Increasing phosphorus fertilizer value of recycled iron phosphates by prolonged flooding and organic matter addition

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ABSTRACT

Iron (Fe) minerals are commonly used to remove phosphorus (P) from waste streams, producing P-loaded Fe(III) oxides or Fe(II)P minerals (e.g. vivianite). These minerals may be used as fertilizers to enhance P circularity if solubilized in soil. Here, we tested the P fertilizer value of recycled iron phosphates (FePs) in a pot trial and in an incubation experiment, hypothesizing that P release from FePs is possible under Fe(III) reducing conditions. First, a pot trial was set up with rice (*Oryza sativa*) in all combinations of soil flooding or not, three P-deficient soils (acid, neutral, calcareous) and six FePs (three Fe(III) and three Fe(II) phosphates) referenced to triple superphosphate (TSP) or zero amendments. Shoot P uptake responded to TSP applications in all treatments but only marginally to FePs. The redox potential did not decrease below 200 mV by flooding for a brief period during the pot trial. A longer incubation experiment (60 days) was performed which included a treatment of glutamate addition to stimulate reductive conditions and P availability was assessed with $CaCl_2$ extraction of soils. Glutamate addition and/or longer incubation lowered soil redox potential to < -100 mV. On the longer term, Fe(III) minerals released P and adequate P was reached in the calcareous soil and in the neutral soil amended with Fe(III)P-sludge. It can be concluded that prolonged soil flooding and organic matter (OM) addition can enhance the P fertilizer efficiency of FePs. Additional treatments showed that application of FeP in powder form may enhance P availability.

Key Words: agronomic efficiency, circular economy, P-loaded Fe(III) oxides, vivianite, paddy soils, redox

INTRODUCTION

The global use of P fertilizers has increased, driven primarily by the growing demand for food and feed (Vance *et al.,* 2003). This large consumption threatens the stock of phosphate rock and, at the same time, leads to local environmental issues where excess fertilizer has been used. To target both issues, P can be removed from P-rich waste streams and potentially be recycled into an effective fertilizer, thereby closing the P cycle in agriculture (Melia *et al.,* 2017).

Currently, most approaches to recover P from wastewater in wastewater treatment plants (WWTPs) focus on the crystallization of struvite, direct application of sewage sludge to agricultural fields, or techniques relying on sludge incineration. Each of these has disadvantages regarding recovery efficiencies and applicability. In contrast, the recovery of P as vivianite ($Fe(II)_3[PO_4]_2 \cdot 8H_2O$), a mineral that is formed when ferrous iron (Fe) is added to wastewaters to remove P, is promising for several reasons. First, it is naturally ubiquitous in sewage sludge, accounting for 70--90% of all phosphates (Wilfert *et al.*, 2016). Second, it has higher P content (Fe:P molar ratio of 1.5) compared to other ferric precipitates in WWTPs. Third, vivianite, owing to its paramagnetic properties, can be separated from sewage sludge, and be collected in relatively pure phase using magnetic separators (Wilfert *et al.,* 2018).

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For the removal of P from diffuse agricultural sources, P removal via adsorption processes using low-cost P-sorbing materials (PSMs) has garnered attention due to the fast immobilization of P (Liu *et al.,* 2018). These PSMs contain metal cations, typically aluminum (Al), calcium (Ca), magnesium (Mg), and Fe, that form an insoluble compound with dissolved P. Specifically, Fe-coated sands and Fe sludges are Fe(III) based minerals that are very effective at removing P (Lambert *et al.,* 2021). These materials are by-products from drinking water production, that are formed during Fe removal to prevent colored and bad-tasting water. Aeration of the groundwater leads to the oxidation of Fe(II) to $Fe(OH)_3$, which coats the sand and gravel filter beds, yielding Fe-coated sand. Alternatively, the Fe-coated sand can be washed for reuse of the sand, producing Fe-sludges (Liu *et al.,* 2018).

The FeP minerals, obtained by trapping P from waste streams using Fe minerals, have the potential to be reused as fertilizers. From the perspective of P recovery, the strong interaction between Fe and P is a clear advantage, however, the poor solubility of P from the FeP materials could strongly limit the plant availability of the recovered P (Samie and Römer, 2001; Kahiluoto *et al.,* 2015), particularly in aerobic conditions. The Fe(II) based ones such as vivianites have been used as P fertilizer but are mostly limited to those of geologic origin (Yaya *et al*., 2015) or of peat origin in various crop experiments in Russia (Gamzikov and Marmulev, 2007). Next to their potential as P source, vivianites can also be valorized as Fe fertilizers to mitigate Fe chlorosis in various crops grown in calcareous soils in the Mediterranean region (Díaz *et al.,* 2009; Rosado *et al.,* 2002). In contrast, Fe(III) based P fertilizers rarely exhibit positive P fertilizer effects, even when added as nanoparticles to soil (Bollyn *et al.,* 2019). However, in some conditions, positive fertilizer values have been found. For example, P sorbed on Fe minerals was used to prepare a P-mineral complex for growing rice in a glasshouse of which 15--31 % of sorbed P was recovered by rice within 3 months (He *et al.,* 1994). Similarly, ochre (hydrous ferrous oxides) from coal mines that were subsequently loaded with P performed well as slow-release P fertilizer in cereals and trees (Dobbie *et al.,* 2005). Synthetic iron phosphates showed a slightly higher biomass yield but lower P uptake in comparison with monocalcium phosphates in ryegrass (Johnston and Richards, 2003), yet no explanation was provided for the relatively good performance of the material. Iron phosphates as by-products of fertilizer reactions in the soil (e.g., hydrogen ammonium iron phosphates and colloidal ferric phosphates) and as impurities of commercial P fertilizers (reported as citrate-insoluble P) were reported to have residual P availability (Lindsay and DeMent, 1961; Sikora and Mullins, 1995) suggesting the release of P in the long term. This could be beneficial for plants requiring frequent P fertilization like tree stands in peatland forests (Nieminen *et al.,* 2011).

Here, we hypothesized that Fe-based P recycling products can be suitable P fertilizers in flooded soils, e.g. in soils used in growing paddy rice. It is well established that soil P availability increases upon soil flooding following reductive dissolution of soil Fe(III) minerals (Rakotoson *et al.,* 2016), and the addition of an organic carbon (OC) source can further stimulate this process (Scalenghe *et al.,* 2002). However, to what extent reductive dissolution in flooded soils can also increase the effectiveness of Fe-based P fertilizers is still unknown. Against this background, a pot trial was set up to identify the relative P use efficiency (RPUE) of six granular recycled FeP products (three vivianites and three P-loaded Fe oxides) in the early stages of rice development, in paddy soils with or without flooding (13 days). The RPUE of FePs was compared to that of triple superphosphate (TSP), a conventional soluble P fertilizer and unamended control treatments. We hypothesized that the recycled FePs would perform better as P fertilizers in the pot trial under flooded conditions due to increased soil P availability after the liberation of P following reductive dissolution of Fe(III) minerals. In addition, a soil incubation experiment was established to determine the P availability of powdered recycled FePs (as measured by 1 mM $CaCl_2$ extraction), exploring also the effect of longer flooding conditions (up to 60 days) and OM addition (glutamate) and thus expanding the range of soil conditions in comparison with those in the pot experiment. With the incubation experiment, we hypothesized that prolonged flooding and OM addition would promote reduced conditions and thus enhance the P availability of FePs in soils.

MATERIALS AND METHODS

Soils

Soil samples were collected from the upper 20--30 cm layer, air-dried, and sieved to <2 mm before use. These soils were chosen as representative of rice-growing areas with low P availability. Soils were analysed for pH, cation exchange capacity (CEC), particle size distribution, total C and N, and oxalate extractable Fe, Mn, and P (Table I).

TABLE I

Selected physicochemical properties of soils from pot experiment

		Acid	Neutral	Calcareous
Site of origin		Cavinti, Laguna, Philippines	Dakawa, Tanzania	Isla Mayor, Seville, Spain
Soil classification[a]		Ferralsol	Vertisol	Fluvisol
Clay[b]	(%)	84	32	8
Silt[b]	(%)	14	56	76
Sand[b]	(%)	2	12	16
pH[c]		4.5; 6.1^c	6.0	7.9
CEC[d]		7.6	20.7	26.4
C/N[e]		10.5	14.6	22.0
Organic C[e]	(%)	0.55	0.86	1.84
Inorganic C[e]	(%)	-	0.2	2.7
P in soil solution[f]	(mg P L^{-1})	<0.008	<0.008	0.01
Fe_{ox}[g]	(g Fe kg^{-1}	0.94	2.62	2.91
Al_{ox}[g]	(g Al kg^{-1})	1.86	0.62	0.72
Mn_{ox}[g]	(g Mn kg^{-1})	0.17	0.39	0.32
P_{ox}[g]	(mg P kg^{-1})	10	40	300
FC[h]	(mL kg^{-1})	410	250	325
K[i]	(L kg^{-1})	1065	136	120
n[i]		0.22	0.29	0.36
PSI[j]	(mg P kg^{-1})	635	70	52
PSC[k]	(mmol kg^{-1})	42.9	35.0	39.4
DPS[l]	(%)	0.7	3.4	24.8

