

Research paper

Desorption of mineral-bound phosphorus across different cropping systems and agronomic strategies to promote efficient input use

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ABSTRACT

Phosphorus (P) is a limiting nutrient to production in various agricultural ecosystems and many soils contain large amounts of legacy P that is strongly adsorbed on iron (Fe) and aluminum oxides and therefore poorly available to plants. Here we investigate how much mineral-bound P is mobilized in different cropping systems and the influence of agronomic practices on this process. Root ingrowth cores filled with a mix of sand and P-saturated goethite were incubated in the topsoil of: 1) sugar beet in Sweden under sufficient or deficient P additions, 2) perennial Kernza (*Thinopyrum intermedium*) in Sweden as monoculture or intercropped with Alfalfa with organic management, contrasted to annual winter wheat with inorganic fertilization, and 3) soybean grown in a P-fixing soil in Kenya with or without fertilizer and biochar addition. After one growing season, desorption was evaluated based on the amount of P that remained in the goethite and its ratio with Fe. Possible relationships between the rate of P desorption and abundance of arbuscular mycorrhizal fungi (AMF) were explored by profiling phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) biomarkers in the sand-goethite mixture collected from the cores.

Significant P desorption was found in the rhizosphere of sugar beets under deficient P supply but not in bulk sand-goethite mixture. No P desorption was observed in Kernza as monocrop or intercropped with alfalfa, nor winter wheat. Soybeans had a strong ability to desorb P under all nutrient regimes but significantly more when no fertilizer was applied. In the soybean experiment a significant positive relationship was found between P removal rates and abundance of AMF.

These results indicate that strategies for downregulating P application rates and promoting crop-AMF association can be a viable avenue to mobilize legacy P and help make more efficient use of nutrient inputs. At the same time, cropping systems and soil conditions have an overriding influence on the potential P desorption and must therefore be carefully considered during implementation.

1. Introduction

Plant growth on 30–40 % of the world's arable soils is limited by P availability (Balemi and Negisho, 2012) and input of P is necessary to sustain food security. In tropical Africa, P use efficiency from fertilizer is especially low at 10–30 % and poses a key barrier to agricultural crop production (Chikowo et al., 2010). The cheap mineral phosphate rock supply is limited to certain geographic regions (Edixhoven et al., 2013). A rapidly escalating threat to the supply comes from global disruptions

and soaring fuel prices which cut off access to fertilizers for farmers, with low-income communities being affected most (World Food Program & Boston Consulting Group, 2022). To avert food shortages, it is critical that farmers utilize P sources more sustainably and efficiently. Such a paradigm shift hinges on recycling of P from geogenic and biological waste streams, and crop and soil management that makes P more available for plants. There are substantial amounts of immobilized inorganic as well as organic phosphorus sources in agricultural soil (Stutter et al., 2012). High P fixing capacity leads to low fertilizer P use

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efficiency, (Dhillon et al., 2017; Syers et al., 2008), and means that farmers must add more P than what is actually required by the crops (Bulgarelli et al., 2013). This is a waste of a valuable P resource and has resulted in the accumulation of P in agricultural soils known as legacy P. Developing and scaling strategies that help to recover fixed pools of P is paramount to make future agriculture more sustainable (Menezes-Blackburn et al., 2018).

Plants have evolved different mechanisms to tap into less accessible fractions of P in soils that are in mineral-bound state. One of these involves rhizosphere acidification mediated by low molecular weight organic acids (LMWOAs) that chelate with metal ions and dissolve phosphorus. Sugar beets (*Beta vulgaris*) have been shown to increase the root to shoot ratio, exudation of root metabolites (Yang, 2016) and production of LMWOAs like citrate, oxalic and oxaloacetic acids (Beissner and Römer, 1998) in response to P-deficiency. When grown without P, soybean was also found to exude higher amounts of oxalate and malate than with a full nutrient regime (Dong et al., 2004). Presence of LMWOAs may result in enhanced dissolution of the goethite, especially if combined with exudation of siderophores (iron chelators) (Reichard et al., 2007). Desorption of phosphate by LMWOAs can equally occur through ligand exchange reactions where the active sites of the organic acids compete with phosphate for the binding sites on the Fe-oxide surfaces (D'Amico et al., 2020; Geelhoed et al., 1999). Therefore, crops that exude LMWOAs in response to P limitation may have the potential to increase P desorption from iron oxides and enhance P nutrition, and this effect should be stronger when the iron oxide is close to the roots in the rhizosphere (Andrino et al., 2021; Sokolova, 2020).

Some crop plants develop large root systems with a specific root architecture and high amounts of root hairs to achieve more efficient P uptake (Doydora et al., 2020; Vance et al., 2003). The domesticated perennial grass *Thinopyrum intermedium*, or Kernza®, is known to produce large root systems (Duchene et al., 2020), which enhance N uptake and reduce nitrate leaching in comparison with conventional annual wheat crops (Jungers et al., 2018; Culman et al., 2013). In light of this, its role in P uptake and the potential to desorb P from iron oxides warrants further investigation. Intercropping legumes, placed in row patterns between the companion crop, is another way to enhance nutrient acquisition (Dimitrova-Mårtensson et al., 2022). Cultivating cereals alongside with alfalfa (*Medicago sativa*) has major benefits to improve nutrient uptake and increase crop yields, notably by biological nitrogen (N) input (Stagnari et al., 2017). Intercropped legumes can facilitate P mineralization and uptake in the companion crop (Li et al., 2007) wherein greater N availability enables plants and microbes to produce more phosphatase enzymes (Png et al., 2017) and LMWOAs (Li et al., 2007; Raghothama, 1999). Thus, line or strip planting of a legume in Kernza production systems can be beneficial for P desorption and uptake from Fe-oxides but this has not been tested yet.

Symbiotic association with microorganisms is another strategy of plants to enhance nutrient uptake from soil (Andrino et al., 2021; Etesami et al., 2021; McKenna et al., 2020). Arbuscular mycorrhizal fungi (AMF) extend their hyphal networks into the soil, providing large surface area for nutrient uptake in close contact with soil minerals. This mutualism involves that a plant transfers part of its photoassimilates to AMF in exchange for nutrients (Smith and Read, 2008). Meta-analysis of agronomic research by Lekberg et al. (2005) revealed a positive relationship between AMF colonization of the roots and foliar P concentration of crops, which suggests a beneficial role in P uptake. When inoculated with AMF, soybean exhibited increased P acquisition, and produced higher grain yields and shoot biomass (Adeyemi et al., 2022). Enhanced microbial activity in the soil can also result in dissolution of iron oxides, which releases P from mineral-bound state (Andrino et al., 2021; Etesami et al., 2021). According to Yu et al. (2019), acid dissolution of hematite, an iron-oxide that strongly adsorbs P, was enhanced when the fungus *Trichoderma* sp. was in physical contact with the mineral. In the case of AMF, recent studies indicate complex trophic relations behind the access of P adsorbed on goethite, possibly through

association with P solubilizing bacteria (PSB) (Andrino et al., 2019, 2021; Etesami et al., 2021). Promoting AMF symbiosis in crop systems thus may open the door to mobilization of P that is bound to iron oxides in soils.

