



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

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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 ($\delta^{15}\text{N}$) 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 $\delta^{15}\text{N}$ 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 alkalization 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 (Marchner 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

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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 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, 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 $\delta^{15}\text{N}$) 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 ($\delta^{15}\text{N}$) 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) was 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. The 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:

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

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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values of maize leaves, shoots, and grains were used to calculate the $\delta^{15}\text{N}$ of the maize AGB based on the respective N concentrations, following He et al. (2009):

$$\delta^{15}\text{N}_{\text{maizeAGB}} = \frac{(\delta^{15}\text{N}_{\text{leaves}} \cdot N_{\text{leaves}} + \delta^{15}\text{N}_{\text{shoots}} \cdot N_{\text{shoots}} + \delta^{15}\text{N}_{\text{grains}} \cdot N_{\text{grains}})}{(N_{\text{leaves}} + N_{\text{shoots}} + N_{\text{grains}})} \quad (2)$$

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

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

The legume-derived N content in maize AGB was calculated based on $\text{PN}_{\text{legume}}$, as follows:

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

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 $4100 \times g$ 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 $1500 \times g$ 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 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

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

NO₃⁻-N and NH₄⁺-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 NO₃⁻ by ion chromatography (Metrohm 881 Compact IC pro, Metrohm AG, Switzerland) and for NH₄⁺ 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).

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).

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).

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. 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 ($^{\circ}p < 0.1$; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$), 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)

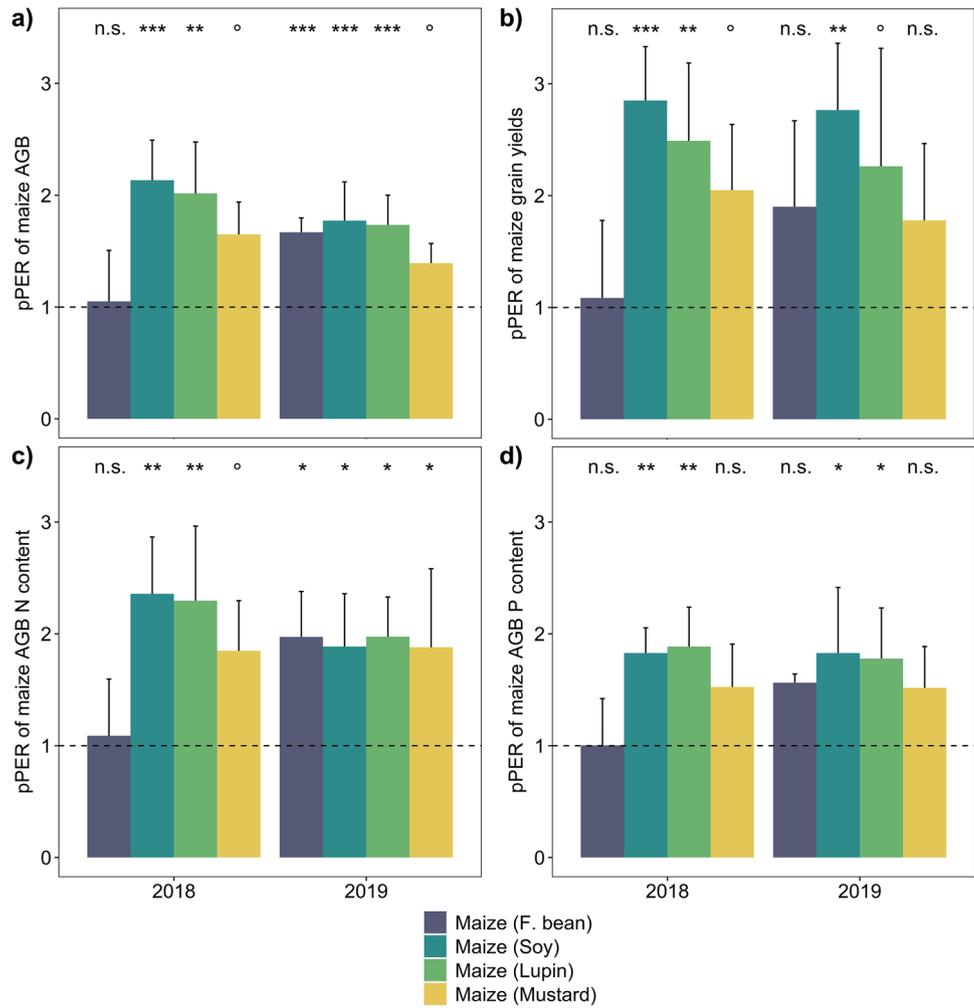


Table 1 Productivity of maize (grown together with the three companions) in terms of aboveground (AGB), belowground (BGB), and total biomass, as well as N and P concentrations of maize AGB and BGB, and N and P contents of maize total biomass, determined 12 weeks after sowing in the rhizobox experiment