[a] Major soil groups (IUSS Working Group WRB, 2022); [b] Clay, silt, sand, and texture class based on particle size distribution determined by laser diffraction method; the texture of acid soil determined by pipette method (Day, 1965); [c] pH (1:5 S:L) in 0.01 M $CaCl_2$ (acid soil was limed to pH 6.1 for the pot trial); [d] Cation exchange capacity (CEC) was determined in a 0.0166 M cobalt hexamine (Cohex) extract (Chhabra et al., 1975), as the difference between total Co added and Co measured in the extract with ICP-MS; [e] C/N and organic C and inorganic C as % of total soil mass determined by the combustion method; [f] P in soil solution determined by indirect sorption/desorption method (0.01 M $CaCl_2$); P concentration in filtrate was determined using malachite green method with detection limit of 8 µg P L^{-1} (Van Veldhoven and Mannaerts, 1987); [g] Ammonium oxalate extractable Fe, Al, Mn and P (Schwertmann, 1964); [h] Field capacity (FC) determined through the glistening effect; [i] Fit parameters for the P sorption isotherms described by the Freundlich equation ($P_{sorbed} = k (P_{solution})^n$); [j] P sorption index (PSI) defined as the amount of added P sorbed on the solid phase at a soil solution concentration of 0.1 mg P L^{-1} (Six et al., 2012); [k] P sorption capacity (PSC) defined as $0.5*(Fe_{ox} + Al_{ox})$; [l] Degree of P saturation (DPS) defined as $\frac{P_{ox}}{PSC}*100\%$

The P adsorption isotherms were also determined for the three soils as detailed by Six et al., (2012). Briefly, soils were mixed with 0.01 M $CaCl_2$ with increasing P concentrations. Soil suspensions were then shaken for 16 hrs, centrifuged (2500 g, 10 min), and filtered (0.45 µm, Chromafil ® Xtra PET -45/25; Macherey- Nagel, Germany). The P concentration in the filtrate was determined using malachite green method (Van Veldhoven and Mannaerts, 1987) and absorbance was measured at 630 nm using a spectrophotometer (Perkin Elmer,

Lambda 20, 1 cm path length). Results showed that the P sorption of the soils ranged acid soil >> neutral soil > calcareous soil (Fig. S1, see Supplementary Material Fig. S1).

Recycled FeP materials

Three vivianites and three Fe(III) phosphates were used (Table II). Two vivianites and two Fe(III) phosphates were collected from different sources. In addition, one extra vivianite was synthesized in the lab by dissolving $FeSO_4$ and $(NH_4)_3PO_4$ and one extra Fe(III) phosphate was produced by loading a Fe(III)-rich product with a saturated P solution. As a reference P fertilizer, the commercial soluble fertilizer TSP was used. The total P content (and other elements) of the FeP materials was determined with Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, Thermo Scientific iCAP 7000 series) after digestion in aqua regia (Table III).

TABLE II

Description of the FeP materials tested in the experiment.

Material	Description
Vivianites (Fe(II) phosphates)	
V-WWTP	Provided by Wetsus (European Center of Excellence for Sustainable Water Technology), a water treatment facility in the Netherlands
V-fert	Provided by Fertiberia, a fertilizer company in Spain
V-syn	Synthesized according to Rosado *et al*., (2002) by dissolving 2.5 % (w/v) of monoammonium phosphate (MAP) with 7.5 % (w/v) ferrous sulfate. The pH is adjusted to 6.5 to 7.5 with 10 M KOH. Suspension is then washed with MilliQ until electrical conductivity (EC) is < 0.2 dS m^{-1}. Precipitates were then dried under anaerobic hood.
Fe(III) phosphates	
Fe(III)P-sludge	Iron sludge from Veghel pumpstation provided by Aquaminerals (company in the Netherlands that finds application of Fe by-products from water companies)
Fe(III)P-gravel	Mixture of iron coated gravels from different pumpstations in the Netherlands provided by Aquaminerals. Due to low P content of the material (0.32%), it was artificially loaded with P by incubating the material for 24 h in a 184 mM KH_2PO_4 solution (L:S ratio of 10 L kg^{-1}, end-over-end shaking). After recovery of the material via centrifugation, it was air-dried and stored until further use.
Fe(III)P-sand	P-loaded iron coated sand provided by Nico Lambert of NuReDrain Project (Lambert *et al*., 2021)

The FeP materials were provided in different forms, i.e., as granules or as powder. Granular materials (i.e., TSP, Fe(III)P-gravel, and Fe(III)P-sand, see Table II for abbreviations) were sieved to obtain granules of 2--4 mm (except the small-sized TSP). The powders (i.e., all vivianites), were made into granular particles via extrusion. For this, a paste was made by mixing ultrapure water (MilliQ) with the powders, which was extruded in a mould with a 4-mm diameter hole. Subsequently, the obtained pellets were cut to 3 mm length and dried at 40 °C overnight.

In the pot trial, all six FePs and TSP applied as granules were tested, while in the incubation experiment only two products were tested, i.e. one Fe(II) phosphate (V-WWTP) and one Fe(III) phosphate (Fe(III)P-sludge).

TABLE III

The elemental composition of the FeP materials, expressed as percentage by weight of air-dried material

Materials	P (%)	Ca (%)	Mg (%)	Al (%)	Fe (%)	Fe:P molar ratio
TSP	18.05	22.40	0.007	2.16	3.87	0.01
Vivianites (Fe(II) phosphates)						
V-WWTP	9.55	0.90	0.96	0.11	25.81	1.50

V-fert	12.65	n.d.	n.d.	n.d.	35.47	1.55
V-syn	13.45	n.d	n.d.	n.d.	38.28	1.58
Fe(III) phosphates						
Fe(III)P-sludge	2.78	7.40	n.d.	0.01	34.70	6.81
Fe(III)P-gravel	3.31	0.002	0.00006	1.23	27.00	4.15
Fe(III)P-sand	1.26	1.90	0.24	0.02	50.18	22.15

n.d. not detected.

Plant growth trial

The experimental design was completely randomized with three factors (soil, amendments, soil flooding) and with three replicates for each treatment. The pot trials were not done simultaneously due to space limitation in the growth cabinet and the late arrival of the calcareous soil. To avoid false positive P response as a result of flooding (raised soil pH under flooded conditions) and the pH neutralizing effect of Fe(III)P fertilizers (mean pH of 7.4) on acid soils (Everaert *et al.,* 2017), the acid soil was limed at a rate of 1.5 g $Ca(OH)_2$ kg^{-1}, which raised the soil pH from pH 4.5 to 6.1; as determined in a liming incubation test. This soil is nevertheless further termed acid soil because of the extremely large P sorption that is typical for ferralsols (Table I, Fig S1).

Soils were mixed with macronutrients (other than P) as dry salts at the rates of 120 mg N kg^{-1} (NH_4NO_3), 120 mg K kg^{-1} (KCl, 254 mg K kg^{-1} for acid soil due to higher P dose), 75 mg Ca kg^{-1} ($CaCl_2$), 30 mg Mg kg^{-1} and 40 mg S kg^{-1} ($MgSO_4·7H_2O$). The N and K were split-applied, partly before sowing as dry salts and at 16 days after planting (DAP) as solution. Micronutrients were applied by spraying the soil with a micronutrient solution (20 ml kg^{-1} soil) with adequate concentration to obtain rates of 1.2 mg Mn kg^{-1} ($MnCl_2·4H_2O$), 0.6 mg Zn kg^{-1} ($ZnCl_2$), 0.6 mg Cu kg^{-1} ($CuCl_2·2H_2O$), 0.2 mg B kg^{-1} ($Na_2B_4O_7·10H_2O$), and 0.2 mg Mo kg^{-1} ($Na_2MoO_4·2H_2O$). After mixing the nutrients with the air-dried soil, soils were transferred individually into 1 L transparent pots (900 g soil for the neutral and calcareous soil, 838 g soil for the acid soil). Then, for each soil, TSP (four doses) and FeP (one P dose; equal to highest TSP dose) granules were added in the center of the pots, 3 cm below the surface with the granules placed in one layer close to each other. For TSP, maximum doses were 24 mg P kg^{-1} for the neutral soil, 50 mg P kg^{-1} for the acid soil, and 40 mg P kg^{-1} for the calcareous soil. This was based on the P adsorption curves of each soil and previous P fertilizer studies where the estimated difference in P uptake between mixed P (KH_2PO_4) and placed P (TSP) application was a factor of 3 for the neutral soil (de Bauw *et al.,* 2019), 4 for the acid soil (de Bauw *et al.,* 2019) and 1.3 for the calcareous soil (Everaert *et al.,* 2017). Control treatments (without P) were also included for all soils. Subsequently, soils were watered until field capacity and were incubated in the phytotron for six days in dark conditions at $25 ± 2$ °C. Then, two pre-germinated seedlings of Malagasy rice variety X265 were transplanted (at root length $±1.5$ cm) to each pot after incubation and thinned to one after 5 DAP. At 8 DAP, the contrasting water regime was imposed by adding water to $±3$ cm on top of the soil surface in the flooded treatments. Water management was maintained daily until the end of pot trial. Until harvest, rice was grown in a plant growth cabinet with day/night temperature setting of 25/20 °C, at relative humidity of 80 % and light intensity of 550--600 µmol cm^{-2} s^{-1}. At 21 DAP, the rice plants were harvested and oven-dried at 60 °C for 3 days. Dry shoots were weighed and digested in 10ml HNO_3 using a microwave (MARS 6 microwave digestion system, CEM). The digests were analyzed for concentrations of P and other plant nutrients by ICP-OES.

Each batch included 2 blank samples, an internal standard, and a certified reference material (hay powder, Joint research centre, European Commission). The average recovery of P, certified concentration 2360 mg P kg^{-1}, was 99%. The internal standard material (rice shoots) is verified relative to certified reference material (hay powder) with P at similar concentrations (e.g., BCR129; Joint research centre European Commission, 2021). For P in the internal standard, verified concentration of 1308 mg P kg^{-1}, the average recovery was 91 %.

