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Do Short-Term Legume–Maize Sequences Outperform Maize Monoculture Regarding Biomass Production and P Use Efficiency Under P-Restricted Conditions?

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ABSTRACT

Background: With their ability to efficiently acquire P, integrating legumes in phosphorus (P)-inefficient cereal-based rotations may enhance P use efficiency (PUE). However, previous studies on crop rotations have not linked growth gains of successive crops to the stimulation of soil P processes by legumes. Thus, knowledge gaps exist in the relevance of changes in soil induced by legumes for the growth and P nutrition of successive crops and the PUE of the entire crop rotation.

Aim: This study aims to address the abovementioned points by testing four short-term crop sequences (alfalfa (*Medicago sativa*)/maize (*Zea mays* cv. Johaninio), faba bean (*Vicia faba* cv. Augusta)/maize, white lupin (*Lupinus albus* cv. Célina)/maize, and maize/maize) under P limitation and with the application of P fertilizers differing in their P availability (rock phosphate, struvite, and triple superphosphate) in a pot trial.

Results: Acid and alkaline phosphomonoesterase activity were strongly and persistently stimulated by white lupin and faba bean (2× for acid and 1.22–1.23× for alkaline phosphomonoesterase activity). However, even under P limitation, biomass and P offtake of successive maize did not benefit from the previous cultivation of legumes. In contrast, lupin strongly inhibited the growth of successive maize (0.4× aboveground biomass of maize after maize), although lupin increased calcium-acetate extractable P. None of the legume-based early growth sequences could compete with maize monoculture regarding PUE due to the low aboveground biomass of legumes compared to pre-crop maize.

Conclusion: This study questions the one-sided positive portrayal of legumes in crop rotations for improving the P nutrition of succeeding maize and can guide the design of future field experiments.

1 | Introduction

Integrating legumes into sustainable production systems may significantly improve current P use efficiency (PUE) of cereal-based rotations. Legumes have a relatively high P requirement due to their energy demand for N fixation and, therefore, react sensitively to P deficiency (Israel 1987). However, they have naturally adopted acquisition strategies to fulfill their demand, even under P limitation. Under P deficiency, and when fixing N, it was shown that legumes acidify the rhizosphere and, thereby, solubilize P close to the roots (Tang et al. 1999, 2001). Compared to other plant species, they exude a larger amount of

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carboxylates, which can act as a chelator and desorb P sorbed to soil minerals (Touhami et al. 2020). Additionally, the release of extracellular enzymes like phosphomonoesterase targets to mineralize organically bound P (Olde Venterink 2011). Therefore, including legumes in cereal-based crop rotations may result in a long-lasting alteration of soil characteristics in favor of a higher P bioavailability. On the other hand, the mineralized, desorbed, and solubilized P is immediately fixed, sorbed, and immobilized in soil, and thus, studies are needed to evaluate the true impact of legumes on P availability in soil.

Through these mechanisms, it was demonstrated that many legumes were capable of using sparingly soluble P sources, such as rock phosphate and organic P fractions, more efficiently than other crops (Hinsinger and Gilkes 1995; Pang et al. 2015; Sepehr et al. 2012; Sulieman and Mühling 2021). As recycled fertilizers showed lower immediate fertilization values than conventional triple super phosphate (Wollmann et al. 2018), the combination of recycled fertilizers with legumes could offer a chance to increase their P availability and their attractiveness for farmers. It was shown that acidification strategies increase the availability of low available fertilizers, such as rock phosphate, and of recycled fertilizers like struvite (Amarasinghe et al. 2022; Hertzberger et al. 2020; Talboys et al. 2016), which is associated with nitrogen fixation by legumes (Wollmann and Möller 2022). Additionally, citrate and other carboxylate exudation by legumes could solubilize more P from struvite (Talboys et al. 2016). However, assessments on the combination of legumes with recycled fertilizers and the impact on succeeding crops are lacking.

Besides possible positive effects of legumes on P cycling and the utilization efficiency of fertilizers, increasing the share of legumes in crop rotations provides opportunities for the sustainable intensification of current agriculture. Previous studies demonstrated that yields of successive crops were increased if legumes were included in the crop rotations compared to cereal-only crop rotations (Zhao et al. 2020, 2022). Moreover, farmers might reduce N fertilizer input and increase their protein harvest due to their N-fixing ability (Notz et al. 2023; Zhao et al. 2020). However, this superiority of legumes compared to other pre-crops is diminished by their relatively low productivity, resulting in a variable economic output for the whole crop rotation. Studies reported negative economic impacts for many legume-based crop rotations (Notz et al. 2023), whereas in other cases, legume-cereal crop rotations, particularly rotations including forage legumes, were able to compete with cereal-based crop rotations due to the higher protein and metabolizable energy harvest (Nemecek et al. 2008; Reckling et al. 2016).

Up to now, the positive impacts of legumes on P availability and P cycling were not considered to the same extent as the benefits of biological N fixation. Little is known about the persistence of soil changes induced by legumes and their benefits for the successive crop. Previous studies demonstrated improved productivity of cereals grown after legumes under P limitations (Jemo et al. 2006; Kamh et al. 2002; Pypers et al. 2007) without linking it to P turnover processes, and, at the same time, other studies came to contradictory conclusions (Nachimuthu et al. 2009; Vandamme et al. 2014). Moreover, assessments of the entire crop sequence are lacking, which are indispensable to critically evaluate the relevance of the legumes' impact on successive crops.