	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

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 $10.9 \pm 9.5\%$ in faba bean/maize, lupin/maize, and soy/maize intercropping,

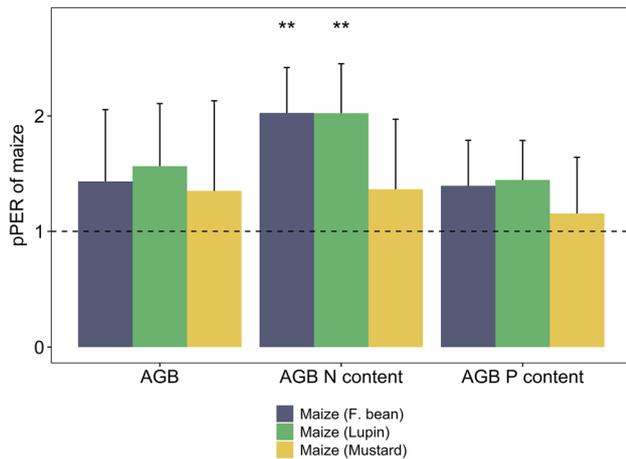


Fig. 2 Partial plant equivalent ratios (pPER) of maize aboveground biomass (AGB), maize AGB N content, and maize AGB P content, determined 12 weeks after sowing in the rhizobox experiment. Columns show means, and error bars indicate standard deviations. Symbols indicate that pPER were significantly different 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)

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

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 9 weeks ($p = 0.078$; Fig. 4). In contrast, mustard increased the rhizosphere pH by 0.7 pH units compared to the bulk soil

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 \pm 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

Fig. 3 $\delta^{15}\text{N}$ 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