Soil analyses after plant growth

One week after harvest, a 1mM $CaCl_2$ soil extraction was performed on two (randomly chosen) out of three replicates of each treatment, the flooded soil was stored and kept flooded between harvest and soil sampling. The

upper 3 cm of the soil column from a pot was sampled and mixed. Ten grams of moist soil was taken and added to a 50 mL polyethylene tube. Next, a 1 mM $CaCl_2$ solution was added to the tube until it was completely filled (i.e., no headspace). Samples were shaken end-over-end for 24 h (0.42 s^{-1}; T = 20 +/- 2 °C), centrifuged (2500 g, 10 min), filtered, and acidified using concentrated HNO_3 to a volume percentage of 1%. Next, the P concentration of the acidified samples was determined with Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Agilent 7700x, Agilent Technologies).

The soil redox potential (E_h) was also measured in control unamended treatments 24 h and 48 h after the harvest using glass fiber probes (8 mm diameter, 10 cm length) (Paleoterra Products, Amsterdam, The Netherlands) inserted vertically into the soil column. The E_h measuring point made of platinum ring was positioned 2 cm from the tip of the probe allowing E_h measurement at 2 cm below the soil surface. The Ag-AgCl reference electrode was positioned in the water layer. The E_h relative to standard hydrogen electrode was calculated from the measured potential with temperature-dependent correction for the potential of the Ag-AgCl electrode. The temperature and pH were also measured using a pH meter.

Incubation Experiment

Soils (air-dried and sieved) were mixed with macronutrients at the same dose used in the pot trial. Two hundred grams of soil were placed into 250 ml plastic containers, after which the soil was flooded with deionized water up to 3 cm above the surface. The pots containing the soils were incubated under water in dark conditions for 60 days at 25 ± 2 °C. Different treatments were set up, varying the type of powdered iron phosphates (Fe(III)P-sludge or V-WWTP applied at P dose of 200 mg P kg^{-1} for acid soil, 70 mg P kg^{-1} for neutral soil, and 52 mg P kg^{-1} for calcareous soil) and glutamate content (0 or 1 g C kg^{-1} soil) for three soils, with three replicates per treatment. Glutamate was added as C source to stimulate reductive dissolution of Fe(III). Control treatments (without FePs) were also included for all soils. After 3, 14, and 60 days of incubation, a 1 mM $CaCl_2$ soil extraction was performed as described above. For each soil, the redox potential values were also monitored weekly in an additional replicate of control treatments.

Data and statistical analysis

The phosphorus uptake (PU) in each treatment was calculated as

PU (mg) = P concentration in shoot (mg g^{-1}) x dry matter yield (g)　　　　　　　　　　(Eq. 1)

The RPUE of the FeP materials was determined as (Cabeza *et al.,* 2011*)*:

$$RPUE, x\ (\%) = \frac{PU_x - PU_{control}}{PU_{TSP} - PU_{control}}\ x\ 100$$ 　　　　　　　　(Eq. 2)

with PU_x = PU obtained by a specific FeP treatment *x* (mg), $PU_{control}$ = mean PU in the unfertilized control (mg), and PU_{TSP} = mean PU in the TSP reference treatment at equal P dose (mg).

Statistical analysis was performed using JMP software (JMP pro 15, SAS Institute Inc.). All data were subjected to analysis of variance (ANOVA) with soil, fertilizer type and water as the main factors. The effect of different FeP fertilizers on P uptake and soil P as extracted with 1 mM $CaCl_2$ (in comparison to the unfertilized control and to the same P dose of TSP) was assessed with the Tukey test following the ANOVA test. This was done for each soil separately, and within each soil, for each water treatment separately at 0.05 level of significance.

For the incubation experiment, the $CaCl_2$-extractable P concentrations were subjected to ANOVA with soil, FeP type and glutamate as the main factors. Then, for each soil the effect of glutamate on the $CaCl_2$-extractable

soil P for each FeP treatment per sampling time was assessed with the Tukey test following the ANOVA test at 0.05 level of significance.

RESULTS

Plant growth trial

    Plant response to TSP application.   The shoot dry matter and shoot P uptake increased largely and significantly with increasing TSP doses in all soils and all water treatments except in the non-flooded calcareous soil (Fig. S2, see Supplementary Material Fig. S2). This supports the fact that the three soils are P deficient, with P deficiency most pronounced in the acid soil in comparison with the neutral and calcareous soils. Shoot P concentrations responded in line with the shoot P uptake, except in the calcareous soil in both water treatments (Fig. S3, see Supplementary Material Fig. S3). The plants grown under non-flooded treatments took up more P than corresponding flooded treatments in the acid and neutral soils (Table SI, see Supplementary Material Table SI). Plant growth and P uptake were distinctly lower in the calcareous soil than in the other two soils (Fig. S3, see Supplementary Material Fig. S3).
    Plant response to application of recycled FeP products.   The application of the different FePs resulted in lower shoot P uptake than in TSP treatments at equal P doses. The FePs did not significantly enhance shoot P uptake in comparison with the unfertilized control in most soils and flooding treatments (Fig. 1) except for V-syn and V-WWTP, and Fe(III)P-gravel in the acid soil for both water treatments. The Fe(III)P-gravel also enhanced P uptake in the flooded treatments of the two other soils but not in the non-flooded ones.
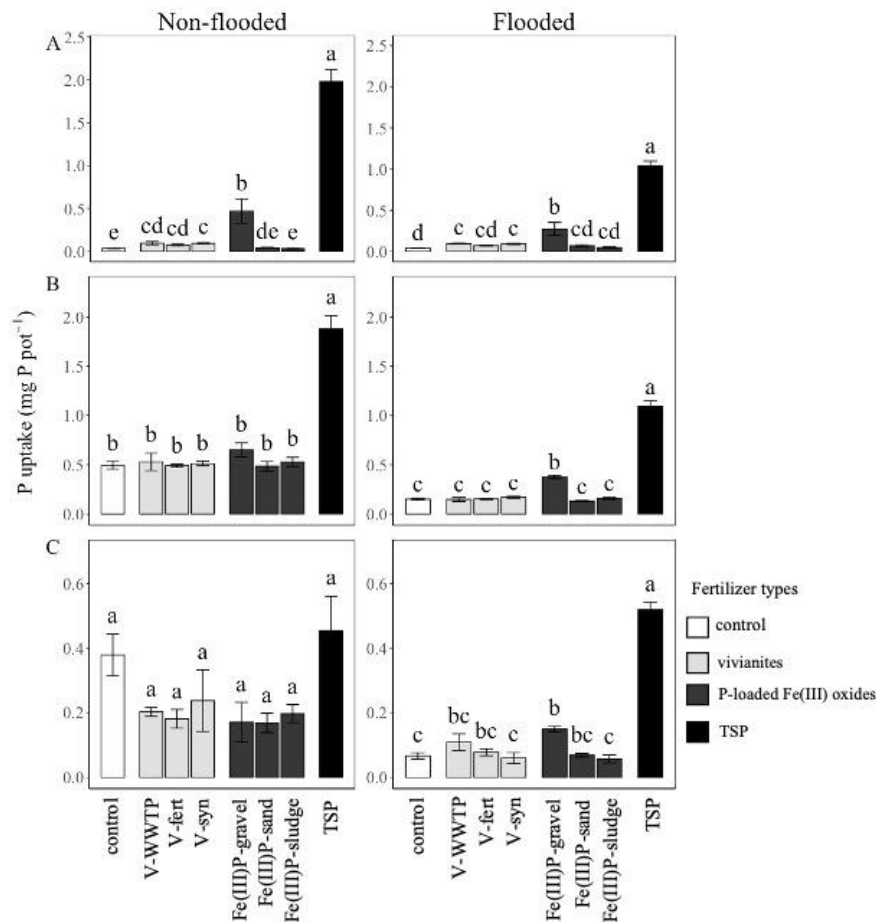

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Fig. 1 The shoot P uptake by rice grown for 3 weeks in the acid (A), neutral (B), and calcareous (C) soils amended by recycled FePs is not significantly different from the unamended control (except Fe(III)P-gravel in A and B) and significantly lower than that of TSP in non-flooded (left) and flooded (right) conditions. Treatments were applied at a dose of 50 mg P kg^{-1} in A, 25 mg P kg^{-1} in B, and 40 mg P kg^{-1} in C. Data is mean of 3 replicates (except for control where n=6) and error bars represent standard error of means. Different letters denote significant differences among treatments (Tukey, $P < 0.05$).

Flooding the soil for a brief period (13 days) did not have any effect on the P uptake of FePs in the acid soil in contrast to the neutral soil where a negative effect of flooding was observed (Table SII, see Supplementary Material Table SII). The average E_h values of the unfertilized control treatments were 280 mV (pH 6) for the acid soil and 370 mV (pH 6) for the neutral soil after harvest indicating soil conditions were not reduced enough to transform Fe(III) to Fe(II) (Fig. 4). In the calcareous soil, flooding induced either a negative or no effect on the P uptake of the treatments. A lower P uptake was observed in the control, V-fert, Fe(III)P-sand and Fe(III)P-sludge while the P uptake of the other FePs were not affected by the water conditions. In the calcareous soil, under non-flooded conditions, all treatments were not significantly different from each other while P uptake in flooded conditions highly differentiated the TSP treatment from the rest of the FePs. The better performance of Fe(III)P-gravel was not observed in this soil (non-flooded treatments) as the material had likely aged by the time the pot experiment for this soil was performed.

