**Fate and bioavailability of phosphorus loaded to iron oxyhydroxide
nanoparticles added to weathered soils**

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10 **Abstract**

11 *Aims* The low phosphorus (P) fertilizer use efficiency in weathered, P deficient soils calls for better
 12 fertilizer formulations. We previously formulated nanoparticles containing P (NP-P) that was successful
 13 fertilizer in nutrient solution. This study was set up to test the fate and the bioavailability of nanofertilizer-
 14 P and of that of native (colloid) P naturally present in soil.

15 *Methods* The NP-P consisted of nano-ferrihydrite (~ 10 nm) loaded with phosphate (P-*n*Fh) and stabilized
 16 with either natural organic matter (NOM) or hexametaphosphate (HMP). Natural colloid concentrations
 17 were increased with KOH addition, as deflocculating agent, to soil; all tests used samples from P deficient,
 18 highly weathered soils.

19 *Results* Pot trials with rice seedlings did not reveal larger P uptake in the NP-P amended soils compared to
 20 equal doses of soluble PO₄ or soluble HMP. Total Fe concentrations in soil solutions were unaffected by
 21 NP-P addition, whereas natural colloidal Fe and P markedly increased by KOH addition. The
 22 bioavailability of native colloidal P, mobilized by KOH addition, could not be assessed due to lack of
 23 growth, likely related to collapse of the soil structure.

24 *Conclusions* This study showed that P-loaded iron oxyhydroxide NPs insufficiently enhanced soluble P in
 25 soil to offer benefits over soluble fertilizers, likely because of a combined effect of lower diffusivity of
 26 NPs compared to P_i and lower bioavailability of NP-P than P_i. Smaller particles or small labile organic
 27 colloids might offer an improvement in both aspects.

28 **Abbreviations**

29 AlOx: aluminum oxides; EDL: electric double layer; FeOx: iron (oxyhydr)oxides; HMEP: humic-metal-
 30 phosphorus; HMP: hexametaphosphate; ICP-MS: inductively coupled plasma – mass spectrometry; LSC:
 31 liquid scintillation counting; *n*Fh: nano-ferrihydrite; NOM: natural organic matter; NPs: nanoparticles;

32 OM: organic matter; PO₄: phosphate; P_p: polyphosphate; TOC: total organic carbon.

34 **Introduction**

35 In strongly weathered soils, low phosphorus (P) availability is a major plant nutritional problem, leading
36 to low yields of agricultural crops (Hinsinger 2001; Sanchez and Jama 2001). The traditional solution to
37 overcome P deficiency in soil is the application of solubilized phosphates (PO_4). However, fertilizer use
38 efficiency of PO_4 fertilizers rarely exceeds 20% due to sorption on iron- and aluminum (oxyhydr)oxides
39 (FeOx/AlOx), dominating soil mineralogy in these soils (Andriamananjara et al. 2018). Moreover, the
40 residual value for the subsequent crops is extremely low due to the slow process of PO_4 fixation (Fageria
41 2008). This leads to low PO_4 concentration in soil solution, i.e., $0.65 - 3 \mu\text{g P L}^{-1}$, (Maertens et al. 2004)
42 while the required concentration for adequate plant nutrition is about $150 - 300 \mu\text{g P L}^{-1}$ (Hinsinger 2001).
43 There is a pressing need for more efficient P fertilizers that can overcome these limitations.