This study aims to address the abovementioned knowledge gaps with the main focus on the influence of legumes in maize-based cropping systems on P availability and the investigation of early plant–soil interactions. For this purpose, three different short-term legume–maize crop sequences in combination with four fertilizer treatments were compared to a maize–maize monoculture in a greenhouse trial. The hypotheses were as follows: (1) Legumes stimulate soil P mineralization processes (phosphomonoesterase activity) and solubilization (acidification). (2) Thereby, the legumes increase the P availability in soil as well as from sparingly available fertilizer sources. (3) From this increased P availability, also maize cropped after the legume benefits in terms of aboveground biomass and P uptake. Finally, (4) the overall PUE is improved in the legume–maize crop sequence compared to the maize–maize sequence.

2 | Materials and Methods

2.1 | Treatments and Experimental Design

We tested four crop sequences with maize (*Zea mays* var. *KWS Johaninio*) as a successive crop. The pre-crops were white lupin (*Lupinus albus* var. *Célina*), faba bean (*Vicia faba* var. *Augusta*), alfalfa (*Medicago sativa*), and maize (*Z. mays* var. *KWS Johaninio*). Additionally, four fertilization treatments were combined with the crop rotations: without P (control), fertilized with rock phosphate (11.34% P), struvite (9.16% P), or triple superphosphate (TSP, 19.64% P). In total, the experiment included four fertilization treatments and four crop rotations, each with four replicates (in total 64 pots), arranged in a randomized complete block design.

2.2 | Growing Conditions

2.2.1 | Greenhouse Trial Setting

A greenhouse trial conducted at the University of Hohenheim's research facility took place from November 2022 to April 2023. The mean temperature was 20.7°C and 21.9°C for the pre-crop and maize growing periods, respectively. Likewise, the mean relative humidity was 36.3% and 35.3%. Additional sodium vapor lamps guaranteed a 15-h day period and provided extra lighting in the morning and evening.

2.2.2 | Soil Characteristics

The soil for the experiment was collected from the research station of the Thuringian State Office for Agriculture and Rural Areas in Heßberg (50°25′ N, 10°46′ E), Thuringia, Germany. Important soil characteristics can be found in Table 1.

According to Wiesler et al. (2018), the soil has a slight P deficiency. The soil was sieved to a grain size of 5 mm before the trial and mixed with 25% quartz sand (0.6–1.2 mm) on a weight basis before conducting the trial. The CAL-P concentration of the substrate was 15 mg kg^{-1}, pH was 6.6, and the total P was 346 mg kg^{-1}.

TABLE 1 | Soil characteristics.

Soil texture[a]	C_{org}[b] (%)	$CaCO_3$[b] (%)	Total N[c] (%)	Total P[d] (g kg^{-1})	Available P[e] (mg kg^{-1})	K[e] (mg kg^{-1})	Mg[e] (mg kg^{-1})	pH[e]
Sandy loam	0.62–0.65	<0.5	0.07	0.479	19–21	63–98	148–219	6.8

[a]Food and Agricultural Organization of the United Nations/World Reference Base.
[b]Dry combustion, DIN ISO 10694 (1996).
[c]Dry combustion DIN ISO 13878 (1995).
[d]DIN ISO 54321 (2021), measured with ICP-OES.
[e]P and K were measured in calcium acetate lactate solution; Mg and pH were measured in $CaCl_2$ according to VDLUFA (1991).

2.2.3 | Establishment of the Experiment and Fertilization

At the beginning of the trial, the soil was fertilized with 200 mg K kg^{-1} dry substrate (0.5 M K_2SO_4 solution) and 100 mg Mg kg^{-1} dry substrate (0.5 M $MgSO_4$ solution). As the struvite fertilizer inherently contains 4.7% N, all other treatments were fertilized with the equivalent amount of N added by the struvite as a 0.5 M $(NH_4)_2SO_4$ solution (15 mg N kg^{-1} dry substrate). The pre-crops did not receive additional N to enable the legumes to fix N. All P fertilizers were solid fertilizers and were ground beforehand to guarantee an even distribution in the substrate. Only a moderate P fertilization (30 mg P kg^{-1}) was chosen. All fertilizers were homogeneously mixed with the soil–sand substrate.

Six kilograms of the fertilized substrate were filled into Mitscherlich pots (Ø 20 cm, height: 21 cm). The water content was adjusted to 70% water holding capacity, which was equivalent to 19% water content on a weight basis. Initially, the weight of the pots was checked every 2 days, and the evapotranspirated water was replenished in the pots. In later stages, watering was done more frequently to avoid drought stress.

The sowing density of the pre-crop was calculated on the basis of the plant density under common farmers' practice for the varieties. Twice the number of seeds was sown to eliminate the possibility of germination failure. After 1 week, surplus plants were removed along with the seeds to achieve the predefined number of plants per pot. This resulted in a final plant number of one plant for maize, two plants for faba bean and white lupin, and 22 plants for alfalfa per pot.

