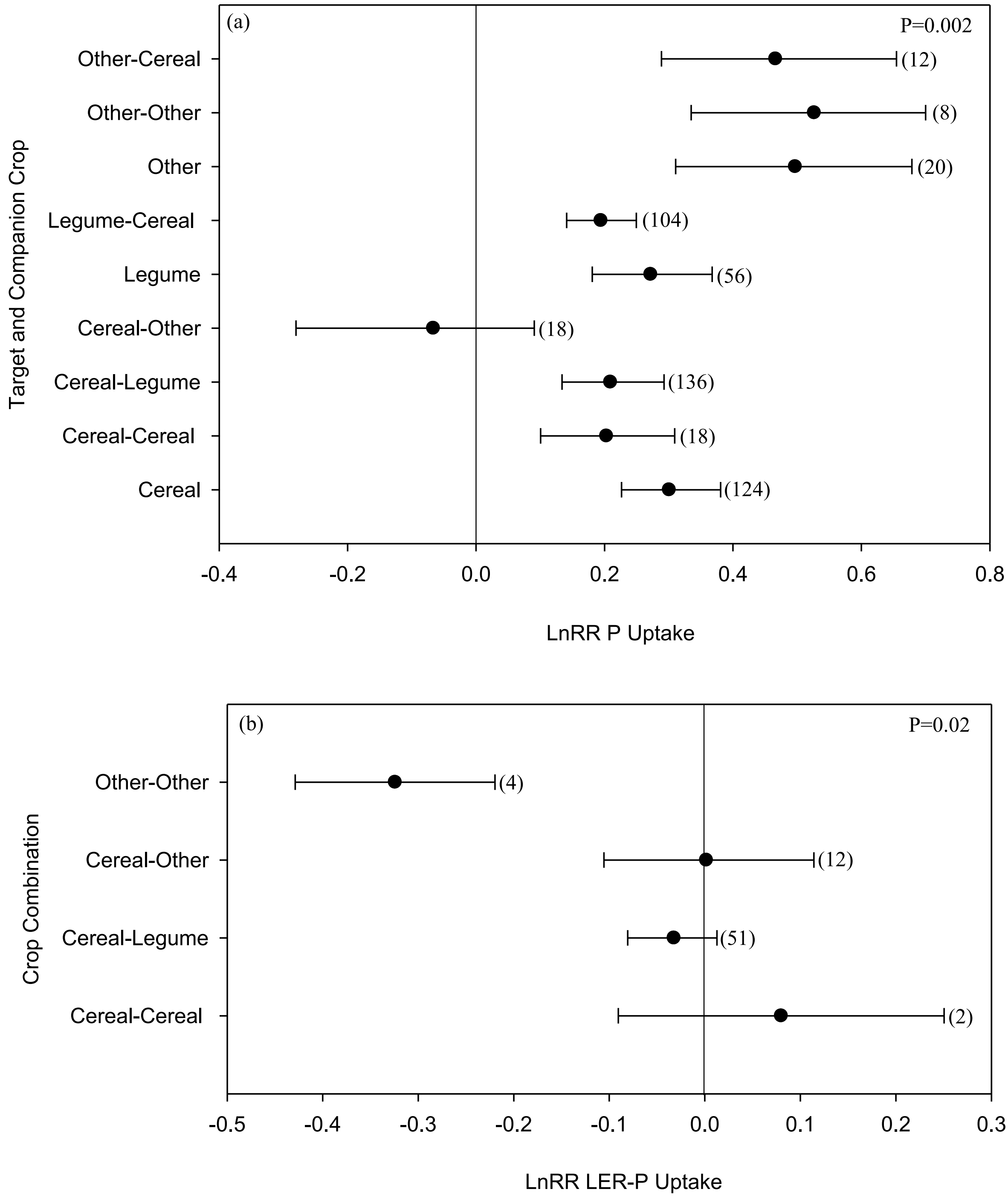


Figure S2. 4. Effect of phosphorus (P) fertilization (LnRR) on the P Uptake by a designated target crop type grown either as a monoculture or in combination with a specific companion crop (a), and on the Land Equivalent Ratio (LER-P Uptake) across different crop combinations (b). Error bars represent 95% bootstrapped confidence intervals (CIs), with the number of observations shown in brackets next to the error bars. The effect of P fertilization is considered significant when the 95% CIs do not overlap with zero. P-values indicate significant differences among the categories ($P < 0.05$).

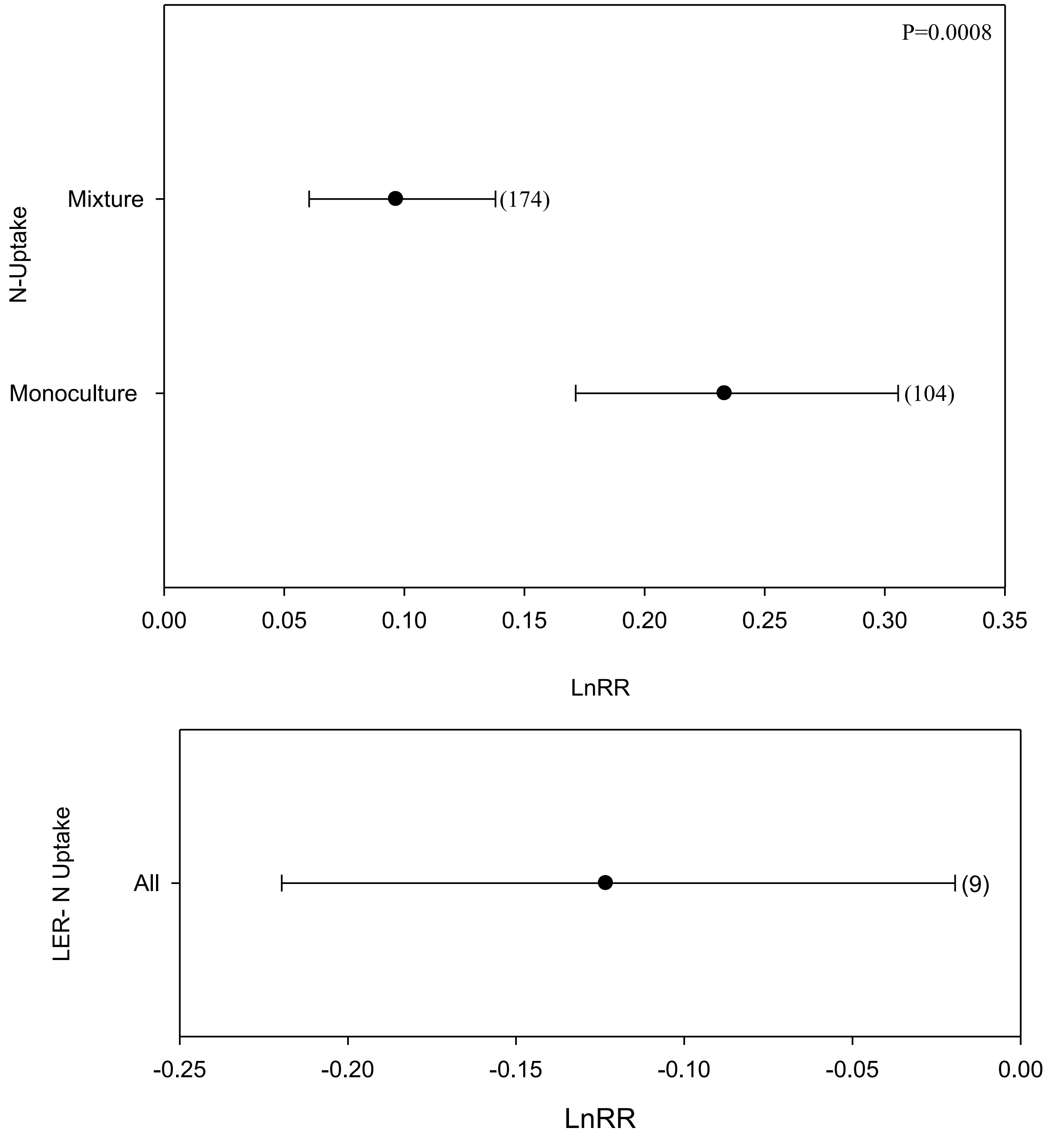
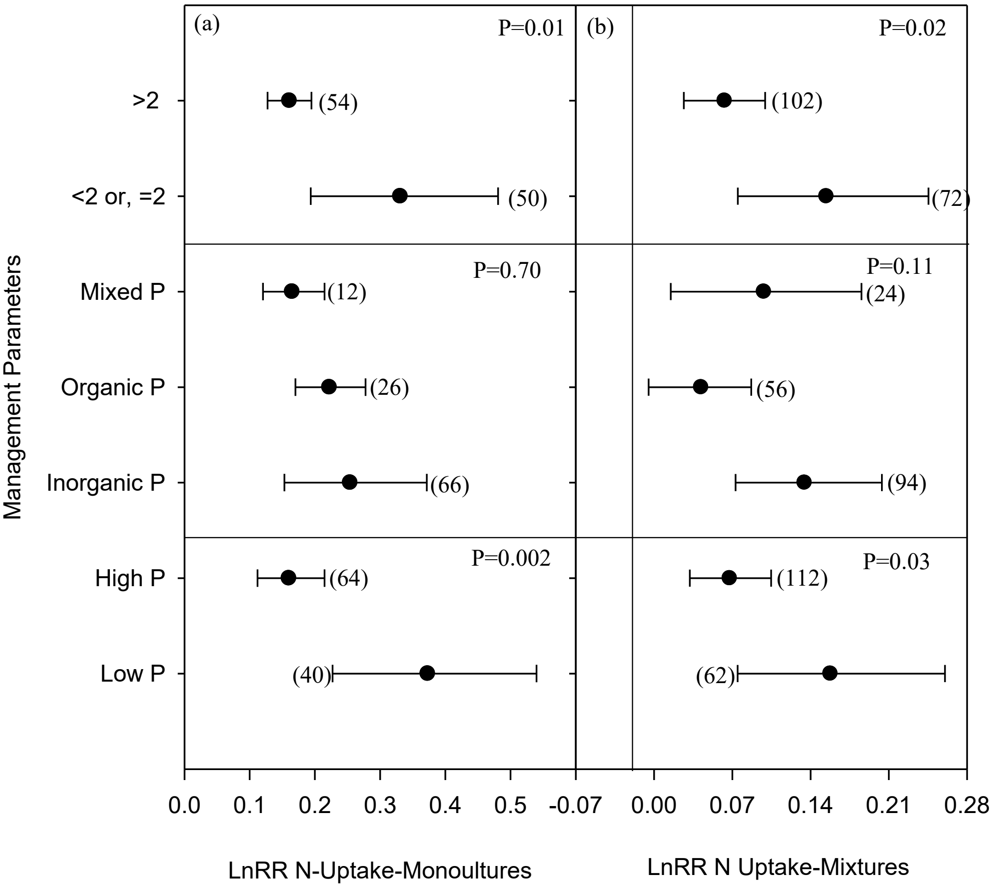


Figure S2. 5. Effect of phosphorus (P) fertilization (LnRR) on N uptake by monocultures and mixtures and on the Land Equivalent Ratio (LER- N uptake). Error bars represent 95% bootstrapped confidence intervals (CIs), and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences between monocultures and mixtures (P<0.05).



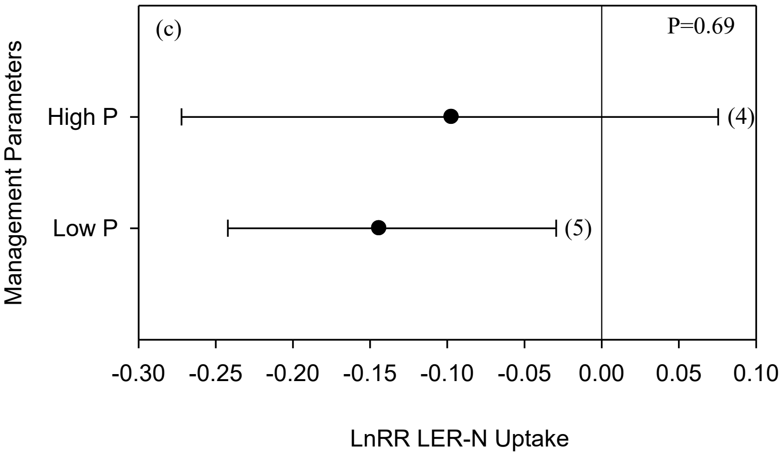


Figure S2. 6. Effect of phosphorus (P) fertilization (LnRR) on N Uptake by monocultures (a) and mixtures (b), and on the Land Equivalent Ratio (LER-N Uptake) (c) separated by management parameters (year based experimental timeframe, phosphorus fertilizer class and phosphorus application levels). Error bars represent 95% confidence intervals (Cls) and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences among categories (P<0.05).

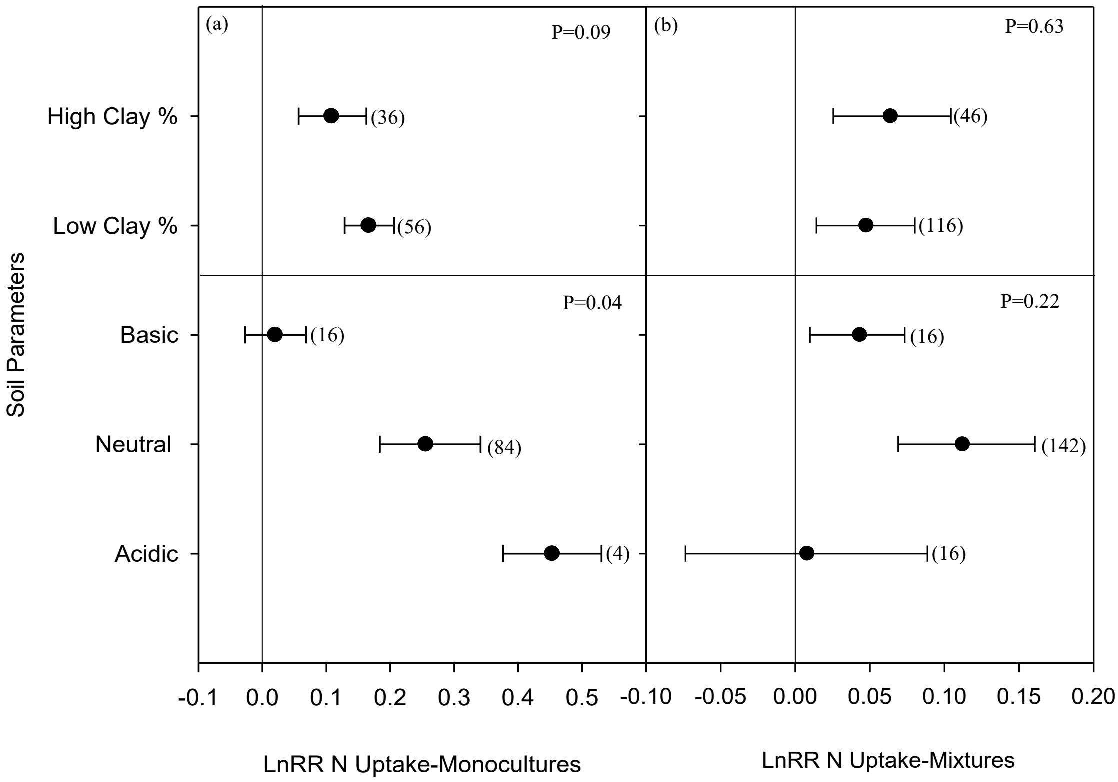


Figure S2. 7. Effect of phosphorus (P) fertilization (LnRR) on N Uptake by monocultures (a) and mixtures (b), and on the Land Equivalent Ratio (LER-N Uptake) (c), separated by soil parameters (clay% and soil pH). Error bars represent 95% bootstrapped confidence intervals (CIs), and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences among categories (P<0.05).

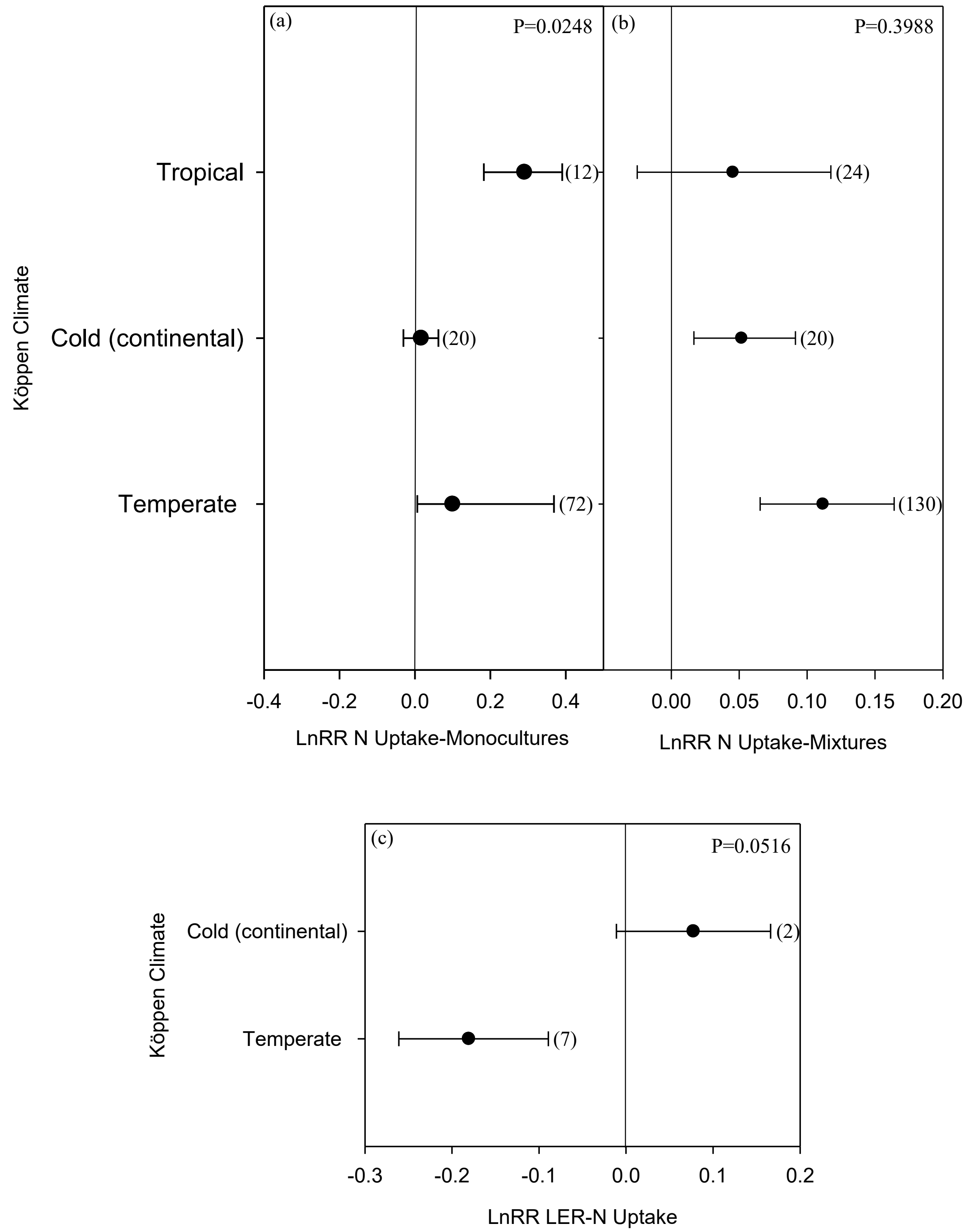
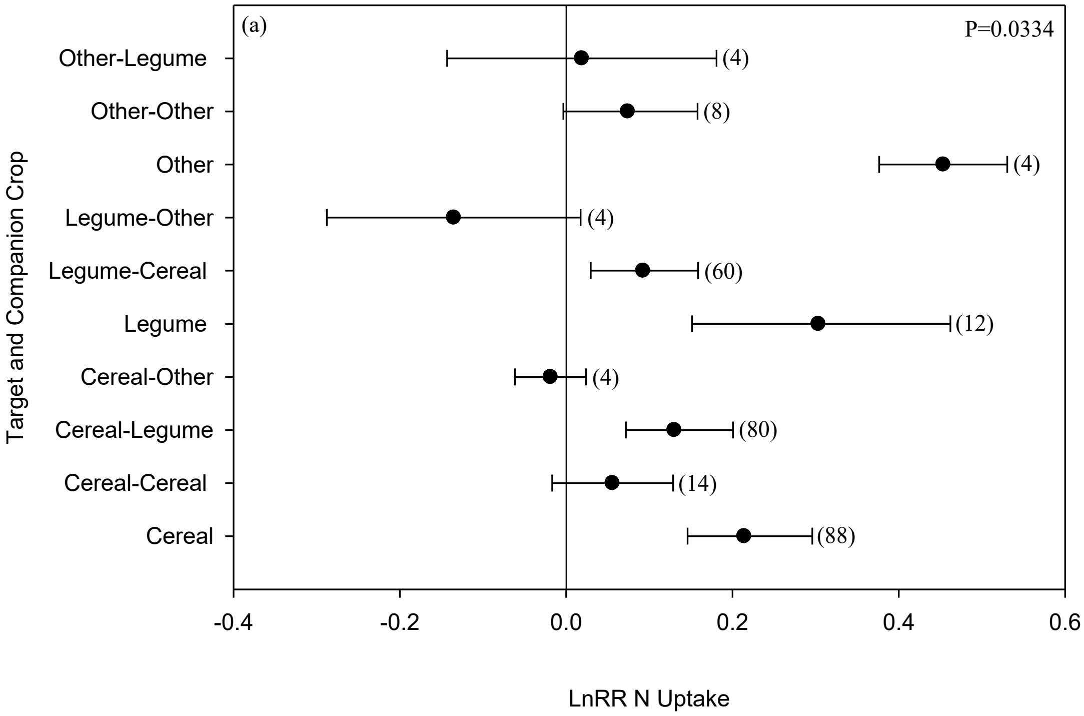


Figure S2. 8. Effect of phosphorus (P) fertilization (LnRR) on N Uptake by monocultures (a) and mixtures (b), and on the Land Equivalent Ratio (LER-N Uptake) (c), separated by different Köppen climate zones, (Tropical, Cold (continental), Temperate, and Arid). Error bars represent 95% bootstrapped confidence intervals (CIs), and the number of observations is shown next to the error bars in brackets. The effect of P fertilization was considered significant if the 95% CIs did not overlap with zero. P-value indicates significant differences among categories (P<0.05).



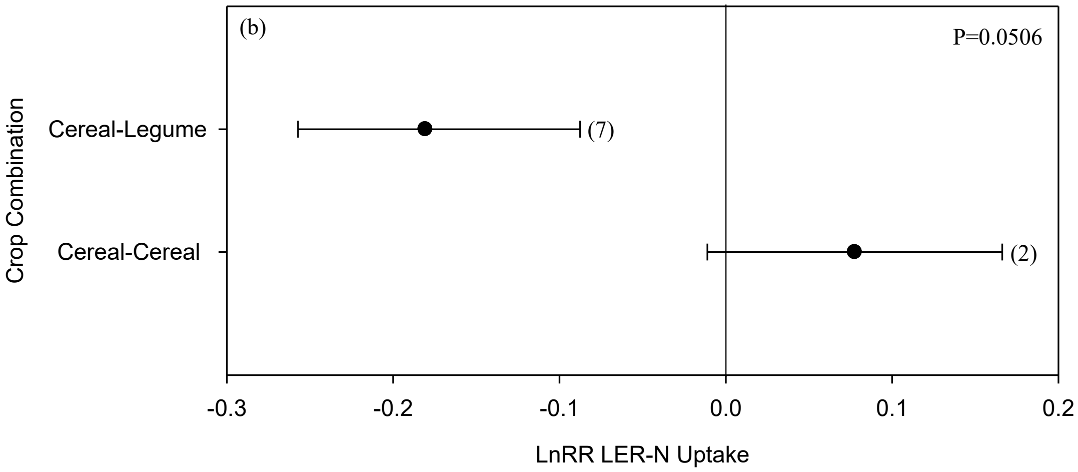


Figure S2. 9. Effect of phosphorus (P) fertilization (LnRR) on the N Uptake by a designated target crop type grown either as a monoculture or in combination with a specific companion crop (a), and on the Land Equivalent Ratio (LER-N Uptake) across different crop combinations (b). Error bars represent 95% bootstrapped confidence intervals (CIs), with the number of observations shown in brackets next to the error bars. The effect of P fertilization is considered significant when the 95% CIs do not overlap with zero. P-values indicate significant differences among the categories ($P < 0.05$).

Chapter 3: Yield, nitrogen fixation and carbon allocation to root biomass and respiration in response to phosphorus fertilization in a wheat-chickpea intercropping system

Plant and Soil

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Abstract

Aims: Phosphorus (P) availability affects carbon (C) allocation to roots for nutrient acquisition and biological nitrogen fixation (BNF), which in turn impacts yield. It is unclear how P availability and inter-specific interactions affect these processes in intercropping systems. Methods: A pot experiment was conducted using a factorial randomized block design with four replicates, where wheat and chickpea were grown alone or together, with 44 mg P kg^{-1} (equivalent to 50 kg P ha^{-1}) or without additional P fertilizer. We evaluated yield, BNF based on ^{15}N natural abundance, plant P content, and C allocation to root biomass and respiration. Results: Phosphorus availability strongly modulated the coupling between C allocation and BNF that depended on interspecific interactions. Under intercropping, chickpea exhibited greater increases in yield and P uptake with P fertilization than in monocropping, reflecting enhanced competition for P when intercropped with wheat. Intercropping significantly increased BNF in chickpea that was further marginally increased by P fertilization, while both intercropping and P fertilization sharply increased specific root respiration, indicating higher metabolic investment to support BNF. However, chickpea allocated less C to root biomass under P fertilization and intercropping, suggesting a strategic shift in C partitioning from root growth to BNF. In contrast, wheat maintained C allocation to roots in both cropping systems under P fertilization, highlighting species-specific strategies for nutrient capture and symbiotic relationships. Conclusion: Our findings highlight the importance of optimizing nutrient management for BNF and P uptake in P-deficient soils and in intercropping systems.

Keywords: Intercropping; Biological Nitrogen Fixation; Seed yield; Belowground C allocation; P fertilizer; Root respiration

3.1. Introduction

Phosphorus (P) ranks second to nitrogen (N) as the most limiting macronutrient for crop production (Vance et al. 2000) and plays a crucial role in energy transfer, photosynthesis, and nutrient transport within the plant (Schulze et al. 2006; Zhang et al. 2014). Phosphorus fertilization is frequently required in agricultural systems, and as P fertilizers are derived from mined phosphate rocks, which are non-renewable, their current application rate is anticipated to considerably deplete these supplies within the forthcoming decades (Cordell et al. 2009; Dawson and Hilton 2011). Enhancing P fertilization is imperative for sustainable crop yields, but its efficiency varies considerably among different cropping systems (Yu et al. 2021).

Intercropping can significantly enhance plant P uptake from the soil, thereby decreasing fertilizer application rates, conserving resources, and safeguarding the environment (Bi et al. 2019; Zhao et al. 2025).