The RPUE (Eq. 2) was calculated and is shown in Fig. 2. The RPUE values of the FePs were all small (2--3%) and RPUE was maximally 24 % compared to TSP for the Fe(III)P-gravel in the acid soil. In some cases, a negative RPUE was obtained, indicating that the P uptake for these treatments is even lower than that of control treatments.
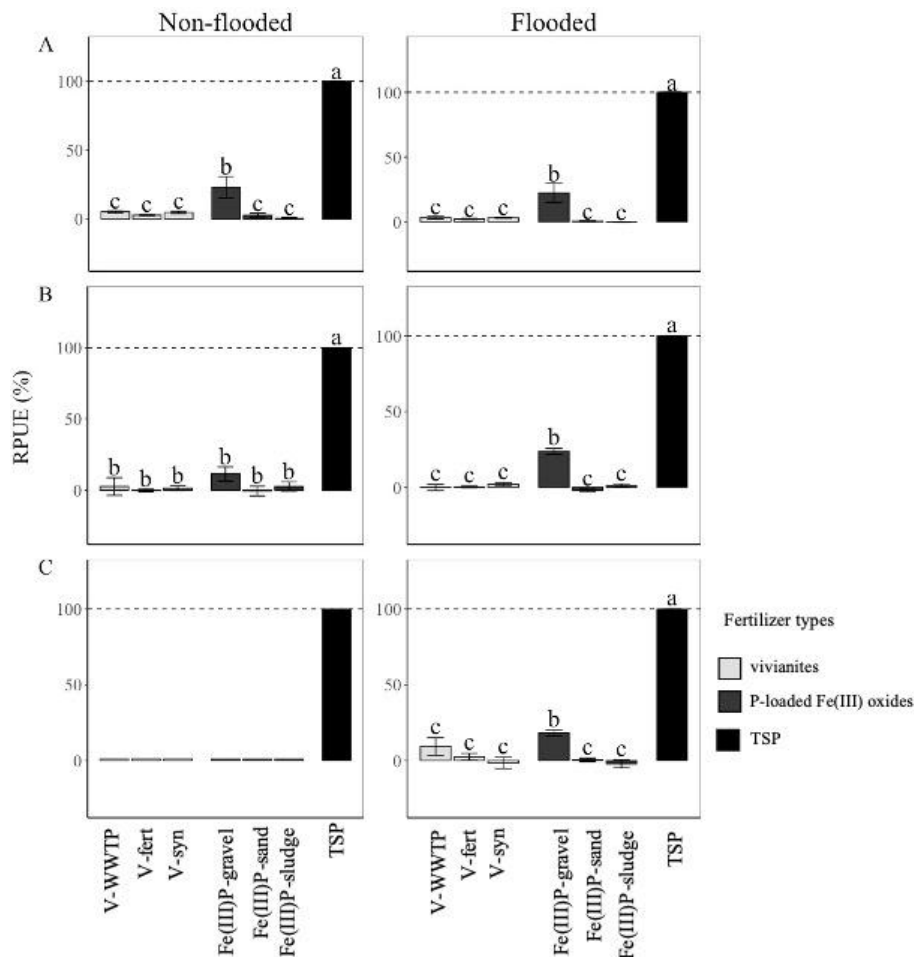
Fig. 2 Relative phosphorus use efficiency (RPUE) of FePs indicates the low effectiveness as P fertilizers in a 3-week rice pot experiment in the acid (A), neutral (B), and calcareous (C) soils under non-flooded (left) and flooded (right conditions). The RPUE of TSP is 100 % by definition. Treatments were applied at a dose of 50 mg P kg^{-1} in A, 25 mg P kg^{-1} in B and 40 mg P kg^{-1} in C. The RPUEs in non-flooded calcareous soil (lowest left graph) were negative and are not shown. Values represent a mean of n=3, except for control treatments where n=6. Error bars represent the standard error of the means. Different letters denote significant differences among treatments (Tukey, $P < 0.05$).

CaCl$_2$-extractable P across soils after plant growth

The majority of the CaCl$_2$-extractable soil P concentrations in the FeP-amended treatments were not statistically different from those of the corresponding unamended control or TSP treatments (Fig. 3). The CaCl$_2$-extractable soil P concentrations confirmed the ranking of soil P deficiency as found in the plant growth assay (acid >> neutral > calcareous soil), since the P concentrations in the acid soil were smallest and all were below the detection limit (0.003 mg P L^{-1}). These concentrations were also low in the neutral soil, averaging at 0.01 mg P L^{-1} in non-flooded and 0.02 mg P L^{-1} in flooded conditions. In the calcareous soil, the values range from 0.03 mg P L^{-1} for the control to about 0.06 mg P L^{-1} for the highest dose of TSP. These values are all below the adequate P concentration values needed for growing rice (0.1 mg P L^{-1}; Beckwith, 1965).
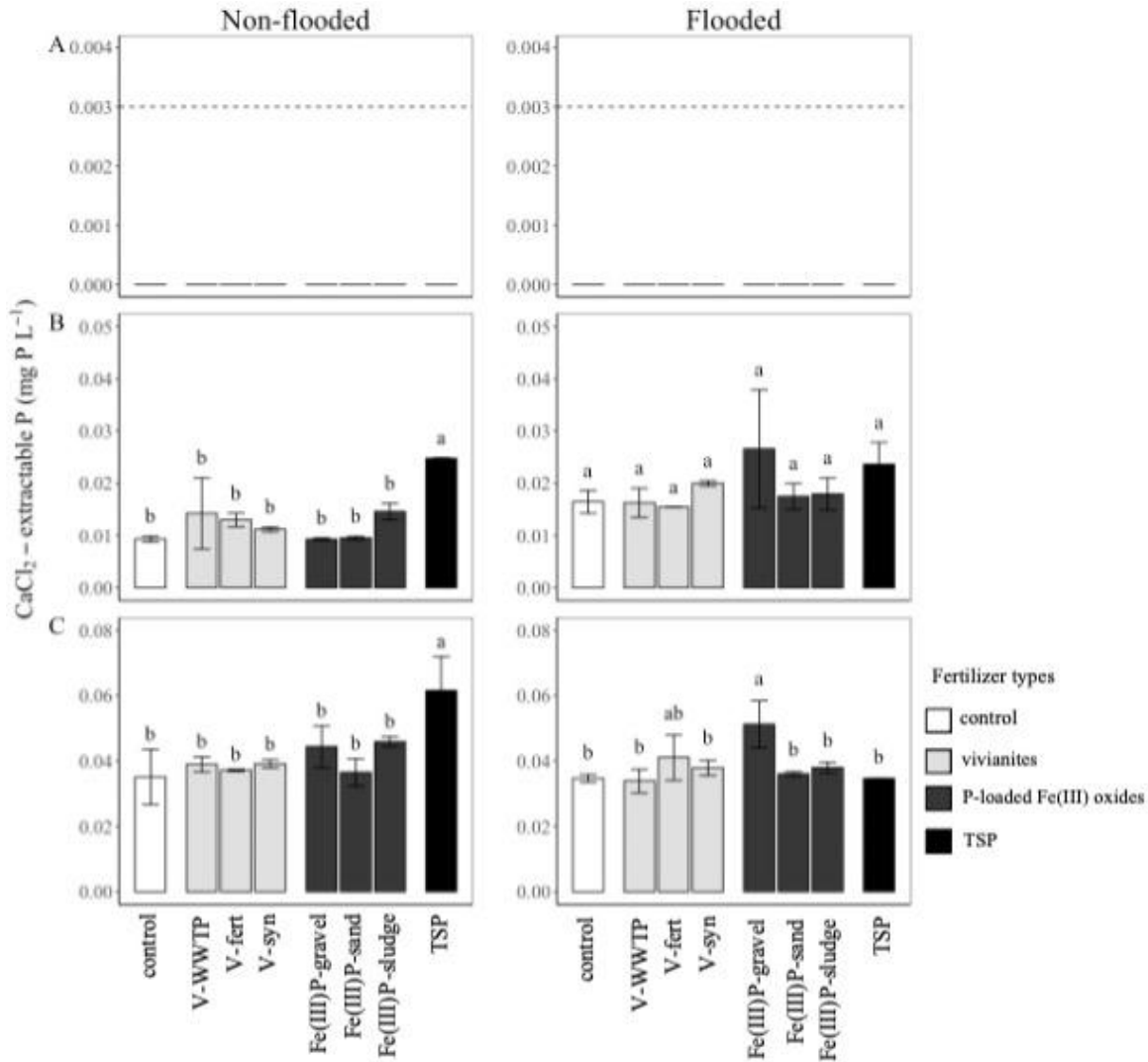
Fig. 3 The CaCl$_2$-extractable P (mg P L^{-1}) in the acid (A), neutral (B), and calcareous (C) soils amended by FePs and TSP that were either flooded (right) or not flooded (left). Note the different scales for the different soils and where values for acid soil were below the limit of detection (0.003 mg P L^{-1}). These data show the low solubility of FePs. All P fertilizers were applied at a dose of 50 mg P kg^{-1} in A, 25 mg P kg^{-1} in B, and 40 mg P kg^{-1} in C. Values represent a mean of n=2, except for control treatments where n=4. Error bars represent the standard error of the means. Different letters denote significant differences among treatments (Tukey, $P < 0.05$).