Amending soils with biochar, i.e., carbon-rich materials produced from biomass through low-oxygen thermal processing, has widely been shown to enhance key soil properties and functions like water retention, nutrient exchange, organic carbon content and pH (Razzaghi et al., 2020; Schmidt et al., 2021). These improvements are known to have positive effects on microbial growth (Li et al., 2020) and activity, and AMF root colonization (Li and Cai, 2021), which can promote P mineralization and uptake (Li and Cai, 2021; Hammer et al., 2014). Moreover, it has been reported that some PSB are benefiting from biochar as it serves as a habitat which enhances phosphatase and LMWOA exudation by PSB (Lu et al., 2023). The use of biochar may thus help to increase P availability and uptake in crops on soils with a high P fixation capacity. In a long-term experiment with maize-soybean rotation in Kenya, where this present study was conducted, it was shown that biochar increased crop yield, soil pH and extractable phosphorus ten years after a single application (Kätterer et al., 2019).

In this paper we evaluate how much P bound to goethite is desorbed in case studies with different crop arrangement (perennial vs annuals, mono-cropped vs intercropped), nutrient acquisition strategies (organic acids exudation vs AMF symbiosis), grown under different fertilizer regimes and contrasting pedoclimatic conditions. On-site incubations were done using root ingrowth cores placed in the 15 cm topsoil layer of: 1) Sugar beet under long-term sufficient or deficient P additions, 2) Kernza (*Thinopyrum intermedium*) (monoculture or intercropped with Alfalfa) under organic management, contrasted to winter wheat with inorganic fertilization and 3) Soybean grown in a P-fixing soil of Kenya in rotation with maize with or without fertilizer and biochar soil addition.

The following hypotheses were tested: 1) P desorption from goethite is positively correlated with fine root biomass production and P desorption will be higher in the rhizosphere than in the bulk sand-goethite mix collected from the ingrowth cores. 2) In the soybean and sugar beet experiment, more P will be desorbed from goethite when P availability is lower. 3) In the mycorrhizal crops (cereals and soybean), P desorption will be positively related to the amount of AMF mycelia colonizing the ingrowth cores.

2. Material and methods

2.1. Site characteristics and experimental design

2.1.1. Sugar beet experiment

The experiment was performed during the growing seasons of 2020 at four long-term fertilization sites in Scania, southern Sweden (Borgeby, Ekebo, Fjärdingslöv and Orup). The treatments started in 1957 and the experimental design and soil characteristics are specified in Carlgren and Mattsson (2013). Sugar beet was the current crop during our experiment (Sugar beet experiment further in the text). Two treatments were selected in a four-year rotation with other crops: 1) Low P fertilization, i.e., the P removed with harvested products during one rotation is replaced with the same amount of mineral P fertilizer added during the next rotation, and 2) as treatment 1 but with additional P fertilization of 30 kg P ha⁻¹ year⁻¹. Two blocks were used in each experimental site (N = 2), but the experimental sites can also be used as replicates (N = 4).

2.1.2. Kernza experiment

The experiment was performed in southern Sweden in the SITES Agroecological Field Experiment (SAFE) at the Lönnstorp field station during the growing season in 2020. We used plots with the perennial cereal Kernza (Intermediate wheat grass, *Thinopyrum intermedium*) grown in monoculture and with Alfalfa (*Medicago sativa*). The crops

received organic fertilization at a rate of 15 kg ha⁻¹ of P during this current season. As a reference we used annual winter wheat cultivated under conventional methods and fertilized with inorganic fertilizer (NPK) at a rate of 18.6 kg ha⁻¹ of P during this current season. Each experimental treatment was replicated in four blocks (N = 4). Details about the experimental design and soil characteristic are specified in [Dimitrova-Mårtensson et al. \(2022\)](#).

2.1.3. Soybean experiment

The third experiment was performed on a farmer field near the town of Nyabeda in Siaya County, Kenya, established in 2006 ([Kätterer et al., 2019](#)). The ingrowth cores were incubated during the 2021 growing season. It consists of an annual maize-soybean rotation cultivated during the two growing seasons in the subhumid region. Our in-growth core experiment included four treatments: No addition, addition of fertilizer, addition of biochar, addition of biochar and fertilizer. Biochar was derived from Acacia wood and applied at a rate of 50 + 50 ton ha⁻¹ during season 1 and 2 of the experiment in 2006. Metal skirts (20 cm high) were installed around the biochar-amended plots after the first growing season to prevent erosional biochar losses and deposition on other plots, which was observed after heavy rains during the first season. Fertilizers were added during the maize phase in the rotation and contained inorganic P at a rate of 68 kg P ha⁻¹ year⁻¹. The soybean phase did not receive any fertilizers. No addition and fertilizer addition treatments were randomly arranged in blocks of 8 by 12 m with three replicates, which had a split-plot design, with and without biochar addition. The study of P dynamics at this site took place from September 2020 to January 2021 coinciding with the soybean phase of the rotation.

2.2. P dynamics study

2.2.1. Goethite-sand mix production

To study the in situ P desorption from an Fe-oxide mineral, quartz sand covered by nano-sized particles of goethite (α -FeO(OH)) was produced and incubated in the soils in the three experiments. The goethite was synthesized in the laboratory as described by [Krumina et al. \(2022\)](#). Briefly, 2.5 M NaOH was slowly mixed with 0.5 M Fe(NO₃)₃·9H₂O and stirred while bubbled with N₂ (gas). The suspension was titrated until it reached a pH of 12. After 12 h the suspension was left at 60 °C for one week before it was dialyzed in Millipore 12–14,000 D tubes. To confirm that the mineral was synthesized successfully and the sample did not contain any other crystalline phases X-ray spectroscopy was used.

Phosphate (in the form of KH₂PO₄) was then added to the goethite to reach a concentration of 18.4 mg of phosphate per gram of goethite. The amount of phosphate was decided to reach a 70 % level of P saturation of the mineral and it was based on a previous P adsorption isotherm experiment. The mix was then stirred and the pH was adjusted (using 1 M HCl) at the beginning and two more times every hour. The sample was left stirring for 48 h before the pH was adjusted one more time.

To confirm that all phosphate had been adsorbed to the mineral, one aliquot of the P-bound goethite was filtrated. The phosphate in the filtered solution was measured with a colorimetric reaction using ammonium molybdate. No detectable phosphate was found in the solution.

The goethite suspension was centrifuged to separate the mineral particles from the water and around 80 % of the clear supernatant was removed. The remaining concentrated suspension was mixed again and then added to previously acid-washed sand (10 % HCl, thereafter rinsed with tap water to remove excess acid) and thoroughly mixed until a homogeneous goethite-sand mix was produced. The concentration of goethite in the suspension was based on the dry weight of the material and used to estimate the amount of suspension necessary to have a goethite-sand mix concentration of 1 % (w/w). The goethite-sand mix was then dried at 60 °C.

2.2.2. In-situ incubations

At each of the three experiments, we installed cylindrical in-growth cores (diameter 4 cm) produced of a 2 mm size plastic mesh, filled with the goethite-sand mix. The ingrowth cores were buried at 15 cm depth within the crop rows and placed in near vicinity (approx. 10 cm) of the plant individuals. A subset of the cores contained sand with goethite without added P and were used to estimate the inflow of P during the incubation in the soil. The average amount of P found in the no-P sand was subtracted from the amount of P in the P-loaded goethite-sand mix when calculating P desorption. Detectable P in the no-P sand was only found in the soybean experiment.