Intercropping is an agroecological practice in which two or more crop species or genotypes grow together on the same land for at least part of their growing cycle (Vandermeer 1989), which has potential benefits like a higher overall productivity, better control of pests and diseases and enhanced biodiversity conservation (Lithourgidis et al. 2011; Malézieux et al. 2009). By cultivating diverse crops together that differ morphologically, the use of resources like radiation, water, space, and nutrients can be more efficient (Raza et al. 2022). Enhanced resource acquisition in intercropping systems is attributed to two key interactive mechanisms among component plant species: niche complementarity and facilitation (Fridley 2002; Loreau et al. 2001). Niche complementarity occurs when component species exploit a certain resource variably across time, space, or chemical forms, thereby weakening competition and increasing resource use efficiency (Fridley 2002). In contrast, facilitation occurs when one component species enhances the growth and production of another species by direct or indirect mechanisms that alter the biotic or abiotic environment, resulting in improved resource availability (Callaway 2007). Facilitation can occur in cereal-legume intercropping system whereby N fixed by the legume can support growth of the cereal and reduce its reliance on soil N (Shen and Chu 2004). For instance, maize intercropped with faba bean exhibited a higher root density in proximity to the faba bean roots compared to maize cultivated in monoculture, which was attributed to increased nutrient availability in the faba bean's root zone, thereby facilitating improved resource acquisition by maize in the intercropping system (Zhang et al. 2016). Nevertheless, the benefits of BNF for plant growth should be larger for the legume with direct access to the N fixed compared to the cereal crop that can only acquire N from BNF through transfer from the legume, particularly when BNF is further enhanced with intercropping (Fan et al. 2006; Mei et al. 2021; Singh et al. 2023; Snapp et al. 1998). However, to what degree this facilitation depends on P fertilization remains unclear.

Phosphorus fertilization often results in increased biological N fixation (BNF) in legumes (Dai et al. 2024). Adequate P availability has been demonstrated to boost nodulation, nodule efficiency, and nitrogenase activity, thereby improving atmospheric N fixation (Cabeza et al. 2024). Since plants can benefit from complementarity effects in mixed stands, legumes may benefit in an intercropping system with cereals from P fertilization boosting BNF more than in a monoculture. In a cowpea-maize intercropping system the BNF by cowpea per unit of land area was the same in intercropping as in monoculture (Vesterager et al. 2008), but to our knowledge, no other study has compared the effect of P fertilization on BNF in monocropping and intercropping systems. Biological N fixation is a carbon (C) expensive process (Minchin and Witty 2005) and therefore any C allocated towards BNF could potentially constrain plant growth and yield when limited by other nutrients from the soil such as P.

Carbon allocation towards root biomass formation and specific root respiration plays critical roles in mediating nutrient acquisition and plant-soil interactions in P-deficient soils. Plants can respond to nutrient scarcity or confined nutrient availability by modifying their root biomass and architecture to effectively explore soil regions with the limited nutrient availability (Giehl and von Wirén 2014). For instance, limited soil P availability alters root architectural traits (Bauer et al. 2024) and generally results in a larger proportion of plant biomass allocated towards roots (i.e., increase in root/total mass ratio) compared to soils with abundant P availability (Lopez et al. 2023; Xiao et al. 2024) so that plants have a greater capacity to scavenge for P. The demand for P will be larger for legumes than for cereals when they obtain extra N from BNF, so that that P fertilization would alleviate P limitation more easily for cereal crops than for legumes in intercropping, but it is unclear whether this also results in stronger reductions in the root/total mass ratio for cereal crops. Similarly, in environments with low P availability, plants often allocate more C towards root respiration (Nielsen et al. 2001; Strock et al. 2018), which reflects their metabolic activity. Phosphorus fertilization may therefore influence root respiration rates in cereal-legume intercropping by altering energy requirements for nutrient absorption, but also for BNF. For instance, BNF in legumes can significantly increase root respiration because of the high energy requirements to fix N in root nodules (Mahon 1977; Ryle et al. 1979). Despite the importance of belowground C allocation towards root growth and respiration for nutrient acquisition, they are also significant C-loss pathways for plants that could potentially affect grain yield.

The overall aim of this study was to elucidate how P fertilization affects yield, BNF, and C allocation to root biomass and respiration in legume-cereal intercropping versus monocropping systems in a P-deficient soil. We conducted a greenhouse experiment where we compared monocultures of wheat and chickpea with their mixtures in pots with two levels of P fertilization (0 and 44 mg P kg^{-1} soil or equivalent to 0 and 50 kg P ha^{-1}, respectively). We hypothesized that: 1) intercropping (mixtures) increases the seed yield of wheat and chickpea (after accounting for plant differences in plant densities) and P fertilization results in a larger increase in yield for chickpea than for wheat; 2) BNF in chickpea increases in mixtures and with P fertilization; and 3) P fertilization reduces wheat root/total biomass ratio more than for chickpea, particularly in monocropping when it is not competing for P with chickpea, reduces specific root respiration of wheat, but increases specific root respiration of chickpea as a result of the increase in C-allocation for BNF.

3.2. Materials and Methods

3.2.1. Study location and soil properties

Soil for this study were obtained in August 2022 from an unfertilized grassland at Westwood Farm, located near Camden, New South Wales, Australia (latitude 33°59′46″S, longitude 150°39′16″E). The average annual precipitation is 790 mm with mean temperatures of 10.4 °C in July and 23.0 °C in January for this area (Australian Bureau of Meteorology). The grassland was primarily composed of *Paspalum dilatatum* Poir., a C4 grass species, and experienced moderate grazing. While this site has not been used for cropping, we chose this site for soil sampling because of its very low available P in soil (Dijkstra et al. 2015).

The soil at the location was classified as a sandy loam red Kurosol (or Acrisol in World Reference Base), noted for its significant P-fixing ability (Dijkstra et al. 2015). Samples were collected from a depth of 0–20 cm following the removal of surface vegetation. The gathered soil was air-dried, pulverized, and sieved through a 2 mm mesh to exclude coarse particles and discernible roots. The characteristics of the soil at the start of the experiment are shown in Table S3.1 (supplementary).

3.2.2. Planting and harvesting

We performed a 100-day pot experiment using a factorial randomized block design with four replicates in a wheat-chickpea intercropping system. The experiment included three cropping systems (monoculture of wheat, monoculture of chickpea, and a wheat-chickpea mixture), two P application levels (0 kg ha^{-1} and 50 kg P ha^{-1}), and three harvests (vegetative, flowering, and maturity) with a total of 72 pots (3 cropping systems × 2 P application levels × 4 replicates × 3 harvests). Each of the rectangular polypropylene pots (height: 40 cm, width at the top: 24 cm, width at the bottom: 18 cm) were filled with 6.5 kg of sieved soil (2 mm). In the monocropping system, four plants were grown per pot. In the intercropping system (or mixture), two plants of each species were grown in each pot. We realise that these planting densities may not correspond to planting densities in field experiments where crops are usually planted in strips in intercropping systems. However, here we are mostly interested in plant-plant interactions, and we therefore decided to have the same planting densities for both species in monoculture and mixture treatments to avoid potential biases caused by variation in planting density.

The wheat variety was Suntop, while the chickpea variety was CBA Captain (desi type). Chickpea seeds were inoculated with *Rhizobium (Mesorhizobium cicero)* by combining the inoculant with deionized water to achieve a paste-like consistency. The seeds were uniformly covered with the mixture and air-dried in the shade for 15 to 20 minutes prior to seeding. After germination, all pots were thinned to retain four plants per pot. Phosphorus was provided as monopotassium phosphate (KH_2PO_4). Alongside P fertilization, N (89 mg kg^{-1}), potassium (232 mg kg^{-1}), sulphur (39 mg kg^{-1}), calcium (35 mg kg^{-1}), magnesium (29 mg kg^{-1}), boron (0.09 mg kg^{-1}), zinc (0.4 mg kg^{-1}), copper (0.09 mg kg^{-1}), iron (0.4 mg kg^{-1}), and manganese (0.7 mg kg^{-1}) were applied to all pots as nutrient solutions after the emergence of both species. Although the N fertilizer could potentially suppress BNF in chickpea, we decided to add N fertilizer to all treatments to avoid potential bias caused by N fertilization. We maintained daytime temperatures within the range of 24-26°C and nighttime temperatures between 18-20°C in the glasshouse. The pots were watered every other day to 70% water holding capacity based on weight loss of the pots. The pots were placed in the glasshouse with at least 30 cm distance from one another, while they were supplemented for 12 hrs with LED lights (55 mW cm^{-2}) when natural sunlight was not enough to ensure that plant growth was not limited by light. The experiment was blocked (4 blocks with each block containing one replicate of each treatment combination) due to spatial variation in lighting within the glasshouse.

3.2.3. Sample collection and analysis

Plants were harvested at three distinct growth stages: vegetative (34 days after sowing), flowering (48 days after sowing), and maturity. Although wheat matured several weeks before chickpea, we decided to harvest plants on the same day at 100 days after sowing after chickpea reached maturity. At each stage, the plants were cut at the soil surface. The pots were transported to the laboratory, where the plants were carefully uprooted, and the soil adhering to the roots was rinsed off using fresh water. In mixtures, roots were separated by species based on their morphology. Excess water on the roots was then gently blotted dry using a paper towel. Because wheat and chickpea have very distinct root morphologies (wheat has a fibrous thinner root system with many nodal, primary, and lateral roots that branch extensively from the base of the stem compared to chickpea with a thick taproot with lateral branches) and color (chickpea roots are much lighter in colour compared to wheat), we are confident that we were able to separate roots for these two species successfully. Moreover, in a separate experiment (unpublished) we pulse-labelled wheat and chickpea separately with $^{13}CO_2$ when grown in mixtures, and where we separated roots by species as described above. We then analysed the root biomass for ^{13}C and observed that root biomass of ^{13}C labelled plants were enriched in ^{13}C and non-labelled plants were not, further confirming that our method of separating roots by species was reliable. For soil analysis, representative soil samples were collected from each pot by thoroughly mixing the entire soil content to ensure uniformity.

After flowering, we determined root respiration immediately after roots were harvested, using excised roots, similar to methods used by others (Guo et al. 2021; Hogan et al. 2023; Muratore et al. 2024). Root respiration rates of wheat and soybean were shown to remain stable for up to 3 hours after excision from shoots (Liu et al. 2006), which was within the timeframe of our measurements. We placed live, fresh roots into 500 ml Mason jars with lids containing an injection port for gas sampling. Gas samples were obtained at three intervals: immediately prior to sealing the jars (time zero), and after one and two hours of incubation (time 1 and time 2, respectively). Each time, 30 mL of gas was taken with a syringe, and after expelling the initial 5 mL to purge the syringe, the residual 25 mL was promptly injected into a pre-evacuated 20 mL vial fitted with an auxiliary needle to facilitate the flushing of the vial with the excess gas. Gas samples were measured for carbon dioxide (CO_2) concentration by injecting them into an infrared gas analyzer (IRGA, Model 6262, Li-Cor, Lincoln, NE, USA). Specific root respiration was measured as the amount of µg CO_2 respired per hour per dry weight of root biomass (µg CO_2 h^{-1} g^{-1}).

The harvested plant samples, including shoots, roots (following respiration measurements at flowering stage), and seeds (at maturity stage), were dried at 60 °C for 72 hours to reach a constant weight. The dried samples were ground into a fine powder using a ball mill (Retsch MM 400, Haan, Germany) and analysed for total N and $\delta^{15}N$ on an isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). To determine plant P, we ashed 0.5 g of plant sample in a muffle furnace at 550 °C for 4 hours. We then added 5 mL of 6 N HCl, which was subsequently heated on a hotplate until the volume diminished to 1-2 mL. The extracts were filtered using Whatman #42 filter paper and diluted to 50 mL with deionized water. The inorganic P concentration was measured colorimetrically at a wavelength of 400 nm on a UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan), using ammonium molybdate and ammonium metavanadate as reagents (Jackson 1958). For plants harvested at vegetative stage, we did not have enough plant material for some samples to measure P concentration accurately, and plant P concentrations were therefore only analyzed for plants harvested and flowering and maturity stage.

Fresh representative bulk soil samples were sieved to <2 mm before determination of available N and available P. The sieved soil was extracted immediately following collection using 1 M KCl. The extraction procedure involved shaking the soil samples on a rotary shaker for 1 hour, followed by centrifugation at 4000 rpm for 5 minutes to separate the supernatant. The supernatant was then filtered through Whatman #42 filter paper and analyzed for nitrate (NO_3^-) and ammonium (NH_4^+) using a Flow Injection Analyzer (FIA automated ion analyzer, Lachat Instruments, Loveland, CO, USA). Total available N was calculated as the sum of NO_3^- and NH_4^+ concentrations. The available P in soil samples was determined by extracting sieved soil from each pot with a 0.03 N NH_4F-0.025 N HCl solution (Olsen and Sommers 1982). The resultant extracts were analysed colorimetrically for inorganic P on a UV-VIS spectrophotometer at a wavelength of 660 nm using ammonium paramolybdate and stannous chloride as reagents.

3.2.4. Calculations

We assessed the biological N fixation (BNF) in chickpea using the ^{15}N natural abundance technique, where we used wheat as the reference plant (Unkovich et al. 2008). The percentage of N derived from atmospheric fixation (Ndfa) was calculated using the following equation:

$$\text{Ndfa} = [(\delta^{15}N_{\text{ref}} - \delta^{15}N_{\text{chickpea}}) / (\delta^{15}N_{\text{ref}} - B) \times 100\% \qquad (1)$$

where $\delta^{15}N_{\text{ref}}$ is the $\delta^{15}N$ value of the reference plants (wheat), $\delta^{15}N_{\text{chickpea}}$ is the $\delta^{15}N$ value of the chickpea plants; B is the $\delta^{15}N$ signature of N-fixing chickpea plants completely relying on BNF. For the B value, we used a value of -1.75 ‰ reported for chickpea (Unkovich et al. 2008).

The amount of N fixed (BNF, mg N plant^{-1}) was calculated using the following equation:

$$\text{BNF} = \text{Ndfa}/100 \times \text{ShootN}_{\text{chickpea}} \qquad (2)$$

where $\text{ShootN}_{\text{chickpea}}$ is the shoot N content in chickpea (mg N plant^{-1}).

3.2.5. Statistical analysis

We first used a mixed model to assess main and interactive effects of P fertilizer application (P fert, 0 *vs.* 50 kg P ha^{-1}), cropping system (CS, monoculture *vs.* mixture), and harvest time (H, vegetative, flowering, maturity) on shoot and root biomass (mg plant^{-1}), and root/total mass ratio, for wheat and chickpea separately, and on Ndfa (%) and BNF (mg plant^{-1}) for chickpea. Block was included as a random effect. We conducted similar mixed models for plant P content (mg plant^{-1}), but with only two harvests (flowering and maturity). Plant biomass and P content were expressed per plant to account for differences in plant number in monocultures and mixtures for a given species. To focus more on treatment effects over the whole growing period, we further conducted mixed models to assess the main and interactive effects of P fertilizer application and cropping system on seed weight (g plant^{-1}) and P content (mg plant^{-1}) at plant maturity for wheat and chickpea and on BNF for chickpea at maturity. We further conducted mixed models to assess main and interactive effects of P fertilizer application, cropping system (wheat monoculture, chickpea monoculture, and wheat-chickpea mixture), and harvest time on available N and P (mg kg^{-1}) in the soil. When necessary, data were log-transformed to improve normal distribution and to reduce heteroscedasticity (examined with the Brown-Forsythe test for equal variance). Upon detecting significant treatment interactive effects at $p < 0.05$, pairwise comparisons among treatment combinations were performed using Tukey's Honest Significant Difference (HSD) test. Results with *p*-values ranging from 0.05 to 0.1 were considered marginally significant. All statistical analyses were conducted utilizing JMP Pro software (version 17.0.0; SAS Institute, Cary, NC, USA).

3.3. Results

Phosphorus fertilizer application markedly enhanced seed weight of wheat (on average by 231% across monocultures and mixtures, $p < 0.0001$, Fig. 3. 1A). Wheat grown in monoculture yielded greater seed weights than in mixtures (on average by 55% across P fertilizer treatments, $p = 0.007$). Chickpea seed weight also significantly increased with P fertilizer application (on average by 44% across monocultures and mixtures, $p = 0.002$, Fig. 3. 1B). However, chickpea seed weight only significantly increased in the mixtures causing a significant P fert × CS interaction ($p = 0.02$). Thus, in terms of seed weight, chickpea had a more pronounced beneficial response to P fertilization when intercropped with wheat, while wheat performed less when intercropped with chickpea, signifying species-specific interactions with P fertilization and cropping systems.

Phosphorus fertilizer application increased root biomass of wheat, and the P fertilizer application effect on wheat root biomass became larger when plants matured (significant P fert × H interaction, Table 3. 1). Chickpea root biomass on the other hand was not affected by P fertilizer application, and there were no significant cropping system effects on root biomass of wheat and chickpea. When we considered root biomass relative to total biomass responses (root/total mass ratio) then P fertilizer application had no effect on wheat root/total mass ratio (Table 3. 1). In contrast, P fertilization caused a significant decrease in chickpea root/total mass ratio (on average by 10%, $p = 0.02$), while the chickpea root/total mass ration was larger in monoculture than in mixture across all three harvests (on average by 15%, $p = 0.04$, Table 3. 1).

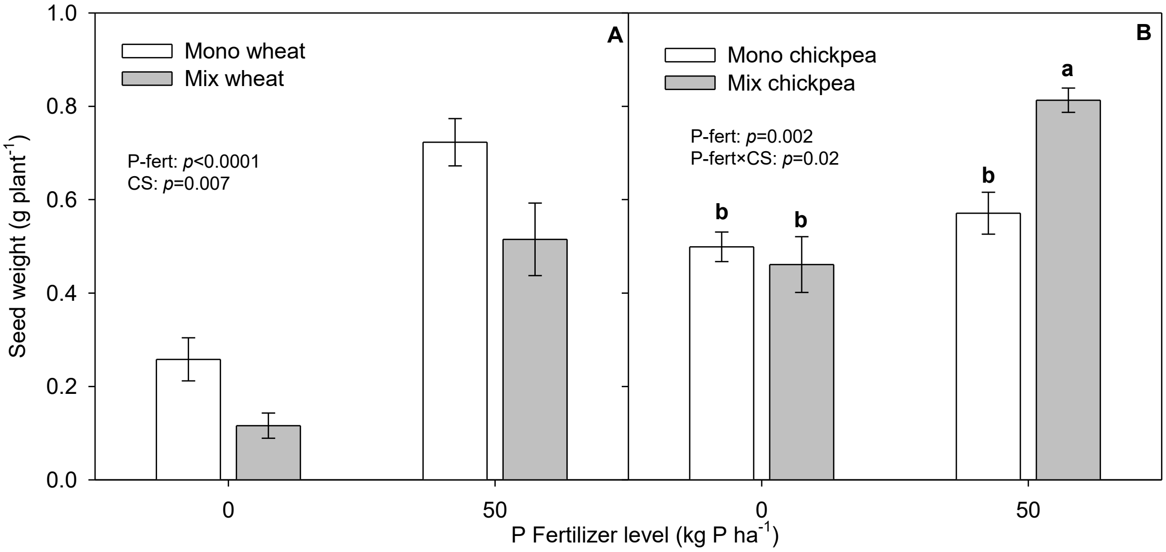

Figure 3.1. Effect of phosphorus (P) fertilizer application (P-fert: 0 and 50 kg P ha-1) and cropping system (CS, Monoculture: Mono and Mixture: Mix) on (A) wheat and (B) chickpea seed weight (g plant^{-1}) at maturity. Error bars represent standard errors. Mixed model *p*-values are shown when <0.05. Letters above bars in panel B indicate differences among treatments based on Tukey’s HSD test.

Table 3. 1. Effect of phosphorus (P) fertilizer application (P fert: 0 and 50 kg P ha-1) and cropping system (Monoculture: Mono and Mixture: Mix) on wheat and chickpea root and shoot biomass (mean ± se), and root/total mass ratio at different harvest times (H1: vegetative stage, H2: flowering stage, H3: maturity stage). Mixed model *p*-values are also shown (H: harvest, P fert: fertilizer application treatment, CS: cropping system).

Harvest	P fert	Cropping system	Root Weight (mg plant^{-1})		Shoot Weight (mg plant^{-1})		Root/total mass ratio	
			Wheat	Chickpea	Wheat	Chickpea	Wheat	Chickpea
H1	0	Mono	20 ± 3	67 ± 2	80 ± 8	383 ± 51	0.20 ± 0.02	0.15 ± 0.02
	50	Mono	43 ± 11	58 ± 8	144 ± 22	354 ± 25	0.22 ± 0.03	0.14 ± 0.02
	0	Mix	26 ± 3	66 ± 7	76 ± 1	335 ± 50	0.25 ± 0.02	0.17 ± 0.03
	50	Mix	46 ± 10	59 ± 3	135 ± 19	408 ± 38	0.25 ± 0.03	0.13 ± 0.01
H2	0	Mono	73 ± 20	346 ± 53	157 ± 17	463 ± 68	0.30 ± 0.03	0.43 ± 0.02
	50	Mono	188 ± 42	421 ± 85	415 ± 124	450 ± 71	0.33 ± 0.03	0.41 ± 0.02
	0	Mix	63 ± 15	384 ± 52	144 ± 19	599 ± 68	0.30 ± 0.04	0.39 ± 0.04
	50	Mix	119 ± 32	289 ± 55	323 ± 73	960 ± 35	0.26 ± 0.02	0.32 ± 0.04
H3	0	Mono	67 ± 7	220 ± 30	322 ± 39	917 ± 71	0.11 ± 0.02	0.15 ± 0.02
	50	Mono	184 ± 27	218 ± 29	809 ± 45	855 ± 26	0.11 ± 0.01	0.13 ± 0.02
	0	Mix	67 ± 7	249 ± 79	191 ± 23	925 ± 103	0.19 ± 0.03	0.15 ± 0.03
	50	Mix	201 ± 65	219 ± 50	619 ± 69	1150 ± 135	0.14 ± 0.03	0.11 ± 0.02
p values		H	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
		P fert	<0.0001	0.6	<0.0001	<0.0001	0.7	0.02
		CS	0.1	0.6	0.01	0.01	0.2	0.04
		H×P fert	0.04	0.9	<0.0001	<0.0001	0.7	0.9
		H×CS	0.3	0.5	0.03	0.03	0.04	0.1
		P fert×CS	0.1	0.1	0.6	0.6	0.2	0.2
		H×P fert×CS	0.5	0.3	0.9	0.9	0.9	0.7

The P content in root and shoot P biomass of wheat and chickpea across the flowering and maturity stages showed variable responses to the P fertilizer and intercropping treatments (Table S3. 2), and here we will focus on P content at maturity as a measure of plant P uptake over the whole growing period. Total P content in wheat at maturity significantly increased with P fertilizer application (on average by 123% across cropping systems, $p = 0.003$) with the majority of P recovered in seed (Fig. 3. 2A). Total P content in wheat was significantly lower in mixtures than in monocultures (on average by 71% across P fertilizer treatment, $p = 0.02$). Root P content at maturity also increased with P fertilization, but particularly in the monocultures (significant P fert × CS interaction, $p = 0.008$). Total P content in chickpea seeds also significantly increased with P fertilizer application (on average by 53% across cropping systems, $p = 0.003$), and the increase was higher in mixtures than in monocultures, although we observed no significant P fert × CS interaction (Fig. 3. 2B). Similarly, root P content at maturity significantly increased with P fertilizer application, particularly in mixtures causing a significant P fert × CS interaction ($p = 0.01$).

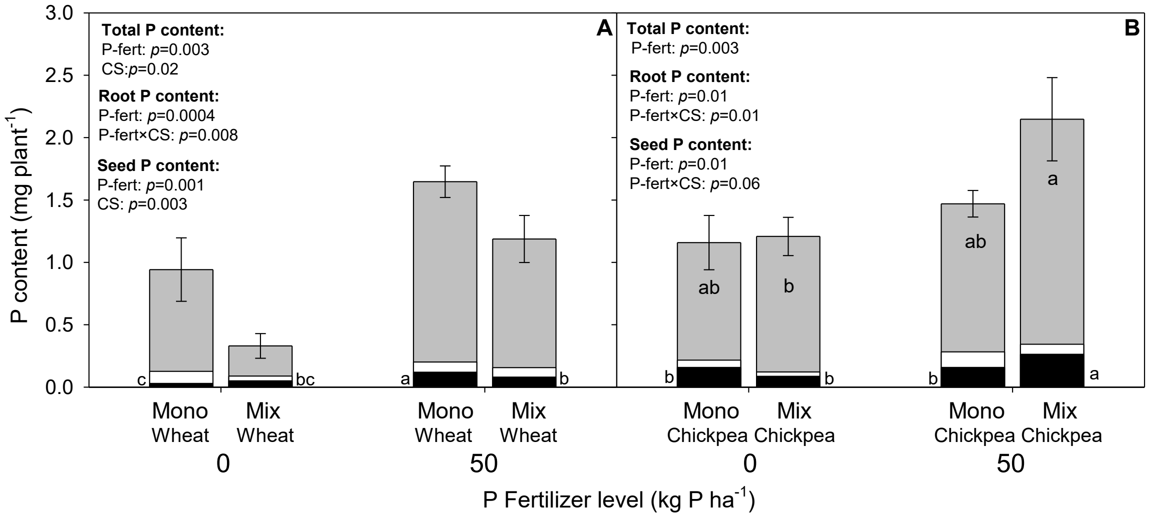

Figure 3. 2. Effect of phosphorus fertilizer application (P-fert: 0 and 50 kg P ha-1) and cropping system (CS, Monoculture: Mono and Mixture: Mix) on root (black), shoot (white), seed (grey) and total P content (mg plant^{-1}) in (A) wheat and (B) chickpea at maturity. Error bars represent standard errors. Mixed model p-values are shown when <0.05. Lettering next to bars in panel A and B indicate differences among treatments in root P content, and inside bars in panel B indicate differences among treatments in seed P content based on Tukey's HSD test.

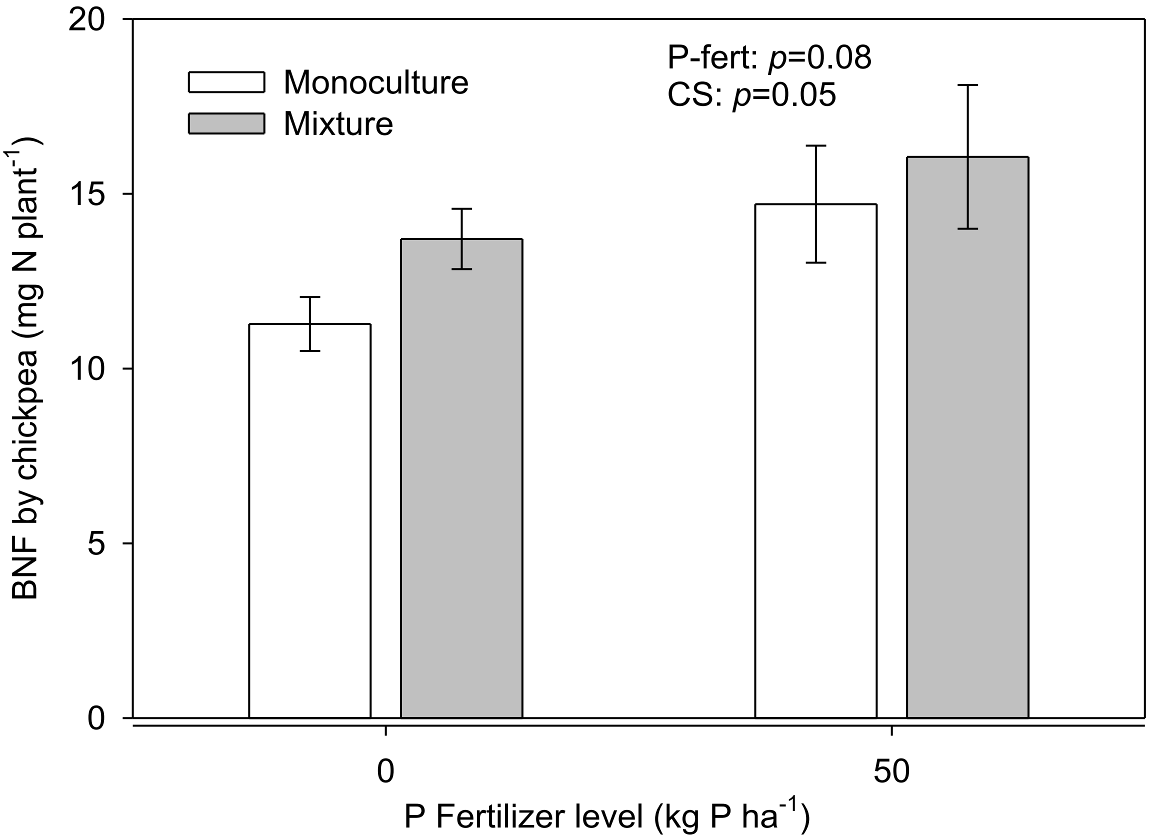

Figure 3. 3. Effect of phosphorus (P) fertilizer application (P-fert: 0 and 50 kg P ha-1) and cropping system (CS: Monoculture and Mixture) on biological nitrogen fixation (BNF, mg N plant-1) by chickpea at maturity. Error bars represent standard errors. Mixed model *p*-values are shown when <0.05.

