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Getting to the roots:
Mechanisms of plant nitrogen and phosphorus acquisition in intercropping

DISSERTATION

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„Diversity in the field requires diversity of thinking and thorough analysis.“

Walter A (2018): A brief history of agriculture. https://bit.ly/3tglU7j (ethz.ch)

Summary

The major challenge of agriculture is to increase food production while simultaneously reducing environmental impacts and resource use. Intercropping, i.e., the simultaneous cultivation of at least two plant species in close proximity, is expected to be a promising approach as it potentially produces higher yields (referred to as overyielding) on less land and with fewer resource inputs (e.g., fertilizers) compared to monocropping. Overyielding in intercropping is supposed to result from above- and belowground interspecific plant interactions comprising the "4C" of competition, compensation, complementarity, and cooperation (facilitation). Intercropping has also been shown to increase plant nutrient contents, although the underlying mechanisms of plant nutrient acquisition are still not fully understood. The present thesis investigated, therefore, how belowground mechanisms of plant nitrogen (N) and particularly phosphorus (P) acquisition contribute to maize overyielding in intercropping.

To investigate the effects of intercropping on plant nutrition and productivity, four case studies were conducted combining different experimental setups and several species combinations with likely contrasting nutrient acquisition mechanisms. A two-year field experiment (Study I, with further explorations in Study II) was accompanied by three greenhouse experiments, of which one was conducted with soil from the field experiment (i.e., with various N and P sources; Study II) and two with mineral substrate (i.e., with defined N and P sources; Studies III and IV). In all experiments, maize (*Zea mays* L.) was cultivated as the main crop, while faba bean (*Vicia faba* L.), soy (*Glycine max* (L.) Merr.), blue lupin (*Lupinus angustifolius* L.), and white mustard (*Sinapis alba* L.) were cultivated as companion crops.

The thesis showed that intercropping resulted in maize overyielding and enhanced maize N and P contents in the field, especially in soy/maize and lupin/maize intercropping, as compared to maize monocropping. Smaller but still positive intercropping effects on maize productivity were also found in faba bean/maize (when simultaneously sown in 2019) and mustard/maize intercropping. Maize overyielding was mainly caused by belowground interspecific interactions in legume/maize intercropping and by aboveground interspecific interactions in mustard/maize intercropping. Legumes enhanced maize N acquisition in intercropping due to their ability to symbiotically fix atmospheric N_2 which was in part transferred to the maize plants, suggesting both N complementarity and N facilitation. Up to 20% of maize aboveground biomass N content was thus derived from legumes in the field. In addition, mustard slightly enhanced maize N acquisition in intercropping compared to monocropping, which was likely associated with compensation and/or complementarity.

Further, the thesis showed that all companion species had generally higher P contents (per plant) and/or higher P concentrations (per gram biomass) than maize, indicating that they mobilized P from sparingly soluble sources more effectively than maize. The three legumes had high phosphomonoesterase activities in the rhizosphere and exuded high amounts of dissolved organic carbon (DOC). The legumes also exuded high amounts of low molecular weight organic acid anions (LMWOA) into the rhizosphere. Faba bean additionally decreased while mustard increased its rhizosphere pH. These changes in the companions' rhizosphere likely mobilized P from organic (via high phosphomonoesterase activities and perhaps stimulation of microorganisms through DOC) and inorganic P sources (via rhizosphere pH changes and high LMWOA exudation). The large root lengths of faba bean, soy, and mustard probably promoted plant P uptake, at least once P was mobilized. Overall, the companion species used species-specific mechanisms of P mobilization, which were likely associated with P mining (exudation of P-mobilizing compounds), root foraging, and stimulation of beneficial microorganisms. In intercropping, these mechanisms were likely also beneficial for maize P acquisition due to P complementarity and P facilitation among the intercropped plant species.

Moreover, the thesis showed for the first time that a high LMWOA concentration in the rhizosphere in intercropping is not only caused by high LMWOA release of the companion species but also by an increased LMWOA exudation of maize, at least when grown together with lupin. With this, the thesis challenges the common view that legume/cereal intercropping is advantageous over monocropping due to the high P mobilization capacity of legumes from which the cereals simply benefit. Hence, the finding that the presence of lupin affected the exudation of maize provides new insights into the mechanisms underlying P acquisition in intercropping.

Taken together, the enhanced maize productivity in intercropping was likely the result of reduced competition for N and P due to the combined effects of compensatory, complementary, and facilitative plant interactions. Hence, intercropping with its positive effects on plant productivity and plant N and P acquisition is promising in achieving food sovereignty and reducing the reliance on industrial fertilizers like those derived from finite phosphate rock. Therefore, intercropping should be considered an integral part of an overall agricultural transformation to meet future needs while staying within humanities' safe (and just) operating space.

Zusammenfassung

Die größte Herausforderung der Landwirtschaft besteht darin, die Nahrungsmittelproduktion zu steigern und gleichzeitig Umweltbelastungen und Ressourcenverbrauch zu reduzieren. Mischkulturen, also der gleichzeitige Anbau von mindestens zwei Pflanzenarten in unmittelbarer Nähe, gelten hier als vielversprechender Ansatz, da sie im Vergleich zu Monokulturen potentiell höhere Erträge (Ertragssteigerungen) bei geringerem Flächen- und Ressourcenverbrauch (z.B. Düngemittel) erzielen. Die Ertragssteigerungen in Mischkulturen sollen aus ober- und unterirdischen interspezifischen Pflanzeninteraktionen resultieren, die die „4K“ von Konkurrenz, Kompensation, Komplementarität und Kooperation (Förderung bzw. „facilitation“) umfassen. Es hat sich auch gezeigt, dass Mischkulturen die Nährstoffgehalte der Pflanzen erhöhen, obwohl die zugrunde liegenden Mechanismen der Nährstoffakquise noch nicht vollständig verstanden sind. Die vorliegende Dissertation untersuchte deshalb, wie unterirdische Mechanismen der pflanzlichen Stickstoff- (N) und insbesondere Phosphor- (P) Aneignung in Mischkulturen zu Ertragssteigerungen von Mais beitragen.

Um die Auswirkungen von Mischkulturen auf Pflanzenernährung und -produktivität zu untersuchen, wurden vier Fallstudien durchgeführt, in denen verschiedene Versuchsarten und mehrere Pflanzenkombinationen mit wahrscheinlich unterschiedlichen Mechanismen der Nährstoffaneignung kombiniert wurden. Ein zweijähriger Feldversuch (Studie I, mit weiteren Untersuchungen in Studie II) wurde durch drei Gewächshausversuche ergänzt, von denen einer mit Boden aus dem Feldversuch (d. h. mit verschiedenen N- und P-Quellen; Studie II) und zwei mit Mineralsubstrat (d. h. mit definierten N- und P-Quellen; Studien III und IV) durchgeführt wurden. In allen Versuchen wurde Mais (*Zea mays* L.) als Hauptkultur angebaut, während Ackerbohne (*Vicia faba* L.), Sojabohne (*Glycine max* (L.) Merr.), blaue Süßlupine (*Lupinus angustifolius* L.) und weißer Senf (*Sinapis alba* L.) als Nebenkulturen angebaut wurden.

Die Dissertation zeigte, dass Misch- gegenüber Monokulturen zu Ertragssteigerungen und erhöhten N- und P-Gehalten der Maispflanzen im Feldversuch führten, insbesondere in Mischkulturen mit Soja und Lupine. Geringere, aber dennoch positive Effekte auf die Maisproduktivität wurden auch in Mischkulturen mit Ackerbohne (bei gleichzeitiger Aussaat in 2019) und Senf festgestellt. Ertragssteigerungen wurden in Mischkulturen mit Leguminosen hauptsächlich durch unterirdische und in Mischkultur mit Senf vor allem durch oberirdische interspezifische Interaktionen verursacht. Leguminosen verbesserten die Mais-N-Aneignung in Mischkulturen durch ihre Fähigkeit, atmosphärischen N_2 symbiotisch zu fixieren, der teilweise zu den Maispflanzen transferiert wurde, was sowohl auf N-Komplementarität als auch auf N-Förderung („facilitation“) hindeutet. Bis zu 20% des N-Gehalts der oberirdischen Mais-Biomasse im Feldversuch stammten somit von Leguminosen. Auch Senf erhöhte die Mais-N-Aneignung in Misch- gegenüber Monokultur leicht, wahrscheinlich durch Kompensation und/ oder Komplementarität.

Darüber hinaus zeigte die Dissertation, dass alle Nebenkulturen generell höhere P-Gehalte (pro Pflanze) und/oder höhere P-Konzentrationen (pro Gramm Biomasse) als Mais aufwiesen, was darauf hindeutet, dass sie P aus schwerlöslichen Quellen effektiver mobilisierten als Mais.

Die drei Leguminosen zeigten hohe Phosphomonoesterase-Aktivitäten in der Rhizosphäre und exsudierten große Mengen an gelöstem organischem Kohlenstoff (DOC). Die Leguminosen exsudierten auch große Mengen an Anionen organischer Säuren mit niedrigem Molekulargewicht (LMWOA) in die Rhizosphäre. Zusätzlich senkte die Ackerbohne den pH-Wert der Rhizosphäre und Senf erhöhte ihn. Diese Veränderungen in der Rhizosphäre der Nebenkulturen mobilisierten wahrscheinlich P aus organischen (über hohe Phosphomonoesterase-Aktivitäten und eventuelle Stimulierung von Mikroorganismen durch DOC) und anorganischen P-Quellen (über pH-Änderungen in der Rhizosphäre und hohe LMWOA-Exsudation). Die hohen Wurzellängen von Ackerbohne, Soja und Senf begünstigten wahrscheinlich die P-Aufnahme der Pflanzen, zumindest nach P-Mobilisierung. Insgesamt verwendeten die Nebenkulturen also spezies-spezifische Mechanismen der P-Mobilisierung, die mit dem P-„Mining“ (Exsudation P-mobilisierender Verbindungen), der Wurzelsuche („root foraging“) und der Stimulierung nützlicher Mikroorganismen assoziiert werden können. In Mischkulturen waren diese Mechanismen wahrscheinlich auch für die P-Aneignung des Mais von Vorteil aufgrund von P-Komplementarität und P-Förderung („facilitation“) zwischen den Pflanzen.

Darüber hinaus zeigte die Dissertation erstmals, dass eine hohe LMWOA-Konzentration in der Rhizosphäre in Mischkultur nicht nur durch eine hohe LMWOA-Freisetzung der Nebenkulturen verursacht wird, sondern auch durch eine erhöhte LMWOA-Exsudation des Mais, zumindest in Mischkultur mit Lupine. Damit stellt die Dissertation die verbreitete Ansicht in Frage, dass Leguminosen/Getreide-Mischkulturen gegenüber Monokulturen aufgrund der hohen P-Mobilisierungskapazität der Leguminosen vorteilhaft sind, von der das Getreide einfach nur profitiert. Folglich liefert der Befund, dass die Anwesenheit der Lupine die Exsudation von Mais beeinflusste, neue Erkenntnisse zu den Mechanismen, die der P-Aneignung in Mischkulturen zugrunde liegen.

Zusammenfassend lässt sich sagen, dass die gesteigerte Maisproduktivität in Mischkulturen wahrscheinlich das Ergebnis einer verringerten N- und P-Konkurrenz infolge der kombinierten Wirkung von kompensatorischen, komplementären und förderlicher Pflanzeninteraktionen war. Folglich sind Mischkulturen mit ihren positiven Auswirkungen auf Produktivität und N- und P-Aneignung der Pflanzen vielversprechend, um Ernährungssouveränität zu erreichen und die Abhängigkeit von Industriedüngern, wie denen aus begrenztem Phosphatgestein, zu verringern. Mischkulturen sollten daher als integraler Bestandteil einer größeren landwirtschaftlichen Transformation betrachtet werden, um künftige Bedürfnisse innerhalb der planetaren Grenzen (gerecht) zu erfüllen.

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SYNOPSIS

1 Introduction

Agricultural production is essential for human survival on Earth, yet it is also a key driver of biodiversity loss, land-use change, resource depletion, and climate change. In this way, agriculture pushes the Earth system over several planetary boundaries beyond which humanity leaves its “safe operating space” (Campbell et al. 2017). In particular, the global expansion of intensive agriculture with its large monocultures has led to an unsustainable use of natural resources at the expense of biodiversity and (agro)ecosystem functions. Consequently, two planetary boundaries have already been exceeded by far, namely biosphere integrity (especially genetic diversity) and biogeochemical flows, i.e., the global nitrogen (N) and phosphorus (P) cycles (Campbell et al. 2017; Steffen et al. 2015). Therefore, agriculture’s major challenge is to increase food production while simultaneously reducing environmental burden and resource use to stay within the planetary boundaries. Nothing less than radical transformations of the agricultural and food systems are needed, with various changes made to all aspects of production (Campbell et al. 2017; Gerten et al. 2020). Against this background, intercropping seems to be a promising way to achieve future food sovereignty without compromising Earth system resilience, as I will elucidate in the following.

1.1 Intercropping and its advantages

Intercropping is defined as “growing two or more crops simultaneously on the same field” (Andrews and Kassam 1976; Vandermeer 1989). More precisely, different plant (crop) species or genotypes are grown simultaneously either without distinct row management (“mixed cropping”), in alternating rows (“row intercropping”), or in strips comprising several rows (“strip intercropping”), with the simultaneity occurring only partly (“relay intercropping”) or during the whole life cycle of associated plant species (Andrews and Kassam 1976; Homulle et al. 2022; Malézieux et al. 2009). Such multispecies cropping systems have been widely applied for millennia by smallholder farmers in Asia, Africa, and Latin America, and are still present around the world. Currently, intercropping attracts worldwide attention because of its potential to produce high yields using less land and fewer inputs than monocropping (Brooker et al. 2015; Homulle et al. 2022; Tang et al. 2021). Often, the intercropped plant species are categorized into “main crop” (e.g., cereals) and “companion crop” (e.g., legumes), assuming that the main crop benefits from the companion crop. The terms are also used in the present thesis whenever the categorization is appropriate. Otherwise, the thesis also refers to “species combinations”, “associated plant species”, or “intercropped plant species”.

1.1.1 Agricultural diversification

Intercropping has been – and in some regions of the world still is – the dominant form of agriculture because it has multiple benefits over monocropping (Brooker et al. 2015). Most obviously, intercropping is characterized by an increased biodiversity. The increased plant diversity in the field may, as a side-effect, host a larger range of species, both above- and belowground, as compared to monocropping (Brooker et al. 2015; Malézieux et al. 2009). For instance, intercropping commonly increases the diversity of pollinators and natural enemies of crop pests, thus simultaneously increasing pollination and reducing plants' susceptibility to pests and diseases (Brooker et al. 2015; Stomph et al. 2020). Moreover, the diversity of soil organisms is influenced by plant species diversity since plants have species-specific effects on the soil microbial community due to specific root exudates. Consequently, intercropping potentially attracts different types of beneficial microorganisms, which might be antagonistic to plant pathogens, provide plants with additional nutrients, or stimulate plant resistance to abiotic stresses and diseases (Brooker et al. 2015; Duchene et al. 2017; Homulle et al. 2022).

By increasing both plant diversity and associated biodiversity, intercropping addresses one of the planetary boundaries that have been exceeded already. However, the biodiversity promotion itself is not the subject of the present thesis, as is also not the positive intercropping effects on soil and water conservation or microclimate regulation (Ehrmann and Ritz 2014; Malézieux et al. 2009). Instead, the thesis focuses on the effects of intercropping and enhanced biodiversity on plant productivity (yields) and plant nutrient acquisition.

1.1.2 Overyielding in intercropping

The relationship between biodiversity and primary productivity has been and continues to be the focus of many ecological studies, and evidence for a positive relationship between them is accumulating (Cappelli et al. 2022; Loreau et al. 2001; Tilman et al. 2014). There is also abundant scientific evidence that intercropping results in higher plant productivity and more stable yields than monocropping, as I will elaborate in the following.

In intercropping research, overall yield increases relative to monocropping are referred to as overyielding, which is often assessed by the land equivalent ratio (LER). The LER is interpreted as the relative land area needed for monocropping to produce the yields attained by intercropping (Stomph et al. 2020; Tang et al. 2021). It is calculated as the sum of the partial land equivalent ratios (pLER) of the intercropped plant species, i.e., their relative yields (the intercrop yield divided by the monocrop yield) (Mead and Willey 1980). If the LER exceeds 1.0, intercropping uses the land more efficiently than monocropping, i.e., higher yields are produced on the same area in intercropping than in monocropping (Stomph et al. 2020; Tang et al. 2021). Similar metrics can be used to characterize the water or nutrient use efficiency in intercropping as compared to monocropping (Li et al. 2020; Stomph et al. 2020).

Recent meta-analyses have reported an average global LER of 1.2 to 1.3, calculated across various species combinations and experimental setups (Bedoussac et al. 2015; Li et al. 2021; Martin-Guay et al. 2018; Yu et al. 2015). Thus, intercropping increased global yields by 20 to 30% as compared to monocropping. Although maize-based intercropping has been shown to result in higher LER than species combinations without maize (Li et al. 2020), intercropping research was not able so far to identify highly efficient species combinations or experimental setups since LER values are largely variable (Bedoussac et al. 2015; Yu et al. 2015). Specifically, single LER ranged between 0.5 and >4.0 in a global meta-analysis, indicating that intercropping can substantially increase yields or affect yields adversely (Martin-Guay et al. 2018). Consequently, it is still a matter of debate under which conditions and with which species combinations intercropping results in overyielding.

1.1.3 The importance of (interspecific) plant interactions

Overyielding in intercropping is supposed to result from (positive) above- and belowground interspecific plant interactions (Li et al. 2014; Zhang et al. 2001). These complex interactions have recently been described by the so-called "4C approach" comprising competition, compensation, complementarity, and cooperation effects (Justes et al. 2021). This approach offers the opportunity to characterize the combination of four effect types produced by various ecological processes occurring simultaneously and dynamically throughout the growing season in intercropping (Justes et al. 2021).

Ever since Darwin, competition has been considered one of the major principles shaping plant communities. Competition occurs when plants (or plant species) depend on the same resources in space and time, and one modifies the environment of the other adversely through, e.g., shading or exploitation of a resource which then becomes limiting (Bedoussac et al. 2015; Justes et al. 2021). Common limiting factors are light, water, temperature, and nutrients, with N, P, and water being often the most limiting resources in agroecosystems (Brooker et al. 2015; Marschner 2012). Since individuals of the same species have nearly identical resource needs, intraspecific competition is generally higher than interspecific competition among individuals of different species, be they plants or microorganisms (Adler et al. 2018; Morris and Blackwood 2015). Hence, resource competition is likely higher in mono- than in intercropping.

In addition to competition, compensation occurs in intercropping when the failure or yield reduction of one plant species is counterbalanced (i.e., compensated) by a yield increase of the other plant species (Horwith 1985; Justes et al. 2021). Especially if a replacement design is used, i.e., plants in monocropping are partly replaced by a companion species giving the same final plant density, resource needs can differ. One plant species might produce less biomass using fewer resources while more resources remain for the associated plant species, which can then produce more biomass, thus compensating the low biomass of the former (Neamatollahi et al. 2013).

Moreover, complementarity occurs in intercropping when plant species differ in their requirements for abiotic resources in space, time, and/or form. Plant species might, for instance, differ in their rooting depth, thus exploiting different soil horizons, or they might use light in different canopy layers (Justes et al. 2021; Malézieux et al. 2009). By contrast, cooperation occurs in intercropping when environmental modifications by one plant species are beneficial to the other plant species. One plant species might, for instance, give physical support to the other species and reduce lodging of the latter, or it might cover the soil surface, thus reducing evapotranspiration and water competition (Brooker et al. 2015; Justes et al. 2021). Cooperation is here synonymous with "facilitation" (Justes et al. 2021), which is also used in the thesis since it is the common term. Both complementarity and facilitation will be further elaborated below since they are crucial for plant nutrient acquisition in intercropping.

Taken together, overyielding in intercropping usually results from a more efficient acquisition and/or conversion of resources into biomass, which is the outcome of interspecific plant interactions occurring above- and belowground (Justes et al. 2021; Stomph et al. 2020). In particular, the yields of the associated plant species in intercropping depend on the relative contributions of competition, compensation, complementarity, and facilitation over the growing season and are then the result of the equilibrium of these interactions (Bedoussac et al. 2015; Justes et al. 2021).

Previous intercropping research further suggested that belowground interspecific interactions are more important for overyielding than aboveground interactions (Duchene et al. 2017; Li et al. 2014). However, only a few studies have quantified the relative contributions of above- and belowground interspecific interactions to overyielding in intercropping, so far. To distinguish between above- and belowground effects, so-called separation or partitioning techniques have been used. They were first established by Donald (1958), who fully partitioned root (belowground) and shoot (aboveground) effects using barriers. Although the partitioning techniques have been used repeatedly since then, a quantification of the relative contributions of above- and belowground interspecific interactions to overyielding remains rare and inconsistent regarding calculation and findings (Chen et al. 2015; Zhang et al. 2001). Consequently, additional research is needed to quantify the relative contributions of above- and belowground interspecific interactions to overyielding.

1.2 Nitrogen and phosphorus in intercropping

N and P are essential macronutrients that often limit plant growth in agriculture (Brooker et al. 2015; Marschner 2012). Yet, human activities have substantially altered the global N and P cycles with the result that the respective planetary boundary is exceeded by far, with agriculture being the main driver (Campbell et al. 2017; Steffen et al. 2015). Numerous research papers have shown that intercropping can contribute to increased plant N and P acquisition, thus simultaneously addressing yield limitations and environmental challenges associated with fertilizer applications (Li et al. 2020; Li et al. 2021; Tang et al. 2021; Xue et al. 2016). However, the underlying mechanisms are still not fully understood. The thesis, therefore, focuses on the mechanisms of plant N and especially plant P acquisition in intercropping, which likely contribute to overyielding.

1.2.1 The importance of N and P in plant nutrition

N is an essential macronutrient for plants since it is an integral component of proteins, nucleic acids, chlorophyll, enzymes, and secondary metabolites (Marschner 2012). By far the largest N pool is atmospheric N_2 comprising 79% of dry air. However, the major N forms taken up by plants are nitrate (NO_3^-) and ammonium (NH_4^+), while atmospheric N_2 is only available to plants living in symbiosis with N_2-fixing soil bacteria (Marschner 2012; Robertson and Vitousek 2009). To increase yields and avoid N limitation, industrial agriculture depends on N fertilizers manufactured in the energy-intensive Haber-Bosch process that was developed in the early 20th century to transform atmospheric N_2 into ammonia (NH_3) under high temperature and very high pressure (Robertson and Vitousek 2009). However, only around half of the N applied to agricultural soils is taken up by plants, while the remaining N contributes to soil, water, and air pollution resulting in soil acidification, eutrophication, ozone depletion, global warming, and biodiversity loss, with negative impacts on both human health and local ecosystems (Bodirsky et al. 2012; Robertson and Vitousek 2009; Sheldrick et al. 2002).

Although N is quantitatively more important than P for plant nutrition, P receives special attention in this thesis due to the finiteness of phosphate rocks and because P is perhaps the plant nutrient with the most limited bioavailability in soils (Menezes-Blackburn et al. 2018; Vance et al. 2003). P is also an essential macronutrient since it is an integral constituent in the structural and cellular metabolism of plants as component of, e.g., nucleic acids, phospholipids of biomembranes, and adenosine triphosphate (ATP) which is the primary carrier of chemical energy in cells (Ashley et al. 2011; Marschner 2012). Many soils contain considerable amounts of total P, but only a small proportion of usually less than 1% is immediately plant-available as plants can only take up P as orthophosphate anions ($H_2PO_4^-$ and HPO_4^{2-}) from the soil solution (Richardson et al. 2009). Soil P comprises a wide range of inorganic and organic P forms that differ substantially in their solubility and hence plant availability. Inorganic P is either adsorbed to soil constituents (e.g., clay minerals, organic matter) or precipitated predominantly with calcium (Ca) and magnesium (Mg) in alkaline soils and with iron (Fe) and aluminum (Al) in acidic soils (Hinsinger 2001; Richardson et al. 2009). By contrast, typically 30–65% of soil P (in some agricultural soils up to 85%) is present as organic P in topsoil, of which the majority (60–90%) exists as orthophosphate monoesters, with *myo*-inositol hexakisphosphate (phytate) being the most abundant form (Menezes-Blackburn et al. 2018; Stutter et al. 2012; Turner et al. 2002). A part of the various soil P forms (e.g., phytate) has been suggested to be only scarcely available to plants. Hence, P must be mobilized, i.e., inorganic P must be either desorbed or solubilized, and organic P must be mineralized, in order to become plant-available as dissolved phosphate anions (Menezes-Blackburn et al. 2018; Richardson et al. 2009).

To increase yields and avoid P limitation, huge amounts of P fertilizers have been and are applied in industrial agriculture, especially since the 1940s to keep up with N fertilization (Ashley et al. 2011; Cordell and White 2014). Most P fertilizer today is produced from phosphate rock, whose application is problematic for several reasons: (1) it is a finite, nonrenewable resource that will likely be exhausted during the next decades, (2) very few countries control around 85% of the world's reserves, (3) remaining reserves contain less P but more impurities and contaminants than at present, (4) mining, processing, and transport currently rely on fossil fuels, whose finiteness will increase fertilizer prices, and (5) already today, many poor farmers cannot access fertilizer markets due to low purchasing power (Ashley et al. 2011; Cordell and White 2014; Elser and Bennett 2011). A further problem is that less than half of the P applied to agricultural soils is taken up by plants, while the remaining P is quickly immobilized in soil (Richardson et al. 2009; Sheldrick et al. 2002). This resulted in a build-up of so-called "legacy P" stocks in many soils of the Global North (Cordell and White 2014; MacDonald et al. 2011). Through soil erosion processes, excessive (legacy) soil P leads both to environmental problems, such as eutrophication and marine "dead zones", and to an acceleration of P resource depletion (Ashley et al. 2011; Elser and Bennett 2011). At the same time, particularly soils in the Global South are still P-deficient (Cordell and White 2014; MacDonald et al. 2011).

Thus, although the element P will never run out per se, phosphate rock-derived fertilizer application is increasingly questioned due to economic, environmental, geopolitical, and social concerns associated with its short- and long-term use (Ashley et al. 2011; Cordell and White

2014). Consequently, mitigating the negative impacts of excessive N and P fertilization while increasing yields is one of the key challenges of global agriculture in this century. This challenge can be addressed through intercropping since some plant species effectively mobilize N and P from which associated plants likely benefit, as I will elaborate next.

1.2.2 Mechanisms of plant N and P acquisition in intercropping

As indicated above, complementarity and facilitation among intercropped plant species can reduce competition (here for nutrients) and thus are crucial for increased plant nutrient acquisition in intercropping. They comprise several mechanisms of plant N and P acquisition, which are discussed below and summarized in Fig. 1 at the end of this section.

Plant N acquisition in intercropping

Previous research on N acquisition in intercropping has mainly focused on processes associated with atmospheric N_2 fixation by legumes (Duchene et al. 2017; Homulle et al. 2022), as is also the case in the present thesis. To break the triple bond between N atoms in atmospheric N_2, much energy is needed, which is naturally provided by lightning and a few specialized prokaryotes that possess the enzyme nitrogenase (Marschner 2012; Robertson and Vitousek 2009). The most significant N-fixers for plants are symbiotic bacteria, and the legume-rhizobia symbiosis is likely the most prominent example of N_2-fixing systems in agriculture (Herridge et al. 2008; Marschner 2012). Legumes provide rhizobia, located in specific root structures called nodules, with photosynthates. In return, rhizobia perform a biological reduction of N_2 to NH_3 at ambient temperature and pressure, thus providing plant-available N to the legumes (Marschner 2012; Robertson and Vitousek 2009). Consequently, legumes are able to use atmospheric N_2 through the symbiotic association with rhizobia.

In intercropping, the legumes' ability to symbiotically fix atmospheric N_2 can enhance plant N acquisition of the main crop through complementarity and/or facilitation. To begin with, the interspecific competition for soil N is likely decreased through complementarity when legumes rely mainly on symbiotically fixed N, with the result that more reactive soil N is available for associated plants, e.g., for intercropped cereals (Duchene et al. 2017; Hinsinger et al. 2011; Homulle et al. 2022). Such a chemical complementarity has been found, for instance, in pea/wheat and pea/barley intercropping (Bedoussac and Justes 2010; Hauggaard-Nielsen et al. 2009; Jensen 1996). Especially when cereals are highly competitive for N, legumes have been reported to rely more on symbiotically fixed N (Fan et al. 2006; Homulle et al. 2022), indicating that interspecific plant interactions influence the N_2 fixation activity. Moreover, complementarity for N might also occur in intercropping if the intercropped plant species differ in or change their preferential uptake of NH_4^+ or NO_3^- (Boudsocq et al. 2012; Homulle et al. 2022).

In addition to complementarity, facilitation for N acquisition occurs in intercropping if a part of the symbiotically fixed N is transferred from the legume to the main crop, thus directly facilitating N acquisition of the main crop (Duchene et al. 2017; Homulle et al. 2022). The (interplant) N transfer can be quantified through natural variation of ^{15}N in plant dry matter among the involved plant species (most evident in biomass $\delta^{15}N$) since legumes that symbiotically fix atmospheric N_2 tend to have a lower $\delta^{15}N$ signature than non-legumes that use reactive soil N (He et al. 2009; Peoples et al. 2015). Several mechanisms are potentially involved in the N transfer: N might be directly relocated via rhizodeposition (i.e., exudation of soluble N compounds by legumes that are taken up by non-legumes) or via mycorrhizal networks. N might also be transferred when legume residues (e.g., root tissues, foliage) decompose and the mineralized N is taken up by associated plants (Homulle et al. 2022; Peoples et al. 2015; Thilakarathna et al. 2016). Faba bean, for instance, has been shown to transfer symbiotically fixed N to intercropped wheat (Wahbi et al. 2016; Xiao et al. 2004). However, N transfer from legumes to cereals has mostly been demonstrated in pot experiments, while evidence for N transfer on a field scale is highly variable (Duchene et al. 2017; Homulle et al. 2022). For instance, a recent literature review found that the N transfer in mixed stands ranged from 0 to 73%, depending on species combinations and abiotic conditions (Thilakarathna et al. 2016).

Consequently, it is still a matter of debate under which conditions and with which companion species the symbiotic N_2 fixation by legumes leads to N complementarity and when N is transferred from legumes to associated non-legumes in intercropping.

Plant P acquisition in intercropping

Since most soil P is present in chemical forms poorly available for plant uptake, plants have evolved several mechanisms to mobilize P from sparingly soluble P sources. They can be summarized as ‘root foraging’ via morphological adaptations and ‘P mining’ via physiological adaptations to P deficiency (Lyu et al. 2016; Richardson et al. 2011; Wen et al. 2021). Root foraging aims at exploring a greater soil volume to minimize the distance between roots and plant-available phosphate anions in soil by extending the root system and increasing the root surface (Ma et al. 2018; Richardson et al. 2011; Wen et al. 2019). Especially *Brassicaceae* exhibit a fibrous root system with thin, long, and intensively branched roots, whereas *Lupinus* species form thick and comparatively short taproots (Lyu et al. 2016; Wen et al. 2019).

In contrast to root foraging, P mining aims at mobilizing phosphate anions from sparingly soluble P sources by exuding various P-mobilizing compounds into the rhizosphere (Richardson et al. 2011; Wen et al. 2021). As mentioned earlier, organic P must be mineralized to release plant-available P into the soil solution. More precisely, mobilizing P from organic sources involves two steps: organic P needs to be released from precipitates and adsorption sites before it can be mineralized via extracellular phosphatase enzymes (Menezes-Blackburn et al. 2018; Richardson et al. 2009; Sulieman and Mühling 2021). Phosphatase enzymes catalyze the hydrolysis of esters and phosphoric acid anhydrides. They have been classified into (1) phosphoric monoester hydrolases (phosphomonoesterases), (2) phosphoric diester hydrolases (phosphodiesterases), (3) triphosphoric monoester hydrolases, and (4) enzymes acting on phosphoryl-containing anhydrides and P–N bonds. Phytases are a specific group of phosphomonoesterases that hydrolyze all six phosphate groups of phytate (Nannipieri et al. 2011). By combining two imaging methods (soil zymography and diffusive gradients in thin films), it has recently been shown that a high phosphatase activity in the rhizosphere of blue lupin was associated with P depletion (Hummel et al. 2021). However, a part of the organic soil P pool, particularly phytate, has been suggested to be recalcitrant and is, therefore, only slowly hydrolyzed by enzymes (Jarosch et al. 2015; Menezes-Blackburn et al. 2018; Nannipieri et al. 2011). Moreover, plant species differ considerably in their capacity to exude phosphomonoesterases and phytases. Legumes and particularly *Lupinus* species are known for a high phosphomonoesterase activity in the rhizosphere, thus likely mobilizing organic P more effectively than *Gramineae* and *Brassicaceae* (Sulieman and Mühling 2021; Wen et al. 2019). However, also legumes differ in their phosphomonoesterase activity, which might affect the P mobilization potential of different legumes (Dong et al. 2020; Gilbert et al. 1999; Tang et al. 2021).

As opposed to organic P mineralization, sparingly soluble inorganic P must be solubilized or desorbed to release plant-available P into the soil solution (Hinsinger 2001; Hinsinger et al. 2003; Richardson et al. 2009). In alkaline soils, P from Ca and Mg phosphates (e.g., apatites, struvites) can be mobilized via rhizosphere acidification since the solubility of such phosphates increases with decreasing pH (Hinsinger 2001; Lindsay et al. 1989; Talboys et al. 2016). In acidic soils, P from Fe and Al phosphates can be mobilized via rhizosphere alkalinization since the solubility of such phosphates increases with increasing pH (Hinsinger 2001; Lindsay 1979; Richardson et al. 2009). Plants can modify the rhizosphere pH by releasing protons or hydroxyls that affect the cation-anion exchange and/or by exuding low molecular weight organic acid anions (LMWOA). In addition, LMWOA can mobilize inorganic P by modifying the surface characteristics of soil colloids, by competing with phosphate for sorption sites, or by chelating cations bound to P (Hinsinger et al. 2003; Richardson et al. 2009; Wang and Lambers 2020). However, only some plant species exude LMWOA at high rates or change the rhizosphere pH substantially. Legumes and particularly *Lupinus* species are known for a high LMWOA exudation (Wang and Lambers 2020; Wen et al. 2019), while different *Brassicaceae* have been shown to substantially alkalize their rhizosphere, thus increasing P mobilization from Fe phosphate (Marschner et al. 2007). A previous study has also demonstrated that LMWOA exudation is not necessarily strongly aligned with a plant species' capacity to mobilize P from Fe, Al, and Ca phosphates (Pearse et al. 2007).

In intercropping, the companions' ability to effectively mobilize P from organic and inorganic sources can enhance plant P acquisition of the main crop through complementarity and/or facilitation. Chemical complementarity has been found, for instance, in lupin/wheat intercropping, where lupin preferentially used soil P mobilized by citrate, whereas wheat preferentially used water-extractable soil P, leading to the exploitation of both P pools (Cu et al. 2005). Moreover, spatial complementarity occurs if plant species differ in their root architecture, thus exploring different soil horizons (Hinsinger et al. 2011; Xue et al. 2016). For instance, maize has been reported to spread its roots in a different soil part than the associated faba bean, thereby increasing the soil volume exploited for nutrients and water (Li et al. 2006).

In addition to complementarity, facilitation for P acquisition occurs in intercropping if P-mobilizing (companion) species increase P availability in the rhizosphere via root exudation, thus directly facilitating the P acquisition of the (non-P-mobilizing) main crop (Hinsinger et al. 2011; Li et al. 2014; Xue et al. 2016). Particularly legumes have been reported to exude high amounts of phosphatases, protons, and LMWOA, which can lead to P mobilization and hence might also be beneficial for the main crop. This has been demonstrated, e.g., for faba bean (Li et al. 2016; Zhang et al. 2016a), lupin (Dissanayaka et al. 2015; 2017), chickpea (Li et al. 2003; Li et al. 2004), alfalfa (Sun et al. 2020), and cowpea (Latati et al. 2014), with positive effects on P uptake and growth of intercropped cereals (mainly maize or wheat). In addition, also *Brassicaceae* can substantially change the rhizosphere pH and exude considerable amounts of LMWOA (Marschner et al. 2007; Zhang et al. 1997). However, previous studies on increased P availability through root exudation and consequent effects on plant P acquisition in intercropping are, to some extent, inconclusive (Pearse et al. 2007; Xue et al. 2016). Several studies found, for instance, no effect of intercropping on P uptake and plant growth of the main crop, although the companions exuded high amounts of carboxylates or changed the rhizosphere pH substantially (e.g., Li et al. 2010; Wang et al. 2007).

Against this background of complementarity and facilitation, the increased productivity and enhanced nutrient contents of cereals in legume/cereal intercropping have often been explained by the P mobilization capacity of legumes, e.g., through the release of LMWOA, from which cereals also benefit (Li et al. 2014; Tang et al. 2021; Zhang et al. 2016a). However, some studies found that cereals also enhanced their LMWOA exudation when grown together with different legumes as compared to cereal monocropping. For instance, an increased concentration of different LMWOA such as malate, citrate, and oxalate has been found in the rhizosphere soil of wheat in intercropping compared to monocropping when grown together with faba bean, white lupin, or pea (Li et al. 2016; Lo Presti et al. 2021). Similarly, the malate concentration in the rhizosphere soil of maize was significantly increased by a factor of 12 in intercropping with faba bean compared to maize monocropping (Li et al. 2013). When maize was intercropped with alfalfa, similar findings were obtained in pot experiments (Wang et al. 2020) and in the field (Sun et al. 2020). The LMWOA composition of maize also differed significantly between mono- and intercropping when maize was grown together with faba bean or white lupin supplied with $FePO_4$ (Li et al. 2010). However, in many of these studies, it cannot be excluded that LMWOA released by the companion species diffused to the roots of the main crop because LMWOA were collected from the rhizosphere soil (Li et al. 2010; Li et al. 2016; Lo Presti et al. 2021; Wang et al. 2020). Thus, it is not clear if the LMWOA are derived from the cereal or the legume. Therefore, research that clearly separates root exudates released by the main and the companion crop could help enlighten the effect that increases P mobilization in intercropping.

Final considerations

Many of the mechanisms of increased nutrient (especially P) acquisition in intercropping may only occur when the intercropped species have intimate root contact, i.e., when root intermingle (Hinsinger et al. 2011; Homulle et al. 2022). However, the processes occurring in the overlapping rhizospheres of different plant species are still not fully understood. This is partly because rhizosphere research has so far often concentrated on studying individual plants kept isolated in pots. Moreover, the mechanisms of nutrient acquisition substantially vary between plant species and even genotypes as, e.g., between legumes and non-legumes regarding N acquisition or between P-mobilizing and non-P-mobilizing plant species (Homulle et al. 2022; Li et al. 2014). Consequently, further research is needed to deepen our understanding of interspecific root interactions and their effect on nutrient availability, plant nutrient uptake, and plant growth in intercropping.

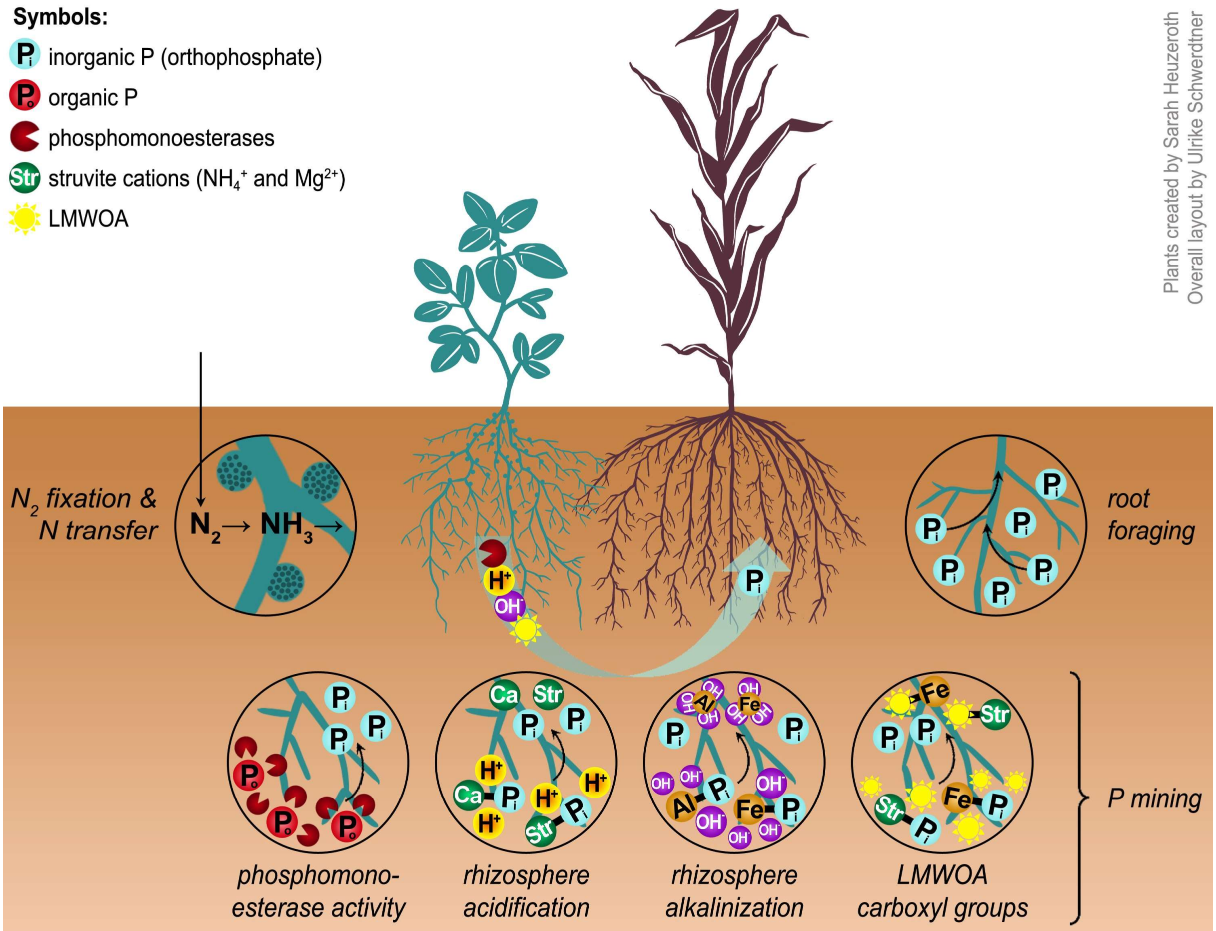


FIGURE 1 Overview of the potential mechanisms of plant N and P acquisition in intercropping comprising N_2 fixation by rhizobia in root nodules and subsequent N transfer from legumes to associated plants (left), root foraging for P via morphological adaptations (right), and P mining via physiological adaptation (below the plants). The latter include high phosphomonoesterase activity in the rhizosphere to mineralize organic P as well as rhizosphere acidification, rhizosphere alkalinization, and high LMWOA exudation to solubilize inorganic P. The symbols used are explained in the upper left corner, except for chemical elements.

1.3 Objectives and hypotheses

The present thesis was driven by the question of how belowground mechanisms of plant N and P acquisition contribute to maize overyielding in intercropping. To investigate this topic, four case studies were conducted combining different experimental setups and several species combinations which likely have contrasting mechanisms of nutrient acquisition. A two-year field experiment (Study I, with further explorations in Study II) was accompanied by three greenhouse experiments, of which one was conducted with soil from the field experiment (i.e., with various N and P sources; Study II) and two with mineral substrate (i.e., with defined N and P sources; Studies III and IV).

The field experiment was conducted to investigate potential intercropping effects on maize under ambient conditions. The rhizoboxes (used in all greenhouse experiments) allowed to measure microscale rhizosphere processes multiple times in the rhizosphere of the same plant using imaging techniques. Using a mineral substrate further allowed us to investigate the rhizosphere processes under controlled conditions with exactly defined nutrient forms.

In all experiments, maize was cultivated as the main crop as it is one of the most important crops for food production worldwide (Xu et al. 2020). Three *Fabaceae* (legumes: faba bean, soy, blue lupin) and one *Brassicaceae* (mustard) were used as companion species. Since positive intercropping effects have mainly been ascribed to the beneficial characteristics of the companion species from which the main crop benefits, the present thesis focused on the effects of intercropping with different companion species on maize.

The main hypotheses of the thesis were:

(1) Intercropping results in maize overyielding and enhanced maize N and P contents as compared to maize monocropping.
(2) Belowground interspecific interactions contribute more than aboveground interspecific interactions to maize overyielding in intercropping.
(3) Legumes enhance maize N acquisition in intercropping due to their ability to symbiotically fix atmospheric N_2, which is in part transferred to the maize plants.
(4) Companion species mobilize P from sparingly soluble sources more effectively than maize due to a higher root exudation of various P-mobilizing compounds.
(5) Companion species enhance maize P acquisition in intercropping due to their ability to effectively mobilize P from sparingly soluble sources.
(6) Maize increases its own root exudation in intercropping compared to monocropping.

2 Materials and methods

The experimental setups of the four case studies are briefly described below, while all analyses are described in detail in the methods section of each study.

2.1 Experimental setup of the field experiment (Studies I and II)

The field experiment was conducted at the University of Bayreuth (Germany) from May to August in 2018 and 2019. The soil texture was loamy sand (10% clay, 23% silt, 67% sand). In the upper 15 cm, the following soil chemical properties were determined (kg^{-1} soil): pH 6.9, 23.9 g total C, 2.2 g total N, and 1.3 g total P. 23.3% of the total P was organic P. According to Hedley fractionation (see Study II), 18.3% of total P was water-soluble, 18.9% was $NaHCO_3$-soluble, 20.8% was NaOH-soluble, 25.8% was HCl-soluble, and 16.2% was residual P.

Five blocks subdivided into six plots (2.5 × 1.7 m) were cultivated in row intercropping, where maize (*Zea mays* L.) was intercropped with one of the following companion species: faba bean (*Vicia faba* L.), soy (*Glycine max* (L.) Merr.), blue lupin (*Lupinus angustifolius* L.), or white mustard (*Sinapis alba* L.). As a control, maize was also cultivated in monocropping. Each plot consisted of eight alternating rows of maize and companion species with twelve plants per row having a distance of 20 cm between plants and rows. Each species combination was replicated five times (25 plots in total). Before seeding, the soil was prepared by plowing, rotary tillage, and surface steaming in both years. In addition, root barriers were installed in one half of each plot in the second year of the field experiment, according to Zhang et al. (2001), to separate the plant rows, thus preventing interspecific root interactions between maize and companions. In the first year, faba bean was sown three weeks earlier than the other plant species (because slower growth was expected), while all seeds were simultaneously sown by hand in the second year. As both summers were dry, the plots were regularly watered to avoid water competition between the plants.

At the end of the growing season, ten mature plants per species (20 maize plants in maize monocropping) were harvested from the four innermost rows of each plot. In the second year, five mature plants were harvested in the plot part with root barriers and five in the part without root barriers. All plants were analyzed for aboveground biomass (AGB). Subsamples of maize leaves, shoots, and grains were also analyzed for N and P concentrations and for the isotopic N signature ($\delta^{15}N$). In the first year, five soil samples per plot were collected between rows at a soil depth of 0–15 cm and homogenized for each plot. Soil samples were analyzed for phosphomonoesterase activity, soil pH, and water-extractable N (NO_3^- and NH_4^+). Further experimental details and photos of the field experiment are provided in Studies I and II.

2.2 Experimental setup of the greenhouse experiment with various N and P sources (Study II)

For the first greenhouse experiment, soil was collected directly next to the field experiment in March 2018 and sieved (< 2 mm); plant residues were removed with tweezers. The soil was filled into rhizoboxes made of PVC with an inner size of 49.2 × 29.3 × 3.0 cm (h × w × d) to a final bulk density of 0.8 g cm^{-3}, and soil water content was adjusted to 50% water holding capacity (WHC). In each box, two plants were sown at a distance of 15 cm. One plant was maize; the other was one of the companion species also used in the field experiment: faba bean, soy, blue lupin, and white mustard. As a control, two maize plants were sown together. Each species combination was replicated five times, but soy failed (except for two rhizoboxes up to the time of the first imaging analysis), resulting in a total of 20 rhizoboxes at harvest. The rhizoboxes were placed in a greenhouse and inclined by 50° on wooden racks to make the roots grow at the bottom wall of the rhizoboxes. The rhizoboxes were arranged in a randomized block design and re-randomized after six weeks. They were watered every two days with tap water to 50% WHC as measured by weight. Plants were sown in April and harvested in July 2018.

Six and nine weeks after sowing, pH imaging and soil zymography were performed to determine the spatial and temporal distribution of pH and phosphomonoesterase activity. Both analyses were conducted at a soil depth of 17–26 cm (from the top, box-centered). Plants were harvested 12 weeks after sowing and analyzed for biomass and N and P concentrations. For this purpose, plants were divided into AGB and roots. In addition, soil was sampled from the area of previous imaging analyses and analyzed for water-extractable N (NO_3^- and NH_4^+). Further experimental details are described in Study II.

2.3 Experimental setup of the greenhouse experiment with single plants and defined P sources (Study III)

In the second greenhouse experiment, single plants were grown in a mineral substrate with one of three P sources. The mineral substrate consisted of 20% (vol.) perlite and 80% (vol.) quartz sand, from which 50% had a grain size of 0.1–0.4 mm and 50% had a grain size of 0.7–1.2 mm. Micronutrients were added to the mineral substrate (320 mg $rhizobox^{-1}$), containing 5.0% MgO, 2.0% Fe, 1.5% Cu, 1.0% Mn, 0.8% Mo, 0.6% B, and 0.5% Zn. One of three P sources was added to the mineral substrate (150 mg P $rhizobox^{-1}$): iron phosphate (iron(III) phosphate dihydrate: $FePO_4 \times 2H_2O$), phytate (phytic acid sodium salt hydrate: $C_6H_{18}O_{24}P_6 \times xNa^+ \times yH_2O$), and struvite (ammonium magnesium phosphate hydrate: $NH_4MgPO_4 \times xH_2O$). The final mineral substrate was filled into rhizoboxes made of PVC with an inner size of 39.2 × 19.2 × 2.2 cm (h × w × d) to a final bulk density of 1.1 g cm^{-3}. Plants were sown at a rate of one seed per rhizobox using the same plant species as before: maize, faba bean, soy, blue lupin, and white mustard. All treatments were replicated four times (60 rhizoboxes in total). However, only two faba beans per P source grew until harvest, and one rhizobox of lupin supplied with struvite failed shortly before harvest. An inoculum, made with soil from the field experiment, was applied to the mineral substrate directly after sowing in order to introduce a soil microbial community. The final soil inoculum had the following chemical properties (l^{-1} inoculum): 15.3 mg organic C, 4.4 mg N, 0.9 mg P, and pH 7.9. Each rhizobox received 180 ml of soil inoculum and 50 ml of tap water to adjust the mineral substrate to 75% WHC. The rhizoboxes were placed and re-randomized in an open greenhouse under ambient conditions and without artificial light, as in Study II. Rhizoboxes were watered every two days with tap water to 75% WHC. In addition, a P-free nutrient solution, i.e., an adapted Ruakura solution

(Smith et al. 1983), was applied regularly, containing (l^{-1}): 220 mg $Mg(NO_3)_2 \times 6 H_2O$, 746 mg $Ca(NO_3)_2 \times 4 H_2O$, 377 mg NH_4NO_3, 189 mg KNO_3, 367 mg K_2SO_4, 27 mg Na_2SO_4, and 15 mg NaCl. Plants were sown in August and harvested in October 2019.

Six weeks after plant emergence, pH imaging and soil zymography were performed to determine the spatial distribution of pH and phosphomonoesterase activity. The pH imaging was conducted to a soil depth of 18 cm and soil zymography to a soil depth of 31 cm (both from the top, box-centered). At harvest, ten weeks after plant emergence, root exudates of intact plants were collected and analyzed for pH, phosphomonoesterase activity, LMWOA, and dissolved organic carbon (DOC). Plants were analyzed for biomass, root morphology, and P concentrations. Further experimental details are described in Study III.

2.4 Experimental setup of the greenhouse experiment with a defined P source (Study IV)

In the third greenhouse experiment, the same mineral substrate as in Study III (section 2.3) was filled into the same rhizoboxes as in Study II (section 2.2) to a final bulk density of 1.1 g cm^{-3}. The only P source in this experiment was $FePO_4$, which was thoroughly mixed to the mineral substrate, amounting to 422 mg P per rhizobox (88.8 mg P kg^{-1} substrate as in Study II). In each rhizobox, two plants were sown at a distance of 15 cm. The same plant species as before were used, except for faba bean. All species were cultivated in monocropping (two plant individuals of the same species), and maize was also cultivated in intercropping, i.e., together with one of the companion species (soy, lupin, and mustard). Each of the seven species combinations was replicated four times (except for soy and mustard in monocropping, of which one rhizobox each failed), resulting in a total of 26 rhizoboxes. Again, an inoculum and a P-free nutrient solution were applied, and all rhizoboxes were treated as in Study III (section 2.3). Plants were sown in March and harvested in May 2020.

At harvest, ten weeks after plant emergence, root exudates of intact plants were collected and analyzed for pH, LMWOA, and DOC. Plants were analyzed for biomass and P and Fe concentrations. Further experimental details are described in Study IV.

2.5 Calculations

In the field experiment, the partial land equivalent ratios (pLER) of maize AGB and grain yield per square meter were calculated according to Mead and Willey (1980), as follows:

$$\mathrm{pLER} = \frac{\mathrm{X_{intercropping}}\ [\mathrm{g\ m^{-2}}]}{\mathrm{mean\ X_{monocropping}}\ [\mathrm{g\ m^{-2}}]} \qquad (1),$$

where X is maize AGB or maize grain yield, respectively. Similarly, the partial N and P equivalent ratios (pNER and pPER) of maize grain yield N and P contents per square meter were calculated, as described in Study I (Equations 2 and 3 therein).