Pre-crop plants were grown for 56 days (BBCH maize 18, white lupin 66, faba bean 63, alfalfa 61), and afterward, above-ground plant parts were harvested. To simulate tillage, the soil and roots were removed from the pots into a larger vessel. Roots were chopped and homogenously mixed with the soil. After that procedure, the substrate was refilled in the pots.

After three weeks, in which the pots remained in the greenhouse and were watered regularly, the substrate was again fertilized with 200 mg K kg^{-1} dry substrate (0.5 M K_2SO_4) and 100 mg Mg kg^{-1} dry substrate (0.5 M $MgSO_4$). Additionally, in total 200 mg N kg^{-1} dry substrate (0.5 M $CaNO_3$) was added in two applications—at the beginning and after four weeks—to the pots to eliminate any N effect by the legume roots. Three maize seeds were sown, and plants were thinned after 1 week to one plant per pot. Maize plants were harvested after 7 weeks (46 days after sowing, BBCH maize 18).

2.3 | Measurements

2.3.1 | Analyses of Soil Samples

Soil samples were taken three times during the trial: after the harvest of the legumes (56 days after the start of the experiment), immediately before sowing the maize (77 days after the start of the experiment), and after the harvest of maize (124 days after the start of the experiment). A representative mixture of the entire soil in the pot was obtained by homogeneously mixing the soil after harvesting the legumes. Fresh soil samples for phosphomonoesterase analysis were stored in the fridge (4°C) until further study, and samples were processed as soon as possible (no longer than three weeks after sampling). Acid phosphomonoesterase activity was measured at all three sampling time points. Alkaline phosphomonoesterase activity was measured twice: at the time of maize sowing and at maize harvest, to assess not only the enzyme activity induced by root exudates (of plant origin) but also the enzyme activity originating from rhizosphere microorganisms.

Soil pH was measured in suspensions of the soil samples using a 0.01 M $CaCl_2$ solution after the pre-crop and maize harvest (VDLUFA 1991). As an indicator of P availability, the calcium-acetate-lactate (CAL) method (Schüller 1969) was conducted according to VDLUFA (1991), an acid extraction commonly used in Germany. Briefly, 5 g of soil was mixed with 100 mL of a CAL extract (pH 4.1) and shaken for 1.5 h before being filtered using P-free filter papers (Macherey-Nagel [MN] 619 1/4 G). The extracts were measured photometrically at 580 nm (U-2900, Hitachi High-Tech Corporation, Tokyo, Japan) using an ammonium molybdate coloring reagent and a reduction solution containing ascorbic acid and tin chloride dissolved in 16% HCl. Total P was determined by the aqua regia digestion with subsequent P measurement via ICP-OES (DIN ISO 54321, 2021). Soil samples for total P, CAL-P, and pH were air-dried in the greenhouse and sieved to a 2-mm grain size before the analysis.

Phosphomonoesterase activity was measured following the procedure of Tabatabai and Bremner (1969), which utilizes *p*-nitrophenyl phosphate. Modifications were adapted according to Rubio et al. (1990) and Redel et al. (2008). Briefly, alkaline

and acid phosphomonoesterase activities were determined using different buffer solutions in the optimal pH range of the enzymes (5.5 and 8.2). A quantity of 2 g of fresh soil samples received 2 mL of 0.115 M *p*-nitrophenyl phosphate and was incubated for 1 h in a water bath at 20°C. The reaction was stopped with 2 mL of 0.5 M $CaCl_2$ solution, and samples received 2 mL double-distilled water. Additionally, two control sets were added: one set received 2 mL double-distilled water, and one received 2 mL nitrophenol (500 µg g^{-1} soil) before the water bath. After the incubation time, they received 2 mL of 0.115 M *p*-nitrophenyl phosphate. After filtering (Macherey-Nagel [MN] 619 1/4 G—phosphate-free filters), 1.2 mL extract was diluted in 13.8 mL 0.014 M NaOH. Samples were centrifuged for 10 min at 2500 *g*, and afterward the concentration of nitrophenol was measured photometrically at 400 nm (U-3300, Hitachi High-Tech Corporation, Tokyo, Japan). Phosphatase activity was calculated on the basis of the equation given by Herrmann et al. (2024).

For a more sophisticated analysis of the legumes' impact on P mineralization, ^{31}P-NMR was used to quantify the organic P compounds in the soil. Three out of four replications were randomly selected from the maize–maize, white lupin–maize, and faba bean–maize rotations in the unfertilized control and the TSP-fertilized soil after maize harvest. The alfalfa–maize rotation was not considered because enzyme activity and P availability were not affected to the same extent. Because the fertilizer–crop sequence interaction was not significant, only the non-fertilized and TSP-fertilized soils were considered. The procedure of Wanke et al. (2024) was followed for the analysis of ^{31}P-NMR samples. Briefly, soil samples were extracted according to Cade-Menun and Preston (1996) with modifications from McLaren et al. (2015). A 1:4 soil-solution (0.25 M NaOH + 0.05 M Na_2EDTA) ratio was chosen. After shaking the suspensions for 16 h and centrifuging them at 3714 *g* for 30 min, the supernatant was filtered (Blue ribbon 589/3, Schleicher and Schuell, Germany). A portion of the extract was kept for analyzing total P content with ICP-OES. Then, 20 mL of the aliquot was freeze-dried. For the ^{31}P-NMR measurement, 80 mg of the freeze-dried sample was dissolved in 600 µL solvent containing 2.7 mM methylene diphosphonic acid as a standard and 0.24 M NaOD in D_2O (Cade-Menun and Liu 2014). After thoroughly mixing, samples were centrifuged again for 30 min at 10,000 *g*. Before starting the measurement, inversion-recovery experiments were performed to determine spin-lattice relaxation times, and the frequency of the scans was adjusted accordingly by multiplying the relaxation times by five. Basic information on the NMR setup is provided in Table S6.