At the end of the growing season, the root ingrowth cores were collected: First, the root connections around the cores were severed using a sharp knife to prevent the roots from being pulled out of the cores once they were lifted from the soil. Then the cores were carefully lifted out of the soil to prevent the sand-goethite mix from falling out. Thick roots protruding from the cores were excised using scissors and the cores were then wrapped in aluminum foil. A paper stopper was introduced in the cores to avoid the sand getting mixed with the soil that had entered the cores during the incubation.

In the laboratory, the mesh of the root cores was opened using a hot rod, and the soil that had entered the cores and sand containing traces of soil were carefully separated to reduce the contribution of P and Fe from outside the cores. Once the sand was separated from the soil, the fine roots were collected using tweezers and the sand that was attached to the roots was collected by gently shaking the roots. This portion of the sand was analyzed as rhizosphere samples while the rest of the sand mix collected was analyzed as core-bulk sand. The fine roots were then freeze-dried and weighted to have an estimation of root colonization inside the cores.

The rhizosphere sand and core-bulk sand collected were further examined under a stereomicroscope, and remaining soil particles were removed if present. Later, the sand was thoroughly mixed in a falcon tube and a subsample was taken for the elemental (Fe and P) and microbial analysis (membrane phospholipids and neutral lipids).

Additionally, yield records of sugar beets, cereal grains and beans were obtained from data holders for the still ongoing long-term experiments for the seasons of the incubation experiments for assessing the effect of the fertilization or nutrient regimes on above-ground production.

2.2.3. Goethite-sand mix measurements

To investigate the fate of the goethite mineral and the P adsorbed on its surface after their incubation in the soil, total Fe and P content in the sand-goethite mixture was measured by Inductively Coupled Plasma (ICP) mass spectroscopy (Optima 7300 DV) after the goethite from a subset of the sand-mix (1 g) had been combined with 10 ml of 6 M HCl and heated in a hot plate for about 20 min. The values were compared with values in the original material that had not been incubated in the field. Additionally, the Fe/P ratio was calculated. This ratio was used to estimate how much P was removed from the goethite surface as the amount of P alone might not necessarily reflect P desorption since losses of the P-bound goethite particles from the sand may also have occurred.

The microbial components of membrane phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFAs) were used as proxies for microbial biomass ([Frostegård et al., 2011](#)) and were extracted from the bulk goethite-sand mix using organic solvents in a method modified from the Bligh & Dyer extraction ([Bligh and Dyer, 1959](#)). Briefly, the samples were subjected to phase separation where the fatty acids were collected using chloroform. NLFAs and PLFAs were then separated using elution columns (Bono-elute®). The esterification process (separation of the hydrophobic fatty acid tails) was done in a methanol matrix using KOH. Finally, the fatty acids were collected in a hexane phase. The extracts were then measured in a gas chromatograph and the abundance of NLFA and PLFA16:1 ω 5 fatty acids (expressed as μ g per g of goethite-sand mix) were used as proxies for the biomass of AMF. The NLFA

16:1 ω 5 is a storage compound specific to AMF while the PLFA 16:1 ω 5 is a membrane compound found in the fungal hyphae although it may also be present in small amounts in some bacteria.

2.2.4. Statistical analysis

To test for differences in fine root biomass, crop yield, Fe, P, and Fe/P ratios between the crops and fertilization regimes, ANOVA and two-way ANOVAs (Fertilization vs Biochar addition in the Soybean experiment) were performed using the CAR package (Fox and Weisberg, 2019) and Dunn's test for non-parametrical samples using the Dunn.test package (Dinno, 2015) respectively in R (R Core Team, 2014). The relation between P amount and Fe/P ratios and AMF biomass Pearson and Spearman correlation tests were used.

3. Results

3.1. Sugar beet experiment

The root dry biomass per gram of goethite-sand mix recovered from the cores in the sugar beet plots was not significantly different between the two P fertilization regimes (additional and no additional P fertilization, Table 1). There was no significant correlation between the fine root biomass and the amount of P remaining in the core-bulk or in rhizosphere sand mix.

The amount of Fe in the rhizosphere sand was significantly higher than in the core-bulk sand (ANOVA, $F = 28.6$; $p < 0.0001$) and even higher than the initial amount of Fe in the non-incubated goethite-sand mix (Fig. 1a). The amount of Fe remaining in the ingrowth cores was not affected by the fertilization regime.

The amount of P remaining in the goethite-sand mix after incubation was significantly lower in both rhizosphere and core-bulk sand in the treatment with no additional P fertilization compared to the full P fertilization (ANOVA, $F = 32.4$; $p < 0.0001$, Fig. 1b). The amount of P in the rhizosphere sand was significantly lower than the amount in the core-bulk sand in the treatment with no additional P fertilization (ANOVA, $F = 8.8$; $p < 0.01$, Fig. 1b).

According to confidence intervals of the response ratios, there was a decrease in the amount of P in the rhizosphere sand of the ingrowth cores incubated in the plots with no additional P fertilization. This loss was on average 20 % of the amount in the reference material (non-incubated goethite-sand mix) (Fig. 2a). Accordingly, the Fe/P ratios of the rhizosphere sand collected from the ingrowth cores were higher than the reference material in the samples from the plots with no additional P fertilization (Fig. 2b). In the core-bulk sand and the rhizosphere sand from the full P fertilization treatment, no significant difference in the Fe/P ratio was found compared to the original material.

The sugar beet biomass yields tended to be higher in the plots that received additional P however these differences were not significantly different (Table 2).

Table 1

Fine root biomass inside the root cores after incubation in the soils. In parenthesis the standard error ($n = 8, 4$) of the sugar beet, Kernza experiment respectively). The lower-case letters represent differences between the treatments according to the posthoc Tukey test.

Kernza experiment		Sugar beet experiment	
	Root biomass mg. g ⁻¹ of sand		Root biomass inside the cores mg. g ⁻¹ of sand
Kernza monocrop	2.2 (± 0.2) a	No additional P added	0.24 (± 0.07) a
Kernza intercrop	0.7 (± 0.2) b	Additional P added	0.21 (± 0.07) a
Winter wheat	0.8 (± 0.2) b		

3.2. Kernza experiment

The fine root biomass per gram of bulk sand recovered from the cores was significantly different between the cereal crops (ANOVA, $F = 16.5$; $p < 0.001$), and the higher amount of fine roots was detected in the Kernza monocrop plots with root biomass >3-fold higher than in the Kernza intercropped with alfalfa and more than double than in the winter wheat crop. There were no significant differences between the fine root biomass in the winter wheat plots and the Kernza intercropped plots (Table 1).

Large amounts of Fe were lost from the ingrowth cores in all treatments in comparison with the reference material (Fig. 3A). The amount of Fe remaining in the goethite-sand mix after incubation was significantly higher in the Kernza-Alfalfa intercrop compared to the Kernza monoculture and the winter wheat (ANOVA, $F = 11$; $p < 0.001$). There were no significant differences between the sand attached to the roots (rhizosphere) and the core-bulk sand in the Kernza and Kernza-Alfalfa crops but for winter wheat the amount of Fe was higher in the core-bulk than in the rhizosphere sand.