Incubation experiment

Stimulating CaCl$_2$-extractable P from FeP-amended soils. Flooding of the soils in the incubation experiment reduced the soil redox potential for all the treatments (Fig. 4) with glutamate-amended treatments reaching the lower limit of -200 mV faster than treatments without OM. The calcareous soil reached negative values within 3 days of flooding, while it took 7 days for the neutral soil. The acid soil treated with glutamate took the longest time (3 weeks) to reach reduced conditions.
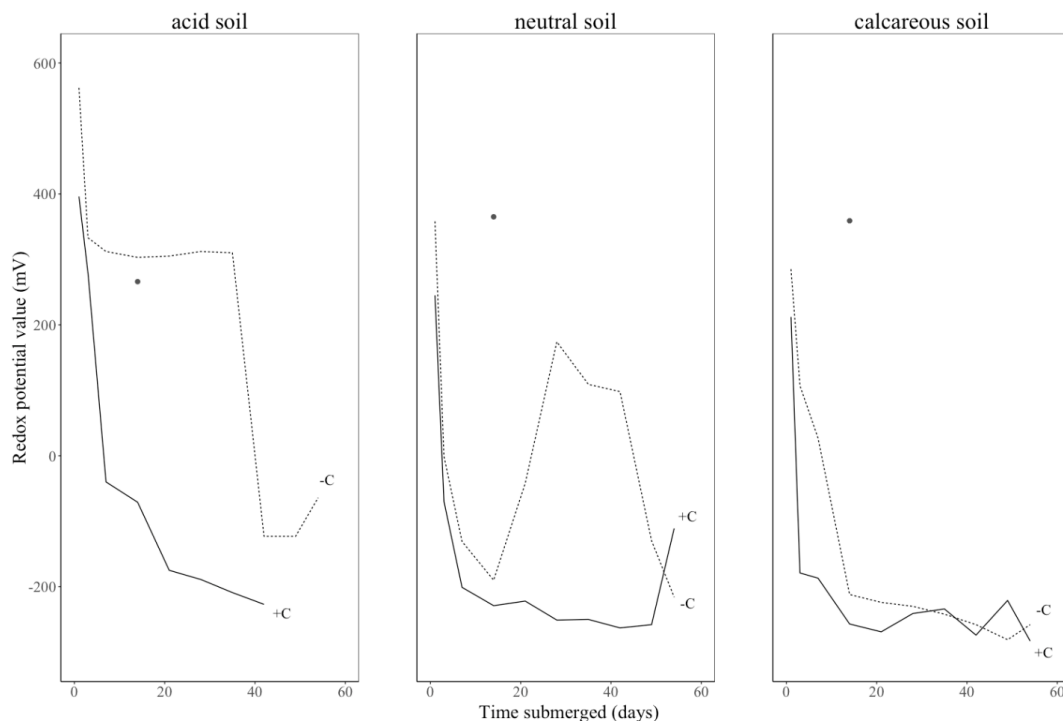

Fig. 4 Changes in soil redox potential (mV) with time after incubating control treatments of acid, neutral, and calcareous soils with (full lines) and without (dotted lines) glutamate under flooded conditions. Dots are the measured redox values on control (no FeP) treatments after harvest in plant growth trial with flooding period of 13 days.

The CaCl$_2$-extractable soil P concentrations are shown in Fig. 5. In the acid soil, the CaCl$_2$-extractable P was lower than the concentration needed to support rice growth (0.1 mg P L^{-1}), even with glutamate addition. In the neutral soil, the P concentration of about 0.1 mg P L^{-1} was only reached in the treatment amended with Fe(III)P-sludge after 60 days. In contrast, in the calcareous soil, this P concentration was already achieved after two weeks for all treatments.
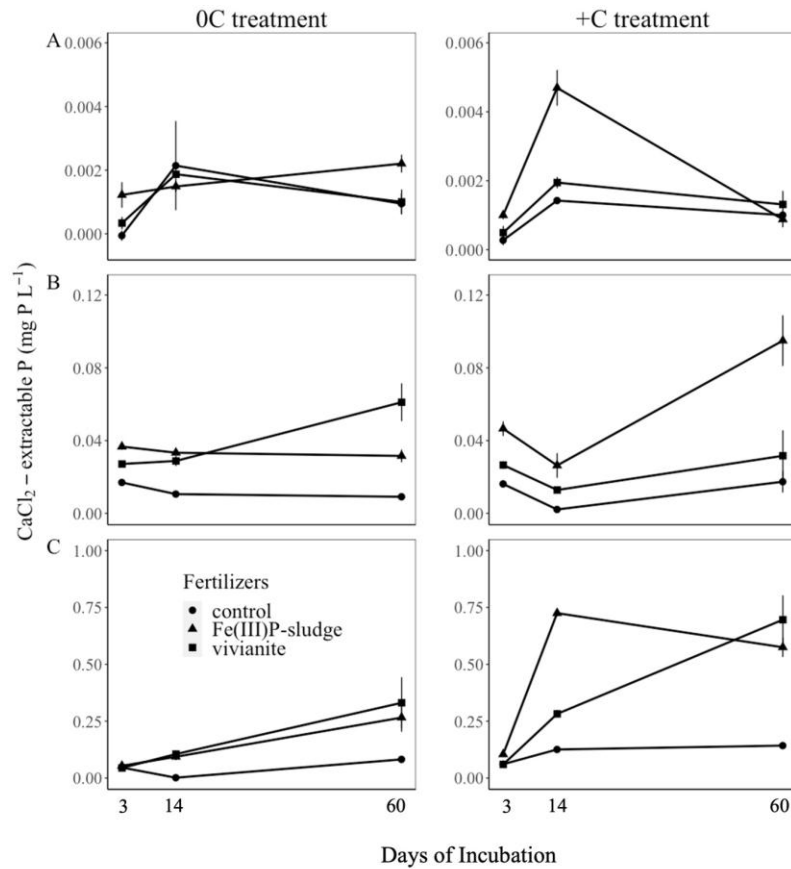
Fig. 5 Changes in mean $CaCl_2$-extractable P concentrations (mg P L^{-1}) with time in acid (A), neutral (B), and calcareous (C) soils amended with recycled FePs with (+C) and without (0C) glutamate. Error bars represent standard error of means (n=3).

The effect of glutamate on stimulating P release was significant in the Fe(III)P-sludge treatments on the 14th and 60th day (Table SIV, see Supplementary Material Table SIV) in the acid soil. In the neutral soil, the addition of glutamate significantly increased the soil P concentrations in FeP treatments but only at 14th day for V-WWTP and only at 60th day for Fe(III)P-sludge. All glutamate-amended treatments in the calcareous soil showed significantly higher P concentrations than those without OM but only the Fe(III)P-sludge treatments had significantly different P values until the end of the incubation period.

DISCUSSION

The different FePs had very low available P in the short-term pot trial here. It is well established that a high Fe:P ratio in a soil amendment lowers its P availability. For example, the low plant P availability of sewage sludges was found to be correlated to the increased concentration of total Fe and Al of the sludge (Lemming *et al.,* 2017; O'Connor *et al.,* 2004). A high Fe content (e.g. Fe:P molar ratio >3) in sewage sludge increased the P sorption capacity of soils, thereby decreasing the P availability (Samie and Römer, 2001). This can partly explain the observed low P uptakes from Fe(III)Ps that have Fe:P molar ratios ranging from 4 to 22 (Table III). An exception was Fe(III)P-gravel, as this material was loaded with P six weeks prior to the pot experiment with the acid and neutral soil. This explains the higher P availability of this material than the other FePs. This was not observed in the calcareous soil as the pot trial with this soil started only 6 months after P loading of the Fe(III)P-gravel. The plant availability of P decreases with time due to diffusion and precipitation reactions, explaining the lower RPUE of the Fe(III)P-gravel in the calcareous soil (Hylander and Simán, 2001). Despite having higher P content, the vivianites (Fe:P molar ratio of 1.5) did not show better P uptakes than Fe(III)Ps. This can be explained by the application of FePs as granules. The FeP fertilizers were still intact as granules in the soil after the plant growth trial indicating reduced solubility of FePs if granulated, which thus explains the poor P supply

from the granules to the soil solution (Fig. 3). The low soil P concentration extracted using $CaCl_2$ implied that the P released (if any), was adsorbed on or precipitated in all soils, becoming unavailable to plants.

The P uptake in the flooded treatments is typically higher than that in the corresponding treatments in non-flooded conditions (Huguenin-Elie *et al.,* 2003; Rabeharisoa *et al.,* 2012). However, this is in contrast with our observations in the pot trial, where P uptake in non-flooded conditions was either higher in the neutral soil or the same in the acid soil than in the flooded counterparts. The Fe(III) transforms to Fe(II) under E_h around +300mV at pH 6, +100 mV at pH 7, and -100 mV at pH 8 (Fageria *et al.,* 2011). The measured redox values indicated that the soils were only at the edge of Fe(III) reduction (Fageria *et al.,* 2011), which most likely was the result of limited flooding time (13 days), low amounts of OC in these soils (Table 1) and/or the presence of plant roots that aerate the rhizosphere. In contrast, soil E_h values were already negative in the soils after two weeks in the incubation experiment (Fig. 4) as the previously mentioned reasons for the positive E_h values in the pot trial were absent. The results from the incubation experiment (Fig. 4), however, should be interpreted carefully. Here the soils were unplanted, a scenario that does not reflect pot or field conditions. Because of their oxidizing potential, rice roots can significantly delay the onset of reduced conditions necessary for reductive dissolution of Fe(III) minerals particularly if the soils contain little OM. The incubation experiment predicts that prolonged flooding and OM addition are necessary to accelerate the release of P from FePs.