In Study II (and here additionally for Study IV), partial plant equivalent ratios (ppER) of maize AGB and maize AGB N and P contents per plant were calculated, as follows (the second P is uncapitalized here to distinguish partial plant and partial P equivalent ratios):

$$\mathrm{ppER} = \frac{\mathrm{X_{intercropping}}\ [\mathrm{g\ plant^{-1}}]}{\mathrm{mean\ X_{monocropping}}\ [\mathrm{g\ plant^{-1}}]} \cdot 0.5 \qquad (2),$$

where X is maize AGB or maize AGB N or P content, respectively. In the thesis, the ppER were multiplied by 0.5 in order to account for the proportion of the plant species in the rhizoboxes, thus allowing a better comparison of the ratios on a square meter and on a plant basis. In addition, the ppER were also calculated for the field experiment (2019 only) to allow a direct comparison of the different experiments. A partial ratio (pLER, pNER, pPER, or ppER) larger than 0.5 indicates intercropping benefits for single maize plants. A partial ratio larger than 1.0 indicates intercropping benefits for maize on an area basis (with twice as many maize plants in monocropping as in intercropping).

Further, the relative contributions of above- (RC_{above}) and belowground (RC_{below}) interspecific interactions to maize overyielding in the different species combinations were calculated based on the barrier treatments in 2019, as described in Study I (Equations 4–7 therein).

The $\delta^{15}N$ values of maize leaves, shoots, and grains were used to calculate the $\delta^{15}N$ of maize AGB, with which the legume-derived N content of maize AGB was calculated, as described in Study II (Equations 2–4 therein).

For the thesis, the rhizosphere pH changes and the phosphomonoesterase activities determined twice by the imaging techniques in Study II were calculated as means of the two analyses conducted six and nine weeks after sowing. The pH in the exudate solutions and the phosphomonoesterase activities determined by soil zymography in Study III were calculated here as means across the three P sources.

Further calculations can be found in detail in the methods sections of the four studies.

2.6 Statistics

Prior to all statistical analyses, normality was checked with Shapiro-Wilk normality tests, and homogeneity of variances was tested with Levene’s tests. When normality and homogeneity assumptions were met, analyses of variance followed by Tukey’s post-hoc test (Tukey honestly significant difference) were conducted to identify significant differences. When normality and homogeneity assumptions were not met, Kruskal-Wallis tests followed by post-hoc tests using the criterium Fisher’s least significant difference and Holm correction for p adjustment were conducted to identify significant differences. The partial (land, N, P, or plant) equivalent ratios were tested for significant differences from 0.5 (corresponding to maize in monocropping). All other data in the thesis were tested for significant differences among the different plant species or among maize in the different species combinations. Statistical groups with n = 2 were included in figures (white-patterned) but excluded from statistical analyses. Details of the statistical analyses and further comparisons can be found in the four studies.

All statistical analyses and data visualization were performed in R (version 3.5.2; R Core Team 2018) using the packages agricolae (version 1.3-2; Mendiburu 2020), car (version 3.0-7; Fox and Weisberg 2019), dplyr (version 0.8.5; Wickham et al. 2020), ggpattern (version 0.4.2; FC et al. 2022), and ggplot2 (version 3.3.0; Wickham 2016).

3 Results and discussion of key findings

3.1 Maize productivity

3.1.1 Maize biomass, grain yield, and N and P contents

In both years of the field experiment (Study I), the pLER of maize AGB were significantly larger than 0.5 in legume/maize intercropping without root barriers ($p \leq 0.002$), except for faba bean/maize intercropping in 2018 (Fig. 2a). The pLER of maize AGB were also slightly larger than 0.5 in mustard/maize intercropping in both years without root barriers ($p \leq 0.071$). No significant difference from 0.5 was found in all species combinations with root barriers (Fig. 2a). The pLER of maize grain yield were significantly larger than 0.5 in soy/maize (both years; $p \leq 0.007$) and lupin/maize intercropping (2018; $p = 0.003$), and slightly larger than 0.5 in lupin/maize (2019; $p = 0.075$) and mustard/maize intercropping (2018; $p = 0.050$) without root barriers (Fig. 2b). With root barriers, the pLER of maize grain yield did not significantly differ from 0.5 in all species combinations (Fig. 2b). Averaged across both years, the pLER of maize grain yield in soy/maize (1.40) and lupin/maize intercropping (1.19) without root barriers equal an overyielding of maize by 40% and 19%, respectively, on an area basis with twice as many maize plants in monocropping than intercropping (Fig. 2b). Maize in mustard/maize and faba bean/maize intercropping overyielded only on a plant basis by 46% (pLER of 0.96) and 45% (pLER of 0.95; 2019 only), respectively (Fig. 2b).

In both years of the field experiment, the pNER of maize grain yield were significantly larger than 0.5 in soy/maize ($p < 0.005$) and lupin/maize intercropping ($p \leq 0.030$), and slightly larger than 0.5 in mustard/maize intercropping ($p \leq 0.097$) without root barriers (Fig. 2c). The pPER of maize grain yield were significantly larger than 0.5 in soy/maize (both years; $p \leq 0.004$) and lupin/maize intercropping (2018; $p = 0.004$), and slightly larger than 0.5 in lupin/maize (2019; $p = 0.070$) and mustard/maize intercropping (2018; $p = 0.078$) without root barriers (Fig. 2d). With root barriers, pNER and pPER of maize grain yield did not significantly differ from 0.5 in all species combinations (Fig. 2c–d). Averaged across both years and compared to maize monocropping, maize grain yield N content was increased by 44%, 38%, and 11% in soy/maize, lupin/maize, and mustard/maize intercropping, respectively, on an area basis (Fig. 2c). Similarly, maize grain yield P content was increased by 49% and 24% in soy/maize and lupin/maize intercropping, respectively, on an area basis (Fig. 2d). On a plant basis, maize grain yield N and P contents were also increased in faba bean/maize (+34% N; +28% P) and mustard/maize intercropping (+47% P), when averaged across both years and compared to maize monocropping (Fig. 2c–d).

In the second year of the field experiment, the ppER of maize AGB N content were significantly larger than 0.5 in all species combinations ($p \leq 0.040$), whereas the ppER of maize AGB P content were significantly larger than 0.5 only in soy/maize and lupin/maize intercropping ($p \leq 0.017$; Table 1). The ppER of maize AGB P content also tended to be larger than 0.5 in faba bean/maize and mustard/maize intercropping in the field ($p = 0.180$; Table 1). In the first greenhouse experiment (Study II), the ppER of maize AGB N content were significantly larger than 0.5 in faba bean/maize and lupin/maize intercropping ($p = 0.007$; Table 1). In contrast, the ppER of maize AGB and maize AGB P content were not significantly different from 0.5 in both greenhouse experiments with species combinations (Studies II and IV; Table 1).

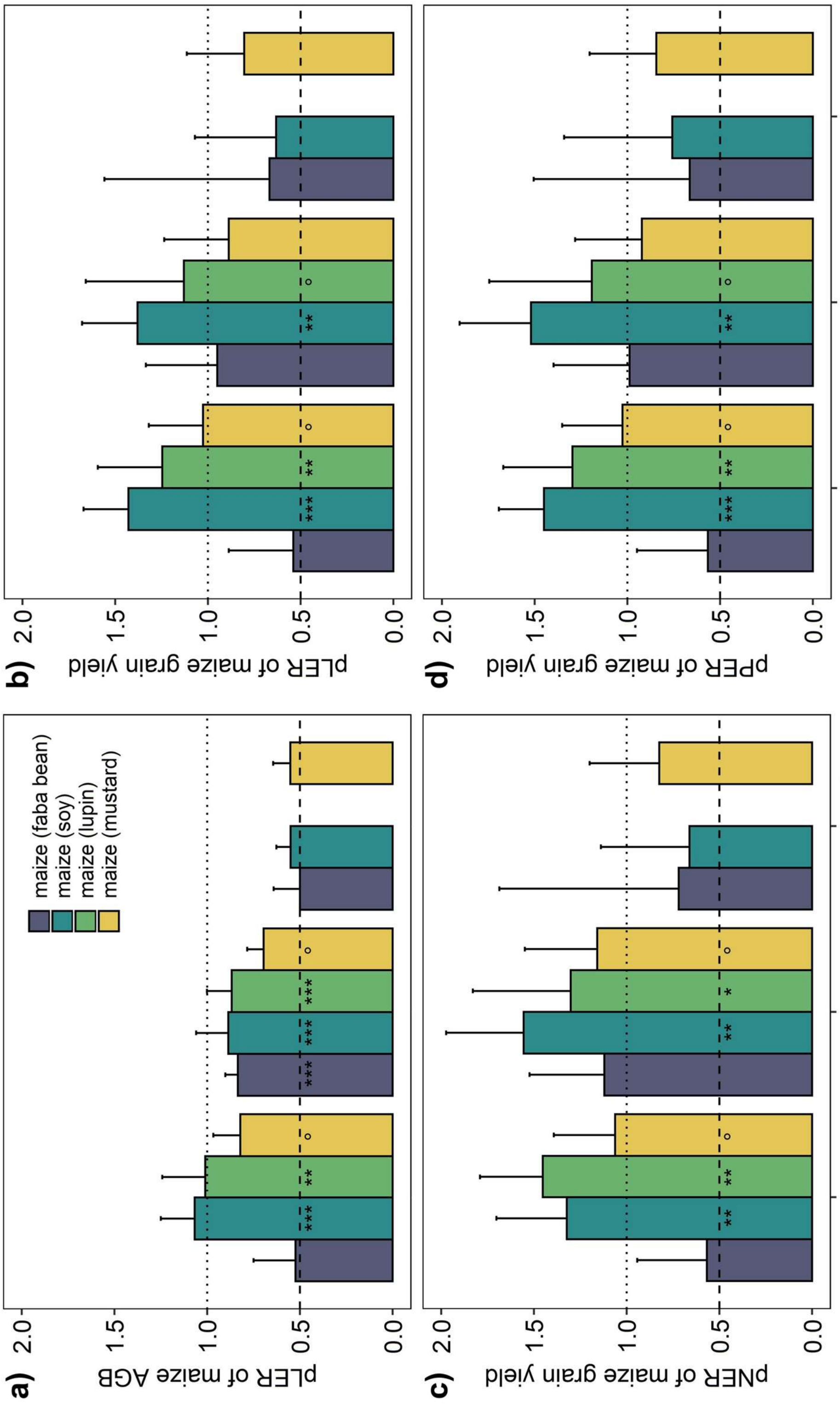


FIGURE 2 Partial land equivalent ratios (pLER) of maize aboveground biomass (AGB; a) and maize grain yield (b) as well as partial nitrogen equivalent ratios (pNER; c) and partial phosphorus equivalent ratios (pPER; d) of maize grain yield, determined in Study I at harvest of the field experiment in 2018 (no root barrier; nb) and 2019 (nb or with root barrier; rb). Columns show means, and error bars indicate standard deviations (n = 5). A ratio larger than 0.50 (dashed line) indicates maize overyielding on a plant basis. A ratio larger than 1.00 (dotted line) indicates maize overyielding on an area basis and overyielding of the whole species combination. Symbols indicate that ratios were significantly different from 0.50 (°: $p < 0.10$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$), tested separately for each ratio, year, and barrier treatment. Absence of symbols indicates that ratios were not significantly different from 0.50. See also Table 1 in Study I.

TABLE 1 Partial plant equivalent ratios (ppER) of maize aboveground biomass (AGB) and maize AGB N and P contents, determined in Study II (field experiment in 2019 and greenhouse experiment with various N and P sources) and for Study IV (greenhouse experiment with defined P sources). See also Figs. 1 and 2 in Study II and Table 1 in Study IV.

	Species combination	AGB	AGB-N	AGB-P
ppER in the field (2019)	maize (f.bean)	0.83 ± 0.07***	0.99 ± 0.20*	0.78 ± 0.04
	maize (soy)	0.89 ± 0.17***	0.94 ± 0.24*	0.91 ± 0.29*
	maize (lupin)	0.87 ± 0.13***	0.99 ± 0.18*	0.89 ± 0.23*
	maize (mustard)	0.70 ± 0.09°	0.94 ± 0.35*	0.76 ± 0.19
ppER in Study II	maize (f.bean)	0.72 ± 0.31	1.01 ± 0.20**	0.70 ± 0.20
	maize (lupin)	0.78 ± 0.27	1.01 ± 0.21**	0.72 ± 0.17
	maize (mustard)	0.68 ± 0.39	0.68 ± 0.30	0.58 ± 0.24
ppER in Study IV	maize (soy)	0.55 ± 0.16	NA	0.63 ± 0.25
	maize (lupin)	0.61 ± 0.18	NA	0.79 ± 0.14
	maize (mustard)	0.52 ± 0.17	NA	0.57 ± 0.23

Numbers show means ± standard deviations (n = 5 in Study II and n = 4 in Study IV). Symbols indicate that ppER were significantly different from 0.50 (°: $p \leq 0.10$; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$), tested separately for each ratio and each study. Absence of symbols indicates that there was no significant difference. NA: not analyzed.

Taken together, the effects of intercropping on maize productivity were more evident in the field experiment than in the greenhouse experiments. Moreover, maize AGB was much higher in the field (about 130 g plant^{-1} without root barriers; Fig. 3 in Study I) than in the rhizoboxes (about 14 g plant^{-1} in Study II (Table 1 therein), and about 3 g plant^{-1} in Study IV (Table 1 therein)). These differences indicate that plant growth was restricted in the greenhouse experiments, probably by the small soil volume available for developing a functional root system that sustains sufficient nutrient acquisition. Hence, it was likely the physical restriction that mitigated the intercropping effects on maize in the greenhouse experiments. I will, therefore, focus on the findings of the field experiment in the following discussion.

The finding that pLER, pNER, and pPER of maize were generally larger than 0.5 without root barriers (Fig. 2) indicates that single maize plants produced more AGB and grain yield and had higher N and P contents in intercropping than in monocropping (except for faba bean/maize intercropping in 2018, which is probably due to the earlier sowing of faba bean, as discussed in Study I). These intercropping benefits for maize plants indicate that interspecific (nutrient) competition in intercropping was less pronounced than intraspecific competition in maize monocropping when roots were intermingled. This could be due to compensation effects (Horwith 1985; Neamatollahi et al. 2013), i.e., maize in intercropping had more resources available than maize in monocropping due to a lower maize plant density in intercropping than in monocropping and because companion species produced less AGB than maize plants (Fig. 4 in Study I). It has been shown for maize monocropping with different planting densities that a lower grain yield per plant was fully compensated by a higher plant density (Testa et al. 2016).

Moreover, the pLER, pNER, and pPER of maize grain yield tended to be equal to or even larger than 1.0 (Fig. 2). This implies that maize in intercropping was similarly productive as in monocropping on an area basis, even though there were twice as many maize plants per area in monocropping than in intercropping (see also Fig. 4 in Study I). LER, NER, and PER of each species combination are then also larger than 1.0, even though companion species have often been shown to result in small partial ratios (Yu et al. 2016). Therefore, a moderate overyielding of both maize and the whole intercropping system can be assumed for all species combinations (except for faba bean/maize intercropping in 2018). Similarly, both maize N and P contents and overall plant N and P uptake are likely higher in intercropping than in monocropping. The moderate overyielding of maize by 19–40% found in soy/maize and lupin/maize intercropping is in the range reported in previous meta-analyses and reviews (Bedoussac et al. 2015; Yu et al. 2016). For instance, soy enhanced AGB production and grain yield of associated maize resulting in a global mean LER of 1.32 for soy/maize intercropping (Chen et al. 2019; Xu et al. 2020). Overyielding has also been found when maize was intercropped with faba bean (Li et al. 2018; Xia et al. 2019), lupin (Lelei and Onwonga 2014), or the *Brassica* turnip rape (Xia et al. 2013; 2019). Similarly, the enhanced maize N and P contents are in accordance with previous studies showing that N and/or P uptake of maize was higher in intercropping with soy (Chen et al. 2019; Xia et al. 2019), lupin (Lelei and Onwonga 2014), and turnip rape (Xia et al. 2013; 2019) than in maize monocropping.

The overyielding of maize without root barriers together with the finding that pLER, pNER, and pPER of maize did not differ significantly from 0.5 with root barriers further indicate that compensation alone cannot fully explain the enhanced productivity of maize in intercropping, which makes the contribution of complementary and facilitative plant interactions very likely, as discussed next.

3.1.2 Contributions of interspecific plant interactions to maize overyielding

Based on the differences in maize yield among the barrier treatments in the second year of the field experiment, the relative contributions of above- and belowground interspecific interactions to maize overyielding were calculated. Maize overyielding in mustard/maize intercropping was to 78% caused by aboveground interspecific interactions (Table 2). In contrast, maize overyielding in soy/maize and faba bean/maize intercropping was mostly caused by belowground interspecific interactions, accounting for 85% of maize overyielding in soy/maize intercropping and for 62% in faba bean/maize intercropping (Table 2).

TABLE 2 Mean relative contributions of above- (RC_{above}) and belowground (RC_{below}) interspecific interactions to maize overyielding in 2019, determined in Study I. See also Table 2 in Study I.

Species combination	RC_{above} [%]	RC_{below} [%]
Faba bean/maize	38	62
Soy/maize	15	85
Mustard/maize	78	22

Since maize overyielding in faba bean/maize and soy/maize intercropping was caused more by belowground than aboveground interspecific interactions, similar findings are assumed for lupin/maize intercropping, which was conducted without root barriers due to time constraints during installation. Thus, belowground interspecific interactions accounted for more than half of maize overyielding in legume/maize intercropping. These findings are in accordance with previous studies that attributed overyielding in faba bean/maize intercropping mainly to belowground interspecific interactions and showed higher plant nutrient acquisition when the roots of both plant species had physical contact (Li et al. 1999; 2007). Overyielding in soy/maize intercropping has also been associated more with below- than aboveground interactions (Lv et al. 2014). The high contribution of belowground interspecific interactions to maize overyielding in legume/maize intercropping points to the importance of belowground complementarity and facilitation of associated plants, as will be discussed in depth in sections 3.2 (for N acquisition) and 3.3.3 (for P acquisition).

Our finding that aboveground interspecific interactions contributed considerably to maize overyielding in mustard/maize intercropping (Table 2) indicates that maize might have benefited from a lower competition for light in intercropping with mustard compared to maize monocropping. A more efficient light use has been reported, e.g., for several legume/maize intercropping systems compared to the respective monocrops (Kermah et al. 2017). In addition, also other aboveground interactions might have positively influenced maize growth, especially in mustard/maize intercropping. These include effects of increased biodiversity on pollinators, pests, and diseases and the suppression of weeds through early soil coverage (Ehrmann and Ritz 2014; Brooker et al. 2015). Moreover, the microclimate regulation through increased soil shading and hence reduced evapotranspiration and more balanced temperatures might have contributed to overyielding in intercropping (Malézieux et al. 2009). However, these aboveground effects were not the subject of the present thesis.

In summary, intercropping resulted in maize overyielding and enhanced maize N and P contents (in AGB and grains), especially in soy/maize and lupin/maize intercropping as compared to maize monocropping. Smaller but still positive intercropping effects on maize productivity were also found in faba bean/maize (2019) and mustard/maize intercropping, provided that both plant species were simultaneously sown in the field. The positive intercropping effects on maize productivity are likely the result of reduced competition due to the combination of compensatory, complementary, and facilitative plant interactions, be they belowground (more important in legume/maize intercropping) or aboveground (more important in mustard/maize intercropping).

3.2 N acquisition in intercropping

In the first year of the field experiment, the $\delta^{15}N$ of maize AGB was significantly decreased by a factor of 0.8 in faba bean/maize intercropping compared to maize monocropping ($p = 0.032$; Fig. 3a). Further, the $\delta^{15}N$ of maize AGB tended to be lower in lupin/maize and soy/maize intercropping compared to maize monocropping, but this was not statistically significant ($p = 0.158$ and $p = 0.448$, respectively; Fig. 3a). The proportion of maize N that was transferred from legumes was 20.3 ± 10.9, 15.2 ± 5.3, and 10.9 ± 9.5% in faba bean/maize, lupin/maize, and soy/maize intercropping, respectively (see also Study II). The legume-derived maize N content was highest in lupin/maize intercropping (Fig. 3b). No significant difference in the $\delta^{15}N$ of maize AGB was found among the species combinations in 2019 (Fig. S3 in Study II).

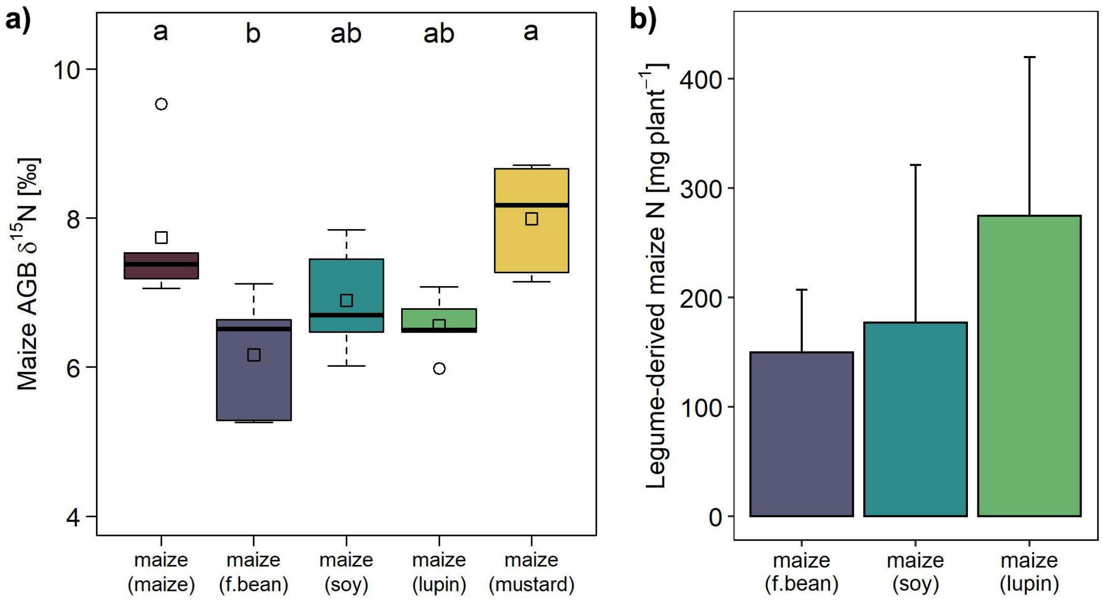


FIGURE 3 δ^{15}N values of maize aboveground biomass (AGB; a) and legume-derived N content of maize AGB (b), determined in Study II at harvest of the field experiment in 2018. Squares in (a) show means, columns in (b) show means, and error bars in (b) indicate standard deviations (n = 5). Different letters indicate significant differences ($p < 0.05$) among the species combinations, tested separately for each panel. Absence of letters indicates that there was no significant difference. See also Figs. 3 and S3 in Study II.

Taken together, 11–20% of maize AGB N content was derived from legumes in legume/maize intercropping (Fig. 3b). This indicates that legumes symbiotically fixed atmospheric N_2 and that a part of this N was transferred to the maize plants, suggesting N complementarity and N facilitation. Thus, both processes likely contributed to the increased maize N contents in legume/maize intercropping, as indicated by both the pNER of maize grain yield in Study I (field; Fig. 2c) and the ppER of maize AGB N content in Study II (field and greenhouse; Table 1). On the one hand, the N_2 fixation by legumes might have reduced the interspecific competition for soil N in legume/maize intercropping compared to maize monocropping through chemical complementarity. Such a chemical complementarity between cereals using mostly reactive soil N and legumes using mostly atmospheric N_2 has also been found in pea/barley (Hauggaard-Nielsen et al. 2009; Jensen 1996) and pea/wheat intercropping (Bedoussac and Justes 2010). On the other hand, a part of the symbiotically fixed N was likely transferred from the legumes to the maize plants through (1) rhizodeposition from legumes, (2) transport via mycorrhizal hyphae, and/or (3) decomposition of legume residues (nodules, roots, foliage) and mineralization of their organic N (Bedoussac et al. 2015; Hupe et al. 2021; Peoples et al. 2015; Thilakarathna et al. 2016). The N transfer found here is in accordance with previous studies reporting N transfer from legumes to non-legumes that was mainly found in pot experiments with lupin/rapeseed, pea/barley, soy/maize, and faba bean/wheat intercropping (Génard et al. 2016; Johansen and Jensen 1996; Meng et al. 2015; Xiao et al. 2004). However, a few studies found evidence for N transfer in a field experiment using the ^{15}N natural abundance method (Duchene et al. 2017; He et al. 2009). Further, only a few studies have so far shown such a high proportion of legume-derived maize N as we found here. For instance, 11%, 13%, and 6% of cereal N were derived from legumes in pea/barley, faba bean/wheat, and kidney bean/wheat intercropping, respectively (Chapagain and Riseman

2014; 2015). The lack of evidence for N transfer in the second year of the field experiment might be due to soil mixing during field preparation, as discussed in Study II.

The pNER of maize grain yield was also slightly larger than 0.5 when maize was intercropped with mustard in the field experiment (Fig. 2c). The reason might be that competition for N in mustard/maize intercropping was lower than in maize monocropping due to compensation or complementarity effects, even though no atmospheric N_2 was fixed as in intercropping with legumes. Compensation would imply that mustard took up less N than maize, with the result that more reactive soil N remained for the associated maize plants. This is supported by low plant N concentrations and hence low N demand of mustard that was reported earlier (Schröder and Köpke 2012). In addition, complementarity would imply that maize and mustard differed in or changed their preferential uptake of N as NH_4^+ or NO_3^- from the soil solution (Boudsocq et al. 2012; Homulle et al. 2022).

In summary, all three legumes likely enhanced maize N acquisition in intercropping due to their ability to symbiotically fix atmospheric N_2, which was in part transferred to the maize plants, suggesting both complementarity and facilitation. In addition, mustard slightly enhanced maize N acquisition in intercropping, probably through compensation and/or complementarity.

3.3 P acquisition in intercropping

3.3.1 Plant P acquisition

In Study III, P concentrations of the total biomass of soy ($p = 0.028$), lupin ($p = 0.052$), mustard ($p = 0.052$), and faba bean (not statistically tested) were higher by a factor of 2.2–2.6 than of maize when P was provided in the form of struvite (Fig. 4a; green columns). When P was provided in the form of phytate, P concentrations of mustard ($p < 0.001$), soy ($p < 0.001$), faba bean (not statistically tested), and lupin ($p = 0.024$) were significantly higher by a factor of 1.4–2.6 than of maize (Fig. 4a; red columns). When P was provided in the form of $FePO_4$, plant P concentrations decreased in the order mustard > lupin > soy > maize, both in Study III (Fig. 4a; plain browny columns) and Study IV (Fig. 4a; striped browny columns), with the P concentration of maize being significantly lower than of the other plant species ($p < 0.003$ in Study III and $p < 0.001$ in Study IV). In addition, the P concentration of faba bean was similar to that of mustard in Study III (Fig. 4a).

Similar to P concentrations, the P content of maize was, in general, significantly lower than of the other plant species (Fig. 4b), with a few exceptions, as follows. When P was provided in the form of phytate, the P content of lupin did not differ significantly from that of maize but was significantly lower than of soy ($p < 0.001$) and mustard ($p = 0.005$; Fig. 4b; red columns). When P was provided in the form of $FePO_4$, no significant difference among the plant species was found in Study III (Fig. 4b; plain browny columns), while P contents of lupin ($p < 0.001$) and mustard ($p = 0.011$) but not soy ($p = 0.228$) were significantly higher than of maize in Study IV (Fig. 4b; striped browny columns). In addition, the P content of faba bean tended to be much higher than of the other plant species in Study III, irrespective of P source (not statistically tested; Fig. 4b).

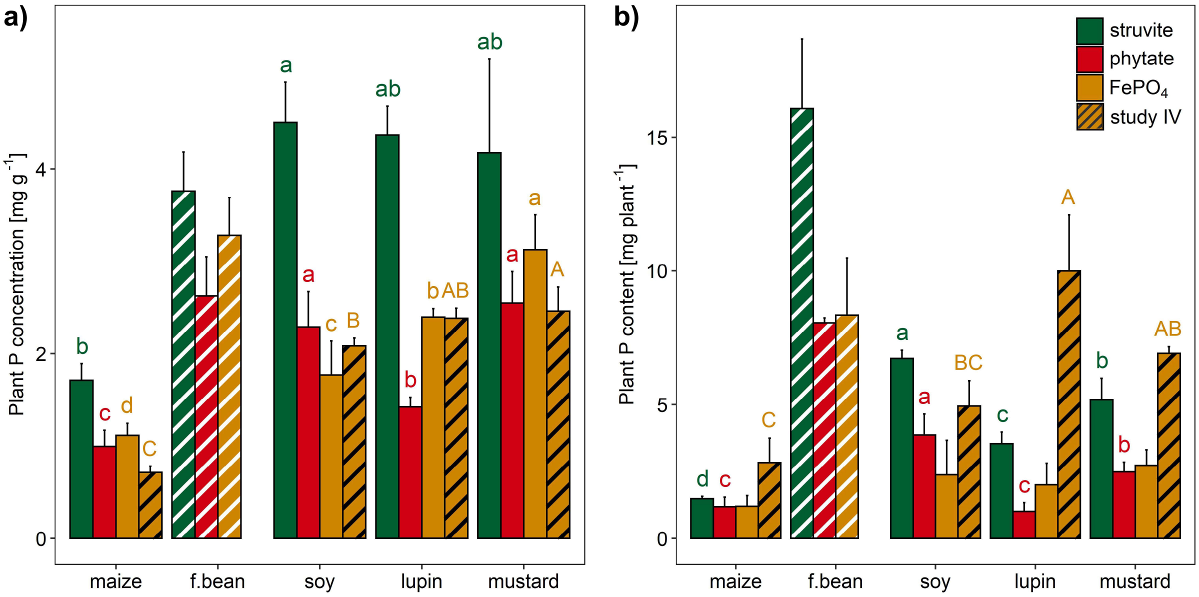


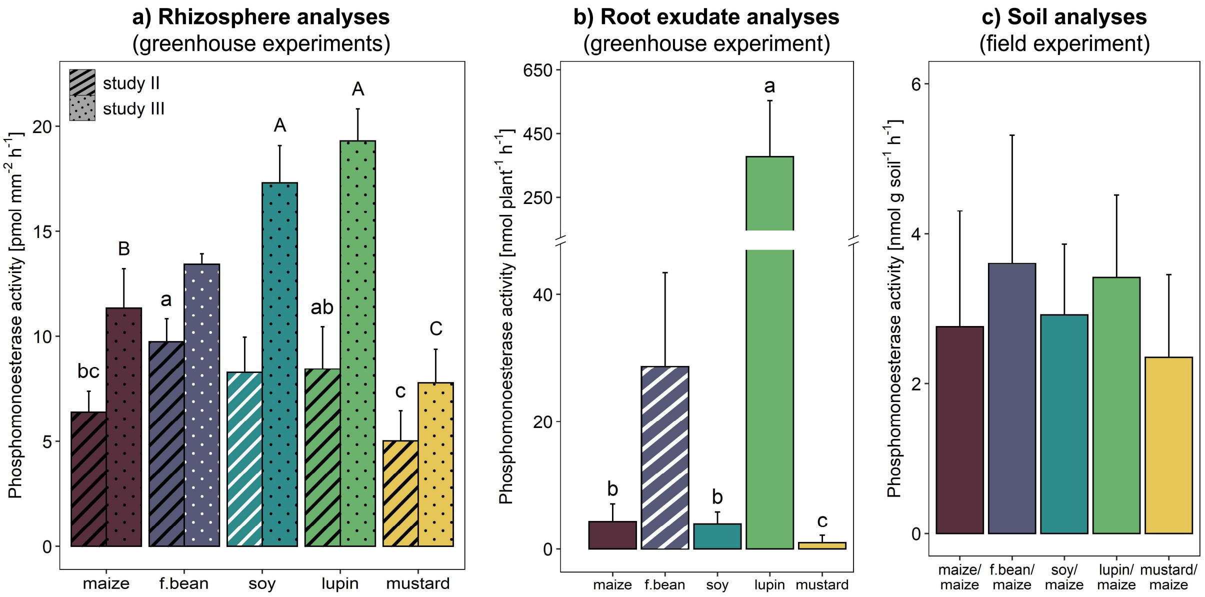
FIGURE 4 Plant P concentration (a) and plant P content (b), determined in Study III (plain columns; P provided as struvite, phytate, or $FePO_4$) and in Study IV (black-striped columns; $FePO_4$ only). Columns show means, and error bars indicate standard deviations (n = 4, except for faba bean as indicated by white stripes). Different lowercase letters indicate significant differences ($p < 0.05$) among the plant species in Study III (faba bean excluded), tested separately for each panel and each P source, as indicated by color. Different capital letters indicate significant differences ($p < 0.05$) among the plant species in Study IV, tested separately for each panel. Absence of letters indicates that there was no significant difference. See also Tables 1 and S2 and Fig. 1 in Study III as well as Tables 1, S2, and S3 and Figs. 1 and S1 in Study IV.

Taken together, the companion species had generally higher P concentrations and higher P contents than maize. Specifically, faba bean and soy had high P contents when supplied with struvite or phytate in Study III. In addition, mustard had a high P concentration in Study III, and lupin had a high P content in Study IV when supplied with $FePO_4$. These findings suggest that the companion species effectively mobilized P from different sources, likely through species-specific mechanisms, as discussed next.

3.3.2 P mobilization by companion species

Phosphomonoesterase activity

In Study II, soil zymography revealed that the phosphomonoesterase activity in the rhizosphere of faba bean was, on average, significantly higher by a factor of 1.5 than of maize ($p = 0.010$). It also tended to be higher in the rhizosphere of lupin ($p = 0.151$) and soy (not statistically tested) than of maize (Fig. 5a; striped columns). In Study III, the phosphomonoesterase activities in the rhizosphere of lupin ($p < 0.001$) and soy ($p = 0.002$) were significantly higher by a factor of 1.7 and 1.5, respectively, than of maize (Fig. 5a; dotted columns). In contrast, the phosphomonoesterase activity in the exudate solution of lupin exceeded that of the other plant species by far, being significantly higher by a factor of 87.7 than of maize when P was provided in the form of phytate (Fig. 5b). The phosphomonoesterase activity in the exudate solution of faba bean also tended to be higher than of maize in Study III (Fig. 5b). No significant difference in the phosphomonoesterase activity was found among the species combinations in the field experiment (Fig. 5c).



a) Rhizosphere analyses (greenhouse experiments)

b) Root exudate analyses (greenhouse experiment)

c) Soil analyses (field experiment)

FIGURE 5 Phosphomonoesterase activity in the rhizosphere, determined by soil zymography in Studies II and III (a), in the exudate solutions in Study III when P was provided in the form of phytate (b), and in soil samples of the field experiment in 2018 in Study II (c). Activities in panel (a) are means of the two analyses conducted six and nine weeks after sowing in Study II and means across the three P sources provided in Study III, respectively. Columns show means, and error bars indicate standard deviations (n = 5 in Study II and n = 4 in Study III, except for soy and/or faba bean in panels (a) and (b), as indicated by white patterns). Different letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each panel and each study (white patterned species excluded). Absence of letters indicates that there was no significant difference. See also Fig. 5 and Table S2 in Study II as well as Figs. 3, S2, and S4 in Study III.

Our finding that phosphomonoesterase activities were higher in Study III than in Study II (Fig. 5a) indicates that all plant species increased their enzyme activity under P scarcity since the soil in Study II contained about three times as much organic P as the mineral substrate in Study III (see also the methods sections in both studies). Particularly lupin might have increased its phosphomonoesterase activity from week 6 (analyzed via soil zymography; Fig. 5a) to week 10 (analyzed in the exudate solution; Fig. 5b) in Study III in response to increasing P scarcity during growth since lupin was barely capable of mobilizing P from phytate in Study III (Fig. 4b), as discussed below. However, we cannot exclude that the results obtained by soil zymography and exudate analyses might not be directly comparable. Regardless, increased enzyme activities in response to P scarcity have been reported earlier for several legumes, cereals, and oilseed crops (Wen et al. 2019; Yadav and Tarafdar 2001). The lack of significant differences in the field experiment (Fig. 5c) meets our expectation that many mechanisms of P acquisition, and hence also the release of phosphomonoesterases by roots, act only locally in the rhizosphere and thus can barely be studied in the field (see also section 1.3). I will, therefore, focus on the findings of the greenhouse experiments in the following discussion.

The phosphomonoesterase activity was generally higher in the rhizosphere of legumes than of maize and mustard (Fig. 5). The reason for this is likely that plants need N to synthesize the N-rich phosphatase enzymes, which might favor legumes due to their ability to symbiotically fix atmospheric N_2 (Marklein and Houlton 2012; Schleuss et al. 2020; Widdig et al. 2019). A higher phosphatase (phosphomonoesterase) activity in the rhizosphere of legumes than of cereals and oilseed crops has been reported earlier, e.g., for faba bean, white lupin, blue lupin, and soy (Nuruzzaman et al. 2006; Wen et al. 2019). A high phosphomonoesterase activity in the rhizosphere has been associated with organic P depletion. For instance, blue lupin has recently been shown to have a high phosphatase activity in the rhizosphere, which went along with soil P depletion (Hummel et al. 2021). Further, faba bean (in contrast to maize) has been shown to respond to P deficiency by increasing phosphatase activity, thus increasing the P availability in the rhizosphere (Liu et al. 2016). Accordingly, we suggest that legumes in our experiments likely mobilized P from organic sources via phosphomonoesterase release.

Our finding that soy but not lupin could effectively mobilize P from phytate in Study III (Fig. 4b) is partially contrary to this preceding suggestion since the phosphomonoesterase activity in the rhizosphere of lupin was substantially higher than that of soy (Fig. 5b). Faba bean also tended to effectively mobilize P from phytate, although its phosphomonoesterase activity tended to be lower than of lupin in Study III (Figs. 4b and 5b). One explanation for the contrasting findings among lupin and soy might be that lupin exuded mainly acid phosphomonoesterases, which do not hydrolyze phytate, while soy exuded more phytases capable of catalyzing phytate hydrolysis. This is in accordance with previous studies showing that the phytase activity of lupin (and other plant species) contributed less than 5% to total phosphatase activity (Gilbert et al. 1999; Hayes et al. 1999; Richardson et al. 2000), while soy has been shown to have a phytase activity higher than acid phosphatase activity (Ramesh et al. 2011). Hence, lupin might mainly mobilize P from readily hydrolyzable phosphomonoesters, which has also been demonstrated for several pasture species (Hayes et al. 2000), while soy (and faba bean) might also be capable of effectively mobilizing P from the more stable phytate.

pH changes

In Study II, pH imaging revealed that faba bean strongly decreased the pH in the rhizosphere by more than one pH unit compared to the bulk soil and more strongly than maize ($p = 0.037$; Fig. 6a). In contrast, lupin barely changed the rhizosphere pH, whereas mustard increased the rhizosphere pH by 0.7 pH units compared to the bulk soil (Fig. 6a). Similarly, the pH in the exudate solution of mustard was significantly higher than that of maize (and the other plant species) in Studies III and IV ($p < 0.001$; Fig. 6b). The pH in the exudate solution of faba bean tended to be much lower than of maize (and the other plant species) in Study III (Fig. 6b; striped columns; not statistically tested). The pH in the exudate solutions of lupin and soy did not significantly differ from that of maize in Studies III and IV (Fig. 6b). No significant difference in soil pH was found among the species combinations in the field experiment (Fig. 6c).

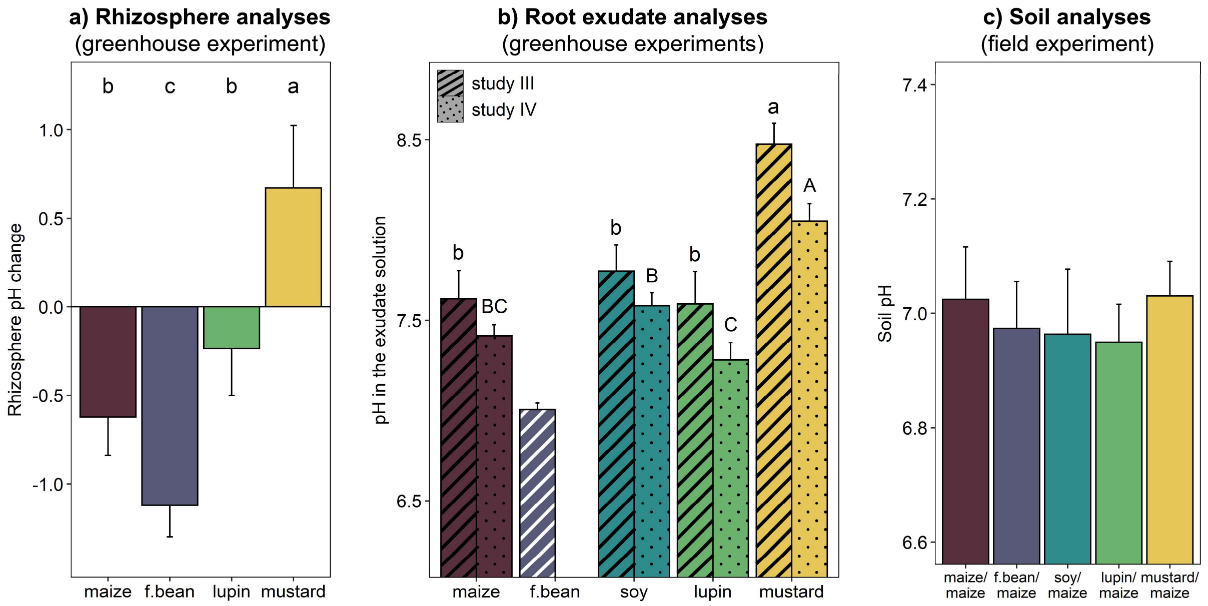


FIGURE 6 Rhizosphere pH changes determined by pH imaging in Study II (a) as well as pH in the exudate solutions in Studies III and IV (b) and in soil samples of the field experiment in 2018 in Study II (c). Values in panel (a) are means of the two analyses conducted six and nine weeks after sowing in Study II. Values of Study III in panel (b) are means across the three P sources provided. Columns show means, and error bars indicate standard deviations (n = 5 in Study II and n = 4 in Studies III and IV, except for faba bean in panel (b) as indicated by white stripes). Different letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each panel and each study (white striped faba bean excluded). Absence of letters indicates that there was no significant difference. See also Fig. 4 and Table S2 in Study II, Figs. 2 and S1 in Study III, and Fig. 4 in Study IV.

Taken together, the three greenhouse experiments yielded consistent results, specifically a rhizosphere alkalinization by mustard and a rhizosphere acidification by faba bean (Fig. 6a–b). The lack of significant differences in the field experiment (Fig. 6c) meets (again) our expectation that many mechanisms of P acquisition, including rhizosphere pH changes, can barely be studied in the field. I will, therefore, focus on the findings of the greenhouse experiments in the following discussion.

Our finding that mustard increased the rhizosphere pH in the greenhouse experiments is likely associated with a higher uptake of anions over cations, which is counterbalanced by OH^- (or HCO_3^-) release (Hinsinger et al. 2003). Rhizosphere alkalinization has been associated with P mobilization from Fe and Al phosphates. For instance, rapeseed has been shown to increase the rhizosphere pH, thereby depleting P from NaOH-extractable pools, i.e., P from Fe and Al phosphates or P bound to Fe and Al oxides (Gahoonia and Nielsen 1992; Hinsinger 2001). Rhizosphere alkalinization has also been found for other *Brassicaceae*, which increased the rhizosphere pH by up to one pH unit compared to the bulk soil (Marschner et al. 2007). A significant positive correlation between pH of rhizosphere extracts and leaf P concentration has also been reported for oilseed rape when supplied with various P sources, including $FePO_4$ (Pearse et al. 2007). Similarly, the rhizosphere alkalinization of durum wheat and the grass *Nassella trichotoma* increased P availability (Devau et al. 2010; Spohn et al. 2020). Hence, mustard in our experiments likely mobilized P from Fe and Al phosphates via rhizosphere alkalinization. This is further supported by our finding that mustard had a high P concentration when supplied with $FePO_4$ in Studies III and IV (Fig. 4a), which went along with a rhizosphere alkalinization in both studies (Fig. 6b). Since the solubility of $FePO_4$ increases with increasing pH (Hinsinger 2001; Lindsay 1979), the rhizosphere alkalinization by mustard likely solubilized $FePO_4$, thereby rendering P available, which explains the comparatively high P concentration of mustard (Fig. 4a). This is also in line with a previous study reporting that oilseed rape mobilized more P from $FePO_4$ than wheat and different legumes (Pearse et al. 2007).

Our finding that faba bean decreased the rhizosphere pH in Studies II and III (Fig. 6a–b) is likely associated with a higher uptake of cations over anions, which is counterbalanced by H^+ release (Hinsinger 2001; Hinsinger et al. 2003). Rhizosphere acidification has been associated with P mobilization from Ca and Mg phosphates (Hinsinger 2001; Hinsinger et al. 2011). For instance, faba bean has been shown to solubilize Ca phosphates via rhizosphere acidification in P-deficient soils, which was also associated with increased biomass Ca concentrations as compared to P-sufficient soils (Houassine et al. 2020). Faba bean has also been reported to acidify the rhizosphere much stronger than soy or maize (Li et al. 2007; Zhou et al. 2009). Hence, faba bean in our experiments likely mobilized P from Ca and Mg phosphates via rhizosphere acidification. This is further supported by our finding that faba bean had by far the highest P content of all investigated plant species when supplied with struvite in Study III (Fig. 4b), which went along with a rhizosphere acidification (Fig. 6b). Since the solubility of struvite (like that of Ca phosphates) increases with decreasing pH (Lindsay et al. 1989; Talboys et al. 2016), the rhizosphere acidification by faba bean likely solubilized struvite, thereby rendering P available, which explains the high P content of faba bean (Fig. 4b). This is in accordance with a previous study reporting that lupin (in contrast to maize) effectively mobilized P from struvite via rhizosphere acidification (Robles-Aguilar et al. 2020).

Exudation of LMWOA and DOC

In Study III, the exudation of LMWOA carboxyl groups by soy was significantly higher by a factor of 7.3 ($p = 0.002$) and that of lupin slightly higher ($p = 0.058$) than of maize when P was provided in the form of struvite (Fig. 7a; green columns). Only faba bean tended to exude even higher amounts of carboxyl groups than soy when supplied with struvite (not statistically tested; Fig. 7a). When P was provided in the form of phytate, the exudation of carboxyl groups by lupin was significantly higher by a factor of 4.2 ($p = 0.011$) and that of soy slightly higher ($p = 0.058$) than of maize (Fig. 7a; red columns). When P was provided in the form of $FePO_4$, soy tended to exude more carboxyl groups than maize in Study III ($p = 0.162$; Fig. 7a; browny columns), whereas lupin exuded significantly more carboxyl groups than maize in Study IV ($p < 0.001$; Fig. 7b).

In Study III, DOC exudation of soy was significantly higher by a factor of 3.2 and 2.4, respectively, than of maize when P was provided in the form of struvite or phytate ($p \leq 0.009$; Fig. 8a; green and red columns). When P was provided in the form of $FePO_4$, no significant difference was found among the plant species in Study III (Fig. 8a; browny columns), whereas DOC exudation of lupin was significantly higher by a factor of 2.9 than of maize in Study IV ($p = 0.001$; Fig. 8b). The DOC exudation of faba bean tended to be higher than of the other plant species in Study III, irrespective of the P source (not statistically tested; Fig. 8a).

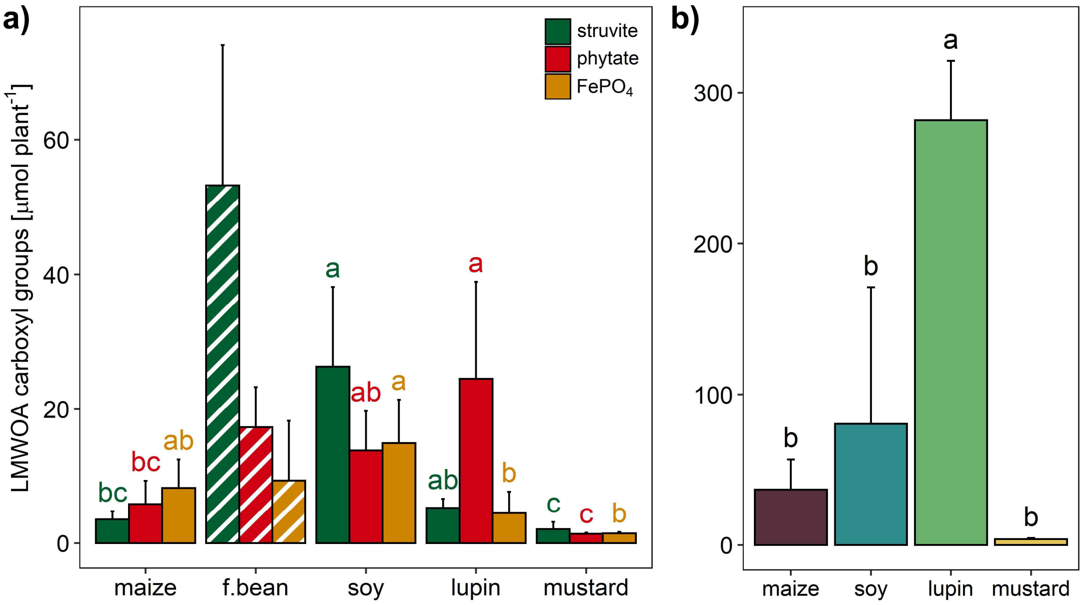


FIGURE 7 LMWOA carboxyl groups in the exudate solutions of Study III (a) and Study IV (b). Columns show means, and error bars indicate standard deviations (n = 4, except for faba bean as indicated by white stripes). Different letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each panel and each P source (faba bean excluded). See also Table 2 and Figs. 4 and S5 in Study III as well as Tables 2 and S4 and Fig. 3 in Study IV. LMWOA: low molecular weight organic acid anions.

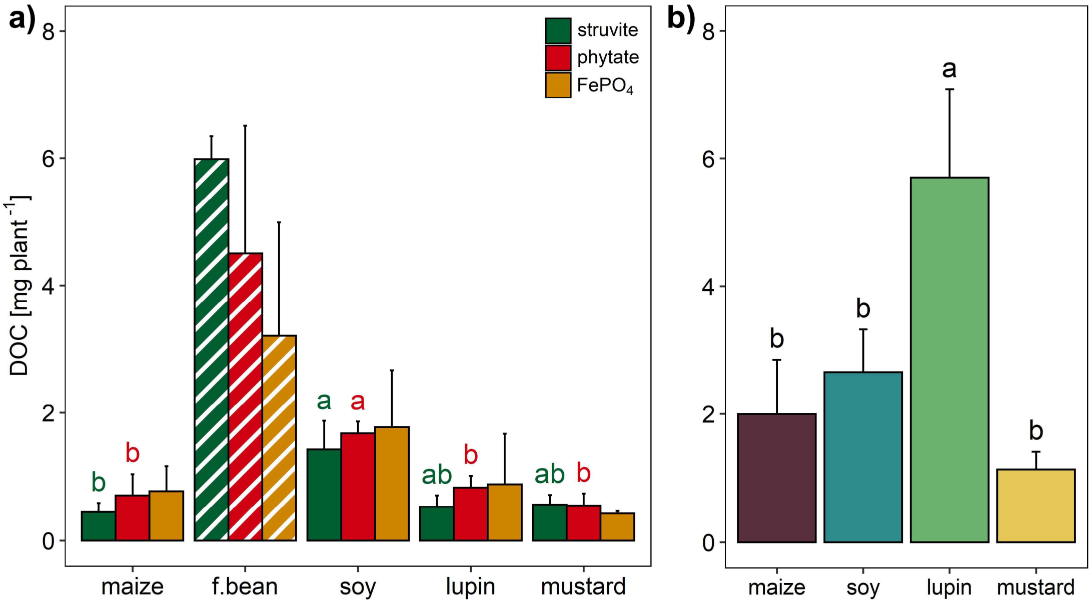


FIGURE 8 Dissolved organic carbon (DOC) in the exudate solutions of Study III (a) and Study IV (b). Columns show means, and error bars indicate standard deviations (n = 4, except for faba bean as indicated by white stripes). Different letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each panel and each P source (faba bean excluded). Absence of letters indicates that there was no significant difference. See also Table 2 in Study III, and Tables 2 and S4 in Study IV.

The exudation of LMWOA carboxyl groups and, to a lesser extent, of DOC was higher in Study IV than in Study III (Figs. 7 and 8), indicating that all plants had more C available for LMWOA synthesis in Study IV. The reason for this is likely the approximately threefold increased biomass production in Study IV compared to Study III (Curl and Truelove 1986; Dechassa and

Schenk 2004). Plants might have grown better in Study IV than in Study III due to insolation differences since Study III was conducted in autumn and Study IV in spring. More to the point, plants in Study IV received about 2.4 times as much N as the plants in Study III (see also the method sections in Studies III and IV), which might have also affected plant growth positively in Study IV. Since the differences between both studies were most obvious for lupin, we further suggest that some other unknown factors restricted lupin's growth in Study III. I will, therefore, focus on lupins' performance in Study IV in the following discussion.

Legumes generally exuded more LMWOA than maize and mustard, although the exudation varied in quantity in response to different P sources. LMWOA exudation was particularly high in faba bean and soy supplied with struvite in Study III and in lupin supplied with $FePO_4$ in Study IV (Fig. 7). Similarly, faba bean and, to a lesser extent, soy exuded high amounts of DOC in Study III (irrespective of P source) and lupin in Study IV (Fig. 8). These findings are in accordance with previous studies showing that legumes (especially several lupins and faba bean) exuded more LMWOA than cereals and oilseed crops (Lyu et al. 2016; Wen et al. 2019). High LMWOA exudation rates have been associated with P mobilization from (sparingly soluble) inorganic P minerals, while DOC (including LMWOA) has been suggested to act as a substrate for microorganisms, which potentially contribute to P mobilization through exuding additional P-mobilizing compounds (Duchene et al. 2017; Richardson et al. 2011; Wang and Lambers 2020). Hence, the legumes in our experiments likely mobilized P from inorganic P minerals via LMWOA exudation and possibly also P from organic P sources via the stimulation of microbial activity through DOC exudation.