Concentrations were then estimated by calculating the integrals of the peaks using TopSpin (Version 3.6.1, Bruker BioSpin GmbH, Germany). These intensities were then converted into concentrations by relating them to the value of the standard with a known concentration (methylene diphosphonic acid). However, in the orthophosphate-monoester region, peaks highly overlap, making it necessary to deconvolute them. For this, the approach of Reusser et al. (2020) was followed. In MATLAB (R2023b), the chemical shift region of the orthophosphate and monoesters was attempted to be portrayed by fitting the peaks, including an underlying broad signal. Compounds were assigned to specific peaks based on previous internal spiking experiments (Table S7) conducted by Wanke et al. (2024).

2.3.2 | Measurements and Analyses of Plant Material

During the growth period of maize, plant height was measured every two weeks. Fresh and dry weights of the aboveground pre-crop and maize plant parts were determined at harvest time.

Plants were dried at 60°C until they reached a constant weight (Binder ED115) and then ground to 1 mm size. Nutrient concentrations were analyzed with the microwave digestion method with 65% HNO_3 and H_2O_2 according to VDLUFA (2011). After coloring the extracts, phosphorus concentrations were determined with a spectrophotometer at 436 nm (U-2900, Hitachi High-Tech Corporation, Tokyo, Japan). Manganese concentrations were measured directly from the microwave digestate with an atomic absorption spectrometer (iCE 3300, Thermo Scientific, Thermo Fisher Scientific, Waltham, USA). Manganese concentration in shoots approximates how intensively plants exude carboxylates (Lambers et al. 2015) and is considered in this study as an indicator of carboxylate exudation.

2.4 | Calculations, Statistical Analysis, and Illustrations

To compare the overall performance of the crop rotations, total biomass production and total P offtake were calculated as the sum of the pre-crop aboveground biomass/P offtake and the aboveground maize biomass/P offtake. These values reflect the efficiency in P use of the crop sequence because all fertilizer treatments received the same amount of P. Additionally, PUE was calculated by the following equation:

$$\mathrm{PUE} = \frac{\mathrm{P}_{\mathrm{offfertilized}} - \mathrm{P}_{\mathrm{offcontrol}}}{180} \times 100\%. \quad (1)$$

With $P_{offfertilized}$ referring to the total P offtake of a crop sequence when fertilized, and $P_{offcontrol}$ being the total P offtake of a crop sequence for the unfertilized control. One-eighty is the P amount given to each pot in mg.

Statistical analysis was performed using the statistical software SAS (Version 9.4; SAS Institute Inc., Cary, NC, USA). For unrepeated measurements, a simple linear model was fitted with *proc mixed* procedure in SAS, including the block (b_i) as a random effect, the pre-crop (c_j) and the fertilizer (f_k) as fixed variables, and the pre-crop × fertilizer ($(cf)_{jk}$) as an interaction term:

$$y_{ijk} = b_i + c_j + f_k + (cf)_{jk} + e_{ijk} \quad (2)$$

with e_{ijk} being the residual error of the mean estimate of y_{ijk}. In the case of repeated measurement, the correlation of measurements was considered by taking the best-fitting variance-covariance matrix based on the Akaike information criterion (Wolfinger 1993) into account by adding the *repeated* statement to the *proc mixed* procedure and adding the sampling time (t_l) and all interactions with sampling time in the model:

$$y_{ijkl} = b_i + c_j + f_k + (cf)_{jk} + t_l + (tc)_{jl} + (tf)_{kl} + (tcf)_{jkl} + e_{ijkl} \quad (3)$$

For enzyme activity, sampling time points could not be analyzed on the same measuring day. To correct for any variation due to

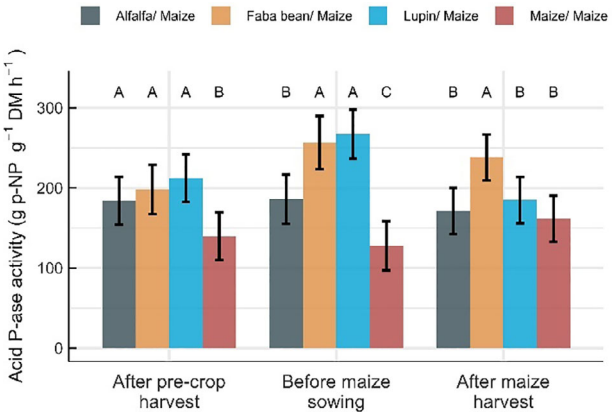

FIGURE 1 | Acid phosphomonoesterase activity (µg *p*-NP g^{-1} DM h^{-1}) at different sampling times and for different crop sequences ($n = 16$). Graphs depict LSMeans ± 95% confidence intervals (CI) pooled over fertilizer treatments (fertilizer × crop sequence not significant, $p > 0.05$). Equal letters indicate no significant difference among the treatments at the respective sampling time point (Fisher's LSD test (Fisher 1936), $\alpha = 0.05$).