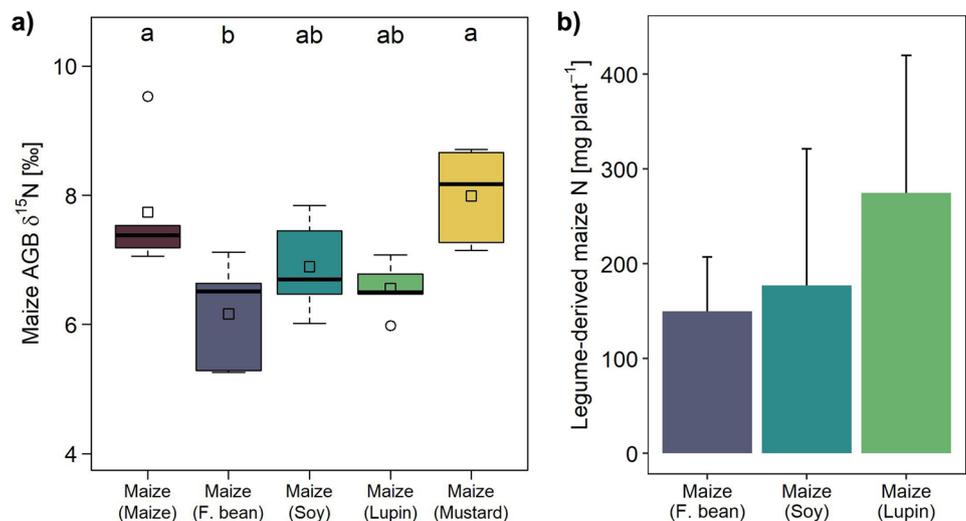
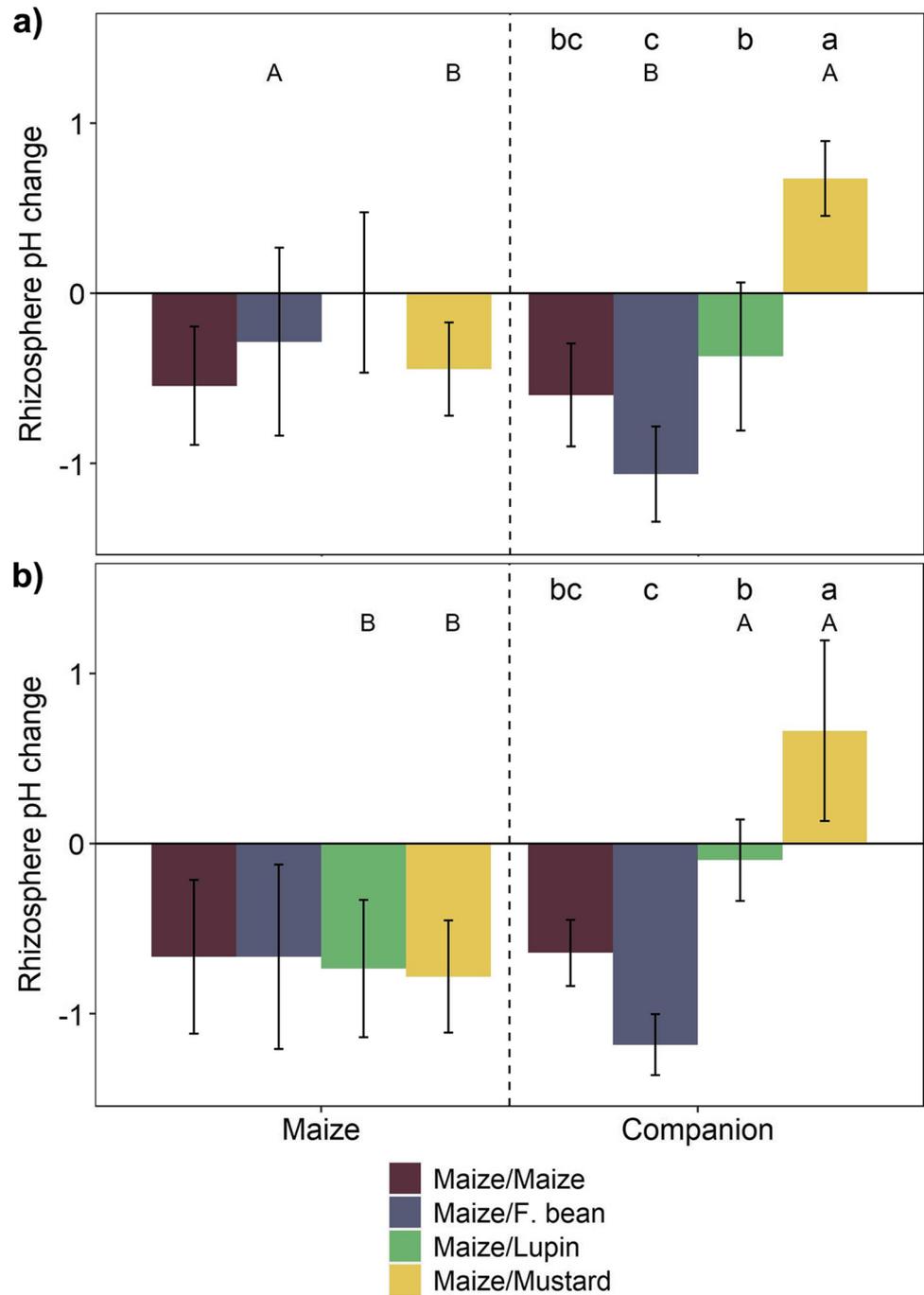


Fig. 4 Rhizosphere pH changes of maize and companions relative to the bulk soil, determined 6 (a) and 9 (b) weeks after sowing in the rhizobox experiment. The zero line corresponds to soil pH of 6.9. Columns show means, and error bars indicate standard deviations. Different lowercase letters indicate 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