Flooding in the short term did not affect P uptake significantly in the calcareous soil for most FePs (Table SII, see Supplementary Material Table SII). In such soil, P precipitates with Ca^{2+} ions to form poorly soluble calcium phosphates (CaPs), restricting P availability. The solubility of CaPs may increase under reduced conditions as soil pH drops after flooding due to CO_2 accumulation and increased dissolved organic matter complexing Ca^{2+} ions (Slaton *et al.,* 2002). However, such reduced conditions were also not observed in this soil as the mean E_h value (360 mV) after harvest in the control treatments still indicates aerobic condition (Fig. 4).

In calcareous soils, the application of vivianites is also of interest because of the possible supply of Fe, that can mitigate Fe chlorosis in crops. Nonetheless, the application of FePs in calcareous soil did neither enhance growth (Fig. S4, see Supplementary Material Fig. S4) nor shoot Fe concentration (Fig. S5, see Supplementary Material Fig. S5; Table SIII, See Supplementary Material Table SIII) compared to the unamended control and TSP treatments. Higher shoot Fe concentrations were found in flooded compared to non-flooded conditions in the acid soil, irrespective of FeP treatments (Fig. S5, see Supplementary Material Fig. S5) indicating that Fe(III) reduction already took place in this soil that was flooded.

The incubation experiment suggested that soil conditions needed to reduce Fe(III) can be met with prolonged flooding. In the presence of native or added OM, E_h values decline more rapidly as OM fuels microorganisms that catalyze the sequential redox reactions following O_2 depletion by mediating the electron transfers involved. Thus, the difference in E_h values between soils with and without glutamate in this study was large. This was particularly true for the acid and neutral soil owing to the inherently low OM content of these soils in contrast to the calcareous soil that contains more OM. The addition of glutamate did not increase the $CaCl_2$-extractable P except in the calcareous soil. The low P values observed in Fe-rich soils, such as the acid and neutral soils used here, may be attributed to the re-sorption of liberated P onto higher P-sorbing secondary ferrous minerals (Holford and Patrick, 1979) and precipitation of Fe(II)P compounds (Amery and Smolders, 2012). However, in the calcareous soil, the P concentration remained sufficient to support rice growth even in the unamended control treatment. This was also demonstrated by the continued increase in the $CaCl_2$-extractable P concentration of vivianite-amended treatments. The continued release of P from vivianite treatments can be attributed to a two-step process. First, Fe(II) in vivianite can oxidize to Fe(III) with time as this mineral is not very stable (Wilfert *et al*., 2018). Second, the Fe(III) can be reduced again in flooded conditions which enhances the solubility of the vivianite.

Despite the poor performance of the FePs compared to TSP for both water conditions observed in the pot trial, some strategies based on the results of the incubation experiment are suggested to circumvent this issue. The pot trial lasted for only 3 weeks during which nonlabile inorganic P in FePs may not have been fully mobilized. This duration may not also have also captured the full picture of changes in the concentration of soluble P following flooding, as our incubation experiment indicated that a flooding period for at least several weeks was necessary to obtain reduced soil conditions. The positive effect of glutamate in increasing P concentration in flooded soils amended by FePs suggests that the application of OM may strongly increase the P fertilizer value of FePs, particularly in the short term. Rice straw, for example, can be applied to accelerate the

reduction of Fe(III) materials (both from FePs and unplanted soils) in submerged conditions (Scalenghe *et al.,* 2022) if recycled iron phosphates are to be further tested and valorized in the field. The use of rice straw or any organic matter amendment should, however, also consider its effect on other plant nutrients particularly on the redox-sensitive ones such as Fe and Mn. Furthermore, it may be best to incubate FePs in flooded soil several weeks before sowing. It is assumed that after the incubation period, the liberation of P from FePs will synchronize with the P demand of rice at its initial growth stages. An incubation experiment testing FePs with or without rice straw in flooded soils for several weeks is also recommended to provide an indication for the potential release of P from FePs with this OM. This can also dictate the timing by which the rice has to be transplanted depending on the peak of the reductive dissolution process as determined by high P or Fe^{2+} concentration in the soil.

The FeP minerals were applied as intact granules in the pot experiment, for which the dissolution was likely slower in comparison to its powdered form, as was shown earlier for struvite fertilizers (Degryse *et al.,* 2017). Lower P availability has also been observed in biosolids-P when pelletized via heat drying (O'Connor *et al.,* 2004). Possibly, the effectiveness of FePs will be higher if applied as powders than as granules as shown in our incubation experiment. The promising results from the incubation experiment highlight the need to further investigate the performance of FePs as P fertilizers in pot and field trials applying the parameters used in the incubation experiment (powdered vs granulated FePs, longer flooding period, and OM addition).

CONCLUSIONS

The P fertilizer efficiency of the different recycled FePs was investigated in a pot trial and an incubation experiment. The results from the pot trial indicated very limited effectiveness of the recycled FePs as P fertilizer for rice grown under both flooded and non-flooded conditions in the three soils. Their PFUEs were negligible compared to that of TSP due to (1) the intrinsically low solubility of the FeP compounds (high Fe content), (2) a subsequent reduction in solubility in soil as result of FeP application as granule, and (3) a short flooding period, low OM in soils and presence of rice roots resulting in a limited reductive dissolution of Fe(III) minerals. These reasons were overcome (except 1) in an incubation experiment where FePs were applied as powder in unplanted soil that was flooded for more than 2 weeks and with (or without) glutamate. The results from the incubation experiment showed that soil conditions needed to reduce Fe(III) and to subsequently release P from FePs, which were not observed during the pot trial, can be met with prolonged flooding and glutamate addition. For the field valorization of FePs, we recommend the use of rice straw as OM, as this is an easily accessible and affordable OM source for rice farmers. Applying FePs as a powder can likely also increase the FeP solubility in comparison with a granular application.

ACKNOWLEDGEMENTS

SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

CONTRIBUTION OF AUTHORS

Rochelle Joie Saracanlao: formal analysis, methodology, investigation, data curation, visualization, writing-original draft, writing- review and editing. Hannah Van Ryckel: formal analysis, investigation, visualization,

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Supplementary Material

METHODOLOGY

Determination of P doses

The P doses used in the pot trial were determined based on the P sorption index (PSI) of the different soils, which represents the amount of adsorbed P (mg P kg^{-1}) equivalent to a P solution concentration of 0.1 mg P L^{-1} (i.e. the solution concentration required for rice growth). The P adsorption isotherms for the different soils were determined by incubating the soil in P solutions with a concentration varying between 0 -- 1500 mg P kg^{-1} at a liquid: solid ratio of 10 L kg^{-1}. The suspensions were shaken end-over-end for 16 h, after which they were centrifuged (2500 g, 10 mins) and filtered (0.45 μm, Chromafil ® Xtra PET -45/25; Macherey- Nagel, Germany). The P concentration of the filtrates was determined using malachite green method and absorbance was read at 630 nm using a spectrophotometer (Perkin Elmer Lambda 20). The adsorption isotherms of the soils were fitted to the Freundlich equation:

$$P_{sorbed} = k\, P_{solution}{}^{n} \tag{Eqn. S1}$$

With P_{sorbed} the P adsorbed in the solid phase (mg P kg^{-1}), $P_{solution}$ the P concentration in the solution (mg P L^{-1}) and k and n the Freundlich parameters.
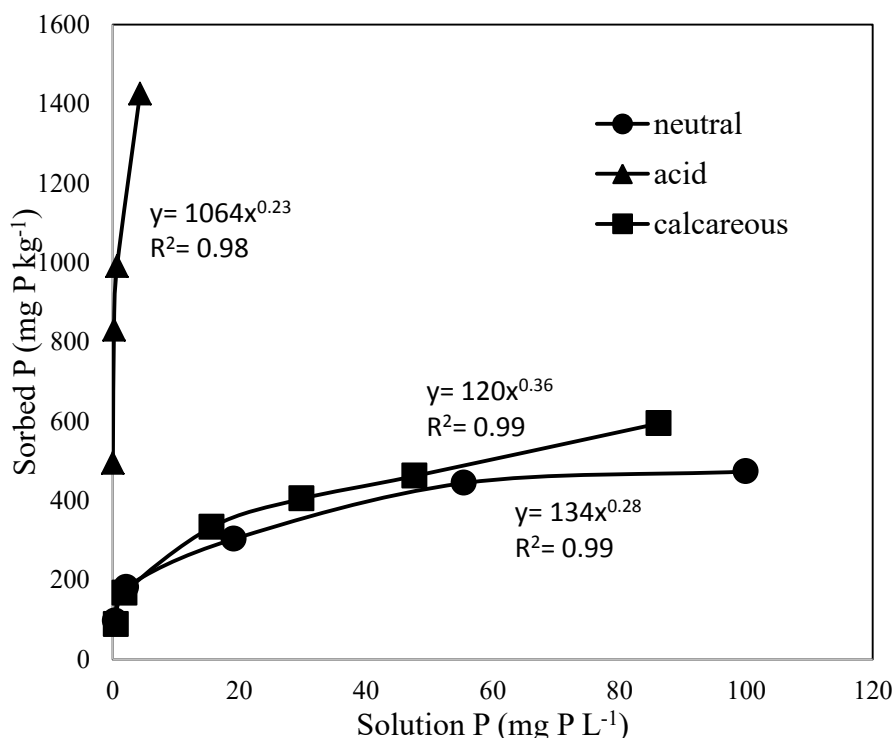
Fig. S1 P sorption isotherms of acid, neutral, and calcareous soil with corresponding Freundlich equations.