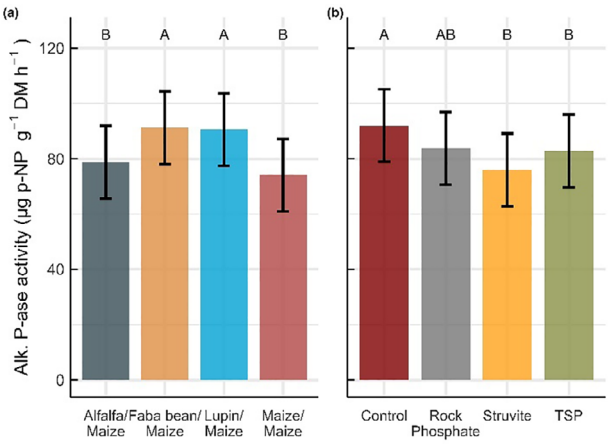

FIGURE 2 | Alkaline phosphomonoesterase activity (µg *p*-NP g^{-1} DM h^{-1}) (a) depending on the crop sequence ($n = 32$) and (b) depending on the fertilizer ($n = 32$). Graph depicts LSMeans ± 95% confidence intervals (CI) pooled over sampling times (interaction with sampling time not significant, $p > 0.05$). Fertilizer × crop sequence was not significant. Equal letters indicate no significant difference among the treatments (Fisher's LSD test (Fisher 1936), $\alpha = 0.05$).

different measuring days, a covariate was added to the model, which is based on the enzyme activity of a standard soil on the measuring day. Normality and variance homogeneity of residuals were checked visually, and no transformation needed to be implemented.

Nonsignificant factors in the ANOVA were removed from the model. Mean comparisons based on the least significant difference (Fisher 1936) were then implemented with the remaining significant terms. The results of the ANOVA can be found in the Supporting Information.

Graphs were designed with the *ggplot2* package in R Studio (Wickham 2016) and depict LSMean values from the statistical models with their respective confidence limits at $\alpha = 0.05$.

3 | Results

3.1 | Legumes' Impact on Soil P Processes and P Plant Availability

Acid phosphomonoesterase activity was unaffected by the fertilizer treatment but was significantly altered by the interaction of crop sequence and sampling time point (Figure 1). Faba bean and white lupin were able to significantly stimulate the acid phosphomonoesterase activity compared to the maize/maize sequence by 41% and 51%, respectively. During the three weeks of root decomposition, acid phosphomonoesterase activity was further promoted by 26%–30% in both sequences, resulting in 100% higher enzyme activity compared to the maize/maize sequence at that sampling time. The acid phosphomonoesterase activity remained stable in the faba bean/maize sequence till the end of the trial, whereas it decreased in the white lupin/maize sequence.

Likewise, both faba bean and white lupin significantly increased alkaline phosphomonoesterase activity by 22%–23% compared to maize (Figure 2a). This impact remained consistent throughout the trial, as the sampling time did not interact with the crop sequence effect on alkaline phosphomonoesterase activity. Additionally, all fertilizers led to a decrease in alkaline phosphomonoesterase activity compared to the unfertilized control (Figure 2b).

The effects on pH were minuscule and hardly relevant for P solubilization, with a maximum difference of 0.2 pH units between the treatments (data not shown).

The impact of the pre-crops on CAL-P stayed constant over time as the interaction of sequence and time was not significant (Figure 3a). In the white lupin/maize sequence, the CAL-P fraction was significantly increased compared to the maize/maize sequence by 1.7 mg P (kg dry soil)$^{-1}$. However, faba bean mined from the CAL-P fraction resulting in a drop of 1 mg P (kg dry soil)$^{-1}$ compared to the maize/maize sequence. Fertilization and sampling time interacted significantly concerning the change of CAL-P (Figure 3b). From the second sampling time, all fertilizer treatments significantly differed in the following order: control < rock phosphate < TSP < struvite.

The ^{31}P-NMR showed no major influence of legume-based short-term crop sequences on organic P compounds in soil (Table 2). Total P in the extracts ranged between 196.2 and 202.5 mg kg^{-1} with no significant differences among crop sequences. P recovery from the ^{31}P-NMR was 42%–58% compared to ICP-OES measurement. Faba beans significantly mined the orthophosphate pool.

3.2 | Plant Growth and Biomass Production

Maize after alfalfa produced a significantly higher aboveground biomass of 21.6 g than maize in other sequences (maize after maize yielded 18.6 g aboveground biomass) (Figure 4). Lupin inhibited the growth of maize, with maize after lupin reaching a