This is further supported by our finding that faba bean and soy had high biomass P contents when supplied with struvite in Study III (Fig. 4b), which went along with a high LMWOA exudation (Fig. 7a). Faba bean and soy likely mobilized P from struvite via LMWOA since in water only 1–2% of struvite P is soluble, while in citric acid solution about 50–100% of struvite P is soluble (Ahmed et al. 2018; Cabeza et al. 2011; Möller et al. 2018; Rech et al. 2019). In a previous study, soy has been shown to mobilize P from struvite nearly as effectively as from the highly soluble triple superphosphate and more effectively than wheat (Rech et al. 2019). By contrast, the low LMWOA exudation of lupin in Study III (Fig. 7a) might explain its comparatively low P content when supplied with struvite (Fig. 4a).

Our finding that lupin had a high P content in Study IV (Fig. 4b), which went along with high LMWOA exudation (Fig. 7b), indicates that lupin likely mobilized P from $FePO_4$ via LMWOA in Study IV. Lupin released mainly citrate and, to a lesser extent, malate (Table 2 in Study IV), which is in accordance with earlier findings (Egle et al. 2003; Pearse et al. 2007). Both citrate and malate have been found to effectively mobilize P from $FePO_4$ (Dissanayaka et al. 2017; Jones 1998), which might explain the high P content of lupin (Fig. 4b). This is in accordance with previous experiments relating biomass and P content of lupin to a high LMWOA exudation (Dissanayaka et al. 2017; Lelei and Onwonga 2014). Especially the citrate exudation by white lupin has been reported to chelate metal cations (Fe^{3+}, Al^{3+}, Ca^{2+}), thus increasing P availability (Cu et al. 2005; Gardner et al. 1983; Gardner and Boundy 1983; Li et al. 2010). It is, therefore, also suggested that lupin might be capable of mobilizing P from struvite when LMWOA exudation is high, as discussed above for soy and faba bean.

Our finding that faba bean and soy had high biomass P contents when supplied with phytate in Study III (Fig. 4b), despite substantially lower phosphomonoesterase activities than lupin (Fig. 5b), indicates that the high DOC exudation of faba bean and soy (Fig. 8a) might have contributed to P mobilization from phytate. DOC might act as a substrate for microorganisms, which likely produce additional phytases that effectively hydrolyze P from phytate in the rhizosphere of faba bean and soy, as demonstrated earlier (Lambers et al. 2008; Wang and Lambers 2020; Wu et al. 2018). For instance, previous studies have shown that the inoculation with phytate-mineralizing bacteria (Ramesh et al. 2011; 2014; Richardson et al. 2000) or the addition of fungal phytases (Hayes et al. 2000; Sun et al. 2021) were effective measures to increase P availability from phytate for soy, wheat, and several pasture species. Similarly, mycorrhizal symbioses have been found to effectively mobilize P from phytate due to hyphae-mediated phytase activity (Wang et al. 2017; Zhang et al. 2016b).

Root foraging

In Study III, the root lengths of faba bean and soy tended to be much larger than those of maize and lupin when P was provided in the form of phytate or struvite (Fig. 9). Also, mustard tended to have a higher root length than maize and lupin when supplied with struvite. When P was provided in the form of $FePO_4$, faba bean, soy, and mustard tended to have higher root lengths than maize and lupin (Fig. 9). Across the greenhouse experiments, mustard and soy had a thin and intensively branched root system, whereas faba bean and lupin formed thick taproots. Moreover, maize exhibited a visibly larger root length in Study II than Study III.

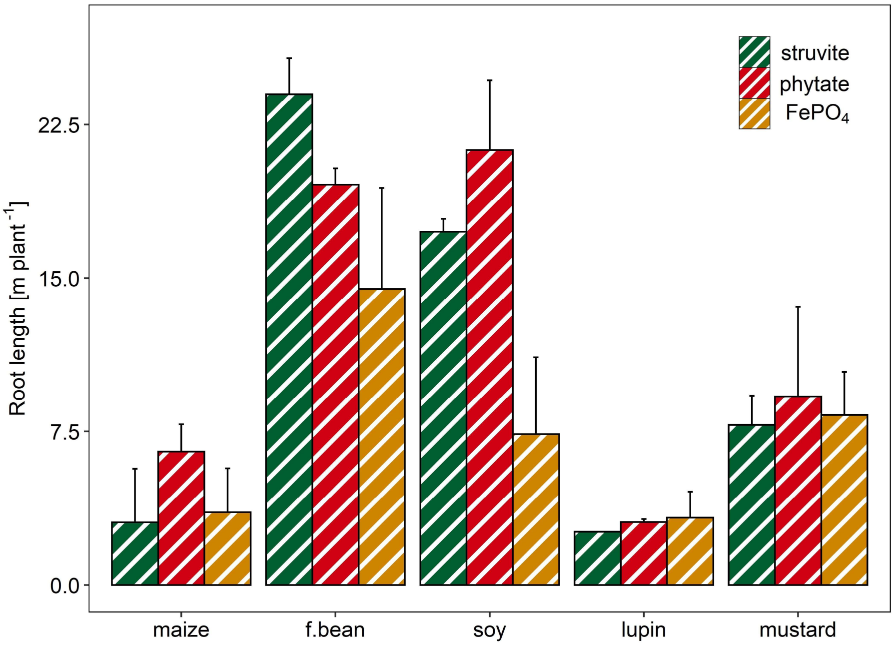


FIGURE 9 Root length determined in Study III. Columns show means, and error bars indicate standard deviations (n = 2 as indicated by white stripes). No statistical analyses were conducted. See also Fig. S3 in Study III.

Our finding that the large root lengths of faba bean, soy, and, to a lesser extent, mustard (Fig. 9) went along with high biomass P contents when supplied with struvite and phytate (Fig. 4b) indicates that a large root length might be advantageous for P uptake, at least once P is mobilized. A strong positive linear correlation between root length and P uptake has been reported earlier (Pang et al. 2015). For instance, the advantage of a large root length for P mobilization from finely ground struvite has been suggested for *Brassicaceae* (Brennan and Bolland 2001; Lyu et al. 2016; Wen et al. 2021), which likely explains the high biomass P content of mustard when supplied with struvite in Study III (Fig. 4b). A significant positive correlation between shoot P uptake and total root length of different *Brassicaceae* and *Poaceae* supplied with $FePO_4$ has also been reported earlier (Marschner et al. 2007; Wang et al. 2007). In some cases, especially for mustard, the root foraging via large root length seems to contribute more to P acquisition than P mining via high root exudation, as suggested earlier (Richardson et al. 2011; Sulieman and Mühling 2021).

Summary

Taken together, all three legumes investigated here showed high phosphomonoesterase activities in their rhizosphere, indicating that they are capable of mineralizing organic P, at least (readily hydrolyzable) phosphomonoesters. However, our findings further suggest that a high DOC exudation (which likely stimulated the activity of beneficial microorganisms) was additionally needed to effectively mobilize P from phytate. Since faba bean and soy (in contrast to non-mycorrhizal lupin) were capable of effectively mobilizing P from phytate in Study III, the so-called tripartite symbiosis, i.e., a double symbiosis with rhizobia and mycorrhizal fungi, might have contributed to phytate mineralization, as reported earlier (Bai et al. 2017; Jia et al. 2004).

Faba bean likely mobilized P from struvite (and Ca phosphates) via rhizosphere acidification and high LMWOA exudation. In contrast, mustard likely mobilized P from $FePO_4$ (and other Fe and Al phosphates) via rhizosphere alkalinization. Soy likely mobilized P from struvite in Study III and lupin P from $FePO_4$ in Study IV, both via high LMWOA exudation. Besides these P mining mechanisms, the large root lengths of faba bean, soy, and mustard were likely advantageous for P uptake via root foraging, at least once P was mobilized.

In summary, the companion species mobilized P from different organic and inorganic P sources more effectively than maize, likely due to high root exudation of specific P-mobilizing compounds in combination with root foraging and stimulation of beneficial microorganisms.

3.3.3 P acquisition by maize in intercropping

Complementarity and facilitation for P in intercropping

The pPER of maize grain yield in the field experiment (Fig. 2d) indicated that maize acquired more P in intercropping than monocropping, especially when maize was grown together with soy and lupin (see section 3.1). The reasons for this are likely complementary and facilitative root interactions, as follows.

About 80% of total soil P in the field experiment was not water-soluble and hence not directly available to plants (section 2.1). This likely resulted in high competition for P among maize plants, which could barely mobilize P from sparingly soluble sources (section 3.3.1), thus restricting maize growth in monocropping. In contrast, the companion species effectively mobilized P from various sources (section 3.3.2). In intercropping, this might have resulted in

P complementarity if companion species and maize use different P forms. That is, companion species might have taken up P mobilized from sparingly soluble sources, while maize might have taken up water-soluble P, which made up 18% of the total soil P. Additionally, the high P mobilization potential of the companion species might have resulted in P facilitation if maize takes up the P that was mobilized by the companion species via exudation of P-mobilizing compounds into the (shared) rhizosphere (Hinsinger et al. 2011; Homulle et al. 2022; Xue et al. 2016). Both processes, i.e., complementarity and facilitation, likely occurred simultaneously and dynamically throughout the growing season (Justes et al. 2021) and resulted in the enhanced P acquisition of intercropped maize in our field experiment.

The pPER of maize grain yield in the field experiment tended to be highest for soy/maize intercropping (Fig. 2d), indicating that interspecific interactions with soy enhanced maize P acquisition more strongly than interactions with other companion species. This likely resulted from the high P mobilization capacity of soy found in Study III (section 3.3.2). Specifically, soy effectively mobilized P from organic (e.g., phytate) and inorganic (e.g., struvite) sources via P mining, from which associated maize plants likely also benefited via complementarity and/or facilitation for P. For instance, soy might have taken up the mineralized organic P, leaving more inorganic P for the maize, or maize might also have taken up part of the mineralized organic P. In addition, the large root length of soy was likely associated with a high probability of root contacts, i.e., intense root intermingling among maize and soy. This likely contributed to P facilitation and maize P acquisition in soy/maize intercropping as the shared rhizosphere with P-mobilizing exudates might be larger and hence P availability higher. Moreover, soy likely established a tripartite symbiosis, which might provide an additional advantage in P acquisition for both plant species in soy/maize intercropping (section 3.3.2). Positive intercropping effects of soy on maize P acquisition have been reported earlier (Fan et al. 2020; Lv et al. 2014; Xia et al. 2019). The lack of intercropping effects on maize P acquisition in Study IV (Table 1) might be explained by the low $FePO_4$ solubilization capacity of soy, indicating that LMWOA exudation and pH changes by soy were not high enough to effectively mobilize P from $FePO_4$ for both species (see also Study IV). This is in accordance with a meta-analysis reporting a low P mobilization ability of soy (Tang et al. 2021). Thus, soy/maize intercropping might enhance maize P acquisition only if soil P comprises P forms, from which soy can effectively mobilize P (e.g., organic P).

Lupin/maize intercropping also resulted in high pPER of maize grain yield in the field experiment (Fig. 2d), indicating that interspecific interactions with lupin enhanced maize P acquisition. This was likely associated with the high P mobilization capacity of lupin found in Studies II and IV. Specifically, lupin likely mobilized P from (readily hydrolyzable) organic and inorganic sources via high phosphomonoesterase activity and high LMWOA exudation (section 3.3.2), from which associated maize plants likely also benefited via complementarity and/or facilitation for P, as discussed above. The intercropping effects on maize P acquisition might, however, be slightly smaller than in soy/maize intercropping since lupin is a non-mycorrhizal plant species (Wen et al. 2019). Since lupin produced little biomass (Fig. 4 in Study I) and forms taproots with only short lateral roots (reducing the likeliness of root contacts with maize), compensation might have also contributed to the enhanced maize productivity in lupin/maize intercropping compared to monocropping. Regardless, positive intercropping effects of lupin on the P acquisition of maize (and other cereals) are in accordance with previous pot and field experiments, although they were mainly conducted with white lupin (known for its ability to form proteoid, i.e., cluster roots) rather than blue lupin (Cu et al. 2005; Dissanayaka et al. 2015; 2017; Lelei and Onwonga 2014).

The pPER of maize grain yield in the field experiment did not differ significantly from 0.5 in faba bean/maize intercropping (Fig. 2d), despite a very high P mobilization potential of faba bean (section 3.3.2). This indicates that faba bean successfully competed for P and likely used most of the P that it mobilized itself instead of facilitating maize P acquisition. This is supported by the high biomass P content of faba bean in Study III, which was substantially higher than of the other companion species (Fig. 4b). Faba bean's competitiveness was even stronger in the first year of the field experiment when it was earlier sown than maize (Fig. 2), which is in accordance with a recent meta-analysis (Yu et al. 2016; see also Study I). Compared to soy, faba bean had a similarly large root length in Study III (Fig. 9) but forms taproots that grow predominantly downward with only a few lateral (secondary) roots. Hence, the likeliness of root contacts with maize was lower in faba bean/maize than soy/maize intercropping, which might have also reduced P facilitation in faba bean/maize intercropping. However, at least on a plant basis, maize P acquisition was enhanced by 49% in the second year of the field experiment in faba bean/maize intercropping compared to maize monocropping (Fig. 2d). This indicates a slightly positive intercropping effect of faba bean on maize P acquisition in intercropping, which was also reported earlier (Li et al. 2007; Li et al. 2018; Xia et al. 2019).

The pPER of maize grain yield in mustard/maize intercropping was slightly larger than 0.5 in 2018 and did not differ significantly from 0.5 in 2019 (Fig. 2d). This might be associated with the comparatively low P mobilization potential of mustard found in Study III (section 3.3.2). Specifically, mustard mainly mobilized P from Fe and Al phosphates via rhizosphere alkalinization, which made up only about 21% of total soil P in the field experiment (section 2.1). Hence, mustard likely competed for plant-available P with maize and might have used most of the P that it mobilized itself instead of facilitating maize P acquisition. This is supported by a previous study reporting that growth and P uptake of intercropped wheat were rather negatively affected by several *Brassicaceae*, suggesting that wheat was a poorer competitor for P than the *Brassicaceae* (Wang et al. 2007). However, at least on a plant basis, maize P acquisition was enhanced by, on average, 47% in the field in mustard/maize intercropping compared to maize monocropping (Fig. 2d). This indicates a slightly positive intercropping effect of mustard on maize P acquisition in intercropping, as has been found earlier (Li et al. 2018; Xia et al. 2013; 2019). Overall, the low P mobilization capacity of mustard, in combination with the lack of mycorrhizal and rhizobia symbioses, likely explains why aboveground interspecific interactions contributed more than belowground interspecific interactions to the slight maize overyielding in mustard/maize intercropping (Table 2).

In summary, companion species (particularly soy and lupin) enhanced maize P acquisition in intercropping, likely due to their high P mobilization ability. Such positive intercropping effects on maize might, however, only occur if soil P is present in forms that match the species-specific P mobilization capacities of the chosen companion species and if total soil P is sufficient to meet the P requirements of both plant species. In addition, intercropping might enhance maize productivity only if complementarity and facilitation in the rhizosphere of associated plant species are stronger than (nutrient) competition.

Maize root exudation in intercropping

In Study IV, the exudation of LMWOA carboxyl groups by maize was increased by a factor of 2.6 in lupin/maize intercropping compared to maize monocropping, although this was only statistically significant when normalized to root biomass ($p = 0.048$; Fig. 10b), not per plant ($p = 0.202$; Fig. 10a). The increase was mainly associated with an enhanced exudation of aconitate, citrate, and malate by maize (Tables 2 and S4 in Study IV). The LMWOA exudation by maize (per g root) was also slightly increased in mustard/maize intercropping compared to maize monocropping ($p = 0.094$; Fig. 10b).

The DOC exudation by maize (plant^{-1}) was significantly increased by a factor of 3.0 in lupin/maize intercropping compared to maize monocropping ($p = 0.030$; Fig. 10c).

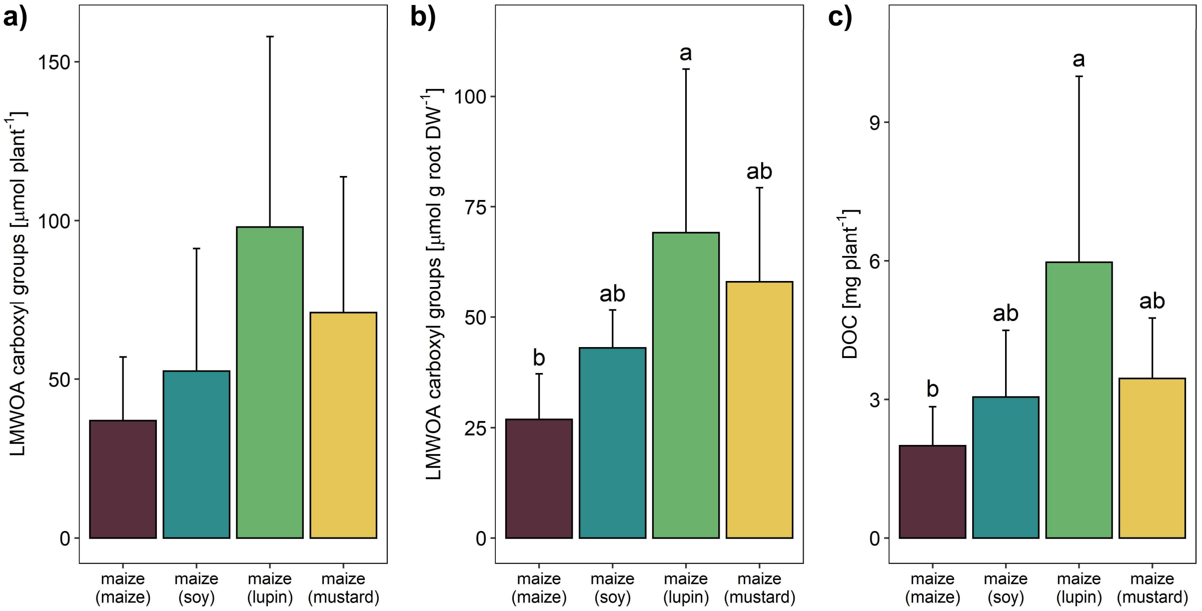


FIGURE 10 LMWOA carboxyl groups in the exudate solutions of maize, determined in Study IV and calculated per plant (a) and per g root dry weight (b), and dissolved organic carbon (DOC; c) in the exudate solutions of maize, determined in Study IV. Columns show means, and error bars indicate standard deviations ($n = 4$). Different letters indicate significant differences ($p < 0.05$) among the species combinations, tested separately for each panel. Absence of letters indicates that there was no significant difference. See also Fig. 3 and Tables 2 and S4 in Study IV. LMWOA: low molecular weight organic acid anions.

Our finding that maize generally exuded more LMWOA and DOC in lupin/maize intercropping than in maize monocropping (Fig. 10) indicates that lupin caused maize to increase its root exudation. This might, however, not be specific to lupin since DOC and LMWOA exudation by maize also tended to be increased when maize was grown together with mustard (compared to maize monocropping; Fig. 10). An increased LMWOA exudation by maize in intercropping with lupin, faba bean, or alfalfa compared to maize monocropping has been reported earlier (Li et al. 2010; 2013; Sun et al. 2020; Wang et al. 2020) and was mainly explained by diffusion of LMWOA from legumes to maize roots (Li et al. 2013; Li et al. 2016). In contrast, our results show that exudation patterns of maize changed through intercropping. Since we collected the root exudates of both plant species separately from intact plants and not from rhizosphere soil, we can exclude that the exudates diffused from companion roots to maize roots. As discussed in more detail in Study IV, it might be the combined effect of improved maize growth and high interspecific competition for P (and Fe) in intercropping that caused maize to increase its root exudation in intercropping compared to maize monocropping. However, it could also be the specific exudation profile of lupin that triggered maize to increase its exudation (see also Study IV). Further research is, therefore, needed to examine in depth the underlying mechanisms of increased maize exudation in intercropping and to test whether this is species-specific.

4 Conclusions and outlook

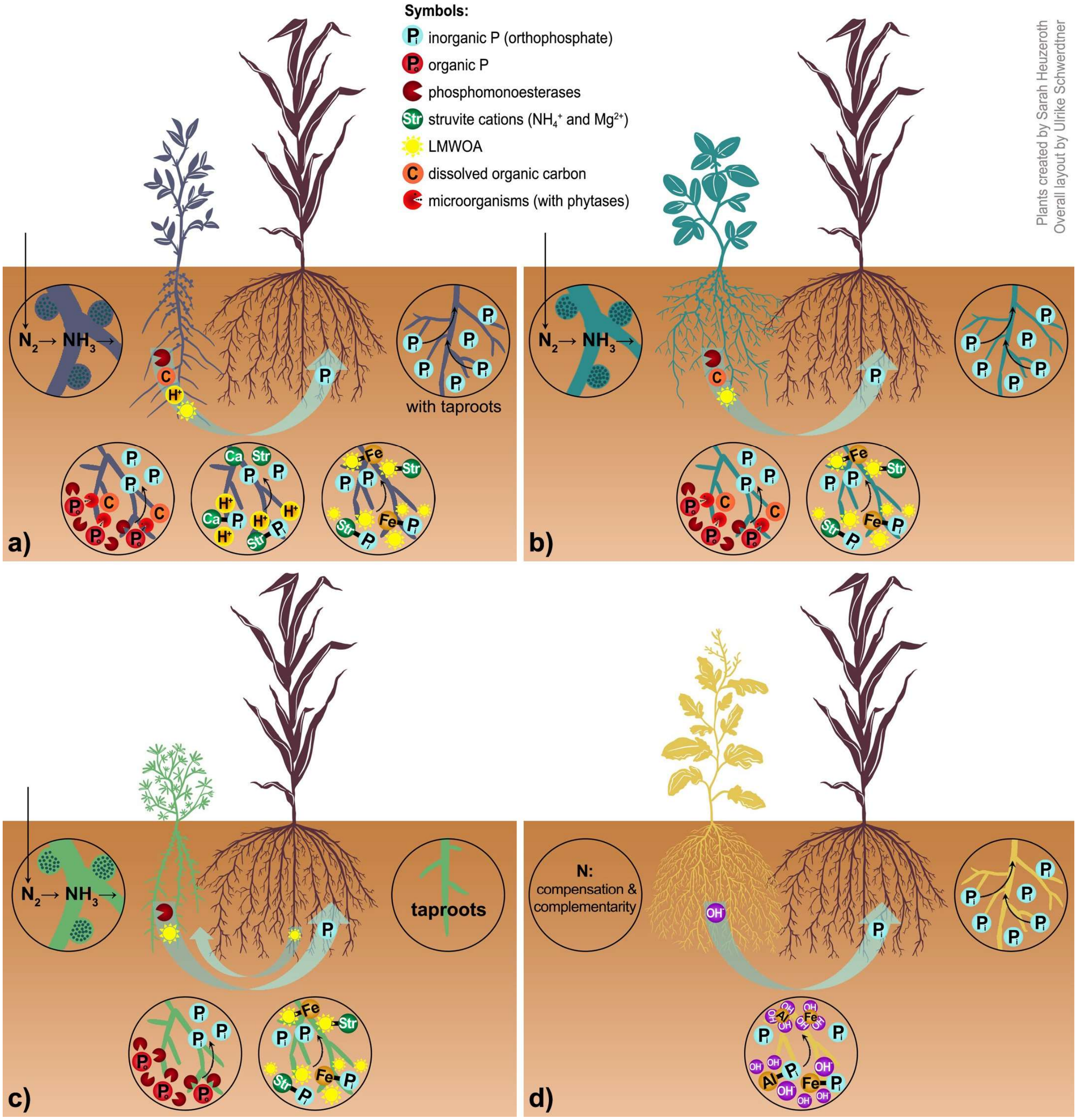
The present thesis showed that intercropping resulted in maize overyielding and enhanced maize N and P contents in the field experiment, especially in soy/maize and lupin/maize intercropping, as compared to maize monocropping. Smaller but still positive intercropping effects on maize plants were also found in faba bean/maize intercropping (when both plant species were simultaneously sown in 2019) and mustard/maize intercropping (section 3.1; Study I). These findings confirm my first hypothesis on maize overyielding in intercropping. Maize overyielding was mostly caused by belowground interspecific interactions in soy/maize and faba bean/maize intercropping (and, hence, probably also in lupin/maize intercropping) and by aboveground interspecific interactions in mustard/maize intercropping (section 3.1; Study I). My second hypothesis on interspecific interactions can, therefore, be confirmed for legume/maize intercropping but needs to be rejected for mustard/maize intercropping. Moreover, the enhanced maize productivity in intercropping was likely the result of reduced N and P competition due to the combined effects of compensatory, complementary, and facilitative plant interactions.

The present thesis further showed that the three legumes enhanced maize N acquisition in intercropping due to their ability to symbiotically fix atmospheric N_2, which was in part transferred to the maize plants, suggesting both complementarity and facilitation. Up to 20% of maize AGB N content was thus derived from legumes in the field experiment, confirming my third hypothesis that legumes enhance maize N acquisition in intercropping. Mustard also slightly enhanced maize N acquisition in intercropping, likely due to compensation and/or complementarity effects (section 3.2; Study II).

Further, the companion species had generally higher P contents and/or higher P concentrations than maize (section 3.3.1; Studies III and IV), indicating that they mobilized P from sparingly soluble sources more effectively than maize. This was likely associated with P mining (i.e., high exudation of P-mobilizing compounds into the rhizosphere), root foraging, and the stimulation of microorganisms (section 3.3.2; Study III). My fourth hypothesis that companion species effectively mobilize P via high root exudation can, therefore, be confirmed but should be complemented by P mobilization via root foraging and beneficial microorganisms in the rhizosphere. Specifically, the legumes likely mineralized organic P or at least readily hydrolyzable phosphomonoesters via high phosphomonoesterase activities in the rhizosphere. The findings further suggest that a high DOC exudation was additionally needed to mobilize P from phytate. Faba bean likely also mobilized inorganic P via rhizosphere acidification and mustard via rhizosphere alkalinization. The legumes likely mobilized inorganic P via high LMWOA exudation. Besides these P mining mechanisms, the large root lengths of faba bean, soy, and mustard were likely advantageous for P uptake via root foraging, at least once P was mobilized. Consequently, the companion species used species-specific mechanisms to mobilize P from sparingly soluble sources (section 3.3.2; Studies II–IV), which are summarized in Fig. 11. In intercropping, these mechanisms of the companion species were likely also beneficial for maize P acquisition due to complementarity and facilitation for P among the intercropped plants. Particularly soy and lupin enhanced maize P acquisition in intercropping, likely due to their high P mobilization ability. My fifth hypothesis that the companion species enhance maize P acquisition in intercropping can, therefore, be confirmed, especially for soy and lupin, with which intercropping had the most significant effects on maize (sections 3.1 and 3.3; Study II). Such positive intercropping effects on maize might, however, only occur when (1) soil P forms match the P mobilization capacities of the chosen companion species, (2) total soil P is sufficient to meet the P requirements of both plant species, and (3) the combined effects of compensation, complementarity, and facilitation are stronger than competition (section 3.3.3).

The thesis further challenged the common view that legume/cereal intercropping is beneficial over monocropping due to the high P mobilization capacity of legumes, from which cereals simply benefit. Instead, we showed for the first time that a high LMWOA concentration in the rhizosphere in intercropping is not only caused by high LMWOA release of the companion species but also by an increased LMWOA exudation of maize, at least when grown together with lupin, but probably also in other species combinations (section 3.3.3; Study IV). Hence, my last hypothesis that maize increases its own root exudation in intercropping compared to maize monocropping proved to be correct, at least in lupin/maize intercropping.

Taken together, intercropping resulted in maize overyielding and enhanced maize N and P contents, which likely resulted from compensatory, complementary, and facilitative plant species interactions, either aboveground (more important in mustard/maize intercropping) or belowground (more important in legume/maize intercropping). Complementarity and facilitation for N, which were mainly associated with the symbiotic N_2 fixation by legumes, likely enhanced maize N acquisition in intercropping (Fig. 11). Complementarity and facilitation for P, which were mainly associated with high species-specific P mobilization capacities of the companion species (through P mining, root foraging, and/or stimulation of microorganisms), likely enhanced maize P acquisition in intercropping (Fig. 11). Moreover, maize also increased its own root exudation in intercropping compared to maize monocropping. Thus, the thesis provides new insights into the mechanisms underlying P acquisition in intercropping.



Plants created by Sarah Heuzeroth
Overall layout by Ulrike Schwerdtner

FIGURE 11 Revised overview of the mechanisms of plant N and P acquisition in intercropping as suggested by the findings in the present thesis for faba bean/maize (a), soy/maize (b), lupin/maize (c), and mustard/maize intercropping (d). See also Fig. 1 in this thesis.

From a methodological point of view, combining a two-year field experiment with three greenhouse experiments has proven beneficial to investigate not only intercropping effects on maize productivity under ambient conditions but also their underlying mechanisms. Maize overyielding was mainly observed in the field, whereas the mechanisms of P mobilization acted mostly locally in the rhizosphere and could best be observed under controlled conditions. Particularly, plant responses to specific P forms could only be observed by growing the plants in a mineral substrate with exactly defined P sources since soils simultaneously contain various inorganic and organic P forms. In addition, the soil-hydroponic-hybrid approach for sampling root exudates was, to my knowledge, used for the first time in an intercropping study and turned out to be a good measure to clearly separate the root exudates released by the main and the companion crop, which might also be useful for future intercropping research. Specifically, further studies are needed to examine in depth the underlying mechanisms of increased maize exudation in intercropping and to test whether this is species-specific.

Retrospectively, some further analyses would have completed the interpretation of the findings of this thesis and should, therefore, be included in future intercropping studies. For instance, growing the companion species in the field also in monocropping would not only allow an estimate of overyielding of the whole intercropping system but would also reveal the respective contributions of the “4C” effects to the overall intercropping outcomes by plotting the pLER of both plant species against each other (Justes et al. 2021). An investigation of the root distribution in the field – preferably in combination with imaging techniques (similar as in Bilyera et al. (2022) but with intercropping), although this is challenging in the field – might clarify how strongly roots of associated plants intermingle or complement each other, and how this affects plant nutrient acquisition. In addition, the potential contribution of (beneficial) microorganisms to nutrient acquisition in intercropping, which has recently been highlighted (Duchene et al. 2017), deserves further investigation. Further, a simultaneous investigation of above- and belowground interspecific interactions in the different species combinations might provide additional insights into the causes of maize overyielding found here.

Based on the present thesis, it seems worthwhile to keep pushing intercropping research forward since intercropping can increase crop yields and simultaneously reduce the need for fertilizer applications, as has been shown here. Such efforts are indispensable in the light of the growing world population with an increasing demand for food, the finiteness of phosphate rock-derived fertilizers, the (near) exceedance of several planetary boundaries with agriculture being a key driver of this, and other future challenges (Campbell et al. 2017; Cordell and White 2014; Gerten et al. 2020; Pe'er et al. 2020). Since radical transformations of the entire agricultural sector are crucial to meet these challenges, intercropping should be considered only one of several solutions (Beillouin et al. 2019; Campbell et al. 2017; George et al. 2022). In the present thesis, we investigated the comparatively simple system of row intercropping with two plant species. However, intercropping can also be more complex, for instance, when more than two plant species grow together in the field. Prominent examples are the Latin-American “milpa” system (mixing the “three sisters” maize, bean, and squash) or agroforestry systems such as the Mediterranean “coltura promiscua” (cereals and vegetables grown under trees) (Brooker et al. 2015; Homulle et al. 2022). In addition, there are abundant interventions that could potentially further enhance the positive effects of intercropping, including the maintenance of persistent ground covers, minimum tillage, the integration of additional cover crops between growing seasons, and the integration of deep-rooting crops (George et al. 2022; Hallama et al. 2019; Malézieux et al. 2009). Hence, future research objectives might also be to investigate the effects of combining various agricultural practices, which aim to build up resilient, sustainable, and diverse agroecosystems, on plant productivity and the environment.

Intercropping and similar practices have been largely replaced by agricultural systems that aim to maximize yields through large monocultures and unsustainable resource uses. Since there is increasing evidence that intercropping has multiple benefits over monocropping, this replacement during the last decades needs to be reversed. Hence, it is reasonable and necessary that intercropping, among other measures, attracts increasing attention in science and practice worldwide, especially in organic farming (Bedoussac et al. 2015; Brooker et al. 2015). Since organic farming is sometimes associated with nutrient limitations and especially reductions in plant-available soil P over time (Cooper et al. 2018; Möller et al. 2018; Reimer et al. 2020), intercropping seems to be a promising practice whose implementation might be very fruitful, from both ecological and economic points of view due to fertilizer savings. Intercropping is, therefore, also suggested as an integral component of the recently standardized biocyclic-vegan agriculture, which simultaneously addresses the urgently needed food system transformation by promoting changes in human diets at both the consumption and the production level (Schwerdtner 2019; Seymour and Utter 2021). Conversely, such dietary shifts towards plant-based (vegan) diets would, among many other positive effects, substantially reduce the overall P demand of food production (Metson et al. 2012). In this regard, our finding that soy and blue lupin most effectively increased maize productivity and maize nutrient acquisition in intercropping holds much promise since both plant species are increasingly used as alternative proteins in human nutrition.

In conclusion, intercropping with its positive effects on plant productivity and nutrient acquisition has been proven here to be promising in achieving food sovereignty and reducing the reliance on industrial fertilizers like those derived from finite phosphate rock. Intercropping should, therefore, be considered an integral part of an overall agricultural transformation to meet future needs while staying within humanities' safe (and just) operating space.

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MANUSCRIPTS

Included are the following manuscripts:

1. Schwerdtner U, Spohn M (2021): Interspecific root interactions increase maize yields in intercropping with different companion crops. Journal of Plant Nutrition and Soil Science 184(5): 596–606.

2. Schwerdtner U, Spohn M (2022): Plant species interactions in the rhizosphere increase maize N and P acquisition and maize yields in intercropping. Journal of Soil Science and Plant Nutrition: accepted.

3. Schwerdtner U, Lacher U, Spohn M (2022): Soy and mustard effectively mobilize phosphorus from inorganic and organic sources. Nutrient Cycling in Agroecosystems: under review.

4. Schwerdtner U, Lacher U, Spohn M (2022): Lupin causes maize to increase organic acid exudation and phosphorus concentration in intercropping. Journal of Sustainable Agriculture and Environment: accepted.

Study I – Field experiment with various N and P sources

Interspecific root interactions increase maize yields in intercropping with different companion crops

Ulrike Schwerdtner and Marie Spohn (2021),

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Abstract

Background: Intercropping is assumed to increase food production while reducing fertilizer needs and environmental impacts of crop production.

Aims: We aimed to (1) investigate the effects of intercropping on yields and nutrient uptake of maize, and (2) determine the relative contributions of above- and belowground interspecific interactions ($RC_{above/below}$) to these effects.

Methods: We conducted a 2-year, small-scale field experiment with maize grown either in monocropping or intercropped with faba bean, soy, blue lupin, or white mustard as companion crop. We included a treatment in which interspecific root interactions were restricted due to barriers in the soil. Maize and companion crops were analyzed for yields, and maize additionally for nutrient uptake. Maize partial land equivalent ratios (pLER), partial nitrogen and phosphorus equivalent ratios (pNER, pPER), and $RC_{above/below}$ were calculated.

Results: Intercropping resulted in a similar productivity of maize as in monocropping on an area basis. Maize pLER, pNER, and pPER were larger than 1.0 in several species combinations, indicating a positive effect of intercropping on maize yields and N and P uptake. Interspecific root interactions accounted for 62–85% of the maize yield increase in legume/maize intercropping, but for only 22% in mustard/maize intercropping.

Conclusion: Our results indicate that intercropping is beneficial for crop production since it increases maize yields and N and P uptake of maize plants, and it also provides yields of a companion crop. A substantial part of these positive effects can be attributed to interspecific root interactions.

Keywords: mixed cropping, nitrogen uptake, phosphorus uptake, root partitioning, species interactions

1 | INTRODUCTION

Intensive agriculture has been increasingly questioned during the last years as it boosts global climate change, land degradation, biodiversity loss, and resource depletion which might result in severe food security and food sovereignty threats for a growing world population (Campbell et al., 2017). The major challenge of agriculture is, therefore, to increase food production while simultaneously reducing environmental burden, resource use, and social threats (Pe'er et al., 2020). Consequently, there is an urgent need to identify and understand agricultural practices that promote increased plant biomass and yield production, thereby lowering environmental problems. Recent research has shown that intercropping (IC) is one of the practices that increases yields and at the same time enhances the crop quality and reduces environmental problems (Duchene et al., 2017; Li et al., 2014).

IC refers to ancient agricultural practices that involve the simultaneous cultivation of at least two crop species or genotypes in close proximity. It is a common practice in peasant farming, and increasingly also in organic farming worldwide (Brooker et al., 2015; Li et al., 2014). IC is commonly assumed to increase above- and belowground biodiversity, soil and water conservation, as well as resilience against diseases, pests, and abiotic stresses (Ehrmann & Ritz, 2014; Malézieux et al., 2009).

IC has also been shown to result in an overall yield increase relative to monocropping which is referred to as overyielding. This is often measured by the land equivalent ratio (LER) (Duchene et al., 2017; Li et al., 2013). LER is defined as the relative land area needed for monocropping to produce the yields attained by IC. It is calculated as the sum of the partial land equivalent ratios (pLER) of the two crop species (Mead & Willey, 1980). If it exceeds 1.0, IC has a positive effect on the area of land needed to produce a given yield, and a larger area is needed in monocropping compared to IC for the same biomass production and yield. Meta-analyses and reviews summarizing between 58 and 939 observations of up to 126 IC studies found a mean LER of 1.17 to 1.30 (Bedoussac et al., 2015; Martin-Guay et al., 2018; Yu et al., 2016). Specifically, for soy/maize IC, a mean LER of 1.32 was reported (Xu et al., 2020). However, it is still a matter of debate under which conditions and with which companion crops IC results in overyielding since single LER ranged between 0.5 and >4.0 in a global meta-analysis (Martin-Guay et al., 2018). Moreover, the underlying mechanisms and the contribution of root interactions to overyielding in IC are not fully understood yet.

Overyielding in IC is supposed to result from positive above- and belowground interspecific plant interactions (Li et al., 2014; Zhang et al., 2001). Positive aboveground interactions may be associated with the light and temperature environment being changed through IC which results in a more efficient light interception of the intercropped species (Lv et al., 2014; Zou et al., 2019). Positive belowground interactions are mostly attributed to a complementary resource use and interspecific facilitation processes resulting in a more efficient acquisition of macronutrients, such as nitrogen (N) and phosphorus (P), microelements, such as iron and zinc, and water by the intercropped species (Chen et al., 2018; Xue et al., 2016). These complementarity and facilitation processes have mostly been studied in legume/cereal IC systems, since legumes can increase N availability through symbiotic N_2 fixation and are also assumed to contribute substantially to plant P acquisition (Li et al., 2013; Xue et al., 2016).

Many IC studies reported that belowground interspecific interactions are more important for overyielding than aboveground interactions (Duchene et al., 2017; Li et al., 2014). However, only a few studies identified the relative contributions (RCs) of above- and belowground interspecific interactions to overyielding in IC, so far. To distinguish between above- and belowground effects, so-called separation or partitioning techniques have been used. They were first established by Donald (1958) who fully partitioned root (belowground) and shoot (aboveground) effects using barriers. Although the partitioning techniques have been used repeatedly since then, a quantification of the RCs of above- and belowground interspecific interactions to overyielding remains rare and inconsistent regarding calculation and findings (Chen et al., 2015; Zhang et al., 2001). Additional research is, therefore, needed to identify the RCs of above- and belowground interspecific interactions to overyielding.

This study aimed to (1) investigate the effects of IC (with four companion crops) on maize aboveground biomass (AGB), grain yields, and nutrient uptake as well as (2) determine the RCs of above- and belowground interspecific interactions to these effects. For this purpose, we conducted a small-scale field experiment for two consecutive years with maize-based IC having a root barrier treatment to (partly) restrict root interactions in the second year. We used maize (*Zea mays* L.) as the main crop as it is one of the most important crops for food production worldwide (Xu et al., 2020). As companion crops, we used three different legumes with contrasting root functional traits and differences in nutrient acquisition mechanisms (Hallama et al., 2019; Wen et al., 2019): faba bean (*Vicia faba* L.) forming taproots, soy [*Glycine max* (L.) Merr.] with a fibrous root system, and blue lupin (*Lupinus angustifolius* L.) forming also taproots but without mycorrhizal symbioses. In addition to these legumes, we used white mustard (*Sinapis alba* L.) as non-leguminous Brassicaceae species which is often cultivated as a cover crop. We decided for an experiment on a small scale because IC is mostly used by peasant farmers rather than on large fields. We hypothesized that (1) AGB production and grain yields of maize are enhanced in IC compared to monocropping, (2) N and P uptake of maize AGB as well as grain yields are higher in IC than in monocropping, (3) the effects of IC on maize AGB, maize grain yields, and maize N and P uptake are stronger in legume/maize than in mustard/maize IC, and (4) positive belowground interspecific interactions contribute more to these effects than aboveground interactions. As our study and the hypotheses focus on the IC effects on maize as the main crop, we decided to have only a maize monocropping control, whereas companion crops were not cultivated in monocropping.

2 | MATERIAL AND METHODS

2.1 | Experimental setup

The field experiment was conducted at the University of Bayreuth (Germany) from May to August in 2018 and 2019. The site is located in the southeast of Bayreuth (49°55′17″ N, 11°35′17″ E). The mean annual rainfall is 756 mm, and the mean annual temperature is 8.0°C (Lüers et al., 2014). The soil texture is loamy sand (10% clay, 23% silt, 67% sand). In the upper 15 cm, the following soil chemical properties were determined: pH 6.9, 23.9 g total C kg^{-1} soil, 2.2 g total N kg^{-1} soil, and 0.5 g plant available P kg^{-1} soil as the sum of water- and $NaHCO_3$-extractable P.

In the first year, five blocks subdivided into six plots (2.5 x 1.7 m) were cultivated in row IC (Supporting information 1), where maize (*Zea mays* L. cv. Damaun, ReinSaat KG, Austria) was intercropped with one of the following companion crops: faba bean (*Vicia faba* L. cv. Hangdown, ReinSaat KG, Austria), soy [*Gylcine max* (L.) Merr. cv. Green Shell, ReinSaat KG, Austria], blue lupin (*Lupinus angustifolius* L. cv. Sonet, Templiner Kräutergarten, Germany), or white mustard (*Sinapis alba* L., ReinSaat KG, Austria). As a control, maize was also cultivated in monocropping (Figure 1C). Each plot consisted of eight alternating rows of maize and companion crop with 12 plants per row having a distance of 20 cm between plants and rows (Figure 2A). As we used a replacement design, the distance between plants and rows was 20 cm in maize monocropping as well, thus, the monocropping plots had twice the number of maize plants as the IC plots (Figure 2A). Each species combination was replicated five times, summing up to a total of 25 plots (Supporting information 1). Before seeding, the soil was prepared by plowing, rotary tillage, and surface steaming. Surface steaming was done by inducing hot steam between the soil surface and a plastic sheet on top of the soil for 4 h (Figure 1A). This was mostly done to kill weed seeds and avoid the application of herbicides. All seeds except mustard were soaked in water for 24 h. Soy and lupin seeds were inoculated with commercial *Bradyrhizobium sp.* inoculants before seeding (lupin: *Bradyrhizobium sp. Lupinus*, Templiner Kräutergarten, Germany; soy: LegumeFix® Soya, Legume Technology Ltd, UK). First, faba bean was sown manually on April 18th 2018 because we expected it to grow more slowly and intended to harvest all plants at the same time. All other seeds were then sown manually 3 weeks later, on May 8th 2018. The five blocks were surrounded by a wire netting to prevent feeding damage (Figure 1C–G). As the summer 2018 was very dry, the plots were weekly watered by hand with a watering spray lance, whenever necessary to avoid competition for water between the plants. All plots were watered for the same period of time until water started to accumulate at the soil surface. After watering all plots once, the watering procedure was repeated twice to ensure that plants received enough water. At the end of the growing season, ten plants per species from the four innermost rows of each plot were harvested (Figure 2A). In the maize monocropping, 20 maize plants were harvested per plot.

In the second year, the same block design was used to cultivate maize (*Zea mays* L. cv. Golden Bantam, Bingenheimer Saatgut AG, Germany) in row IC with faba bean (*Vicia faba* L. cv. Hangdown, Bingenheimer Saatgut AG, Germany), soy [*Gylcine max* (L.) Merr. cv. Lica, Naturland, Germany], blue lupin (*Lupinus angustifolius* L. cv. Rumba, Templiner Kräutergarten, Germany), or white mustard (*Sinapis alba* L., Bingenheimer Saatgut AG, Germany) or in monocropping. In addition, in one half of each plot (i.e., six plants in each row) root barriers according to Zhang et al. (2001) were installed to separate the plant rows to prevent interspecific root interactions between maize and companion crops (Figure 2B). For this purpose, impermeable plastic sheets made of plexiglass (3 mm thick) were inserted into the soil between the rows to a depth of 50 cm prior to sowing (Figure 1I). Root barriers were also installed in the maize monocropping plots to check whether the root barriers influenced the maize AGB. The lupin/maize IC was done without such a root barrier treatment due to time constraints during installation. All seeds were simultaneously sown by hand on May 8th and 9th, 2019. All seeds except for mustard were soaked in water for 24 h prior to sowing. As the summer 2019 was also very dry, the plots were regularly watered. At the end of the growing season, ten plants per species and plot were harvested (Figure 2B). Five of them were harvested in the plot part with root barriers (rb) and five in the part with no barriers (nb). All plants were analyzed for AGB production; maize plants were also analyzed for N and P uptake.

FIGURE 1 Photos of the field experiment in 2018 and 2019 showing the steaming process as part of the field preparation (A), the plot preparation in late April 2018 (B), plant interactions in the different species combinations in late June 2018 (C–G), plots before harvest in August 2018 (H), plot preparation with root barriers in late April 2019 (I), and plots before harvest in August 2019 (J)

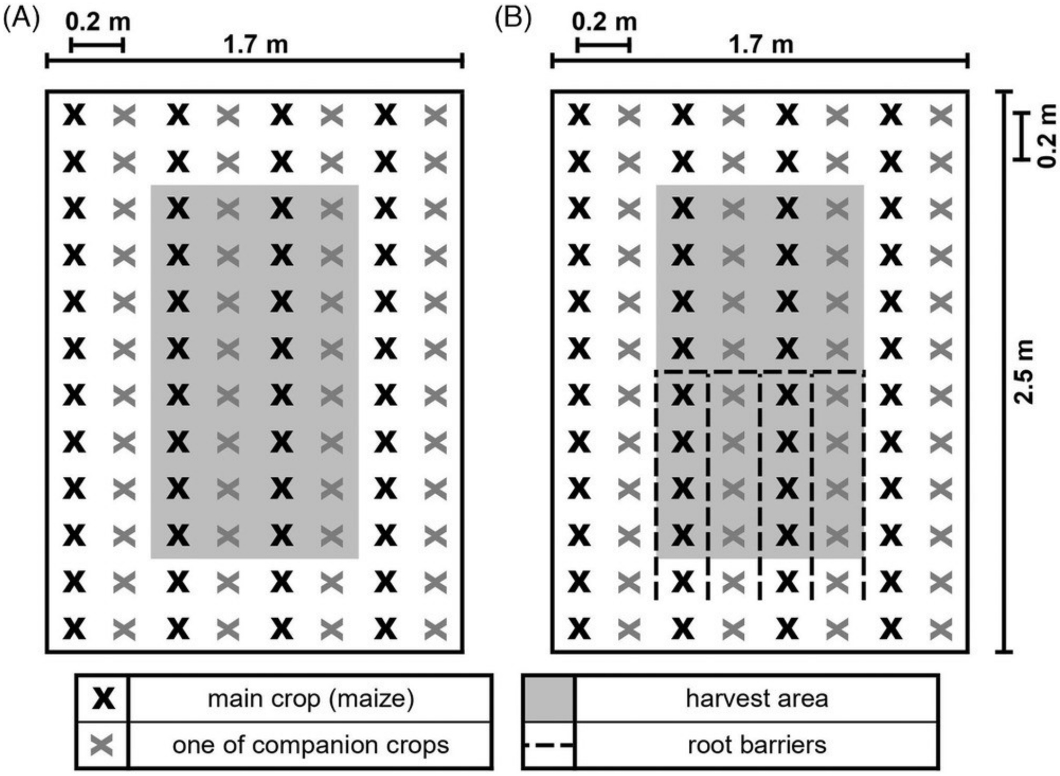

FIGURE 2 Experimental setup of the plots in 2018 without root barriers (A) and in 2019 with and without root barriers (B)

2.2 | Biomass analyses

Harvested companion crops were dried at 60°C and weighed. Harvested maize plants were divided into leaves, shoots, and grains, dried at 60°C and weighed. Dried leaves and shoots were cut with scissors, and subsamples of maize leaves, shoots, and grains were milled (MM400, Retsch, Haan, Germany). Subsamples were then analyzed for total N concentrations using an element analyzer (Vario Max, Elementar, Hanau, Germany). For total P concentrations, 100 mg of each subsample were dissolved in 1 mL of concentrated nitric acid at 170°C for 12 h (pressure digestion) before being analyzed with an inductively coupled plasma-optical emission spectroscopy (Vista-Pro radial, Varian Inc., Palo Alto, USA).

2.3 | Calculations

Maize AGB was calculated as the sum of the dry mass of leaves, shoots, and grains per single plant. For scaling up the AGB production to the square meter, maize AGB was multiplied by 12.5 (as 12.5 maize plants were cultivated per square meter in IC), or by 25 in the case of maize monocropping. Maize leaf, shoot, and grain N and P uptake was calculated by multiplying the dry mass of leaves, shoots, and grains with the corresponding N and P concentrations. Total maize N and P uptake per square meter was calculated as the sum of leaf, shoot, and grain N and P uptake.

The pLER of maize grain yield per square meter was calculated according to Mead and Willey (1980), as follows:

$$\mathrm{pLER} = \frac{\text{grain yield}_{\text{intercropping}}\ (\mathrm{g\ m^{-2}})}{\text{mean grain yield}_{\text{monocropping}}\ (\mathrm{g\ m^{-2}})}\ . \tag{1}$$

Similarly, maize AGB N and P uptake was used to calculate partial N and P equivalent ratios (pNER and pPER; Equations 2 and 3) as a measure of the increase in N and P uptake of maize in IC compared to monocropping, as follows:

$$\mathrm{pNER} = \frac{\text{grain yield } \mathrm{N}_{\text{intercropping}}\ (\mathrm{g\ m^{-2}})}{\text{mean grain yield } \mathrm{N}_{\text{monocropping}}\ (\mathrm{g\ m^{-2}})}\ , \tag{2}$$

$$\mathrm{pPER} = \frac{\text{grain yield } \mathrm{P}_{\text{intercropping}}\ (\mathrm{g\ m^{-2}})}{\text{mean grain yield } \mathrm{P}_{\text{monocropping}}\ (\mathrm{g\ m^{-2}})}\ . \tag{3}$$

We further calculated the RC of above- (RC$_{\text{above}}$) and belowground (RC$_{\text{below}}$) interspecific interactions to maize yield increases in the different IC systems. For this purpose, we first calculated the relative yield increases (RYI) of single maize plants in IC compared to monocropping both with root barriers (rb; Equation 4) and with no root barriers (nb; Equation 5), as follows:

$$RYI1 = \frac{mean\ grain\ yield_{intercropping}(rb)}{mean\ grain\ yield_{monocropping}(rb)} - 1\ , \tag{4}$$

$$RYI2 = \frac{mean\ grain\ yield_{intercropping}(nb)}{mean\ grain\ yield_{monocropping}(nb)} - 1\ . \tag{5}$$

In the rb treatment, interspecific root interactions were largely excluded, thus, we assume that the RYI in this treatment is only derived from aboveground interspecific interactions. In the nb treatment, we assume that the RYI is derived from both aboveground and belowground interspecific interactions. The RCs were, therefore, calculated, as follows:

$$RC_{above} = \frac{RYI1}{RYI2} \times 100\%\ , \tag{6}$$

$$RC_{below} = 100\% - RC_{above}\ . \tag{7}$$

The calculation of RC also accounted for the small influence that the root barrier installation had on maize grain yields.

2.4 | Statistics

Data were tested separately for significant differences between species combinations, years, and barrier treatments. For this purpose, normality was checked with Shapiro-Wilk normality tests, and homogeneity of variances was tested with Levene's tests. Where normality and homogeneity assumptions were met, analyses of variance (ANOVA) followed by Tukey's post-hoc tests (Tukey HSD) were used to identify significant differences between species combinations, years, or barrier treatments. Where normality and homogeneity assumptions were not met, log-transformations of data were performed prior to ANOVA and Tukey HSD. All statistical analyses were performed in R (version 3.5.2; R Core Team, 2018) using the packages *agricolae* (version 1.3-2; de Mendiburu, 2020), *car* (version 3.0-7; Fox & Weisberg, 2019), *dplyr* (version 0.8.5; Wickham et al., 2020), and *ggplot2* (version 3.3.0; Wickham, 2016).

3 | RESULTS

3.1 | Biomass and grain yields

On a single plant basis, maize AGB ranged between 81.8 ± 12.6 g plant^{-1} (maize monocropping, 2018) and 174.8 ± 29.4 g plant^{-1} (soy/maize IC, 2018) across all species combinations, years, and barrier treatments (Figure 3). Maize grain yields ranged between 4.9 ± 3.2 g plant^{-1} (maize monocropping, 2019, rb) and 36.2 ± 6.2 g plant^{-1} (soy/maize IC, 2018) across all species combinations, years, and barrier treatments (Figure 3). In 2018, maize AGB and grain yields were significantly higher in soy/maize and lupin/maize IC than in maize monocropping. In 2019, maize AGB was significantly higher in faba bean/maize, soy/maize, and lupin/maize IC than in maize monocropping (nb), whereas maize grain yields were significantly higher only in soy/maize IC compared to maize monocropping (nb). In 2019, maize AGB in faba bean/maize and soy/maize IC, and maize grain yields in soy/maize IC were significantly higher in the nb than in the rb treatment. Maize AGB and grain yields in maize monocropping did not differ significantly between nb and rb in 2019, showing that the installation of the root barriers did not negatively impact maize growth. In the rb treatment, maize AGB and grain yields showed no significant differences between the species combinations.