The percentage of N derived from atmospheric fixation in chickpea shoot biomass (Ndfa) increased with time (significant harvest treatment effect, $p < 0.0001$) but was not significantly affected by P fertilization and cropping system (Table 3. 2). At maturity between 44 and 55% of chickpea shoot N content was derived from N fixation. Likewise, the BNF increased with time ($p < 0.0001$), but was also higher for chickpea in mixtures (p = 0.04) across the three harvests (Table S3). When focussing over the whole growing period, the BNF in chickpea at maturity slightly increased with P fertilization (on average by 23% across cropping system, $p = 0.08$) and increased in mixtures compared to monocultures (on average by 15% across P fertilizer treatments, $p = 0.05$, Fig. 3.3).

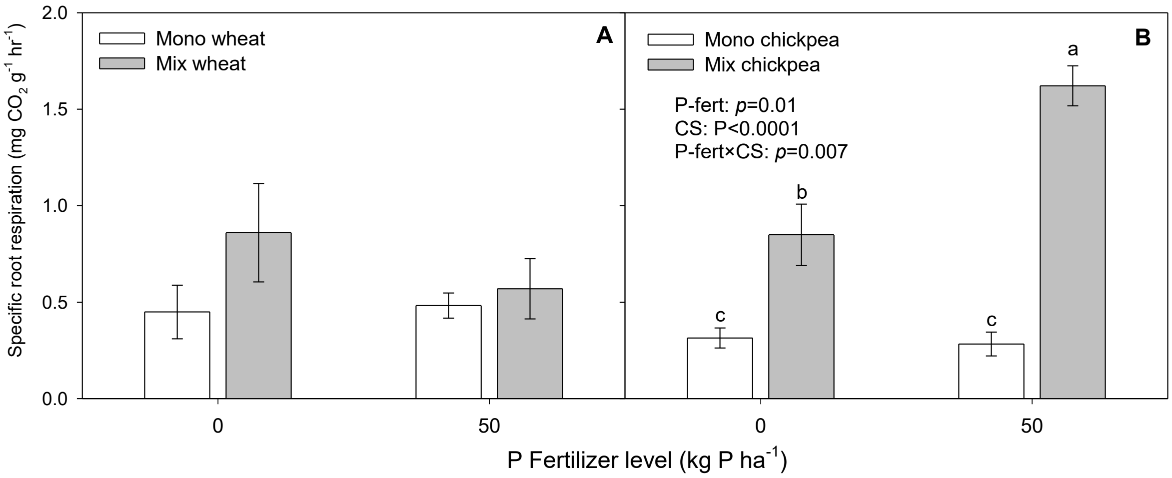

Figure 3. 4. Effect of phosphorus (P) fertilizer application (P-fert: 0 and 50 kg P ha-1) and cropping system (CS, Monoculture: Mono and Mixture: Mix) on specific root respiration (mg CO_2 g-1 hr^{-1}) by (A) wheat and (B) chickpea at flowering. Error bars represent standard errors. Mixed model *p*-values are shown when <0.05. Letters above bars in panel B indicate differences among treatments based on Tukey's HSD test.

Specific root respiration in wheat was not significantly influenced by P fertilization (Fig. 3. 4A). Although specific root respiration by wheat on average increased by 54% in mixtures compared to monocultures, the increase was not significant. In contrast, specific root respiration in chickpea significantly increased with P fertilization (p = 0.01, Fig. 3. 4B). However, similar to seed weight, the increase only occurred in mixtures, causing a significant P fert × CS interaction (p = 0.007). Notably, specific root respiration rates in chickpea in the mixtures with P fertilization were the highest between species and treatments.

Available N in the soil decreased with harvest time (p = 0.05), and it decreased with P fertilizer application (p = 0.009), particularly at later stages of plant growth (significant P fert × Harvest interaction, p = 0.04, Table 3. 2). However, available N was not affected by cropping system (wheat monoculture, chickpea monoculture, wheat-chickpea mixture). Not surprisingly, available P significantly increased with P fertilizer application (p < 0.0001), and it decreased with harvest time (p = 0.02), but as with available N, cropping system had no effect on available P in soil (Table 3. 2).

Table 3. 2. Effect of phosphorus (P) fertilizer application (P fert: 0 and 50 kg P ha-1) and cropping system (C: chickpea monoculture, W: wheat monoculture, W-C wheat-chickpea mixture) on extractable nitrogen, extractable phosphorus in the bulk soil (mean ± se) and nitrogen derived from atmospheric fixation (Ndfa) (mean ± se) by chickpea at different harvest times (H1: vegetative stage, H2: flowering stage, H3: maturity stage). Mixed model *p*-values are also shown (H: harvest, P: fertilizer application treatment, CS: cropping system treatment).

Harvest	P fert	Cropping System	Extractable N (mg kg^{-1})	Extractable P (mg kg^{-1})	Ndfa (%)
H1	0	C	56.4 ± 9.6	1.70 ± 0.2	34 ± 6
	50	C	36.9 ± 3.9	5.10 ± 0.4	26 ± 6
	0	W	52.8 ± 2.9	2.50 ± 0.9	-
	50	W	50.5 ± 7.7	4.20 ± 0.7	-
	0	W-C	46.4 ± 4.3	1.70 ± 0.3	44 ± 10
	50	W-C	46.9 ± 4.4	5.50 ± 0.9	33 ± 8
H2	0	C	34.4 ± 3.3	0.60 ± 0.2	30 ± 2
	50	C	37.0 ± 3.1	4.90 ± 0.5	34 ± 5
	0	W	58.7 ± 4.8	0.90 ± 0.1	-
	50	W	47.0 ± 9.5	4.00 ± 1.0	-
	0	W-C	39.2 ± 6.7	0.60 ± 0.2	32 ± 2
	50	W-C	36.7 ± 5.8	4.30 ± 0.9	22 ± 6
H3	0	C	35.6 ± 2.4	1.20 ± 0.2	44 ± 2
	50	C	28.2 ± 4.4	3.50 ± 0.8	55 ± 2
	0	W	53.5 ± 3.2	1.10 ± 0.3	-
	50	W	43.5 ± 2.7	4.20 ± 0.9	-
	0	W-C	39.0 ± 5.8	1.40 ± 0.3	49 ± 1
	50	W-C	35.3 ± 5.5	4.40 ± 0.4	54 ± 2
p values		H	0.05	0.02	<0.0001
		P	0.009	<0.0001	0.6
		CS	<0.0001	0.9	0.6
		H×P	0.04	0.4	0.06
		H×CS	0.3	0.9	0.2
		P×CS	0.4	0.4	0.2
		H×P×CS	0.1	0.5	0.2

3.4. Discussion

3.4.1. Seed yield and P acquisition

Contrary to expectations, wheat seed yield decreased under intercropping relative to monocultures (Fig. 3. 1A), therefore only partially corroborating our hypothesis that intercropping improves seed yield in both wheat and chickpea through complementary resource utilisation. This indicates that chickpea demonstrated competitive superiority over wheat, perhaps owing to its enhanced capacity to acquire or access P and other resources. The lower P content in wheat under intercropping (Fig. 3. 2) further substantiated its reduced capacity for P uptake. These findings contest the prevalent idea that cereals gain advantage from intercropping with legumes due to less N competition (Hauggaard-Nielsen and Jensen 2005). Although legumes might diminish interspecific competition for N via BNF, our data suggest that chickpea was a strong competitor for other resources that reduced growth of wheat in intercropping. We should note that we used the same planting density for chickpea and wheat in our study, but that under field conditions wheat planting densities are usually higher compared to chickpea, and that competition by chickpea may therefore be less strong under such conditions.

We further hypothesized that yield of chickpea in intercropping would increase more with P fertilization. This was corroborated by our results, where chickpea yield was higher in mixtures than in monoculture only with P fertilization (Fig. 3. 1B), suggesting that its competitive advantage over wheat was heightened with increased P availability. This is consistent with other research indicating that legumes, with their capacity to fix atmospheric N, particularly exhibit sensitivity to P deficiency (Divito and Sadras 2014). The largest P accumulation occurred in the seed, which was further increased by P fertilization, highlighting the significance of P in producing grain (Fig. 3. 2A). These results confirm earlier studies demonstrating that P fertilization facilitates P movement from root to aboveground organs (Sun et al. 2008), thereby augmenting seed yield and nutritional composition. The increase in P content in chickpea under intercropping (Fig. 3. 2) with P fertilization provides evidence that chickpea performance is closely linked to P availability, and that P fertilization enhances its competitiveness within the mixture.

3.4.2. Biological nitrogen fixation

We observed a small and marginally significant increase in BNF with P fertilization (Fig. 3. 3), providing limited support for our second hypothesis (BNF in chickpea increases with P fertilization), although P fertilization did not significantly enhance the percentage of N derived from the atmosphere (Ndfa) in chickpea shoots (Table 3. 2). Phosphorus is considered vital for nodulation and N fixation in legumes (Chen et al. 2019; Mitran et al. 2018; Qin et al. 2012), however, its small effect in this study may indicate the influence of other variables, such as N availability in the soil because P enhanced N fixation in legumes only at low N availability (Leidi and Rodríguez-Navarro 2000). For instance, available N remained high throughout the experiment (Table 3. 2), which may have suppressed BNF, particularly at early stages of plant growth, as indicated by the relatively low Ndfa (no more than 44% of shoot N was derived from BNF during vegetative and flowering stage, Table 3. 2). Nevertheless, the small increase in BNF likely contributed to the increased chickpea yield with P fertilization. This corresponds with findings from previous studies indicating that P might indirectly enhance BNF by facilitating root and nodule development, encouraging plant growth, and supporting rhizobial symbiosis (Almeida et al. 2000; Ge et al. 2025; Peoples et al. 1998; Qiang et al. 2021; Yeremko et al. 2025).

In support of our second hypothesis, chickpea in mixtures demonstrated significantly enhanced BNF at maturity relative to monocultures (Fig. 3. 3). This result was supported by previous studies indicating increased N fixation in intercropping systems (Singh et al. 2023; Snapp et al. 1998), presumably attributable to higher nutrient availability, synergistic root interactions, and beneficial soil microbial populations. In our study, the reduced growth of wheat when intercropped with chickpea may have provided chickpea with more resources to fix N than when grown alone. Our findings are in line with previous research, which indicated significantly greater reliance on BNF by legumes when intercropped with cereals (Bedoussac et al. 2015). Although chickpea yield in mixtures responded more strongly to P fertilization than in monoculture, we observed no significant interaction between cropping system and P fertilization for BNF. This suggests that the chickpea yield response to P fertilization in mixtures cannot be solely contributed to BNF, but that other factors have played a role.

Not only was BNF increased at maturity, but the percentage of N obtained via atmospheric fixation (Ndfa) in chickpea shoots considerably increased over time, demonstrating the dynamic characteristics of BNF throughout growth. Our results support earlier research (Yun et al. 2023) highlighting that nodule production and BNF in legumes intensify during the vegetative phase and reach their peak at reproductive stage (maturity), resulting from an increased N demand for growth and reproduction.

3.4.3. Carbon allocation to root biomass and respiration

In contrast to our third hypothesis, P fertilization did not decrease root/total mass ratio of wheat, but instead, decreased the root/total mass ratio of chickpea, with overall smaller ratios in mixtures than in monocultures (Table 3. 1). We expected that P fertilization would relieve P limitation to wheat, which would translate into proportionally smaller C allocation towards roots as has been observed by others (Lopez et al. 2023; Xiao et al. 2024). It is possible that increased competition for P with chickpea caused wheat to maintain the same root/total mass ratio as without P fertilization in mixtures, but this does not explain why root/total mass ratio of wheat was not affected by P fertilization in monoculture. It is also possible that plant growth remained P limited after P fertilization. All pots were fertilized with other nutrients, so it is possible that with the amount of P that was added did not switch limitation to another nutrient. Although the extractable P was elevated with P fertilization, values remained low and were considerably lower than extractable N (Table 3. 2), suggesting that plant growth may have remained P limited even with P fertilization.

We further expected that P demand by chickpea would be sustained with P fertilization, particularly in mixtures, because of an increase in BNF that would maintain P limitation in chickpea with P fertilization. We are therefore unclear why the root/total mass ratio of chickpea decreased with P fertilization and in mixture, but it suggests that there may be a trade-off between C allocation towards BNF and root growth (Friel and Friesen 2019). Despite the reduced root/total mass ratio with P fertilization and in mixtures, as discussed above, chickpea yield and P uptake benefited more from P fertilization when grown in mixtures, and suggests that other factors played a role. One possibility to improve P availability and uptake is through root exudation (Dakora and Phillips 2002). Unfortunately, we were not successful in measuring root exudation rates accurately. We also did not assess mycorrhizal colonization in our study, which may have played a significant role in the effect of P fertilization on wheat and chickpea yield, P uptake and belowground C allocation towards root growth in intercropping systems (Song et al. 2021; Xu and Liu 2024).

Similar to root/total mass ratio, we observed no effect of P fertilization on specific root respiration in wheat (Fig. 3. 2A), which contrasts with what we hypothesized. This finding indicates that wheat may exhibit a relatively stable respiratory activity to different P levels. It was suggested that under low P availability, common bean allocate a greater proportion of C to root respiration due to an increase in maintenance respiration (Nielsen et al. 1998; Nielsen et al. 2001). We are not aware of studies where this was assessed for wheat, but our results suggest that specific root respiration by wheat may not be as sensitive to P fertilization. Although not significant, specific root respiration in wheat was higher when grown in mixtures than in monoculture, which could indicate stress adaptation in competitive intercropping systems, consistent with previous research on the role of root respiration related to maintenance, growth and nutrient uptake by the root (Poorter et al. 1991; Reich et al. 1998; Rewald et al. 2016).

Conversely, as we hypothesized, chickpea increased specific root respiration in response to P fertilization, but this increase was limited to mixtures only (Fig. 3. 4b). The increase in specific root respiration in chickpea could be associated with BNF, which increased with P fertilization and intercropping (Fig. 3. 3). This corresponds with earlier research indicating that BNF is a C-intensive process, wherein a significant fraction of photo-assimilates is directed towards nodules and roots (Gordon 1987), much of which is ultimately released as CO_2 via root respiration (Voisin et al. 2003). Therefore, the notably increased specific root respiration revealed in chickpea mixtures likely indicates enhanced BNF. While chickpea yield may benefit from BNF with P fertilization and intercropping, they also lose a significant amount of assimilated C through root respiration.

3.5. Conclusion

This study highlights important mechanisms that influence the balance between C costs for acquiring nutrients and producing yield in intercropping systems and how this is affected by P fertilization. We showed that when grown on a P-deficient soil, wheat will underperform when grown with chickpea, and performance will not improve with P fertilization. While P fertilization significantly increased wheat yield, both in monoculture and mixtures, the C allocated to root biomass relative to the total plant biomass remained unchanged, suggesting the wheat growth remained P limited with P fertilization. However, wheat yield was significantly reduced when grown with chickpea, potentially as a result of more allocation of C to processes with the view to enhance competitiveness with chickpea such as specific root respiration, at the expense of yield. In contrast, chickpea yield only increased with P fertilization when grown with wheat, despite a decrease in C allocated to root biomass relative to the total plant biomass. Chickpea yield was higher with P fertilization in mixtures likely because of its proportionally greater C allocation to support in BNF, as evidenced by higher BNF and specific root respiration in mixtures, at the expense of C allocation for root growth.

Our findings underscore the necessity for optimised, species-specific P management to enhance yield and resource use efficiency in intercropping systems in P deficient conditions and highlight the importance of assessing these responses in other soil types and legume-cereal crop combinations.

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Competing Interests

Authors declare no competing interests

3.6. References

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Supplementary for Chapter 3

Table S 3. 1. Soil properties and nutrient composition at the start of the experiment.

Soil Property	**Value**	**Soil Property**	**Value**
Sand (%)	63	Extractable NH_4^+ (mg N kg^{-1})	4.2
Silt (%)	19	Extractable NO_3^- (mg N kg^{-1})	17.2
Clay (%)	18	Extractable P (mg kg^{-1})	1.9
pH (H_2O)	5.8		
Total C (%)	5.6		
Total N (%)	0.4		
Total P (%)	0.14		

Chapter 4: Ammonium-nitrate uptake in cereal-legume intercropping varies with phosphorus availability: A ^{15}N labelling approach

Abstract

Optimizing nitrogen (N) uptake in cereal-legume intercropping systems is critical for improving nutrient use efficiency in nutrient-deficient soils. A ^{15}N labelling pot experiment was conducted using a factorial randomized block design in a wheat–chickpea intercropping system. In this study we investigated species-specific ^{15}N recovery in wheat and chickpea after ^{15}N-labelled ammonium ($^{15}NH_4^+$) and nitrate ($^{15}NO_3^-$) addition under two phosphorus (P) fertilization levels in intercropping systems. We hypothesized that (H1) wheat will exhibit greater competitiveness for $^{15}NH_4^+$ and $^{15}NO_3^-$ than chickpea, as chickpea can depend on biological nitrogen fixation (BNF) to meet its N requirements; (H2) wheat will particularly enhance its competitiveness for $^{15}NH_4^+$ with P fertilization, due to its inherently more fibrous root architecture in comparison to chickpea. Our findings partially support H1, as wheat consistently exhibited greater ^{15}N recovery after $^{15}NO_3^-$ addition than chickpea, particularly with P fertilization, consistent with chickpea's capacity to supplement N demand through BNF. However, wheat and chickpea showed similar ^{15}N recovery after $^{15}NH_4^+$ addition. H2 was also partially supported where P fertilization enhanced ^{15}N recovery in both species, but where wheat's competitive advantage was reinforced primarily for $^{15}NO_3^-$ rather than $^{15}NH_4^+$, suggesting that P availability strengthens overall mineral N acquisition without shifting uptake preference. Root traits such as higher specific root length (SRL) and root tip density in wheat were positively associated with both $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake, underscoring the importance of fine root morphology for accessing both N forms. In conclusion, our results highlight how species-specific root strategies and nutrient interactions shape N uptake, providing guidance for optimizing cereal–legume intercropping and breeding for improved nutrient efficiency in low-input cropping systems.

Keywords: Phosphorus, ^{15}N labelling, ammonium, nitrate, biological nitrogen fixation, root morphology

4.1. Introduction

Intercropping is the cultivation of two or more crops on the same piece of land simultaneously for at least part of their life cycle. It is considered a cornerstone of ecological intensification and sustainable agriculture. Among various intercropping systems, cereal-legume combinations are widely recognized for their potential to enhance resource use efficiency, increase profitability, improve soil fertility, and sustain crop productivity with reduced external inputs (Malézieux et al., 2009, Chen et al., 2015; Hong et al., 2017, Zhang and Li, 2003). These systems capitalize on functional diversity between plant species, particularly in nutrient acquisition and root architecture. Intercropping a cereal with a legume, can result in more efficient use of soil nutrients such as nitrogen (N) and phosphorus (P), especially in resource-limited environments. For instance, in cereal-legume mixtures, non-legume species benefit from reduced N competition due to the legume's reliance on biological N fixation (BNF) (Ramirez-Garcia et al. 2014). However, it remains unclear how cereals and legumes in mixtures compete for available N in the soil under variable P availability.

Nitrogen is a critical macronutrient for plant growth and development and is taken up by plants mainly in two inorganic forms: ammonium (NH_4^+) and nitrate (NO_3^-). These forms differ significantly in their chemical behavior, mobility in soil, and interaction with plant root systems. Nitrate is usually the most abundant source of N in anionic form, is readily dissolved in soil water, and being more mobile, can move easily through the soil matrix to reach plant roots through mass flow (Lambers et al. 2008; Li et al. 2013). In contrast, NH_4^+ is less mobile due to its tendency to bind to soil particles and plant uptake is more limited by diffusion (Lambers et al. 2008; Li et al. 2013), while a closer root proximity and more intensive soil exploration allows for more effective uptake. Therefore, the efficiency of resource acquisition of NH_4^+ and NO_3^- is primarily influenced by root morphological traits (root length and diameter) and root architecture (like number of root tips, branching pattern) (Barber 1995; Caldwell 1987; Wang et al. 2006). Cereal crops like wheat tend to have a fibrous root system which is structurally equipped to explore soil taking up water and nutrients in a greater extent (Zadworny 2011). Legumes like chickpea are characterized by a taproot-dominated structure, featuring first-order lateral roots along with densely or sparsely distributed second-order branches (Chen et al. 2017). Typically, legumes may rely more on atmospheric N fixation by the symbiosis with *Rhizobium* bacteria (Guo et al. 2023), but the extent of biological N fixation by legumes is highly sensitive to environmental conditions and varies across legume species

(Guinet et al. 2018; Romanyà and Casals 2020). Among these factors, phosphorus (P) availability is critical, as BNF is an energy-intensive process dependent on adequate ATP supply. Under sufficient P, plants allocate more photosynthetically derived carbohydrates to root nodules, promoting nodule formation and function, enhancing BNF efficiency, and ultimately increasing plant N acquisition (Chen et al., 2019; Pueyo et al., 2021). Owing to this context dependency, legumes may not consistently offer benefits to companion crops in mixture systems and, in some cases, may compete with them for resources. In addition to monocropping, intercropping legumes with other species can enhance competition for N by non-legumes (Ramirez-Garcia et al. 2014) or result in complementarity in N use in the long-term, as more of the fixed N is shared with companion crops over time (Kebede 2021).

Phosphorus is a key macronutrient for plants, playing a significant role in growth and development, the regulation of enzymatic activities, and energy transfer processes (Lambers et al. 2008), but also in regulating the efficiency of BNF and associated plant–microbe interactions. Consequently, variation in P availability has the potential to alter N acquisition strategies and competitive dynamics in cereal-legume intercropping systems. In legumes, a strong association exists between P availability and BNF and root nodulation (Almeida et al. 2000; Schulze et al. 2006). Consequently, supplementing P-deficient soils with P has shown to significantly enhance nodulation and biomass accumulation in legume species (Míguez-Montero et al. 2020), including chickpea in intercropping (Xia et al. 2013). When P fertilization increases BNF in the legume, then this could potentially relieve competition for available N in the soil with the cereal in cereal-legume intercropping systems. However, N uptake may also depend on how plant root growth responds to P fertilization in P-deficient soils. The more fibrous root systems of cereal crops are particularly conducive for P uptake and their root growth may therefore respond more strongly to P fertilization compared to the more taproot-dominated structures of legumes (Lai and Lawton 1962; Zhao et al. 2025). Cereal crops may therefore benefit more from P fertilization than legumes, which could then potentially also increase their demand and competitiveness for available N in the soil.

Understanding how P availability modulates N uptake dynamics and plant competitiveness is therefore crucial for designing efficient intercropping systems. The influence of P availability on the forms of N uptake, specifically NH_4^+ and NO_3^-, as well as its role in mediating competitive interactions between cereals and legumes for these N forms and their contribution to N cycling within intercropping systems, remains insufficiently understood. In this context, the application of ^{15}N-labelled fertilizers offers a powerful isotopic approach to trace and quantify N acquisition and competition in plants. By supplying either $^{15}NH_4^+$ or $^{15}NO_3^-$ under controlled P availability in cereal-legume mixtures, it will be possible to measure the relative influence of each N form to plant N nutrition and find out species-specific acquisition patterns and their competition in intercropping systems. Measuring ^{15}N recovery in both shoots and roots enables a more complete understanding of N partitioning, mobility, and assimilation.

The aims of this study were to investigate the ^{15}N recovery in root and shoot tissues of intercropped wheat and chickpea without (0 mg P kg^{-1} soil or equivalent to 0 kg P ha^{-1}) and with P fertilization (50 kg P ha^{-1} or equivalent to 44 mg P kg^{-1} soil) to compare the uptake of $^{15}NH_4^+$ and $^{15}NO_3^-$ between the two species, and to relate these uptake patterns to species-specific root traits to elucidate the physiological basis of competitive nutrient acquisition in a P-limited soil. We hypothesize that (H1) Wheat would be more competitive for $^{15}NH_4^+$ and $^{15}NO_3^-$ compared to chickpea because chickpea can rely on BNF for its N demand; (H2) with P fertilization, wheat would become more competitive for $^{15}NH_4^+$ in particular, due to its inherently more fibrous root structure compared to chickpea. Findings from this study will contribute to optimizing cereal-legume intercropping strategies for nutrient-limited environments and guide management practices targeting improved nutrient use efficiency.

4.2. Materials and Methods

4.2.1. Soil sampling and processing

The soil was collected from a grassland at Westwood farm, Camden, New South Wales, Australia (latitude 34.033290S, longitude 150.658110E) dominated by *Paspalum dilatatum* Poir., a C4 grass species, which was subjected to moderate grazing. Although the site has not been used for cropping, it was selected for soil sampling due to its exceptionally low levels of inherent available P (Dijkstra et al. 2015). The area receives an average annual precipitation of 790 mm, with mean temperatures of 10.4 °C in July and 23.0 °C in January (Australian Bureau of Meteorology). The soil was taken from the upper horizon (0-0.2 m). The texture was 18% clay, 19% silt, 63% sand with a pH of 5.8. The soil contained extractable concentrations of 17.2 mg kg^{-1} NO_3^--N, 4.2 mg kg^{-1} NH_4^+-N, and 1.9 mg kg^{-1} P. The soil total carbon (C), N, and P contents were 5.6%, 0.4%, and 0.14%, respectively. Collected soils were air dried and sieved through a 2 mm mesh to remove roots, gravel, stones, and other debris. All pots were filled with approximately 2.5 kg of sieved soil prior to planting. The experiment was conducted in a glasshouse at the University of Sydney in Camden between the months of May and September in 2023.

4.2.2. Experimental setup

For the ^{15}N labelling experiment we conducted a pot experiment using a factorial randomized block design with a wheat-chickpea intercropping system. The experimental treatments included a wheat-chickpea mixture with two P application levels (0 kg P ha^{-1} and 50 kg P ha^{-1}) which were 0 and 44 mg P kg^{-1} soil or equivalent, respectively and two ^{15}N forms applied ($^{15}NH_4^+$ $^{15}NO_3^-$). There were 16 experimental units (four treatments and four replicates). We further included 8 extra pots grown with the same wheat-chickpea mixture (4 pots with 0 kg P ha^{-1} and 4 pots with 50 kg P ha^{-1}) for measuring background ^{15}N concentrations and estimating BNF in chickpea. These plants were harvested at 48 hours after ^{15}N labelling (after 47 days of planting). Seeds of wheat (*Triticum Aestivum* L. cv Hispanic) and chickpea (*Cicer arietinum* L.) were sown on May 20, 2023, to a P-deficient soil on the plastic pot. The pots had an inner diameter of 20 cm and 15 cm height. The wheat variety used was 'Suntop', and the chickpea variety was 'CBA Captain' (desi type). Chickpea seeds were inoculated with *Rhizobium* (*Mesorhizobium ciceri*) by mixing the inoculant with deionized water to form a paste-like consistency. Seeds were uniformly coated with this mixture and air-dried in the shade for 15 to 20 minutes before planting. Following germination, all pots were thinned to retain two wheat and two chickpea plants per pot.