Data and statistical analysis for TSP treatments

Statistical analysis was performed using JMP software (JMP pro15, SAS Institute Inc.). All data were subjected to analysis of variance (ANOVA) with soil, fertilizer type, and water as the main factors. Then, for each soil the effects of P dose and water treatments on P uptake and $CaCl_2$-extractable soil P in the TSP treatments (each indicated as Y) were assessed using multiple linear regression with the resulting model:

$$Y = \beta_0 + \beta_1*(P\ dose) + \beta_2*(Flooded) + \beta_3*(P\ dose - c)2 + \beta_4*((P\ dose - c)*Flooded) \quad (Eq.\ S2)$$

with β_0, β_1, β_2, β_3 and β_4 the parameter estimates of this model and c the mean P dose. The parameter estimates for this equation is shown in Table SI.

RESULTS

TABLE SI

Parameter estimates of the model describing the P uptake of X265 rice plants for TSP treatments in each soil. P uptake $= \beta_0 + \beta_1*(P\ dose) + \beta_2*(Flooded) + \beta_3*(P\ dose - c)^2 + \beta_4*((P\ dose - c)*Flooded)$

	β_0	β_1	β_2	β_3	β_4	c
Acid soil	0.33*	0.04*	-0.18*	-0.0006*	-0.009*	18.5
Neutral soil	0.52*	0.06*	-0.26*	-0.003*	-0.01*	9
Calcareous soil	0.19*	0.007*	-0.01n.s.	/	0.002n.s.	15

*: significant, n.s.: not significant (P < 0.05).

TABLE SII

Effect of flooding on the P uptake of X265 rice shoots grown in acid, neutral, and calcareous soils for FePs and TSP

	P uptake (mg P pot^{-1})							
	Control	V-WWTP	V-fert	V-syn	Fe(III)P-gravel	Fe(III)P-sand	Fe(III)P-sludge	TSP
Acid soil	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Neutral soil	*	*	*	*	*	*	*	*
Calcareous soil	*	*	n.s.	n.s.	n.s.	*	*	n.s.

*: significant effect (P < 0.05); n.s.: not significant.

TABLE SIII

Mean iron concentration (mg Fe kg^{-1} tissues) of rice shoots in the calcareous soil under flooded and non-flooded conditions. Standard error of the three replicates (except for control where n=6) between brackets. Mean values with the same letter are not significantly different (TUKEY, P < 0.05).

Fertilizer treatment	Non-flooded	Flooded
Control	82.2 (3.0)a	59.5 (3.4) a
TSP	60.4 (13.6) a	56.8 (3.6) a
V-WWTP	66.5 (4.1) a	51.1 (6.4) a
V-fert	69.3 (6.6) a	54.6 (0.8) a
V-syn	66.2 (9.8) a	56.5 (4.8) a
Fe(III)P-gravel	49.2 (15.4) a	55.8 (4.1) a
Fe(III)P-sludge	70.9 (5.6) a	57.4 (5.3) a
Fe(III)P-sand	67.5 (7.8) a	66.4 4.5) a

TABLE SIV

Statistical significance of glutamate addition on the CaCl$_2$-extractable P for each FeP treatment per sampling time (TUKEY, P < 0.05).

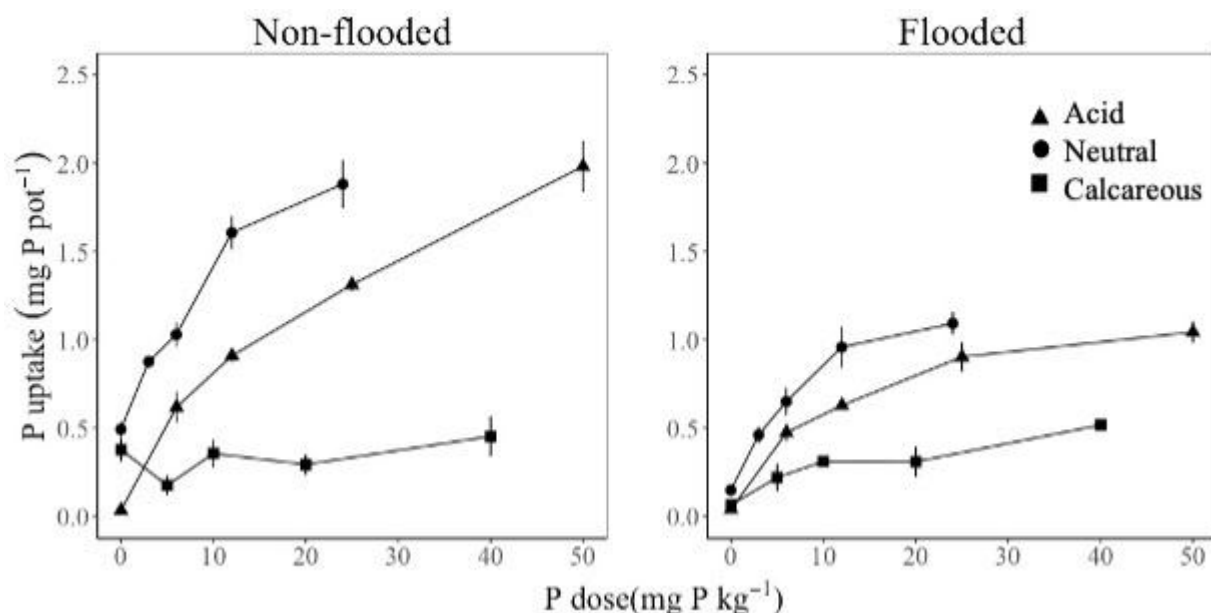| Soil	Recycled FePs	3rd day	14th day	60th day
Acid soil	V-WWTP	n.s.	n.s.	n.s.
	Fe(III)P-sludge	n.s.	*	*
	control	n.s.	n.s.	n.s.
Neutral soil	V-WWTP	n.s.	*	n.s.
	Fe(III)P-sludge	n.s.	n.s.	*
	control	n.s.	n.s.	n.s.
Calcareous soil	V-WWTP	*	*	n.s.
	Fe(III)P-sludge	*	*	*
	control	*	*	*

*: significant; n.s: not significant (P < 0.05)

Fig. S2 The shoot P uptake by rice grown in the acid (▲), neutral (●) and calcareous

(■) soils with increasing TSP dose. Data are means of 3 replicates (except for control where n=6) and error bars represent standard errors.
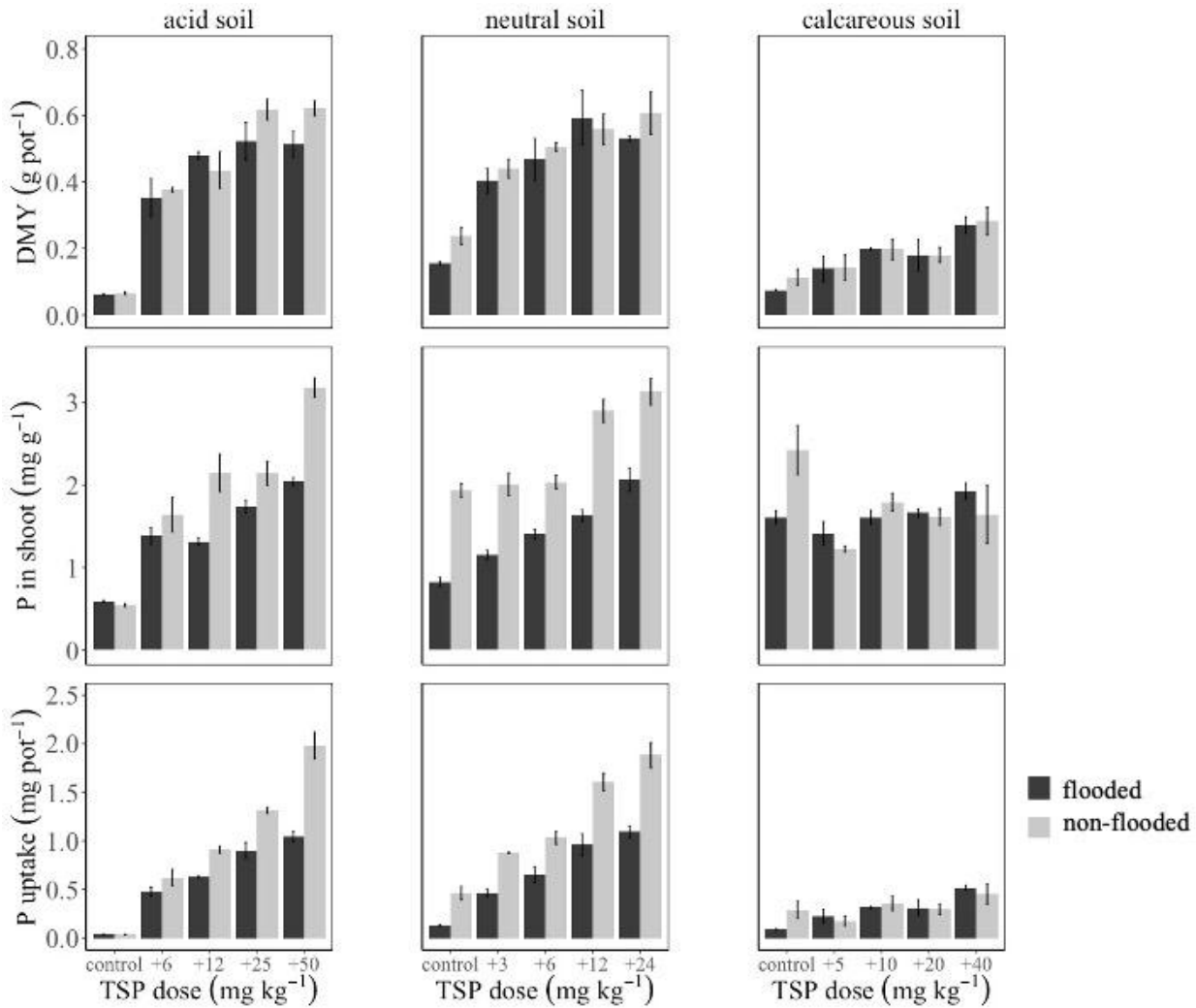