During the incubation, P remained to a much greater extent in the ingrowth cores compared to Fe. In most cases, the amount of P per gram goethite-sand mix tended to be higher in the ingrowth cores compared to the original material, except for the core-bulk sand in the Kernza-alfalfa intercrop plots which tended to be lower (Fig. 3B). There were no differences between the crops regarding the amount of P remaining in the goethite-sand mix after incubation, but the amount of P was higher in the sand collected from the rhizosphere in comparison with the core-bulk sand in the mixed crops (Fig. 3B).

The Fe/P ratios of the core-bulk or the rhizosphere sand after incubation were not significantly different between the crops.

The AMF biomass (using the 16:1 ω 5 NLFA marker) in the core-bulk sand was significantly higher in Kernza growing in monoculture compared to Kernza intercropped with Alfalfa (ANOVA, $F = 11.01$). The AMF biomass was intermediate in wheat, and not significantly different from the other two treatments (Fig. 4). The amount of Phospholipid Fatty Acid 16:1 ω 5 (PLFA) in the ingrowth cores was not significantly different between the crops. There were no significant correlations between the amount of P remaining in the sand mix and the 16:1 ω 5 NLFA or the 16:1 ω 5 PLFA.

The grain yields of the Kernza intercropped with alfalfa were on average 2 times higher than when Kernza was grown alone but these differences were not significant. The yields of the perennial cereals were substantially lower than the annual winter wheat as Kernza is a novel crop that is still undergoing breeding efforts to reach similar yields as the annual counterparts (Table 2).

3.3. Soybean experiment

In contrast to the other two experiments, very small amounts of roots could be collected in the cores from the soybean experiment and the roots collected lacked root hairs, while there were plenty of root hairs in the Kernza and sugar beet experiment. In most cases, there was one root (with rhizobium nodules) colonizing the cores with fungal hyphae, probably of AMF origin, visible in the sand.

There was no significant loss of Fe from the core-bulk sand in the ingrowth cores compared to the reference material for any of the treatments, and thus, no apparent effects from fertilization or biochar addition on the amount of Fe remaining in the goethite-sand mix. Significant amounts of P were removed from core-bulk sand in the ingrowth cores as compared to the reference material (non-incubated goethite-sand mix) under all treatments. The desorption was highest in the non-fertilized plots with biochar amendment where there was an average P loss of 40 % (Fig. 5A). This also resulted in an increase of the Fe/P ratios compared to the reference material and the highest increase was detected in the non-fertilized plots (Fig. 5B). The amounts of roots collected were small and the sand/goethite mixture did not attach to the

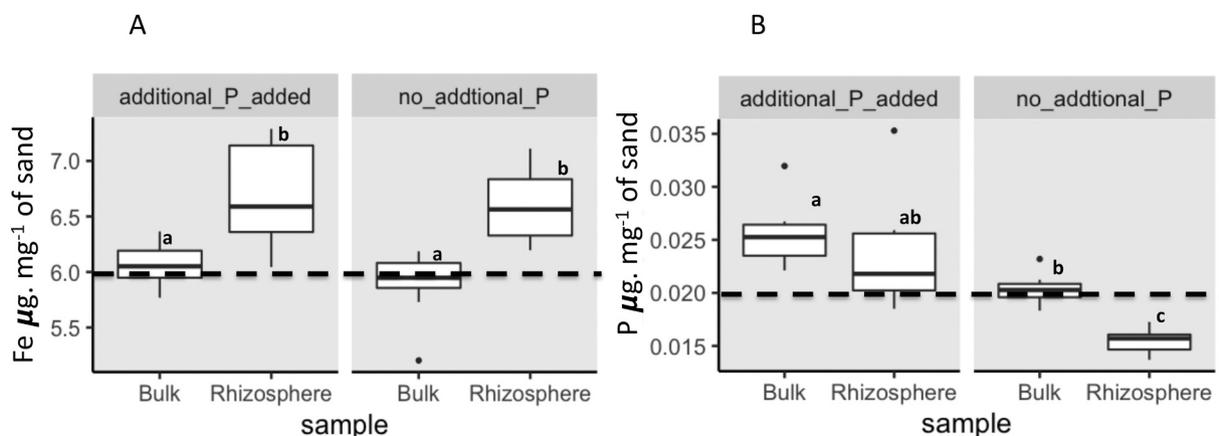


Fig. 1. Fe ($\mu\text{g. mg}^{-1}$, A) and P ($\mu\text{g. mg}^{-1}$, B) in the goethite-sand mix from the root cores after incubation in the soil from the Sugar beet plots. The low case letters indicate significant differences between the crops according to the pairwise Dunn test. The dashed line corresponds to the Fe and P concentration in the non-incubated reference sand-mix.

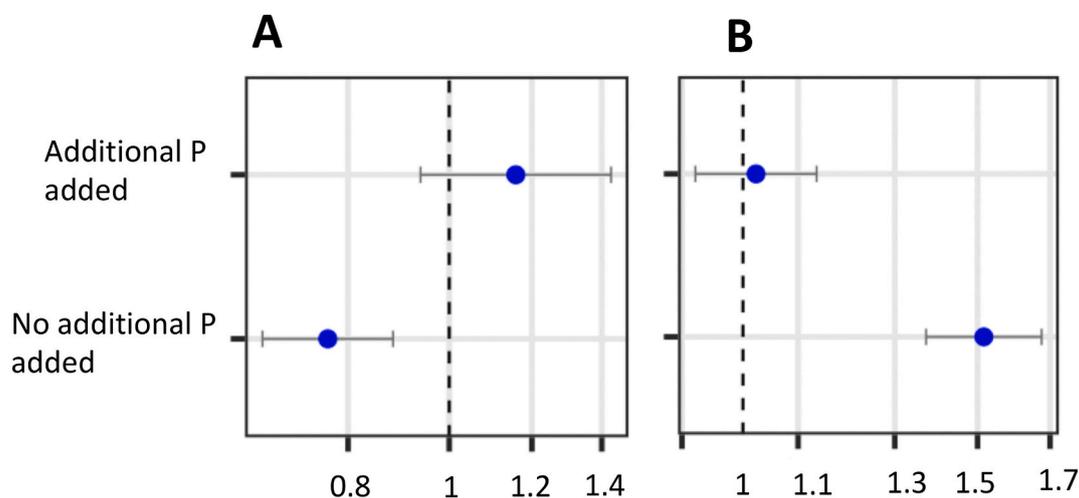


Fig. 2. Response ratios of the P concentration A) and Fe/P ratios B) in the goethite-sand mix from the sand attached to the roots (rhizosphere) in comparison with the non-incubated goethite-sand mix from the sugar beet plots. The horizontal lines correspond to the 95% confidence interval for each treatment. The dashed line corresponds to the P concentration and Fe/P ratio in the non-incubated reference sand-mix.

Table 2

Yield measured for the three experiments for the growing season when the root-cores were incubated. In parenthesis the standard error ($n = 8, 4, 3$ for the sugar beet, Kernza and soybean experiment respectively). The lower-case letters represent differences between the treatments according to the posthoc Tukey test.