Phosphorus was supplied as monopotassium phosphate (KH_2PO_4) to half the pots (50 kg P ha^{-1}). Alongside P fertilization, all pots received supplementary nutrients after seedling emergence, including nitrogen (89 mg kg^{-1}), potassium (232 mg kg^{-1}), sulphur (39 mg kg^{-1}), calcium (35 mg kg^{-1}), magnesium (29 mg kg^{-1}), boron (0.09 mg kg^{-1}), zinc (0.4 mg kg^{-1}), copper (0.09 mg kg^{-1}), iron (0.4 mg kg^{-1}), and manganese (0.7 mg kg^{-1}) supplied as nutrient solutions. Although the addition of nitrogen can potentially reduce biological nitrogen fixation (BNF) in chickpea, a uniform N supply was applied across treatments to maintain consistent nutrient conditions and prevent bias attributable to N availability. The glasshouse conditions were maintained at daytime temperatures of 24-26 °C and nighttime temperatures of 18-20 °C. The pots were watered every other day to maintain 70% of water holding capacity throughout the growing period of the experiment by manual watering each pot based on weight loss. Soil moisture was maintained at 70% of maximum water holding capacity (WHC), which was determined using the funnel and filter paper method as described by Robertson and Hamilton (2015).

4.2.3. ^{15}N pulse labelling

To trace N uptake pathways, different ^{15}N-labelled N sources were applied 45 days after planting when all plants were still at vegetative stage. ^{15}N was injected to each of the pots on the same day. We injected 10ml ^{15}N solution per pot (including 5 injections of 2 ml to 5 cm soil depth). Half of the pots received 4 mg N pot^{-1} as $(^{15}NH_4)_2SO_4$ (98 atom% ^{15}N) and 2 mg N pot^{-1} as $K^{14}NO_3$. The other half received 2 mg N pot^{-1} as $K^{15}NO_3$ (98 atom% ^{15}N) and 4 mg N pot^{-1} as $(^{14}NH_4)_2SO_4$. We decided to add more ^{15}N labelled NH_4^+ than ^{15}N labelled NO_3^-, because NH_4^+ is less mobile in the soil compared to NO_3^-. The ^{15}N labelling period lasted 24 hours. Two days after ^{15}N injection, plants were clipped and shoots and roots were collected separately for each species. A limitation of this study was that, despite applying NH_4^+ and NO_3^- treatments separately, rapid nitrification in the soil may have converted some of the applied NH_4^+ to NO_3^- prior to plant uptake, potentially reducing the distinction between nitrogen forms. Root samples were separated by species based on differences in morphology. Roots were analysed for root architecture (see below) and then, together with shoot biomass, oven-dried at 60 °C for 72 hours. After weighing the root and shoot biomass, the dried samples were ground with a ball mill and packed into tins and then analysed for ^{15}N and total N on a Delta V Advantage isotope ratio mass spectrometer (IRMS) with a Conflo IV interface (Thermo Fisher Scientific, Bremen, Germany). The extra 8 pots for estimating background ^{15}N concentrations and BNF were harvested after 47 days of planting. After clipping aboveground plant biomass was separated into shoot and seed biomass for each species and analysed for ^{15}N and total N as above.

4.2.4. Soil nutrient analysis

Fresh representative soil samples were sieved to <2 mm prior to analysis of available nitrogen (N) and phosphorus (P). Immediately after collection, sieved soil was extracted with 1 M KCl to determine available N (extractable NH_4^+ and NO_3^-). The extraction procedure involved shaking the soil on a rotary shaker for 1 h, followed by centrifugation at 4000 rpm for 5 min. The supernatant was filtered through Whatman No. 42 filter paper and analyzed for NO_3^- and NH_4^+ concentrations using a Flow Injection Analyzer (FIA automated ion analyzer, Lachat Instruments, Loveland, CO, USA). Total available N was calculated as the sum of NO_3^- and NH_4^+.

Available P was determined following the Olsen method (Olsen and Sommers 1982). Briefly, sieved soil from each pot was extracted with a 0.03 N NH_4F–0.025 N HCl solution, and the extracts were analyzed colorimetrically on a UV-VIS spectrophotometer at 660 nm using ammonium paramolybdate and stannous chloride as reagents.

4.2.5. Root Morphological Traits

The roots from the 16 labelled pots were washed with deionized water. Regarding root sampling, we clarify that a single intact root system per species was selected and scanned from the two plants grown per species in each pot. Care was taken to excavate and recover the entire root system, which was scanned without subsampling with an Epson V700 flatbed scanner at 1200 dpi and analysed with WinRhizo v. 2009c software (Regent Instruments Inc., Quebec, Canada). Root attributes such as average root diameter (mm), number of root tips per gram of root, and specific root length (SRL) were measured.

4.2.6. Calculations

We calculated ^{15}N recovery in shoot and root biomass ($^{15}N_{rec}$ in µg N plant^{-1}) as follows:

$$^{15}N_{rec} = N_{cont} \times (A\%^{15}N_{labelled} - A\%^{15}N_{unlabelled})/(A\%^{15}N_{fert} - A\%^{15}N_{unlabelled}) \qquad (1)$$

where N_{cont} is the N content in shoot or root biomass (µg N plant^{-1}), $A\%^{15}N_{labelled}$ is the ^{15}N atom% in shoot or root biomass of ^{15}N labelled pots, $A\%^{15}N_{unlabelled}$ is the ^{15}N atom% in shoot or root biomass of unlabelled pots (also used for BNF, see below), and $A\%^{15}N_{fert}$ is the ^{15}N atom% of the $^{15}NH_4^+$ or $^{15}NO_3^-$ label added. We then calculated total plant ^{15}N recovery as the sum of ^{15}N recovery in shoot and root biomass for each species.

Biological N fixation (BNF) in chickpea was estimated using the natural abundance of ^{15}N measured in the 8 extra pots. We followed the methodology outlined by (Unkovich et al. 2008) where we used wheat as the non-fixing reference crop. The proportion of N derived from atmospheric fixation (Ndfa) was determined using the equation:

$$Ndfa = [(\delta^{15}N_{ref} - \delta^{15}N_{chickpea}) / (\delta^{15}N_{ref} - B) \qquad (2)$$

where $\delta^{15}N_{ref}$ is the $\delta^{15}N$ value of the reference plants (wheat), $\delta^{15}N_{chickpea}$ is the corresponding $\delta^{15}N$ value for chickpea plants, and B is the isotopic signature of chickpea when solely dependent on BNF. A B value of -1.75‰ was used, as reported by Unkovich et al. (2008).

To quantify the actual N fixed (BNF, in mg N plant^{-1}), the following equation was applied:

$$\mathrm{BNF} = \mathrm{Ndfa} \times \mathrm{N}_{\mathrm{cont,\ chickpea}} \quad (3)$$

where $\mathrm{N}_{\mathrm{cont,\ chickpea}}$ refers to the N content in shoot or seed biomass of the chickpea plant, expressed in mg N plant^{-1}. We then calculated total BNF in chickpea as the sum of BNF in shoot and seed biomass.

4.2.7. Statistical analysis

A two-way analysis of variance (ANOVA) was conducted to evaluate the main and interactive effects of P fertilizer application (0 vs. 50 kg P ha^{-1}) and species identity (wheat vs. chickpea) on shoot, root, and total biomass, their N content and ^{15}N recovery under intercropping conditions. Because variation in background concentrations of NH_4^+ and NO_3^- in the soil and different amounts of label added would cause differences in the ^{15}N enrichment in the soil, and therefore would influence ^{15}N recovery, we did the ANOVAs on each ^{15}N form for each ^{15}N form ($^{15}NH_4^+$ and $^{15}NO_3^-$) separately. Biomass, N content and ^{15}N recovery were expressed on a per-plant basis to facilitate evaluation of individual plant performance within the intercrop system. Furthermore, two-way ANOVAs were performed to assess the effects of P fertilizer application and species identity on SRL (cm g^{-1}), average root diameter (mm), and number of root tips (g^{-1} root), and one-way ANOVAs to assess the effect of P fertilizer application on NH_4^+, NO_3^-, and available P concentrations in the soil. Where appropriate, data were log-transformed to improve normality and reduce heteroscedasticity, as assessed by the Brown–Forsythe test for homogeneity of variances. Following detection of significant treatment effects ($p < 0.05$), pairwise comparisons among treatment combinations were conducted using Tukey’s Honest Significant Difference (HSD) test. Effects with p-values between 0.05 and 0.1 were interpreted as marginally significant. All statistical analyses were performed using JMP Pro software (version 18.0.0; SAS Institute Inc., Cary, NC, USA).

4.3. Results

Phosphorus fertilizer application significantly influenced shoot and root biomass production, with varying responses observed between chickpea and wheat. For shoot biomass, both P fertilization ($p = 0.0002$) and species ($p < 0.0001$) had significant effects, and a significant interaction ($p = 0.006$) indicating species-specific responses (Table 4. 1). Chickpea had higher shoot biomass than wheat at both P levels, while the increase with P fertilizer was more significant in wheat than in chickpea. P fertilization ($p = 0.01$) and species ($p < 0.0001$) also significantly affected root biomass, but with no significant interaction ($p = 0.5$). Chickpea consistently produced greater root biomass than wheat at both P levels, and both species showed modest increases in response to P fertilizer. Treatment effects on N content in both shoot and root biomass followed similar patterns as for treatment effects on shoot and root biomass (Table 4. 1).

Table 4. 1. Effect of phosphorus (P) fertilizer application (P fert: 0 and 50 kg P ha^{-1}) and wheat-chickpea mixture on shoot biomass, root biomass, shoot N content and root N content (mean ± se). ANOVA *p*-values are also shown (P-fert: P fertilizer application treatment, S: Species in mixture).

P fert	Component crop	Shoot biomass (g plant^{-1})	Root biomass (g plant^{-1})	Shoot N content (mg plant^{-1})	Root N content (mg plant^{-1})
0	C	0.28 ± 0.03	0.23 ± 0.02	12.3 ± 1.13	6.43 ± 0.70
50	C	0.31 ± 0.02	0.27 ± 0.03	12.7 ± 1.04	6.16 ± 0.76
0	W	0.08 ± 0.01	0.08 ± 0.01	2.45 ± 0.25	1.50 ± 0.19
50	W	0.22 ± 0.01	0.14 ± 0.01	10.9 ± 0.68	2.91 ± 0.23
ANOVA					
	P-fert	0.0002	0.01	<0.0001	0.2
p values	Species	<0.0001	<0.0001	<0.0001	<0.0001
	P-fert×Species	0.006	0.5	<0.0001	0.3

Application of P fertilizer significantly influenced ^{15}N recovery in shoot tissues for both $^{15}NH_4^+$ and $^{15}NO_3^-$ forms (P-fert effect). For $^{15}NH_4^+$, shoot ^{15}N recovery increased significantly with the addition of P ($p = 0.004$), while there were no significant differences between species ($p = 0.69$) (Figure 4. 1a). However, the interaction between P fertilization and species was marginally significant ($p = 0.06$), suggesting a possible differential response. Under 50 kg P ha^{-1}, both chickpea and wheat exhibited increased ^{15}N recovery, with wheat showing a somewhat superior mean recovery compared to chickpea. For $^{15}NO_3^-$, ^{15}N recovery was significantly affected by P fertilization ($p < 0.0001$), plant species ($p = 0.001$), and their interaction ($p = 0.0004$) (Figure 4. 1b). The use of P significantly improved ^{15}N recovery in wheat for $^{15}NO_3^-$, exhibiting an increase of almost fourfold compared to chickpea at a rate of 50 kg P ha^{-1}. At 0 kg P ha^{-1}, both species exhibited relatively small ^{15}N recovery from $^{15}NO_3^-$.

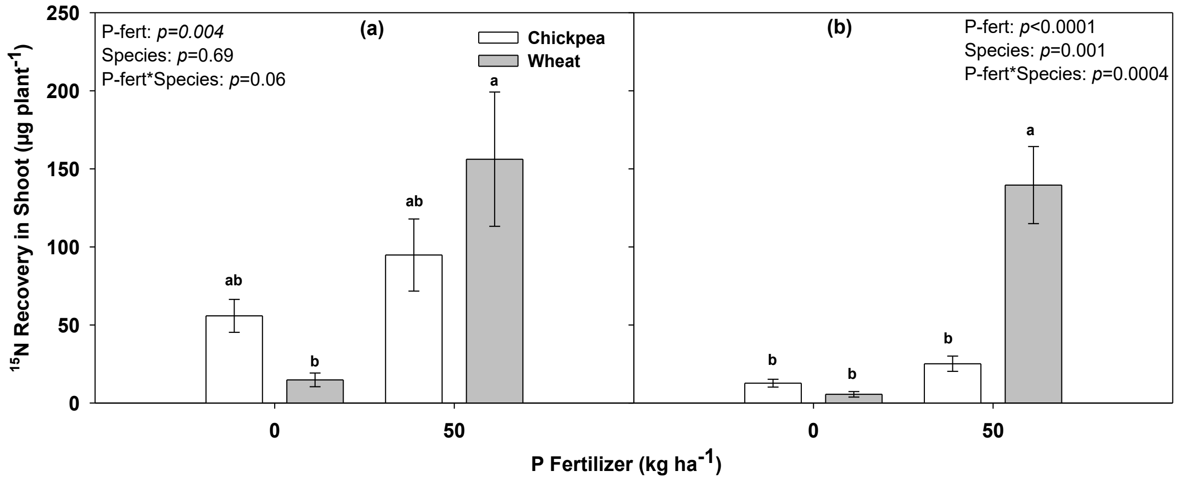

Figure 4. 1. Effect of P fertilization on shoot ^{15}N recovery from (a) labelled ammonium ($^{15}NH_4^+$) and (b) labelled nitrate ($^{15}NO_3^-$) by chickpea and wheat. Bars represent mean ± SE. Different letters above bars indicate significant differences among treatments based on post-hoc tests ($p < 0.05$).

The recovery of root ^{15}N was markedly affected by P fertilization (P-fert) and plant type, exhibiting differing patterns between $^{15}NH_4^+$ and $^{15}NO_3^-$ sources. For $^{15}NH_4^+$, P fertilization ($p = 0.01$) and species ($p < 0.0001$) significantly influenced root ^{15}N recovery, although their interaction was not significant ($p = 0.11$) (Figure 4. 2a). In contrast to the ^{15}N recovery in shoots, chickpea had much stronger ^{15}N recovery in roots compared to wheat at both P levels, and P fertilization further improved recovery in both species, with chickpea exhibiting a stronger response. Conversely, for $^{15}NO_3^-$, P fertilization exerted a significant influence ($p = 0.0005$), whereas species alone did not significantly influence root ^{15}N recovery ($p = 0.2$) (Figure 4. 2b). A significant interaction between P and species ($p = 0.04$) suggested a species-specific response to P application. At 0 kg P ha^{-1}, ^{15}N recovery in roots was higher than in wheat. The results show that chickpea exhibits superior efficiency compared to wheat in the root uptake of $^{15}NH_4^+$, and higher root uptake of $^{15}NO_3^-$ under low P availability.

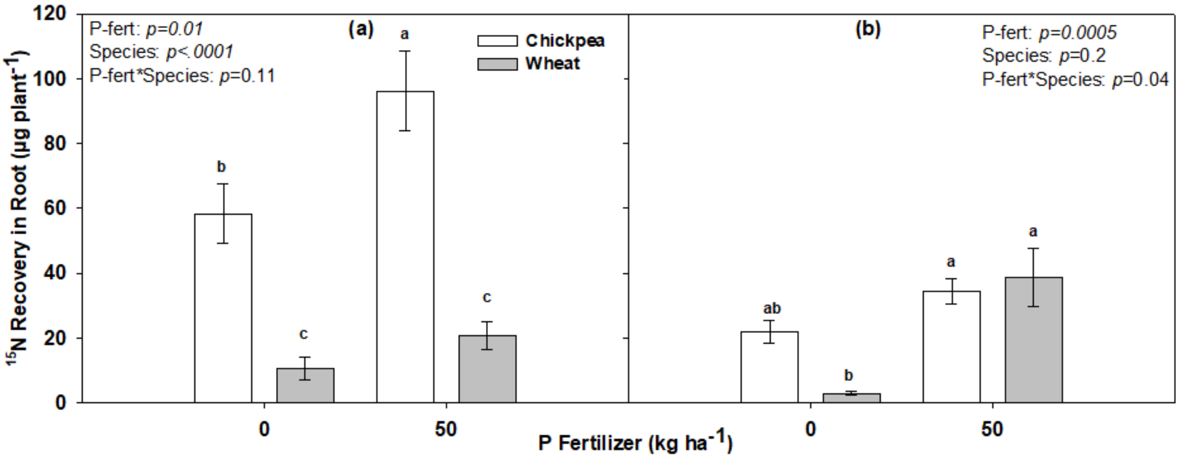

Figure 4. 2. Effect of P fertilization on root ^{15}N recovery from (a) labelled ammonium ($^{15}NH_4^+$) and (b) labelled nitrate ($^{15}NO_3^-$) in chickpea and wheat. Bars represent mean ± SE. Different letters above bars indicate significant differences among treatments based on post-hoc tests (*p* < 0.05).

Application of P fertilizer significantly enhanced total plant ^{15}N recovery of both $^{15}NH_4^+$ and $^{15}NO_3^-$ forms across wheat and chickpea. For $^{15}NH_4^+$, the total plant ^{15}N recovery was significantly influenced by P fertilizer ($p = 0.0009$), with chickpea exhibiting greater recovery compared to wheat, although the species effect was marginally significant ($p = 0.07$) (Figure 4. 3a). No significant interaction was revealed ($p = 0.2$), although the greater ^{15}N recovery in chickpea was mostly observed without P fertilization. In contrast, for $^{15}NO_3^-$, P fertilizer ($p = 0.0001$), species ($p = 0.02$), and their interaction ($p = 0.0001$) exhibited significant effects (Figure 4. 3b). Wheat exhibited much more ^{15}N recovery in NO_3^- form with P fertilization compared to chickpea, while chickpea showed greater ^{15}N recovery in NO_3^- form compared to wheat without P fertilization. These outcomes illustrate differential uptake and redistribution of N between species when applied in different forms, with more pronounced species-specific responses for $^{15}NO_3^-$.

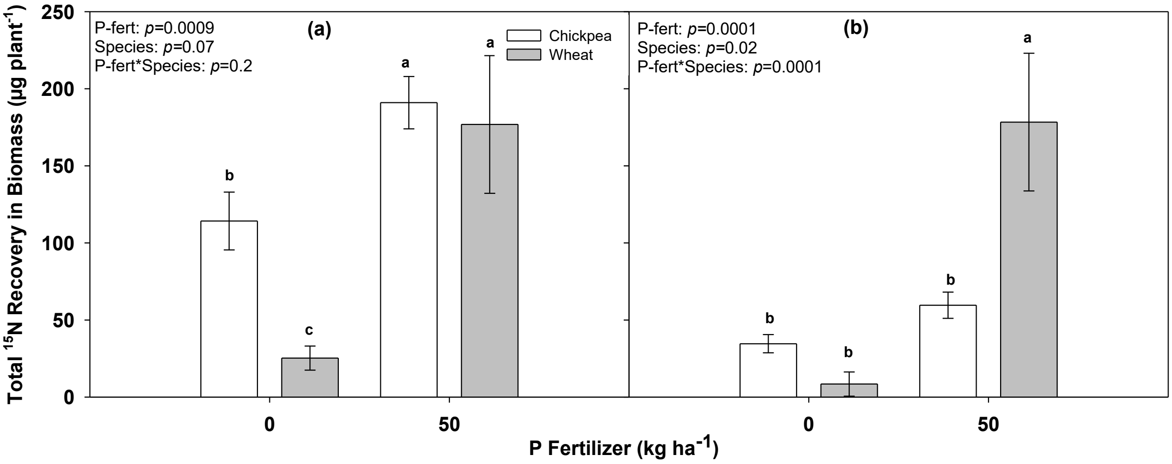

Figure 4. 3. Effect of P fertilization on total biomass (µg plant^{-1}) ^{15}N recovery from (a) labelled ammonium ($^{15}NH_4^+$) and (b) labelled nitrate ($^{15}NO_3^-$) in chickpea and wheat. Bars represent mean ± SE. Different letters above bars indicate significant differences among treatments based on post-hoc tests ($p < 0.05$).

Soil NO_3^- and NH_4^+ concentrations were not significantly influenced by P fertilization ($p = 0.7$ and $p = 0.9$, respectively). Mean NO_3^- levels were 23.1 ± 2.7 mg kg^{-1} at 0 P and 21.0 ± 4.3 mg kg^{-1} at 50 P, while NH_4^+ concentrations remained stable across treatments (2.47 ± 0.23 vs. 2.42 ± 0.09 mg kg^{-1}). By contrast, extractable P was strongly affected by P fertilization ($p = 0.007$), increasing from 1.28 ± 0.32 mg kg^{-1} under 0 P to 4.90 ± 0.85 mg kg^{-1} with 50 P application.

Table 4. 2. Effects of phosphorus (P) treatments (P fert: 0 and 50 kg P ha-1) on soil ammonium (NH_4^+), nitrate (NO_3^-), and available phosphorus (P) concentrations at harvest under intercropping conditions.

P fert	Nitrate (mg kg^{-1})	Ammonium (mg kg^{-1})	Extractable P (mg kg^{-1})
0	23.1 ± 2.7	2.47 ± 0.23	1.28 ± 0.32
50	21.0 ± 4.3	2.42 ± 0.09	4.90 ± 0.85
ANOVA *p* values	0.7	0.9	0.007

Specific root length varied significantly between species ($p < 0.0001$), with wheat demonstrating a significantly higher SRL than chickpea across both P fertilizer levels (Figure 4. 4a). However, there was no significant effect of P fertilization ($p = 0.8$) or the interaction between P fertilization and species ($p = 0.9$). Average root diameter (mm) was also significantly affected by species ($p = 0.003$), with chickpea consistently demonstrating greater diameter than wheat at both P levels (Figure 4. 4b). Phosphorus fertilization ($p = 0.1$) and the interaction between P levels and species ($p = 0.2$) did not show a significant influence. Similarly, the quantity of root tips (number g^{-1} root) was significantly greater in wheat compared to chickpea ($p < 0.0001$), regardless of P fertilization (Figure 4. 4c). Phosphorus fertilization ($p = 0.8$) and the interaction between phosphorus and species ($p = 0.7$) did not have a significant influence. These outcomes demonstrate intrinsic species variations in SRL, diameter and number of root tips, irrespective of P fertilization.

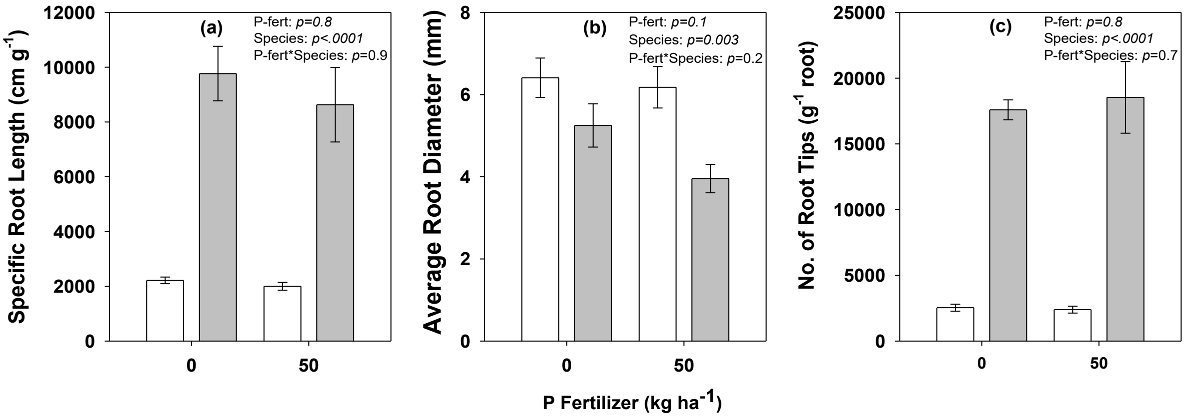

Figure 4. 4. Effect of P fertilization on (a) specific root length density (cm g-1), (b) average root diameter (mm), and (c) number of root tips (g^{-1} root) in chickpea (white bar) and wheat (gray bar) under two P fertilizer levels (0 and 50 kg P ha^{-1}) at 47 DAS. Bars represent mean ± SE. Different letters above bars indicate significant differences among treatments based on post-hoc tests ($p < 0.05$).

Phosphorus fertilization had no significant effect on BNF or Ndfa by chickpea under mixture. The Ndfa in chickpea shoots at maturity was 0.85 ± 0.18 without P fertilization and 0.83 ± 0.11 with P fertilization ($p = 0.6$). Similarly, BNF in chickpea shoot biomass at maturity was 14.5 ± 2.6 mg $plant^{-1}$ without P fertilization and 16.2 ± 2.6 mg $plant^{-1}$ with P fertilization ($p = 0.7$).

4.4. Discussion

Phosphorus availability and intercropping jointly shaped N acquisition strategies in wheat-chickpea systems, revealing clear functional divergence between the two species. Chickpea maintained reliance on BNF across P levels, whereas wheat showed strong dependence on soil mineral N, with enhanced NO_3^- uptake under adequate P supply. Importantly, intercropping reduced direct competition for mineral N by reinforcing this complementarity: chickpea sustained BNF, while wheat more effectively exploited available NO_3^-. These results demonstrate that sufficient P availability is critical for maintaining this functional separation, thereby improving overall N-use efficiency in cereal–legume mixtures.

4.4.1. Competitiveness for $^{15}NH_4^+$ and $^{15}NO_3^-$ between wheat and chickpea

We hypothesized that wheat would be more competitive than chickpea for $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake due to the legume's reliance on BNF. Our findings only partially support this hypothesis for $^{15}NO_3^-$ (although this only occurred with P fertilization discussed below), but not for $^{15}NH_4^+$ (Fig. 4. 3). Chickpea's lower total ^{15}N recovery with labelled NO_3^- compared to wheat may stem from its dependence on BNF for N resources and leaving the soil N pool available to adjacent companion plants in mixture (Ashworth 2018; Singh et al. 2023). In our study up to 90% of N in shoot biomass of chickpea at maturity came from BNF, and this may have significantly reduced its demand for available N from the soil.

In contrast to what we hypothesized, chickpea showed a marginally higher total ^{15}N recovery and a consistently higher ^{15}N recovery in roots compared to wheat for $^{15}NH_4^+$, across P levels (Fig. 4. 2). This suggests that chickpea possesses a stronger capacity or affinity for NH_4^+ uptake compared to wheat, despite an overall lower demand for soil N due to BNF. Consistent with our results, Daryanto et al. (2018) observed greater preference for NH_4^+ in soybean (legume) compared to maize (cereal), while wheat prefers NO_3^- uptake, particularly in non-acidic soils (Zhao and Shen 2018). It requires less energy for plants to take up and assimilate NH_4^+, which is already in reduced form, compared to NO_3^- (Britto and Kronzucker 2013) and plants often preferentially take up NH_4^+ over NO_3^- when both are applied (Gazzarrini et al. 1999; Gojon et al. 1986; Sasakawa and Yamamoto 1978), although this also depends on plant developmental stage and soil conditions (Britto and Kronzucker 2013).