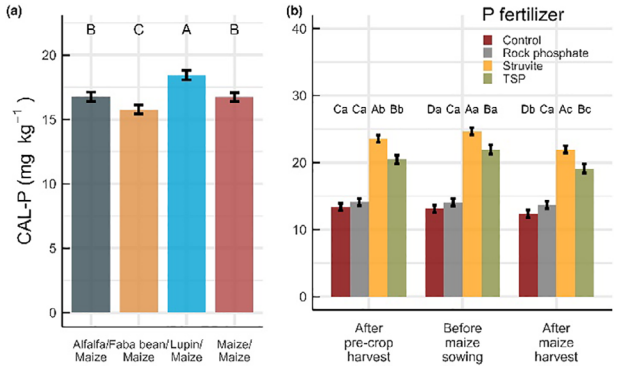

FIGURE 3 | CAL-P (mg kg^{-1} DM) (a) depending on the crop sequence ($n = 48$) (pooled over sampling time (interaction with sampling time not significant, $p > 0.05$)), and (b) depending on the fertilizer and sampling time point ($n = 16$). Graphs depict LSMeans ± 95% confidence intervals (CI). Fertilizer × crop sequence was not significant. Equal capital letters indicate no significant difference among the treatments (at the respective sampling time point) (Fisher's LSD test (Fisher 1936), $\alpha = 0.05$). In (b), in addition, equal small letters indicate no significant difference between the sampling time points for the respective fertilizer treatment (Fisher's LSD test (Fisher 1936), $\alpha = 0.05$). TSP, triple superphosphate.

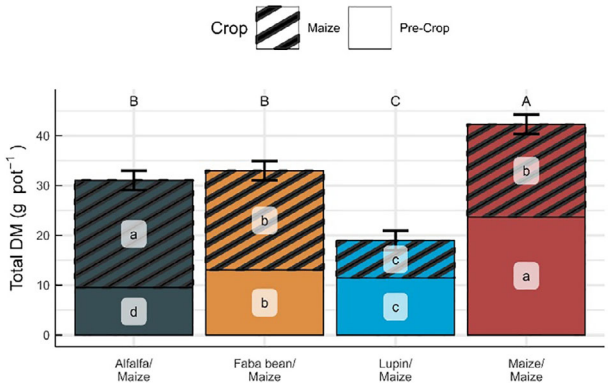

FIGURE 4 | Total dry mass (sum of pre-crop and maize) (g pot^{-1}) depending on the crop sequence ($n = 16$). The graph depicts LSMeans ± 95% confidence intervals (CI) of total dry mass pooled over fertilizer treatments (fertilizer × crop sequence not significant, $p > 0.05$). Equal capital letters indicate no significant difference among the treatments concerning total biomass, and equal small letters indicate no significant difference among the sequences concerning the pre-crop or maize biomass, respectively (Fisher's LSD test (Fisher 1936), $\alpha = 0.05$).

biomass of only 7.5 g. The effect of the sequence on maize biomass was of higher magnitude than the fertilization treatment, and the interaction of sequence and fertilizer was not significant (Figure S6).

Regarding total biomass production as the sum of pre-crop and maize dry mass, none of the legume-based crop sequences could compete with the maize/maize sequence due to the strong growth of the pre-crop maize (25.6 g), which was 2–2.5× higher than all the legumes (alfalfa: 9.5 g, faba bean: 13.1 g, white lupin: 11.5 g) (Figure 4).

3.3 | P Concentration and P Offtake

Due to the high P concentrations of the legumes' shoot compared to the pre-crop maize (Figure S3), the performance of the crop sequences regarding total P accumulation did not follow the trend of total biomass production (Figure 5). The faba bean/maize

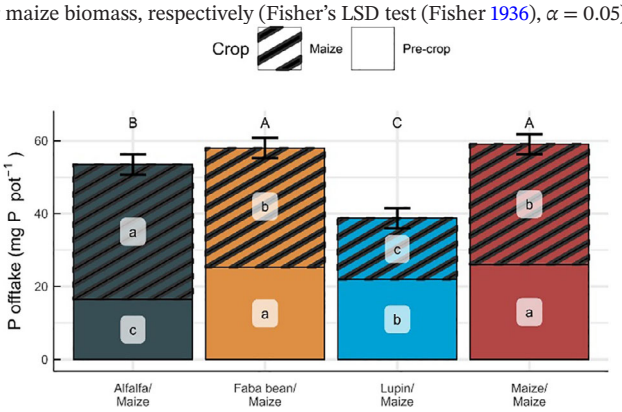

FIGURE 5 | P offtake of the crop sequence (sum of P uptake of pre-crop and maize) (mg P pot^{-1}) depending on the crop sequence ($n = 16$). The graph depicts LSMeans ± 95% confidence intervals (CI) of total P offtake pooled over fertilizer treatments (fertilizer × crop sequence not significant, $p > 0.05$). Equal capital letters indicate no significant difference among the treatments concerning total P offtake, and equal small letters indicate no significant difference among the sequences concerning pre-crop or maize P-offtake, respectively (Fisher's LSD test (Fisher 1936), $\alpha = 0.05$).

TABLE 2 | P compounds in the soil at harvest time of maize measured with the ^{31}P-NMR depending on the crop sequence ($n = 6$).