Kernza experiment			Sugar beet experiment		Soyabean experiment	
	Kernel yield t.ha ⁻¹	Straw yield t.ha ⁻¹		Root yield t.ha ⁻¹		Bean yield t.ha ⁻¹
Kernza monocrop	0.2 (± 0.1) ^a	4.5 (± 0.5) ^a	No additional P added	67 (± 12) ^a	Fert-Bio	4.1 (± 0.4) ^a
Kernza intercrop	0.3 (± 0.1) ^a	5.1 (± 1.0) ^a			Fert-no bio	3.4 (± 0.2) ^{ab}
Winter wheat	8.7 (± 0.4) ^b	6.0 (± 0.4) ^a	Additional P added	75 (± 10) ^a	No fert-Bio	2.9 (± 0.2) ^{ab}
					No fert-no bio	1.1 (± 0.4) ^b

root. Therefore, we could not measure Fe and P removals from rhizosphere sand.

Significantly larger amounts of P were desorbed in core-bulk sand under soybean, where the maize phase in the rotation did not receive fertilizer, than with annual doses of N and P, both with and without biochar (ANOVA, $F = 5.6$; $p < 0.05$, Fig. 5C). Soils amended with biochar but receiving no fertilization had the lowest mean P concentration of ingrowth cores, yet no significant biochar effect or interaction with fertilizer addition was found on the amount of remaining P due to the high variability and limited replication.

There was a negative correlation between the NLFA marker

corresponding to AMF (*16:1 ω 5*) and the amount of P remaining in the goethite-sand mix (Pearson, $\text{Cor} = -0.73$, $p < 0.05$). The correlation between PLFA *16:1 ω 5* and amount of P was marginally significant (Pearson, $\text{Cor} = -0.56$, $p = 0.06$) (Figs. 6 A and B). A significant positive correlation was exhibited between NLFA *16:1 ω 5* and the Fe/P ratios (Pearson, $\text{Cor} = 0.58$, $p < 0.05$), while the correlation between PLFA *16:1 ω 5* and Fe/P ratio was marginally significant ($\text{Cor} = 0.54$, $p = 0.07$) (Figs. 6 C and D). The abundance for PLFA *16:1 ω 5* tended to be higher in the plots amended with biochar (with and without inorganic fertilization) (ANOVA, $F = 3.9$; $p = 0.08$). Individual and interactive effects of fertilization or biochar addition on the abundance of the NLFA *16:1 ω 5*

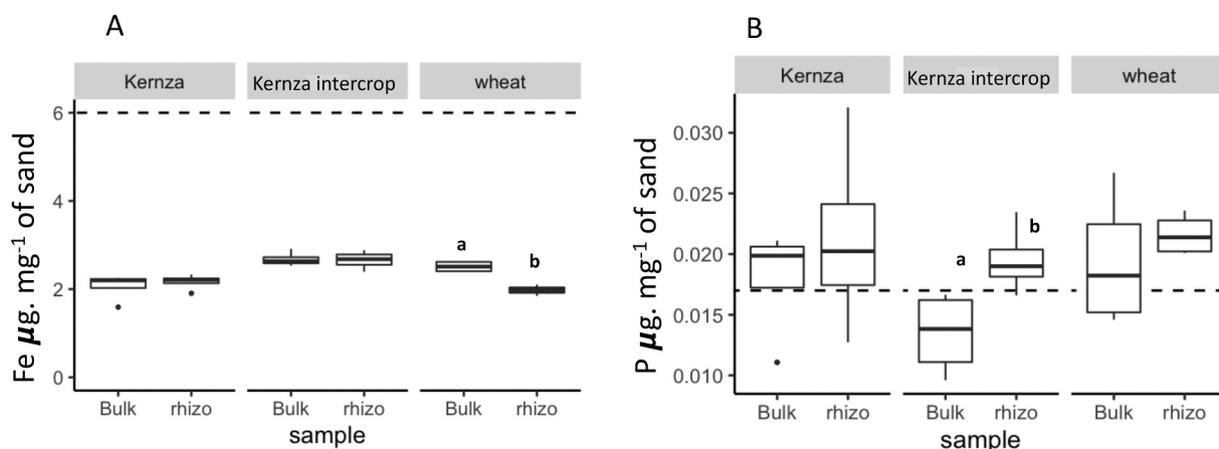


Fig. 3. Fe ($\mu\text{g mg}^{-1}$, A) and P ($\mu\text{g mg}^{-1}$, B) in the goethite-sand mix from the root cores after incubation in the soil from the Kernza experiment. The lower case letters indicate significant differences between the core-bulk and rhizosphere sand according to the Kruskal-Wallis test. The dashed line corresponds to the Fe and P amount in the non-incubated reference sand-mix.

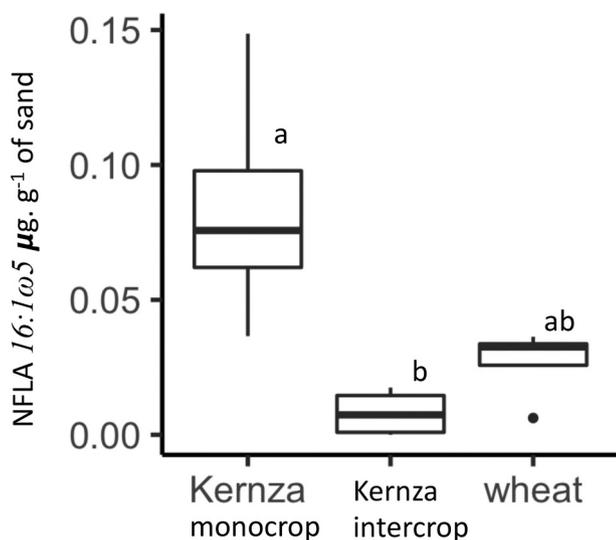


Fig. 4. Neutral Lipid Fatty Acids (NLFAs, $\mu\text{g g}^{-1}$) extracted from the goethite-sand mix from the root-cores after incubation in the soil from the Kernza experiment. The lower case letters indicate significant differences between the crops according to the pairwise Dunn test.

and PLFA 16:1 ω 5 were not statistically significant.

Both fertilization and biochar amendment significantly affected soybean yields during the incubation period (ANOVA, $F = 32.7$ and $F = 16.6$, respectively; $p < 0.001$) (Table 2). The highest yields were detected in the plots where a combination of fertilization and biochar was added, followed by the plots that received fertilization only, which in turn were followed by the plots that received biochar amendment only. The yield for the plots that received no fertilization or biochar were significantly lower than those for all other treatments according to the post-hoc tests. The yield in the biochar-only plots was lower than the fertilized biochar plots but the difference was marginally significant ($p = 0.08$) (Table 2).

4. Discussion

4.1. Sugar beet experiment

The results from the sugar beet experiment partially support the first hypothesis since the P concentration of the sand goethite mix was lower

in the rhizosphere compared to the core-bulk sand indicating that the roots drive P mobilization. However, this relationship was significant only in the plots where no additional P was added which indicates that an increase in soil available P reduced uptake of P adsorbed on the goethite. Indeed, P remaining in the cores was significantly lower in the plots with no extra P addition in comparison with the plots that received additional P. This supports our second hypothesis stating that more P will be mobilized under lower P availability. Moreover, only the plots without additional fertilization had P amounts lower than the non-incubated reference material suggesting that soil labile P regulates the P desorption capacity of roots. Our results are consistent with those by (Bhadoria et al. 2002), who found a higher influx of P per unit root length of sugar beet when grown in oxisols under limiting compared to sufficient P conditions.