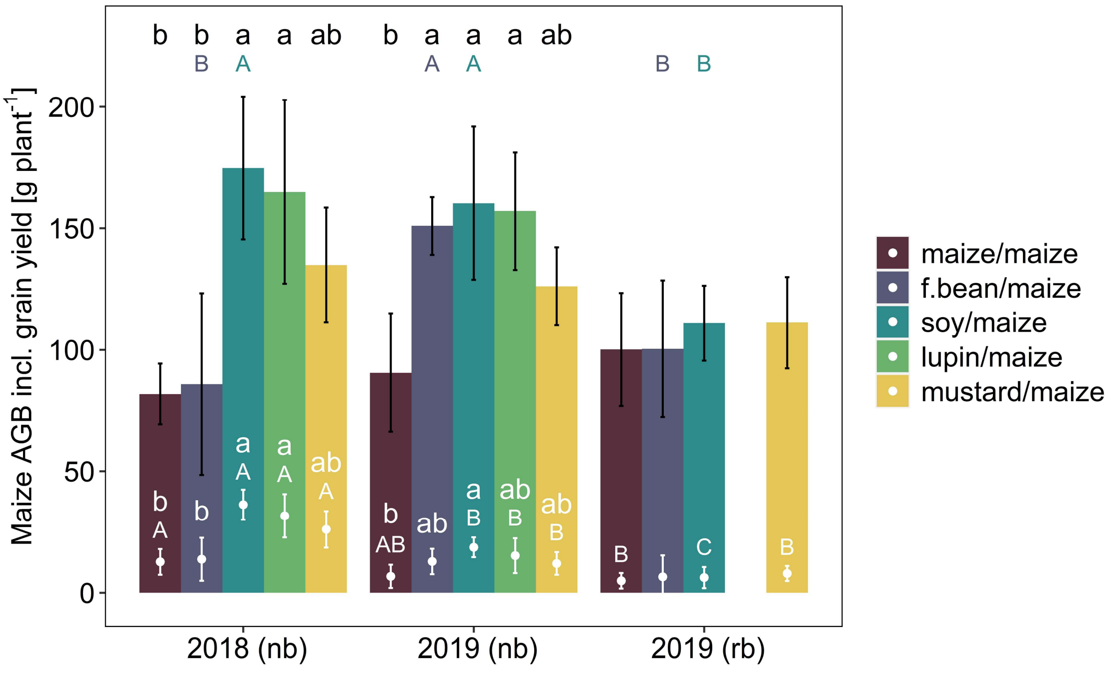

FIGURE 3 Aboveground biomass (AGB) per maize plant as the sum of grain yield and nongrain biomass, harvested in 2018 (no root barrier; nb) and 2019 (nb or with root barrier; rb). Columns show AGB means, white dots show grain yield means, and error bars indicate standard deviations (n = 5). A one-way ANOVA was conducted followed by Tukey post-hoc test. Lowercase letters indicate significant differences ($p < 0.05$) in the maize AGB (black lowercase letters) and grain yields (white lowercase letters) between the species combinations, tested separately for each year and barrier treatment. Capital letters indicate significant differences ($p < 0.05$) in the maize AGB (colored capital letters) and grain yields (white capital letters) between years and barrier treatments, tested separately for each species combination. Absence of letters indicates that there were no significant differences

In contrast, on an area basis we observed a significantly lower maize AGB in faba bean/maize IC than in maize monocropping and in soy/maize and lupin/maize IC in 2018 (Figure 4). The total biomass (as the sum of maize and companion AGB) was significantly higher in soy/maize IC than in faba bean/maize IC in 2018. In the rb treatment in 2019, maize AGB and total biomass were significantly higher in maize monocropping than in all IC systems. In the nb treatment, we found no significant differences in maize AGB and total biomass between the species combinations in 2019. Maize AGB and total biomass in faba bean/maize IC were significantly higher in 2019 (nb) than in 2018. Maize AGB in faba bean/maize IC in 2019 was also significantly higher in the nb than in the rb treatment. Maize AGB and total biomass in soy/maize IC were significantly higher in the nb treatment (both years) than in the rb treatment. The companion crop AGB was not affected by year or barrier treatments, except for mustard, showing a significantly higher AGB in 2018 than in 2019.

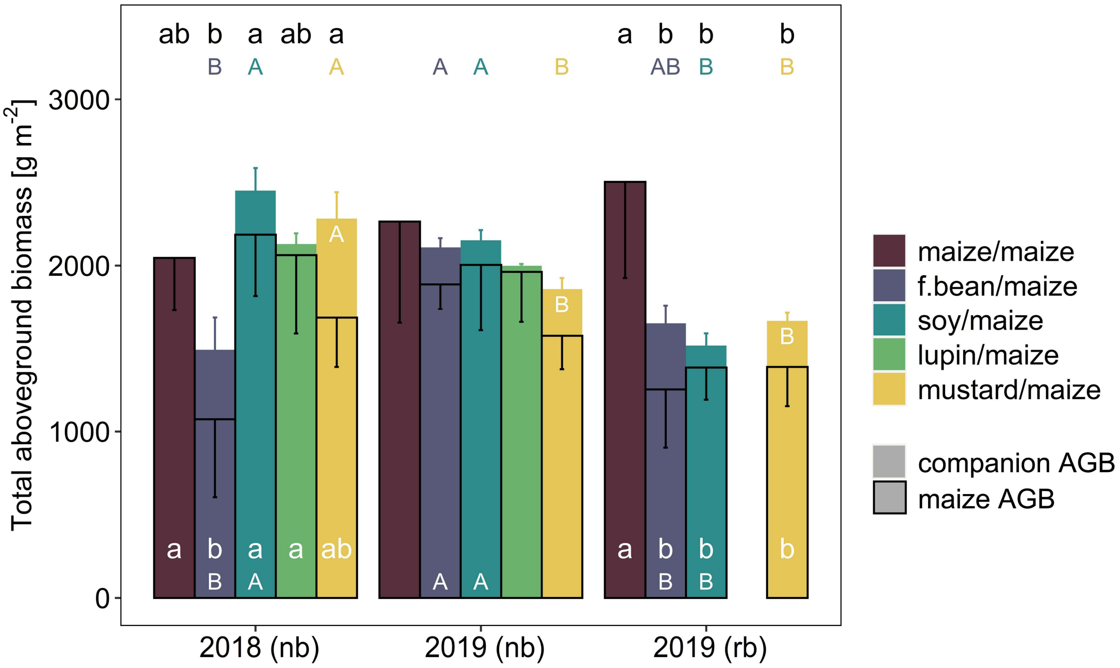

FIGURE 4 Total aboveground biomass (AGB) per area as the sum of maize AGB (black-rimmed) and companion AGB (rim-less on top), harvested in 2018 (no root barrier; nb) and 2019 (nb or with root barrier; rb). Columns show means and error bars indicate standard deviations (n = 5). A one-way ANOVA was conducted followed by Tukey post-hoc test. Lowercase letters indicate significant differences ($p < 0.05$) in the total AGB (sum of maize and companion AGB; black lowercase letters) and in the maize AGB (white lowercase letters) between the species combinations, tested separately for each year and barrier treatment. Capital letters indicate significant differences ($p < 0.05$) in the total AGB (colored capital letters), in the maize AGB (white capital letters; bottom of columns), and in the companion AGB (white capital letters; top of columns) between years and barrier treatments, tested separately for each species combination. Absence of letters indicates that there were no significant differences

The pLER of maize grain yields in IC were higher than 0.5 in all species combinations in both years, except for faba bean/maize IC in 2018 (Table 1). They were even larger than 1.0 in soy/maize and lupin/maize IC in both years (nb). The pLER was significantly higher in soy/maize and lupin/maize IC than in faba bean/maize IC in 2018. We found no significant difference between the species combinations in 2019 for both barrier treatments. The pLER of maize grain yields in soy/maize IC was significantly higher in the nb treatment (both years) than in the rb treatment.

TABLE 1 Partial land equivalent ratios (pLER) and partial nitrogen and phosphorus equivalent ratios (pNER and pPER) of maize grain yields, calculated for maize harvested in 2018 (no root barrier; nb) and 2019 (nb or with root barrier; rb)

		pLER	pNER	pPER
Faba bean/maize	2018 (nb)	0.54 ± 0.35^{b}	0.57 ± 0.37^{b}	0.57 ± 0.38^{b}
	2019 (nb)	0.95 ± 0.38	1.12 ± 0.41	0.99 ± 0.41
	2019 (rb)	0.67 ± 0.89	0.72 ± 0.97	0.66 ± 0.84
Soy/maize	2018 (nb)	1.43 ± 0.24^{aA}	1.32 ± 0.38^{aAB}	1.45 ± 0.24^{aAB}
	2019 (nb)	1.38 ± 0.30^{A}	1.56 ± 0.42^{A}	1.52 ± 0.38^{A}
	2019 (rb)	0.63 ± 0.44^{B}	0.66 ± 0.48^{B}	0.76 ± 0.58^{B}
Lupin/maize	2018 (nb)	1.25 ± 0.35^{a}	1.45 ± 0.34^{a}	1.30 ± 0.37^{a}
	2019 (nb)	1.13 ± 0.53	1.30 ± 0.53	1.19 ± 0.55
Mustard/maize	2018 (nb)	1.02 ± 0.29^{ab}	1.06 ± 0.33^{ab}	1.03 ± 0.32^{ab}
	2019 (nb)	0.89 ± 0.34	1.16 ± 0.39	0.92 ± 0.36
	2019 (rb)	0.80 ± 0.31	0.82 ± 0.37	0.84 ± 0.36

Note: Numbers show means ± standard deviations (n = 5). A one-way ANOVA was conducted followed by Tukey post-hoc test. Lowercase letters indicate significant differences ($p < 0.05$) between the species combinations, tested separately for each year and barrier treatment. Capital letters indicate significant differences ($p < 0.05$) between the years and barrier treatments, tested separately for each species combination. Absence of letters indicates that there were no significant differences.

Yield increases in mustard/maize IC resulted mostly (78%) from aboveground interspecific interactions (Table 2). In contrast, yield increases in soy/maize and faba bean/maize IC were caused mostly by belowground interspecific interactions, accounting for 85% of yield increases in soy/maize IC and for 62% in faba bean/maize IC.

TABLE 2 Mean relative contributions of above- (RC_{above}) and belowground (RC_{below}) interspecific interactions to yield increases of maize in 2019

Species combination	RC_{above} [%]	RC_{below} [%]
Faba bean/maize	38	62
Soy/maize	15	85
Mustard/maize	78	22

3.2 | Nutrients (N and P)

On a single plant basis, N uptake of maize AGB and grains was significantly higher in soy/maize and lupin/maize IC than in maize monocropping in 2018 (Table 3, Supporting information 2). N uptake of maize AGB and grains was also significantly higher in faba bean/maize (AGB), soy/maize (grains), and lupin/maize IC (AGB) than in maize monocropping in 2019 (nb). On an area basis, however, we found no significant differences in N uptake of maize AGB and grains between monocropping and IC. N uptake of maize AGB and grains was significantly higher in soy/maize IC (AGB and grains) and in lupin/maize IC (grains) than in faba bean/maize IC in 2018. Maize N uptake in soy/maize and lupin/maize IC (AGB and grains), and in mustard/maize IC (grains) were significantly higher in 2018 than in 2019. Similarly, N concentrations of maize AGB in all species combinations were significantly higher in 2018 than in 2019, except for mustard/maize IC in 2019 (nb), but showed no significant differences between the species combinations (Supporting information 1). N concentrations of maize grain yields did not differ significantly between species combinations, years, and barrier treatments, except that they were significantly higher in mustard/maize IC than in maize monocropping in 2019 (nb).

On a single plant basis, P uptake of maize AGB and grains was significantly higher in soy/maize and lupin/maize IC than in maize monocropping in 2018 (Table 3, Supporting information 1). P uptake of maize AGB and grains was also significantly higher in soy/maize (AGB and grains) and lupin/maize IC (AGB) than in maize monocropping in 2019 (nb). On an area basis, however, we found no significant differences in P uptake of maize AGB and grains between monocropping and IC in both years (nb), except for faba bean/maize IC in 2018. In the rb treatment, P uptake of maize AGB was significantly higher in maize monocropping than in IC (Supporting information 1). P uptake of maize AGB and grains in soy/maize IC (both years; AGB and grains), and in faba bean/maize IC (2019; AGB) was significantly higher in the nb than in the rb treatment. P concentrations of maize AGB did not differ significantly between species combinations, years, and barrier treatments (Supporting information 1). P concentrations of maize grains did not differ significantly between species combinations, but were significantly higher in soy/maize, lupin/maize, and mustard/maize IC in 2018 than in 2019 (nb).

Similar to pLER, the pNER and pPER of maize grain yields in IC were higher than 0.5 across years and barrier treatments (Table 1). They were even larger than 1.0 in soy/maize and lupin/maize IC in both years (nb). The pNER was also larger than 1.0 in mustard/maize IC (both years) and in faba bean/maize IC (2019; nb). Both ratios were significantly higher in soy/maize and lupin/maize IC than in faba bean/maize IC in 2018. We found no significant differences between the species combinations in 2019 for both barrier treatments. In 2019, pNER and pPER of soy/maize IC were significantly higher in the nb than in the rb treatment.

TABLE 3 N and P concentrations per unit plant biomass (dry weight) as well as N and P uptake of maize grains per plant and per area, harvested in 2018 (no root barrier; nb) and 2019 (nb or with root barrier; rb)

		N [g kg^{-1}]	P [g kg^{-1}]	N [mg plant^{-1}]	P [mg plant^{-1}]	N [g m^{-2}]	P [g m^{-2}]
Maize/maize	2018 (nb)	21.5 ± 1.3	4.3 ± 0.4	278.6 ± 133.3^{bA}	56.6 ± 28.6^{b}	7.0 ± 3.3^{abA}	1.4 ± 0.7^{abA}
	2019 (nb)	13.3 ± 7.8$^{(b)}$	2.7 ± 1.5	115.7 ± 91.8^{bAB}	23.5 ± 17.8^{b}	2.9 ± 2.3^{AB}	0.6 ± 0.4^{AB}
	2019 (rb)	14.6 ± 8.9	2.6 ± 1.6	90.6 ± 61.9^{B}	16.2 ± 11.3	2.3 ± 1.5^{B}	0.4 ± 0.3^{B}
F. bean/maize	2018 (nb)	22.7 ± 2.6	4.5 ± 0.4^{A}	315.5 ± 208.5^{b}	64.1 ± 43.1^{bA}	3.9 ± 2.6^{b}	0.8 ± 0.5^{b}
	2019 (nb)	20.4 ± 3.5$^{(ab)}$	3.6 ± 1.0^{AB}	258.8 ± 93.9^{ab}	46.3 ± 19.3^{abAB}	3.2 ± 1.2	0.6 ± 0.2
	2019 (rb)	17.7 ± 11.7	2.7 ± 1.5^{B}	130.4 ± 175.1	21.4 ± 27.2^{B}	1.6 ± 2.2	0.3 ± 0.3
Soy/maize	2018 (nb)	20.9 ± 7.3	4.5 ± 0.1^{A}	736.5 ± 212.3^{aA}	164.3 ± 27.7^{aA}	9.2 ± 2.7^{aA}	2.1 ± 0.3^{aA}
	2019 (nb)	19.1 ± 2.0$^{(ab)}$	3.8 ± 0.4^{B}	360.6 ± 96.4^{aB}	71.4 ± 18.0^{aB}	4.5 ± 1.2^{B}	0.9 ± 0.2^{B}
	2019 (rb)	20.3 ± 4.9	3.9 ± 0.5^{AB}	120.0 ± 86.5^{B}	24.5 ± 18.9^{C}	1.5 ± 1.1^{B}	0.3 ± 0.2^{C}
Lupin/maize	2018 (nb)	27.1 ± 9.7	4.6 ± 0.2^{A}	809.0 ± 187.4^{aA}	146.8 ± 42.1^{aA}	10.1 ± 2.3^{aA}	1.8 ± 0.5^{abA}
	2019 (nb)	20.3 ± 2.6$^{(ab)}$	3.7 ± 0.5^{B}	301.9 ± 122.1^{abB}	55.9 ± 25.9^{abB}	3.8 ± 1.5^{B}	0.7 ± 0.3^{B}
Mustard/maize	2018 (nb)	22.6 ± 1.3	4.4 ± 0.3^{A}	590.9 ± 185.4^{abA}	116.0 ± 36.8^{abA}	7.4 ± 2.3^{abA}	1.5 ± 0.5^{abA}
	2019 (nb)	23.1 ± 3.7$^{(a)}$	3.6 ± 0.4^{B}	267.6 ± 90.0^{abB}	43.4 ± 16.8^{abB}	3.3 ± 1.1^{B}	0.5 ± 0.2^{B}
	2019 (rb)	18.7 ± 3.0	3.4 ± 0.5^{B}	149.1 ± 67.9^{B}	27.3 ± 11.6^{B}	1.9 ± 0.8^{B}	0.3 ± 0.1^{B}

Note: Numbers show means ± standard deviations ($n = 5$). A one-way ANOVA was conducted followed by Tukey post-hoc test. Lowercase letters indicate significant differences ($p < 0.05$) between the species combinations, tested separately for each year and barrier treatment. Capital letters indicate significant differences ($p < 0.05$) between the years and barrier treatments, tested separately for each species combination. Brackets indicate that data were not normally distributed (log-transformation was not possible). Absence of letters indicates that there were no significant differences.

4 | DISCUSSION

We found that IC had positive effects on biomass production and grain yields of maize on a plant basis, particularly when intercropped with legumes. On an area basis, IC with interspecific root interactions resulted in a similar productivity of maize as in monocropping, even though there were twice as many maize plants per area in monocropping than in IC.

4.1 | IC effects on single maize plants

On a single plant basis, we found that maize AGB and grain yields were significantly increased in IC compared to monocropping, especially when maize was intercropped with soy and lupin (Figure 3). Similarly, N and P uptake of maize AGB and grains were significantly increased in IC compared to monocropping, especially when maize was intercropped with soy and lupin (Table 3, Supporting Information 1). These findings indicate that resource competition between maize plants and companion crops in IC was less pronounced than competition between maize plants in maize monocropping. This could be due to a compensation effect (Horwith, 1985; Neamatollahi et al., 2013), that is, maize in IC had more resources available than maize in monocropping due to a lower maize plant density in IC than in monocropping and because companion crops produced less AGB than maize plants (Figure 4). It has been shown for maize monocropping with different planting densities that a lower grain yield per plant was fully compensated by a higher plant density (Testa et al., 2016). However, in the rb treatment, maize AGB, grain yields, and N and P uptake did not differ significantly between the species combinations on a single plant basis (Figure 3, Table 3, Supporting Information 1). This indicates that mainly interspecific root interactions caused the IC effects and that companion crops might use complementary and facilitative processes that provide maize in IC with additional nutrients (see Section 4.3).

The observation that there was no positive effect of faba bean/maize IC on maize AGB production, grain yields, and nutrient uptake of maize in 2018 (Figure 3, Table 3, Supporting Information 1) can be explained by the earlier sowing of faba beans in 2018, leading to a higher competition between the species for light, water, and nutrients when maize was sown later. This is in accordance with a global meta-analysis on legume/cereal IC showing that the species competitiveness and consequently the pLER were increased for earlier sown species, but decreased for later sown species (Yu et al., 2016).

4.2 | IC effects on an area basis

On an area basis, we found that maize in IC was similarly productive as in monocropping, even though there were twice as many maize plants per area in monocropping than in IC. Neither total biomass, maize AGB (Figure 4), maize grain yields nor maize N and P uptake (Table 3, Supporting Information 1) differed significantly between monocropping and IC in the nb treatments, except for maize AGB and maize AGB P uptake in faba bean/maize IC in 2018 (Figure 4, Supporting Information 1). This indicates again a lower competition in IC than in maize monocropping and, therefore, compensation effects, as discussed above. We also found that maize pLER was larger than 1.0 in soy/maize and lupin/maize IC in both years in the nb treatments (Table 1). Hence, LER of the whole IC system (which is the sum of the pLER of both species) is also larger than 1.0 in soy/maize and lupin/maize IC. Although companion crops have been shown to be less competitive than cereals resulting in low pLER values (Yu et al., 2016), LER of the whole IC system is likely also larger than 1.0 in mustard/maize IC (both years; nb) and in faba bean/maize IC (2019; nb) since maize pLER is already 0.9 to 1.0 (Table 1). We, therefore, assume a moderate overyielding of all species combinations (except for faba bean/maize IC in 2018) indicated by LER being larger than 1.0. The moderate overyielding is in the range reported in previous meta-analyses and reviews (Bedoussac et al., 2015; Yu et al., 2016). For example, soy enhanced AGB production and grain yields of maize in IC resulting in a global mean LER of 1.32 for soy/maize IC (Chen et al., 2019; Xu et al., 2020). Overyielding has also been found when maize was intercropped with soy, faba bean (Li et al., 2018; Xia et al., 2019), or lupins (Lelei and Onwonga, 2014). Similarly, overyielding has been found when maize was intercropped with turnip rape (*Brassica campestris* L.; Xia et al., 2013, 2019). In addition, another meta-analysis revealed that Brassicaceae, including mustard, increased maize AGB and grain yields when used as a cover crop (Hallama et al., 2019).

Moreover, we found that maize pNER and pPER in soy/maize and lupin/maize IC, and pNER in mustard/maize and faba bean/maize IC (the latter only in 2019) were larger than 1.0 in the nb treatments (Table 1). Moreover, we found that maize pPER in mustard/maize IC (both years; nb) and in faba bean/maize IC (2019; nb) were 0.9 to 1.0 (Table 1). Hence, NER and PER of the whole IC system (which is the sum of the pNER or pPER of both species, respectively) are very likely larger than 1.0 in all species combinations (nb), except for faba bean/maize IC in 2018. These findings indicate that N and P uptake in IC is probably higher than in monocropping. This is in accordance with previous studies showing that N and/or P uptake of maize were higher when intercropped with soy (Chen et al., 2019; Xia et al., 2019), lupin (Lelei & Onwonga, 2014), or turnip rape (Xia et al., 2013, 2019) than in monocropping. Our finding that total biomass, maize AGB, and maize AGB P uptake were significantly higher in maize monocropping than in IC in the rb treatment (Figure 4 and Supporting Information 1) further indicates that interspecific root interactions were important for IC effects on maize, as discussed above.

We further found that N uptake (Table 3, Supporting Information 1) of maize AGB and grains tended to be lower in 2019 than in 2018 in all species combinations, except for faba bean/maize IC. In contrast, P uptake (Table 3, Supporting Information 1) of maize AGB tended to be slightly higher in 2019 than in 2018. These findings indicate that the smaller AGB and yield increases in 2019 compared to 2018 might be due to a nutrient (mainly N) scarcity in the second year of the experiment. One reason for the decline in yield, and in N uptake could be the lack of nutrient inputs since only root biomass was left in our field experiment and no fertilizers were applied. A decline in maize AGB and N uptake over time along with decreases in soil N concentrations have also been found in a 3-year soy/maize IC study (Chen et al., 2017). Similarly, IC maize with groundnut, cowpea, soy, or nonleguminous species over 11 years resulted in maize grain yield decreases over time (Agyare et al., 2006).

4.3 | Above- and belowground interactions

We found that belowground interspecific interactions accounted for more than half of the maize yield increases in legume/maize IC. These findings are in accordance with previous studies that attributed overyielding in faba bean/maize IC mainly to belowground interspecific interactions and showed higher nutrient acquisition when roots of both species had physical contact (Li et al., 1999, 2007). Overyielding in soy/maize IC has also been shown to be associated more with belowground than with aboveground interactions (Lv et al., 2014). The high contribution of belowground interspecific interactions to maize yields in legume/maize IC may be explained by two major factors, that is, niche complementarity and interspecific facilitation, that both comprise several mechanisms. Complementarity refers to the complementary use of resources which decreases competition because nutrients are taken up in different parts of the soil, during different times or in different forms. In contrast, facilitation refers to beneficial interactions between the intercropped species that increase resource availability and improve environmental conditions for both species (Duchene et al., 2017; Xue et al., 2016). The fact that most mechanisms of complementary and facilitative nutrient acquisition are associated with a high N demand and/or the ability to symbiotically fix atmospheric N_2 might be the reason why the RC of belowground interspecific interactions to maize yields was much higher in legume/maize than in mustard/maize IC. Moreover, N availability for maize in mustard/maize IC was probably lower since mustard does not fix atmospheric N_2. However, it needs to be further investigated which of these potential mechanisms caused the increase in maize yields.

Furthermore, we found that aboveground interspecific interactions contributed considerably to maize yield increases in mustard/maize IC (Table 2). This indicates that a part of the yield increase of maize intercropped with mustard might be due to a lower competition for light in IC than in monocropping. A more efficient light use has been reported, for example, for several legume/maize IC systems compared to the respective monocrops (Kermah et al., 2017). In addition, also other aboveground interactions might positively influence plant growth. These include the effects of an increased overall biodiversity on pollinators, pests, and diseases, and the suppression of weeds through an early soil coverage (Brooker et al., 2015; Ehrmann & Ritz, 2014). Moreover, the microclimate regulation through increased soil shading and, therefore, reduced evapotranspiration and more balanced temperatures might contribute to overyielding in IC (Malézieux et al., 2009).

5 | CONCLUSIONS

We found that IC resulted in a similar productivity of maize as in monocropping on an area basis, and in an increase in maize AGB, grain yield, and N and P uptake on a plant basis, when interspecific root interactions were not restricted. Maize pLER, pNER, and pPER (and therefore also LER, NER, and PER of the whole IC system) were larger than 1.0 in several species combinations in the nb treatments, indicating a positive effect of IC on maize, especially in soy/maize and lupin/maize IC. This confirms our first hypothesis about maize AGB and grain yields and our second hypothesis about maize N and P uptake for the species combinations soy/maize and lupin/maize. The highest pLER, pNER, and pPER of maize were found for soy/maize and lupin/maize IC confirming our third hypothesis. We further found that interspecific root interactions contributed considerably to maize yield increases in legume/maize IC, whereas aboveground interspecific interactions were more important in mustard/maize IC. Our fourth hypothesis can, therefore, be confirmed for legume/maize IC, but needs to be rejected for mustard/maize IC. Taken together, our results indicate that IC is beneficial for maize production.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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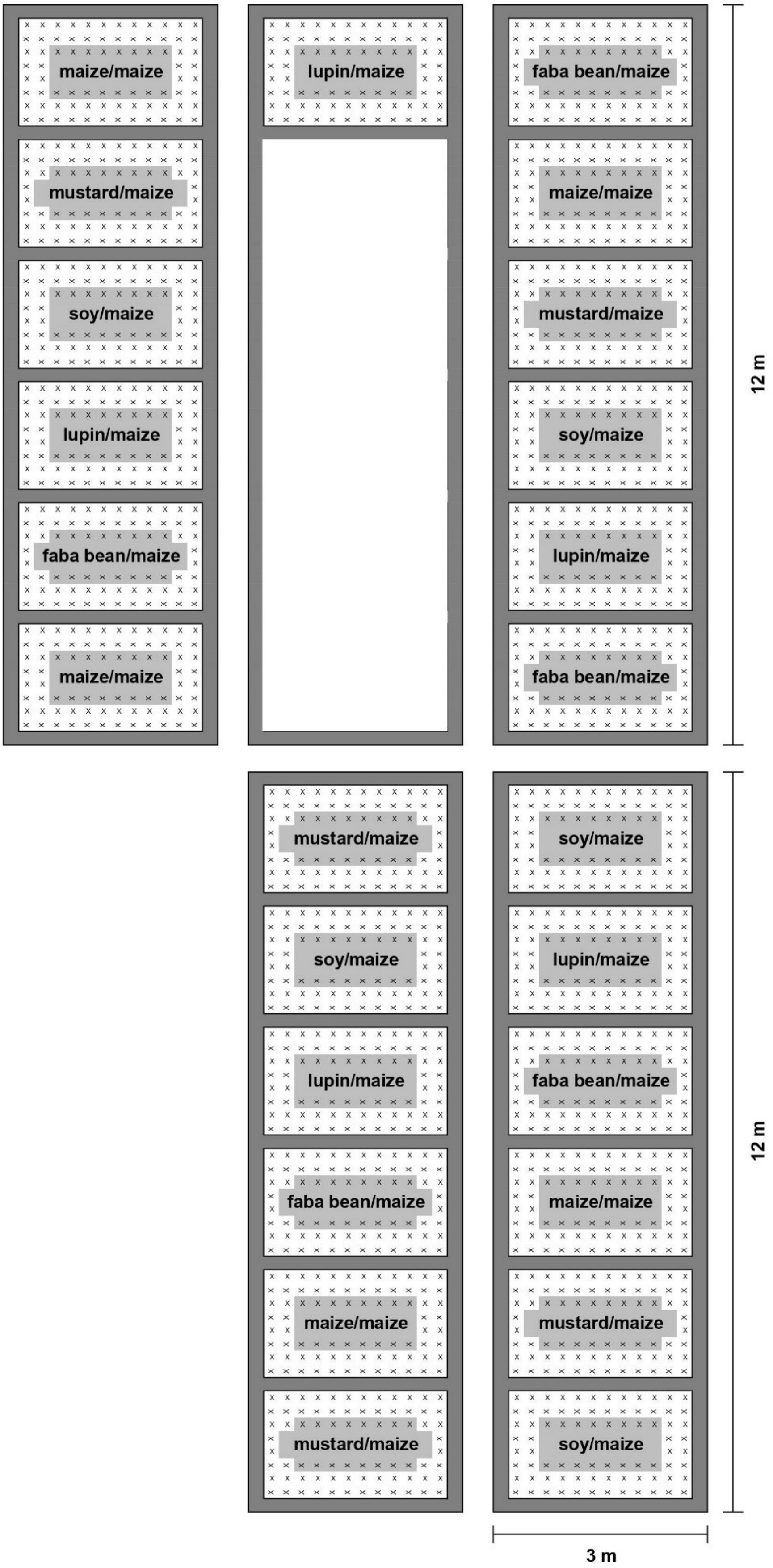
SUPPORTING INFORMATION



SUPPLEMENT 1 Experimental setup of the five blocks subdivided into six plots (2.5 x 1.7 m). For experimental setup of the single plots, see Figure 1.

SUPPLEMENT 2 N and P concentrations per unit plant biomass (dry weight) as well as N and P uptake of maize aboveground biomass (AGB) per plant and per area, harvested in 2018 (no root barrier; nb) and 2019 (nb or with root barrier; rb)

		N [g kg^{-1}]	P [g kg^{-1}]	N [mg plant^{-1}]	P [mg plant^{-1}]	N [g m^{-2}]	P [g m^{-2}]
Maize/maize	2018 (nb)	10.7 ± 3.6^{A*}	3.5 ± 0.5	904.6 ± 441.9^{b*}	286.9 ± 76.4^{b}	22.6 ± 11.0^{ab}	7.2 ± 1.9^{a}
	2019 (nb)	6.7 ± 1.1^{B*}	3.6 ± 0.5	613.7 ± 241.4^{b}	323.1 ± 108.4^{b}	15.3 ± 6.0	8.1 ± 2.7
	2019 (rb)	6.1 ± 0.9^{B*}	3.2 ± 0.4	618.3 ± 189.9	312.8 ± 61.2	15.5 ± 4.7	7.8 ± 1.5^{a}
F. bean/maize	2018 (nb)	11.3 ± 1.8^{A}	3.4 ± 0.1	983.9 ± 460.2^{b*}	287.3 ± 119.9^{bB}	12.3 ± 5.8^{b}	3.6 ± 1.5^{bB}
	2019 (nb)	8.0 ± 1.6^{B}	3.4 ± 0.3	1210.8 ± 248.8^{a}	505.3 ± 25.6^{abA}	15.1 ± 3.1	6.3 ± 0.3^{A}
	2019 (rb)	6.6 ± 1.7^{B}	3.1 ± 0.6	690.4 ± 343.5	310.3 ± 113.5^{B}	8.6 ± 4.3	3.9 ± 1.4^{bB}
Soy/maize	2018 (nb)	12.2 ± 1.6^{A}	3.0 ± 0.3	2133.2 ± 459.0^{a*A}	524.8 ± 64.8^{aA}	26.7 ± 5.7^{aA}	6.6 ± 0.8^{aA}
	2019 (nb)	7.2 ± 1.0^{B}	3.7 ± 0.9	1157.3 ± 289.8^{abB}	591.3 ± 189.2^{aA}	14.5 ± 3.6^{B}	7.4 ± 2.4^{A}
	2019 (rb)	6.2 ± 1.5^{B}	2.8 ± 0.5	701.1 ± 263.1^{B}	308.2 ± 71.3^{B}	8.8 ± 3.3^{B}	3.9 ± 0.9^{bB}
Lupin/maize	2018 (nb)	12.5 ± 1.1^{A}	3.3 ± 0.4	2077.3 ± 605.8^{a*A}	541.4 ± 101.0^{a}	26.0 ± 7.6^{abA}	6.8 ± 1.3^{a}
	2019 (nb)	7.7 ± 0.6^{B}	3.6 ± 0.7	1212.6 ± 218.3^{aB}	574.5 ± 146.4^{a}	15.2 ± 2.7^{B}	7.2 ± 1.8
Mustard/maize	2018 (nb)	12.4 ± 2.0^{A}	3.2 ± 0.3	1670.5 ± 406.4^{ab*A}	437.0 ± 109.5^{ab}	20.9 ± 5.1^{abA}	5.5 ± 1.4^{ab}
	2019 (nb)	9.0 ± 2.8^{AB}	3.8 ± 0.7	1153.5 ± 432.3^{abAB}	489.9 ± 120.0^{ab}	14.4 ± 5.4^{AB}	6.1 ± 1.5
	2019 (rb)	6.8 ± 0.9^{B}	3.3 ± 0.4	770.8 ± 211.5^{B}	367.0 ± 74.7	9.6 ± 2.6^{B}	4.6 ± 0.9^{b}

Note: Numbers show means ± standard deviations (SD; n = 5). A one-way ANOVA was conducted followed by Tukey post-hoc test. Lowercase letters indicate significant differences ($p < 0.05$) between the species combinations, tested separately for each year and barrier treatment. Capital letters indicate significant differences ($p < 0.05$) between the years and barrier treatments, tested separately for each species combination. Stars indicate that statistics were made after log-transformation of the data. Absence of letters indicates that there were no significant differences.

CORRECTIONS

After publication of the manuscript, some minor errors have been detected which are corrected here, as follows. Corrections and errors are italicized.

Own negligence:

- The scientific name of soy is misspelled twice in section 2.1 (Experimental setup). It should be *Glycine max* rather than *Gylcine max*.
- The description of Equations 2 and 3 is still in a former version. It should read “Similarly, maize *grain yield* N and P uptake […]” rather than “Similarly, maize *AGB* N and P uptake [...]”. Equations 2 and 3 are, however, correct.
- In the heading of Supporting Information 1 (Supplement 1), we cross-reference to *Figure 1* for the experimental setup of the single plots. This should, however, be *Figure 2*.

Third-party negligence:

- From section 3.2 on (after the first sentence), it is repeatedly referred to *Supporting Information 1* regarding maize N and P concentrations/ uptake. This should, however, be *Supporting Information 2*.

Study II – Field and greenhouse experiment with various N and P sources

Plant Species Interactions in the Rhizosphere Increase Maize N and P Acquisition and Maize Yields in Intercropping

Ulrike Schwerdtner and Marie Spohn (2022),

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Abstract

The aim of the study was to examine interspecific plant interactions that contribute to plant nitrogen (N) and phosphorus (P) acquisition and are likely the reason for overyielding in intercropping. We conducted a field and a rhizobox experiment with the same soil. Maize (*Zea mays* L.) was grown alone or intercropped with the companions faba bean (*Vicia faba* L.), soy (*Glycine max* (L.) Merr.), blue lupin (*Lupinus angustifolius* L.), or white mustard (*Sinapis alba* L.). We determined the isotopic N signature ($\delta^{15}N$) of maize as well as soil parameters (pH, phosphatase activity, nitrate) in the field experiment. We analyzed phosphatase activities and rhizosphere pH by soil zymography and pH imaging in the rhizobox experiment. Maize N and P contents were larger in intercropping than monocropping, especially with soy and lupin in the field, indicating intercropping advantages for maize N and P acquisition. Intercropping with legumes decreased maize $\delta^{15}N$ in the field, suggesting that 11–20% of maize aboveground biomass N was transferred from legumes to maize. Soil zymography revealed high phosphatase activities in the rhizosphere of lupin and faba bean. pH imaging showed a rhizosphere alkalinization by mustard, and a rhizosphere acidification by faba bean. These changes in the companions' rhizosphere likely mobilized P and were also beneficial for maize in intercropping. Taken together, our study provides evidence that the companions' ability to mobilize N and P in the rhizosphere promotes increases in maize nutrient contents and causes maize overyielding in intercropping and thus can contribute to fertilizer savings.

Keywords: plant nitrogen acquisition, plant phosphorus acquisition, rhizosphere, nitrogen transfer, phosphatase activity, pH changes

1 Introduction

The major agricultural challenges in the next decades are to increase food production and to simultaneously reduce environmental burden of agriculture as well as its dependence on industrial fertilizers. Numerous research papers have shown that intercropping can contribute to increased nutrient acquisition by plants resulting in higher yields and improved grain nutritional and environmental quality without increased fertilizer application (Li et al. 2020, 2021; Tang et al. 2021; Xue et al. 2016). However, the underlying mechanisms that cause overyielding in intercropping are still not fully understood. In particular, the contribution of interspecific root interactions to overyielding in intercropping is still a matter of debate since previous findings are to some extent inconsistent (Duchene et al. 2017; Homulle et al. 2022).

Nitrogen (N) and phosphorus (P) are essential macronutrients that often limit plant growth in agriculture (Marschner 2012). Industrial agriculture depends on N inputs manufactured in the energy-intensive Haber–Bosch process, and on P fertilizers often obtained from limited reserves of phosphate rock (Elser and Bennett 2011; MacDonald et al. 2011; Robertson and Vitousek 2009). However, fertilizer N that is not taken up by plants can pollute groundwater and contaminate air resulting in eutrophication, soil acidification, air pollution, and global warming (Chen et al. 2019; Robertson and Vitousek 2009). Similarly, the excessive use of P fertilizers results in environmental problems such as eutrophication and in an acceleration of P resource depletion (Ashley et al. 2011; Elser and Bennett 2011).

Although intercropping has been shown to increase plant nutrient acquisition and productivity, the underlying mechanisms are still not fully understood (Duchene et al. 2017; Homulle et al. 2022; Xue et al. 2016). Previous research suggested that increases in plant nutrient acquisition in intercropping might be caused by two major ecological processes: niche complementarity and interspecific facilitation (Brooker et al. 2015; Duchene et al. 2017). Complementarity can be understood as a decreased competition between the intercropped species compared to monocultured species through differences in their spatial and temporal use of resources or the chemical form of nutrients used. By contrast, facilitation refers to beneficial interspecific interactions that increase resource availability and improve environmental conditions for both species in intercropping compared to monocropping (Duchene et al. 2017; Hinsinger et al. 2011; Homulle et al. 2022; Xue et al. 2016).

A chemical complementarity has been found, for example, for P acquisition in lupin/wheat intercropping, in which lupin preferentially used soil P mobilized by citrate, whereas wheat preferentially used water-extractable soil P, leading to the exploitation of both P pools (Cu et al. 2005). Chemical complementarity also plays a role for N acquisition, for instance, when crops differ in their preferential uptake of ammonium (NH_4^+) or nitrate (NO_3^-) (Boudsocq et al. 2012; Homulle et al. 2022). Moreover, the interspecific competition for soil N is likely decreased in intercropping since legumes are able to symbiotically fix atmospheric N_2 (Duchene et al. 2017; Hinsinger et al. 2011), which results in more reactive soil N remaining for the intercropped cereals (Duchene et al. 2017; Hinsinger et al. 2011). Such a chemical complementarity has been found in pea/barley (Hauggaard-Nielsen et al. 2009; Jensen 1996) and pea/wheat intercropping (Bedoussac and Justes 2010). Simultaneously, plant N nutrition in the form of either NO_3^-, NH_4^+, or N_2 fixation strongly affects cation-anion relationships in plants and thus rhizosphere processes (Hinsinger et al. 2003; Marschner 2012).

Besides complementarity, intercropping might also benefit from facilitation that increases the amount of available N and/or P for the main crop. Facilitation with regard to N acquisition in intercropping includes the transfer of (symbiotically fixed) N from the companion to the main crop via rhizodeposition, decomposing legume residues, and/or mycorrhizal networks (Bedoussac et al. 2015; Duchene et al. 2017; Homulle et al. 2022; Peoples et al. 2015; Thilakarathna et al. 2016). For example, faba bean has been shown to transfer symbiotically fixed N to intercropped wheat (Wahbi et al. 2016; Xiao et al. 2004). However, N transfer from legumes to cereals has mostly been demonstrated in pot experiments, while evidence for N transfer on a field scale is highly variable (Duchene et al. 2017; Homulle et al. 2022). For instance, a recent literature review found that the N transfer in mixed stands ranged from 0 to 73%, depending on species combinations and abiotic conditions (Thilakarathna et al. 2016). The N transfer can be quantified through natural variation of ^{15}N in plant dry matter among the involved plant species (most obvious in biomass $\delta^{15}N$) since legumes that symbiotically fix atmospheric N_2 tend to have a lower ^{15}N natural abundance than non-legumes that use reactive soil N (He et al. 2009; Peoples et al. 2015).

Potential facilitative mechanisms that increase P acquisition in intercropping include (i) high phosphatase activities and (ii) high proton, hydroxyl, and/or carboxylate exudation in the companions' rhizosphere, from which the main crop also benefits. High phosphatase activity can increase the availability of inorganic P in the rhizosphere since these enzymes catalyze the hydrolysis of organic P forms (Hinsinger et al. 2011; Spohn et al. 2013; Spohn and Kuzyakov 2013). In contrast, protons, hydroxyls, and carboxylates (low molecular weight organic acid anions) can mobilize P from sparingly soluble inorganic soil P pools such as calcium, iron, and aluminum phosphates (Hinsinger 2001; Hinsinger et al. 2003). Particularly legumes have been reported to exude high amounts of phosphatases, protons, and carboxylates, which can lead to P mobilization and hence might also be beneficial for the main crop. This has been demonstrated, e.g., for faba bean (Li et al. 2007, 2016), lupin (Cu et al. 2005; Dissanayaka et al. 2015), chickpea (Li et al. 2004), alfalfa (Sun et al. 2020), and cowpea (Latati et al. 2014). In addition, also *Brassicaceae* can substantially change the rhizosphere pH and exude considerable amounts of carboxylates (Marschner et al. 2007; Pearse et al. 2007; Zhang et al. 1997). However, causal relationships between root exudation and changes in P availability and P uptake in intercropping remain to be established since former findings are not conclusive (Pearse et al. 2007; Xue et al. 2016). For instance, several studies found no effect of intercropping on P uptake and plant growth of the main crop, although the companions exuded high amounts of carboxylates or changed the rhizosphere pH (e.g., Li et al. 2010; Wang et al. 2007).

Many of the mechanisms of increased nutrient (especially P) acquisition in intercropping may only occur when the intercropped species have intimate root contact, i.e., when roots intermingle (Hinsinger et al. 2011; Homulle et al. 2022), but the processes occurring in the overlapping rhizospheres of different plant species are rather poorly understood. This is in part because rhizosphere research has so far mostly concentrated on studying individual plants kept isolated in pots. Moreover, the mechanisms of nutrient acquisition substantially vary between plant species and even genotypes, for instance between legumes and non-legumes regarding N acquisition and between P-mobilizing and non-P-mobilizing plant species (Homulle et al. 2022; Li et al. 2014). Consequently, further research is needed to improve our understanding of interspecific root interactions and their effect on soil P availability, plant P uptake, and plant growth in intercropping.

In a 2-year field experiment, we found that intercropping of maize with different companions was advantageous over monocropping in terms of biomass production, grain yields, and N and P uptake of maize (Schwerdtner and Spohn 2021). Furthermore, we showed with root barriers that increased maize yields were mainly caused by interspecific root interactions of the intercropped species, particularly in legume/maize intercropping (Schwerdtner and Spohn 2021). However, the underlying mechanisms have not been explored yet. Therefore, the present study aims to examine the mechanisms of plant N and P acquisition in intercropping, which were not addressed in our previous study. For this purpose, we further explored the mentioned field experiment. In addition, we conducted a rhizobox experiment with the same soil and the same plant species as in the field experiment. The rationale behind this is that many mechanisms of N and P acquisition act only locally in the rhizosphere in close vicinity of the roots and thus can only be studied with in situ imaging techniques. The rhizobox approach allowed us to measure rhizosphere processes multiple times in the rhizosphere of the same plant using imaging techniques. In both experiments, maize (*Zea mays* L.) was the main crop and was intercropped with four companions: faba bean (*Vicia faba* L.), soy (*Glycine max* (L.) Merr.), blue lupin (*Lupinus angustifolius* L.), or white mustard (*Sinapis alba* L.). We selected contrasting companions for the experiments (i.e., legume and non-legume; fibrous roots and taproots) with potential differences in N and P acquisition (Homulle et al. 2022; Wen et al. 2019). We hypothesized that (i) the legumes complement and facilitate N acquisition of maize in legume/maize intercropping due to the legumes' ability to symbiotically fix atmospheric N_2 which is transferred to maize and (ii) the companions complement and facilitate P acquisition of maize in intercropping due to their ability to change the rhizosphere pH and to exude high amounts of phosphatases that both mobilize otherwise-unavailable P forms. To test these hypotheses, we determined the partial plant equivalent ratios (pPER) for maize biomass and maize N and P contents in both experiments. A pPER larger than 1.0 indicates increased biomass, yields, or nutrient contents, respectively, of maize plants in intercropping compared to monocropping. Furthermore, we analyzed the isotopic N signature ($\delta^{15}N$) of maize in the field experiment, and we determined phosphatase activities and rhizosphere pH by soil zymography and pH imaging in the rhizobox experiment.

2 Materials and Methods

2.1 Field Experiment

The field experiment was conducted at the University of Bayreuth (Germany) for two consecutive years from May to August in 2018 and 2019. The site is located in the southeast of Bayreuth (49°55′17″ N, 11°35′17″ E). The mean annual rainfall is 756 mm and the mean annual temperature is 8.0 °C (Lüers et al. 2014). The soil was classified as loamy sand (10%

clay, 23% silt, 67% sand). It has previously been cultivated with various crops and fertilized with compost, not with mineral fertilizers. In the upper 15 cm, the following soil chemical properties were determined: pH 6.9, 23.9 g total C kg^{-1} soil, 2.2 g total N kg^{-1} soil, and 1.3 g total P kg^{-1} soil. A total of 23.3% of the total P was organic P. Moreover, 18.3% of total P was water-soluble, 18.9% was $NaHCO_3$-soluble, 20.8% was NaOH-soluble, 25.8% was HCl-soluble, and 16.2% was residual P.

In the first year, five blocks subdivided into six plots (2.5 × 1.7 m) were cultivated in row intercropping, where maize (*Zea mays* L. cv. Damaun, ReinSaat KG, Austria) was intercropped with one of the following companions: faba bean (*Vicia faba* L. cv. Hangdown, ReinSaat KG), soy (*Glycine max* (L.) Merr. cv. Green Shell, ReinSaat KG), blue lupin (*Lupinus angustifolius* L. cv. Sonet, Templiner Kräutergarten, Germany), or white mustard (*Sinapis alba* L., ReinSaat KG) (Supplementary Fig. S1a, d, e, f, g, h). As a control, maize was also cultivated in monocropping. Each plot consisted of eight alternating rows of maize and companion with twelve plants per row having a distance of 20 cm between plants and rows (Supplementary Fig. S1c). Each species combination was replicated five times, summing up to a total of 25 plots. Before seeding, the soil was prepared by plowing, rotary tillage, and surface steaming. Surface steaming was done by inducing hot steam between the soil surface and a plastic sheet on top of the soil for 4 h. This was mostly done to kill weed seeds and avoid the application of herbicides. All seeds except mustard were soaked in water for 24 h. Soy and lupin seeds were inoculated with commercial *Bradyrhizobium sp.* inoculants before seeding (lupin: *Bradyrhizobium sp. Lupinus*, Templiner Kräutergarten; soy: LegumeFix® Soya, Legume Technology Ltd, UK). First, faba bean was sown manually on April 18th, 2018, because we expected it to grow more slowly and intended to harvest all plants at the same time. All other seeds were then sown manually 3 weeks later, on May 8th, 2018. The five blocks were surrounded by a wire netting to prevent feeding damage. As the summer 2018 was very dry, the plots were watered by hand with a watering spray lance, whenever necessary to avoid competition for water between the plants. At the end of the growing season, ten mature plants per species were harvested from the four innermost rows of each plot (Supplementary Fig. S1c). In maize monocropping, 20 maize plants were harvested per plot. In addition, five soil samples per plot were collected between rows at a soil depth of 0–15 cm and homogenized for each plot (Supplementary Fig. S1c).

In the second year, the same block design was used to cultivate maize (*Zea mays* L. cv. Golden Bantam, Bingenheimer Saatgut AG, Germany) in row intercropping with faba bean (*Vicia faba* L. cv. Hangdown, Bingenheimer), soy (*Glycine max* (L.) Merr. cv. Lica, Naturland, Germany), blue lupin (*Lupinus angustifolius* L. cv. Rumba, Templiner Kräutergarten), or white mustard (*Sinapis alba* L., Bingenheimer), or in monocropping (Supplementary Fig. S1b). Before seeding, the soil was again prepared by plowing, rotary tillage, and surface steaming. All seeds were simultaneously sown by hand on May 8th and 9th, 2019. All seeds except for mustard were soaked in water for 24 h prior to sowing. As the summer 2019 was also very dry, the plots were regularly watered. At the end of the growing season, five mature plants per species and plot were harvested (ten plants in maize monocropping).

For the present study, dried and milled subsamples of maize leaves, shoots, and grains were analyzed for the isotopic N signature ($\delta^{15}N$) as described below. Moreover, pPER of maize aboveground biomass (AGB), maize grain yields, and maize AGB N and P contents on a single plant basis were calculated (see below). Soil samples collected in 2018 were analyzed for soil pH, phosphatase activity, and water-extractable N (NO_3^- and NH_4^+) as described below.

2.2 Rhizobox Experiment

Soil for the rhizobox experiment was collected directly next to the field experiment in March 2018. The soil was sieved (< 2 mm); plant residues were removed with tweezers. The soil was filled into rhizoboxes made of PVC with an inner size of 49.2 × 29.3 × 3.0 cm to a final bulk density of 0.8 g cm^{-3} similar as in Hofmann et al. (2016). Directly after filling the soil into the rhizoboxes, soil subsamples were dried, milled, and analyzed for element concentrations and soil pH (see below). Prior to sowing, soil water content was adjusted to 50% water holding capacity (WHC). In each box, two plants were sown at a distance of 15 cm. One plant was maize (*Zea mays* L. cv. Damaun, ReinSaat KG); the other plant was one of the following companions that were also used in the field experiment: faba bean (*Vicia faba* L. cv. Hangdown, ReinSaat KG), blue lupin (*Lupinus angustifolius* L. cv. Sonet, Templiner Kräutergarten), or white mustard (*Sinapis alba* L., ReinSaat KG). Soy (*Glycine max* (L.) Merr.) failed in the rhizobox experiment shortly before harvest, probably due to pest infestation. As a control, two maize plants were sown together. Each species combination was replicated five times, resulting in a total of 20 rhizoboxes (neglecting the rhizoboxes with soy). All seeds except mustard were soaked in water for 24 h, and lupin (and soy) were inoculated with commercial *Bradyrhizobium sp.* inoculants before seeding, as in the field experiment. The rhizoboxes were placed in a greenhouse and inclined by 50° on wooden racks (Supplementary Fig. S1i) to make the roots grow at the bottom wall of the rhizoboxes (Supplementary Fig. S1j). The rhizoboxes were placed in a randomized block design in the greenhouse and re-randomized after 6 weeks. The rhizoboxes were watered every 2 days with tap water to 50% WHC as measured by weight. The plants were sown in April 2018 and harvested after 12 weeks in July 2018. The greenhouse was continuously shaded by a net, and windows opened automatically when temperatures were above 20 °C. No further climate control was performed.

Six and nine weeks after sowing, pH imaging and soil zymography were performed to determine the spatial and temporal distribution of pH and phosphatase activity as described below. Both analyses were conducted at a soil depth of 17 to 26 cm (from the top, box-centered; Supplementary Fig. S2). Plants were harvested 12 weeks after sowing and analyzed for biomass production, and N and P concentrations. For this purpose, AGB was dried at 60 °C, weighed, and milled. Belowground biomass (BGB) was sampled and separated per plant species. BGB not assignable to one plant species was collected as mixed BGB. All BGB was washed with deionized water, dried at 60 °C, weighed, and milled. In addition, soil was sampled from the area of previous imaging analyses at a soil depth of 17 to 26 cm and equally split into three samples, one dominated by roots of maize (left side of the box), one by roots of the companion (right side of the box), and one by roots of both (middle of the box; referred to as “mixed”; Supplementary Fig. S2). Soil samples were analyzed for water-extractable N (NO_3^- and NH_4^+).

2.2.1 pH imaging

The distribution of pH in the rhizosphere was analyzed in situ by pH imaging, following Marschner and Römheld (1983) with modifications. The pH indicator bromocresol purple (Sigma-Aldrich, Merck KGaA, Germany) was dissolved in deionized water (0.6%). NaOH was added dropwise for better dissolution as described by Nkebiwe et al. (2016). The day before analysis, a boiled agar solution (1.3% agarose; Sigma-Aldrich) was mixed with the pH indicator solution (final pH indicator concentration of 0.006%), adjusted to soil pH with NaOH, and cast in glass systems usually used for gel electrophoresis with an inner size of 24.5 × 18.5 × 0.1 cm. Gels were plastic-wrapped to prevent drying and stored overnight in the 20 °C climate chamber where the analyses took place to allow acclimatization. Rhizoboxes were transferred to the climate chamber 1 h before analyses to allow acclimatization of the soil. After removing the bottom wall of the rhizoboxes, the exposed plant roots were photographed (Supplementary Fig. S1j). The pH indicator gels were cut into two pieces, each with a size of 9 × 24 cm. Each gel was attached to the soil surface of one rhizobox at a soil depth of 17 to 26 cm (from the top, box-centered) and covered with a plastic sheet. After 12 min of incubation in the dark at 20 °C, gels were removed from the soil surface, cut into two pieces, washed carefully with deionized water to remove adhering soil particles, and photographed with a digital camera (D60, Nikon) in front of a white background (Supplementary Fig. S2). For the quantitative image analysis, the two photographs of one gel were merged again using the software GIMP (version 2.10.18).