P COMPOUNDS (MG P kg^{-1})/TREATMENT	Maize–maize	Faba bean–maize	White lupin–maize
Orthophosphate	74.5 ± 7.1 (A)	67.2 ± 7.2 (B)	73.5 ± 7.5 (AB)
Inositol-6-phosphate	1.99 ± 0.33	2.07 ± 0.33	2.10 ± 0.34
Broad peak	21.9 ± 3.2	22.5 ± 3.2	23.7 ± 3.4
Total monoester peak	27.8 ± 3.7	27.9 ± 3.7	29.8 ± 4.0
Phospholipids	1.62 ± 0.59	1.31 ± 0.63	1.66 ± 0.60
DNA	2.01 ± 0.59	1.62 ± 0.65	1.90 ± 0.70
Pyrophosphate	1.49 ± 0.37	1.27 ± 0.37	1.42 ± 0.40

Note: LSMeans with their 95% confidence intervals pooled over fertilizer treatments (fertilizer × crop sequence not significant, $p > 0.05$) are shown. Equal letters indicate no significant difference between treatments with Fisher's LSD test at $\alpha = 0.05$. In rows with no letters, treatments did not differ significantly.

sequence accumulated P in an equal amount as the maize/maize sequence (58 mg P pot^{-1} compared to 59.1 mg P pot^{-1}) because the low biomass of faba bean, being only half as much as the one of maize, was counterbalanced by a P concentration, which was nearly twice as high. In the alfalfa/maize sequence, total P offtake was 5.6 mg P pot^{-1} lower than in the maize/maize sequence, which was especially related to the low biomass production of alfalfa. The white lupin/maize sequence had a significantly lower P offtake compared to the maize/maize sequence with 38.7 mg P pot^{-1}, although white lupin, as well as maize after white lupin, showed both a higher P concentration compared to pre-crop maize and maize after maize. PUE followed the same trend as total P offtake (Table S5).

4 | Discussion

4.1 | Processes Increasing P Availability by Legumes

With this pot trial, an exploratory pre-study was designed aligned to common farming practice, in which legumes are harvested for grain or as fodder before maize is planted and aboveground plant parts are removed from the field. All legumes, especially white lupin and faba bean, were able to stimulate phosphomonoesterase activity but differed in their effectiveness in maintaining these levels over time. Nuruzzaman et al. (2006) underlined this outcome by showing a more than 2× higher acid phosphatase activity of white lupin and faba bean compared to wheat. The induced changes in activity endured over a longer period in the case of faba bean and white lupin sequences (in this trial two months), possibly still shaping the environment for successively grown maize under field conditions. In general, phosphomonoesterase enzymes are relatively stable in soil under temperate conditions and can last in soil for at least four weeks without considerable changes in activity (Pettit et al. 1977).

The increase in acid phosphomonoesterase activity after the tillage and three weeks of decomposition of white lupin and faba bean roots may be attributed to the relevant enzymes present in the roots, which become active upon release into the soil (Gilbert et al. 1999). Additionally, soil microorganisms may be stimulated through the addition of energy-rich carbon compounds to produce and release more phosphomonoesterase into the soil, as there is a close link between soil organic carbon and phosphomonoesterase activity (Hegyi et al. 2021; Margalef et al. 2017; Sinsabaugh et al. 2008). However, microbial biomass P was also assessed through chloroform fumigation, and no impact of the crop sequence was detected (data not shown).

Further observations emphasize the influence of legumes on P cycling in this trial. Legume roots, especially of faba bean, showed nodules implying an active N fixation. Previous studies have linked the increase in P availability by legumes with their biological N fixation (Aguilar and van Diest 1981; Wollmann and Möller 2022). Additionally, we observed a high Mn concentration in white lupin shoots (Figure S1), which positively correlates with the carboxylate release and negatively with the pH value in soil (Lambers et al. 2015, 2021). The pH measurements showed no considerable changes in the samples, as the soil was mixed prior to sampling, which may have diluted the effect of acidification by legumes.

The stimulation of phosphomonoesterase and the increase of carboxylate exudation may lead to an increased P availability for the successive maize plants. Active stimulated P turnover pathways resulted in an increase of CAL-P in the white lupin/maize sequence. Due to low biomass production and low P removal from pots by maize and white lupin, the impact on CAL-P remained consistent over time. Faba bean, on the contrary, appears to mine from the CAL-P fraction as the P removal from the system was equivalent to the offtake of the maize/maize sequence. Faba beans are capable of solubilizing and using P fractions, which are hardly available for other plant species, and can be extracted with the acidic CAL-solution (Barberis et al. 1995; Li et al. 2015; Liao et al. 2020; Steinfurth et al. 2021). However, measuring P availability can only give a hint of the mineralization and solubilization of P, as the freshly available P is immediately fixed and immobilized in soil. Additionally, organic P is also sorbed to soil surfaces, which may hinder its degradation. Especially because this experiment was a short-term crop sequence, changes in P turnover and cycling may not be visible, yet. Previous field trials indeed found evidence for an increase of P availability in legume crop rotations (Chen et al. 2025).

Similarly, organic P compounds in soil analyzed with the ^{31}P-NMR were not significantly depleted or altered by legume-based crop sequences. One explanation may be that the organic matter content in this soil was below average in Germany (Poeplau et al. 2020), resulting in a relatively low mineralizable organic P pool. Additionally, the experimental time was short, sample size was limited, and no changes in total P content over time could be identified (Table S3). Nevertheless, in tendency, legumes could decrease the phospholipids, DNA, and pyrophosphate concentrations, which are all easily degradable. The results of this study should be seen as an exploratory indicator, and further trials under field conditions are needed to verify this tendency.

4.2 | Impact on Maize Growth and P Concentrations

The increase of maize growth in the alfalfa/maize sequence compared to the maize/maize sequence may be linked to the continuous ground cover of alfalfa and the improved soil structure. Moreover, previous field trials observed beneficial impacts of forage crops like alfalfa on successive plants compared to pulses like soybean, for example, through an increase of soil organic carbon and a reduction of the N demand (Ma et al. 2012; Riedell and Osborne 2017).