However, this does not explain why chickpea would take up more N as NH_4^+ compared to wheat when grown together, and why more of the N was retained in chickpea roots than in wheat roots. A possible reason is that chickpea had more root biomass compared to wheat (Table 4. 1) allowing for a denser root system and greater access to $^{15}NH_4^+$. However, the chickpea roots also had a lower SRL, were larger in diameter, and had a smaller density of root tips (Fig. 4. 4), root characteristics that are normally negatively associated with root uptake

(Wen et al. 2022) and that do not favour scavenging of relatively immobile nutrients such as NH_4^+ (Dallstream et al. 2025; Ito et al. 2023). Instead, chickpea's larger root diameter may relate to its investment in BNF, as N-fixing bacteria are positively associated with greater average root diameter (Wang et al. 2022). In all, these root traits cannot explain the greater ^{15}N recovery in chickpea roots with $^{15}NH_4^+$ addition compared to wheat. Another reason could be that chickpea had a greater affinity to mycorrhiza that tend to have a greater efficiency in taking up NH_4^+ than NO_3^- (Kobae et al. 2010; Kronzucker et al. 1997), as well as rhizobial symbiosis influencing N acquisition pathways. Unfortunately, we did not measure mycorrhizal associations in our study, but legumes often show great affinity with mycorrhiza, and this could explain the greater ^{15}N recovery in chickpea roots, particularly under low P availability where mycorrhiza tend to proliferate more (Smith and Read 2008). In legumes, NH_4^+ is actively incorporated into amides that remain mostly in roots (Weissman 1972), which could further explain the much larger ^{15}N recovery in roots of chickpea compared to wheat.

The total ^{15}N recovery in plant biomass of wheat and chickpea were in the same range for $^{15}NH_4^+$ and $^{15}NO_3^-$ (Fig. 4. 3). This gives the impression that both forms of N were taken up in similar amounts, but we would like to point out that this was most likely not the case. We added twice as much ^{15}N in the form of NH_4^+ compared to NO_3^- while background concentrations of NH_4^+ in the soil were much lower than for NO_3^- (Table 4. 2 or Table S4. 1). As a result, the NH_4^+ in the soil after labelling with $^{15}NH_4^+$ must have been more enriched in ^{15}N compared to the ^{15}N enrichment of NO_3^- in the pots that received $^{15}NO_3^-$. Therefore, uptake of NH_4^+ most likely was lower compared to uptake of NO_3^-, reflecting the abundance of these pools in the soil (Wang and Macko 2011). Because in our study we focussed on assessing competitiveness for NH_4^+ and NO_3^- uptake separately in wheat-chickpea mixtures, we did not assess ^{15}N enrichment in the NH_4^+ and NO_3^- pools after labelling, and we are therefore not able to examine preference between the two forms by wheat and chickpea.

4.4.2. Effects of P fertilization

We hypothesized that wheat would become more competitive for $^{15}NH_4^+$ with P fertilization, but instead we found that wheat was significantly more competitive for $^{15}NO_3^-$ with P fertilization (Fig. 4. 3). This was somewhat of a surprise, given that wheat has a more fibrous root structure with higher SRL and root tips compared to chickpea (Fig. 4. 4), and where wheat root biomass significantly increased with P fertilization (Table 4. 1). Because of its lower mobility, we expected that $^{15}NH_4^+$ uptake by wheat would increase more with P fertilization than $^{15}NO_3^-$ uptake. Although total ^{15}N recovery in wheat biomass with $^{15}NH_4^+$ addition did increase with P fertilization (on average by 600%), the P fertilization effect was stronger and larger for $^{15}NO_3^-$addition (on average by 2013%, Fig. 4. 3). The stronger effect for $^{15}NO_3^-$ may be because wheat has a preference for NO_3^- over NH_4^+, particularly in non-acidic soils (Zhao and Shen 2018). Our results also suggest that acquisitive root traits such as SRL and root tips may be as important for NO_3^- uptake as for NH_4^+ uptake. It is worth noting that much of the increase in ^{15}N recovery in wheat biomass with P fertilization after $^{15}NO_3^-$ addition ended up in shoots rather than roots (Fig. 4. 1, 4. 2), suggesting that an increase in $^{15}NO_3^-$ uptake with P fertilization was particularly important for the large increase in wheat shoot biomass (Table 4. 1).

Phosphorus fertilization often increases BNF fixation in legumes (Almeida et al. 2000; Schulze et al. 2006), which could potentially reduce competition for N from the soil with its companion crop in intercropping systems (Ramirez-Garcia et al. 2014). However, in this study, P fertilization had no significant effect on %Ndfa and BNF in chickpea at maturity, suggesting that P fertilization did not change the relative reliance on N uptake from the soil *vs* BNF for its N demand. This could be a reason why ^{15}N recovery in chickpea, both with $^{15}NH_4^+$ and $^{15}NO_3^-$ addition, did not respond strongly to P fertilization. It further suggests that BNF did not play an important role in altering the competitiveness for N uptake from the soil between wheat and chickpea in response to P fertilization.

The marked enhancement in ^{15}N recovery following P fertilization suggests that P promoted the uptake of both $^{15}NO_3^-$ and $^{15}NH_4^+$, consistent with earlier findings that P deficiency reduces the uptake rates of both N forms (De Magalhães et al. 1998; Gniazdowska and Rychter 2000; Rufty et al. 1990; Schjørring 1986). Phosphorus availability directly regulates ATP production, which subsequently affects the uptake and metabolism of N (Veneklaas et al. 2012). Additionally, once the P demand of plants is met through P fertilization, plants can assimilate and use more N to maintain stoichiometric homeostasis (Ament et al. 2018; Blanes et al. 2012; Xiao et al. 2022) leading to an increase in N content within plant tissues (Wullaert et al. 2010). It is noteworthy that much of the increased N uptake with P fertilization went into shoots, particularly for wheat (Table 4. 1), likely because the majority of shoot N in chickpea came from BNF (up to 90%).

Interestingly, P fertilization did not significantly influence any root morphological traits measured, including SRL, root diameter, or root tip density, nor did it interact with species

(Fig. 4. 4). These findings suggest that the observed differences in root traits are governed by genetics than by plastic responses to P availability. There is ample evidence that plants increase their root surface area when P availability in the soil becomes scarce by growing more root hairs and thinner roots (Péret et al. 2011; Richardson et al. 2009). While we did not assess root hairs, we found no support for thinner roots. Possibly, our study did not last long enough for plants to develop strong root plasticity responses to P fertilization. Furthermore, we used a soil that was very deficient in available P at the start of the experiment (1.9 mg kg^{-1} Bray-P), and that remained low in available P by the time of harvesting, even with P fertilization (Table 4. 2). Therefore, the small increase in available P in the soil with P fertilization may have had limited effect on changing root traits.

4.5. Conclusion

Our study provides partial support for the proposed hypotheses. Consistent with H1, wheat showed greater competitiveness for $^{15}NO_3^-$ uptake than chickpea under low-P conditions, reflecting its higher specific root length (SRL) and limited reliance on BNF. Contrary to H2, P fertilization did not shift wheat's preference toward $^{15}NH_4^+$ but instead further amplified its recovery of $^{15}NO_3^-$, suggesting that P availability strengthens wheat's overall mineral N acquisition rather than altering the uptake form. Moreover, higher SRL particularly in wheat was positively associated with both $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake, underscoring the importance of fine root traits for accessing both N forms.

Together, our results show that wheat's finer root system and greater root length density enable more efficient inorganic N uptake in response to P fertilization, whereas whereas chickpea showed lower recovery of mineral N and maintained a high dependence on BNF.. Our data further demonstrate that chickpea derived more than 80% of shoot N from atmospheric fixation under both P treatments, with no significant effect of P supply on Ndfa or BNF. These findings suggest contrasting nutrient acquisition strategies between the two species under differing P availability. Our results further highlight the importance of adequate P supply in shaping N acquisition patterns in cereal-legume intercropping systems. Increased P availability enhanced wheat's recovery of NO_3^-, while chickpea maintained substantial reliance on BNF, potentially reducing direct competition for soil mineral N. These species-specific responses should be considered when designing intercropping systems and nutrient management strategies aimed at improving nutrient use efficiency and productivity in low-input agroecosystems.

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Competing Interests

Authors declare no competing interests

4.6. References

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Supplementary for Chapter 4

Table S4. 1.Soil properties and nutrient composition at the start of the experiment

Soil Property	**Value**	**Soil Property**	**Value**
Sand (%)	63	Extractable NH_4^+ (mg N kg^{-1})	4.2
Silt (%)	19	Extractable NO_3^- (mg N kg^{-1})	17.2
Clay (%)	18	Extractable P (mg kg^{-1})	1.9
pH (H_2O)	5.8		
Total C (%)	5.6		
Total N (%)	0.4		
Total P (%)	0.14		

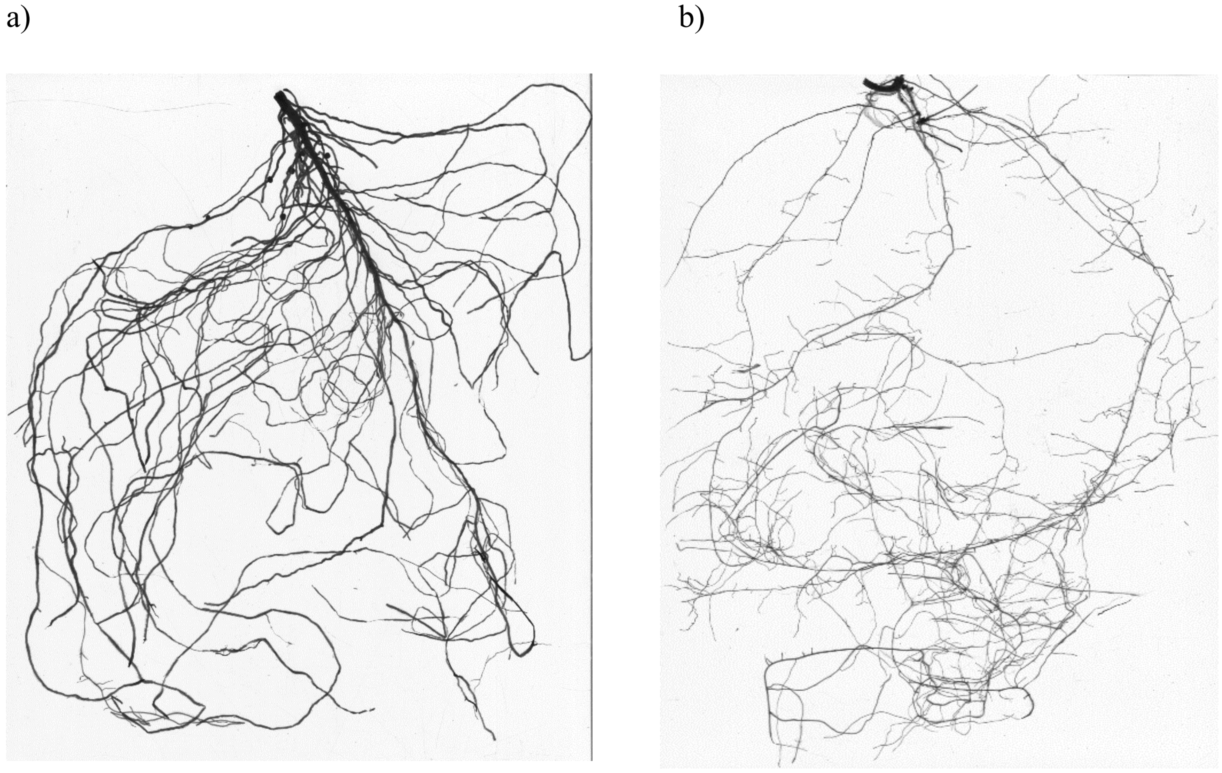

Figure S4.1. Root morphological characteristics of chickpea (*Cicer arietinum* L.) (a) and wheat (*Triticum aestivum*) (b).

Chapter 5: Linking photosynthetic carbon uptake, belowground allocation, and phosphorus acquisition across species and cropping systems

Abstract

Improving phosphorus (P) use in cereal-legume intercropping systems requires mechanistic understanding of how plants allocate carbon (C) belowground and acquire P under contrasting nutrient regimes. This study investigated how P fertilization (0 vs 50 kg P ha^{-1}) and cropping system (monocropping vs wheat-chickpea intercropping) influence photosynthate allocation, rhizodeposition, and P uptake in wheat (*Triticum aestivum* L.) and chickpea (*Cicer arietinum* L.). A $^{13}CO_2$ pulse-labelling experiment was conducted under controlled glasshouse conditions to quantify excess ^{13}C in shoots, roots, and rhizodeposited C, alongside plant P content and biomass responses. Chickpea exhibited consistently high P content and yield, and minimal changes in ^{13}C allocation across P levels and cropping systems, indicating a limited dependence on short-term rhizosphere C release for P acquisition. Conversely, wheat showed clear P limitation: without P fertilization, wheat had low P content, reduced belowground ^{13}C allocation, and suppressed yield, particularly in monocropping. P fertilization increased wheat P content more than twofold, enhanced root ^{13}C enrichment and rhizodeposition, and nearly doubled yield. Intercropping partially alleviated wheat P stress by slightly increasing P uptake and root ^{13}C allocation relative to monocropping. Species-specific ^{13}C-P relationships further revealed that chickpea showed no noticeable coupling between rhizodeposited ^{13}C and P uptake, whereas wheat displayed strong positive ^{13}C-P linkages. Overall, the results demonstrate that chickpea maintains P acquisition independently of rapid rhizosphere C fluxes, while wheat relies more strongly on P fertilization to support root C investment and P uptake. These findings provide mechanistic insights into C-P interactions and highlight the potential of strategic P inputs and intercropping design to improve P-use efficiency in cereal-legume systems.

Keywords: Photosynthate, rhizodeposition, P uptake, ^{13}C, P content, nitrogen fixation, cereal, legume

5.1. Introduction

Intercropping is an agroecological approach where two or more crops are growing together in the same field for at least part of their growing cycle (Vandermeer 1989). Globally it has long been recognized for its potential to enhance resource use efficiency, a higher overall productivity, better control of pests and diseases, improve biodiversity, and increase system resilience (Lithourgidis et al. 2011; Malézieux et al. 2009). Different combinations of companion crops are practised in intercropping approaches, but cereal-legume intercropping has gained significant attention due to the complementary nutrient strategies of this combination, which can lead to enhanced yields and better soil health (Lithourgidis et al. 2011). By combining species with complementary traits, cereal-legume intercropping can improve the productivity by capturing and utilizing light, water, and nutrients, including phosphorus (P), compared to monoculture crops (Latati et al. 2016). In cereal–legume systems, legumes release root exudates that acidify the rhizosphere to mobilize inorganic P and secrete phosphatase enzymes that hydrolyze organic forms of P (Dai et al. 2025), thereby enhancing P availability for plant uptake benefiting both components of the mixture. Additionally, interspecific belowground interactions play a crucial role in enhancing P uptake in cereal-legume intercropping systems (Dai et al. 2025). Such enhanced P uptake, however, may require greater belowground carbon (C) inputs to support root growth, exudation, and microbial interactions that facilitate nutrient acquisition. Legumes may also contribute more photosynthetically fixed C to belowground processes, as they generally have higher shoot nitrogen (N) concentrations derived from biological N fixation (BNF), which supports greater photosynthetic capacity and CO_2 assimilation (Evans 1989; Field and Mooney 1986). Despite these potential advantages, it remains unclear how belowground C inputs and P uptake are jointly influenced by cereal-legume intercropping systems and varying levels of soil P availability.

Belowground C inputs by plants play a pivotal role in enhancing P acquisition, particularly under intercropping systems where species interactions can modify root growth and rhizosphere processes. One major pathway through which C inputs promote P uptake is by stimulating root proliferation, allowing plants to explore a larger soil volume and access available P pools. For instance, cereal-legume intercropping often enhances total root length density to improve uptake of available resources in the soil profile (Gao et al. 2010; Ren et al. 2017). Cereals such as wheat primarily invest in developing a fine, fibrous root system with vigorous root branching, high specific root length and root hair density to acquire soluble forms of soil P (Costa et al. 2021; Li et al. 2006; Siegwart et al. 2023; Zhou et al. 2024). In contrast, legumes such as faba bean and chickpea allocate more C to root exudation processes that acidify the rhizosphere, thereby mobilizing inorganic P and releasing phosphatases to hydrolyze organic P (Hinsinger 2001; Li et al. 2004a; Mahajan and Gupta 2009; Zhang et al. 2016). These rhizosphere-driven processes underpin overyielding and facilitative P uptake in cereal-legume intercropping systems (Li et al. 2016). Furthermore, legumes allocate a greater proportion of C into rhizosphere respiration to meet the additional energy and C requirements of biological N_2 fixation by rhizobia (Rahman et al. 2025; Warembourg et al. 2003).

Uptake of nutrients like P is typically more C demanding than N uptake because soil P is less available and mobile than N (Vitousek and Howarth 1991). Under P deficiency, plants release carboxylates to desorb inorganic P from mineral surfaces or produce extracellular phosphatases, a C-costly process, to mobilize organic P through hydrolysis (Lambers et al. 2008; Richardson et al. 2011; Wen et al. 2019). Additionally, plants may invest more C belowground to sustain arbuscular mycorrhizal fungi, which facilitate P acquisition under low-P conditions (Smith et al. 2011; van der Heijden et al. 2015; Ven et al. 2019). Consequently, P limitation often leads to increased belowground C allocation in the form of root exudation or rhizosphere respiration. Because legumes can fix atmospheric N_2, their growth is more frequently constrained by P availability (Png et al. 2017), making their belowground C allocation generally more responsive to soil P supply than that of cereals. These findings suggest that P availability not only governs total root biomass but also mediates species-specific C allocation strategies that determine belowground complementarity and nutrient acquisition in mixed cropping systems. These plant strategies-including root morphological adjustments, secretion of P-mobilizing compounds, and mycorrhizal associations-are typically upregulated under P deficiency but downregulated or inhibited when soil P availability is high (Raven et al. 2018). However, it remains unclear what the magnitude and direction of these belowground interactions are under intercropping system influenced by P availability.

The capacity of plants to allocate C belowground is closely linked to their photosynthetic performance, which determines the supply of assimilates available for root growth, rhizodeposition, and symbionts. Photosynthetic capacity of a plant is strongly associated with leaf N content, which plays a substantial role in plant growth and productivity. A substantial proportion of leaf N (up to 70%) is allocated to photosynthetic enzymes and pigments such as Rubisco and chlorophyll (Field & Mooney, 1986). Consequently, higher leaf N typically supports greater photosynthetic capacity (Evans 1989). Species with higher shoot N concentrations can sustain greater photosynthetic rates and consequently allocate more C to roots and rhizosphere processes. Legumes, through their symbiotic association with N-fixing bacteria, generally maintain higher leaf N content and photosynthetic capacity compared to cereals. For example, legume crops contained 40% more leaf N than the cereal crops (Adams et al. 2018). We know that cereals are generally more competitive than legumes in acquiring available soil N (Chamberlin et al. 1986); therefore, legumes grown in intercropping systems tend to rely more heavily on atmospheric N fixation to meet their N requirements (Rahman et al. 2025; Schipanski and Drinkwater 2012). This difference may influence their relative belowground C investment and nutrient acquisition strategies. However, it remains unclear how photosynthetic capacity and thus the balance of C allocation belowground-is modulated by intercropping systems and varying soil P availability.

This study examined the coupling between P fertilization, C allocation, and nutrient (N and P) dynamics in a cereal-legume intercropping system using a controlled pot experiment. Wheat and chickpea were grown either as monocultures or as intercrops under contrasting P fertilization levels. To trace the flow of recently assimilated C within the plant-soil system, a ^{13}C pulse-labelling approach was employed, complemented by measurements of C allocation in shoot and root, rhizodeposition, N and P acquisition, and seed yield. Our novel ^{13}C tracer approach, where specific species were temporarily exposed to $^{13}CO_2$, enabled us to track newly assimilated C as it moves into different plant biomass components and is released into the soil, providing valuable insights into C allocation dynamics in intercropping systems.

The objective of this study was to investigate how P fertilization and interspecific interactions in cereal-legume intercropping influence C allocation and nutrient acquisition. Specifically, this study aimed to answer the following questions: i) How much of the photosynthesized C is allocated belowground (fraction of excess ^{13}C allocated to root biomass and rhizodeposition) and how does belowground C allocation vary with species, cropping system, and P fertilization? ii) How does plant P uptake vary with cropping system, species and P fertilization and to what degree can this be explained by variation in belowground C allocation to root growth and rhizodeposition?; iii) How does photosynthetic uptake capacity of C vary with cropping system, species and P fertilization and to what degree can this be explained by variation in shoot N concentration?

5.2. Materials and Methods

5.2.1. Study area and soil characteristics

The experiment was conducted under controlled glasshouse conditions at the University of Sydney, Camden, Australia, between May and September 2023. Soil was collected from the upper horizon (0-20 cm) of a moderately grazed grassland (Westwood Farm, Camden, latitude 33°59′46″S, longitude 150°39′16″E), dominated by Paspalum dilatatum Poir. (C4 species) and not previously cropped. The region receives an average annual precipitation of 790 mm, with mean monthly temperatures ranging from 8.4 °C in July to 24.9 °C in January (Australian Bureau of Meteorology). The soil was classified as a sandy loam Red Kurosol according to the Australian Soil Classification, and as an Abruptic Acrisol under the World Reference Base system. The site was chosen due to its exceptionally low inherent phosphorus (P) availability (Dijkstra et al., 2015). The soil texture was 18% clay, 19% silt, and 63% sand, with a pH of 5.8. Extractable nutrient concentrations were 17.2 mg kg^{-1} NO_3^--N, 4.2 mg kg^{-1} NH_4^+-N, and 1.9 mg kg^{-1} P. The bulk soil contained 39 g kg^{-1} SOC, 4.0 g kg^{-1} total N, and 1.38 g kg^{-1} total P, corresponding to a C: N: P ratio of 28:3:1. After collection, the soil was air-dried and sieved (<2 mm) to remove roots, shoot, gravel, and debris. Each pot (20 cm inner diameter, 15 cm height) was filled with 2.5 kg of processed soil. Soil moisture was measured from a subsample of sieved soil to calculate the dry soil weight for each pot. Pots were then transferred to a glasshouse.

5.2.2. Experimental design and treatments

A factorial randomized block design was used with four replicates for each treatment combination. Treatments consisted of three cropping systems (wheat monocropping, chickpea monocropping, and wheat-chickpea intercropping) and two P application levels (0 and 50 kg P ha^{-1}). For the intercropping treatments we included 3 sets of four replicates allowing us to ^{13}C pulse label both wheat and chickpea in one set, wheat only (by covering chickpea plants) in a second set, and chickpea only (by covering wheat plants) in the third set (Fig. 5. 1). This allowed us to assess belowground C allocation for both species combined and, in each species separately in the intercropping treatment. We further included non-planted treatments with both P application levels. Thus, in total we planted 40 pots (wheat monocropping × 2 P levels: 8 pots, chickpea monocropping × 2 P levels: 8 pots, wheat-chickpea intercropping × 2 P levels × 3 sets: 24 pots) and another 8 pots without plants. In all planted pots we maintained 4 plants during the experiment (4 plants in monocropping and 2 plants of each species in intercropping).

Daytime temperatures in the glasshouse were maintained at 24-26 °C, while nighttime temperatures were kept between 18-20 °C along with 60% relative humidity. Pots were supplemented for 12 hrs with LED lights (55 mW cm^{-2}). Fertilizers were applied after seedling emergence according to treatment. Phosphorus (44 mg P kg^{-1} soil, equivalent to 50 kg P ha^{-1}) was supplied as monopotassium phosphate (KH_2PO_4). In addition, all pots received nutrient solutions providing N (89 mg kg^{-1}), K (232 mg kg^{-1}), S (39 mg kg^{-1}), Ca (35 mg kg^{-1}), Mg (29 mg kg^{-1}), B (0.09 mg kg^{-1}), Zn (0.4 mg kg^{-1}), Cu (0.09 mg kg^{-1}), Fe (0.4 mg kg^{-1}), and Mn (0.7 mg kg^{-1}) after the emergence of both species. Soil moisture was regulated by weighing pots daily and adjusting with water to maintain target weights across all treatments. To ensure uniform water availability, soils were maintained at 70% of water-holding capacity (WHC) throughout the growing period, with irrigation withheld 15 days prior to final harvest at maturity. Soil moisture (%) was maintained at 70% of maximum water holding capacity (WHC), following the protocol funnel and filter paper method described by Robertson and Hamilton (2015).

Seeds of wheat (*Triticum aestivum* L. cv. Suntop) and chickpea (*Cicer arietinum* L. cv. CBA Captain, desi type) were sown on 20 May 2023 in plastic pots (20 cm inner diameter, 15 cm height). Chickpea seeds were inoculated with *Mesorhizobium ciceri* prior to sowing. The inoculant was mixed with deionised water to form a slurry, applied to seeds, and air-dried in the shade for 15-20 min before planting. Following germination, seedlings were thinned to maintain four plants per pot, ensuring equal plant density across treatments.

5.2.3. $^{13}CO_2$ Pulse-labelling and measurements

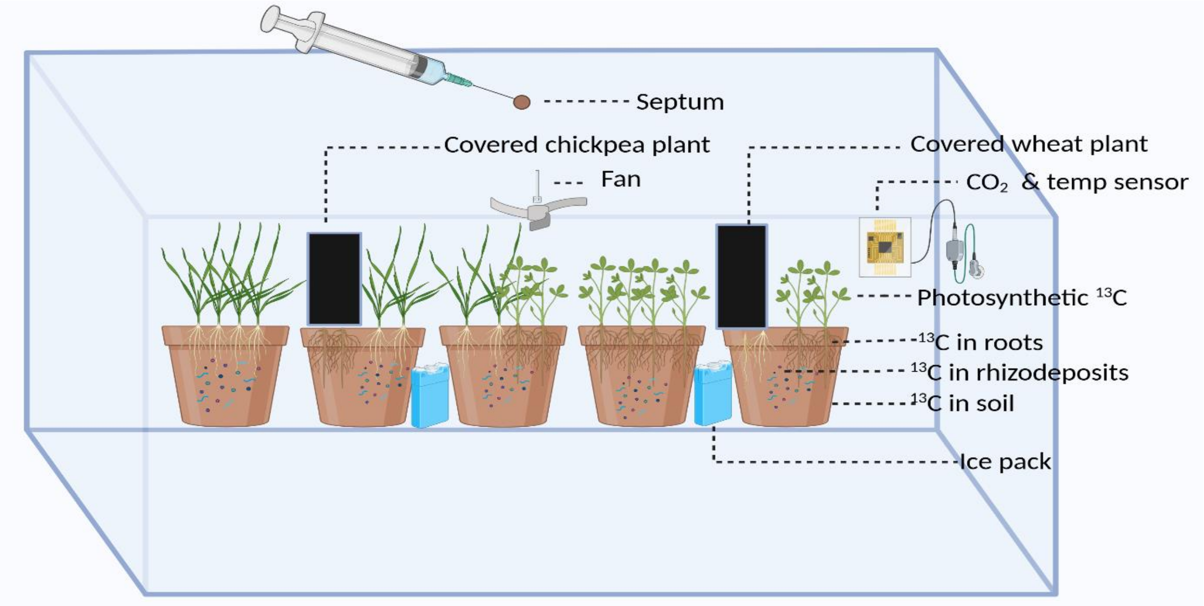

Figure 5. 1. A schematic diagram showing the experimental setup for tracing belowground carbon (C) allocation in a wheat monocropping, chickpea monocropping and wheat–chickpea intercropping system using ^{13}C pulse labelling. Plants grown in pots were exposed to $^{13}CO_2$ for 2 hours in a transparent chamber equipped with a fan, CO_2 sensor, and septum for $^{13}CO_2$ gas injection. Intercropping treatments included 3 separate sets where in one set both species were exposed to $^{13}CO_2$, and in the other two sets either wheat was exposed (by covering chickpea with a non-transparent bag to stop photosynthesis) or chickpea was exposed to $^{13}CO_2$ (by covering wheat with a non-transparent bag). Ice packs were included in the chamber to maintain stable temperature conditions during the 2-hour labelling period.