For calibration, the agar-indicator solution was adjusted to different pH values (4.5, 5.5, 6.0, 6.5, and 7.5), cast in the same glass systems as before, and stored overnight before being photographed. The color channels of each photograph were split, and the green channel was used for analyses resulting in a linear correlation between the different pH values and the corresponding gray values.

2.2.2 Soil zymography

Directly after pH imaging, the distribution of phosphatase activity was measured in situ by soil zymography following Spohn and Kuzyakov (2013) with modifications. No agarose gels were used as in Holz et al. (2019) as the soil had a low organic matter content and thus, the gel, which is thought to protect the membrane from staining with organic material, was not required. The substrate 4-methylumbelliferyl phosphate (Sigma-Aldrich) was dissolved in deionized water to a concentration of 2 mM. Membrane filters of nylon (0.45 µm pore size; Nantong FilterBio Membrane Co. Ltd., China) with a size of 9 × 28 cm were coated with this solution. The membranes were allowed to dry flat for 1 min at room temperature (20 °C) on aluminum foil, before being attached to the soil surface. The studied soil area in the rhizoboxes was the same as for the pH imaging. After 30 min of incubation at 20 °C in the dark, the membrane was removed from the soil surface, cut into three equal pieces, and each piece was photographed with a digital camera (D60, Nikon) on an epi-UV-desk (Desaga, Germany) at 366 nm wavelength (Supplementary Fig. S2). The cutting was done to ensure equal distribution of UV light all over the zymogram. For the quantitative image analysis, the three photographs of one zymogram were merged again using the software GIMP (version 2.10.18).

For calibration, membranes were soaked in 4-methylumbelliferone (MUF; Sigma-Aldrich) of different concentrations (0, 25, 75, 125, 200 µM). The membranes were also allowed to dry for 1 min and then photographed as described for the zymograms. Phosphatase activity was calculated based on a linear correlation between the different MUF concentrations and the corresponding gray values of the images (Spohn and Kuzyakov 2013).

2.2.3 Quantitative Image Analyses

All images were analyzed using the open-source software ImageJ (version 1.52a; Rasband 2018). For the pH images, color channels were split and only the green channel image was used for further analyses, because color (pH) changes were most pronounced here. Background (soil) values were gathered in six areas of 250 × 250 pixels per image, in which no roots were visible. Rhizosphere values were gathered in three areas of 50 × 50 pixels per plant species using areas with maximum pH changes. The corresponding pH values were calculated based on the calibration line and the means of the three measured areas. Rhizosphere pH changes were calculated separately for maize and companions as the difference between rhizosphere pH and soil pH.

For the image analyses of the zymograms, the photographs were converted into 8-bit (grayscale) images. Background (soil) values were gathered in six areas of 150 × 150 pixels per image, in which no roots were visible. Rhizosphere values were gathered in three areas of 15 × 15 pixels per species using areas with maximum grayscale values. The corresponding phosphatase activities were calculated based on the calibration line, the incubation time, and the means of the three measured areas. Phosphatase activities were calculated separately for maize and companions as the difference between rhizosphere and bulk soil.

2.3 Biomass Analyses and Calculations

Dried and milled maize AGB and BGB samples of the rhizobox experiment were analyzed for the total N concentration with an element analyzer (Vario Max, Elementar, Germany) and for total P concentration after pressure digestion in concentrated nitric acid with an inductively coupled plasma-optical emission spectroscopy (ICP-OES; Vista-Pro radial, Varian Inc., USA). Maize N and P contents per plant in AGB and BGB were calculated by multiplying the dry mass of AGB and BGB with the corresponding N and P concentrations. Total maize biomass was calculated as the sum of maize AGB and BGB, and total maize element contents were calculated as the sum of the AGB and BGB element contents.

The pPER of maize AGB and grain yields as well as maize AGB N and P contents were calculated for the field experiment, as follows:

$$\mathrm{pPER\ (X)} = \frac{\mathrm{X_{intercropping}\ [g{\cdot}plant^{-1}]}}{\mathrm{mean\ X_{monocropping}\ [g{\cdot}plant^{-1}]}} \qquad (1)$$

where X is either maize AGB, maize grain yield, or maize AGB N or P content. Similarly, pPER of maize AGB, BGB, and total biomass, as well as the respective maize N and P contents, were calculated for the rhizobox experiment. As stated above, a pPER larger than 1.0 indicates increased biomass, yields, or nutrient contents, respectively, of maize plants in intercropping compared to monocropping.

Milled subsamples of dried maize leaves, shoots, and grains from the field experiment were used to determine maize $\delta^{15}N$ using an EA-IRMS coupling (Element analyzer: NA 1108, CE Instruments, Italy; Interface: ConFlo III, Finnigan MAT, Germany; Isotope ratio mass spectrometer: delta S, Finnigan MAT). The $\delta^{15}N$ values of maize leaves, shoots, and grains were used to calculate the $\delta^{15}N$ of the maize AGB based on the respective N concentrations, following He et al. (2009):

$$\delta^{15}\mathrm{N}_{\mathrm{maize\ AGB}} = \frac{\left(\delta^{15}\mathrm{N}_{\mathrm{leaves}} \cdot \mathrm{N}_{\mathrm{leaves}} + \delta^{15}\mathrm{N}_{\mathrm{shoots}} \cdot \mathrm{N}_{\mathrm{shoots}} + \delta^{15}\mathrm{N}_{\mathrm{grains}} \cdot \mathrm{N}_{\mathrm{grains}}\right)}{\left(\mathrm{N}_{\mathrm{leaves}} + \mathrm{N}_{\mathrm{shoots}} + \mathrm{N}_{\mathrm{grains}}\right)} \quad (2).$$

The proportion of maize N transferred from legumes (PN_{legume}) in the field experiment was determined according to Peoples et al. (2015), as follows:

$$\mathrm{PN}_{\mathrm{legume}}[\%] = \left(1 - \frac{\delta^{15}\mathrm{N}_{\mathrm{maize\ AGB\ (intercropping)}}}{\mathrm{mean}\ \delta^{15}\mathrm{N}_{\mathrm{maize\ AGB\ (monocropping)}}}\right) \cdot 100 \quad (3).$$

The legume-derived N content in maize AGB was calculated based on PN_{legume}, as follows:

$$\mathrm{maize\ N}_{\mathrm{legume}}\ [\mathrm{mg} \cdot \mathrm{plant}^{-1}] = \frac{\mathrm{PN}_{\mathrm{legume}}}{100\%} \cdot \mathrm{maize\ AGB\ N\ content}\ [\mathrm{mg} \cdot \mathrm{plant}^{-1}] \quad (4).$$

2.4 Soil Analyses

2.4.1 Element Concentrations

Dried and milled soil subsamples of the rhizobox experiment were analyzed for the total N using an element analyzer (Vario Max, Elementar) and for the total P by ICP-OES after pressure digestion in *aqua regia*. Soil P fractions were determined by Hedley fractionation (Hedley et al. 1982) modified by Tiessen and Moir (2007). In brief, 0.5 g of dried and milled soil samples were shaken in 30 ml deionized water for 16 h on an overhead shaker and centrifuged at 4100 × g for 15 min. Inorganic P in water extracts was measured colorimetrically by a multiplate reader (Infinite® 200 PRO, Tecan Trading AG, Switzerland), using the molybdenum blue method (Murphy and Riley 1962). The remaining soil was subsequently extracted in 30 ml 0.5 M $NaHCO_3$, followed by an extraction with 30 ml 0.1 M NaOH and 30 ml 1 M HCl. The total P of $NaHCO_3$, NaOH, and HCl extracts was determined using ICP-OES. Residual P was measured after pressure digestion in *aqua regia*, as described above. In addition, the total organic P was determined by the ignition method according to Saunders and Williams (1955) modified by Walker and Adams (1958). In brief, an aliquot of the dried soil samples was ignited at 550 °C in a muffle furnace. Both ignited and non-ignited aliquots were extracted in 0.5 M H_2SO_4 for 16 h on a horizontal shaker followed by centrifugation at 1500 × g for 15 min. Inorganic P in the extracts was determined by the molybdenum blue method (Murphy and Riley 1962) using an UV–VIS spectrophotometer (UV-1800, Shimadzu Corporation, Japan). Total organic P was calculated as the difference between ignited and non-ignited samples.

2.4.2 Soil pH

Soil pH was measured in soil subsamples in a ratio (w/v) of 1:2.5 in deionized water using a pH electrode (WTW SenTix 51, Xylem Analytics Germany Sales GmbH & Co. KG, Germany).

2.4.3 Phosphatase Activity

Phosphatase activity in fresh soil samples was measured directly after harvest of the field experiment using the fluorogenic substrate 4-methylumbelliferyl phosphate (Sigma-Aldrich) following Marx et al. (2001), German et al. (2011), and Herold et al. (2014). In brief, 1 g of fresh soil and 50 ml of sterile deionized water were weighed into a sterilized beaker. The sample was homogenized on an overhead shaker for 20 min. The soil homogenates (50 µl) were pipetted into black polystyrene 96-well microplates (Brand GmbH & Co. KG, Germany) having four replicates. Sterile deionized water (50 µl) and substrate solution (100 µl) were added to the soil homogenates. Microplates were covered and pre-incubated in the dark at 15 °C for 30 min and measured fluorometrically after 0, 60, and 180 min with 360-nm excitation and 460-nm emission filters (Herold et al. 2014) by a microplate reader (Infinite® 200 PRO, Tecan). Between measurements, microplates were incubated in the dark at 15 °C. Phosphatase activities were calculated according to German et al. (2011) modified by Widdig et al. (2019). Fluorescence values were corrected for soil quenching, homogenate fluorescence, and substrate fluorescence.

2.4.4 Water-Extractable N

NO_3^--N and NH_4^+-N were determined as described in Schleuss et al. (2019). In brief, 20 g dry-mass equivalents of soil subsamples were extracted in 80 ml deionized water by shaking for 1 h on an overhead shaker. The extracts were passed through 0.45 µm cellulose acetate filters by means of an underpressure filtration device and subsequently analyzed for NO_3^- by ion chromatography (Metrohm 881 Compact IC pro, Metrohm AG, Switzerland) and for NH_4^+ by flow injection analysis (FIA-LAB, MLE GmbH, Germany).

2.5 Statistical analyses

Data were tested separately for significant differences among species combinations. Prior to all statistical analyses, normality was checked with Shapiro–Wilk normality tests, and homogeneity of variances was tested with Levene’s tests. Where normality and homogeneity assumptions were met, analyses of variance (ANOVA) followed by Tukey’s post-hoc test were used to identify significant differences among species combinations. Where normality and homogeneity assumptions were not met, Kruskal–Wallis tests followed by post-hoc tests using the criterium Fisher’s least significant difference and Holm correction for *p* adjustment were conducted to identify significant differences. In addition, the pPER of both experiments were tested separately for significant differences from 1.0 using ANOVA (or Kruskal–Wallis tests). All statistical analyses were performed in R (version 3.5.2; R Core Team 2018) using the packages agricolae (version 1.3–2; Mendiburu 2020), car (version 3.0–7; Fox and Weisberg 2019), dplyr (version 0.8.5; Wickham et al. 2020), and ggplot2 (version 3.3.0; Wickham 2016).

3 Results

3.1 Biomass Production

In the field experiment, the pPER of single maize plants' AGB was significantly larger than 1.0 in soy/maize and lupin/maize intercropping in both years ($p \leq 0.002$), and in faba bean/maize intercropping in 2019 ($p < 0.001$). It was also slightly larger than 1.0 in mustard/maize intercropping in both years ($p \leq 0.069$, respectively; Fig. 1a). The pPER of maize grain yields was significantly larger than 1.0 in soy/maize (both years; $p \leq 0.007$) and lupin/maize intercropping (2018; $p = 0.003$). It was also slightly larger than 1.0 in mustard/maize (2018; $p = 0.051$) and lupin/maize intercropping (2019; $p = 0.073$; Fig. 1b).

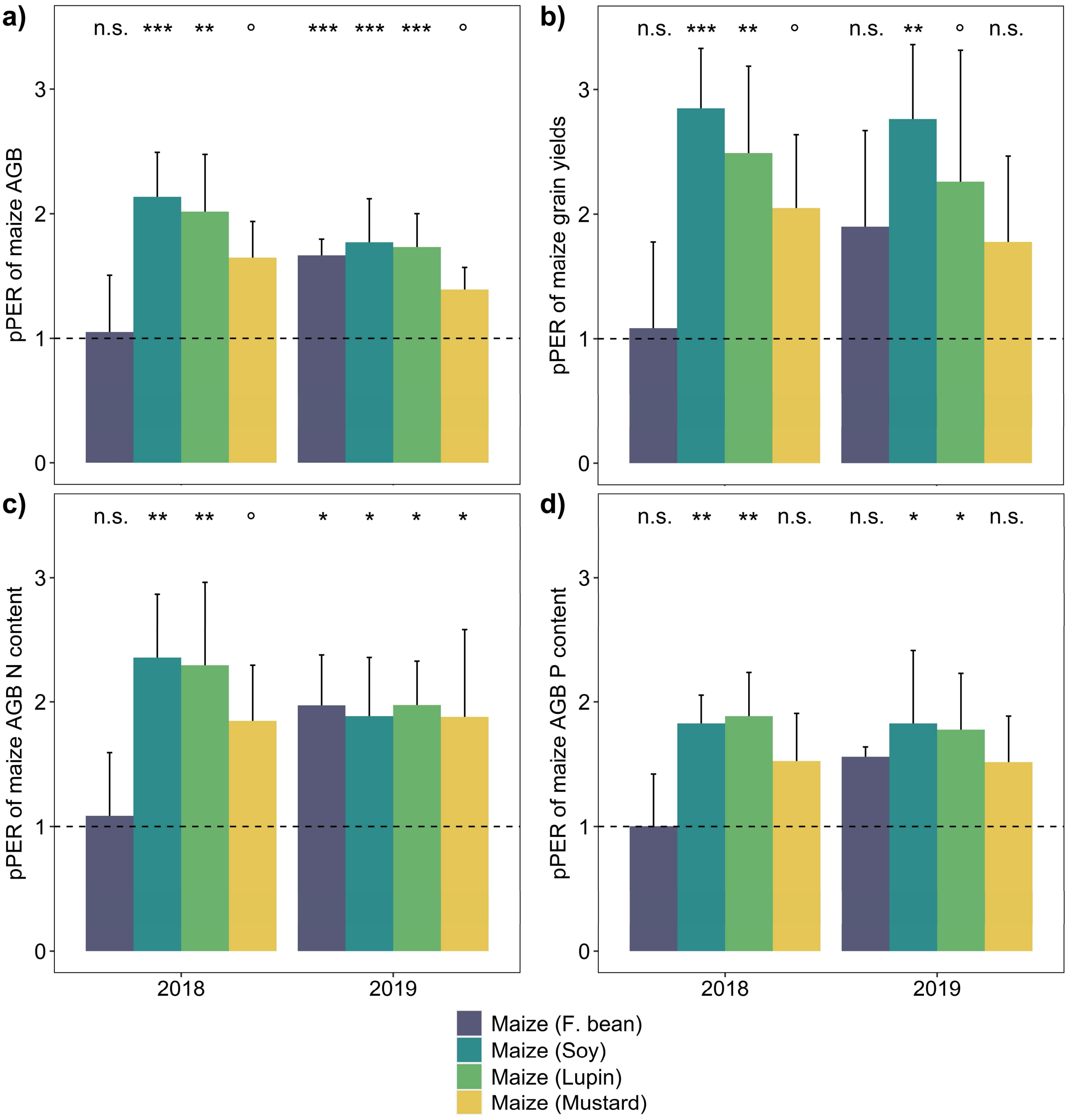

Fig. 1 Partial plant equivalent ratios (pPER) of **a** maize aboveground biomass (AGB), **b** maize grain yields, **c** maize AGB N contents, and **d** maize AGB P contents, determined at harvest of the field experiment in 2018 and 2019. Columns show means, and error bars indicate standard deviations. Symbols indicate that pPER were significantly different from 1.00 (°$p < 0.1$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$), tested separately for each panel and year. "n.s." indicates that pPER was not significantly different from 1.00 (indicated by dashed line; equal to maize monocropping)

In the rhizobox experiment, maize plants had a mean AGB of 14.5 ± 6.1 g plant^{-1} and a mean BGB of 1.6 ± 0.6 g plant^{-1} amounting to a total biomass of 16.1 ± 6.6 g plant^{-1} averaged across all four species combinations without significant differences among them (Table 1). The pPER of maize AGB, maize BGB, and maize total biomass were 1.4, 1.0, and 1.4, respectively, averaged across the three species combinations. They were not significantly different from 1.0 (Fig. 2; Supplementary Table S1).

Table 1 Productivity of maize (grown together with the three companions) in terms of aboveground (AGB), belowground (BGB), and total biomass, as well as N and P concentrations of maize AGB and BGB, and N and P contents of maize total biomass determined 12 weeks after sowing in the rhizobox experiment

	Maize (maize)	**Maize (F. bean)**	**Maize (lupin)**	**Maize (mustard)**
AGB [g plant^{-1}]	10.83 ± 1.41	15.52 ± 6.75	16.94 ± 5.90	14.62 ± 8.45
BGB [g plant^{-1}]	1.62 ± 0.43	1.53 ± 0.66	1.81 ± 0.56	1.37 ± 0.69
Total biomass [g plant^{-1}]	12.44 ± 1.78	17.05 ± 7.34	18.75 ± 6.44	15.99 ± 9.12
AGB N concentration [mg g^{-1}]	7.18 ± 1.41	11.50 ± 5.18	10.75 ± 6.07	7.95 ± 2.04
BGB N concentration [mg g^{-1}]	7.04 ± 1.52	8.11 ± 1.02	8.89 ± 3.23	6.93 ± 0.65
Total N content [mg plant^{-1}]	88.91 ± 19.63^{b}	168.45 ± 32.04^{a}	170.97 ± 33.73^{a}	114.68 ± 50.57^{ab}
AGB P concentration [mg g^{-1}]	3.76 ± 0.79	3.85 ± 0.63	3.64 ± 0.70	3.65 ± 1.00
BGB P concentration [mg g^{-1}]	3.19 ± 1.01	2.67 ± 0.30	2.81 ± 0.68	2.68 ± 0.50
Total P content [mg plant^{-1}]	45.67 ± 11.21	60.71 ± 17.53	63.49 ± 15.24	50.22 ± 20.98

Numbers show means ± standard deviations (*n* = 5). Different lowercase letters indicate significant differences ($p < 0.05$) among the species combinations, tested separately for each row. The absence of letters indicates that there was no significant difference

In the rhizobox experiment, the companions had lower AGB, BGB, and total biomass than the maize plants (Tables 1 and 2). Faba bean had a significantly higher BGB than lupin ($p = 0.010$) and mustard ($p = 0.030$). BGB not assignable to one species (mixed BGB) accounted for 0.9 to 1.1 g per rhizobox (Table 2).

Table 2 Productivity of companions (grown together with maize) in terms of aboveground (AGB), belowground (BGB), and total biomass, determined 12 weeks after sowing in the rhizobox experiment. BGB not assignable to one species is included as mixed BGB

	Faba bean	**Lupin**	**Mustard**
Companion AGB [g plant^{-1}]	2.43 ± 1.36	0.78 ± 0.52	3.16 ± 2.54
Companion BGB [g plant^{-1}]	0.43 ± 0.21^{a}	0.10 ± 0.08^{b}	0.16 ± 0.13^{b}
Companion total biomass [g plant^{-1}]	2.86 ± 1.56	0.88 ± 0.60	3.32 ± 2.66
Mixed BGB [g rhizobox^{-1}]	1.14 ± 0.64	0.94 ± 0.48	0.92 ± 0.41

Numbers show means ± standard deviations (*n* = 5). Different lowercase letters indicate significant differences ($p < 0.05$) among the species combinations, tested separately for each row. The absence of letters indicates that there was no significant difference

3.2 Maize N and P Contents

In the field experiment, the pPER of maize AGB N and P contents were significantly larger than 1.0 in soy/maize and lupin/maize intercropping in both years ($p \leq 0.039$; Fig. 1c–d). The pPER of maize AGB N content was also significantly larger than 1.0 in faba bean/maize and mustard/maize intercropping in 2019 ($p \leq 0.040$), and slightly larger than 1.0 in mustard/maize intercropping in 2018 ($p = 0.077$; Fig. 1c). Furthermore, the pPER of maize AGB P content tended to be larger than 1.0 in in faba bean/maize (2019; $p = 0.186$) and mustard/maize intercropping (both years; $p = 0.104$ in 2018 and $p = 0.186$ in 2019; Fig. 1d). The pPER of maize AGB N contents were higher than the pPER of maize AGB P contents in all species combinations and both years (Fig. 1c–d).

In the rhizobox experiment, maize total biomass N content was significantly increased by a factor of 1.9 in faba bean/maize and lupin/maize intercropping compared to maize monocropping ($p \leq 0.014$), while there was no significant difference in N and P concentrations and P contents among the species combinations (Table 1). The pPER of maize AGB N content and maize total biomass N content were significantly larger than 1.0 in faba bean/maize and lupin/maize intercropping ($p \leq 0.010$; Fig. 2; Supplementary Table S1). We found no significant differences in the pPER of maize AGB P content, maize BGB P content, and maize total biomass P content among the species combinations (Fig. 2; Supplementary Table S1).

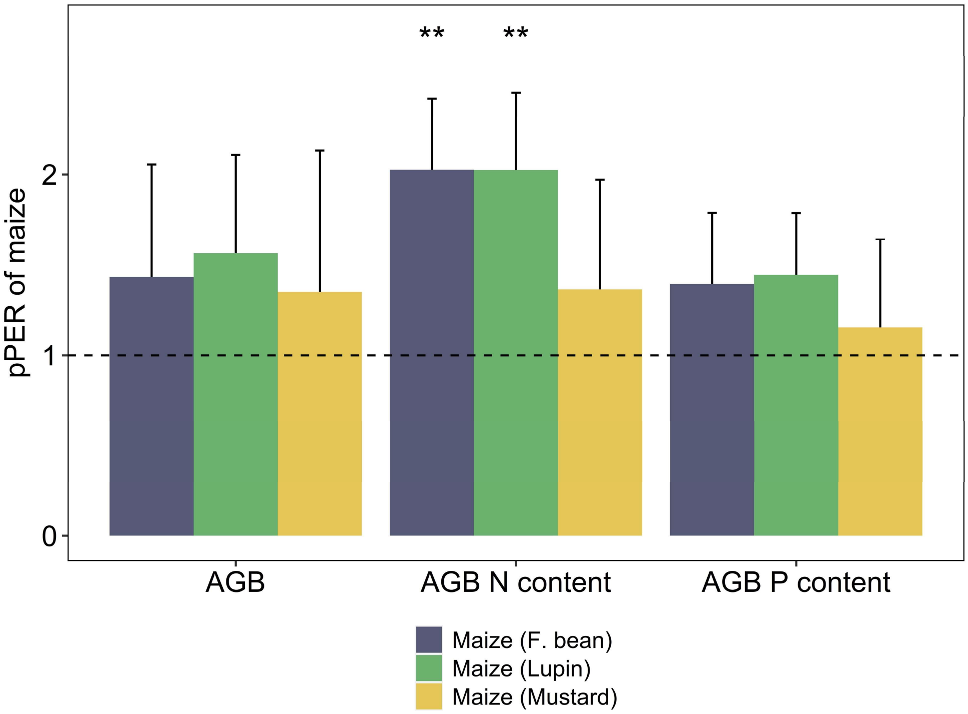

Fig. 2 Partial plant equivalent ratios (pPER) of maize aboveground biomass (AGB), maize AGB N content, and maize AGB P content, determined 12 weeks after sowing in the rhizobox experiment. Columns show means, and error bars indicate standard deviations. Symbols indicate that pPER were significantly different from 1.00 (**$p < 0.01$), tested separately for each pPER. The absence of symbols indicates that pPER were not significantly different from 1.00 (indicated by dashed line; equal to maize monocropping)

3.3 Isotopic N Signatures and N Transfer

In the first year of the field experiment, the $\delta^{15}N$ of maize AGB was significantly decreased in faba bean/maize intercropping compared to maize monocropping ($p = 0.032$; Fig. 3a). Furthermore, $\delta^{15}N$ of maize AGB tended to be lower in soy/maize and lupin/maize intercropping compared to maize monocropping, but this was not statistically significant ($p = 0.448$ and $p = 0.158$, respectively; Fig. 3a). The proportion of maize N transferred from legumes

(PN_{legume}) was 20.3 ± 10.9, 15.2 ± 5.3, and 10.9 ± 9.5% in faba bean/maize, lupin/maize, and soy/maize intercropping, respectively. The legume-derived maize N content was highest in lupin/maize intercropping (Fig. 3b). Maize AGB $\delta^{15}N$ was generally lower in 2019 than in 2018, particularly in maize monocropping, in which $\delta^{15}N$ was significantly decreased by a factor of 0.8 in 2019 compared to 2018 (p = 0.014). No significant difference in maize AGB $\delta^{15}N$ was found among the species combinations in 2019 (Supplementary Fig. S3).

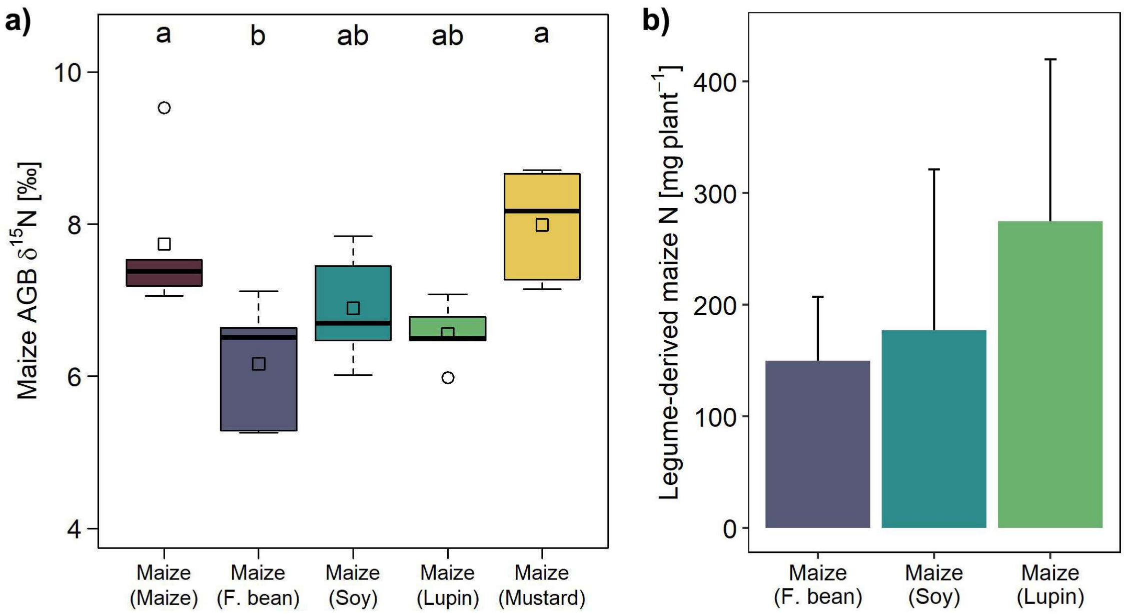

Fig. 3 $\delta^{15}N$ values of maize aboveground biomass (AGB) **a** and legume-derived N content of maize AGB **b**, determined at harvest of the field experiment in 2018. Squares in **a** show means, columns in **b** show means, and error bars in **b** indicate standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among the species combinations, tested separately for each panel. The absence of letters indicates that there was no significant difference

3.4 pH changes

In the field experiment, soil pH was, on average, 7.0 ± 0.1 across all species combinations showing no significant differences among them (Supplementary Table S2).

In the rhizobox experiment, faba bean strongly decreased the pH in the rhizosphere by more than one pH unit compared to the bulk soil 6 and 9 weeks after sowing (Fig. 4). Faba bean acidified the rhizosphere significantly more than maize in faba bean/maize intercropping after 6 weeks (p = 0.023), and slightly more after nine weeks (p = 0.078; Fig. 4). In contrast, mustard increased the rhizosphere pH by 0.7 pH units compared to the bulk soil 6 and 9 weeks after sowing. The changes in rhizosphere pH differed significantly between mustard and maize in mustard/maize intercropping 6 and 9 weeks after sowing ($p < 0.001$; Fig. 4). Maize generally decreased the rhizosphere pH compared to the bulk soil, particularly 9 weeks after sowing. Maize acidified the rhizosphere significantly more than lupin in lupin/maize intercropping 9 weeks after sowing (p = 0.016; Fig. 4b).

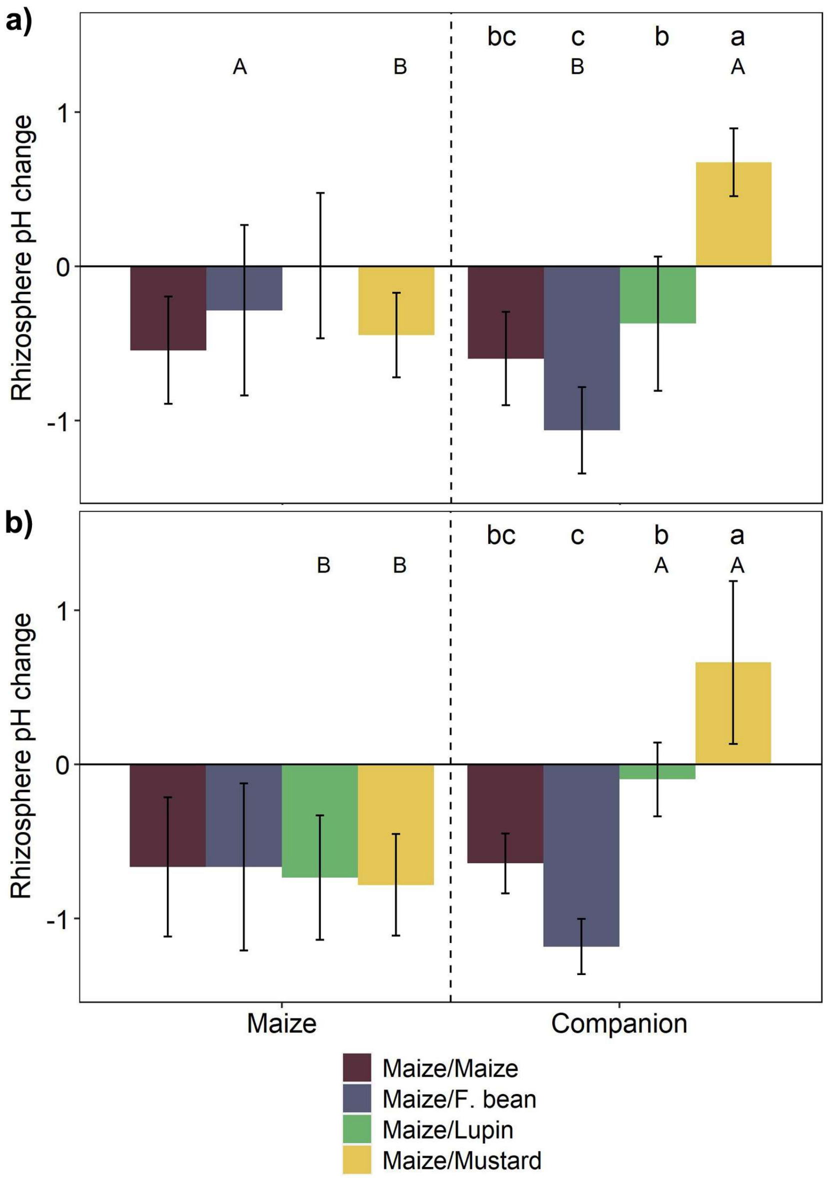

Fig. 4 Rhizosphere pH changes of maize and companions relative to the bulk soil, determined 6 (**a**) and 9 (**b**) weeks after sowing in the rhizobox experiment. The zero line corresponds to soil pH of 6.9. Columns show means, and error bars indicate standard deviations. Different lowercase letters indicate significant differences (p < 0.05) among the species combinations, tested separately for maize and companion. Different capital letters indicate significant differences (p < 0.05) between maize and companion, tested separately for each species combination. The absence of letters indicates that there was no significant difference

3.5 Phosphatase Activity

In the field experiment, soil phosphatase activity was, on average, 3.0 ± 1.3 nmol g soil^{-1} h^{-1} across all species combinations showing no significant differences among them (Supplementary Table S2).

In the rhizobox experiment, faba bean and lupin showed significantly higher phosphatase activities in the rhizosphere than mustard, both 6 and 9 weeks after sowing ($p \leq 0.045$; Fig. 5). Phosphatase activities in the rhizosphere of lupin after 6 weeks and of faba bean after 9 weeks were significantly higher than of intercropped maize ($p \leq 0.005$), while phosphatase activity in the rhizosphere of mustard was significantly lower than in the rhizosphere of intercropped maize during both analyses ($p \leq 0.043$). Phosphatase activity in the rhizosphere of faba bean after 6 weeks was also slightly higher than in the rhizosphere of intercropped maize ($p = 0.092$; Fig. 5). No significant difference in the phosphatase activity in the rhizosphere of maize was found among the species combinations both 6 and 9 weeks after sowing (Fig. 5).

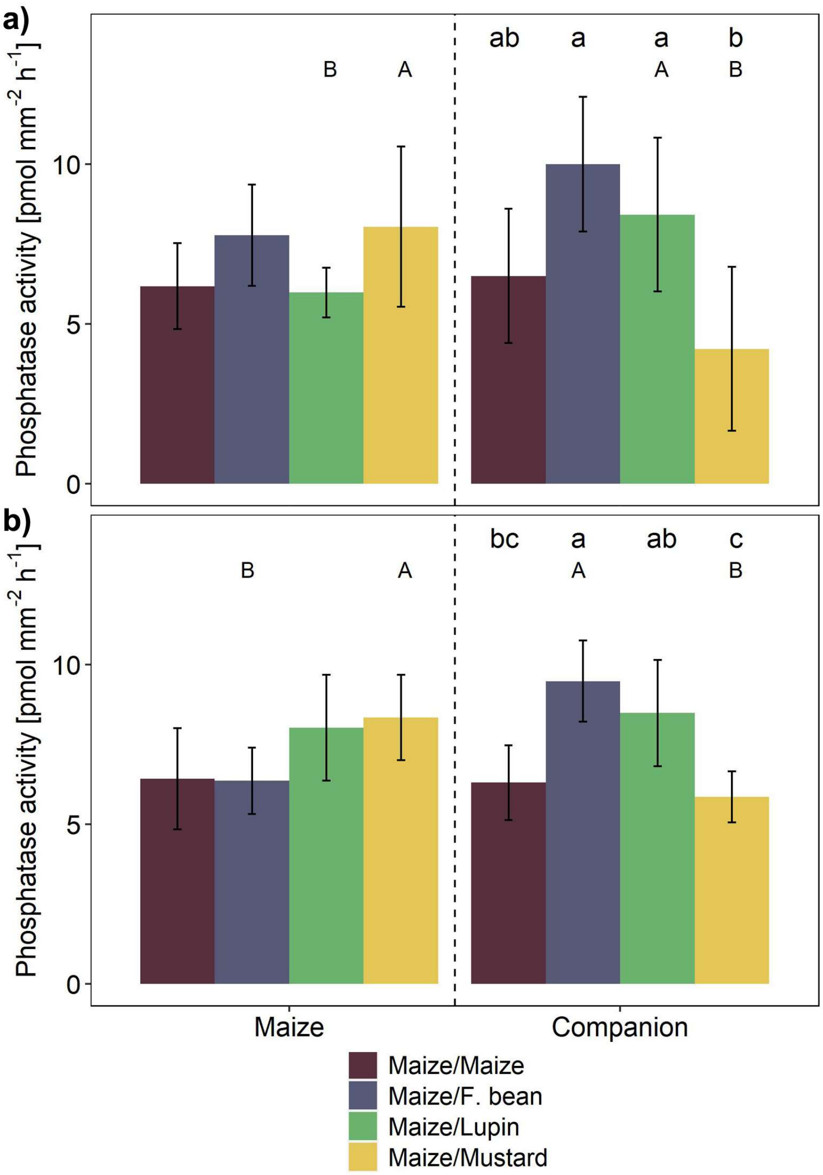

Fig. 5 Phosphatase activities of maize and companions relative to the bulk soil, determined 6 (**a**) and 9 (**b**) weeks after sowing in the rhizobox experiment. Columns show means, and error bars indicate standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among the species combinations, tested separately for maize and companion. Different capital letters indicate significant differences ($p < 0.05$) between maize and companion, tested separately for each species combination. The absence of letters indicates that there was no significant difference

3.6 Water-Extractable Soil N

In the field experiment, water-extractable NO_3^--N was, on average, 3.8 ± 1.1 µg N g soil^{-1} across all species combinations, with no significant difference among them (Supplementary Table S2).

In the rhizobox experiment, water-extractable NO_3^--N was significantly higher in lupin/maize intercropping than in maize monocropping, both in the maize-dominated and the mixed soil area ($p \leq 0.037$; Supplementary Fig. S4). No significant difference among the species combinations was found in the companion-dominated soil area, although NO_3^--N tended to be higher in the rhizosphere of lupin ($p = 0.174$) and mustard ($p = 0.187$) than of maize (Supplementary Fig. S4).

NO_3^--N was higher in the field than in the rhizobox experiment (Supplementary Table S2; Fig. S4). In both experiments, water-extractable NH_4^+-N was near the detection limit and hence negligible in all species combinations and soil areas (data not shown).

4 Discussion

We found indications of complementarity and facilitation in N and P acquisition, which were likely the reason for the increased nutrient uptake and biomass production of intercropped maize, especially when grown together with soy and lupin in the field. The mechanisms of N acquisition were mostly associated with N transfer from legumes to maize. The mechanisms of P acquisition were associated with high phosphatase activities and micro-scale pH changes in the immediate vicinity of (intermingled) roots.

In the field experiment, legumes symbiotically fixed atmospheric N_2, of which a part was transferred to the maize plants, as indicated by the decreased maize $\delta^{15}N$. The N_2 fixation by legumes might have reduced the competition for soil N in legume/maize intercropping compared to maize monocropping through chemical complementarity. Such a chemical complementarity between cereals using mostly reactive soil N and legumes using mostly atmospheric N_2 has also been found in pea/barley (Hauggaard-Nielsen et al. 2009; Jensen 1996) and pea/wheat intercropping (Bedoussac and Justes 2010). In addition, a part of the symbiotically fixed N was transferred from the legumes to the maize plants, likely through (1) rhizodeposition from legumes, (2) transport via mycorrhizal hyphae, and/or (3) decomposition of legume nodules and roots and mineralization of their organic N (Bedoussac et al. 2015; Hupe et al. 2021; Peoples et al. 2015; Thilakarathna et al. 2016), thus facilitating maize N acquisition in legume/maize intercropping. Our findings are in accordance with previous studies reporting N transfer from legumes to non-legumes that was found particularly in pot experiments with lupin/rapeseed, pea/barley, soy/maize, and faba bean/wheat intercropping (Génard et al. 2016; Johansen and Jensen 1996; Meng et al. 2015; Xiao et al. 2004). However, few studies found evidence for N transfer in a field experiment using the ^{15}N natural abundance method (Duchene et al. 2017; He et al. 2009). Furthermore, only few studies have so far shown such a high proportion of legume-derived maize N as we found here. For instance, 11% and 13% of cereal N were derived from legumes in pea/barley and faba bean/wheat intercropping, respectively (Chapagain and Riseman 2014; 2015). The lack of N transfer in the second year of our field experiment might be due to soil mixing during field preparation in autumn 2018 (first year) and spring 2019 (second year). This might have decreased the soil $\delta^{15}N$ in the non-leguminous plots due to legume roots decomposing in the soil over winter resulting in the observed, significantly lower $\delta^{15}N$ of maize AGB in maize monocropping in 2019 than in 2018.

Overall, the legumes' ability to symbiotically fix atmospheric N_2 and the transfer of a part of this N to the maize plants are likely the reason for the pPER of maize AGB N content being generally larger than 1.0 in legume/maize intercropping in both experiments.

Nearly a quarter of total P in the soil used in both experiments was present in organic forms, which is not directly available to plants. However, faba bean and lupin likely mobilized P from the organic P pool through high phosphatase activities in their rhizosphere (relative to the bulk soil and the rhizosphere of maize and mustard). Moreover, the legumes likely exuded further organic compounds, such as for example sugars, into the soil, which stimulated the release of phosphatases by microorganisms (Duchene et al. 2017; Richardson et al. 2011; Spohn et al. 2013). The hydrolysis of organic P by legumes (and associated microorganisms) might result in P complementarity in intercropping if legumes and maize use different P forms. Legumes might have taken up the mineralized organic P, while maize might have taken up water-soluble P, which made up 18% of the total P in our soil. Previous studies showed such a complementary use of different P forms between intercropped species in lupin/wheat, chickpea/wheat, chickpea/maize, and common bean/durum wheat intercropping (Cu et al. 2005; Li et al. 2003, 2004, 2008). In addition, the hydrolysis of organic P by legumes might result in P facilitation in intercropping if maize takes up the P mobilized by the legumes' phosphatase release (Duchene et al. 2017; Xue et al. 2016). Different cereals have been suggested to benefit from enhanced phosphatase activities of companions as has been reported, for instance, for lupin/maize (Dissanayaka et al. 2015), faba bean/maize (Zhang et al. 2016), faba bean/barley (Mouradi et al. 2018), and chickpea/maize intercropping (Li et al. 2004). Hence, the high phosphatase activities in the rhizosphere of faba bean and lupin (and perhaps also of soy) likely contributed to maize P acquisition in legume/maize intercropping in our experiments.

In addition, faba bean (but not lupin) strongly acidified the rhizosphere (relative to the bulk soil and the rhizosphere of the other species), which likely resulted from an excess uptake of cations over anions that was counterbalanced by proton release (Hinsinger 2001; Hinsinger et al. 2003). The acidification of the rhizosphere might cause a dissolution of P minerals, such as calcium phosphates (Ca-P), thereby increasing P availability in the rhizosphere (Hinsinger 2001; Hinsinger et al. 2011). In our experiments, about a quarter of soil P was HCl-soluble (i.e., Ca-associated P; Tiessen and Moir 2007), which might have been mobilized by faba bean via rhizosphere acidification. This might either result in P complementarity in intercropping if faba bean and maize access different P forms (i.e., Ca-P by faba bean and water-soluble P by maize) or in P facilitation if maize takes up P that has been mobilized by faba bean (Duchene et al. 2017; Xue et al. 2016). A strong rhizosphere acidification by faba bean has been observed earlier, which was associated with organic acid and proton exudation and resulted in higher P uptake of intercropped maize (Li et al. 2007). Similarly, faba bean has been reported to acidify the rhizosphere much more than soy or maize, thereby mobilizing sparingly soluble P from that soil, which might partly explain the interspecific facilitation of P uptake in faba bean/maize intercropping found in that study (Zhou et al. 2009). Faba bean (in contrast to maize) has also been shown to respond to P deficiency with high phosphatase activity and rhizosphere acidification, which both increased P availability in the rhizosphere (Liu et al. 2016). However, despite the high phosphatase activity and the strong rhizosphere acidification by faba bean found here, faba bean did not significantly enhance maize P acquisition in our experiments. This indicates that faba bean successfully competed for P and likely used most of the P that it mobilized itself instead of facilitating maize P acquisition. Faba beans' competitiveness was even stronger in the first year of the field experiment when it was earlier sown than maize, which is in accordance with a recent meta-analysis (Yu et al. 2016), as discussed in more detail in a previous study (Schwerdtner and Spohn 2021). However, in the second year of the field experiment, the pPER of maize AGB P content was about 1.6, indicating at least small intercropping benefits for maize plants in faba bean/maize intercropping as compared to maize monocropping. In contrast, lupin and soy significantly enhanced maize P acquisition in intercropping (as indicated by pPER), indicating that lupin and soy were less competitive than faba bean. Lupin did not acidify its rhizosphere in our experiments (pH changes were smaller than in the rhizosphere of maize) but might have mobilized P from inorganic soil P pools via exudation of carboxylates in addition to organic P mineralization (Dissanayaka et al. 2017). Since soy failed in our rhizobox experiment, the mechanisms of P acquisition by soy remained unclear.

Since maize plants were planted very narrowly in maize monocropping (as compared to agricultural practice), an additional potential explanation of maize overyielding in intercropping could be that intraspecific competition among maize plants in monocropping was high and that positive intercropping effects were due to compensation, as discussed in more detail in a previous study (Schwerdtner and Spohn 2021). Moreover, the soil in both experiments was rich in nutrients suggesting that competition for light might have limited maize growth more than competition for nutrients since the companions are likely weak competitors for light (in intercropping) in comparison to the tall maize plants (in monocropping). A better light utilization in intercropping than in monocropping has been reported earlier and was associated with plant growth promotion (Brooker et al. 2015; Kermah et al. 2017). However, when root barriers were installed in the second year of the field experiment, maize plants produced similar biomass in monocropping and intercropping (Schwerdtner and Spohn 2021), indicating that increased nutrient uptake of maize and maize overyielding were caused by belowground processes in the intermingled rhizosphere and not by competition for light. Specifically, by comparing the treatments with and without root barriers, we estimated that maize overyielding was mainly caused by interspecific root interactions in legume/maize intercropping, while aboveground interspecific interactions contributed more to maize overyielding in mustard/maize intercropping (Schwerdtner and Spohn 2021).

Maize N and P acquisition in mustard/maize intercropping likely differed from that in legume/maize intercropping since mustard belongs to the *Brassicaceae*. Maize N acquisition in mustard/maize intercropping was slightly enhanced in our field experiment, as indicated by pPER. The reason might be that competition for N in mustard/maize intercropping was lower than in maize monocropping since mustard is likely a weak competitor for N. This is supported by low N concentrations and, therefore, low N demand of mustard that was reported earlier (Schröder and Köpke 2012). Hence, our findings suggest that competition for N in mustard/maize intercropping was lower than in maize monocropping, even though no atmospheric N_2 was fixed as in intercropping with legumes. Moreover, mustard strongly increased the rhizosphere pH (relative to the bulk soil and the rhizosphere of the other species), which likely resulted from a higher uptake of anions than cations. The rhizosphere alkalinization might cause P desorption from iron and aluminum phosphates (Fe-P, Al-P) via ligand exchange reactions (Hinsinger 2001; Hinsinger et al. 2003). In our experiments, about 20% of soil P was NaOH-soluble (i.e., Fe- and Al-associated P), which might have been mobilized by mustard via rhizosphere alkalinization. Thus, mustard likely increased soil P availability for both species (either complementary or facilitative, as discussed above) through changes in the rhizosphere pH. Rhizosphere alkalinization has also been found for other *Brassica* genotypes (Marschner et al. 2007). For instance, rapeseed has been shown to increase the rhizosphere pH, thereby depleting P from NaOH-extractable pools (Gahoonia and Nielsen 1992; Hinsinger 2001). Similarly, the rhizosphere alkalinization of durum wheat and the grass *Nassella trichotoma* increased P availability (Devau et al. 2010; Spohn et al. 2020). Hence, the rhizosphere alkalinization by mustard might have contributed slightly to maize P acquisition in mustard/maize intercropping, although the pPER of maize AGB P content was not significantly enhanced and compensation effects likely also occurred.

5 Conclusions

We found species-specific mechanisms of plant N and P acquisition, which likely explain the higher maize N and P contents in intercropping than monocropping. Maize benefited particularly from intercropping with lupin and soy, while intercropping effects of faba bean and mustard on maize were comparatively small.

Our findings indicate that a high proportion of maize N was derived from the intercropped legumes. This confirms our first hypothesis that legumes complement and facilitate maize N acquisition in legume/maize intercropping due to the legumes' ability to symbiotically fix N_2 from the atmosphere and to transfer a part of it to maize. Our findings also indicate reduced competition for N in mustard/maize intercropping compared to maize monocropping.

Furthermore, we found indications that the companions have larger capacities to mobilize P than maize. We observed high phosphatase activities in the rhizosphere of faba bean and lupin, a rhizosphere acidification by faba bean, and a rhizosphere alkalinization by mustard. These changes in the rhizosphere mobilize P from less plant-available soil P pools (organic P, Ca-P, Fe-P, Al-P), from which maize likely benefited in intercropping when roots were intermingled. This confirms our second hypothesis that the companions complement and facilitate maize P acquisition in intercropping due to rhizosphere processes that mobilize otherwise unavailable P forms.

Taken together, our study provides evidence that the companions' ability to mobilize N and P can promote maize overyielding in intercropping if facilitative and complementary rhizosphere processes are stronger than nutrient competition. Thus, intercropping can contribute to fertilizer savings and promote agricultural sustainability.

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Data Availability

The data generated and analyzed during the current study are available from the corresponding author on reasonable request.

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SUPPLEMENTARY MATERIALS

Table S1 Partial plant equivalent ratios (pPER) of maize belowground (BGB) and total biomass (TBM), as well as N and P contents of maize BGB and TBM determined 12 weeks after sowing in the rhizobox experiment

	Maize (F. bean)	**Maize (Lupin)**	**Maize (Mustard)**
pPER of maize BGB	0.95 ± 0.41	1.12 ± 0.35	0.85 ± 0.43
pPER of maize TBM	1.37 ± 0.59	1.51 ± 0.52	1.29 ± 0.73
pPER of maize BGB N	1.04 ± 0.39	1.26 ± 0.20	0.78 ± 0.35
pPER of maize TBM N	1.89 ± 0.36**	1.92 ± 0.38**	1.29 ± 0.57
pPER of maize BGB P	0.82 ± 0.37	0.96 ± 0.32	0.68 ± 0.27
pPER of maize TBM P	1.33 ± 0.38	1.39 ± 0.33	1.10 ± 0.46

Numbers show means ± standard deviations (n = 5). Symbols indicate that pPER was significantly different from 1.00 (**: $p < 0.01$), tested separately for each row.

Table S2 Soil pH, soil phosphatase activity, and soil nitrate (NO_3^--N) determined at harvest in the field experiment in 2018

	Maize/ Maize	**Maize/ F. bean**	**Maize/ Soy**	**Maize/ Lupin**	**Maize/ Mustard**
pH	7.02 ± 0.09	6.97 ± 0.08	6.96 ± 0.11	6.95 ± 0.06	7.03 ± 0.06
Phosphatase activity [nmol g soil^{-1} h^{-1}]	2.76 ± 1.55	3.61 ± 1.71	2.92 ± 0.94	3.42 ± 1.10	2.36 ± 1.10
NO_3^--N [µg N g soil^{-1}]	3.25 ± 1.10	4.27 ± 1.15	3.17 ± 0.98	4.01 ± 1.07	4.26 ± 0.91

Numbers show means ± standard deviations (n = 5). No significant difference was found among the species combinations, tested separately for each row.

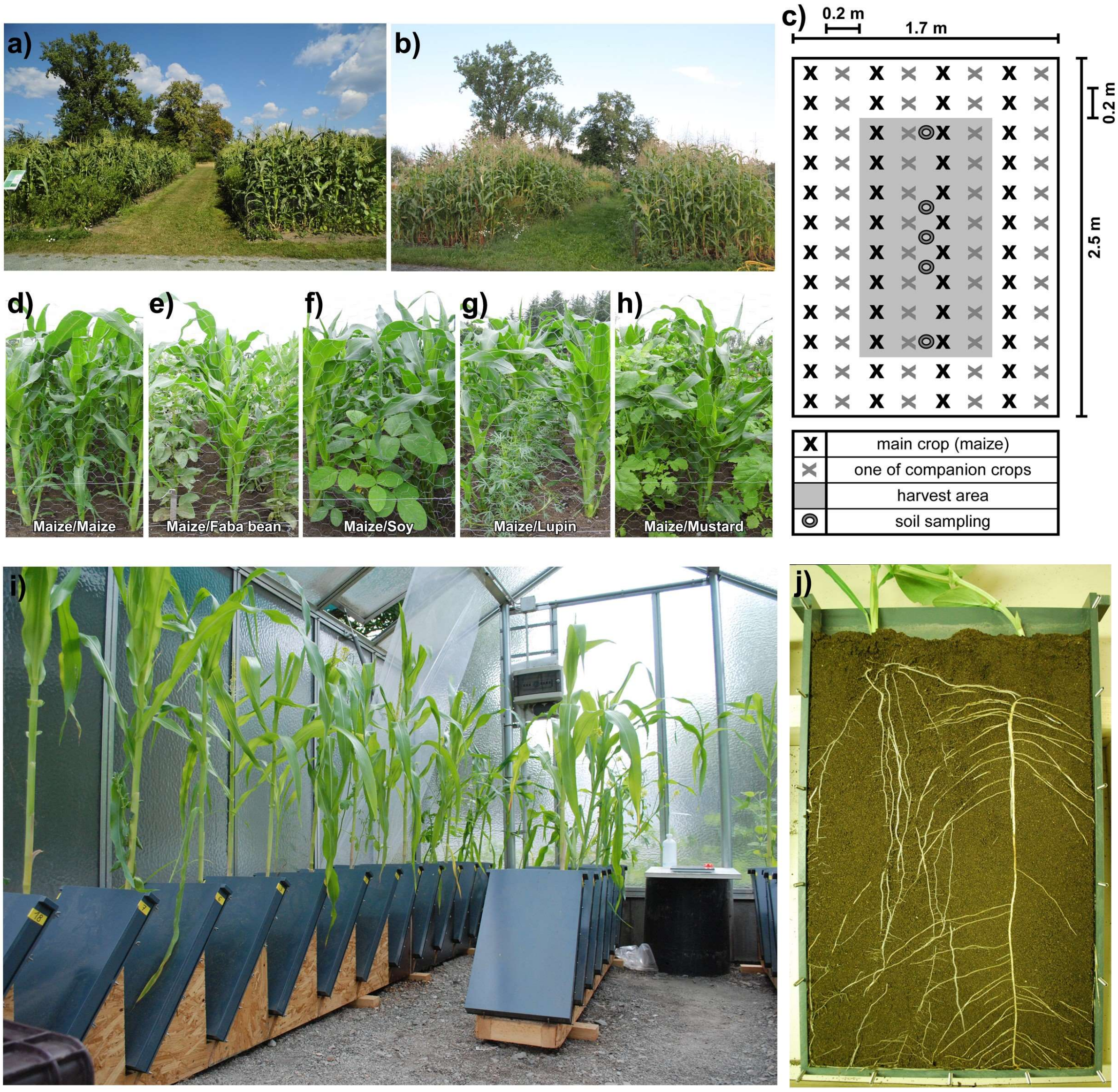

Fig. S1 Photos of the field experiment in July 2018 (a) and August 2019 (b), the experimental setup of one of the 25 plots in the field experiment in 2018 (c) as well as photos of the plant interactions in the different species combinations in the field experiment in late June 2018 (d–h). Further, photos of the rhizoboxes in the greenhouse in June 2018 (i) and of the roots in one faba bean/maize rhizobox in May 2018 (j).