Fig S3 Dry matter yield, shoot P concentration, and P uptake of rice grown amended by increasing doses of TSP in three different soils under flooded and non-flooded conditions. Data are means of 3 replicates (except for control where n=6) and error bars represent standard error of means.
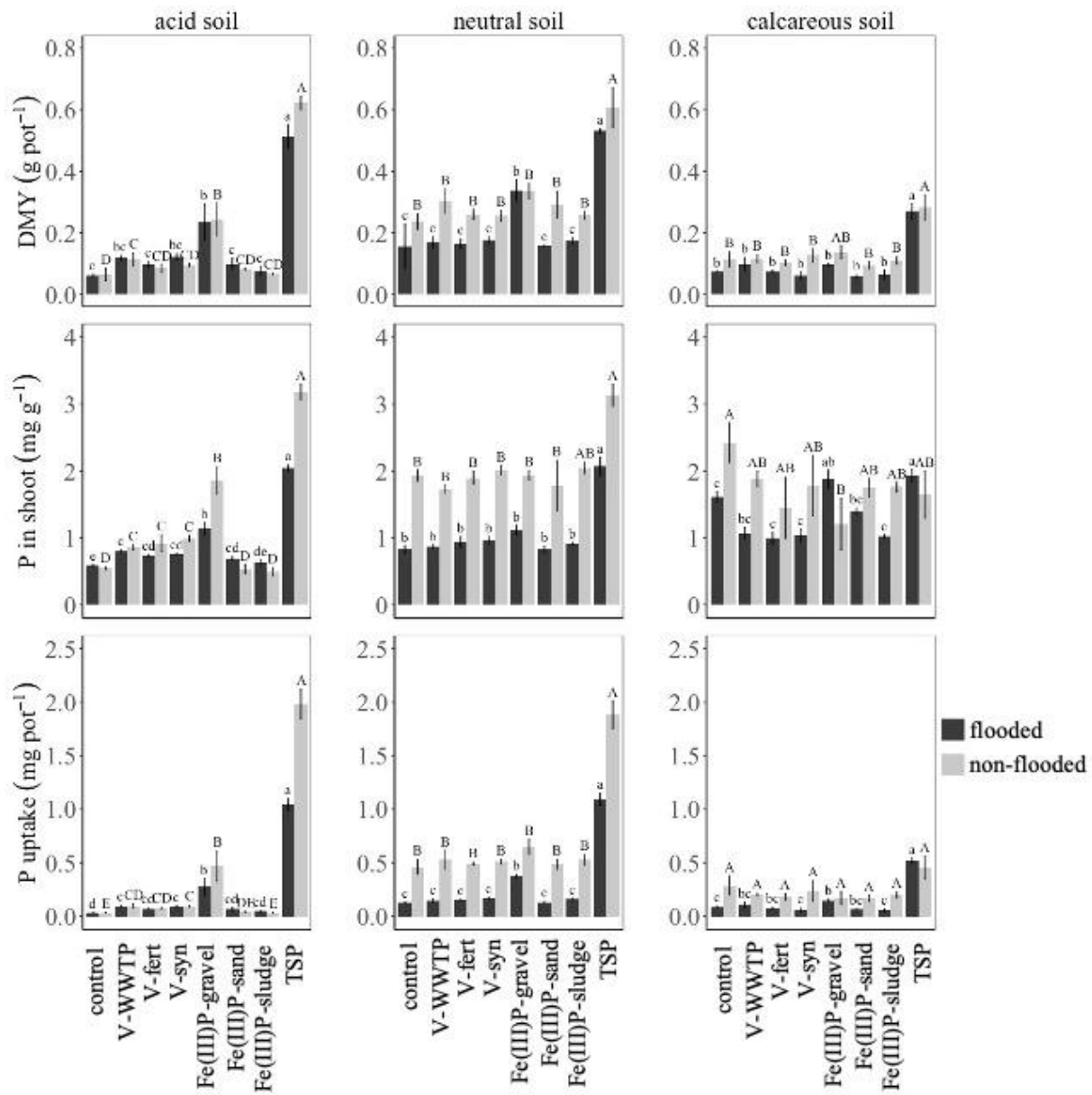
Fig. S4 Dry matter yield, P concentration in shoots and P uptake of rice grown for 3 weeks amended by different FePs in comparison with TSP (applied at 50 mg P kg^{-1} in acid soil, 25 mg P kg^{-1} in neutral soil, and 40 mg P kg^{-1} in calcareous soil) and control treatments in three different soils under flooded and non-flooded conditions. Data are means of 3 replicates (except for control where n=6) and error bars represent standard error.
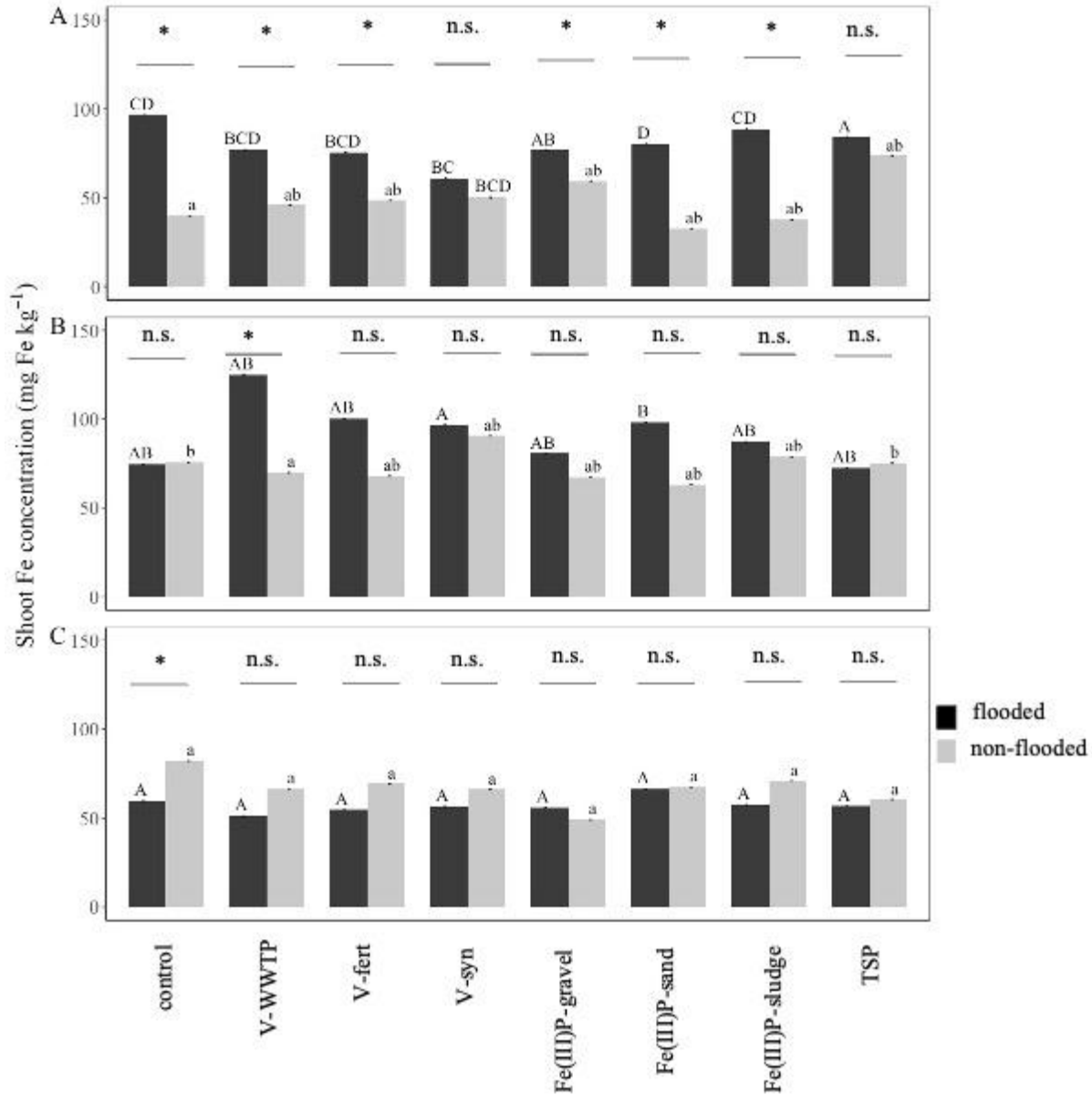
Fig. S5 Mean iron concentration (mg Fe kg $^{-1}$ tissues) of rice shoots in the acid (A), neutral (B), and calcareous (C) soils under flooded and non-flooded conditions. Treatments were applied at a dose of 50 mg P kg $^{-1}$ in A, 25 mg P kg $^{-1}$ in B, and 40 mg P kg^{-1} in C. Data are means of 3 replicates (except for control where n=6) and error bars represent standard error of means. Different letters denote significant differences among treatments (Tukey, $P < 0.05$).