Plants were grown for 45 days after sowing (DAS) before initiating the $^{13}CO_2$ pulse labelling. At 45 DAS, pots were placed in transparent chambers (0.4 × 0.4 × 0.7 m; 5 pots per chamber, one block consisting of one replicate for each treatment) equipped with a circulating fan (Fig. 5. 1). Ice packs were added to stabilise chamber temperature, while sensors continuously monitored CO_2 concentration and temperature. Plants were exposed to ^{13}C-enriched CO_2 (99 atom% ^{13}C; Cambridge Isotope Laboratories, Andover, MA, USA) for 2 h, with injections raising the chamber CO_2 concentration to 1000 ppm. Over the 2-h labelling period, CO_2 levels inside the chamber declined to approximately 600 ppm. During the labelling period, CO_2 concentration, temperature, and humidity were continuously monitored. Temperature and humidity were measured using a BME280 sensor (Core Electronics, Australia), while CO_2 concentration was measured using an MG811 CO_2 gas module (DFRobot, Shanghai, China). Both sensors were connected to an Arduino Nano board (Shanghai, China), and the data were recorded onto an SD card by using a laptop. For the intercropping treatment, three separate pots were placed in the chamber where in one pot, both species were exposed to ^{13}C-enriched CO_2, while in the other two pots either wheat only or chickpea only was exposed to ^{13}C-enriched CO_2. This was established by covering the companion species with a non-transparent bag to stop photosynthesis in these plants (Fig. 5. 1).

Following the pulse labelling, plants were given two days to allocate recently fixed ^{13}C belowground. Shoots were then clipped to 5 mm above the soil surface. Soil with roots were removed from the pots and all roots were separated from the soil through handpicking. Roots were gently washed with water to remove soil. In mixtures, roots were separated by species based on their morphology (wheat has a fibrous thinner root system with many nodal, primary, and lateral roots that branch extensively from the base of the stem compared to chickpea with a thick taproot with lateral branches that are lighter in colour). When we analysed root biomass for ^{13}C (see below) in the intercropping treatment where only one of the species was ^{13}C labelled, we observed that root biomass of ^{13}C labelled plants were enriched in ^{13}C and non-labelled plants were not (Table S5. 1), and we are therefore confident that we were able to separate roots for these two species successfully.

As an indicator of C allocation towards rhizodeposition, we assessed the excess ^{13}C in soil respiration from the soil after harvesting. The soil after harvesting (without roots but containing rhizodeposition in planted treatments) was thoroughly mixed and sieved (2 mm) prior to measuring soil respiration. After careful removal of all live and dead roots, 100 g of sieved soil was placed in 16 oz. The soil was carefully sieved to remove all roots and plant debris and minimize potential contamination. (≈473 mL) Mason jars fitted with lids containing an injection port for gas collection within one hour of sampling. Gas was sampled at three time points at 25°C: immediately before sealing (0 h), and after 1 h and 2 h of incubation. At each sampling, 30 mL of headspace gas was withdrawn with a syringe; the first 5 mL was discarded to flush the syringe, and the remaining 25 mL was transferred into a pre-evacuated 20 mL vial equipped with an auxiliary needle to allow excess gas to escape during filling. CO_2 and ^{13}C concentrations (atom%) were determined using a Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS; Thermo Fisher Scientific, Bremen, Germany) connected to a GasBench system (Thermo Fisher Scientific). In addition, dried and finely ground root and shoot samples were analysed for total C, total N, and ^{13}C on a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 IRMS (Sercon Ltd., Cheshire, UK).

5.2.4. Plant and soil available P analyses

Dried plant material was finely ground to a homogeneous powder using a ball mill (Retsch MM 400, Haan, Germany) for phosphorus determination. Approximately 0.5 g of ground sample was ashed in a muffle furnace at 550 ^{0}C for 4 h. The ash was treated with 5 ml of 6 N HCl and heated on a hotplate until the solution was reduced to 1-2 ml. The resulting digest was filtered through Whatman No. 42 filter paper and made up to 50 ml with deionized water. Inorganic phosphorus concentration was quantified colorimetrically at 400 nm using a UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan) with ammonium molybdate and ammonium metavanadate as color-developing reagents (Jackson 1958).

Fresh representative soil samples were sieved to <2 mm prior to analysis of available P. Available P was extracted from each pot using a 0.03 N NH_4F-0.025 N HCl solution, following (Olsen and Sommers 1982). The extracts were then analyzed colorimetrically for inorganic P on a UV-VIS spectrophotometer at 660 nm, employing ammonium paramolybdate and stannous chloride as reagents.

5.2.5. Yield

For seed yield we included a separate set of pots where chickpea and wheat were grown under the same combinations (but with only one set of four replicates for the intercropping treatments) and conditions as described above, to evaluate productivity in sole and intercropped systems under abovementioned two levels of P availability. All plants from each pot were harvested at maturity after 137 DAS. In both monocultures and intercropping systems, crops were sampled separately and partitioned into grain and shoot fractions. The samples were oven-dried at 60 ^{0}C for 72 h to reach a constant weight and subsequently weighed.

5.2.6. Calculations and statistical analyses

The ^{13}C atom% of CO_2 derived from soil respiration of planted pots containing rhizodeposits, and non-planted pots were estimated using the Keeling plot approach (Pataki et al. 2003). Soil respiration rates (R_{soil}, μg C kg^{-1} soil d^{-1}) were determined from the temporal increase in CO_2 concentration in planted pots using linear regressions. We then calculated the excess ^{13}C in soil respiration ($Excess^{13}C_{resp\ per\ kg}$, μg ^{13}C kg^{-1} soil d^{-1}):

$$Excess^{13}C_{resp\ per\ kg} = (A\%_{planted} - A\%_{non\text{-}planted}) \times R_{soil}/100 \quad (1)$$

where $A\%_{planted}$ and $A\%_{non\text{-}planted}$ are the ^{13}C atom% in soil respiration from individual planted treatments and the average of non-planted treatments, respectively. The excess ^{13}C in soil respiration was originally derived from the plant after the pulse labelling and therefore represents decomposition of rhizodeposits. Given that most rhizodeposition is rapidly decomposed after being released into soil (Kuzyakov 2005) we assumed that the $Excess^{13}C_{resp\ per\ kg}$ is a good indicator of C allocation towards rhizodeposition (Wang et al. 2021). To relate C allocation to rhizodeposition more closely to each plant, we expressed the $Excess^{13}C_{resp}$ per plant in each pot for each species ($Excess^{13}C_{resp\ per\ plant}$, in μg ^{13}C plant^{-1} d^{-1}):

$$Excess^{13}C_{resp\ per\ plant} = Excess^{13}C_{resp\ per\ kg} \times W_{soil,\ pot}/P\# \quad (2)$$

where $W_{soil,\ pot}$ is the total dry soil weight in each pot (kg pot^{-1}) and P# is the number of labelled plants in each pot (# pot^{-1}).

Similar to root respiration we calculated the excess ^{13}C in shoot and root biomass of labelled wheat and chickpea plants ($Excess^{13}C_{shoot/root\ per\ plant}$ in mg ^{13}C plant^{-1}):

$$Excess^{13}C_{shoot/root\ per\ plant} = (A\%_{labelled} - A\%_{non\text{-}labelled}) \times B_{shoot/root\ per\ plant}/100 \quad (3)$$

where $A\%_{labelled}$ and $A\%_{non\text{-}labelled}$ are the ^{13}C atom% in shoot/root biomass of individual labelled plants and the average of non-labelled wheat or chickpea plants (i.e., plants that were covered), respectively, and where $B_{\text{shoot/root per plant}}$ represents the shoot/root biomass of the labelled plants of each species (mg plant^{-1}). As a measure of belowground C allocation, we calculated the fraction of excess ^{13}C allocated to roots (Excess^{13}C$_{\text{fraction to roots}}$) by dividing Excess^{13}C$_{\text{root per plant}}$ by the total excess ^{13}C in plant biomass (sum of Excess^{13}C$_{\text{shoot per plant}}$ and Excess^{13}C$_{\text{root per plant}}$). When expressed per unit of shoot biomass, the excess^{13}C in shoot biomass two days after labelling can be regarded as a measure of photosynthetic C uptake efficiency by the plant (Excess^{13}C$_{\text{shoot, conc}}$, in mg ^{13}C g^{-1} shoot):

$$\text{Excess}^{13}\text{C}_{\text{shoot, conc}} = \text{Excess}^{13}\text{C}_{\text{shoot}} / \text{B}_{\text{shoot per plant}} \quad (4)$$

where $B_{\text{shoot per plant}}$ is now expressed in g plant^{-1}. To relate belowground C allocation to soil P availability we used Excess^{13}C$_{\text{resp per kg}}$ and Excess^{13}C$_{\text{root per kg}}$, both expressed per kg soil for a better comparison with soil P availability, which was also expressed per kg soil. To convert Excess^{13}C$_{\text{root per plant}}$ (expressed in mg ^{13}C plant^{-1}) to Excess^{13}C$_{\text{root per kg}}$ (expressed in mg ^{13}C kg^{-1} soil) values were divided by the total dry soil weight per pot (kg pot^{-1}) and multiplied by the total root biomass per pot (g pot^{-1}). For the intercropping treatment we used the sum of wheat and chickpea measured in pots where both species were labelled.

All statistical analyses were conducted in JMP (SAS Institute Inc., Cary, NC, USA). We used three-way analysis of variance (ANOVA) to assess the main and interactive effects of species (wheat and chickpea), P treatment (0 and 50 kg P ha^{-1}), and cropping system (monocropping and intercropping) on Excess^{13}C$_{\text{shoot, conc}}$ (measure of plant C uptake efficiency), Excess^{13}C$_{\text{root per plant}}$, Excess^{13}C$_{\text{fraction to roots}}$, and Excess^{13}C$_{\text{resp per plant}}$ (all measures of belowground C allocation), and biomass and P content of shoot and root, and seed yield (plant performance traits). Block was included as a random factor. Post-hoc tests (Tukey HSD) were applied when significant interactions were observed ($p < 0.05$). Regression analyses were performed separately for wheat and chickpea to examine relationships between plant C uptake efficiency (Excess^{13}C$_{\text{shoot, conc}}$) and shoot N concentration, and between plant P content and belowground C allocation (Excess^{13}C$_{\text{root per plant}}$ and Excess^{13}C$_{\text{resp per plant}}$). We further performed regression analyses between available P in the soil and belowground C allocation (Excess^{13}C$_{\text{root per kg}}$ and Excess^{13}C$_{\text{resp per kg}}$) combining the different crop combinations (wheat monocropping, chickpea monocropping, wheat-chickpea intercropping). Scatterplots with fitted regression lines are presented when significant ($p < 0.05$). When any of these relationships differed among cropping system (monocropping and intercropping) or crop combination, we conducted separate regression analyses for each of the cropping systems or crop combinations. To assess whether relationships differed among cropping systems or crop combinations, we performed analysis of covariance (ANCOVA) where cropping system (CS) was included as a main and interactive factor for the relationships with $\text{Excess}^{13}\text{C}_{\text{shoot, conc}}$ or plant P content, and where crop combination (CC) was included as a main and interactive factor for the relationships with available P in the soil. Covariates were selected based on their known relationships with $\text{Excess}^{13}\text{C}_{\text{shoot, conc}}$ or plant P content. Cropping system (CS) was included for plant-level variables ($\text{Excess}^{13}\text{C}_{\text{shoot, conc}}$, plant P content) to test how relationships differ between sole and intercropping. Crop combination (CC) was used for soil available P, as it reflects species interactions driving soil processes. This approach distinguishes plant-level from soil-level responses and allows testing of intercropping effects.

5.3. Results

Plant C uptake efficiency, assessed by the incorporation of labelled ^{13}C in shoot tissue ($\text{Excess}^{13}\text{C}_{\text{shoot, conc}}$) varied significantly with species, P fertilization that, to a lesser extent, depended on cropping system (Table 5. 1). Chickpea had relatively low ^{13}C enrichment (4-5 mg excess ^{13}C g^{-1} shoot) irrespective of P fertilization or cropping system (Fig. 5. 2). In contrast, wheat showed a strong response to P fertilization: ^{13}C uptake efficiency nearly doubled with P fertilization compared with no P fertilization. Cropping system (monocropping vs. intercropping) had no influence on ^{13}C uptake efficiency for each species, but P fertilization tended to increase ^{13}C uptake in intercropping and decrease in monocropping systems.

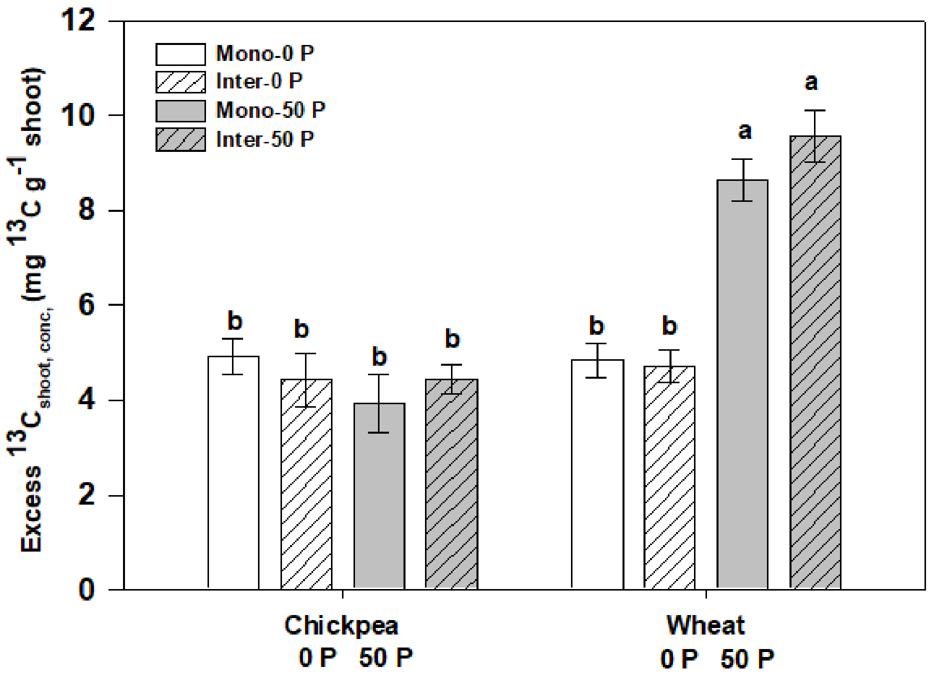

Figure 5. 2. Plant carbon (C) uptake efficiency (Excess$^{13}C_{shoot, conc}$, mg 13C g^{-1} shoot) of wheat and chickpea grown under monocropping (Mono) and intercropping (Inter) with 0 or 50 kg P ha^{-1}. Bars represent mean ± SE. Different letters above bars indicate significant differences among treatments within each species based on post-hoc tests ($p < 0.05$).

In chickpea no relationship was observed between shoot N concentration and excess ^{13}C in the shoot, with both monocropping and intercropping treatments showing scattered patterns (Fig. 5. 3a). In contrast, a strong positive relationship was observed for wheat, where increasing N concentration in shoots was associated with greater ^{13}C enrichment across both monocropping and intercropping treatments (Table 5. 2, Fig. 5. 3b).

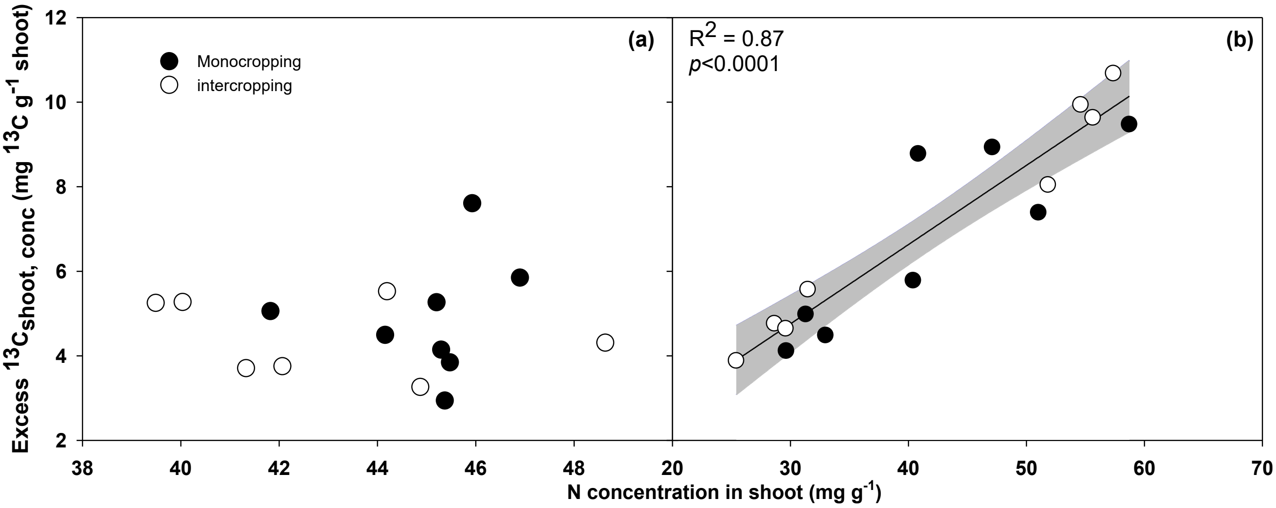

Figure 5. 3. Relationship between shoot nitrogen (N) concentration (mg g^{-1}) and plant carbon (C) uptake efficiency (Excess13Cshoot, conc, mg 13C g^{-1} shoot) in (a) chickpea and (b) wheat under monocropping and intercropping. Regression line with 95% confidence interval bands is shown for wheat.

The recently fixed C allocated belowground, as indicated by the fraction of excess ^{13}C allocated to roots (Excess$^{13}C_{fraction\ to\ roots}$) varied with species and P fertilization that depended on cropping system (Table 5. 1, Fig. 5. 4a). Across treatments, chickpea allocated a relatively stable fraction of excess ^{13}C to roots (0.43-0.47), with no significant differences between P levels or cropping systems (Fig. 5. 4a). In contrast, wheat showed a marked reduction in the fraction of excess ^{13}C allocated to roots with P fertilization, particularly when intercropped with chickpea, where the fraction decreased to 0.2 ($p < 0.05$). The excess ^{13}C in roots per plant differed significantly between species and P fertilization levels but was not affected by cropping system (Table 5. 1, Fig. 5. 4b). Chickpea exhibited higher excess ^{13}C in roots compared with wheat across all treatments. In contrast, excess ^{13}C in roots of wheat strongly increased with P fertilization. The ^{13}C allocated to rhizodeposition (Excess$^{13}C_{resp\ per\ plant}$) showed clear species- and P-dependent responses (Table 5. 1, Fig. 5. 4c). In chickpea, Excess$^{13}C_{resp\ per\ plant}$ remained low (on average 99.7 µg ^{13}C plant d^{-1}) and did not differ significantly between P levels or cropping systems. By contrast, wheat exhibited an overall larger Excess$^{13}C_{resp\ per\ plant}$ compared to chickpea and that increased with P fertilization, reaching 373 µg ^{13}C plant d^{-1}. Across species and P fertilization, the Excess$^{13}C_{resp\ per\ plant}$ was 38% higher in monocropping than in intercropping, although the main effect was only marginally significant (p = 0.06, Table 5. 1).

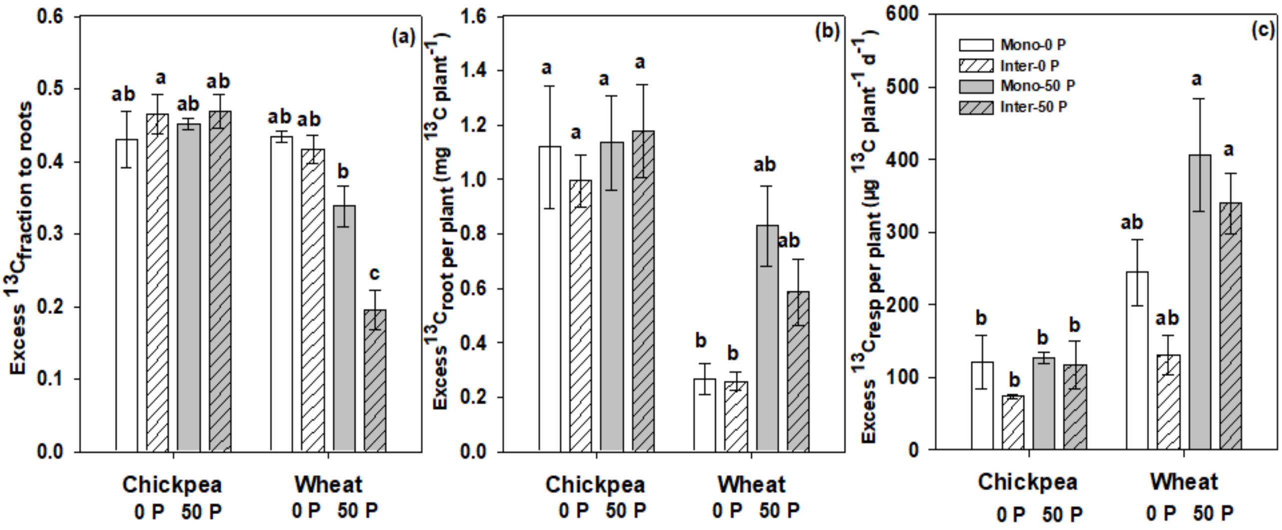

Figure 5. 4. Belowground allocation of recently fixed carbon (C) in chickpea and wheat grown under monocropping (Mono) and intercropping (Inter) with 0 or 50 kg P ha^{-1}. (a) Fraction of excess ^{13}C allocated to roots (Excess$^{13}C_{fraction\ to\ roots}$), (b) excess ^{13}C retained in root tissue (Excess$^{13}C_{root\ per\ plant}$, mg ^{13}C plant^{-1}), and (c) excess ^{13}C respired from rhizodeposition (Excess$^{13}C_{resp\ per\ plant}$, µg ^{13}C plant^{-1} d^{-1}). Bars represent mean ± SE. Different letters above bars indicate significant differences among treatments within each species based on post-hoc tests ($p < 0.05$).

Phosphorus content in plants after 45 days of growth differed significantly between species, P fertilization levels, and cropping systems (Table 5. 1, Fig. 5. 5a). Chickpea consistently showed high P content in shoots and roots (0.77-1.03 mg P plant^{-1}) regardless of P fertilization or cropping system, with no significant differences across treatments ($p > 0.05$). In contrast, wheat exhibited a strong P response: P content was low without P fertilization, particularly in monoculture (0.26 mg P plant^{-1}), but increased more than twofold with P fertilization, reaching values comparable to chickpea ($p < 0.05$). Intercropping slightly enhanced wheat P content compared with monocropping, although the effect was smaller than that of P fertilization. Seed yield followed similar patterns (Fig. 5. 5b). Chickpea maintained stable yields (1.83-2.55 g plant^{-1}) across treatments, indicating limited yield responsiveness to external P supply. Wheat, however, showed a strong dependence on P fertilization, with seed yield nearly doubling with P fertilization compared to without P fertilization. Without P fertilization, wheat yield was particularly low in monocropping (1.05 g plant^{-1}), while in intercropping wheat produced a somewhat higher yield (1.38 g plant^{-1}). With P fertilization, wheat yield improved substantially (up to 2.03 g plant^{-1}), although it remained below chickpea yields.

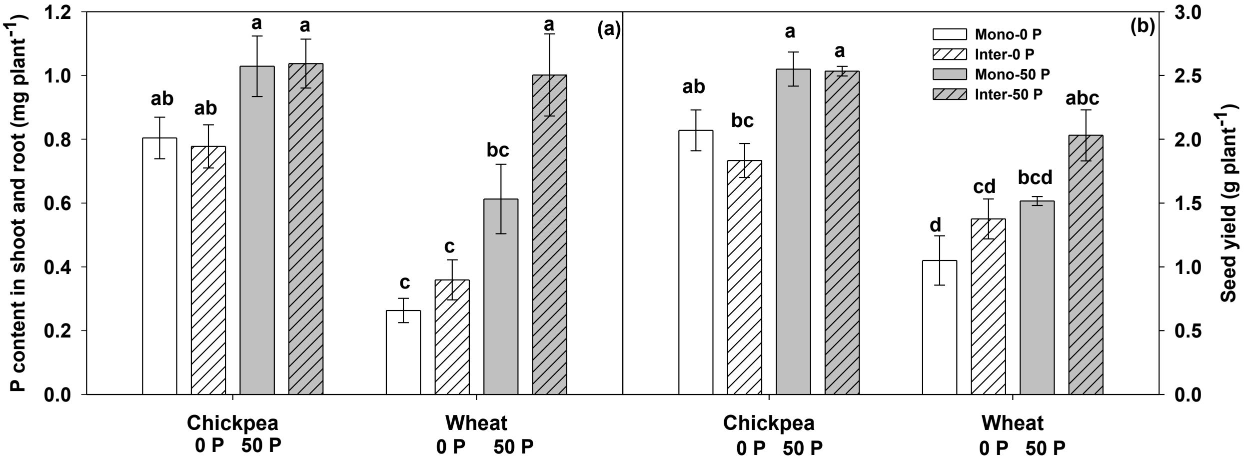

Figure 5. 5. Phosphorus (P) content at 45 days after sowing and seed yield of chickpea and wheat grown under monocropping (Mono) and intercropping (Inter) with 0 or 50 kg P ha^{-1}. (a) P content in total biomass (shoot and root, mg P plant^{-1}) and (b) seed yield (g plant^{-1}). Bars represent mean ± SE. Different letters above bars indicate significant differences among treatments within species based on post-hoc tests ($p < 0.05$).

The relationship between excess ^{13}C in roots (Excess^{13}C$_{\text{root per plant}}$) and plant P content was evaluated separately for chickpea (Fig. 5. 6a) and wheat (Fig. 5. 6b) under monocropping and intercropping systems. A positive relationship was observed for chickpea across both cropping systems (Table 5. 2, Fig. 5. 6a). In wheat, excess ^{13}C in roots was also positively associated with P content in shoots and roots (Table 5. 2, Fig. 5. 6b). The relationship was significant in monocropping ($R^2 = 0.69$, $p = 0.01$) and marginally significant in intercropping ($R^2 = 0.48$, $p = 0.05$), but when intercropped, wheat showed a larger plant P content per unit of excess ^{13}C allocated to roots (Table 5. 2, Fig. 5. 6b).

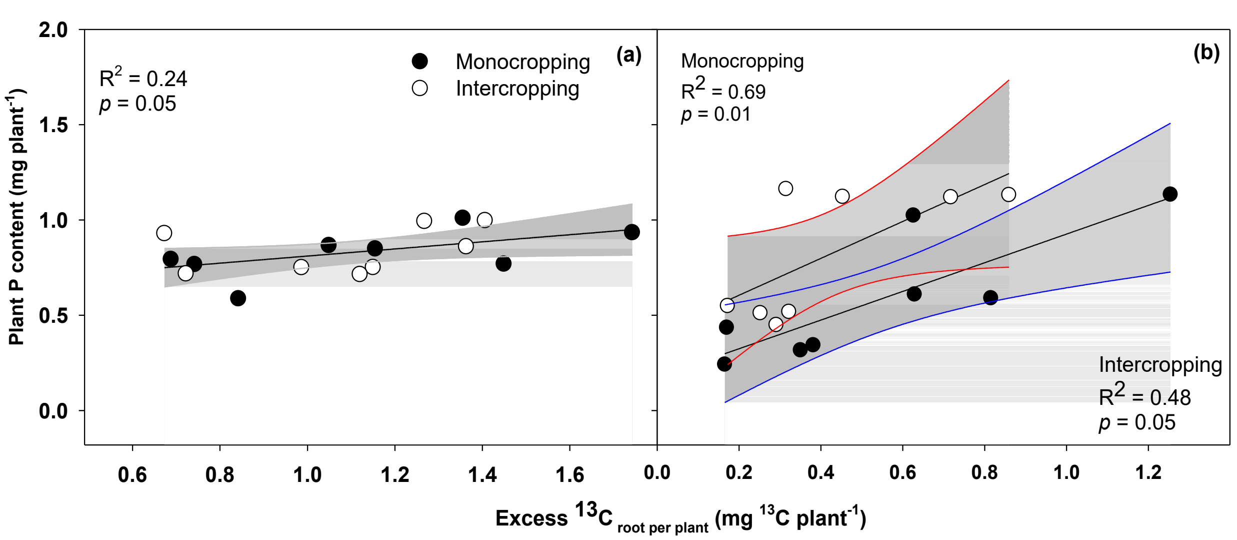

Figure 5. 6. Relationships between excess ^{13}C in roots (Excess^{13}C$_{\text{root per plant}}$, mg ^{13}C plant^{-1}) and plant phosphorus (P) content in (a) chickpea and (b) wheat under monocropping and intercropping. Regression lines (black lines) with 95% confidence interval bands are shown for wheat (blue lines for monocropping and red lines for intercropping).