The Fe enrichment in the rhizosphere (in both fertilization regimes) in comparison with the core-bulk sand (Fig. 1) might suggest movement of goethite particles towards the root during water uptake. If this had been the case the P removal in the rhizosphere may have been underestimated as this movement of goethite must have carried P-adsorbed Fe to the rhizosphere. Still, the P amount in the rhizosphere remained lower than in the core-bulk sand suggesting that more P was taken up by the root than the amount accounted for.

Partially in contrast with hypothesis 1, there was no relationship between fine root biomass and P desorption from the core-bulk sand. Furthermore, there were no differences in root biomass between the P addition treatments, which indicates that the plants did not stimulate P desorption through increasing root biomass but probably by increasing the production of LMWOA. It has been shown that sugar beets exuded citrate, oxalic and oxaloacetic acids to enhance P availability (Beissner and Römer, 1998). The presence of these acids may result in the desorption of phosphate from goethite due to competition for the same adsorption sites (D'Amico et al., 2020; Geelhoed et al., 1999; Sokolova, 2020; Parfitt, 1979). It has been estimated that the concentration of LMWOA surrounding the root surface could be considerably higher than concentrations in the soil solution (especially under P limitation) reaching values high enough to promote Fe dissolution and mobilization of P (Sokolova, 2020; Jones et al., 1996). This could have been the case in the current study since P removal from the Fe-oxide mineral was found in the rhizosphere samples but not in the core-bulk sand. This might suggest that an effect of LMWOA on the goethite was only significant in the rhizosphere area where root exudates are more concentrated. Further measurements of LMWOA concentration in relation to P desorption from goethite under field conditions are strongly encouraged.

Despite the significant effect of the P fertilization regime on P

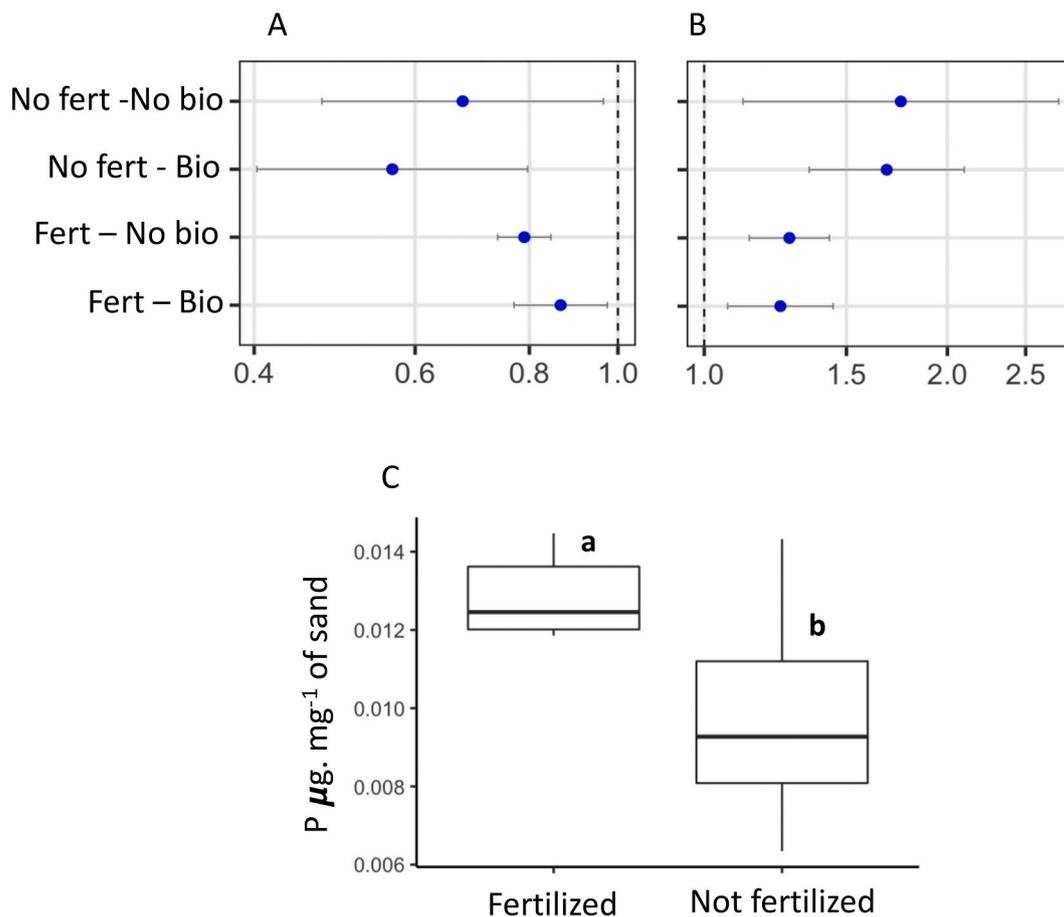


Fig. 5. Response ratios of the P amount ($\mu\text{g mg}^{-1}$, A) and Fe/P ratios (B) in the goethite-sand mix from the root-cores after incubation in the soil in comparison with the non-incubated goethite-sand mix in the Soybean plots. The horizontal bars correspond to the 95% confidence interval for each treatment. The treatments correspond to fertilization plus biochar additions (Fert-Bio), fertilization alone (Fert-No bio), no fertilization but biochar additions (No fert-Bio), and no fertilization and no biochar additions (No fert-No bio). The P amount ($\mu\text{g mg}^{-1}$) remaining in the goethite-sand mix after incubation in the soil, comparison between fertilized (with and without biochar) and unfertilized plots (with or without biochar) (C). The lower case letters indicate significant differences between the crops according to the post hoc Tukey test.

dynamics inside the root cores, the difference in sugar beet yield between the treatments was not statistically different at the current growing season. Different effects of P additions on sugar beet yields have been reported where gradients of labile P (phosphate) from 30 to 336 kg P ha^{-1} have increased sugar beet yields linearly without reaching a plateau (see Kusi et al., 2021) while other studies have shown that phosphate concentrations higher than 120 kg P ha^{-1} did not significantly increase sugar beet yields (see Bouras et al., 2021). The response of sugar beets to labile P additions can depend on soil type, soil properties and the amount of soil available P (Kusi et al., 2021; Bouras et al., 2021; Hergert, 2012). Subsoils in the province in Sweden where these experimental plots are established have been reported to contain iron oxides (Ulén, 2006) with a high P adsorption capacity (Ulén, 2006). Roots in the plots that did not receive extra P additions could possibly compensate for this by accessing P adsorbed in the soil lower layers, as our results show that under these conditions (no additional P) roots can desorb and take up P from iron oxides.