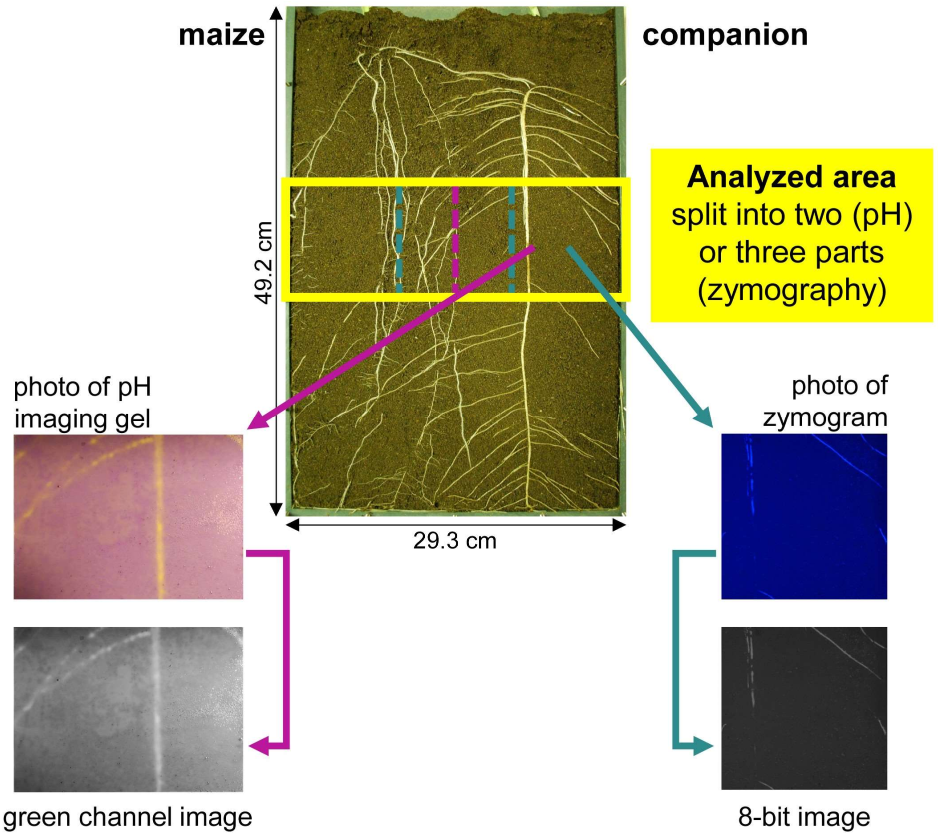

Fig. S2 Overview of image processing steps to analyze the images obtained from pH imaging (left side) and soil zymography (right side) including exemplary images of the first analysis of a faba bean/maize rhizobox. The dashed lines within the yellow-rimmed area of analyses indicate how the pH imaging gels (purple) and the zymograms (blue) were cut into (two or three, respectively) equal pieces to ensure high-quality photographs.

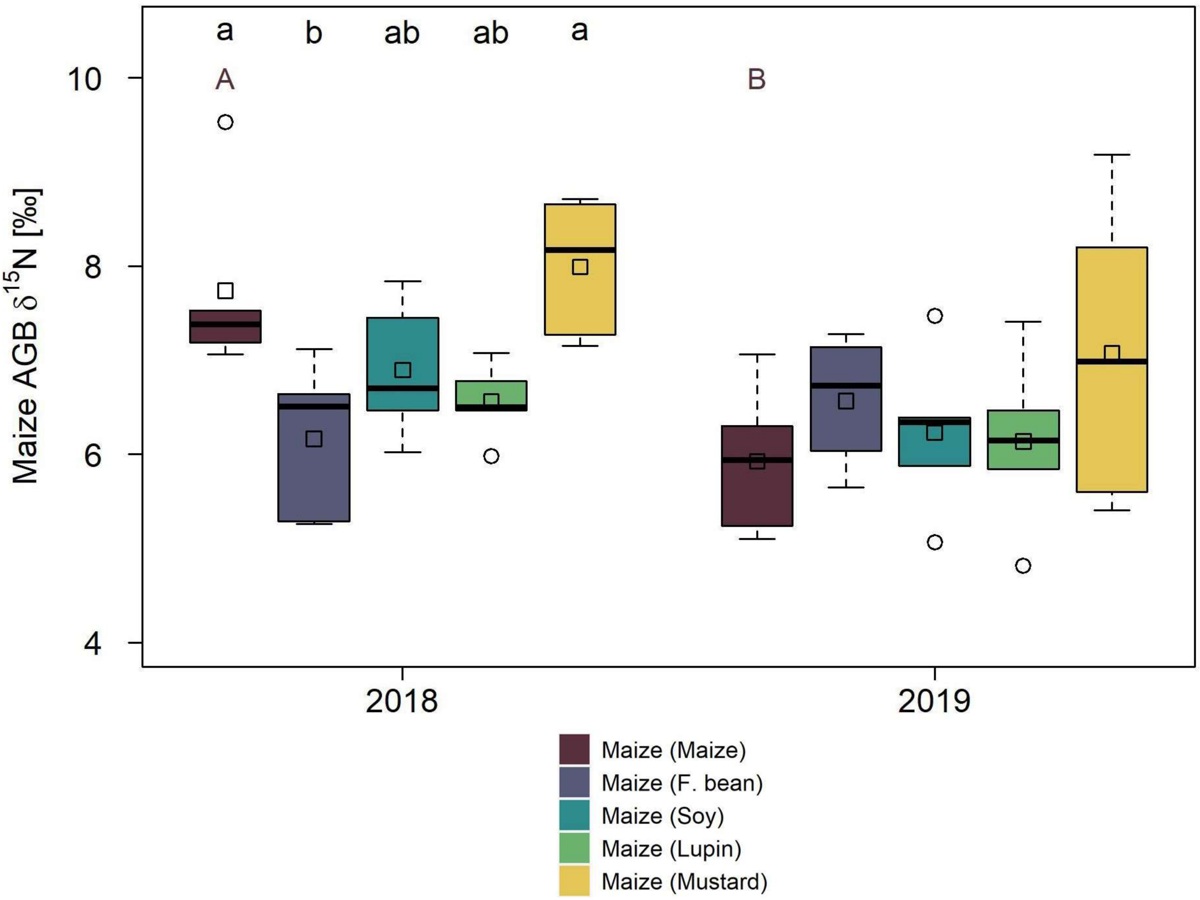

Fig. S3 δ^{15}N values of maize aboveground biomass (AGB) in the field experiment in 2018 and 2019. Squares show means. Different lowercase letters indicate significant differences ($p < 0.05$) among the species combinations, tested separately for each year. Different capital letters indicate significant differences ($p < 0.05$) between years, tested separately for each species combination. Absence of letters indicates that there was no significant difference.

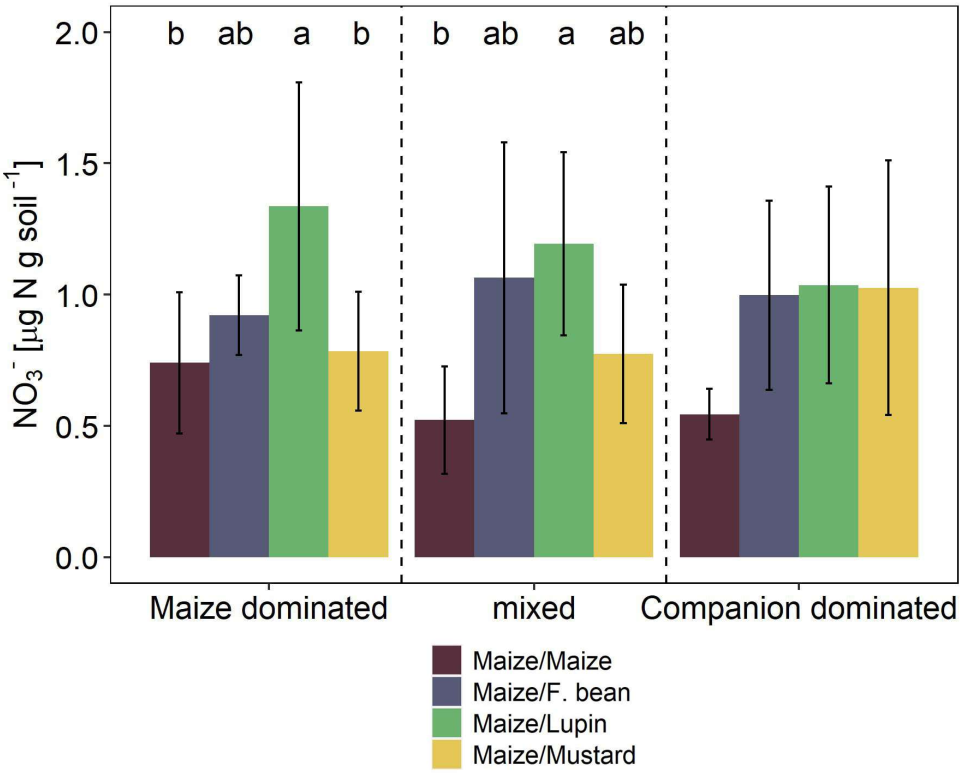

Fig. S4 Water-extractable nitrate (NO_3^-) in the rhizobox experiment derived from soil samples at harvest after twelve weeks in the soil areas dominated by roots of maize (left), by roots of one of the companions (right), or by roots of both (middle, referred to as “mixed”). Columns show means and error bars indicate standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among the species combinations, tested separately for each soil area. Absence of letters indicates that there was no significant difference.

Study III – Greenhouse experiment with single plants and defined P sources

Soy and mustard effectively mobilize phosphorus from inorganic and organic sources

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Abstract

We aimed to investigate phosphorus (P) mobilization by different plant species from organic and inorganic sources in relation to different P mobilization mechanisms. Knowledge about P mobilization is important for producing crops on P sources other than phosphate rock-derived fertilizers. We conducted a greenhouse experiment with four plant species (maize, soy, lupin, mustard) and three P sources ($FePO_4$, phytate, struvite). We determined pH and phosphomonoesterase activity in the rhizosphere using pH imaging and soil zymography. At harvest, root exudates were analyzed for phosphomonoesterase activity, pH, organic acids, and dissolved organic carbon (DOC). Plants were analyzed for biomass, root length, and P content. Struvite was more plant-available than phytate and $FePO_4$ as indicated by higher plant P contents. Soy had the highest biomass and P content, irrespective of P source. Soy exuded up to 12.5 times more organic acids and up to 4.2 times more DOC than other plant species. Lupin had a 122.9 times higher phosphomonoesterase activity than other plant species with phytate. The pH in the exudate solution of mustard was on average 0.8 pH units higher than of the other plant species. P uptake by mustard and soy seemed to have also benefited from large root lengths. Taken together, our study indicates that soy has a particularly high potential to mobilize P from struvite and phytate, while mustard has a high potential to mobilize P from $FePO_4$. Therefore, soy and mustard seem to be good options for agricultural production that relies less on phosphate rock-derived fertilizers.

Keywords: plant phosphorus mobilization, struvite, phytate, iron phosphate, organic acids, phosphomonoesterase activity

Introduction

Phosphorus (P) is essential for crop production since it is an integral constituent in the structural and cellular metabolism of plants as component of, e.g., adenosine triphosphate (ATP), nucleic acids, and phospholipids of biomembranes, and is thus involved in transferring information (DNA, RNA) and energy (ATP, ADP) (Ashley et al. 2011; George et al. 2016; Marschner 2012). To increase crop yields and avoid P limitation, huge amounts of P fertilizers, i.e., about 46 million metric tons of P_2O_5 per year, mainly derived from phosphate rock, are applied globally in agricultural production (Cordell and White 2014; IFA 2022). However, the application of phosphate rock-derived fertilizer is increasingly problematic since phosphate rock represents a finite and geographically unevenly distributed resource (Ashley et al. 2011; George et al. 2016). Thus, there is a need to reduce the reliance on phosphate rock-derived fertilizers, for instance through cultivation of crops that effectively mobilize less easily available soil P forms and recycled P sources (George et al. 2016; Sulieman and Mühling 2021).

Plants have developed various mechanisms to mobilize P from different sources, which might be used to reduce the reliance on phosphate rock-derived fertilizers in agriculture. These mechanisms can be summarized as ‘root foraging’ via morphological adaptations and ‘P mining’ via physiological adaptations to P deficiency (Lyu et al. 2016; Richardson et al. 2011; Wen et al. 2021). Root foraging allows plants to acquire nutrients from a greater soil volume and minimizes the distance between roots and plant-available orthophosphate anions in soil by extending the root system and increasing the root surface (Ma et al. 2018; Richardson et al. 2011; Wen et al. 2019). Especially *Brassicaceae* exhibit a ramified root system with thin, long, and intensively branched roots, whereas *Lupinus* species form thick and comparatively short taproots (Lyu et al. 2016; Wen et al. 2019). In contrast to root foraging, P mining refers to the mobilization of phosphate anions from sparingly soluble P sources by exuding various substances, such as phosphatases and low molecular weight organic acid anions (LMWOA), into the soil (Richardson et al. 2011; Wen et al. 2021).

Up to 80% of soil P is present as organic P in the topsoil, of which the majority (60–90%) exists as orthophosphate monoesters, with *myo*-inositol hexakisphosphate (phytate) being the most abundant form (Dalal 1977; Liu et al. 2022; Turner et al. 2002). Mobilizing P from phytate involves two steps: organic P needs to be released from precipitates and adsorption sites before it can be mineralized through hydrolysis of the ester bonds via extracellular phosphomonoesterase enzymes, namely phytases (Liu et al. 2022; Menezes-Blackburn et al. 2018; Richardson et al. 2009). However, a part of the organic soil P pool and particularly phytate is stabilized against mineralization by adsorption to mineral surfaces and is therefore only slowly hydrolyzed by enzymes (Jarosch et al. 2015; Menezes-Blackburn et al. 2018; Nannipieri et al. 2011). Moreover, plant species differ considerably in their capacity to exude phosphomonoesterases including phytases. Legumes, and particularly *Lupinus* species, are known for a high phosphomonoesterase activity in the rhizosphere, thus likely mobilizing organic P more effectively than *Gramineae* and *Brassicaceae* (Sulieman and Mühling 2021; Wen et al. 2019). However, the amount of released phytases differ among different legumes, both in absolute terms and relative to other phosphatases (Dong et al. 2020; Gilbert et al. 1999; Tang et al. 2021).

Orthophosphate anions have a high affinity for metal cations, e.g., of iron (Fe^{2+} and Fe^{3+}), with which they precipitate, forming sparingly soluble P minerals such as iron phosphate ($FePO_4$) (Chang and Jackson 1958; Richardson et al. 2009). $FePO_4$ is also formed during wastewater treatment and its use in agriculture as a fertilizer can thus increase P recycling (Li et al. 2020b; Wilfert et al. 2015). Mobilizing P from $FePO_4$ requires changes in the precipitation-dissolution equilibrium, which can be achieved by increases in the soil pH changes (Hinsinger 2001; Lindsay 1979; Richardson et al. 2009). Plants can modify the rhizosphere pH by releasing protons or hydroxyl ions and/or by exuding LMWOA. The latter can also mobilize inorganic P by modifying the surface characteristics of soil colloids, by successfully competing with phosphate for sorption sites, or by chelating cations bound to P (Hinsinger et al. 2003; Richardson et al. 2009; Wang and Lambers 2020). However, only some plant species exude LMWOA at high rates or change the rhizosphere pH substantially. Legumes, and particularly *Lupinus* species, are known for a high LMWOA exudation (Wang and Lambers 2020; Wen et al. 2019), while different *Brassicaceae* have been shown to substantially alkalize their rhizosphere, thus increasing P mobilization from $FePO_4$ (Marschner et al. 2007). However, former research has also shown that LMWOA exudation is not necessarily strongly aligned with a plant species' capacity to mobilize P from $FePO_4$ (Pearse et al. 2007).

Struvite (ammonium magnesium phosphate; NH_4MgPO_4) is frequently formed as a byproduct of wastewater and sludge treatments (Kataki et al. 2016; Talboys et al. 2016). Compared to other recycling products, struvite has a high P content, contains also N, and tends to have a low concentration of heavy metals and other contaminants (Faucon et al. 2015; Schneider et al. 2019). Yet, the capacities of different plant species to mobilize P from struvite are still not fully understood. Lupin and buckwheat have been shown to mobilize P from struvite through the exudation of LMWOA (Robles-Aguilar et al. 2019; Talboys et al. 2016). Moreover, lupin has been shown to be more efficient in P uptake from struvite than maize due to rhizosphere acidification (Robles-Aguilar et al. 2020). However, the capacities of several other common crop species to mobilize P from struvite have not yet been investigated.

Our study aimed to investigate the mechanisms of plant P mobilization from different P sources in relation to plant P uptake by different plant species in order to identify plant species that effectively use P sources other than phosphate rock-derived fertilizers. For this purpose, we conducted a greenhouse experiment with three different P sources (phytate, $FePO_4$, and struvite) that require different P mobilization mechanisms and four different plant species (maize, soy, lupin, and mustard) that likely have contrasting mechanisms of P mobilization. We hypothesized that (i) plant species with a high LMWOA exudation effectively mobilize P from struvite and $FePO_4$, (ii) rhizosphere alkalinization mobilizes P from $FePO_4$, and (iii) plant species with a high phosphomonoesterase activity in the rhizosphere effectively mobilize P from phytate. To test these hypotheses, we analyzed the spatial distribution of rhizosphere pH and phosphomonoesterase activities during plant growth using the *in situ* techniques pH imaging and soil zymography. Additionally, we collected root exudates at harvest, in which pH, phosphomonoesterase activity, LMWOA, and dissolved organic carbon (DOC) were measured, before plants were analyzed for biomass production, P content, and root length.

Materials and methods

Experimental setup

We conducted a greenhouse experiment with four plant species grown in rhizoboxes in mineral substrate with three different P sources. The mineral substrate consisted of 20% (vol.) perlite and 80% (vol.) quartz sand, from which 50% (wt.) had a grain size of 0.1–0.4 mm and 50% (wt.) had a grain size of 0.7–1.2 mm. A mix of micronutrients (RADIGEN ® Micronutrient mixed fertilizer, Terraflor GmbH, Iserlohn, Germany) was added to the mineral substrate (320 mg rhizobox^{-1}), containing 5.0% MgO, 2.0% Fe, 1.5% Cu, 1.0% Mn, 0.8% Mo, 0.6% B, and 0.5% Zn. One of the following P sources was added to the mineral substrate (150 mg P rhizobox^{-1}): iron phosphate (iron(III) phosphate dihydrate: $FePO_4 \times 2H_2O$; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), phytate (phytic acid sodium salt hydrate: $C_6H_{18}O_{24}P_6 \times xNa^+ \times yH_2O$; Sigma-Aldrich), and struvite (ammonium magnesium phosphate hydrate: $NH_4MgPO_4 \times xH_2O$; Sigma-Aldrich). Further nutrients were supplied with a P-free nutrient solution as described below. The mineral substrate was filled into the rhizoboxes to a final bulk density of 1.1 g cm^{-3} which equals 1.7 kg of mineral substrate (dry weight) per rhizobox. Rhizoboxes were made of PVC and had an inner size of 39.2 × 19.2 × 2.2 cm (h × w × d).

In each rhizobox, one out of four plant species was sown: maize (*Zea mays* L. cv. Golden Bantam, Bingenheimer Saatgut AG, Echzell, Germany), soy (*Glycine max* (L.) Merr. cv. Lica, Marktgesellschaft der Naturland Bauern AG, Hohenkammer, Germany), blue lupin (*Lupinus angustifolius* L. cv. Rumba, Templiner Kräutergarten, Templin, Germany), and white mustard (*Sinapis alba* L., Bingenheimer Saatgut AG). All seeds except for mustard were soaked in water for 24 h before seeds of a consistent size were sown at a rate of one seed per rhizobox. All treatments were replicated four times summing up to a total number of 48 rhizoboxes (four plant species × three P sources × four replicates). However, one rhizobox of lupin supplied with struvite failed shortly before harvest. The plants were sown in August 2019 and harvested after 10 weeks in October 2019.

An inoculum was applied to the mineral substrate at the beginning of the experiment in order to introduce a soil microbial community. For this purpose, fresh soil with a loamy sandy texture was sampled from an agricultural soil cultivated with the same plant species used here. In the field, soy and lupin seeds had been inoculated with commercial *Bradyrhizobium* sp. inoculants, which are assumed to be also part of the microbial community introduced here (for details, see Schwerdtner and Spohn 2021). The soil was sieved (< 2 mm), mixed with tap water (1:2), and shaken on an overhead shaker for 1 h before being filtered through cellulose filters (Rotilabo®, type 113P, Carl Roth GmbH & Co. KG, Karlsruhe, Germany). The filtrate was mixed with tap water to a final soil:water ratio of 1:4 and stored at 20 °C over night before being applied to all rhizoboxes. The final soil inoculum had the following chemical properties (l^{-1} inoculum): 15.3 mg organic C, 4.4 mg N, 0.9 mg P, and pH 7.9. Each rhizobox received 180 ml of soil inoculum and 50 ml of tap water to adjust the mineral substrate to 75% water holding capacity (WHC).

The rhizoboxes were placed in an open greenhouse at the University of Bayreuth under ambient conditions and without artificial light. The rhizoboxes were placed in a randomized block design on a wooden rack that kept them inclined by 50° throughout the experiment, and they were rearranged randomly after 5 weeks. The inclination of the rhizoboxes made the roots grow along the bottom wall of the rhizoboxes, which made it possible to conduct imaging analyses (see below) and to remove the entire plant at harvest with very limited damage to the root system. Rhizoboxes were watered every two days with tap water to 75% WHC as measured by weight. In addition, a P-free nutrient solution was applied regularly. For this purpose, an adapted Ruakura solution (Smith et al. 1983) was used, where KH_2PO_4 was substituted by KNO_3 and K_2HPO_4 by K_2SO_4. The final nutrient solution applied to the rhizoboxes contained (l^{-1}): 220 mg $Mg(NO_3)_2 \times 6\ H_2O$, 746 mg $Ca(NO_3)_2 \times 4\ H_2O$, 377 mg NH_4NO_3, 189 mg KNO_3, 367 mg K_2SO_4, 27 mg Na_2SO_4, and 15 mg NaCl. In total, 9.2 mg N kg^{-1} substrate were applied in the form of inoculum and nutrient solution.

Six weeks after plant emergence, pH imaging and soil zymography were performed to determine the spatial distribution of pH and phosphomonoesterase activity (see below). At harvest, 70 days after plant emergence, root exudates were collected, and plants were analyzed for biomass production, root length, and P concentrations (see below).

pH imaging

The distribution of pH in the rhizosphere was analyzed *in situ* by pH imaging, following Marschner and Römheld (1983) with modifications. The pH indicator bromocresol purple (Sigma-Aldrich) was dissolved in deionized water (0.6%). NaOH was added dropwise for better dissolution as described by Nkebiwe et al. (2016). The day before analysis, a boiled agar solution (1.3% agarose; Sigma-Aldrich) was mixed with the pH indicator solution (final pH indicator concentration of 0.006%), adjusted to mineral substrate pH with NaOH, and cast in glass systems usually used for gel electrophoresis with an inner size of 24.5 × 18.5 × 0.1 cm. Gels were plastic-wrapped to prevent drying and stored overnight at 20 °C to allow acclimatization. Rhizoboxes were transferred to the 20 °C climate chamber 1h before analyses to allow acclimatization of the mineral substrate. The pH indicator gels were cut into three pieces, each with a size of 8 × 18 cm. Each gel was attached to the soil surface of one rhizobox to a soil depth of 18 cm (from the top, box-centered) and covered with a plastic sheet. After 12 min of incubation in the dark at 20 °C, gels were photographed with a digital camera (EOS 1100D, Canon). No quantitative image analysis was performed since the mineral substrate gave no uniform background values due to the mixing with perlite. Instead, representative pH images of each plant species supplied with one of the three P sources are presented in Fig. S1 (Supplement). Photos of the root systems are not included in the study as it was practically impossible to photograph the roots accordingly since mineral substrate and roots had very similar colors.

Soil zymography

Directly after pH imaging, the distribution of phosphomonoesterase activity was measured *in situ* by soil zymography following Spohn and Kuzyakov (2013) with modifications. No agarose gels were used as in Holz et al. (2019) as we used a mineral substrate and thus the gel, which is thought to protect the membrane from staining with organic material, was not required. The substrate 4-methylumbelliferyl phosphate (Sigma-Aldrich) was dissolved in deionized water to a concentration of 2 mM. Membrane filters of nylon (0.45 µm pore size; Nantong FilterBio Membrane Co. Ltd., Jiangsu, China) with a size of 8 × 31 cm were coated with this solution. The membranes were allowed to dry flat for 1 min at room temperature (20 °C) on aluminum foil, before being attached to the soil surface of one rhizobox to a soil depth of 31 cm (from the top, box-centered). After 30 min of incubation at 20 °C in the dark, the membrane was removed from the soil surface, cut into two equal pieces, and each piece was photographed with a digital camera (D60, Nikon) on an epi-UV-desk (Desaga, Heidelberg, Germany) at 366 nm wavelength. The cutting was done to ensure equal distribution of UV light all over the zymogram.

For calibration, membranes were soaked in 4-methylumbelliferone (MUF; Sigma-Aldrich) of different concentrations (0, 25, 75, 125, 200 µM). The membranes were also allowed to dry for 1 min and then photographed as described for the zymograms. Phosphomonoesterase activity was calculated based on a linear correlation between the different MUF concentrations and the corresponding gray values of the images (Spohn and Kuzyakov 2013).

The zymograms were analyzed using the open-source software ImageJ (version 1.52a; Rasband 2018). For this purpose, the photographs were converted into 8-bit, i.e., grayscale images, and a digital grid with cells of 10 × 10 pixels was laid on the images, similar as in Hofmann et al. (2016). The mean gray value of each grid cell was determined, and the twenty highest gray values of each rhizobox (considering both pieces of the zymogram) were arithmetically averaged to obtain one average value per rhizobox, i.e., per plant. The corresponding phosphomonoesterase activities were calculated based on the calibration line and the incubation time. Representative zymograms of each plant species supplied with phytate are presented in Fig. S2 (Supplement).

Root exudate collection

Root exudates were collected in sterile deionized water using the soil-hydroponic-hybrid sampling approach (Oburger and Jones 2018). For this purpose, the bottom walls of the rhizoboxes were opened at harvest and plants were removed as carefully as possible to prevent root damage. Roots were gently shaken and washed with deionized water to remove adhering substrate particles and potential metabolites (Oburger and Jones 2018). The entire root system of the intact plant was then transferred to a sterile beaker that was filled with a known volume of sterile deionized water (between 50 and 125 ml) so that roots were completely submerged. We used sterile deionized water instead of a $CaCl_2$ solution since this reduces the background matrix for the analyses while not altering exudation patterns (Egle et al. 2003; Oburger and Jones 2018). Plants in beakers were stored at 20 °C in a climate chamber with artificial lighting (650 µmol m^{-2} s^{-1}). After 4 h, plants were removed, and beakers were swayed to homogenize the exudates in the solution. The exudate solutions were filtered through 0.2 µm syringe filters and four aliquots were frozen for subsequent analyses of pH, phosphomonoesterase activity, LMWOA, and DOC. All plants were sampled in a way ensuring that exudate collection took place during peak metabolic activity, i.e., collection started 3.5 ± 1 h after sunrise, as recommended in Oburger and Jones (2018). Since we used a relatively short exudate collection period and maintained very similar temperatures during plant growth and exudate collection, we assume that plant metabolism and therefore exudation patterns do not differ between growth and sampling conditions (Oburger and Jones 2018).

Biomass analyses

After the plants were removed from the exudate collection beakers, aboveground biomass (AGB) was separated from belowground biomass (BGB). AGB was immediately dried at 60 °C for 24 h, then weighed and milled. The BGB was washed again with deionized water and stored over night at 2 °C. For root length determination, two plant individuals per species and P source (24 individuals in total) were chosen (due to time constraints during harvest). The root length was determined using a flatbed scanner (Epson Perfection V700, Seiko Epson Corporation, Nagano, Japan) and the software WinRhizo™ 2008 (Regent Instruments Inc., Québec, Canada). The BGB was submerged in a water bath, neatly arranged to avoid root overlapping, and scanned at 400 dpi resolution. After root length analysis, BGB of all plants was dried at 60°C, weighed, and milled.

The biomass samples (AGB and BGB of each plant) were analyzed for total P concentrations after pressure digestion in concentrated nitric acid using inductively coupled plasma-optical emission spectroscopy (Vista-Pro radial, Varian Inc., Palo Alto, USA).

Exudate analyses

pH The pH in the exudate solution was measured with a pH electrode (WTW SenTix 51, Xylem Analytics GmbH & Co. KG, Weilheim, Germany).

Phosphomonoesterase activity Phosphomonoesterase activity in the exudate solution was measured using the fluorogenic substrate 4-methylumbelliferyl phosphate following Marx et al. (2001), German et al. (2011), and Herold et al. (2014). In brief, the exudate samples (50 µl) were pipetted into black polystyrene 96-well microplates (BRANDplates®, Brand GmbH & Co. KG, Wertheim, Germany) having four replicates. Sterile deionized water (50 µl) and substrate solution (100 µl) were added. Microplates were covered and pre-incubated in the dark at 15°C for 30 min and measured fluorometrically after 0, 60, and 180 min with 360 nm excitation and 460 nm emission filters (Herold et al. 2014) by a microplate reader (Infinite® 200 PRO, Tecan Trading AG, Männedorf, Switzerland). Between measurements, microplates were incubated in the dark at 15 °C. Enzyme activities were calculated according to German et al. (2011) modified by Widdig et al. (2019). Fluorescence values were corrected for quenching, sample fluorescence, and substrate fluorescence.

LMWOA LMWOA were analyzed using high-performance liquid chromatography-mass spectrometry (HPLC-MS). For this purpose, the exudate samples were loaded on a HPLC RP-C18 column (Luna Omega 1.6 µm PS C18, 100 Å, 100 × 2.1 mm, Phenomenex Inc., Torrance, USA; operated as part of an Ultimate 3000 HPLC, Thermo Fisher Scientific GmbH, Bremen, Germany), which was connected to a Q Exactive mass spectrometer (Thermo Fisher Scientific GmbH) equipped with a hybrid quadrupole orbitrap mass analyzer (maximum mass range 50–6000 Da, resolution 140.000 @ m/z = 200). A 10 min isocratic elution with pure water (HPLC-grade, spiked with 0.2% formic acid) at a flow rate of 0.3 ml min^{-1} was applied. Mass spectra were acquired after electrospray ionization (ESI negative) in full scan mode ($50 < m/z < 750$) recording the total ion current. For evaluation (i.e., identification and integration/quantitation) of the LMWOA, their characteristic mass traces were used (Table S1; Supplement).

DOC Dissolved organic carbon (DOC) was analyzed using a Total Carbon Analyzer (TOC-TN Analyzer, multi N/C 2100, Analytik Jena GmbH, Jena, Germany).

Calculations

The total biomass (TBM) was calculated as the sum of the dry weights (DW) of AGB and BGB for each plant. The plant P concentration (in mg P g^{-1} DW) was calculated based on the P concentrations of AGB and BGB and the DW of AGB and BGB for each plant. The plant P content (in mg P $plant^{-1}$) was calculated as the sum of AGB and BGB P content (calculated by multiplying the P concentrations of AGB and BGB with the DW of AGB and BGB, respectively).

The pH in the exudate solution was converted into the H^+ concentration, and subsequently the H^+ concentration was multiplied by the volume of sterile deionized water and reconverted into pH in order to correct for the different volumes. The phosphomonoesterase activity and DOC concentration in the exudate solution were multiplied by the volume of sterile deionized water, in which roots were submerged in order to correct for the different volumes and gain results per plant.

The exudation of each LMWOA (in µmol $plant^{-1}$) was calculated by multiplying the LMWOA concentrations in the exudate solution (in mg l^{-1}) with the volume of sterile deionized water and dividing by the molar mass of the respective LMWOA. The concentration of each LMWOA (in µmol $plant^{-1}$) was multiplied with the number of carboxyl groups (1, 2, or 3, respectively; Table S1; Supplement), and all numbers were totaled up to calculate the total number of carboxyl groups in the exudate solution. The carboxyl groups (in µmol $plant^{-1}$) were divided by the BGB DW in order to gain results per g root DW. For two plant individuals per treatment, the carboxyl groups (in µmol $plant^{-1}$) were also divided by the root length in order to gain results per cm root length.

Statistical analyses

Data were tested for significant differences both among plant species (tested separately for each P source) and among P sources (tested separately for each plant species). Prior to all statistical analyses, normality was checked with Shapiro–Wilk normality tests, and homogeneity of variances was tested with Levene's tests. Where normality and homogeneity assumptions were met, analyses of variance followed by Tukey's post-hoc tests were conducted to identify significant differences. Where normality and homogeneity assumptions were not met, Kruskal–Wallis tests followed by post-hoc tests using the criterium Fisher's least significant difference and Holm correction for p adjustment were conducted to identify significant differences. All statistical analyses were performed in R (version 3.5.2; R Core Team 2018) using the packages agricolae (version 1.3–2; Mendiburu 2020), car (version 3.0–7; Fox and Weisberg 2019), dplyr (version 0.8.5; Wickham et al. 2020), and ggplot2 (version 3.3.0;

Wickham 2016). Data on root length were not tested for significant differences since the sample size was too low (n = 2).

Results

Biomass production

AGB was 870 ± 353 mg plant^{-1} and BGB was 244 ± 104 mg plant^{-1}, averaged across all plant species and P sources (Table 1). When P was provided in the form of phytate, TBM of soy was highest and was significantly higher, by a factor of 2.5, than TBM of lupin ($p = 0.005$; Table 1). When P was provided in the form of struvite, TBM of soy was also highest, and TBM of soy and mustard were significantly higher than of lupin and maize ($p \leq 0.007$; Table 1). When P was provided in the form of $FePO_4$, no significant difference in TBM among the plant species was found (Table 1).

Table 1 Aboveground (AGB), belowground (BGB), and total biomass (TBM) as well as plant P concentrations of maize, soy, lupin, and mustard grown with three different P sources

Species	P source	AGB [mg plant^{-1}]	BGB [mg plant^{-1}]	TBM [mg plant^{-1}]	Plant P [mg g^{-1}]
Maize	$FePO_4$	889.3 ± 397.3	234.8 ± 122.5	1124.0 ± 510.7	1.11 ± 0.13^{dB}
	Phytate	962.4 ± 370.7^{a}	281.8 ± 152.1	1244.1 ± 521.9^{ab}	1.00 ± 0.17^{cB}
	Struvite	674.6 ± 73.1^{b}	203.5 ± 68.2	878.1 ± 122.2^{b}	1.71 ± 0.18^{bA}
Soy	$FePO_4$	1017.4 ± 487.1	273.4 ± 147.7	1290.8 ± 630.7	1.77 ± 0.37^{cB}
	Phytate	1389.6 ± 477.0^{a}	365.4 ± 106.8	1755.0 ± 581.3^{a}	2.29 ± 0.38^{aB}
	Struvite	1211.6 ± 74.1^{a}	290.6 ± 71.9	1502.3 ± 140.2^{a}	4.51 ± 0.43^{aA}
Lupin	$FePO_4$	606.1 ± 176.6	239.0 ± 186.9	845.1 ± 350.5	2.40 ± 0.09^{bB}
	Phytate	495.1 ± 140.7^{b}	211.3 ± 58.1	706.4 ± 197.2^{b}	1.42 ± 0.11^{bC}
	Struvite	636.7 ± 77.1^{b}	180.0 ± 64.8	816.7 ± 140.2^{b}	4.37 ± 0.31^{abA}
Mustard	$FePO_4$	699.8 ± 187.4^{B}	186.0 ± 27.8^{B}	885.8 ± 210.1^{B}	3.13 ± 0.38^{aAB}
	Phytate	785.9 ± 70.6^{abAB}	193.0 ± 19.0^{AB}	978.9 ± 66.1^{abAB}	2.55 ± 0.34^{aB}
	Struvite	1011.5 ± 154.6^{aA}	250.8 ± 43.1^{A}	1262.3 ± 121.0^{aA}	4.18 ± 1.02^{abA}

Numbers show means ± standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each P source. Different capital letters indicate significant differences among the P sources, tested separately for each plant species. Absence of letters indicates that there was no significant difference

Root length of soy, and to a lesser extent of mustard, tended to be larger than of maize and lupin, especially when supplied with phytate or struvite (Fig. S3; Supplement). Irrespective of the P source, mustard and soy had a thin and intensively branched root system that already filled the whole rhizobox 6 weeks after plant emergence. Maize had thicker roots that penetrated almost exclusively the upper third of the substrate in the rhizoboxes, whereas lupin formed thick tap roots that reached the bottom of the rhizoboxes but did not fill the whole volume of the rhizoboxes 6 weeks after plant emergence.

Plant phosphorus

Plant P concentrations (in mg g^{-1}) differed significantly among all plant species and decreased in the order mustard > lupin > soy > maize when P was provided in the form of $FePO_4$ (p <

0.003; Table 1). More precisely, plant P concentration of mustard was significantly higher, by a factor of 2.8, compared to maize ($p < 0.001$; Table 1). When P was provided in the form of phytate, plant P concentrations of mustard and soy were significantly higher than of maize ($p < 0.001$) and lupin ($p \leq 0.009$; Table 1). When P was provided in the form of struvite, plant P concentrations of soy ($p = 0.028$), lupin ($p = 0.052$), and mustard ($p = 0.052$) were higher, by a factor of 2.4 to 2.6, than those of maize (Table 1). Lupin was the only plant species for which the P concentration was significantly higher when supplied with $FePO_4$ than when supplied with phytate ($p < 0.001$; Table 1).

Plant P content (in mg plant^{-1}) of soy was significantly higher, by a factor of 3.8, 3.3, and 1.6, respectively, than of lupin ($p < 0.001$), maize ($p < 0.001$), and mustard ($p = 0.009$) when P was provided in the form of phytate. Moreover, plant P content of mustard was significantly higher, by a factor of 2.5 and 2.1, respectively, than of lupin ($p = 0.005$) and maize ($p = 0.013$) when P was provided in the form of phytate (Fig. 1). When P was provided in the form of struvite, plant P contents significantly differed among all plant species and decreased in the order soy > mustard > lupin > maize ($p < 0.007$). More precisely, plant P content of soy was significantly higher, by a factor of 4.5, than of maize ($p < 0.001$; Fig. 1). When P was provided in the form of $FePO_4$, no significant difference in plant P content was found among the plant species. Plant P contents of soy, lupin, and mustard were significantly higher when supplied with struvite than when supplied with phytate ($p < 0.004$) and $FePO_4$ ($p \leq 0.020$). Soy was the only plant species for which the plant P content was slightly higher with phytate than $FePO_4$ ($p = 0.097$; Fig. 1). The P contents of all plant species were higher in AGB than in BGB (Table S2; Supplement).

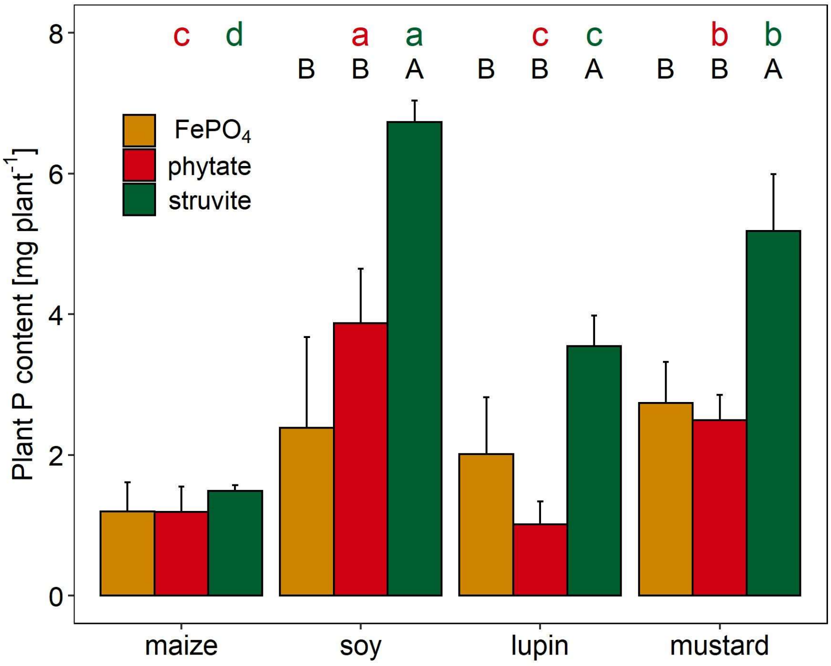

Fig. 1 Plant P content of the total biomass which is the sum of P in AGB and BGB. Columns show means and error bars indicate standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each P source. Different capital letters indicate significant differences among the P sources, tested separately for each plant species. Absence of letters indicates that there was no significant difference

Exudate pH and rhizosphere pH

The pH in the exudate solution of mustard was 0.8 pH units higher than in the exudate solution of the other plant species, when averaged across P sources (Fig. 2). When P was provided in the form of phytate or struvite, the pH in the exudate solution of mustard was significantly higher, by 1.0 and 0.7 pH units, respectively, than the pH of all other plant species ($p \leq 0.001$; Fig. 2). When P was provided in the form of $FePO_4$, the pH in the exudate solution of mustard was significantly higher, by 1.0 and 0.8 pH units, respectively, than of lupin ($p = 0.004$) and maize ($p = 0.014$), and it tended to be higher than of soy ($p = 0.117$; Fig. 2). The pH in the exudate solution of mustard was slightly higher when supplied with phytate than when supplied with $FePO_4$ ($p = 0.061$) or struvite ($p = 0.060$; Fig. 2).

In addition, the pH images, taken 6 weeks after plant emergence, revealed a comparatively strong rhizosphere acidification by maize and an intermediate rhizosphere acidification by soy, irrespective of the P source (Fig. S1; Supplement).

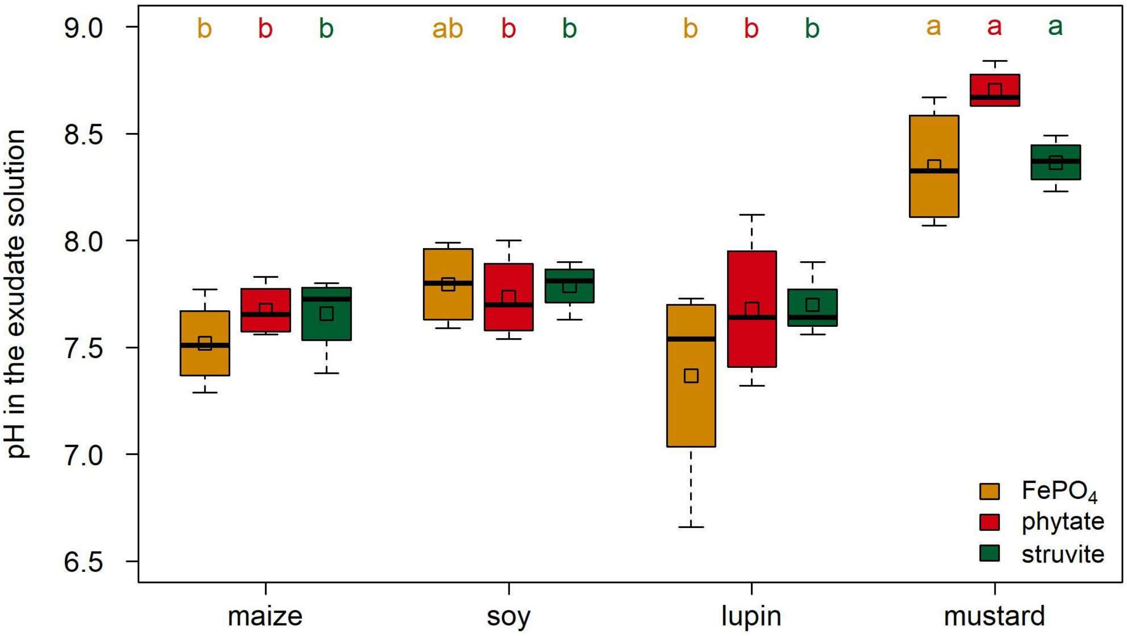

Fig. 2 pH in the exudate solution. Squares show means. Different lowercase letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each P source. No significant difference was found among the P sources, tested separately for each plant species

Phosphomonoesterase activity

When P was provided in the form of phytate, phosphomonoesterase activity of lupin was significantly higher, by a factor of on average 122.9, compared to the other plant species ($p <$ 0.022; Fig. 3). When P was provided in the form of $FePO_4$, phosphomonoesterase activity of lupin was significantly higher, by a factor of 33.8, compared to soy and mustard ($p \leq 0.011$), and slightly higher than of maize ($p = 0.084$; Fig. 3). When P was provided in the form of struvite, phosphomonoesterase activity of lupin was also significantly higher, by a factor of 8.5, compared to the other plant species ($p < 0.001$; Fig. 3).

Soil zymography, conducted 6 weeks after plant emergence, revealed that lupin and soy had similarly high maximum phosphomonoesterase activities in the rhizosphere (Figs. S2 and S4;

Supplement). When P was provided in the form of phytate, the phosphomonoesterase activities of lupin and soy were higher, by a factor of on average 1.8, compared to mustard (*p* < 0.001 and *p* = 0.005, respectively) and maize (*p* = 0.002 and *p* = 0.126, respectively; Fig. S4; Supplement).

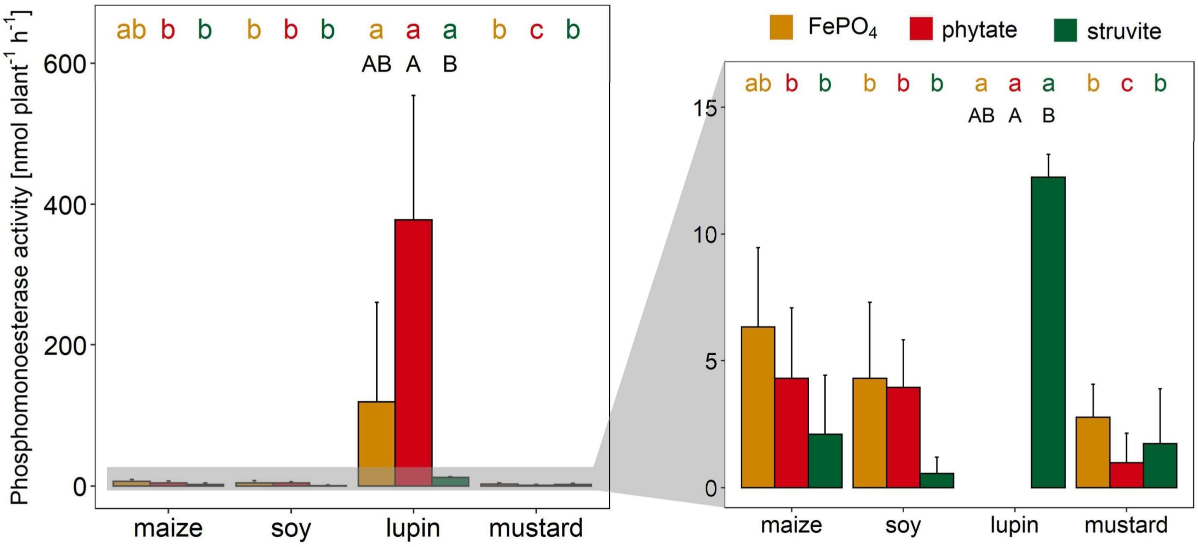

Fig. 3 Phosphomonoesterase activity in the exudate solution, including a zoom onto low values (right). Columns show means and error bars indicate standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each P source. Different capital letters indicate significant differences among the P sources, tested separately for each plant species. Absence of letters indicates that there was no significant difference

Exudation of LMWOA

When P was provided in the form of struvite, the exudation of carboxyl groups by soy was significantly higher, by a factor of 12.5 and 7.3, respectively, compared to mustard (*p* < 0.001) and maize (*p* = 0.002), and slightly higher than of lupin (*p* = 0.061; Fig. 4). When P was provided in the form of $FePO_4$, the exudation of carboxyl groups by soy was significantly higher, by a factor of 10.1 and 3.3, respectively, compared to mustard (*p* = 0.003) and lupin (*p* = 0.019; Fig. 4). When P was provided in the form of phytate, the exudation of carboxyl groups by lupin was significantly higher, by a factor of 17.5 and 4.2, respectively, compared to mustard (*p* < 0.001) and maize (*p* = 0.011), while LMWOA exudation did not differ significantly among lupin and soy (*p* = 0.253; Fig. 4). Moreover, lupin exuded significantly more carboxyl groups when supplied with phytate than when supplied with $FePO_4$ (*p* = 0.008) or struvite (*p* = 0.024; Fig. 4). Similar findings were obtained when LMWOA data were normalized to BGB DW (Fig. S5a; Supplement). In contrast, maize, soy, and lupin exuded similar amounts of carboxyl groups per cm root length, which tended to be higher than of mustard, when P was provided in the form of struvite or $FePO_4$. When P was provided in the form of phytate, lupin tended to exude substantially more carboxyl groups per cm root length than the other plant species (Fig. S5b; Supplement).

Irrespective of P source, maize exuded mainly aconitate, followed by citrate and malate. Soy exuded mainly malonate, followed by citrate and malate. Lupin exuded mainly citrate, followed by malate. Mustard exuded mainly malate, followed by citrate. Succinate, fumarate, and gluconate played a minor role in all plant species (Table 2).

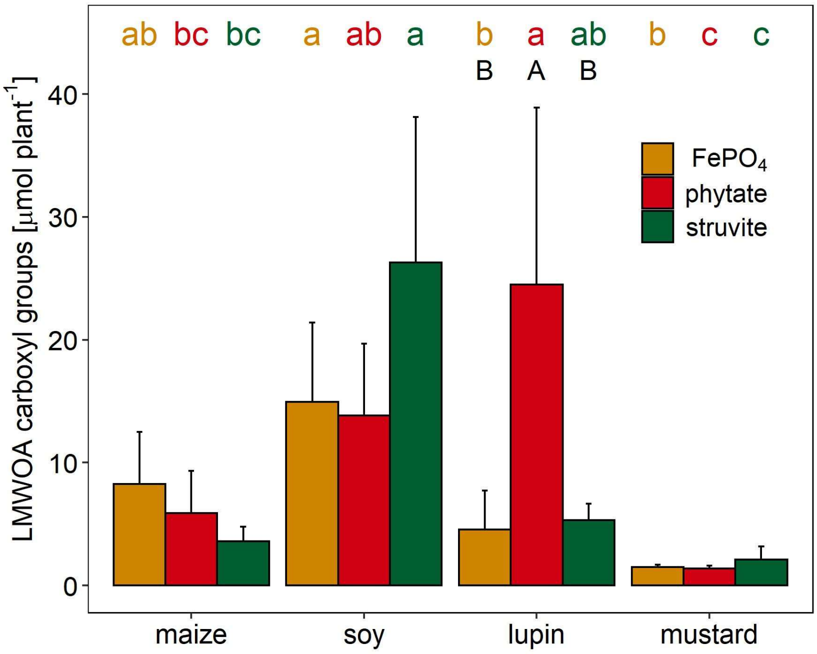

Fig. 4 Total number of LMWOA carboxyl groups in the exudate solution. Columns show means and error bars indicate standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each P source. Different capital letters indicate significant differences among the P sources, tested separately for each plant species. Absence of letters indicates that there was no significant difference

DOC exudation

When P was provided in the form of phytate, DOC exudation of soy was significantly higher, by a factor of 3.1, 2.4, and 2.0, respectively, than of mustard, maize, and lupin ($p < 0.001$; Table 2). When P was provided in the form of struvite, DOC exudation of soy was significantly higher, by a factor of 3.2, than DOC exudation of maize ($p = 0.009$), and slightly higher than DOC exudation of lupin and mustard ($p = 0.052$; Table 2). When P was provided in the form of $FePO_4$, DOC exudation of soy was slightly higher than of mustard ($p = 0.056$; Table 2).