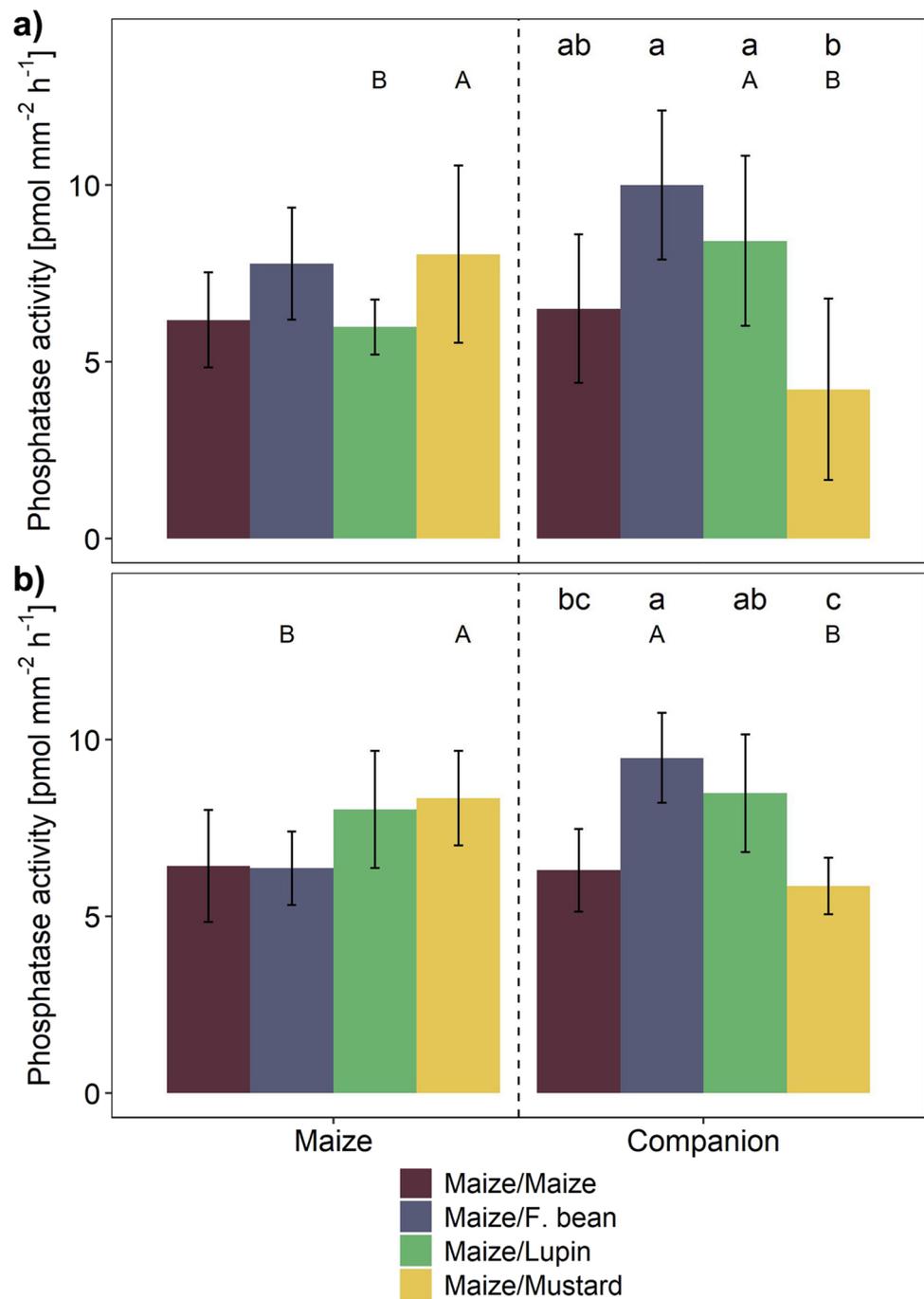
6 and 9 weeks after sowing. The changes in rhizosphere pH differed significantly between mustard and maize in mustard/maize intercropping 6 and 9 weeks after sowing ($p < 0.001$; Fig. 4). Maize generally decreased the rhizosphere pH compared to the bulk soil, particularly 9 weeks after sowing. Maize acidified the rhizosphere significantly more than lupin in lupin/maize intercropping 9 weeks after sowing ($p = 0.016$; Fig. 4b).

3.5 Phosphatase Activity

In the field experiment, soil phosphatase activity was, on average, $3.0 \pm 1.3 \text{ nmol g soil}^{-1} \text{ h}^{-1}$ 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

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



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).

3.6 Water-Extractable Soil N

In the field experiment, water-extractable NO_3^- -N was, on average, $3.8 \pm 1.1 \mu\text{g N g soil}^{-1}$ 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 $\delta^{15}\text{N}$. 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 $\delta^{15}\text{N}$ in the non-leguminous plots due to legume roots decomposing in the soil over winter resulting in the observed, significantly lower $\delta^{15}\text{N}$ 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 the 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 N₂ 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 alkalization 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 alkalization. Thus, mustard likely increased soil P availability for both species (either complementary or facilitative, as discussed above) through changes in the rhizosphere pH. Rhizosphere alkalization 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 alkalization of durum wheat and the grass *Nassella trichotoma* increased P availability (Devau et al. 2010; Spohn et al. 2020). Hence, the rhizosphere alkalization 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 N₂ 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 alkalization 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.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42729-022-00936-3>.

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Author Contribution All authors contributed to the study conception and design. US performed the experiments and the analyses. 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.

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

Declarations

Competing Interests The authors declare no competing interests.

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