Chickpea showed no clear relationship between ^{13}C allocated to rhizodeposition (Excess^{13}C$_{\text{resp per plant}}$) and plant P content under either cropping system (Table 5. 2, Fig. 5. 7a). In contrast, for wheat, a strong positive relationship was observed in monocropping ($R^2 = 0.74$, $p = 0.006$, Fig. 5. 7b). In intercropping, the relationship was positive but weaker and not statistically significant ($R^2 = 0.37$, $p = 0.1$). However, the ANCOVA indicated that wheat showed a larger plant P content per unit of excess ^{13}C allocated to rhizodeposition when intercropped with chickpea (Table 5. 2).

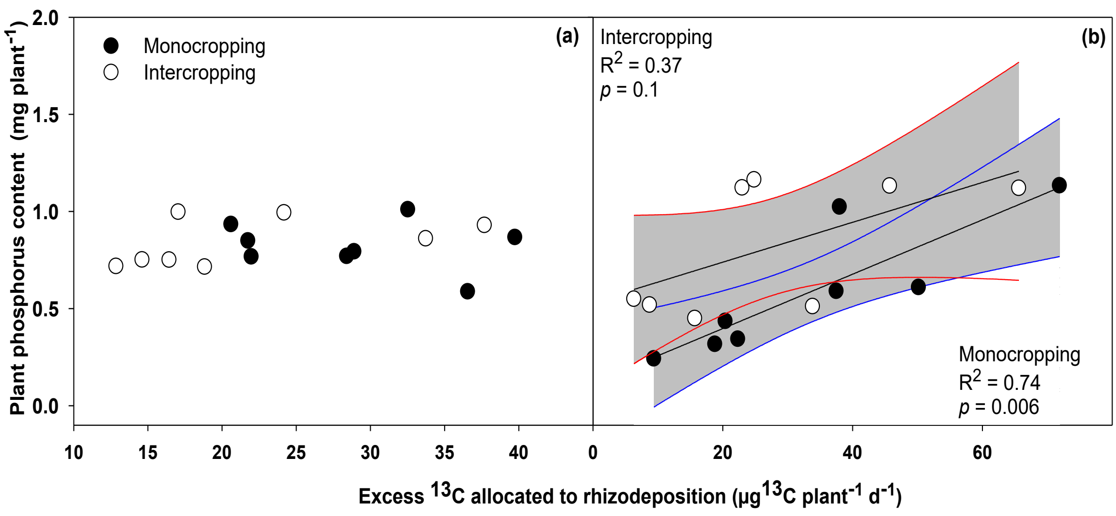

Figure 5. 7. Relationships between excess 13C allocated to rhizodeposition (Excess^{13}C$_{\text{resp per plant}}$ µg ^{13}C plant^{-1} d^{-1}) and plant phosphorus (P) content in (a) chickpea and (b) wheat under

monocropping and intercropping. Regression lines with 95% confidence interval bands are shown for wheat (blue lines for monoculture and red lines for intercropping).

The association between belowground C inputs and soil available P was evaluated using excess ^{13}C in root biomass (Excess^{13}C$_{\text{root per kg}}$) and rhizodeposition (Excess^{13}C$_{\text{resp per kg}}$) across different crop combinations (wheat monocropping, chickpea monocropping, and wheat-chickpea intercropping). Soil available P increased significantly with both Excess^{13}C$_{\text{root per kg}}$ and Excess^{13}C$_{\text{resp per kg}}$ under different cropping systems (Table 5. 2, Fig. 5. 8a, b). A positive linear relationship was observed between soil available P and excess ^{13}C in roots (R^2 = 0.23, p = 0.007; Fig. 5. 8a). Excess ^{13}C in rhizodeposition showed an even stronger correlation with soil available P (R^2 = 0.37, p = 0.001; Fig. 5. 8b).

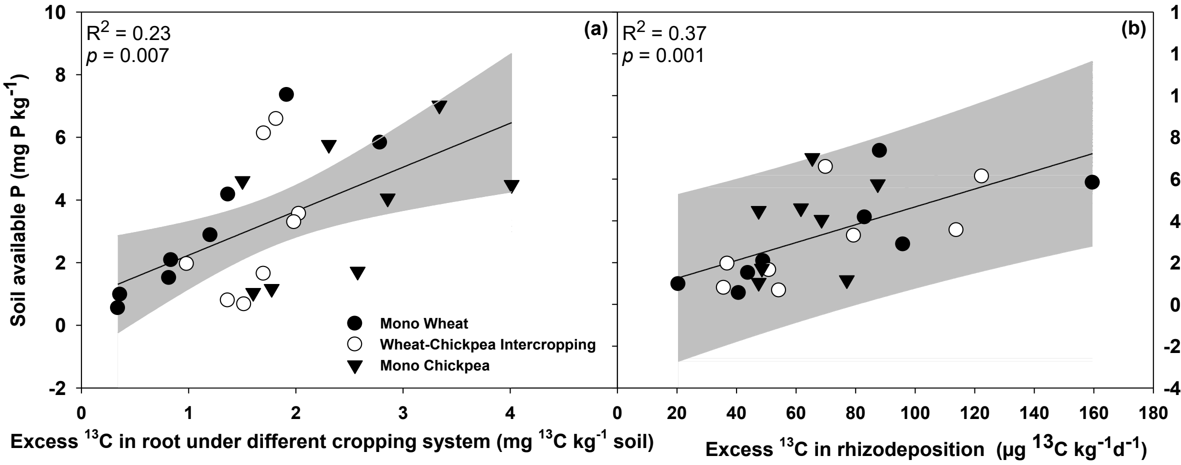

Figure 5. 8. Relationships between soil available phosphorus (P) and (a) excess ^{13}C in root biomass (Excess^{13}C$_{\text{root per kg}}$, mg ^{13}C kg^{-1} soil) and (b) excess ^{13}C in rhizodeposition (Excess^{13}C$_{\text{resp per kg}}$, µg ^{13}C kg^{-1} d^{-1}) across wheat monocropping, chickpea monocropping, and wheat–chickpea intercropping systems. Regression line with 95% confidence interval bands are shown.

Table 5. 1. Analysis of variance (ANOVA) results (p values) for the effects of species (Sp), phosphorus fertilization (P-fert), and cropping system (CS) on plant parameters related to carbon (C) allocation and P uptake.

Plant parameters ANOVA *p* values	Excess$^{13}C_{shoot, conc}$ (mg ^{13}C g^{-1} shoot)	Excess$^{13}C_{fraction\ to\ roots}$	Excess $^{13}C_{root\ per\ plant}$ (mg ^{13}C $plant^{-1}$)	Excess$^{13}C_{resp\ per\ plant}$ (µg ^{13}C $plant^{-1}$ d^{-1})	P content in shoot and root (mg P $plant^{-1}$)	Seed yield (g $plant^{-1}$)
Sp	<0.001	<0.001	<0.001	<0.0001	<0.001	<0.001
P-fert	<0.001	0.006	0.010	0.005	<0.001	<0.001
CS	0.300	0.100	0.400	0.060	0.050	0.100
SP×P-fert	<0.001	0.001	0.090	0.004	0.030	0.800
SP×CS	0.200	0.010	0.600	0.400	0.030	0.400
P-fert×CS	0.010	0.050	0.800	0.400	0.200	0.400
Sp×P-fert×CS	0.700	0.200	0.300	0.900	0.200	0.900

Table 5. 2. Analysis of covariance (ANCOVA) results for the main effect of cropping system (CS, monocropping, intercropping) and different covariates on excess ^{13}C in shoot (Excess^{13}C$_{\text{shoot, conc}}$) and plant P content, and the main effect of crop combination (CC, wheat monocropping, chickpea monocropping, and wheat-chickpea intercropping) on soil available P.

Response variable	Main effect / covariate	*p*-value
Excess^{13}C$_{\text{shoot, conc}}$ in wheat (mg ^{13}C g^{-1} shoot)	CS	0.40
	N conc. wheat shoot (mg g^{-1})	<0.0001
	CS × N conc. wheat shoot	0.80
Excess^{13}C$_{\text{shoot, conc}}$ in chickpea (mg ^{13}C g^{-1} shoot)	CS	0.60
	N conc. chickpea shoot (mg g^{-1})	0.80
	CS × N conc. chickpea shoot	0.40
P content in wheat (mg plant^{-1})	CS	0.01
	Excess ^{13}C$_{\text{root per plant}}$ (mg ^{13}C plant^{-1})	0.01
	CS × Excess ^{13}C$_{\text{root per plant}}$ (mg ^{13}C plant^{-1})	0.60
P content in chickpea (mg plant^{-1})	CS	0.60
	Excess ^{13}C$_{\text{root per plant}}$ (mg ^{13}C plant^{-1})	0.009
	CS × Excess ^{13}C$_{\text{root per plant}}$ (mg ^{13}C plant^{-1})	0.30
Plant P content in wheat (mg plant^{-1})	CS	0.03
	Excess ^{13}C$_{\text{resp per plant}}$ (µg ^{13}C plant^{-1} d^{-1})	0.003
	CS × Excess ^{13}C$_{\text{resp per plant}}$ (µg ^{13}C plant^{-1} d^{-1})	0.60
Plant P content in chickpea (mg plant^{-1})	CS	0.70
	Excess ^{13}C$_{\text{resp per plant}}$ (µg ^{13}C plant^{-1} d^{-1})	0.10
	CS × Excess ^{13}C$_{\text{resp per plant}}$ (µg ^{13}C plant^{-1} d^{-1})	0.20
Soil available P (mg kg^{-1})	CC	0.30
	Excess ^{13}C in root biomass (mg ^{13}C kg^{-1} soil)	0.007
	CC × Excess ^{13}C in root biomass (mg ^{13}C kg^{-1} soil)	0.40
Soil available P (mg kg^{-1})	CC	0.60
	Excess ^{13}C in rhizodeposition (µg ^{13}C kg^{-1} d^{-1})	0.001
	CC × Excess ^{13}C in rhizodeposition (µg ^{13}C kg^{-1} d^{-1})	0.50

5.4. Discussion

The marked difference in ^{13}C enrichment between wheat and chickpea reflects species-specific variation in photosynthetic C uptake efficiency and P responsiveness. Wheat showed nearly a twofold increase in ^{13}C incorporation in shoot tissue under P fertilization, indicating that P availability strongly limited its photosynthetic C assimilation efficiency. Phosphorus plays a crucial role in adenosine triphosphate (ATP) production, which provides energy for photosynthesis; thus, its limited availability can restrict photosynthetic rate, leading to reduced C fixation, plant growth, and productivity (Carstensen et al. 2018; Malhotra et al. 2018). Thus, enhanced ^{13}C uptake under low P supply likely influenced ATP production which reflects the alleviation of the abovementioned limitations. In contrast, chickpea maintained relatively low and stable ^{13}C enrichment across treatments, suggesting a more buffered photosynthetic response to P. Legumes employ multiple strategies to cope with P deficiency. These include the activation of high-affinity phosphate transporters to enhance P uptake, the secretion of organic acids that dissolve Ca-bound phosphate, and the release of phosphatase enzymes that mineralize organic P forms (George et al. 2010; Richardson et al. 2011). Legumes are often able to maintain photosynthetic activity under low P conditions by employing the aforementioned mechanisms that mobilize otherwise insoluble forms of P.

Lower ^{13}C enrichment in chickpea can be justified by its physiological allocation priorities under N-fixing conditions. Atmospheric N fixation is a highly C-demanding process, as legumes must supply substantial photosynthate to root nodules to sustain their symbiotic bacteria (White et al. 2007). This C investment supports the energy-intensive reduction of atmospheric N_2 to ammonia but simultaneously diverts assimilated C away from shoot growth and CO_2 fixation processes (White et al. 2007). Consequently, the relatively low ^{13}C enrichment observed in chickpea reflecting reduced aboveground C fixation efficiency is likely due to its greater C allocation belowground for sustaining symbiotic N fixation. Cropping system had little effect on ^{13}C uptake, indicating that short-term photosynthetic responses were governed mainly by species traits and nutrient status rather than interspecific interactions. While intercropping can alter resource capture and canopy microclimate (Hailu Gebru 2015), such effects may require longer durations to influence C assimilation. Overall, these findings highlight that wheat depends more strongly on external P supply for maintaining photosynthetic efficiency, whereas chickpea exhibits greater physiological resilience under low-P conditions.

The contrasting relationships between shoot N concentration and excess ^{13}C in the shoot observed in wheat and chickpea suggest fundamental differences in their photosynthetic N use efficiency and C assimilation strategies. The strong positive association in wheat implies that higher shoot N concentration reflecting greater investment in photosynthetic enzymes, such as Rubisco and light harvesting complexes, supports enhanced C fixation and assimilation (Evans

1989; Zhu et al. 2008). In contrast, although legumes generally exhibit higher leaf N levels than most other crops (Adams et al. 2016; Mathesius 2022), the absence of a clear correlation in chickpea suggests that its C fixation may be less tightly coupled with shoot N concentration. This decoupling likely reflects a strategic allocation of assimilated C toward supporting nodule activity and biological N_2 fixation, a process that is itself highly C demanding. Consequently, the energy and C costs associated with maintaining nodules may constrain the proportional increase in aboveground ^{13}C enrichment despite higher N accumulation. Furthermore, a substantial proportion of leaf N is allocated to compounds not directly associated with photosynthesis (e.g., cell wall proteins, amino acids, nucleic acids, alkaloids, and inorganic N, (Funk et al. 2013), which may have obscured any relationship between shoot N concentration and $Excess^{13}C_{shoot,\ conc}$ in chickpea. Unfortunately, we did not measure the non-photosynthetic N fraction in chickpea. Nevertheless, these findings reinforce that cereals and legumes differ in how N assimilation interacts with photosynthetic C uptake.

The belowground allocation of freshly assimilated C differed markedly between wheat and chickpea, but critically, these responses were modified by cropping system. Chickpea maintained a relatively stable allocation of ^{13}C to roots across P levels in both sole and intercropping systems (Fig. 5. 4a, b), suggesting that its C investment is regulated primarily by the metabolic demands of BNF, rather than external P supply. This stability may correspond with earlier research indicating that BNF is a C-intensive process, wherein a significant fraction of photo-assimilates is directed towards nodules and roots (Gordon 1987), much of which is ultimately respired (Voisin et al. 2003). In contrast, wheat showed a pronounced reduction in root ^{13}C allocation under P fertilization, and this response was substantially stronger under intercropping than in sole cropping. This indicates that interspecific interactions alter how wheat adjusts its C allocation in response to improved nutrient availability. While it is well established by multiple studies that P-deficient plants typically have higher root:shoot ratios than high-P plants, either as a consequence of allometric scaling (Niklas 1994) or because plants allocate more biomass to roots when P is limiting (Ciereszko et al. 1996; Gutschick 1993; Nielsen et al. 2001), our results suggest that this response is amplified in intercropping systems, potentially due to complementary resource use or competitive shifts in belowground interactions. Additionally, increased P availability through fertilization can reduce the C investment required for nutrient acquisition (Lu et al. 2022). The stronger reduction in root C allocation in intercropped wheat implies that plants may rely more on shared or redistributed nutrient acquisition processes when grown with legumes, thereby reducing the need for individual root investment. Together, these findings highlight that C allocation patterns are not solely governed by nutrient availability, but are strongly modulated by species interactions, emphasising the importance of intercropping context in regulating belowground carbon dynamics.

Wheat's greater and P-responsive rhizodeposition contrasts sharply with the consistently low ^{13}C losses from chickpea, but importantly, this divergence becomes more pronounced under intercropping. Chickpea maintained minimal variation in rhizodeposition across both P levels and cropping systems, indicating a tightly regulated C economy prioritising nodulation and N_2 fixation over rhizosphere carbon release (Voisin et al. 2003), rather than allocating it to exudation-driven nutrient foraging. In contrast, wheat exhibited a strong increase in ^{13}C - derived rhizodeposition under P fertilization, and this response was amplified when grown in mixture. For instance, while increased rhizodeposition under higher N supply is a well-established response associated with enhanced biomass production and photosynthetic capacity (Ge et al. 2015; Qiao et al. 2017), this effect is driven by greater C fixation in shoots and its subsequent transfer to roots, coupled with increased root metabolic activity that promotes the release of soluble C compounds into the rhizosphere. Our results show that this response is not uniform across cropping systems. The stronger P-induced increase in rhizodeposition in intercropped wheat suggests that species interactions modify how assimilated C is channelled into the rhizosphere. This may reflect a shift towards greater rhizosphere engagement in mixed systems, potentially to exploit complementary nutrient pools or to respond to competitive signalling from neighbouring plants.

While increased rhizodeposition with improved nutrient supply is a known response driven by enhanced photosynthesis and plant growth (e.g. Ge et al., 2015; Qiao et al., 2017), our results show that this response is not uniform across cropping systems. The stronger P-induced increase in rhizodeposition in intercropped wheat suggests that species interactions modify how assimilated C is channelled into the rhizosphere. This may reflect a shift towards greater rhizosphere engagement in mixed systems, potentially to exploit complementary nutrient pools or to respond to competitive signalling from neighbouring plants. Together, these findings indicate that rhizodeposition is not solely a function of plant C status but is context-dependent and shaped by interspecific interactions. The contrasting strategies responsive rhizosphere investment in wheat versus conservative allocation in chickpea highlight how intercropping can drive functional divergence in belowground C fluxes, with implications for nutrient cycling and rhizosphere processes.

The contrasting P dynamics between species highlight fundamentally different acquisition strategies. Chickpea maintained consistently high shoot and root P content across all treatments, reflecting its well-documented ability to mobilise organic P through rhizosphere acidification, which buffers the plant against short-term P scarcity (Li et al. 2004) (Li et al. 2003; Liu et al. 2021). This stability suggests that external P supply and cropping system had limited leverage over chickpea's internal P status. Wheat, however, displayed a classical P-limited profile: P content was markedly depressed under no-P fertilization, especially in monoculture, but increased more than twofold with P addition, reflecting the strong fertilizer responsiveness of cereals (Akpinar and Ortas 2023), which aligns with their lower capacity to mobilise soil P compared with legumes (Li et al. 2003). The modest improvement under intercropping indicates that chickpea likely facilitated wheat P uptake-potentially through localised rhizosphere modification or complementary rooting patterns-but this effect remained secondary to direct fertilizer inputs. Overall, these patterns emphasise that P fertilization is essential for optimising wheat nutrition, while chickpea's P economy remains robust across management contexts. These patterns are further reflected in similar yield patterns (Fig. 5. 5) indicating the strong dependence on P for producing yield in this P-poor soil.

The positive association between belowground C allocation and plant P content observed in both species (Fig. 5. 6) indicates that greater investment of recent photosynthate into roots supports enhanced P acquisition, consistent with the central role of root C expenditure in nutrient foraging and rhizosphere modification (Lu et al. 2022; Ven et al. 2019). In chickpea, this relationship was similar between monocropping and intercropping systems, reflecting its capacity to maintain C allocation patterns and P uptake irrespective of competitive context-typical of legumes with efficient nutrient acquisition and symbiotic strategies. Wheat, however, exhibited a cropping-system–dependent response: wheat accumulated more P per unit C invested into roots in intercropping than in monocropping. This suggests that intercropping altered wheat's foraging efficiency, possibly because legumes mobilise organic soil P through root-induced processes (Chen et al. 2019), thereby increasing P availability for the companion crop (Gong et al. 2020) under cereal–legume intercropping.

Only wheat displayed a clear dependence on rhizodeposition for P acquisition (Fig. 5. 7). This was surprising, given that legumes in particular have shown to allocate more C to root exudation and phosphatase production to hydrolyse organic P (Hinsinger 2001; Li et al. 2004a; Mahajan and Gupta 2009; Zhang et al. 2016). Our results suggest that wheat strongly relied on rhizodeposition for P acquisition, and similar to C allocation to roots, that wheat obtained more P per unit of rhizodeposited C when grown with chickpea under intercropping (Fig. 5. 7b), suggesting facilitative effects likely through legume-driven changes in rhizosphere that improved P availability. Overall, these findings highlight contrasting C-P acquisition strategies, with chickpea relying mostly on root growth for P acquisition regardless of cropping system, while wheat relying both on root growth and rhizodeposition for P acquisition, with greater returns when intercropped with chickpea.

Soil available P increased with both excess ^{13}C in root biomass and rhizodeposition (Fig. 5. 8), indicating that greater belowground C allocation to root growth and rhizodeposition enhanced P mobilisation across cropping systems. The positive association between soil P and excess ^{13}C in roots ($R^2 = 0.23$) suggests that increased root biomass inputs supported microbial processes that release P from soil minerals and organic pools. This interpretation aligns with evidence that soil microbes play a central role in P cycling through the decomposition of organic matter and mobilisation of P, with microbial biomass acting as a temporary reservoir for released P (Solangi et al. 2024). In parallel, root-mediated changes in the rhizosphere-particularly through exudates that alter pH are known to enhance P availability (Richardson 1994; Richardson et al. 2009). Root exudates also provide microbes with readily degradable energy-rich substrates, stimulating extracellular enzyme production and promoting the mineralisation of organic P compounds (Bilyera et al. 2021;Spohn et al. 2013). Moreover, this suggests that optimised P fertilization can enhance rhizosphere activity and nutrient cycling, improving overall P use efficiency and system productivity in intercropping systems.

5.5. Conclusion

This study demonstrates that cereal-legume intercropping fundamentally modifies how carbon allocation and P acquisition are coordinated between species. While chickpea maintained a stable and conservative C-P strategy regardless of P supply or cropping system, wheat exhibited a highly plastic response that was strongly influenced by both P availability and interspecific interactions. Notably, intercropping altered wheat's belowground C investment and strengthened its linkage with P acquisition, indicating that species interactions can reshape nutrient acquisition strategies beyond the effects of fertilization alone.

These findings highlight that the benefits of intercropping are not simply additive but arise from functional complementarity in C allocation and nutrient use. In this context, chickpea acts as a stable component, whereas wheat adjusts its strategy in response to both resource availability and neighbouring plants. Overall, this work provides mechanistic evidence that integrating legumes into cereal systems can enhance P-use efficiency through shifts in belowground carbon dynamics, offering a pathway to reduce fertilizer dependence and improve the sustainability of cropping systems. Lastly, the combined findings provide mechanistic insights into C-P interactions and highlight opportunities to design more P-efficient cereal-legume systems for sustainable agriculture in the future.

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Data availability

The data supporting the findings of this study are available from the corresponding author upon reason able request.

Competing interests

Authors declare no competing interests.

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Supplementary for Chapter 5

Table S5. 1. Atom% ^{13}C in root biomass of wheat and chickpea grown in intercropping under 0P and 50P, where only one species was ^{13}C pulse-labelled.

Species / P level	Wheat		Chickpea	
	Non-labelled	^{13}C Labelled	Non-labelled	^{13}C Labelled
0 P	1.09 ± 0.05	1.84 ± 0.03	1.09 ± 0.01	1.93 ± 0.13
50 P	1.09 ± 0.01	2.13 ± 0.18	1.10 ± 0.01	1.80 ± 0.06

Chapter 6: General Discussion and Conclusions

6.1. General Discussion

This thesis integrates evidence from meta-analysis, controlled glasshouse experiments, and isotope trace methods to unravel how P availability shapes nutrient acquisition and belowground C dynamics in cereal-legume intercropping systems. Across chapters, the work links system-level patterns of yield and P responsiveness to mechanistic insights into C allocation, root respiration, and N uptake of different forms under contrasting P regimes. Using ^{15}N and ^{13}C pulse-labelling, the thesis advances a process-based understanding of how interspecific interactions and P supply govern the balance between nutrient foraging, C investment, and plant performance under cereal-legume intercropping in P-limited soils.

6.1.1. Intercropping improves resource use efficiency, not necessarily yield response

I hypothesized that intercropping would show greater yield gains than monocropping in response to P fertilization, leading to higher LER values (Chapter 2: meta-analysis). Across all observations, monocultures showed a greater yield response to P fertilization than mixtures, even though LER consistently increased (Figure 2. *1*). This contradicts the expectation that P fertilizers play an important role in intercropping systems (An et al., 2023; Hinsinger et al., 2011; Tang et al., 2021) and that interspecific competition and facilitation in mixture should amplify nutrient use efficiency thereby increase crop productivity (Zhang & Li, 2003). The weaker yield responsiveness of mixtures is likely due to interspecific competition, particularly from cereals, as the cereal component, typically taller with faster growth and a more extensive root system including a greater fine-root mass (Lehmann et al., 1998), tends to be highly competitive for nutrients such as N (Carr et al., 2004; Carruthers et al., 2000). Yet, despite lower yield increases, LER improved, confirming more efficient land use under P fertilization. This aligns with evidence that P fertilization substantially enhances resource use efficiency under mixture (An et al., 2023; Hinsinger et al., 2011; Tang et al., 2021). Moreover, P fertilization can enhance N fixation in legumes, supplying additional N to the cereal and intensifying rhizosphere acidification and P acquisition (Hinsinger et al., 2011; Stern, 1993), processes that may ultimately influence overall LER. The key insight is that intercropping can enhance resource-use efficiency, not necessarily fertilizer-driven yield gains.

6.1.2. Soil, climate, and crop identity as key determinants of p fertilization benefits

The meta-analysis demonstrates that P fertilization effects are highly context dependent. Fertilization of P increased yield across both low- and high-clay soils, but LER gains were greater in low clay soils (Figure 2. 3c). This aligns with known P sorption dynamics: clay-rich soils bind P strongly (Gérard, 2016), while sandy or low-clay soils enable higher mobility and diffusion (Lambers et al., 2008), so that the P fertilizer can be taken up more efficiently in mixtures. Similarly, LER increased most in alkaline soils, possibly due to legume-mediated rhizosphere acidification mobilising P under alkaline soil (Latati et al., 2014). This contradicts expectations that neutral soils would show the strongest response as it typically provides the highest P availability and supports the greatest plant P uptake (Marschner et al., 2005) and highlights the functional importance of rhizosphere processes.

Yield responses were lowest in arid regions due to water constraints on P uptake which are in line with the observation which revealed that water availability strongly shapes P uptake, even without P inputs (He et al., 2002) and highest in temperate and tropical regions where crops may likely benefit from higher baseline precipitation, which supports greater nutrient mobility and nutrient uptake (Rahman et al., 2025). Interestingly, LER gains were largest in cold (continental) climates (Figure 2. 4c). Slow P mineralisation at low temperatures (Grierson et al., 1999) means that fertilizer additions disproportionately increase availability for both species in mixtures. Moreover, given the low precipitation typical of cold (continental) climates, P fertilization may support better growth of both crops in mixtures.

6.1.3. Crop combinations and their competitiveness

In contrast to our hypothesis, cereal-legume intercrops did not show an increase in LER-Yield with P fertilization despite conceptual expectations of facilitation, as I assumed that adding P would enhance biological N fixation in the legume, enabling cereals to access more soil N (Hauggaard-Nielsen & Jensen, 2005b; Raseduzzaman & Jensen, 2017) and thereby improve resource use and overall productivity. Intercropping systems of legumes with non-cereal crops produced the strongest LER improvements, indicating highly complementary root architectures and reduced competitive asymmetry for ensuring maximum resource use efficiency. On the other hand, non-cereal and legume yields were suppressed when grown with cereal crops under added P (Figure 2. 5a), leading to similar impact on LER-Yield consistent with the observation that cereals often dominate cereal–legume intercrops because of their dominance by rapid growth, height, and larger roots. (Kamara et al., 2019). This highlights that functional traits, rather than species identity alone, shape how fertilization influences intercropping performance.