Table 2 Gluconate (Glu), malate (Mal), malonate (Mao), succinate (Suc), fumarate (Fum), citrate (Cit), and aconitate (Aco) as well as dissolved organic carbon (DOC) in the exudate solution

Species	**P source**	**Glu [µmol plant^{-1}]**	**Mal [µmol plant^{-1}]**	**Mao [µmol plant^{-1}]**	**Suc [µmol plant^{-1}]**	**Fum [µmol plant^{-1}]**	**Cit [µmol plant^{-1}]**	**Aco [µmol plant^{-1}]**	**DOC [mg plant^{-1}]**
Maize	$FePO_4$	0.03 ± 0.01^{b}	0.32 ± 0.19^{b}	0.05 ± 0.01^{c}	0.04 ± 0.01	0.00 ± 0.00^{b}	0.26 ± 0.14^{b}	2.212 ± 1.218^{a}	0.77 ± 0.39
	Phytate	0.04 ± 0.02^{b}	0.34 ± 0.18^{b}	0.06 ± 0.01^{b}	0.05 ± 0.01	0.00 ± 0.00^{b}	0.44 ± 0.33^{c}	1.215 ± 0.686^{a}	0.70 ± 0.34^{b}
	Struvite	0.02 ± 0.01^{b}	0.26 ± 0.06^{c}	0.05 ± 0.01^{b}	0.04 ± 0.01	0.00 ± 0.00^{b}	0.34 ± 0.09^{c}	0.615 ± 0.420^{a}	0.44 ± 0.14^{b}
Soy	$FePO_4$	0.14 ± 0.07^{a}	2.19 ± 0.90^{a}	3.34 ± 1.77^{a}	0.06 ± 0.00	0.03 ± 0.02^{a}	1.19 ± 0.78^{a}	0.000 ± 0.000^{b}	1.77 ± 0.89
	Phytate	0.20 ± 0.05^{a}	1.49 ± 0.57^{a}	2.70 ± 1.31^{a}	0.06 ± 0.02	0.03 ± 0.01^{a}	1.69 ± 1.19^{b}	0.000 ± 0.000^{c}	1.68 ± 0.18^{a}
	Struvite	0.12 ± 0.05^{a}	2.58 ± 0.90^{a}	5.17 ± 3.09^{a}	0.06 ± 0.01	0.02 ± 0.00^{a}	3.50 ± 1.51^{a}	0.000 ± 0.000^{b}	1.43 ± 0.45^{a}
Lupin	$FePO_4$	0.04 ± 0.02^{ab}	0.60 ± 0.67^{b}	0.11 ± 0.07^{b}	0.08 ± 0.06	0.00 ± 0.00^{b}	0.97 ± 0.55^{aB}	0.000 ± 0.000^{bB}	0.87 ± 0.80
	Phytate	0.04 ± 0.02^{b}	0.72 ± 0.39^{b}	0.08 ± 0.03^{b}	0.06 ± 0.02	0.00 ± 0.00^{b}	7.57 ± 4.52^{aA}	0.005 ± 0.003^{bA}	0.82 ± 0.18^{b}
	Struvite	0.03 ± 0.00^{ab}	0.50 ± 0.13^{b}	0.07 ± 0.01^{ab}	0.05 ± 0.01	0.00 ± 0.00^{b}	1.35 ± 0.37^{bB}	0.004 ± 0.001^{aAB}	0.52 ± 0.18^{ab}
Mustard	$FePO_4$	0.02 ± 0.00^{b}	0.44 ± 0.07^{b}	0.00 ± 0.00^{d}	0.05 ± 0.01	0.00 ± 0.00^{b}	0.16 ± 0.02^{b}	0.000 ± 0.000^{b}	0.42 ± 0.04
	Phytate	0.02 ± 0.00^{b}	0.42 ± 0.10^{b}	0.00 ± 0.00^{c}	0.06 ± 0.01	0.00 ± 0.00^{b}	0.14 ± 0.01^{d}	0.000 ± 0.000^{c}	0.54 ± 0.19^{b}
	Struvite	0.04 ± 0.02^{b}	0.63 ± 0.38^{b}	0.04 ± 0.05^{b}	0.07 ± 0.03	0.00 ± 0.00^{b}	0.19 ± 0.06^{d}	0.000 ± 0.000^{b}	0.56 ± 0.15^{ab}

Numbers show means ± standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each P source. Different capital letters indicate significant differences among the P sources, tested separately for each plant species. Absence of letters indicates that there was no significant difference.

Discussion

In the present study, we found indications that soy had a particularly high potential to mobilize P from struvite and phytate, while mustard had a high potential to mobilize P from $FePO_4$. The underlying mechanisms of plant P mobilization from the different P sources are discussed in the following.

Soy effectively mobilized P from struvite likely through LMWOA exudation

Our finding that soy had the highest P content of all investigated plant species when supplied with struvite (Fig. 1) is in accordance with a recent study showing that soy mobilized P from struvite nearly as effectively as from the highly soluble triple superphosphate and more effectively than wheat (Rech et al. 2019). We further found that soy exuded more LMWOA, mostly malonate, citrate, and malate, than the other plant species when supplied with struvite (Fig. 4; Table 2). Thus, soy might have mobilized P from struvite via a high LMWOA exudation, since in water only 1–2% of struvite P is soluble while in citric acid solution about 50–100% of struvite P is soluble (Ahmed et al. 2018; Cabeza et al. 2011; Möller et al. 2018; Rech et al. 2019). Moreover, dicarboxylic LMWOA (malate and oxalate) have been shown to mobilize slightly more P from struvite than citrate (Talboys et al. 2016). Further, the low LMWOA exudation of lupin found here (Fig. 4) might explain the comparatively low P content of lupin when P was provided in the form of struvite (Fig. 1).

We further found that the root length of soy tended to be larger than of the other plant species (Fig. S3; Supplement). This might have been advantageous for P uptake by soy once P is mobilized, since a strong positive linear correlation between root length and P uptake has been reported earlier (Pang et al. 2015). This is further supported by our finding that mustard had both the second highest plant P content (Fig. 1) and the second largest root length when supplied with struvite (Fig. S3; Supplement), while LMWOA exudation of mustard was low (Fig. 4). The advantage of a large root length for P mobilization from finely ground struvite has also been suggested for other *Brassicaceae* (Brennan and Bolland 2001; Lyu et al. 2016; Wen et al. 2021). By contrast, maize and lupin had comparatively low root lengths (Fig. S3; Supplement), which might be the reason for the lower P content of maize and lupin (Fig. 1). Our finding that mustard had the second highest plant P content when supplied with struvite (Fig. 1) is in accordance with studies on other *Brassicaceae* such as canola, in which P mobilization from struvite was high (Ahmed et al. 2018; Brennan and Bolland 2001; Katanda et al. 2016).

Mustard effectively mobilized P from $FePO_4$ likely through rhizosphere alkalinization

Our finding that mustard had the highest P concentration of all investigated plant species when supplied with $FePO_4$ (Table 1) indicates that mustard has a high potential to mobilize P from $FePO_4$. This is in accordance with a previous study reporting that the *Brassica* oilseed rape mobilized more P from $FePO_4$ than wheat and different legumes (Pearse et al. 2007). We further found that the pH in the exudate solution of mustard was higher than of the other plant species (Fig. 3). Thus, mustard might have mobilized P from $FePO_4$ via rhizosphere alkalinization since the solubility of $FePO_4$ increases with increasing pH (Hinsinger 2001; Lindsay 1979). Such pH increases in the rhizosphere have also been found for other

Brassicaceae, which increased the rhizosphere pH by up to one pH unit compared to bulk soil (Marschner et al. 2007). Moreover, a significant positive correlation between the pH of rhizosphere extracts and leaf P concentration has been reported for the *Brassica* oilseed rape when supplied with various P sources including $FePO_4$ (Pearse et al. 2007). We further found that the root length of mustard tended to be larger than of maize and lupin when supplied with $FePO_4$ (Fig. S3; Supplement), which might have been advantageous for P uptake by mustard, as discussed above. A significant positive correlation between shoot P uptake and total root length of different *Brassicaceae* and *Poaceae* supplied with $FePO_4$ has been reported earlier (Marschner et al. 2007; Wang et al. 2007a). The authors suggested that the large root length allowed the *Brassicaceae* to access a greater soil volume than wheat, resulting in root foraging for P in addition to P mining. However, it was also reported that P uptake and root length of the *Brassicaceae* correlated mainly in early growth stages (Marschner et al. 2007; Wang et al. 2007a).

The P concentration of lupin supplied with $FePO_4$ was significantly higher than of lupin supplied with phytate (Table 1). A higher capacity to mobilize P from $FePO_4$ than from phytate has also been reported for white lupin and chickpea (Shu et al. 2007; Wang et al. 2007b). However, since pH in the exudate solution of lupin was significantly lower than of mustard (Fig. 3) and LMWOA exudation of lupin was significantly lower than of soy and lower with $FePO_4$ than phytate (Fig. 4), the mechanisms of P mobilization from $FePO_4$ by lupin remained largely unclear. This indicates that LMWOA amount and composition as well as pH in the exudate solution alone do not explain lupins' ability to mobilize P from $FePO_4$, as has also been found in another experiment comparing different plant species and their utilization of different P sources (Pearse et al. 2007). However, lupin has been shown to effectively mobilize P from $FePO_4$ in another experiment, likely through a high LMWOA exudation (Schwerdtner et al. 2022).

Legumes differed in their response to phytate

When P was provided in the form of phytate, plant P content of lupin was significantly lower than of soy (Fig. 1), while phosphomonoesterase activity was considerably higher in the rhizosphere of lupin than of soy (Fig. 3). One explanation for the contrasting findings among lupin and soy might be that soy exuded more phytases capable of catalyzing phytate hydrolysis, whereas lupin exuded mainly other phosphomonoesterases, not capable of hydrolyzing phytate. This is in accordance with former studies showing that the phytase activity of lupin (and other plant species) contributed less than 5% to total phosphatase activity (Gilbert et al. 1999; Hayes et al. 1999; Richardson et al. 2000), whereas soy has been shown to have a high phytase activity relative to other phosphatases (Ramesh et al. 2011). We further found that the maximum phosphomonoesterase activity per root surface, determined by soil zymography, was similar in the rhizosphere of lupin and soy (Figs. S2 and S4; Supplement), indicating that soy also had a few root regions with very high phosphomonoesterase activities. We cannot exclude that the phytase activity by soy was not (fully) detected by our analyses since (some) phytases might specifically catalyze the hydrolysis of phytate, but not of other phosphomonoesters (such as 4-methylumbelliferyl phosphate used in our analyses), thus potentially underestimating the total phosphomonoesterase activity of soy (German et al. 2011; Oh et al. 2004; Turner et al. 2002).

We further found that soy exuded significantly more DOC than the other plant species (Table 2). These organic substances (including LMWOA) might act as substrate for microorganisms, which produce additional phytases that effectively hydrolyze phytate in the rhizosphere of soy, as demonstrated earlier (Lambers et al. 2008; Wang and Lambers 2020; Wu et al. 2018). This is supported by previous studies showing that the addition of commercial fungal phytases (Hayes et al. 2000; Sun et al. 2021) as well as the inoculation with phytate-mineralizing bacteria (Ramesh et al. 2011; 2014; Richardson et al. 2000) increased P availability from phytate for soy, wheat, and several pasture species. Similarly, mycorrhizal symbionts have been found to effectively mobilize P from phytate (Wang et al. 2017; Zhang et al. 2016). Thus, in the case of soy (but not of non-mycorrhizal lupin), the so-called tripartite symbiosis, i.e., a double symbiosis with rhizobia and mycorrhizal fungi, might have contributed to phytate mineralization, as reported earlier (Bai et al. 2017; Jia et al. 2004).

Taken together, the P content of soy was highest among the investigated plant species in our study indicating effective P mobilization by soy. Soy mobilized P from phytate likely via high enzyme activity and high DOC exudation, and from struvite likely via high LMWOA exudation.

Implications

Overall, our study indicates that plant responses to different P sources were plant species-specific rather than P source-specific. The plant species-specific differences in P mobilization could be utilized to design multi-species plant communities that sustainably improve plant P nutrition in agriculture. For instance, the reliance on phosphate rock-derived fertilizers in agriculture could be reduced in intercropping systems with plant species that have complementary P mobilization capacities (Homulle et al. 2022; Honvault et al. 2021; Sulieman and Mühling 2021). The plant P content of maize was generally lower than of soy, mustard, and lupin (Fig. 1) and maize P concentrations (Table 1) indicate that maize P acquisition was relatively low (Reuter and Robinson 1997). Thus, maize plants could potentially benefit from intercropping with one of the other plant species if the different plant species have a joint rhizosphere in which complementary and facilitative interactions can occur. Such positive intercropping effects on maize have been proposed earlier in various studies on intercropping (Homulle et al. 2022; Li et al. 2020a; Schwerdtner and Spohn 2021; Tang et al. 2021). Moreover, plant species that effectively mobilize P from different soil P pools (such as soy and mustard) could potentially be used in crop rotations and/or as cover crops with likely positive effects on the P uptake of subsequent crops (Hallama et al. 2019).

Conclusion

In the present study, we found plant species-specific responses to different P sources. In particular, we found that soy had a high potential to mobilize P from struvite and phytate, while mustard had a high potential to mobilize P from $FePO_4$. Our findings suggest that soy effectively mobilized P from struvite via a high LMWOA exudation, which might be further promoted by its long roots. This partly confirms our first hypothesis on P mobilization via

LMWOA from struvite, while it needs to be rejected for $FePO_4$. Our findings further suggest that mustard effectively mobilized P from $FePO_4$ via a rhizosphere alkalinization, confirming our second hypothesis. We further found that soy but not lupin was capable of effectively mobilizing P from phytate, while phosphomonoesterase activity was considerably higher in the rhizosphere of lupin than of soy, indicating that the phosphomonoesterases that were determined here likely do not hydrolyze phytate. Our third hypothesis that a high phosphomonoesterase activity in the rhizosphere effectively mobilizes P from phytate needs, therefore, to be rejected.

Taken together, particularly soy and mustard were capable of mobilizing P from inorganic and organic sources through species-specific mechanisms. Thus, these plant species with their specific P mobilization mechanisms offer a chance to reduce the reliance of agricultural production on phosphate rock-derived fertilizers.

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Data Availability

The data generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Supplementary Information

Table S1 LMWOA with number of carboxyl groups and their characteristic retention times and mass traces used for HPLC-MS analyses in both experiments

LMWOA	Number of carboxyl groups	Retention time [min]	Recorded mass traces [(M-H)$^-$]
Gluconic acid	1	1.55	195.00–195.10
Malic acid	2	1.70	133.00–133.10
Malonic acid	2	1.92	103.00–103.10
Succinic acid	2	2.30	117.00–117.10
Fumaric acid	2	2.55	115.00–115.10
Citric acid	3	2.15	191.00–191.10
Aconitic acid	3	3.05	173.00–173.10

Table S2 Aboveground (AGB) and belowground biomass (BGB) P concentrations and P contents of maize, soy, lupin, and mustard grown with three different P sources

Species	P source	AGB P [mg g^{-1}]	BGB P [mg g^{-1}]	AGB P [mg plant^{-1}]	BGB P [mg plant^{-1}]
Maize	$FePO_4$	1.22 ± 0.17^{cB}	0.72 ± 0.06^{bB}	1.03 ± 0.33	0.17 ± 0.08^{b}
	phytate	1.09 ± 0.18^{cB}	0.66 ± 0.13^{bB}	1.01 ± 0.29^{bc}	0.18 ± 0.08^{b}
	struvite	1.92 ± 0.19^{bA}	1.02 ± 0.12^{bA}	1.29 ± 0.05^{d}	0.20 ± 0.05^{b}
Soy	$FePO_4$	1.97 ± 0.37^{bB}	1.02 ± 0.39^{bB}	2.07 ± 1.07^{B}	0.32 ± 0.24^{abB}
	phytate	2.69 ± 0.54^{aB}	0.81 ± 0.23^{bB}	3.56 ± 0.70^{aB}	0.31 ± 0.16^{bB}
	struvite	4.68 ± 0.64^{aA}	3.56 ± 1.11^{aA}	5.64 ± 0.41^{aA}	1.09 ± 0.53^{aA}
Lupin	$FePO_4$	2.70 ± 0.13^{aB}	1.55 ± 0.42^{bB}	1.64 ± 0.52^{B}	0.37 ± 0.31^{ab}
	phytate	1.56 ± 0.14^{bC}	1.11 ± 0.14^{bB}	0.78 ± 0.25^{cC}	0.23 ± 0.07^{b}
	struvite	4.46 ± 0.63^{aA}	3.86 ± 1.01^{aA}	2.81 ± 0.08^{cA}	0.74 ± 0.44^{ab}
Mustard	$FePO_4$	2.95 ± 0.41^{aAB}	3.79 ± 0.73^{aAB}	2.02 ± 0.41^{B}	0.72 ± 0.22^{aB}
	phytate	2.44 ± 0.37^{aB}	3.00 ± 0.37^{aB}	1.91 ± 0.34^{bB}	0.58 ± 0.10^{aB}
	struvite	4.07 ± 1.20^{aA}	4.80 ± 0.39^{aA}	3.98 ± 0.65^{bA}	1.20 ± 0.22^{aA}

Numbers show means ± standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each P source. Different capital letters indicate significant differences among the P sources, tested separately for each plant species. Absence of letters indicates that there was no significant difference.

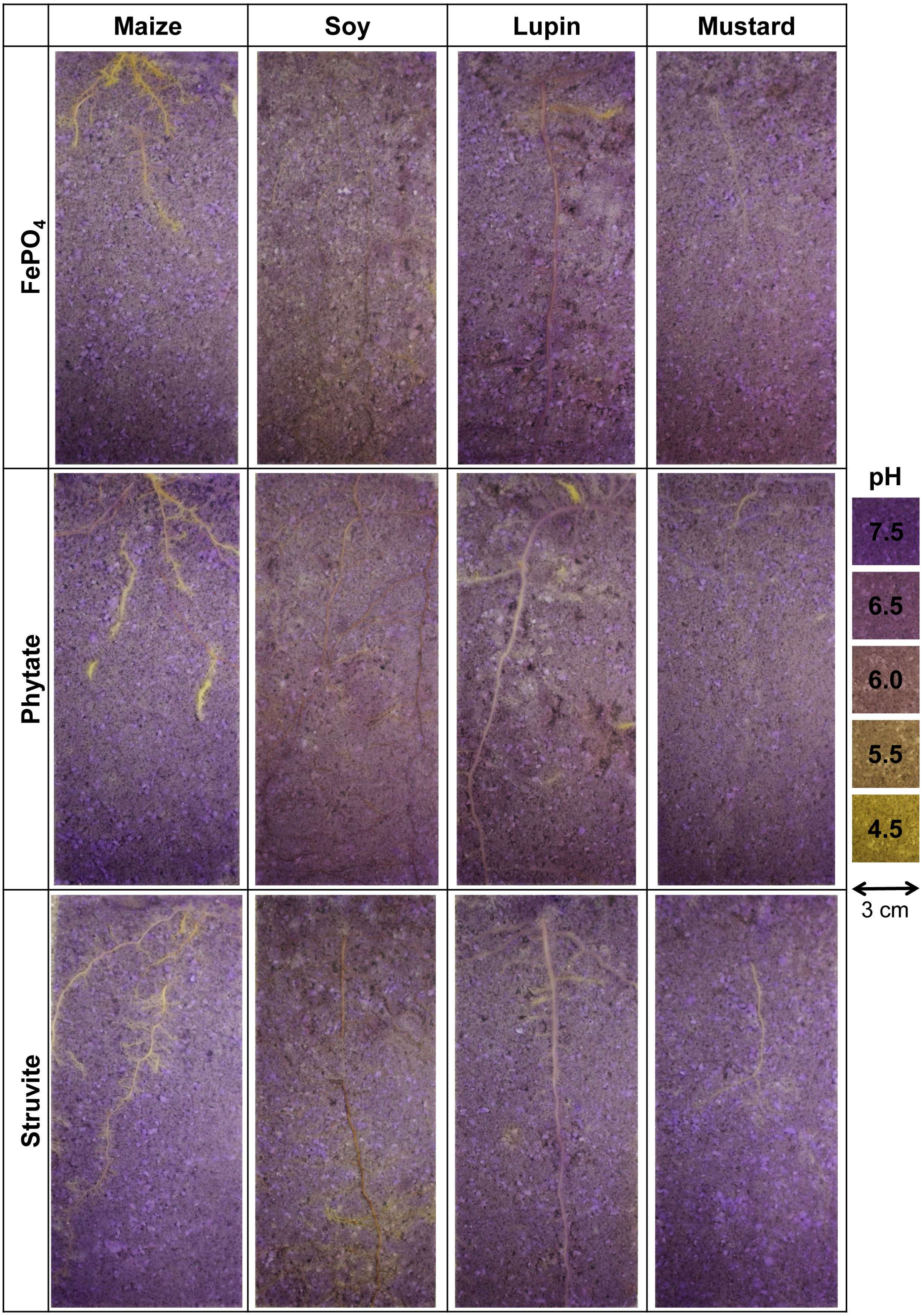

Fig. S1 Representative pH images of each plant species supplied with one out of the three P sources.

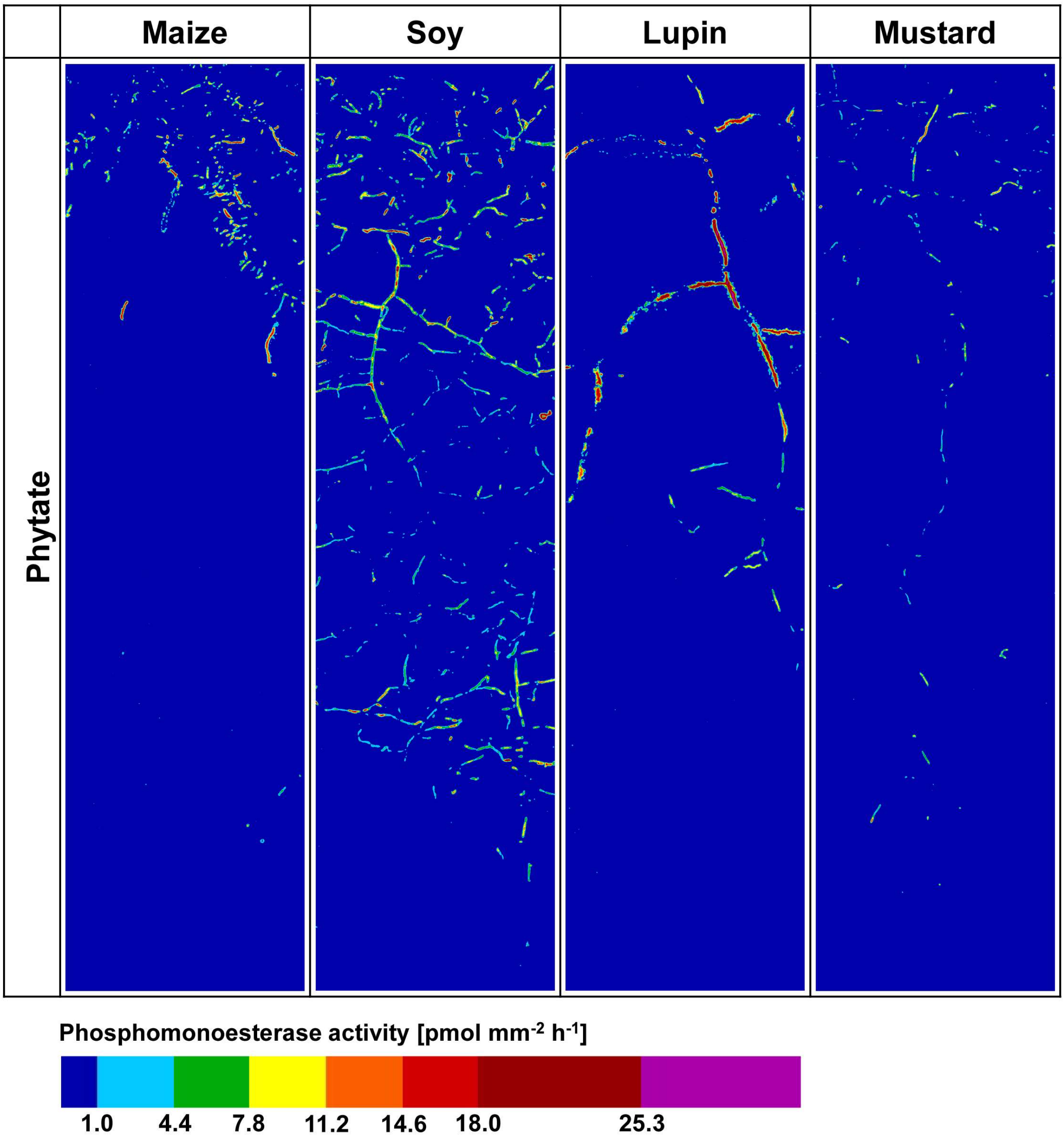

Fig. S2 Representative zymograms of each plant species when P was provided in the form of phytate.

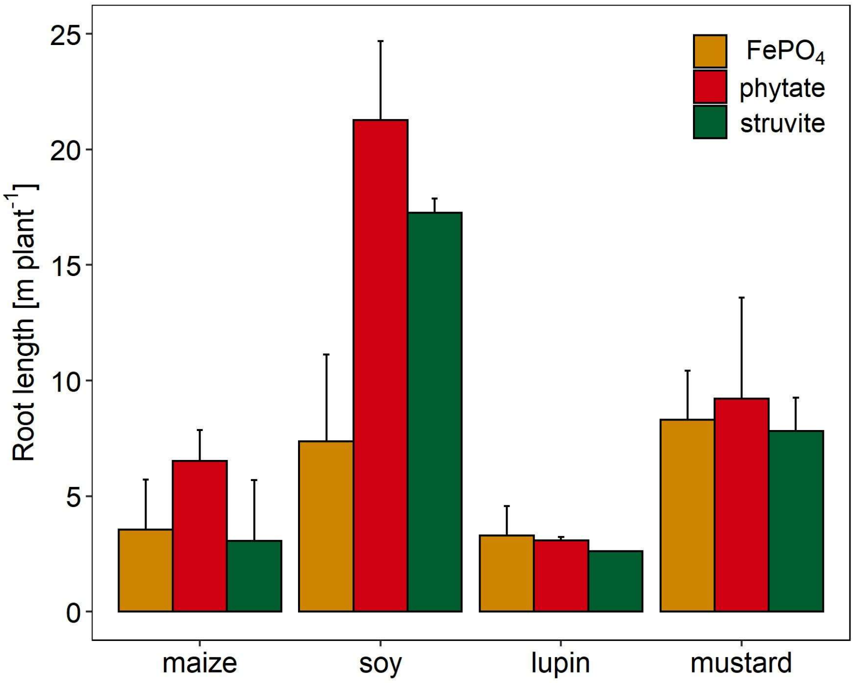

Fig. S3 Root length of two individuals per plant species and P source. Columns show means and error bars indicate standard deviations. No statistical analyses were conducted.

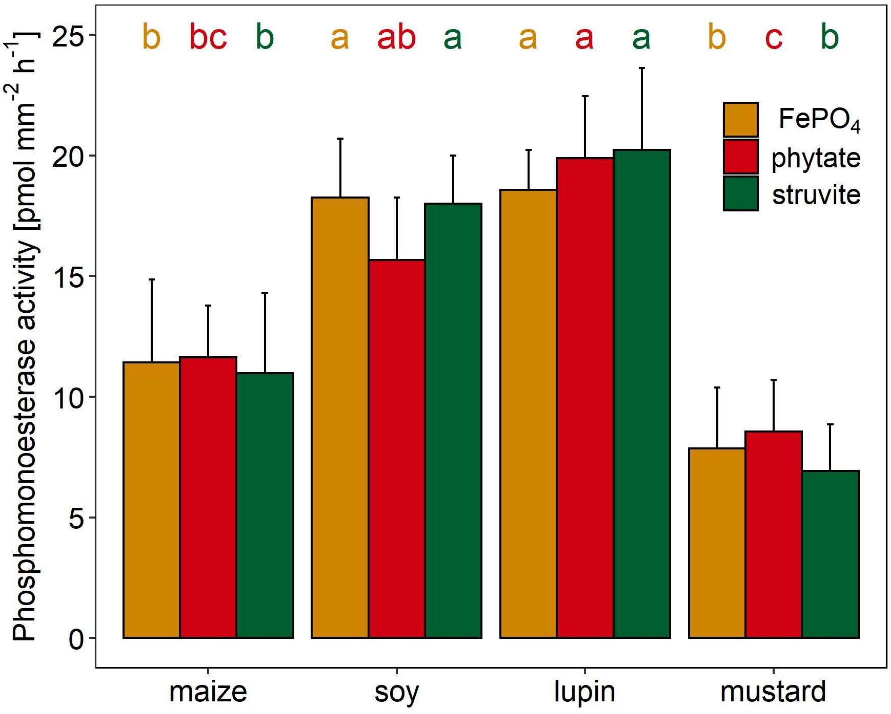

Fig. S4 Maximum phosphomonoesterase activity in the rhizosphere, determined by soil zymography six weeks after plant emergence. Columns show means and error bars indicate standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each P source. No significant difference was found among the P sources, tested separately for each plant species.

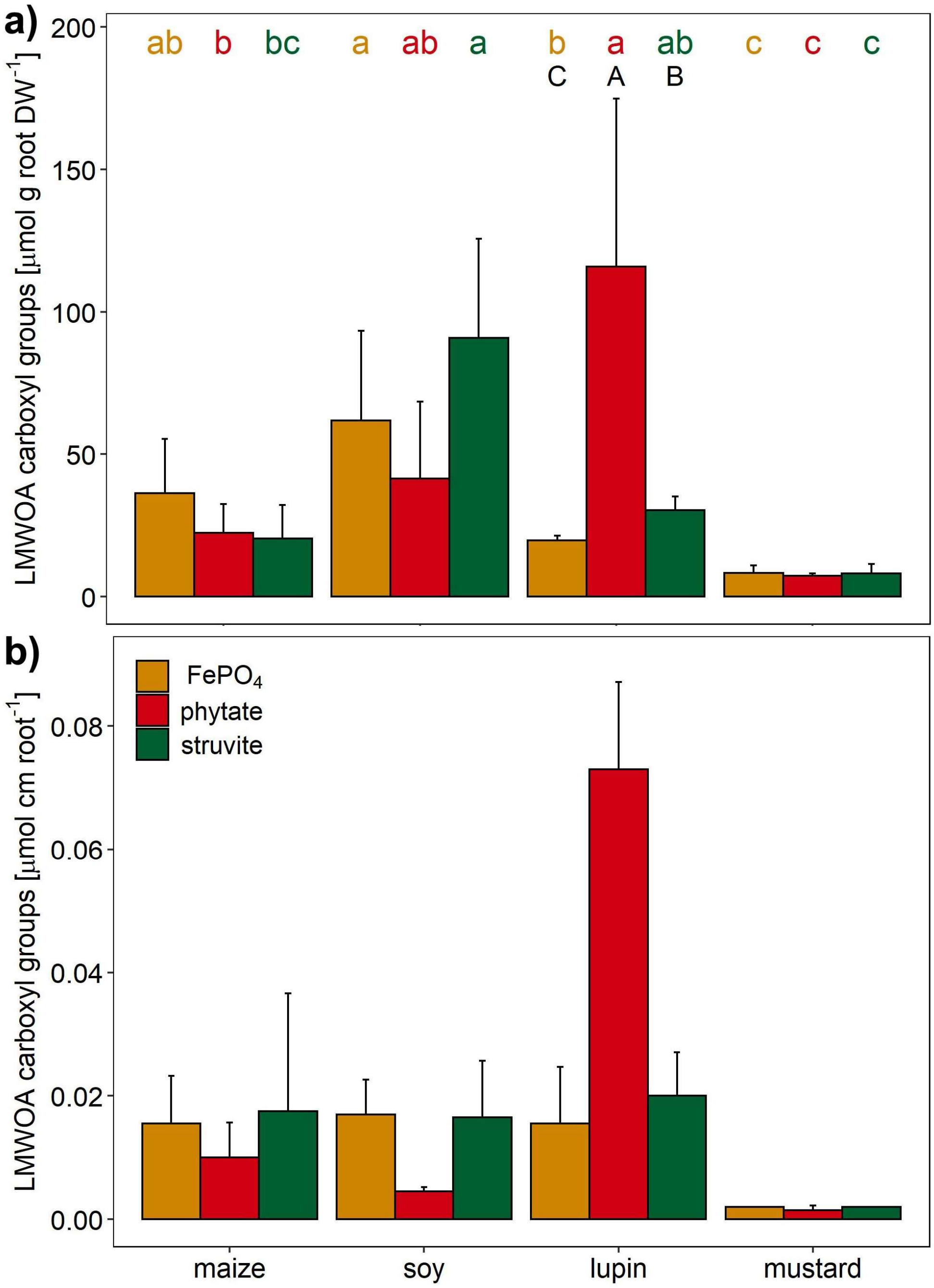

Fig. S5 Total number of LMWOA carboxyl groups in the exudate solution, calculated per gram root dry weight (a) and per cm root length (b). Columns show means and error bars indicate standard deviations. In panel a, different lowercase letters indicate significant differences ($p < 0.05$) among the plant species, tested separately for each P source. Different capital letters indicate significant differences among the P sources, tested separately for each plant species. Absence of letters indicates that there was no significant difference. In panel b, no statistical analyses were conducted ($n = 2$).

Study IV – Greenhouse experiment with a defined P source

Lupin causes maize to increase organic acid exudation and phosphorus concentration in intercropping

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Abstract

Purpose: There is a need to develop agricultural practices that mobilize sparingly soluble soil phosphorus (P) due to increasing scarcity of P fertilizer. Interactions of different plant species in the rhizosphere might increase P mobilization, but the underlying mechanisms are still not fully understood.

Methods: We conducted a pilot study with four plant species (maize, soy, lupin, mustard) grown alone and in combination with maize (intercropping) to investigate how species interact to mobilize P from iron phosphate ($FePO_4$). Root exudates of individual plants were collected and analyzed for low molecular weight organic acid anions (LMWOA) and pH.

Results: Maize increased its exudation of LMWOA and its biomass P concentration in intercropping, especially when grown together with lupin. This is the first study to show unequivocally that a high LMWOA concentration in the rhizosphere in intercropping is not only caused by high LMWOA release of the companion but also by an increased LMWOA exudation of the main crop. The high release of LMWOA was associated with a higher maize P concentration, indicating that enhanced LMWOA release in intercropping is beneficial for P acquisition of maize. Moreover, lupin and mustard mobilized more P from $FePO_4$ than maize and soy likely through high LMWOA exudation (lupin) and rhizosphere alkalinization (mustard).

Conclusion: Taken together, we reveal that intercropping with lupin increases the release of LMWOA by maize and concurrently the maize P concentration, suggesting that intercropping is useful for the mobilization of P from $FePO_4$ because it affects the exudation of maize.

Keywords: intercropping, iron phosphate, organic acid anions, pH, phosphorus mobilization

1 | INTRODUCTION

Phosphorus (P) is an essential macronutrient that often limits plant growth in agriculture if not provided as fertilizer. Most P fertilizer today is produced from phosphate rock which is a finite resource that will likely be exhausted during the next decades (Cordell & White, 2014). Thus, there is a need to reduce the reliance on phosphate rock-derived fertilizers (Ashley et al., 2011; Cordell & White, 2014). As iron phosphate ($FePO_4$) is formed in municipal wastewater treatment plants (Wilfert et al., 2015), it could potentially be used as a renewable P fertilizer, thus increasing P recycling. Several studies have already investigated $FePO_4$ as potential alternative, yet sparingly soluble P source for plants (e.g., H. Li et al., 2010; L. Li et al., 2007; Marschner et al., 2007; Pearse et al., 2007; Sega et al., 2019). Since likely only some plant species are capable of mobilizing P from $FePO_4$, intercropping could be beneficial for the P acquisition of associated non-P-mobilizing plant species. However, the underlying mechanisms of plant P mobilization from $FePO_4$ are still not fully understood, especially not in intercropping.

Plants have developed several mechanisms to mobilize P from sparingly soluble sources (Hinsinger, 2001; Richardson et al., 2011; Tang et al., 2021). Among others, alkalinization of the rhizosphere caused by the exudation of OH^- (or HCO_3^-) potentially mobilizes P from $FePO_4$ since the solubility of $FePO_4$ increases with increasing pH (Hinsinger, 2001; Lindsay, 1979). Moreover, the exudation of low molecular weight organic acid anions (LMWOA) potentially mobilizes P from $FePO_4$ via ligand exchange reactions by replacing P and chelating (i.e., complexing) metal cations, such as Fe^{3+}, which prevent them from precipitating with phosphate ions (Richardson et al., 2011; Y. Wang & Lambers, 2020). LMWOA possess one to three carboxyl groups (COOH or COO^-) that are crucial for P mobilization. Generally, the P mobilization capacity of LMWOA increases with the number of carboxyl groups, making citrate very efficient in mobilizing P from $FePO_4$ (Jones, 1998; Tsado et al., 2014; Y. Wang & Lambers, 2020). However, only some plant species exude LMWOA at high rates or change the rhizosphere pH substantially. For instance, legumes (*Fabaceae*) and especially lupins (*Lupinus* L. spp.) commonly have high LMWOA exudation, while many mustard species (*Brassicaceae*) cause rhizosphere alkalinization (Marschner et al., 2007; Pearse et al., 2007; Y. Wang & Lambers, 2020).

Intercropping, that is, the simultaneous cultivation of at least two plant species, has been shown to increase grain yields as it elevates nutrient availability, and has other positive effects (L. Li et al., 2014; C. Li et al., 2020). Intercropping might also be beneficial for plant P mobilization from $FePO_4$ since plant species with a low capacity to mobilize sparingly available nutrients might benefit from a high LMWOA exudation or changes of the rhizosphere pH by P-mobilizing companions when roots intermingle (L. Li et al., 2014; Xue et al., 2016). For instance, different lupins have been shown to solubilize sparingly available P through a high LMWOA exudation causing increased P uptake of intercropped cereals (Dissanayaka et al., 2017). Especially white lupin has been reported to chelate metal cations (Fe^{3+}, Al^{3+}, Ca^{2+}) through the release of citrate, thus increasing P availability (Cu et al., 2005; Gardner et al., 1983; Gardner & Boundy 1983; H. Li et al., 2010). Moreover, different *Brassicaceae* have been shown to substantially alkalize their rhizosphere which increases P mobilization from $FePO_4$

(Marschner et al., 2007; Pearse et al., 2007). However, P uptake of wheat was decreased or not affected by intercropping with these different *Brassicaceae* (D. Wang et al., 2007). Taken together, even though intercropping might be a promising approach to improve plant P uptake, it is still not fully understood how different plant species interact to mobilize P in intercropping.

Increased growth and yields of cereals in legume/cereal intercropping have often been explained by the P mobilization ability of legumes, for example, through the release of LMWOA, from which cereals also benefit (L. Li et al., 2014; Tang et al., 2021; Zhang et al., 2016). However, some studies found that cereals also enhanced their exudation of LMWOA in intercropping with different legumes compared to cereal monocropping. For instance, an increased concentration of different LMWOA such as malate, citrate and oxalate has been found in the rhizosphere soil of wheat in intercropping compared to monocropping when grown together with faba bean, white lupin or pea (C. Li et al., 2016; Lo Presti et al., 2021). Similarly, the malate concentration in the rhizosphere soil of maize was significantly increased by a factor of 12 in intercropping with faba bean compared to maize monocropping (H. Li et al., 2013). When maize was intercropped with alfalfa, similar findings were obtained, both in pot experiments (L. Wang et al., 2020) and in the field (Sun et al., 2020). Moreover, the LMWOA composition of maize also differed significantly between mono- and intercropping when maize was grown together with faba bean or white lupin supplied with $FePO_4$ (H. Li et al., 2010). However, in many of these studies it cannot be excluded that LMWOA released by the companion plants diffused to the roots of the main crop because LMWOA were collected from the rhizosphere soil (H. Li et al., 2010; C. Li et al., 2016; Lo Presti et al., 2021; L. Wang et al., 2020). Thus, it is not known if the LMWOA are derived from the cereal or from the companion plant. Therefore, an experiment that unequivocally separates root exudates released by the main and the companion crop is required to enlighten the effect that increases P mobilization in intercropping.

The aim of this pilot study was to investigate P mobilization from $FePO_4$ in intercropping. We hypothesized that maize increases its root exudation when grown together with a companion, and that maize P uptake from $FePO_4$ is increased in intercropping compared to monocropping. To test this hypothesis, we conducted a greenhouse experiment with four different crop species, and $FePO_4$ as the only P source. The species were maize (*Zea mays* L.), soy (*Glycine max* (L.) Merr.), blue lupin (*Lupinus angustifolius* L.) and white mustard (*Sinapis alba* L.). Maize was grown in rhizoboxes in combination with one of three companions (two individuals of different species in one rhizobox; intercropping), and in addition all species were grown alone (two individuals of the same species; monocropping). We selected contrasting companions for this experiment, two legumes (lupin and soy) and the *Brassica* white mustard. Root exudates were collected separately from all plant individuals using the soil-hydroponic-hybrid sampling approach, in contrast to previous studies that extracted LMWOA only from rhizosphere soil (H. Li et al., 2010; C. Li et al., 2016; Lo Presti et al., 2021; L. Wang et al., 2020). Root exudates were analyzed for LMWOA, pH and dissolved organic carbon (DOC). In addition, plant biomass, and P and Fe concentrations of the plants were determined.

2 | MATERIALS AND METHODS

2.1 | *Experimental setup*

We conducted an intercropping experiment with four plant species grown in rhizoboxes. The only P source available for the plants was iron phosphate, except for 0.5 mg P per rhizobox supplied with an inoculum (see below). Iron phosphate (iron(III) phosphate dihydrate: $FePO_4 \times 2\ H_2O$; Sigma-Aldrich, Merck KGaA) was thoroughly mixed to the mineral substrate, amounting to 422 mg P per rhizobox (88.8 mg P kg^{-1} substrate). The mineral substrate consisted of 20% (vol.) perlite and 80% (vol.) quartz sand, from which 50% (wt.) had a grain size of 0.1–0.4 mm and 50% (wt.) had a grain size of 0.7–1.2 mm. A mix of micronutrients (RADIGEN® Micronutrient mixed fertilizer; Terraflor GmbH) was added to the mineral substrate (900 mg $rhizobox^{-1}$), containing 5.0% MgO, 2.0% Fe, 1.5% Cu, 1.0% Mn, 0.8% Mo, 0.6% B and 0.5% Zn (further nutrients were supplied with a P-free nutrient solution as described below). The mineral substrate was filled into the rhizoboxes to a final bulk density of 1.1 g cm^{-3} which equals 4.75 kg of mineral substrate (dry weight [DW]) per rhizobox. Rhizoboxes were made of PVC and had an inner size of 49.2 × 29.3 × 3.0 cm (h × w × d).

In each rhizobox, two plants were sown at a distance of 15 cm. We used the following plant species: maize (*Z. mays* L. cv. Golden Bantam; Bingenheimer Saatgut AG), soy (*G. max* (L.) Merr. cv. Lica, Marktgesellschaft der Naturland Bauern AG), blue lupin (*L. angustifolius* L. cv. Rumba; Templiner Kräutergarten) and white mustard (*S. alba* L.; Bingenheimer Saatgut AG). All species were cultivated in monocropping, that is, two plant individuals of the same species. In addition, maize was cultivated in intercropping, that is, maize was grown together with one out of three companions (soy, lupin and mustard) in one rhizobox. All seeds except mustard were soaked in water for 24 h before seeds of a consistent size were sown in the rhizoboxes. Each of the seven combinations (maize/maize, soy/soy, lupin/lupin, mustard/mustard, maize/soy, maize/lupin, maize/mustard) was replicated four times (except for soy and mustard in monocropping of which one rhizobox each failed), resulting in a total of 26 rhizoboxes. The plants were sown in March and harvested in May 2020 after 70 days.

The rhizoboxes were placed in an open greenhouse at the University of Bayreuth under ambient conditions (i.e., around 20°C day temperature, automatic aeration above 23°C, no artificial light, automatic shading in case of strong sun exposure). The rhizoboxes were placed in a randomized block design on a wooden rack that kept them inclined by 50° throughout the experiment, and they were rearranged randomly after 5 weeks. The inclination of the rhizoboxes made the roots grow along the bottom wall of the rhizoboxes, which made it possible to remove the entire plant at harvest with very limited damage to the root system.

An inoculum was applied to the mineral substrate at the beginning of the experiment to introduce a soil microbial community. For this purpose, fresh soil (with a loamy sandy texture) was sampled from an agricultural field (for details see Schwerdtner & Spohn, 2021). The soil was sieved (< 2 mm), mixed with tap water (1:2) and shaken on an overhead shaker for 1 h before being filtered through cellulose filters (Rotilabo®, type 113P; Carl Roth GmbH & Co. KG). The filtrate was mixed with tap water to a final soil:water ratio of 1:4 and stored at 20°C over night before being applied to all rhizoboxes. The final soil inoculum had the following chemical properties (l^{-1} inoculum): 15.3 mg organic C, 4.4 mg N, 0.9 mg P and pH 7.9. Each rhizobox received 507 ml of soil inoculum and 140 ml tap water to adjust the mineral substrate to 75% water holding capacity (WHC). Priorly, the WHC of the mineral substrate was determined gravimetrically. For this purpose, an aliquot of the mineral substrate was oversaturated with water, drained for 24 h on a sand bath and weighed before and after drying at 105°C.

Rhizoboxes were watered every second day with tap water to 75% WHC as measured by weight. For this purpose, each rhizobox was weighed and water was added until the desired weight (which is the sum of the weights of the rhizobox, the mineral substrate and the water amount equivalent to 75% WHC) was reached. In addition, a P-free nutrient solution was applied regularly. For this purpose, an adapted Ruakura solution (Smith et al., 1983) was used where KH_2PO_4 was substituted by KNO_3 and K_2HPO_4 by K_2SO_4. The final nutrient solution applied to the rhizoboxes contained (l^{-1}): 220 mg $Mg(NO_3)_2 \times 6\ H_2O$, 746 mg $Ca(NO_3)_2 \times 4\ H_2O$, 377 mg NH_4NO_3, 189 mg KNO_3, 367 mg K_2SO_4, 27 mg Na_2SO_4, and 15 mg NaCl. In total, 15.8 mg N kg^{-1} substrate were applied in the form of inoculum and nutrient solution.

At harvest, 10 weeks (70 days) after emergence, root exudates were collected, and plants were analyzed for biomass production and P and Fe concentrations (see below).

2.2 | Root exudate collection

Root exudates were collected in sterile deionized water using the soil-hydroponic-hybrid sampling approach (Oburger & Jones, 2018). For this purpose, the bottom walls of the rhizoboxes were opened, and plants were removed as carefully as possible to prevent root damage. Roots were gently shaken and washed with deionized water to remove adhering substrate particles and potential metabolites (Oburger & Jones, 2018). The entire root system of the intact plant was then transferred to a sterile beaker that was filled with a known volume of sterile deionized water (between 75 and 175 ml) so that roots were completely submerged. We used sterile deionized water instead of a $CaCl_2$ solution since this reduces the background matrix for the analyses while not altering exudation patterns (Egle et al., 2003; Oburger & Jones, 2018). Three blanks, that is, three beakers with known volumes of sterile, deionized water but without plants, were also included and treated in the same way as all beakers. Plants in beakers were stored at 20°C in a climate chamber with artificial lighting (650 $\mu mol\ m^{-2}\ s^{-1}$) for 4 h. Subsequently, plants were removed from beakers, and beakers were swayed to homogenize the exudates in the solution. The exudate solutions were filtered through 0.2 µm syringe filters and three aliquots were frozen for subsequent analyses of LMWOA, pH and DOC. All plants were sampled in a way ensuring that exudate collection took place during peak metabolic activity, that is, collection started 3.5 ± 1 h after sunrise, as recommended in Oburger and Jones (2018). Since we used a relatively short exudate collection period and maintained very similar temperatures during plant growth and exudate collection, we assume that plant metabolism and, therefore, exudation patterns do not differ between growth and sampling conditions (Oburger & Jones, 2018).

2.3 | Biomass analyses

After the plants were removed from the exudate collection beakers, aboveground biomass (AGB) was separated from belowground biomass (BGB), dried at 60°C, weighed and milled. BGB was washed again with deionized water, dried at 60°C, weighed and milled.

The biomass samples (AGB and BGB of each plant) were analyzed for total P and Fe concentrations after pressure digestion in concentrated nitric acid using an inductively coupled plasma-optical emission spectroscopy (Vista-Pro radial, Varian Inc.).

2.4 | Exudate analyses

LMWOA were analyzed using high-performance liquid chromatography-mass spectrometry (HPLC-MS). For this purpose, the exudate samples were loaded on a HPLC RP-C18 column (Luna Omega 1.6 µm PS C18, 100 Å, 100 × 2.1 mm, Phenomenex Inc.; operated as part of an Ultimate 3000 HPLC, Thermo Fisher Scientific GmbH) which was connected to a Q Exactive mass spectrometer (Thermo Fisher Scientific GmbH) equipped with a hybrid quadrupole orbitrap mass analyzer (maximum mass range 50–6000 Da, resolution 140,000 @ *m/z* = 200). A 10 min isocratic elution with pure water (HPLC-grade, spiked with 0.2% formic acid) at a flow rate of 0.3 ml min^{-1} was applied. Mass spectra were acquired after electrospray ionization (ESI negative) in full scan mode (50 < *m/z* < 750) recording the total ion current. For evaluation (i.e., identification and integration/quantitation) of the LMWOA, their characteristic mass traces were used (Supporting Information: Table S1).

The pH of the exudate solution was measured with a pH electrode (WTW SenTix 51; Xylem Analytics GmbH & Co. KG).

DOC was analyzed using a Total Carbon Analyzer (TOC-TN Analyzer, multi N/C 2100, Analytik Jena GmbH).

2.5 | Calculations

The total biomass (TBM) was calculated as the sum of the DWs of AGB and BGB for each plant. The P and Fe concentrations of the TBM (plant P concentration in mg P g^{-1} TBM and plant Fe concentration in mg Fe g^{-1} TBM) were calculated based on the element concentrations of AGB and BGB and the DW of AGB and BGB for each plant. The element concentrations were used as means of assessing the nutritional status of the plants since the element concentrations mainly depend on the element availability in the growth medium (Mengel et al., 2001). To additionally show the total uptake of P and Fe by the plants, plant P and Fe contents (in mg plant^{-1}) were calculated by multiplying the plant element concentrations with the TBM.

The DOC concentration in the exudate solution (in mg l^{-1}) was multiplied by the volume of sterile deionized water in which roots were submerged to correct for the different volumes and gain results in mg per plant.

The exudation of each LMWOA (in µmol plant^{-1}) was calculated by multiplying the LMWOA concentrations in the exudate solution (in mg l^{-1}) with the volume of sterile deionized water and dividing by the molar mass of the respective LMWOA. The LMWOA exudation was also divided by the DW of BGB to gain results per g root DW. The concentration of dicarboxylic LMWOA (in µmol plant^{-1} or µmol g root DW^{-1}) was calculated as the sum of malate, malonate, succinate and fumarate. The concentration of tricarboxylic LMWOA was calculated as the sum of citrate and aconitate. The concentrations of mono- (gluconate), di- and tricarboxylic LMWOA (in µmol plant^{-1} or µmol g root DW^{-1}) were multiplied with the number of carboxyl groups (1, 2, or 3, respectively; Supporting Information: Table S1) to calculate the total number of carboxyl groups in the exudate solution.

The pH in the exudate solution (including blanks) was converted into the H^+ concentration, and subsequently the H^+ concentration was multiplied by the volume of sterile deionized water and reconverted into pH to correct for the different volumes.

2.6 | Statistical analyses

Before all statistical analyses, normality was checked with Shapiro–Wilk normality test, and homogeneity of variances was tested with Levene's test. To test for significant differences ($p < 0.05$) among maize in the different species combinations, we conducted an analysis of variance (ANOVA) followed by Tukey's post-hoc test (Tukey honestly significant difference) when normality and homogeneity assumptions were met, or a Kruskal–Wallis test followed by a post-hoc test using the criterium Fisher's least significant difference and Holm correction for *p* adjustment when normality and homogeneity assumptions were not met. To test for significant differences ($p < 0.05$) of the single companions between mono- and intercropping, we conducted a Wilcoxon rank sum test. Further, we conducted simple regressions (i.e., linear models) to identify correlations between response variables. All statistical analyses were performed in R (version 3.5.2; R Core Team, 2018) using the packages agricolae (1.3-2; Mendiburu, 2020), car (3.0-7; Fox & Weisberg, 2019), dplyr (0.8.5; Wickham et al., 2020), ggpattern (0.4.2; FC et al., 2022) and ggplot2 (3.3.0; Wickham, 2016).

3 | RESULTS

3.1 | Plant P and Fe

Maize P concentration was significantly increased in intercropping with lupin, by a factor of 1.4, compared to maize in monocropping ($p = 0.045$; Figure 1). P concentrations of soy, lupin and mustard were higher by a factor of 2.3–3.7 compared to maize, irrespective of cropping treatment (Figure 1). Maize P content tended to be increased by a factor of 1.4 in intercropping with lupin compared to maize monocropping, although the difference was not statistically significant ($p = 0.187$; Supporting Information: Figure S1). P contents of lupin and mustard were higher by a factor of 2.4–3.5 compared to maize, irrespective of cropping treatment (Table 1; Supporting Information: Figure S1). More P was allocated in AGB than BGB by all species (Supporting Information: Tables S2–S3).

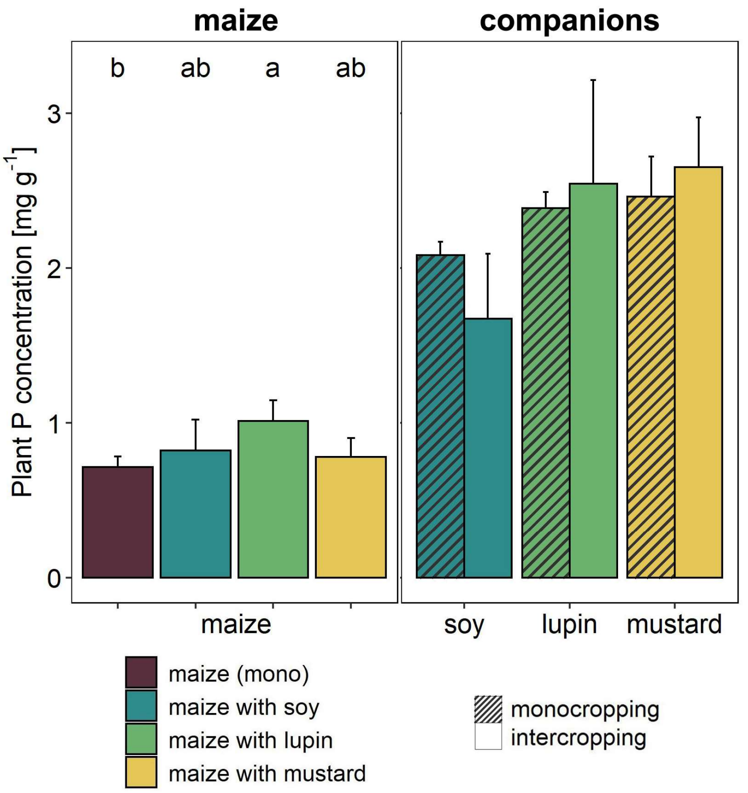

FIGURE 1 Plant P concentration of maize (left) and companions (right). Columns show means and error bars indicate standard deviations. Different lowercase letters indicate significant differences (p < 0.05) among maize in the different species combinations. There was no significant difference for single companions between mono- and intercropping.