These findings are very important for sustainable and P-efficient agriculture as sugar beets can be used in crop rotations to mobilize legacy P from Fe-oxides. Moreover, depending on soil type, extra addition of P (above the amounts taken out with the harvest) might not be needed in some crops of this region which can help to mitigate P losses by leaching or adsorption in Fe and Al oxides (Bergström et al., 2015). In addition, minimizing the need for P additions can be important for farmers and stakeholders as it can help to reduce their costs.

4.2. *Kernza* experiment

Perennial *Kernza* (monocrop) produced considerably higher amounts of fine roots than annual wheat, sugar beet and soybean crops in the present study. This is not surprising as *Kernza* is a perennial plant that commonly produces larger root systems than annuals (see Sprunger et al., 2019). The high root production in the *Kernza* monocrop did however not result in P removal from goethite in the *Kernza* plots in our study. Thus, hypothesis 1 stating that P desorption is related to root biomass was not supported. It is however still likely that enhanced root growth will result in better P uptake since a larger soil volume will be explored, but poorly available P sources like goethite bound P are probably not the main source of this P uptake, at least not in our experiments. Surprisingly, root ingrowth produced in the cores from the *Kernza*-alfalfa intercrop system was substantially lower than when the cereals were grown alone producing as much root biomass as the annual winter wheat. These results might be due to the higher N resources provided by the legume. When N is not limiting in the soil, the C partitioning to the shoots is prioritized over the roots (functional equilibrium) (Zhang et al., 2022; Kleemola et al., 1996). Indeed, both grain and straw yields in the intercrop tended to be higher than in the *Kernza* monocrop. Therefore, a reduction in belowground C allocation can be expected in the intercrop in comparison with the monocrops as the plants decrease the investment in soil exploration and fine roots.

The P amount in the core-bulk sand collected from the intercrop was lower than the cores from the *Kernza* in monoculture and had lower

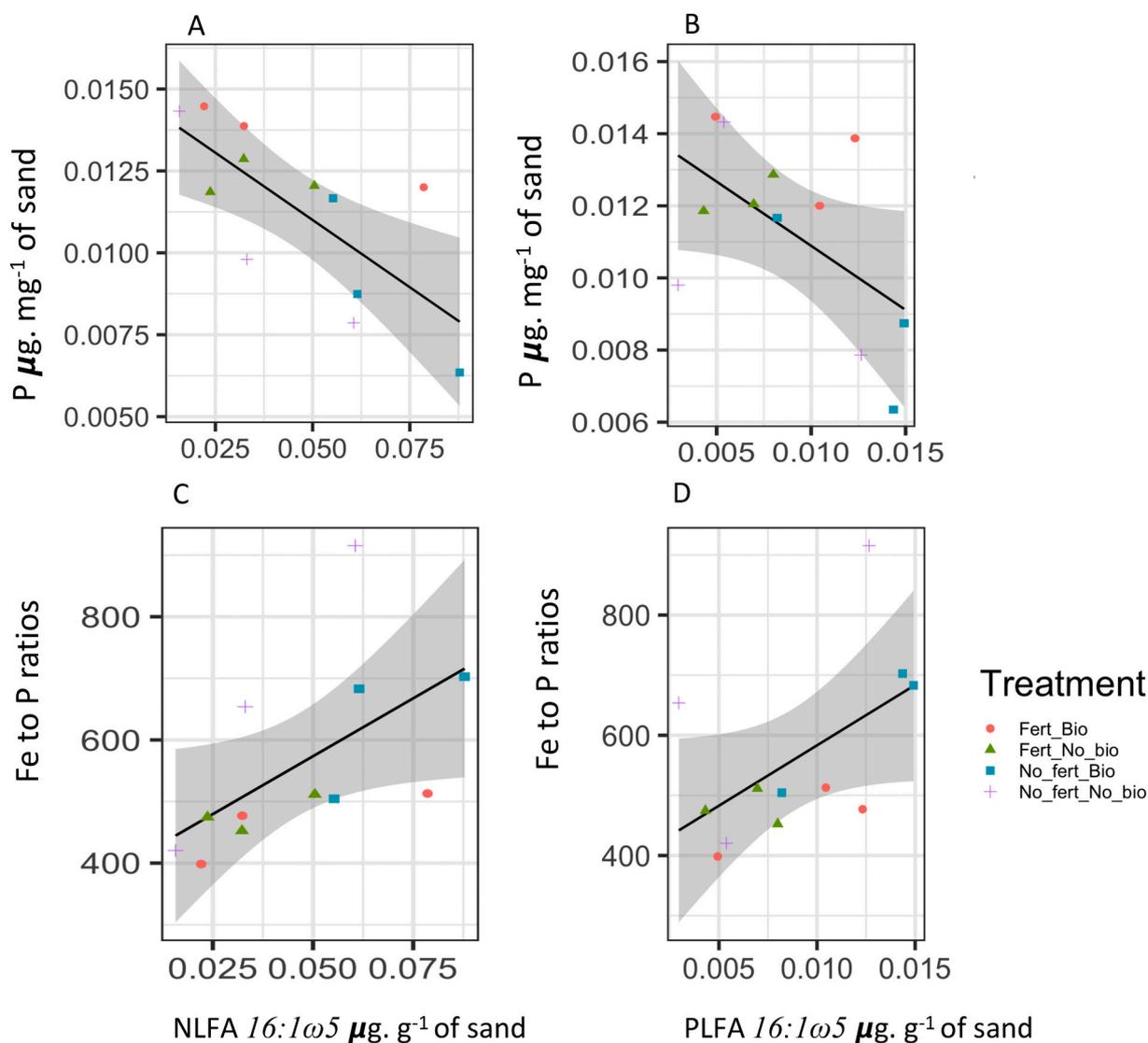


Fig. 6. Correlation between the arbuscular mycorrhizal markers (*16:1 ω 5*) NLFAs ($\mu\text{g mg}^{-1}$, A) and PLFAs ($\mu\text{g mg}^{-1}$, B) and the P amount remaining in the goethite-sand mix from the root-cores after incubation in the soil of the Soybean plots. Correlation between the arbuscular mycorrhizal markers (*16:1 ω 5*) NLFAs ($\mu\text{g mg}^{-1}$, C) and PLFAs ($\mu\text{g mg}^{-1}$, D) with the Fe/P ratios in the goethite-sand mix from the root-cores after incubation in the soil. The treatments correspond to fertilization with biochar addition (Fert_Bio), fertilization without biochar addition (Fert_No_bio), no fertilization with biochar addition (No_fert_Bio), and no fertilization without biochar addition (No_fert_No_bio).

values than the non-incubated reference material. This would support the hypothesis that legume intercropping enhances P uptake from iron oxides. However, a substantial amount of Fe was lost from the cores after incubation, suggesting losses of goethite from the sand probably by water runoff (some of the sand from these plots looked bleached suggesting losses of goethite). Thus, the low P values could be attributed to a general loss of Fe loaded with P. Moreover, P concentrations in the core-bulk sand were lower than in the rhizosphere which makes it difficult to draw conclusions about the role of the roots in P uptake. It should be noted however, that we did not identify the roots in the cores. They may have been dominated by Kernza roots. Possibly, P was removed from the core-bulk sand not by the root itself but by AMF hyphae in the Kernza intercrop plots. Nevertheless, the amount of AMF biomass was higher in the Kernza monocrop in comparison with the Kernza intercrop and no association between AMF and P amount in the sand mix was found which contradict our hypothesis. This prevents us from making further conclusions about the role of AMF mycelia for P removal from the core-bulk sand. The higher abundance of AMF in the Kernza plots in comparison with the annual winter wheat is expected as perennial roots

allocate more C belowground (Sumiyoshi et al., 2017; Anderson-Teixeira et al., 2013) that can sustain more root symbionts (Duchene et al., 2020). In addition, the lack of tillage in this system can preserve mycelial connections (Van Groenigen et al., 2010). These results are consistent with another study performed in the same Kernza plots as the current paper showing that soils from Kernza monocrop plots harbor more AMF biomass than both Kernza-alfalfa intercrop or annual winter wheat (Meijer, 2020). This higher AMF colonization in the root cores of the perennial Kernza monocrop suggests a soil-foraging advantage for nutrients like P. The lower AMF colonization seen in the Kernza intercrop could be explained as a reduction in belowground investments due to more N resources as suggested earlier and might indicate that N is the limiting nutrient in these systems as it is common for high latitude soils (Vitousek et al., 2010).