6.1.4. Yield responses are tightly coupled with changes in P and N uptake

Across monoculture and intercropping systems, increases in yield were strongly correlated with increases in both P and N uptake (Figure 2. 6). Although the slope difference for P uptake *versus* yield was only marginally significant, it indicates that intercrops may convert applied P into yield more efficiently than the monocrops. This observation also supports the finding that interspecific competition and facilitation in intercropping systems can enhance nutrient use efficiency, thereby increasing overall crop productivity (Zhang & Li, 2003). Our analysis also revealed a significant relationship between the effect size of P uptake and N uptake (Figure 2. 6c), supporting the well-documented role of P fertilization in enhancing N uptake (Jiang et al., 2019). These relationships further indicate that the interaction between P and N availability is highly interconnected and plays a critical role in regulating growth-related processes in plant production systems. These relationships reinforce intercropping's role in improving nutrient use efficiency rather than absolute fertilizer responsiveness. Although the meta-analysis provides essential global insights, it does not explain why species behave differently under P fertilization, nor how P availability regulates BNF, C allocation, and belowground interactions at the physiological level. Therefore, mechanistic experimental studies are needed to unravel how cereals and legumes adjust their growth, C allocation, and N fixation strategies in response to P availability.

6.1.5. Yield and P uptake responses reveal asymmetric competition between wheat and chickpea

I first hypothesised that intercropping would increase seed yield for both species and that P fertilization would enhance chickpea yield more strongly than wheat (Chapter 3). The results only partly supported the hypothesis as wheat yield declined significantly in mixtures regardless of P input (Figure 3.1A), demonstrating competitive suppression by chickpea, a pattern reinforced by the substantially lower wheat P content in mixtures (Figure 3. 2 A). These findings challenge the traditional assumption that cereals gain advantage in mixtures due to reduced N competition via BNF by the legume component (Hauggaard-Nielsen & Jensen, 2005a). However, our results are based on one growing season only, while it is possible that cereals could benefit from BNF by the legume component in subsequent years when N-rich litter of the legume is mineralised in the soil. By contrast, chickpea yield increased with P fertilization only in mixtures (Figure 3.1B), showing a synergistic effect of P addition and intercropping on chickpea by exerting competitive advantage over wheat. The enhanced chickpea P uptake in mixtures (Figure 3. 2B) corroborates this advantage and aligns with the understanding that legumes are particularly sensitive to P deficiency because of their high P demand to sustain BNF (Divito & Sadras, 2014). Overall, the results indicate that when P is scarce, chickpea outcompetes wheat and gains more from P addition and invests those resources into higher seed yield. For yield estimation, plants in both experiments (Chapters 3 and 5) were harvested at maturity; however, seasonal variation in background photoperiod and solar angle influenced wheat growth trajectories, particularly tillering, resulting in differing yield outcomes in wheat. The experiment established in September (Chapter 3) exhibited markedly reduced effective tiller formation compared with the May planting (Chapter 5), resulting in lower wheat biomass and yield in mixture treatments. These seasonal effects likely amplified competitive interactions during early vegetative development, thereby contributing to the contrasting yield responses observed between experiments.

6.1.6. BNF is enhanced in intercropping but only marginally stimulated by P fertilization

I expected that BNF by chickpea would increase with both intercropping and P fertilization. Only the intercropping effect supported the hypothesis. Chickpea BNF was significantly higher in mixtures than monocultures (Figure 3. 3), consistent with previous evidence that legumes increase reliance on atmospheric N fixation when intercropped (Singh et al., 2023; Snapp et al., 1998). P fertilization produced only a marginal increase in BNF (23%; $p = 0.08$) and had no significant effect on Ndfa (% of N derived from fixation) (Table 3. 2). This limited response is explained by persistently high available soil N (Table 3. 2) which may discourage the BNF process and aligns with prior findings that P enhances N_2 fixation primarily when soil N is low (Leidi & Rodríguez-Navarro, 2000). Nonetheless, the small increase in BNF under P fertilization likely contributed to improved chickpea yield in mixtures. P-mediated indirect influences on root growth, nodulation and plant growth along with rhizobium assisted N fixation are well documented (Almeida et al., 2000; Ge et al., 2025; LI et al., 2021; Peoples et al., 1998; Yeremko et al., 2025) and similar mechanisms may have operated here though dampened by high soil N. Furthermore, BNF increased substantially from vegetative to reproductive stages (Table 3. 2), consistent with the recognised pattern that legumes' N fixation demand peaks at maturity (Yun et al., 2023). Thus, intercropping had a robust positive effect on BNF by chickpea, whereas P fertilization had only a secondary, modest influence under the residual nutrient conditions of this experiment.

6.1.7. Belowground C allocation reveals contrasting species strategies under P limitation

I hypothesised that P fertilization would reduce wheat's root/total biomass ratio more than chickpea's especially in monoculture where wheat faces no P competition and that P fertilization would lower root respiration in wheat but increase root respiration in chickpea due to greater C allocation to BNF. Contrary to these expectations, the wheat root/total mass ratio did not decline with P fertilization (Table 3. 1), suggesting that wheat remained P-limited even after P fertilization, a conclusion supported by persistently low extractable soil P (Table 3. 2) and unchanged respiration rates (Figure 3. 4A). Prior research indicates that plants with low-P conditions often maintain high root investment compared to high P availability (Lopez et al., 2022; Xiao et al., 2024), which does not align with wheat's inflexible response. Although wheat respiration tended to be higher in mixtures (Figure 3. 4A), the increase was not significant. This trend may indicate competitive-related maintenance costs for growth and nutrient acquisition (Poorter et al., 1991; Reich et al., 1998; Rewald et al., 2016), potentially contributing to wheat's reduced yield in mixtures.

6.1.8. Chickpea reallocates C away from root biomass and toward respiratory support for BNF

Chickpea root/total mass ratio declined significantly with P fertilization and in mixtures (Table 3. 1), demonstrating reduced structural root allocation even as yield and P uptake increased. This behaviour suggests a trade-off between root growth and metabolic investment in BNF which is consistent with earlier work showing that BNF is highly C-intensive where plant distribute a substantial portion of assimilates to roots and nodules (Gordon, 1987) and where a big portion is released as part of respiratory demand (Voisin et al., 2003). This interpretation is reinforced by the sharp rise in chickpea specific root respiration under P-fertilized mixtures (Figure 3. 4), the highest across all treatments. Enhanced root respiration under low P status underpins the increased C investment for maintaining BNF and other processes observed in legumes (Nielsen et al., 1998; Nielsen et al., 2001). Thus, chickpea adopted a strategy of reduced structural root investment paired with increased respiratory C allocation, enabling greater BNF and yield benefits when competing with wheat. Together, these mechanisms highlight that intercropping does not guarantee mutual yield improvement; rather, species-specific nutrient and C economies determine system outcomes.

While these results provide insights on how P availability affects yield, BNF and C allocation, it does not address how cereal and legume species partition inorganic N as ammonium (NH_4^+) versus nitrate (NO_3^-) or how P availability modifies competition for these forms of N under intercropping system. To understand the full N economy of intercropping systems, a direct quantification of NH_4^+ vs NO_3^- uptake using ^{15}N tracers is required (Chapter 4).

6.1.9. Competitive uptake patterns reflect divergent nitrogen acquisition strategies under mixture

Although I predicted that wheat would dominate acquisition of both ^{15}N forms, the results only supported this for $^{15}NO_3^-$ and only under P fertilization (Figure 4. 3). Wheat recovered more $^{15}NO_3^-$ than chickpea under high P, a pattern consistent with wheat's known physiological preference for NO_3^- in non-acidic soils (Zhao & Shen, 2018) and with its higher specific root length and greater number of root tips could allow wheat to forage form NO_3^- more efficiently. In contrast, chickpea displayed consistently higher $^{15}NH_4^+$ recovery in roots than wheat across P treatments (Figure 4. 2) despite relying heavily on BNF for its N requirements (up to 90% of shoot N at maturity) and therefore having a reduced dependence on soil N (Ashworth et al., 2018; Singh et al., 2023). Several mechanisms likely explain chickpea's superior $^{15}NH_4^+$ recovery. For instance, chickpea roots had greater diameter and lower specific root length (Figure 4. 4), characteristics that discourage the uptake by root (Wen et al., 2022) and disfavour the absorption of less mobile nutrients such as NH_4^+ (Dallstream et al., 2025; Ito et al., 2023). N-fixing legumes often develop larger root diameters correlated with rhizobial colonisation which may relate to its investment in BNF (Wang et al., 2022). Additionally, legumes frequently exhibit stronger mycorrhizal associations, and mycorrhizae preferentially absorb NH_4^+ over NO_3^- (Kobae et al., 2010; Kronzucker et al., 1997). Although mycorrhiza was not measured in our study, legumes often form strong associations that may explain chickpea's higher ^{15}N recovery, particularly under low P where mycorrhiza could proliferate (Smith & Read, 2008)**.** Lastly, NH_4^+ is rapidly assimilated in legume roots into amides (Weissman, 1972), resulting in more ^{15}N retained in roots. These results demonstrate that BNF dependence does not diminish chickpea's competitive ability for NH_4^+, contradicting our initial assumption. Instead, chickpea's physiological and morphological specialisation positions it as a strong competitor for reduced N forms when intercropped.

6.1.10. Nitrogen form availability and soil pools influence apparent uptake competitiveness

Although total ^{15}N recovery were similar between species across N forms, this was misleading because the soil was labelled with double the amount of $^{15}NH_4^+$ relative to $^{15}NO_3^-$. Since the initial NO_3^- pool was much larger than the NH_4^+ pool (Table 4. 2; Table S4. *1*), $^{15}NH_4^+$ enrichment of the soil pool must have been substantially higher than that of $^{15}NO_3^-$. Therefore, absolute uptake of NO_3^- likely exceeded that of NH_4^+, as expected given its mobility and abundance (Wang & Macko, 2011). This highlights an important conceptual point for intercropping systems: competition outcomes depend not only on root traits or species identity but also on the relative abundance, mobility, and background concentration of each nutrient pool.

6.1.11. Phosphorus fertilization shifts competitive dynamics towards nitrate acquisition

I predicted that P fertilization would enhance wheat competitiveness for $^{15}NH_4^+$, given the immobility of NH_4^+ and wheat's fine root system. Instead, P fertilization strongly increased wheat's competitiveness for $^{15}NO_3^-$, not $^{15}NH_4^+$ (Figure 4. 3). Wheat's $^{15}NO_3^-$ recovery increased by more than 2000% with P fertilization, and this increase was most pronounced in shoot tissues (Figure 4. 1 & Figure 4.2), indicating that improved NO_3^- uptake was a key driver of enhanced shoot biomass of wheat (Table 4. 1). I observed a strong P × NO_3^- interaction, which likely reflects wheat's inherent preference for NO_3^- (Zhao & Shen, 2018). The P availability influence the ATP production, which may enhance N uptake and assimilation (Veneklaas et al., 2012). The sharp rise in ^{15}N recovery with P fertilization suggests improved uptake of both $^{15}NO_3^-$ and $^{15}NH_4^+$, consistent with studies showing that P deficiency limits absorption of both N forms (De Magalhães et al., 1998; Gniazdowska & Rychter, 2000; Rufty et al., 1990; Schjørring, 1986) and improved stoichiometric balance that allows greater N assimilation once P limitation is relieved (Ament et al., 2018; Blanes et al., 2012; Xiao et al., 2022). Notably, chickpea's ^{15}N recovery from both NO_3^- and NH_4^+ was unaffected by P fertilization, consistent with the absence of a P effect on its BNF or %Ndfa (Table 4. 2).

6.1.12. Root trait-N uptake relationships and limited trait plasticity under low P

Although wheat exhibited higher specific root length and more root tips, P fertilization did not alter any measured root morphological traits (Figure 4. 4). This suggests that the contrasting root architectures of the two species are mostly inherent genetic differences that show no or small plastic responses to P availability. This is consistent with the literature showing that plants expand their root surface area by developing thinner roots and more abundant root hairs to enhance soil exploration under phosphorus-limited conditions (Péret et al., 2011; Richardson et al., 2009). In this study, initial soil P was extremely low (1.9 mg kg^{-1} Bray-P), and even after post-fertilization, P availability remained low (Table 4. 2), explaining why morphological responses were minimal. Thus, root traits were major determinants of N uptake outcomes, but their expression was largely unaffected by P fertilization.

6.1.13. Species-specific controls on photosynthetic ^{13}C assimilation

While Chapter 4 elucidates inorganic N uptake pathways, it does not address how photosynthetic assimilation and belowground C allocation drive nutrient acquisition, nor how P availability influences rhizodeposition, root respiration, or rhizosphere interactions. To connect C assimilation with nutrient acquisition, a ^{13}C tracer method was used under mono- and intercropping systems (Chapter 5). First, species identity overwhelmingly controlled photosynthetic ^{13}C incorporation and belowground C distribution. I assumed that P availability would differentially regulate photosynthetic C assimilation in wheat and chickpea, with wheat showing a stronger dependence on external P supply than chickpea. Wheat displayed strong P responsiveness, with fertilization nearly doubling ^{13}C assimilation (Figure 5. 2), consistent with the pivotal role of P in ATP production and photosynthetic capacity, which can influence plant growth and productivity (Carstensen et al., 2018; Malhotra et al., 2018). Chickpea maintained low and stable ^{13}C enrichment across treatments, reflecting its well-documented physiological capacity to mitigate P deficiency through high-affinity transporters, organic-acid secretion, and phosphatase activity to mineralise organic P forms (George et al., 2010; Richardson et al., 2011). Moreover, its large C requirement to support BNF (White et al., 2007) constrained aboveground ^{13}C accumulation and stabilised belowground allocation regardless of P level or cropping system. The absence of cropping-system effects on short-term ^{13}C uptake confirms that photosynthetic C acquisition was driven primarily by species traits rather than immediate interspecific interactions. Overall, these findings show that wheat relies more heavily on external P inputs to sustain photosynthetic function, whereas chickpea is physiologically more resilient under low-P conditions.

Belowground C investment revealed fundamental differences in how cereals and legumes manage C-nutrient trade-offs. I hypothesised that P availability would differentially regulate belowground C allocation in wheat and chickpea, with chickpea maintaining stable root C investment across P regimes and wheat reducing root C allocation under P fertilization, particularly in intercropping systems. Chickpea displayed remarkably stable allocation of ^{13}C to roots and rhizodeposition across P treatments and cropping systems (Figure 5. 4 a, b), indicating a conservative and persistent belowground C strategy. This stability aligns with its continuous demand for photosynthate to support nodules and N-fixing bacteria, a metabolically expensive process that dominates its C economy (Voisin et al., 2003). Because the energetic cost of symbiotic N fixation remains high regardless of external P supply, chickpea's root-nodule system likely maintains a relatively constant C draw even when P availability fluctuates. Wheat, in contrast, exhibited a flexible and highly responsive belowground strategy. Under P deficiency, wheat allocated a greater share of recent assimilates to roots (Figure 5. 4 a, b), which is consistent with well-established increases in root: shoot ratios under nutrient limitation (Ciereszko et al., 1996; Nielsen et al., 2001). Once P supply improved, wheat sharply reduced ^{13}C investment in roots and intensified aboveground C use (Figure 5. 4a), suggesting that P fertilization alleviated the need for C-intensive nutrient foraging (Lu et al., 2022). This reallocation was particularly pronounced in intercrops, implying that interspecific interactions may further reduce wheat's belowground C demand when chickpea enhances rhizosphere P mobilisation.

Rhizodeposition patterns strengthened this contrast. Wheat displayed a strong P-induced rise in ^{13}C losses from rhizodeposits (Figure 5. 4c), consistent with increased C flow to the rhizosphere when photosynthetic activity and root metabolism are stimulated (Qiao et al., 2017). Chickpea, however, maintained minimal fluctuations in rhizodeposited ^{13}C, reflecting a strategy centred on internal C allocation to nodules rather than exudate-driven P foraging. Together, these results show that cereals modulate belowground C investment in tight correspondence with P availability, whereas legumes employ a physiologically anchored, fixation-oriented C strategy that is resilient to management and nutritional fluctuations.

6.1.14. Nutrient acquisition patterns shaped by C allocation and interspecific facilitation

Nutrient acquisition responses demonstrated clear divergence between cereals and legumes under mixture. Chickpea maintained high P uptake across all conditions (Figure 5. 5a), reflecting its ability to mobilise sparingly soluble soil P forms by rhizosphere acidification (Li et al., 2003; Li et al., 2004). Wheat, by contrast, showed a pronounced P-fertilization effect, with shoot P content more than doubling following P fertilization (Figure 5. 5a). Importantly, both species showed positive associations between P content and belowground C allocation, supporting the fundamental principle that root C expenditure enhances nutrient foraging by root and rhizosphere processes to make P available (Lu et al., 2022; Ven et al., 2019). Wheat's P acquisition particularly benefitted from rhizodeposition, and this efficiency increased when intercropped with chickpea, suggesting facilitative rhizosphere effects typical of legume–cereal systems (Chen et al., 2019; Gong et al., 2020). Chickpea, however, relied more consistently on root growth than rhizodeposits for P capture, underscoring distinct uptake pathways.

The ^{13}C pulse-labelling experiment validates the P acquisition trends reported in the third chapter and advances them by resolving the C allocation pathways that govern species-specific P uptake. Consistent with the earlier study, wheat showed a lower inherent capacity for P acquisition under intercropping, while chickpea maintained high P content and competitiveness, particularly when P was supplied. In the third chapter, this manifested as reduced wheat P accumulation and growth in mixtures, challenging the assumption that cereals automatically benefit from legume neighbours via reduced N competition. The ^{13}C pulse-labelling results provide a mechanistic explanation for this outcome by demonstrating that chickpea sustains stable belowground C investment and P acquisition regardless of cropping system, whereas wheat remains strongly dependent on external P inputs and facilitative rhizosphere effects. However, unlike the cumulative P content patterns observed previously, the ^{13}C data reveal that wheat can achieve higher P acquisition efficiency per unit of root or rhizodeposited C under intercropping, suggesting short-term facilitation that does not necessarily translate into greater whole-plant P accumulation or yield. These differences likely arise from methodological scale: the 3rd chapter integrates longer-term biomass partitioning and competitive outcomes, whereas ^{13}C pulse labelling captures immediate C allocation strategies and nutrient foraging efficiency. Together, the two studies indicate that although intercropping can transiently enhance wheat P foraging efficiency, chickpea remains the dominant competitor for P over longer timescales, particularly under equal planting densities.

Finally, increases in soil-available P with rising ^{13}C in roots and rhizodeposits (Figure 5. 8) indicate that increased root biomass inputs stimulated microbial processes that mobilise P from soil minerals and organic pools, consistent with evidence that microbes drive P cycling and temporarily store released P (Solangi et al., 2024). The above mechanisms involve exudate-driven shifts in pH and stimulation of microbial enzyme activities (Bilyera et al., 2021; Richardson, 1994; Spohn et al., 2013) which provides ecosystem-level evidence that C allocation strategies feed back into soil P dynamics.

6.2. Practical Implications

Collectively, the findings from the four research chapters provide a coherent, mechanistic re-interpretation of how P availability regulates productivity in cereal-legume intercropping systems, not merely through direct effects on nutrient uptake, but by reshaping whole-plant C economies and belowground investment strategies. The meta-analysis demonstrates that although P fertilization increases yield in both monocropping and intercropping systems, its greatest benefit in LER under intercropping indicates improving land-use efficiency by stronger interspecific resource sharing. This finding reframes P fertilization as a driver of interspecific interaction strength, with implications for designing input-efficient cropping systems rather than maximising single-crop responses. The experimental chapters extend this insight by demonstrating that P availability fundamentally alters how plants allocate photosynthate between shoots, roots, rhizosphere processes, and BNF. Chickpea maintains stable C allocation to BNF and belowground processes across P regimes, underpinning its resilience in low-P soils, whereas wheat shows a stronger dependence on external P supply to sustain photosynthetic efficiency, N uptake, and growth. These findings challenge nutrient-centric models of crop performance by showing that P availability governs productivity indirectly via C allocation trade-offs and metabolic costs associated with nutrient acquisition. The ^{13}C and ^{15}N tracing approaches indicate that belowground C allocation is closely associated with P acquisition, N-form uptake, and interspecific interactions, suggesting a potentially active role in regulating these processes, although alternative explanations such as surplus C disposal cannot be excluded. Enhanced root biomass, respiration, and rhizosphere activity under P limitation indicate that intercropping systems can mobilise soil P through biologically driven processes, but these responses impose measurable carbon costs that differ between cereals and legumes. In practical terms, this suggests that P management in intercropping should move away from uniform fertilizer application toward functionally targeted strategies. For example, moderate P limitation can be maintained to stimulate rhizosphere activity in legumes, which enhances P mobilisation, while growth phase-based P placement can be used to support cereal growth without suppressing belowground interactions. Also, using phosphorus based on the crop combination and soil phosphorus level, instead of applying the same amount everywhere, can improve nutrient uptake efficiency while reducing unnecessary carbon use. These results demonstrate that optimising intercropping performance depends on exploiting functional complementarity through differentiated P supply, rather than maximising fertilizer inputs. More broadly, the integration of meta-analysis with isotopic and physiological approaches provides a transferable framework for linking agronomic outcomes to underlying plant–soil–microbe mechanisms, enabling more predictive and system-specific nutrient management under P-limited conditions. Lastly, these findings indicate that phosphorus availability regulates key physiological and ecological processes underpinning productivity in cereal–legume intercrops, including biological nitrogen fixation, root carbon allocation, nutrient acquisition, and interspecific resource partitioning. Strategic P fertilization, particularly in P-limited soils, can maximise the complementary functioning of wheat and chickpea, resulting in greater land-use efficiency and improved nutrient-use efficiency at the system level. Therefore, optimising P management according to soil conditions and crop composition represents an important pathway for enhancing productivity while reducing reliance on synthetic N inputs and improving the long-term sustainability of intercropping systems.

6.3. Conclusions

1. Phosphorus fertilization generally increases productivity, but the magnitude and direction of responses vary with soil P availability, climate conditions, and crop composition, reflecting shifts between cereal-driven competition for soil P and legume-mediated rhizosphere processes that enhance P mobilisation.
2. Intercropping improves nutrient-use efficiency and land productivity, but its response to phosphorus fertilization varies with soil P status and crop composition: benefits are strongest under low P where legumes enhance P mobilisation, while high P availability reduces facilitation and increases competition, diminishing the intercropping advantage.
3. Legume competitive strength under P fertilization is context- and scale-dependent. In the longer-term, yield-integrated responses reported in Chapter 3, P fertilization amplified chickpea competitiveness, resulting in greater P accumulation and yield gains relative to wheat under equal planting densities. However, the ^{13}C pulse-labelling results in Chapter 5 reveal that this dominance is not driven by superior short-term carbon foraging efficiency. Instead, chickpea maintained stable carbon allocation and phosphorus acquisition regardless of cropping system, whereas wheat exhibited increased P acquisition efficiency per unit of root and rhizodeposited C when intercropped. These findings indicate that while P fertilization enhances legume competitive outcomes over the full growing season, cereals can transiently benefit from legume-induced rhizosphere modifications without achieving comparable cumulative yield or nutrient capture. Together, the two chapters demonstrate that apparent legume dominance reflects sustained, conservative uptake strategies rather than dynamic short-term plasticity in C allocation.
4. BNF enhancement is primarily intercropping-driven, with P supply providing only secondary benefits under high soil N.

5. Wheat relies on soil mineral N and thus responds strongly to P-mediated increases in NO_3^- uptake. Chickpea can rely on BNF and preferentially stores NH_4^+ in roots.
6. Increased NO_3^- uptake by wheat under P fertilization reduces direct N competition with chickpea, supporting greater complementarity.
7. Wheat's fine extended root system increases access to mobile NO_3^-. Chickpea's thicker roots and likely mycorrhizal affinity favour NH_4^+ uptake and retention.
8. Belowground C dynamics fundamentally shape species performance in cereal-legume intercropping systems. Chickpea consistently channels substantial C into biological N fixation and P-mobilising processes, enabling stable C and nutrient allocation patterns regardless of P supply. Wheat, by contrast, shows far less metabolic flexibility, relying heavily on external P to adjust its C allocation between shoots, roots, and the rhizosphere. Consequently, P fertilization and interspecific interactions do more than alter nutrient uptake and they restructure whole-plant C economies in species-specific ways that determine how each crop acquires and utilises nutrients in P-limited soils.
9. Wheat depends heavily on external P to sustain photosynthesis and relies on both root growth and rhizodeposition for P foraging, with intercropping enhancing acquisition efficiency.
10. Collectively, these findings strengthen the mechanistic basis for designing cereal-legume systems that exploit complementary carbon–nutrient strategies to improve nutrient-use efficiency in P-limited soils.

6.4. Key Limitations to Address

1. Glasshouse and pot experiments cannot fully replicate field heterogeneity (soil structure, rooting depth, water fluxes, mycorrhizal networks). This constrains the generalisability of belowground C allocation, root traits, and isotopic uptake patterns under real cropping systems.
2. Short experimental durations limit insight into multi-season nutrient cycling, interspecific interactions, and long-term C investment dynamics.
3. Using only a few discrete P levels (e.g., 0 vs 50 kg ha^{-1}) shows basic contrasts but does not capture the full response curve. As a result, it becomes difficult to identify threshold or saturation points for P effects on N uptake, biological N fixation, rhizodeposition, and root respiration.

4. Labelling by ^{13}C and ^{15}N labelling only gives short-term scenarios, not continuous measurements, so they may miss changes over time in rhizodeposition, root respiration, and switches between N forms.
5. Root interaction zones, competition intensity, and facilitation mechanisms were inferred from plant-level measurements, not directly visualised (e.g., via root imaging, mycorrhizal colonisation).
6. Phenological mismatches between wheat and chickpea were controlled experimentally but not quantified at high temporal resolution.
7. Although pot experiments were conducted under controlled temperature and light regimes, seasonal differences in background photoperiod and solar angle still influenced wheat growth trajectories, particularly tillering. One experiment (September) showed markedly reduced tiller formation compared with the other (May), leading to lower wheat biomass and yield in mixture treatments. This indicates that the glasshouse controls could not fully decouple plant development from ambient seasonal cues which is an inherent limitation that affects comparability across experiments and restricts the interpretation of yield differences.

6.5. Future Research Direction

1. Translate pot and glasshouse findings into multi-site field trials to test yield, nutrient uptake, and belowground C allocation under realistic soil and climate variability. Long-term field studies are needed to evaluate system stability, P-use efficiency, and soil C sequestration potential.
2. Assess a wider range of cereals and legumes to determine species- and genotype-specific competitiveness, facilitation mechanisms, and nutrient-acquisition strategies under contrasting P availability.
3. Implement multi-level and phenology-specific P treatments across multiple seasons to map P response curves, quantify mixture advantages under P-limited and P-sufficient soils, and understand how sustained P regimes influence productivity and soil function.
4. Deploy high-resolution root imaging across growth stages and P treatments to link root traits with nutrient uptake patterns, interspecific competition, and the physiological basis of mixture performance in P-deficient soils.

5. Quantify AMF colonisation, hyphal P transfer, and fungal responses to species interactions and P supply to clarify how mycorrhiza shape P uptake, nutrient complementarity, and the C costs of nutrient foraging in intercropping systems.
6. Combine repeated ^{15}N labelling with soil N monitoring to characterise temporal switching between NH_4^+ vs NO_3^- uptake and how this competitivity changes with P supply.
7. Parameterise process-based models with empirical C allocation, root traits, and nutrient uptake data to predict mixture outcomes and identify management levers that maximise facilitation and minimise competition.

6.6. References

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