TABLE 1 Dry weights of aboveground (AGB), belowground (BGB) and total biomass (TBM) as well as plant phosphorus content (plant P) of the four species grown in mono- and intercropping.

Species	Companion	AGB (g plant^{-1})	BGB (g plant^{-1})	TBM (g plant^{-1})	Plant P (mg plant^{-1})
Maize	Maize	2.55 ± 0.51	1.38 ± 0.54	3.93 ± 1.00	2.84 ± 0.91
	Soy	2.81 ± 0.84	1.14 ± 0.65	3.95 ± 1.49	3.27 ± 1.31
	Lupin	3.11 ± 0.91	1.50 ± 0.50	4.61 ± 1.29	4.56 ± 0.95
	Mustard	2.68 ± 0.89	1.18 ± 0.48	3.86 ± 1.35	3.07 ± 1.25
Soy	Soy	1.87 ± 0.41	0.51 ± 0.12	2.38 ± 0.53	4.94 ± 0.96
	Maize	1.93 ± 0.26	0.59 ± 0.08	2.52 ± 0.24	4.23 ± 1.17
Lupin	Lupin	1.86 ± 0.14	2.34 ± 0.84	4.20 ± 0.94	10.00 ± 2.10
	Maize	2.49 ± 1.93	1.99 ± 1.48	4.48 ± 3.39	10.00 ± 4.83
Mustard	Mustard	2.16 ± 0.18	0.67 ± 0.04	2.83 ± 0.21	6.92 ± 0.26
	Maize	2.56 ± 0.25	0.58 ± 0.06	3.14 ± 0.30	8.32 ± 1.12

Note: Numbers show means ± standard deviations. There was no significant difference among maize in the different species combinations or for single companions between mono- and intercropping.

Maize Fe concentration was significantly higher in in intercropping with lupin than with soy (p = 0.008; Figure 2). It also tended to be increased by a factor of 1.4 in intercropping with lupin compared to maize monocropping, albeit not statistically significantly (p = 0.274; Figure 2). Plant Fe content of lupin was higher by a factor of 3.8 compared to maize, irrespective of cropping treatment (Supporting Information: Figure S2). More Fe was allocated in BGB than AGB by all species (Supporting Information: Tables S2–S3).

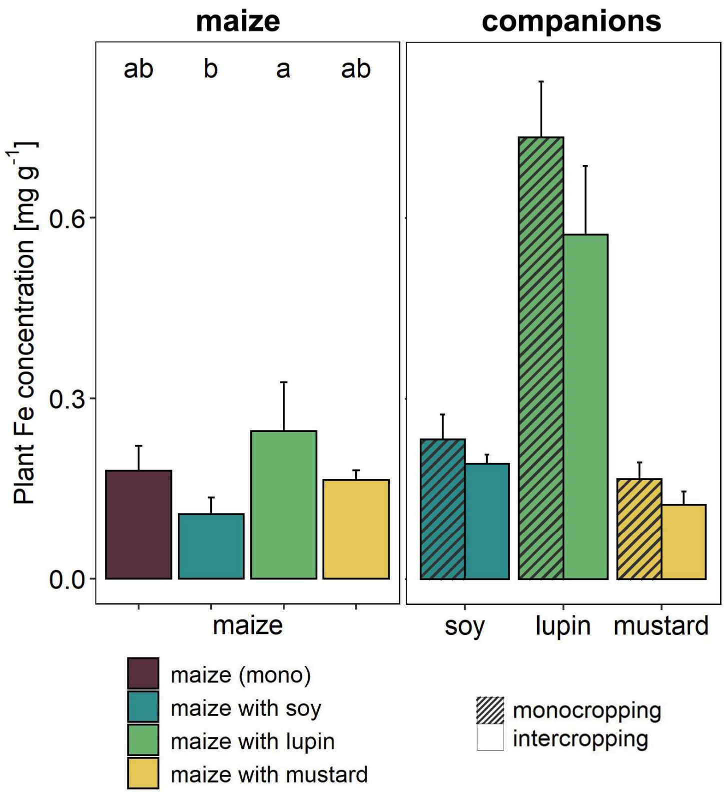

FIGURE 2 Plant Fe concentration of maize (left) and companions (right). Columns show means and error bars indicate standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among maize in the different species combinations. There was no significant difference for single companions between mono- and intercropping.

3.2 | Biomass

No significant difference in biomass production was found among maize in the different species combinations (Table 1). Only maize AGB and TBM in intercropping with lupin tended to be increased by a factor of 1.2 when compared to maize monocropping, although the differences were not statistically significant ($p = 0.765$ for AGB; $p = 0.879$ for TBM; Table 1).

3.3 | Exudation of LMWOA and DOC and pH

In intercropping with lupin, dicarboxylic LMWOA exudation by maize was significantly increased by a factor of 3.6 compared to maize monocropping ($p = 0.018$; Figure 3a; Table 2). In intercropping with lupin, tricarboxylic LMWOA exudation by maize tended to be increased by a factor of 2.6 compared to maize monocropping, albeit not statistically significantly ($p = 0.258$; Figure 3b; Table 2). The total number of carboxyl groups exuded by maize tended to be increased by a factor of 2.6 in intercropping with lupin compared to maize monocropping, albeit not statistically significantly ($p = 0.202$; Table 2).

Similar trends in maize exudation were observed when LMWOA were calculated on BGB rather than plant basis (Figure 3c; Supporting Information: Table S4). The total number of carboxyl groups exuded by maize per gram root DW was significantly increased by a factor of

2.6 in intercropping with lupin compared to maize monocropping (*p* = 0.048; Figure 3c). It was also slightly increased by a factor of 2.2 in intercropping with mustard compared to maize monocropping (*p* = 0.094; Figure 3c). Moreover, tricarboxylic LMWOA exuded by maize per gram root DW was slightly increased by a factor of 2.6 in intercropping with lupin (*p* = 0.066), and tended to be increased by a factor of 2.2 in intercropping with mustard (*p* = 0.128), both compared to maize monocropping (Supporting Information: Table S4).

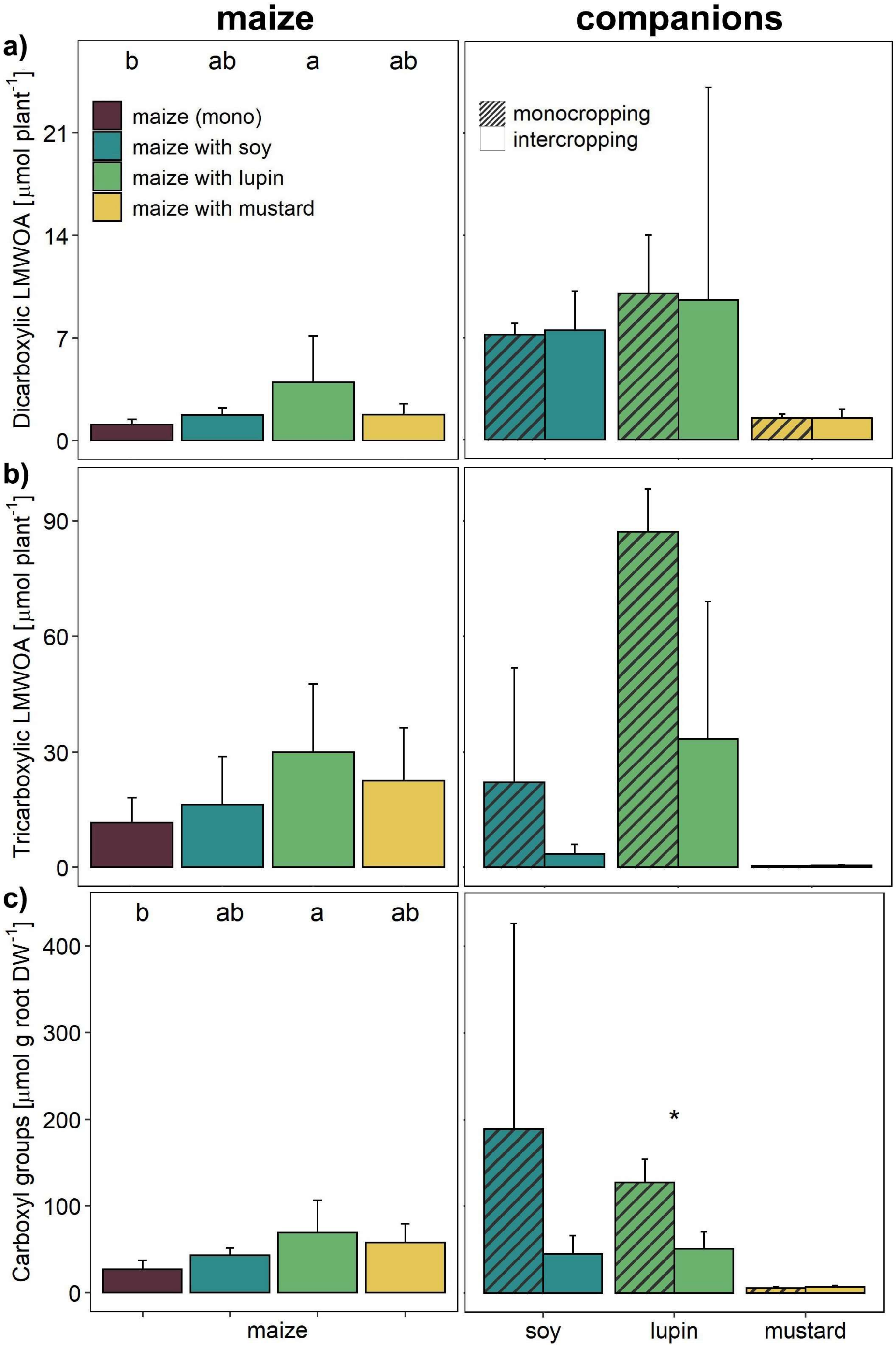

FIGURE 3 Dicarboxylic (a) and tricarboxylic (b) LMWOA in the exudate solution of maize (left) and companions (right), calculated per plant, as well as total number of carboxyl groups (c), calculated per gram root dry weight (see also Table 2 and Supporting Information: Table S4). Columns show means and error bars indicate standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among maize in the different species combinations, tested separately for each panel. Asterisks indicate significant differences ($p < 0.05$) between mono- and intercropping of single companions. LMWOA, low molecular weight organic acid anions.

TABLE 2 Gluconate (Glu), malate (Mal), malonate (Mao), succinate (Suc), fumarate (Fum), citrate (Cit) and aconitate (Aco) as well as the total number of carboxyl groups (COOH) and dissolved organic carbon (DOC), analyzed in the exudate solutions and calculated per plant (for data per gram root dry weight see Supporting Information: Table S4).

Species 1	**Species 2**	**Glu (µmol plant^{-1})**	**Mal (µmol plant^{-1})**	**Mao (µmol plant^{-1})**	**Suc (µmol plant^{-1})**	**Fum (µmol plant^{-1})**	**Cit (µmol plant^{-1})**	**Aco (µmol plant^{-1})**	**COOH (µmol plant^{-1})**	**DOC (mg plant^{-1})**
Maize	Maize	0.06 ± 0.02	0.93 ± 0.29^{b}	0.08 ± 0.05	0.07 ± 0.03^{b}	0.02 ± 0.01	4.49 ± 2.94	7.09 ± 3.65	37.0 ± 20.0	2.00 ± 0.85^{b}
	Soy	0.09 ± 0.03	1.50 ± 0.47^{ab}	0.11 ± 0.02	0.10 ± 0.03^{ab}	0.02 ± 0.00	3.48 ± 2.40	12.89 ± 10.31	52.6 ± 38.5	3.05 ± 1.44^{ab}
	Lupin	0.20 ± 0.19	3.63 ± 3.14^{a}	0.15 ± 0.08	0.17 ± 0.05^{a}	0.02 ± 0.00	12.26 ± 11.84	17.68 ± 6.25	98.0 ± 59.9	5.97 ± 4.03^{a}
	Mustard	0.12 ± 0.04	1.54 ± 0.72^{ab}	0.10 ± 0.03	0.11 ± 0.03^{ab}	0.02 ± 0.00	7.01 ± 9.68	15.42 ± 5.68	70.9 ± 42.8	3.45 ± 1.31^{ab}
Soy	Soy	0.17 ± 0.05	5.68 ± 1.11	1.33 ± 0.63	0.10 ± 0.01	0.13 ± 0.02	21.98 ± 29.89	0.02 ± 0.01	80.6 ± 90.4	2.65 ± 0.67
	Maize	0.17 ± 0.02	5.53 ± 2.11	1.73 ± 0.53	0.11 ± 0.02	0.16 ± 0.06	3.04 ± 2.74	0.39 ± 0.31	25.5 ± 8.7	3.19 ± 0.59
Lupin	Lupin	0.20 ± 0.06	9.51 ± 3.87	0.28 ± 0.06	0.15 ± 0.03	0.12 ± 0.03	87.01 ± 11.14	0.05 ± 0.01	281.5 ± 39.5	5.70 ± 1.38
	Maize	0.26 ± 0.40	9.09 ± 14.17	0.28 ± 0.27	0.09 ± 0.01*	0.12 ± 0.12	32.50 ± 34.92	0.87 ± 0.88	119.5 ± 136.6	7.90 ± 12.09
Mustard	Mustard	0.04 ± 0.01	1.19 ± 0.21	0.12 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.29 ± 0.06	0.01 ± 0.00	3.9 ± 0.7	1.13 ± 0.28
	Maize	0.04 ± 0.00	1.21 ± 0.53	0.11 ± 0.01	0.10 ± 0.03	0.08 ± 0.03	0.31 ± 0.05	0.13 ± 0.14	4.3 ± 1.1	1.05 ± 0.16

Note: Numbers show means ± standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among maize in the different species combinations. Asterisks indicate significant differences ($p < 0.05$) for single companions between mono- and intercropping. Absence of letters or asterisks indicates that there was no significant difference.

Maize exuded mainly aconitate and citrate, irrespective of cropping treatment. Soy in monocropping and lupin in both cropping treatments exuded mainly citrate, while soy in intercropping exuded mainly malate and citrate. Mustard exuded mainly malate, irrespective of cropping treatment (Table 2). Intercropping maize with the different companions also affected the composition of LMWOA exuded by maize. When maize was grown with lupin, the contribution of citrate and malate to the total number of carboxyl groups increased, while the contribution of aconitate decreased compared to maize monocropping. In contrast, when maize was grown with soy or mustard, the contribution of aconitate increased and the contribution of citrate decreased compared to maize monocropping (Table 2; contributions not shown).

DOC exudation of maize was significantly increased by a factor of 3.0 in intercropping with lupin compared to maize monocropping ($p = 0.030$; Table 2). The pH in the exudate solution of mustard was higher by 0.6 pH units compared to all other species, irrespective of cropping treatment (Figure 4). Mustard and, to a lesser extent, soy increased the pH in the exudate solution compared to the blanks (Figure 4).

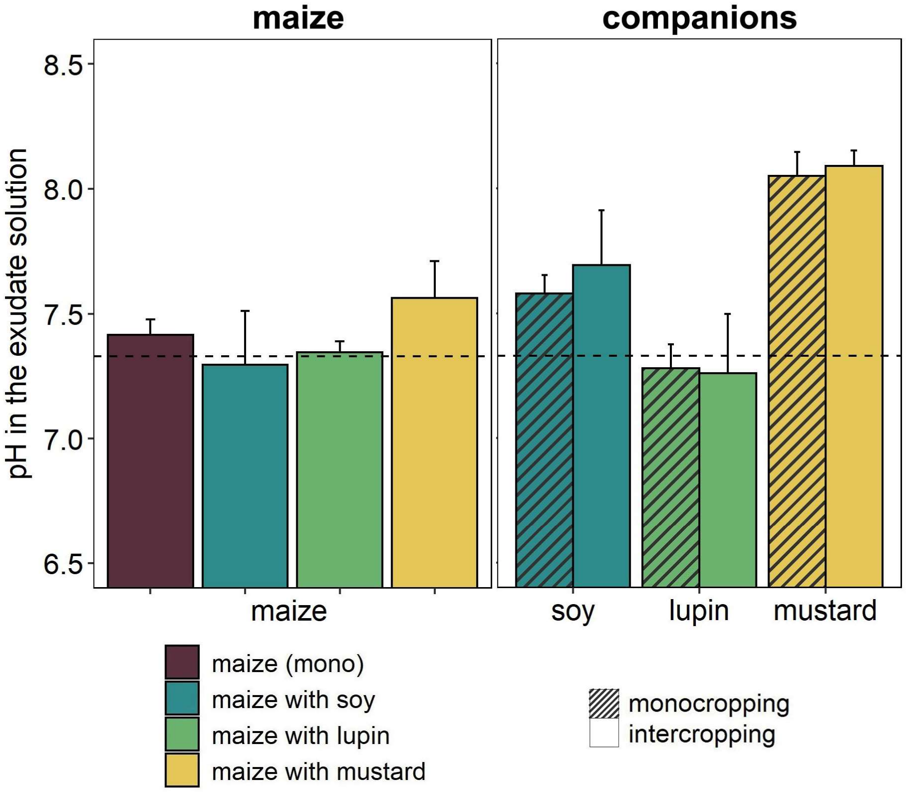

FIGURE 4 Mean pH in the exudate solution of maize (left) and companions (right). Columns show means and error bars indicate standard deviations. The dashed line shows the solution pH of blanks. There was no significant difference among maize in the different species combinations or for single companions between mono- and intercropping.

3.4 | Correlations

The total number of carboxyl groups exuded by maize was positively related with maize biomass, and the correlation was stronger for maize AGB ($R^2 = 0.582$; $p < 0.001$) and TBM ($R^2 = 0.487$; $p = 0.002$) than maize BGB ($R^2 = 0.248$; $p = 0.029$; Figure S3). We also found a positive correlation between the total number of carboxyl groups exuded by maize and the total plant P content per rhizobox which is the sum of maize P content and companion P content (R^2 = 0.578; *p* = 0.003; Supporting Information: Figure S4a). Further, maize P content increased with increasing LMWOA exudation by maize (R^2 = 0.407; *p* = 0.005; Supporting Information: Figure S4b).

4 | DISCUSSION

We found that lupin caused maize to increase its root exudation and its biomass P concentration in intercropping. This is the first study to show unequivocally that a high LMWOA concentration in the rhizosphere in intercropping is not only caused by high LMWOA release of the companion but also by an increased LMWOA exudation of maize when grown together with lupin. In addition, our study suggests that P from $FePO_4$ was likely mobilized via LMWOA exudation (particularly by lupin) and rhizosphere alkalinization (by mustard).

4.1 | Intercropping caused maize to increase root exudation and P concentration

We found that maize exuded generally more DOC (Table 2) and LMWOA (Figure 3) in intercropping with lupin than in maize monocropping, irrespective of the calculation basis (per plant or per g root DW) (Figure 3; Table 2 and Supporting Information: Table S4). These findings indicate that lupin caused maize to increase specific root exudates. This might, however, not be specific to lupin since DOC and LMWOA exudation by maize also tended to be enhanced when maize was grown together with mustard (compared to maize monocropping). Moreover, DOC and LMWOA exudation by maize were not significantly different among the intercropped maize plants in intercropping with lupin, soy and mustard (Table 2 and Supporting Information: Table S4; Figure 3). An increased LMWOA exudation by maize in intercropping with lupin, faba bean or alfalfa compared to maize monocropping has been reported earlier (H. Li et al., 2010, 2013; Sun et al., 2020; L. Wang et al., 2020). However, these previous studies explained the elevated LMWOA concentrations mainly by diffusion of LMWOA from legumes to maize roots, and their analysis of rhizosphere soil did not allow a distinction between the exudates of the two different plants (H. Li et al., 2013; C. Li et al., 2016). In contrast, our results show that exudation patterns of maize changed through intercropping. Since we collected the root exudates of both species separately from different plants and not from rhizosphere soil, we can exclude that the exudates diffused from companion roots to maize roots.

The main reason for the high LMWOA exudation of maize in intercropping with lupin is likely that maize tended to produce the largest biomass in this species combination, which was likely associated with more carbon being available for LMWOA synthesis (Curl & Truelove 1986; Dechassa & Schenk 2004). This is supported by a positive correlation between maize biomass and maize LMWOA exudation (Supporting Information: Figure S3). A positive relationship between maize biomass and maize exudation has been reported earlier (Groleau-Renaud et al., 1998). However, the higher maize biomass alone does not fully explain the enhanced LMWOA exudation by maize in intercropping since the relationship between maize biomass and LMWOA exudation was not very strong (R^2 = 0.487; Supporting Information: Figure S3). Moreover, maize TBM tended to be slightly increased only in intercropping with lupin (Table

1), whereas the LMWOA exudation by maize also tended to be slightly enhanced in intercropping with mustard (Table 2; Figure 3) where the maize biomass was not increased.

A second reason for the high LMWOA exudation of maize in intercropping was likely P (and Fe) deficiency. Maize has been found earlier to respond to P (and Fe) deficiency by exuding higher amounts of the same LMWOA for which we found the largest increases in intercropping (Carvalhais et al., 2011; Gaume et al., 2001). The exudation of LMWOA is commonly increased in response to P (and Fe) deficiency by many plant species (Canarini et al., 2019; Spohn et al., 2020; Y. Wang & Lambers, 2020). Hence, the increased LMWOA exudation by maize in intercropping compared to monocropping might additionally be explained by high interspecific competition for P (and Fe) since the companion plants took up more P than maize. Lupin took up 3.5 times more P than maize (Table 1; Supporting Information: Figure S1), which likely decreased soil P availability and thus triggered increased LMWOA exudation by maize (Figure 3). Further, mustard took up 2.7 times more P than maize (Table 1; Supporting Information: Figure S1), which likely decreased P availability in the rhizoboxes and promoted the slight increase in LMWOA exudation by maize in intercropping with mustard compared to maize monocropping (Figure 3). This is also indicated by our finding that the total number of carboxyl groups exuded by maize was positively correlated with the total P content, that is, the sum of maize P content and companion P content (Supporting Information: Figure S4a). Moreover, lupin took up significantly more Fe than all other species (Supporting Information: Figure S2), which likely caused Fe scarcity in the rhizosphere and further triggered increased LMWOA exudation by maize (Figure 3), as discussed above for P. LMWOA exudation of maize was likely more affected by lupin than mustard and soy (Figure 3) since mustard and soy took up substantially less Fe than lupin (Supporting Information: Figure S2). Thus, taken together, it might be the combined effect of improved maize growth and high interspecific competition for P (and Fe) in intercropping that caused maize to increase its root exudation in intercropping compared to maize monocropping. However, maize plants in monocropping likely also competed for P (and Fe) without affecting maize LMWOA exudation. Thus, it could also be the specific exudation profile of lupin that triggered maize to increase its exudation. Further research is, therefore, needed to examine in depth the underlying mechanisms of increased maize exudation in intercropping, and to test whether this is species-specific.

Our finding that maize P concentrations were significantly increased in intercropping with lupin compared to maize monocropping (Figure 1) indicates that maize P acquisition benefited from the presence of lupin. Similar beneficial effects of intercropping with lupin on maize biomass production and P uptake have been reported before and were related to a high LMWOA exudation by lupin that mobilized P from sparingly available P sources from which intercropped maize also benefited (Dissanayaka et al., 2017). Our results show that maize did not only benefit from the high LMWOA release by lupin, but also increased its own exudation. Direct evidence that maize P was increased due to the enhanced exudation of maize rather than of lupin cannot be provided by our pilot study. However, we found a positive correlation between LMWOA exudation by maize and maize P content (irrespective of companion species). This indicates that maize P content likely increased with an increasing number of carboxyl groups exuded by maize (Supporting Information: Figure S4b). However, the LMWOA concentration alone does not fully explain maize P contents since the relationship between maize exudation and maize P contents was not very strong ($R^2 = 0.407$; Supporting Information: Figure S4b), probably because LMWOA are only efficient in P mobilization once a specific threshold concentration in the rhizosphere is reached (McKay Fletcher et al., 2021). Root intermingling might, therefore, also play an important role since intercropped species could exude LMWOA into the same soil regions. This would create even higher LMWOA concentrations in these regions causing significant P mobilization which likely results in P facilitation in intercropping (McKay Fletcher et al., 2021). Thus, the enhanced maize P acquisition from $FePO_4$ in intercropping with lupin can potentially reduce the reliance of agricultural production on phosphate rock-derived fertilizers.

Further, our results show no intercropping effect of mustard on maize P uptake, indicating that mustard was a strong competitor for P that did not facilitate P uptake of maize. This is supported by a previous study reporting that several *Brassica* genotypes benefited from intercropping with wheat in terms of P uptake and biomass production, whereas growth and P uptake of intercropped wheat were rather negatively affected by the *Brassicaceae*, suggesting that wheat was a poorer competitor for P than the *Brassicaceae* (D. Wang et al., 2007). Similarly, our results show no significant effect of intercropping on maize P uptake in intercropping with soy (Figure 1; Table 1). Soy took up substantially less P than lupin and mustard (except for mustard in monocropping; Table 1). We further found that soy exuded substantially less tricarboxylic LMWOA than lupin (Figure 3b), and that the rhizosphere alkalinization by soy was lower than by mustard (Figure 4). This indicates that LMWOA exudation and pH changes by soy were not high enough to have beneficial effects on maize P or Fe acquisition. This is in accordance with a meta-analysis reporting a low P mobilization ability to soy (Tang et al., 2021).

4.2 | P mobilization by the companions

P concentrations of all companions (soy, lupin and mustard) were substantially higher than of maize (Figure 1) indicating their competitive advantage in P acquisition. Our findings suggest that lupin mobilized P from $FePO_4$ through a high exudation of LMWOA (Figure 3). The efficiency of different LMWOA to mobilize P depends, among other factors, on the type of LMWOA (Jones, 1998; Tsado et al., 2014; Y. Wang & Lambers, 2020). Lupin released mainly citrate and, to a lesser extent, malate (Table 2), which is in accordance with earlier findings (Egle et al., 2003; Pearse et al., 2007). Both citrate and malate have been found to efficiently mobilize P from $FePO_4$ (Dissanayaka et al., 2017; Jones, 1998), which might, at least partially, explain the high P concentration of lupin (Figure 1). This is in accordance with former experiments relating the biomass and P content of lupin to a high LMWOA exudation (Dissanayaka et al., 2017; Lelei & Onwonga, 2014). Especially the citrate exudation by white lupin has been reported to chelate metal cations (Fe^{3+}, Al^{3+}, Ca^{2+}), thus increasing P availability (Cu et al., 2005; Gardner et al., 1983; Gardner & Boundy, 1983; H. Li et al., 2010).

Our findings further suggest that mustard mobilized P from $FePO_4$ through rhizosphere alkalinization (Figure 4). Since the solubility of $FePO_4$ increases with increasing pH (Hinsinger, 2001; Lindsay, 1979), the rhizosphere alkalinization by mustard likely solubilized $FePO_4$

thereby rendering P available, which explains the relatively high biomass P concentration of mustard (Figure 1). Moreover, the rhizosphere alkalinization by mustard is in accordance with former experiments showing that *Brassica* genotypes increase the rhizosphere pH resulting in enhanced P mobilization from $FePO_4$ (Marschner et al., 2007; Pearse et al., 2007). Oilseed rape, for instance, mobilized more P from $FePO_4$ than wheat and different legumes (Pearse et al., 2007). However, $FePO_4$ precipitates in soil might include isomorphous substitutions which likely change the solubility compared to pure crystalline minerals which were used in the present study (Lindsay et al., 1989).

5 | CONCLUSIONS

Our pilot study challenges the common view that legume/cereal intercropping is advantageous over monocropping due to the high nutrient mobilization capacity of legumes (e.g., through high LMWOA exudation) from which the cereals simply benefit. Instead, it suggests that cereals themselves increase their LMWOA exudation in intercropping. Further, we showed that maize increases its P concentration in intercropping with lupin indicating that maize P acquisition benefited from the presence of lupin, which might be associated with the increased LMWOA release of both species. Thus, our results provide new insights into the mechanisms underlying P facilitation in intercropping which should be reaffirmed with larger sample sizes and additional plant species.

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DATA AVAILABILITY STATEMENT

The data generated and analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The authors confirm that they have adhered to the ethical policies of the journal.

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SUPPORTING INFORMATION

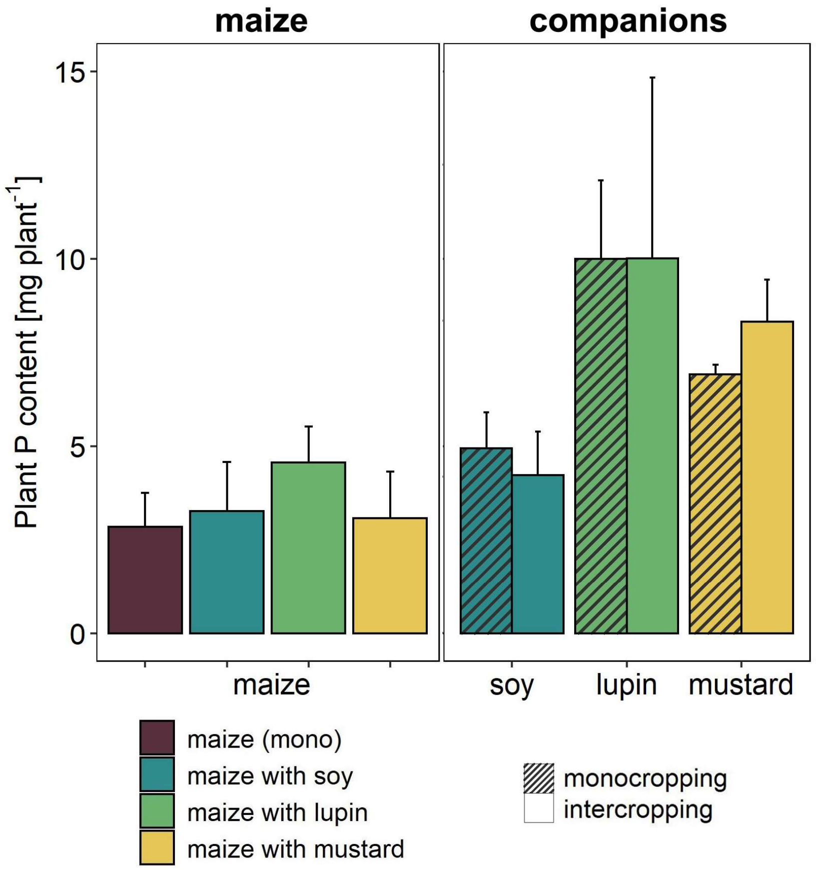

FIGURE S1 Plant P content of maize (left) and companions (right). Columns show means and error bars indicate standard deviations. There was no significant difference among maize in the different species combinations or for single companions between mono- and intercropping.

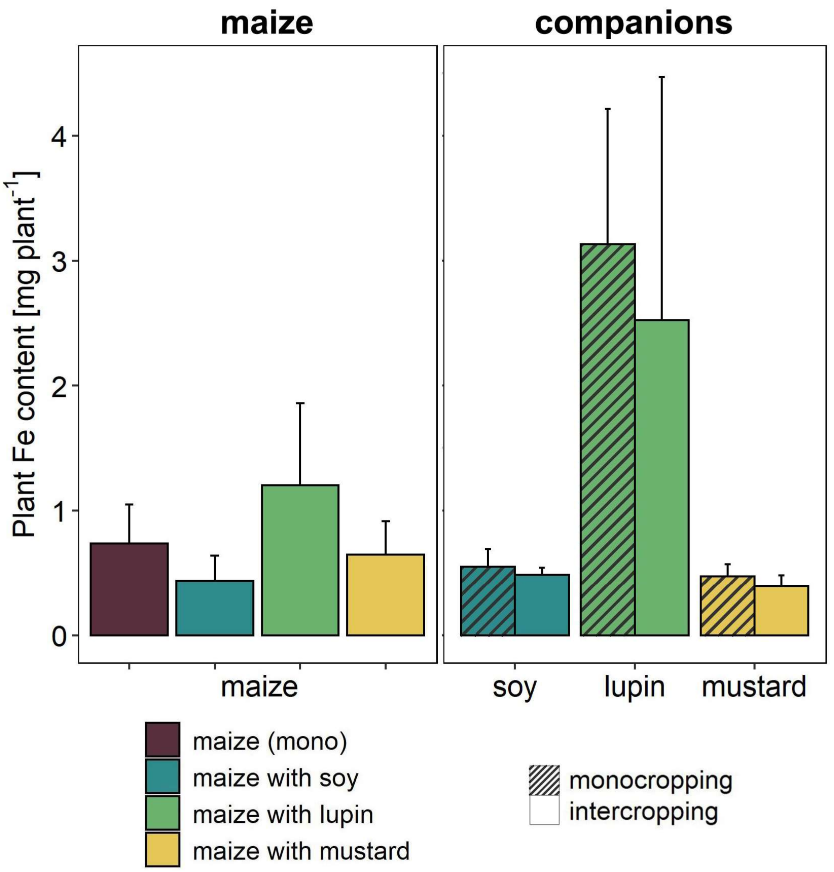

FIGURE S2 Plant Fe content of maize (left) and companions (right). Columns show means and error bars indicate standard deviations. There was no significant difference among maize in the different species combinations or for single companions between mono- and intercropping.

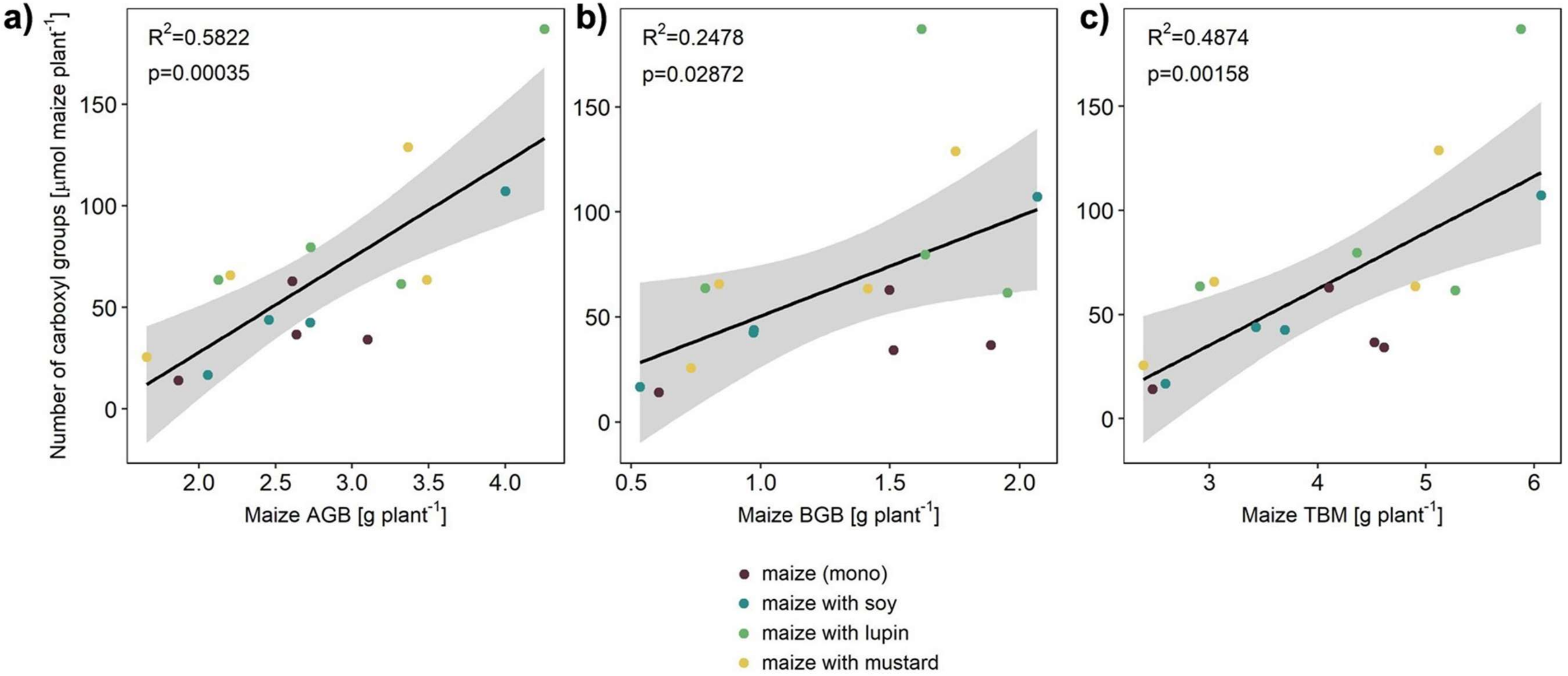

FIGURE S3 Total number of carboxyl groups in the exudate solutions of maize as a function of maize aboveground (a), belowground (b), and total biomass (c). Dots depict individual data points. Black line shows linear regression estimate, and the grey shade indicates the standard error of the estimate.

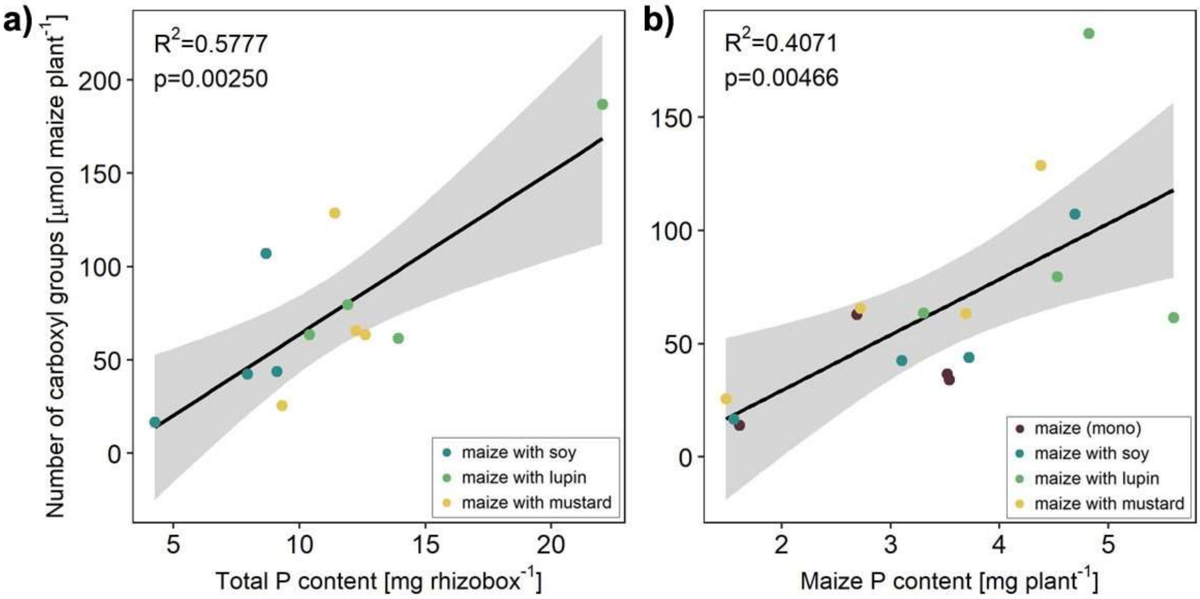

FIGURE S4 The total number of carboxyl groups exuded by maize as a function of total P content which is the sum of maize P content and companion P content (a) and maize P content (b). Dots depict individual data points. Black line shows linear regression estimate, and the grey shade indicates the standard error of the estimate.

TABLE S1 LMWOA with number of carboxyl groups and their characteristic retention times and mass traces used for HPLC-MS analyses

LMWOA	Number of carboxyl groups	Retention time [min]	Recorded mass traces [$(M-H)^-$]
Gluconate	1	1.55	195.00–195.10
Malate	2	1.70	133.00–133.10
Malonate	2	1.92	103.00–103.10
Succinate	2	2.30	117.00–117.10
Fumarate	2	2.55	115.00–115.10
Citrate	3	2.15	191.00–191.10
Aconitate	3	3.05	173.00–173.10

TABLE S2 Phosphorus (P) and iron (Fe) concentrations of aboveground (AGB) and belowground biomass (BGB) of the four species grown in mono- and intercropping

Species	Companion	AGB P [mg g^{-1}]	BGB P [mg g^{-1}]	AGB Fe [mg g^{-1}]	BGB Fe [mg g^{-1}]
Maize	Maize	0.82 ± 0.13	0.52 ± 0.09^{b}	0.038 ± 0.003	0.45 ± 0.09^{a}
	Soy	0.93 ± 0.27	0.58 ± 0.06^{b}	0.042 ± 0.010	0.28 ± 0.06^{b}
	Lupin	1.11 ± 0.21	0.83 ± 0.05^{a}	0.042 ± 0.006	0.69 ± 0.33^{a}
	Mustard	0.87 ± 0.14	0.58 ± 0.10^{b}	0.038 ± 0.003	0.46 ± 0.03^{a}
Soy	Soy	2.39 ± 0.16	0.96 ± 0.36	0.125 ± 0.014	0.63 ± 0.14
	Maize	1.91 ± 0.54	0.95 ± 0.15	0.100 ± 0.008	0.49 ± 0.05
Lupin	Lupin	3.79 ± 0.30	1.24 ± 0.07	0.615 ± 0.162	0.85 ± 0.09
	Maize	3.75 ± 1.09	1.07 ± 0.21	0.488 ± 0.177	0.68 ± 0.11
Mustard	Mustard	2.76 ± 0.31	1.49 ± 0.26	0.044 ± 0.001	0.57 ± 0.14
	Maize	2.88 ± 0.37	1.66 ± 0.26	0.040 ± 0.002*	0.49 ± 0.12

Numbers show means ± standard deviations. Different lowercase letters indicate significant differences ($p < 0.05$) among maize in the different species combinations. Asterisks indicate significant differences ($p < 0.05$) between mono- and intercropping for single companions. Absence of letters or asterisks indicates that there was no significant difference.

TABLE S3 Phosphorus (P) and iron (Fe) contents of aboveground (AGB) and belowground biomass (BGB) of the four species grown in mono- and intercropping

Species	Companion	AGB P [mg $plant^{-1}$]	BGB P [mg $plant^{-1}$]	AGB Fe [mg $plant^{-1}$]	BGB Fe [mg $plant^{-1}$]
Maize	Maize	2.10 ± 0.61	0.74 ± 0.33	0.10 ± 0.01	0.64 ± 0.30
	Soy	2.64 ± 1.05	0.63 ± 0.28	0.12 ± 0.03	0.32 ± 0.18
	Lupin	3.33 ± 0.59	1.23 ± 0.40	0.13 ± 0.03	1.07 ± 0.64
	Mustard	2.37 ± 0.96	0.70 ± 0.30	0.10 ± 0.03	0.54 ± 0.24
Soy	Soy	4.47 ± 0.98	0.47 ± 0.14	0.23 ± 0.05	0.32 ± 0.09
	Maize	3.66 ± 1.08	0.57 ± 0.15	0.19 ± 0.02	0.29 ± 0.05
Lupin	Lupin	7.09 ± 1.07	2.91 ± 1.10	1.16 ± 0.40	1.97 ± 0.69
	Maize	8.01 ± 3.56	1.99 ± 1.27	1.10 ± 0.66	1.43 ± 1.31
Mustard	Mustard	5.93 ± 0.17	0.98 ± 0.14	0.10 ± 0.01	0.38 ± 0.09
	Maize	7.36 ± 1.00	0.96 ± 0.14	0.10 ± 0.01	0.29 ± 0.08

Numbers show means ± standard deviations. There was no significant difference among maize in the different species combinations or between mono- and intercropping for single companions.

TABLE S4 Gluconate (Glu), malate (Mal), malonate (Mao), succinate (Suc), fumarate (Fum), citrate (Cit), and aconitate (Aco) as well as dissolved organic carbon (DOC), analyzed in the exudate solutions and calculated per gram root dry weight

Species 1	**Species 2**	**Glu [µmol g root DW^{-1}]**	**Mal [µmol g root DW^{-1}]**	**Mao [µmol g root DW^{-1}]**	**Suc [µmol g root DW^{-1}]**	**Fum [µmol g root DW^{-1}]**	**Cit [µmol g root DW^{-1}]**	**Aco [µmol g root DW^{-1}]**	**DOC [mg g root DW^{-1}]**
Maize	Maize	0.05 ± 0.03	0.82 ± 0.50	0.08 ± 0.07	0.07 ± 0.06	0.02 ± 0.02	3.09 ± 1.61	5.19 ± 1.85	1.65 ± 0.83
	Soy	0.09 ± 0.02	1.48 ± 0.46	0.12 ± 0.05	0.10 ± 0.03	0.02 ± 0.01	2.92 ± 0.97	10.26 ± 2.92	2.79 ± 0.27
	Lupin	0.14 ± 0.11	2.46 ± 1.83	0.11 ± 0.05	0.12 ± 0.05	0.02 ± 0.01	8.40 ± 7.04	12.81 ± 5.08	4.16 ± 2.41
	Mustard	0.10 ± 0.01	1.28 ± 0.31	0.09 ± 0.03	0.09 ± 0.02	0.02 ± 0.01	4.70 ± 5.11	13.61 ± 5.35	2.96 ± 0.69
Soy	Soy	0.34 ± 0.06	11.78 ± 4.60	2.53 ± 0.82	0.20 ± 0.03	0.26 ± 0.04	52.96 ± 76.61	0.05 ± 0.04	5.21 ± 0.22
	Maize	0.29 ± 0.07	9.41 ± 3.25	2.94 ± 0.77	0.18 ± 0.03	0.28 ± 0.14	5.79 ± 6.24	0.62 ± 0.48	5.46 ± 1.05
Lupin	Lupin	0.09 ± 0.02	4.02 ± 0.41	0.12 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	39.51 ± 8.84	0.02 ± 0.01	2.52 ± 0.41
	Maize	0.09 ± 0.08	3.06 ± 2.83	0.13 ± 0.03	0.06 ± 0.03	0.05 ± 0.01	14.34 ± 4.48*	0.47 ± 0.42*	2.72 ± 2.37
Mustard	Mustard	0.06 ± 0.01	1.80 ± 0.41	0.17 ± 0.04	0.14 ± 0.03	0.12 ± 0.03	0.43 ± 0.10	0.01 ± 0.00	1.70 ± 0.45
	Maize	0.07 ± 0.00	2.05 ± 0.74	0.19 ± 0.03	0.17 ± 0.03	0.13 ± 0.04	0.54 ± 0.13	0.21 ± 0.23	1.80 ± 0.22

Numbers show means ± standard deviations. There was no significant difference ($p < 0.05$) among maize in the different species combinations. Asterisks indicate significant differences between mono- and intercropping for single companions. Absence of asterisks indicates that there was no significant difference.

Contributions to the included manuscripts

Study I

Authors: Ulrike Schwerdtner, Marie Spohn

Title: Interspecific root interactions increase maize yields in intercropping with different companion crops

Status: published in 2021 in the *Journal of Plant Nutrition and Soil Science*

Own contribution: concept and study design 70%, experiment realization 85%, laboratory works 80%, data analyses 90%, discussion of results 60%, manuscript writing 70%

US and MS designed the study. US performed the experiment and the analyses with support from four Hiwis and four technicians (see acknowledgements). Parts of the chemical analyses were conducted by the chemical analytics (CAN) of the BayCEER (see acknowledgements). US and MS discussed the results. US created the figures and tables. US wrote a first draft of the manuscript. US and MS worked on the manuscript. All authors read, contributed to, and approved the final version of the manuscript. US is the corresponding author.

Study II

Authors: Ulrike Schwerdtner, Marie Spohn

Title: Plant species interactions in the rhizosphere increase maize N and P acquisition and maize yields in intercropping

Status: accepted for publication in the *Journal of Soil Science and Plant Nutrition*

Own contribution: concept and study design 70%, experiment realization 90%, laboratory works 85%, data analyses 90%, discussion of results 60%, manuscript writing 70%

US and MS designed the study. US performed the experiments and the analyses with support from four Hiwis, four technicians, and three colleagues (see acknowledgements). Parts of the analyses were conducted by the CAN and the Laboratory of Isotope Biogeochemistry of the BayCEER (see acknowledgements). US and MS discussed the results. US created the figures and tables. US wrote a first draft of the manuscript. US and MS worked on the manuscript. All authors read, contributed to, and approved the final version of the manuscript. US is the corresponding author.

Study III

Authors: Ulrike Schwerdtner, Ulrike Lacher, Marie Spohn

Title: Soy and mustard effectively mobilize phosphorus from inorganic and organic sources

Status: submitted to the journal *Nutrient Cycling in Agroecosystems* (under review)

Own contribution: concept and study design 75%, experiment realization 85%, laboratory works 70%, data analyses 90%, discussion of results 60%, manuscript writing 65%

US and MS designed the study. US performed the greenhouse experiment with support from three Hiwis and four technicians (see acknowledgements). US and UL performed plant and exudate analyses with support from one Hiwi and one technician (see acknowledgements). Parts of the chemical analyses were conducted by the CAN (see acknowledgements). US and MS discussed the results. US created the figures and tables. US wrote a first draft of the manuscript. US and MS worked on the manuscript. All authors read, contributed to, and approved the final version of the manuscript. US is the corresponding author.

Study IV

Authors: Ulrike Schwerdtner, Ulrike Lacher, Marie Spohn

Title: Lupin causes maize to increase organic acid exudation and phosphorus concentration in intercropping

Status: accepted for publication in the *Journal of Sustainable Agriculture and Environment*

Own contribution: concept and study design 75%, experiment realization 85%, laboratory works 70%, data analyses 90%, discussion of results 60%, manuscript writing 65%

US and MS designed the study. US performed the greenhouse experiment with support from one Hiwi and four technicians (see acknowledgements). US and UL performed plant and exudate analyses with support from one Hiwi and one technician (see acknowledgements). Parts of the chemical analyses were conducted by the CAN (see acknowledgements). US and MS discussed the results. US created the figures and tables. US wrote a first draft of the manuscript. US and MS worked on the manuscript. All authors read, contributed to, and approved the final version of the manuscript. US is the corresponding author.

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Publications (peer-reviewed)

Publications included in this thesis

Schwerdtner U, Spohn M (2021): Interspecific root interactions increase maize yields in intercropping with different companion crops. Journal of Plant Nutrition and Soil Science 184(5): 596–606.

Schwerdtner U, Spohn M (2022): Plant species interactions in the rhizosphere increase maize N and P acquisition and maize yields in intercropping. Journal of Soil Science and Plant Nutrition: accepted.

Schwerdtner U, Lacher U, Spohn M (2022): Soy and mustard effectively mobilize phosphorus from inorganic and organic sources. Nutrient Cycling in Agroecosystems: under review.

Schwerdtner U, Lacher U, Spohn M (2022): Lupin causes maize to increase organic acid exudation and phosphorus concentration in intercropping. Journal of Sustainable Agriculture and Environment: accepted.

Further publications

Aguilera N, Aburto F, Bustamante-Sánchez M, Acevedo M, González M, Oelmann Y, Schoebitz M, Spohn M, Schwerdtner U (in preparation): Effects of the co-cultivation of tree species with complementary nutrient acquisition strategies on nutrient availability and acquisition in a post-fire soil.

Schwerdtner U (2019): Gutes Essen für alle! Grundlagen einer antispeziesistischen Landwirtschaft von morgen. Zeitschrift für kritische Tierstudien 02: 155–179.

Schwerdtner U, Spohn M (2019): Mechanismen der Phosphor-Akquise von Nutzpflanzen in Mischkulturen: Die Rolle von Phosphatasen und pH-Änderungen in der Rhizosphäre. In: Mühlrath D, Albrecht J, Finckh M, Hamm U, Heß J, Knierim U, Möller D (Hrsg.): Innovatives Denken für eine nachhaltige Land- und Ernährungswirtschaft – Beiträge zur 15. Wissenschaftstagung Ökologischer Landbau, 05.–08. März 2019, Universität Kassel. Verlag Dr. Köster, Berlin: 20–21.

Further scientific contributions

Posters

Schwerdtner U, Spohn M (2018): Mechanisms of plant phosphorus acquisition in intercropping: The role of phosphatase activity and pH in the rhizosphere – Preliminary results. Poster at the 6th Symposium on Phosphorus in Soils and Plants (PSP6), KU Leuven (Belgium), September 2018.

Schwerdtner U, Spohn M (2018): Mechanisms of plant phosphorus acquisition in intercropping: The role of phosphatase activity and pH in the rhizosphere – Preliminary results. Poster at the 10th BayCEER Workshop, University of Bayreuth (Germany), October 2018.

Schwerdtner U, Spohn M (2019): Mechanismen der P- und N-Akquise von Nutzpflanzen in Mischkulturen: Die Rolle von Phosphatasen und pH-Änderungen in der Rhizosphäre. Poster auf der 15. Wissenschaftstagung Ökologischer Landbau, Universität Kassel, März 2019.

Schwerdtner U, Spohn M (2019): Nutrient acquisition in intercropping: Insights from a rhizobox study and a field experiment. Poster at the 9th International Phosphorus Workshop (IPW9), ETH Zurich (Switzerland), July 2019.

Aguilera N, Aburto F, Salazar F, Bustamante M, Acevedo M, Gonzalez M, Schwerdtner U, Oelmann Y (2020): Effect of tree native species assemblages on C, N & P contents of burned soils. Poster at the EGU General Assembly, online, May 2020.

Talks

Schwerdtner U (2019): Mechanismen der Phosphor-Akquise von Nutzpflanzen in Mischkulturen. Oral presentation at DBU-Stipendiat*innen-Seminar, Georgsmarienhütte, October 2019.

Schwerdtner U (2020): Mechanismen der Phosphor-Akquise in Mischkulturen. Oral presentation at DBU-Jour Fixe, online, September 2020.

Schwerdtner U, Spohn M (2021): Overyielding in maize-based intercropping through species-specific mechanisms of plant nutrient acquisition. Oral presentation at the Agricultural Chemistry Winter School on Interactions between biogeochemical cycles of elements in plant-soil-microbe systems, University of Torino (Italy), online, February 2021.

Declarations

(Eidesstattliche) Versicherungen und Erklärungen

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Hiermit versichere ich eidesstattlich, dass ich die Arbeit selbstständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe (vgl. Art. 64 Abs. 1 Satz 6 BayHSchG).

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Hiermit erkläre ich, dass ich die Dissertation nicht bereits zur Erlangung eines akademischen Grades eingereicht habe und dass ich nicht bereits diese oder eine gleichartige Doktorprüfung endgültig nicht bestanden habe.

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