We cannot rule out that P uptake by Kernza, and the possible role of AMF, may be different under more P limiting conditions. For instance, the positive effects of AMF seen in the Kenya experiment but not in the Kernza crops might be due the intrinsic P limitation present in tropical crops as these soils are severely P limited (Vitousek et al., 2010) due to

high P fixing capacity of Fe-oxides (Chacon et al., 2006). It has been reported that different host plants harbor different AMF species and microbe associate with different capacities to take up nutrients (Fester and Sawers, 2011; Säle et al., 2021). Thus, it is possible that roots, AMF and associated microbes from P limited soils like in Kenya have developed strategies to dissolve P from Fe-oxide minerals. In the organically fertilized perennial crops in Sweden on the other hand, it could be expected that plant and microbes are more reliant on a P economy based on nutrient mineralization from organic sources; as perennial root systems are known to promote highly fertile soils in terms of organic matter, root and microbial biomass (Duchene et al., 2020).

4.3. Soybean experiment

The significant removal of P from the sand-goethite mix irrespective of fertilizer and biochar amendment demonstrates that soybean has a strong capacity to interact with Fe-oxides, even when more P is available. The low soil pH, characteristic of high sesquioxide content and strong affinity for binding phosphate, may explain why the crop is adapted to dissolution of mineral-bound states. The significantly lower amount of P remaining in the non-fertilized plots (both with and without biochar amendment) support our second hypothesis and demonstrate that labile P in the soil influences the degree of nutrient release through dissolution reactions. This negative effect of P fertilization is likely a result of a downregulation of the transfers of resources (lipids and carbohydrates) to AMF and associated bacteria which restricts microbially-mediated desorption activity. This is consistent with findings by Abdel-Fattah et al. (2014) who found that input of inorganic P led to lower AMF colonization in soybean roots.

Unexpectedly, biochar did not have a significant positive influence on P desorption. Dissolution of legacy P by crops on soils that received biochar may have also been lower due to reversible exchange that prevents nutrients from binding with iron oxides resulting in higher P availability (Ahmed et al., 2020). This mechanism was not evaluated in this study, primarily because in-growth cores did not contain biochar. The greater nutrient availability in biochar amended soil is evidenced by three times higher soybean yield compared to the non-enriched plots, when no fertilizer was added. Kätterer et al. (2019) similarly found greater production and extractable P in the first 10 years after biochar addition for the unfertilized treatment. Yield patterns for fertilized plot without and with biochar also corresponded to what has been reported by Kätterer et al. (2019) for previous years. Results from this paper confirm that the positive effect of biochar persists 14 years after addition, proving longevity of benefits on nutrient regulation.

The higher abundance of biomarker for AMF hyphae (*16:105*) in biochar enriched plots without or with fertilizer agrees with previous studies that found greater mycorrhizal and microbial growth and ascribed it to improvement of soil conditions (Li and Cai, 2021; Li et al., 2020). The significant positive relationship of P removal from goethite with abundance of AMF biomarkers in the Kenyan site support hypothesis 3. Such plant-microbe-soil interaction is also supported by Andriano et al. (2019, 2021) who found significant P mobilization from goethite in mesocosm experiments with tomato (*Solanum lycopersicum*) associated with AMF (*Rhizophagus irregularis*). In those studies, transfers of desorbed P to the plants via AMF mycelium were confirmed. The exact mechanisms by which AMF facilitate the re-mobilization of legacy P remains unclear. For instance, Andriano et al. (2021) detected LMWOA in root-free soil which suggests that the mechanism of P desorption is governed by microbial communities. The authors could not distinguish if the LMWOA were produced by AMF or other microbes, and there is also no direct evidence of LMWOA production by AMF (Campos et al., 2018). A growing body of literature recognizes that AMF rely on associations with other soil microorganisms like PSB to dissolve P from mineral-bound state. This association consists of AMF hyphae shuttling PSB and providing sugars and other resources to obtain mobilized P by the bacteria for onward exchange with the crop (Fall et al., 2022;

Etesami et al., 2021). Previous studies also found greater bacterial abundance and richer composition in the hyphosphere, ascribing it to exudation of labile and C-rich compounds (Qin et al., 2016; Andrade et al., 1997). Hence, the high AMF abundance found in this study could have promoted the growth of different bacterial groups including PSB which have contributed to P desorption. The microbial complex involved with desorption of mineral-bound P, along with the influence of agronomic practices on these associations, may lead to variable effectiveness of implemented strategies.

5. Conclusion

The three investigated cropping systems demonstrated explicit differences in the amount of P desorbed from goethite in incubated ingrowth cores, which point out that plant traits and soil conditions have an overriding influence on the effectiveness of utilizing legacy P. In sugar beet, a non-AMF host, long-term application of reduced P rates led to higher P removal from goethite, which may be due to enhanced exudation of LMWOA. Absence of P mobilization by the Kernza crop may be an effect of better accessibility of available P due to its extensive root system and the organic amendments.

For soybeans on highly weathered tropical farms, P fertilization decreased the desorbing capacity of the crop. Thus, the use of biochar in combination with small amounts of P could be recommended as an alternative for farmers to increase grain yields and at the same time enhance the access and mobilization of P bound to iron oxides in the soil.

The suitability of crop arrangement, P application rate and organic matter input for specific soil types must be determined to concurrently realize efficient use of legacy P and satisfactory crop yield. Moreover, the significant relationship between AMF and P observed in this study and novel findings on the role of PSB require further investigation so that practices are well formulated. Findings from our field experiments demonstrate the possibility of recovering fixed P in arable land through crop and soil management for sustainable crop production and reduced fertilizer use, both in temperate and tropical regions. Researchers and policymakers should pay closer attention to such strategies for improving nutrient use efficiency and tackling fertilizer shortages.

CRedit authorship contribution statement

Juan Pablo Almeida: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Dries Roobroeck:** Writing – review & editing, Investigation. **Linda-Maria Dimitrova Mårtensson:** Writing – review & editing, Investigation. **Pedro Rosero:** Methodology. **Geoffrey Kimutai:** Methodology, Investigation. **Thomas Kätterer:** Writing – review & editing, Project administration, Investigation. **Håkan Wallander:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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