Right from sowing, a visual inhibition of maize growth after white lupin was apparent and remained until the end of the trial (Figure S5). Moreover, P concentrations in maize plants grown after lupin were considerably higher and in the range of the critical P concentration (on average 2.3 mg P g^{-1} with an assumed critical level of 2–2.5 mg P g^{-1}) (Figure S4) (Morel et al. 2021; Wen et al. 2017), indicating that P was not the growth-limiting factor. White lupin may have released allelopathic compounds or precursors of inhibitory compounds, which accumulated in the soil after the destruction of the roots. White lupin is known to exude a significant amount of various isoflavonoids, such as genistein, under P deficiency to protect carboxylates from degradation (Weisskopf et al. 2006). Genistein, for example, is

known for its cytotoxicity, antifungal, and antibacterial action (Kikuta 2020; Sharifi-Rad et al. 2021). Therefore, increasing the fallow period between legumes and maize is critical in order not to risk an early growth depression of successive plants. In Middle European countries, this is typically the case in common agricultural practices. Moreover, legumes were harvested in the middle of the generative phase, whereas senescent plants would be harvested under field conditions. Root aging is linked to the relocation of nutrients from roots to aboveground plant parts (Liu et al. 2019) and a reduced carboxylate release by roots compared to the vegetative phase (Santangeli et al. 2024). Thus, allelopathic impacts as well as positive P mineralization effects on the succeeding crops may be mitigated under field conditions. However, further field trials are needed to conclusively evaluate the impact under realistic conditions.

4.3 | Legumes' Impact on the Availability of P Fertilizers

The crop sequence and fertilization never interacted significantly, which implies that P mineralization and solubilization, as well as the effect of legumes on maize growth, are independent of the P status in the soil. However, we decided to apply a low P dosage, which still exposes plants to a P-deficient environment.

In this trial, we only tested mineral phosphate fertilizers. As the high exudation of phosphomonoesterase enzymes attacks organic P forms and legumes are considered efficient organic P utilizers (Sulieman and Mühling 2021), the situation may differ if fertilizers provide a larger share of organic phosphorus.

Struvite showed its comparability with TSP and especially its action as a slow-release fertilizer (Talboys et al. 2016). The residual influence of struvite on maize growth and P concentration was stronger than TSP, and the CAL-P remained higher in soils fertilized with struvite than in soils fertilized with TSP. However, the initial values were on the same level.

4.4 | Evaluation of the Performance of the Crop Sequence

Legume-based crop sequences could not reach the productivity level of the maize/maize sequence even though the gap was narrowed regarding total P offtake. Eventually, the limiting factor is that legumes cannot compete with the efficient growth of maize plants. The plants in the pre-crop growing period, including maize, were not sufficiently fertilized with N. With additional N fertilization, which farmers would typically apply in maize, the maize/maize sequence would even extend its lead compared to other sequences. A previous field study confirmed this assumption under realistic conditions; however, lower productivity of legumes could be overcompensated by higher market prices (Zhang et al. 2025). The higher biomass of the pre-crop maize compared to the successive maize can be related to lower temperatures in the greenhouse during the growing period of the successive maize.

Although it is highly unlikely that faba bean and white lupin can reverse this observed tendency in later growth stages, the situation may differ for alfalfa, because this forage crop can be cut several times within one vegetation period in the field, and alfalfa can be grown for more than 1 year. At the same time, the growth of maize was significantly improved after alfalfa in this trial. Therefore, this crop sequence promises to achieve the goal of a higher PUE in terms of biomass production and P uptake under field conditions. But further investigations are needed to confirm this assumption.

5 | Conclusion

In all legume-based crop sequences, higher phosphomonoesterase activity and higher carboxylate exudation were observed, confirming the stimulation of P mineralization and desorption processes by legumes. The magnitude and persistence in soil are species-specific, which needs to be considered in the future design of crop rotations.

However, these processes were not linked to P availability in soil expressed as CAL-P, and in the short-term, legumes (except white lupin) could not increase P availability in soil. Additionally, legumes were not able to increase P fertilizer availability from sparingly available P fertilizers, such as rock phosphate.

Only alfalfa, as a pre-crop, benefited successive maize growth. In the specific case of white lupin, the increase in P availability comes at the price of a growth inhibition for the subsequent crop and a strong reduction in PUE.

Ultimately, none of the legume-based crop sequences could compete with the maize/maize monoculture in terms of total biomass and total P offtake due to the low biomass production of the legumes during the 8-week growing period. With this, the efficiency of P use could not be optimized by integrating legumes in the crop sequence.

This trial could provide valuable insights into the stimulating effect of legumes on P cycling and its persistence in a legume-based crop sequence, which is also relevant for further long-term studies. It questions the one-sided positive portrayal of legumes pre-crops effects due to their lower productivity and partly inhibitory effect on succeeding maize. Further studies are needed to assess the PUE of real crop rotation and to re-evaluate legumes' impact on P pathways and P availability under field conditions.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The dataset, SAS scripts, and R scripts of the current study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: jpln70060-sup-0001-SuppMat.pdf.