44 Ideally, special fertilizers should be designed that respond to the plant's need to limit nutrient loss; these
45 "rhizosphere-controlled fertilizers" could react to rhizosphere acidification or the release of organic acids
46 (Erro et al. 2007). Some techniques are already in use to deliver a nutrient or pesticide in a controlled
47 fashion, for example, polymer-coated pellets, polymer films or hydrogel matrices (Davidson and Gu 2012;
48 Bortolin et al. 2016). However, nutrient release from such formulation is often not controlled by the plant,
49 but, for example, by biodegradation of the polymer coating, which is also affected by soil moisture and
50 could thus be unsynchronized with plant demand (Davidson and Gu 2012). Layered double hydroxides
51 (LDHs) are being designed to release PO_4 by the ion exchange reaction between intercalated PO_4 in the
52 layered structure and CO_3 anions present in the soil (Everaert et al. 2016). Initial results showed a positive
53 effect of LDH compared to soluble P (as KH_2PO_4) in acid soils, although this was likely related to a
54 liming effect (Everaert et al. 2016); further pot trials did not show an advantage of LDHs over granular
55 mono-ammonium phosphate in P uptake (Everaert et al. 2017). Nanomaterials, defined as particles with at
56 least one dimension below 100 nm, have been proposed by several authors to serve as an environmental
57 nano-vector for nutrients (Gogos et al. 2012; Bindraban et al. 2015; Hamon et al. 2015; Wang et al. 2016).

By combining a large surface area with strong sorption properties, nanofertilizers have the potential for sustained and slow release (Bindraban et al. 2015).

Recent observations highlighted the possible contribution of P associated with colloidal FeOx and AlOx to the plant bioavailable P pool. These colloids are naturally present in all soils. Santner et al. (2012) showed that supplementing a low-P nutrient solution, containing only $0.65 \mu\text{g P L}^{-1}$ as truly dissolved P, with 30 - 2000 $\mu\text{g P L}^{-1}$ as PO_4 -coated AlOx nanoparticles (NPs), enhanced P uptake by plants compared to the treatments with only the truly dissolved P. The suspected mode of action is that PO_4 ions are released from the colloids near actively PO_4 absorbing plant roots, i.e., that the colloids act as a mobile PO_4 buffer in the root bathing solution. Montalvo et al. (2015) found a larger P uptake in plants grown in a solution containing natural colloids (nonfiltered), compared to plants grown in the same, colloid-free solution (3 kDa-filtered). Along the same lines, a nutrient solution culture showed that organic-P and polyphosphate(P_p)-P bound to FeOx nanoparticles were bioavailable (Bollyn et al. 2017). These findings open up possibilities for the use of P-loaded colloids as nanofertilizers, which could act as a carrier of PO_4 , buffering the truly dissolved PO_4 outside the root cells and limiting PO_4 fixation by soil minerals. Nevertheless, in soil, several obstacles are present that could lead to a loss of efficiency of colloidal P as a fertilizer compared to that in hydroponics. Three main processes could hinder the mobility/bioavailability of P-loaded colloids: (i) loss of PO_4 from the colloids toward soil particles, if soil solution PO_4 concentration is lower than the equilibrium concentration of the prepared colloids; (ii) homo-aggregation of colloids due to the exposure to multivalent cations, e.g., Ca^{2+} and Al^{3+} ; (iii) retention of colloids on positively charged soil sites (hetero-aggregation), which will be especially prevalent in weathered soils with low PO_4 status and low organic matter.

A wide array of studies exists on the exploration of nanofertilizers with a main focus on micronutrients, for examples the use of ZnO to alleviate Zn deficiency (Gogos et al. 2012; Servin et al. 2015). Few studies address the use of nanofertilizers for alleviation of P deficiency. Apatite nanoparticles have been tested as a nanofertilizer by two different research groups. Liu et al. (2014) found that nano-apatite performed

83 better as a P fertilizer compared to soluble $\text{Ca}(\text{H}_2\text{PO}_4)_2$, however, *Glycine max.* (soybean) was grown in an
84 artificial substrate somewhat remote from soils. In contrast, Montalvo et al. (2015b) found that granular
85 triple superphosphate (TSP) was still a more efficient P fertilizer than nano-apatite for *Triticum aestivum*
86 (wheat) in two Ferralsols and two Andosols. The latter used a more realistic substrate than the former,
87 suggesting that little is to be gained from nano-apatite in soils. Here, we chose to work with FeOx
88 nanoparticles that are loaded with P on their surface. Hence, a different mechanism is at play here,
89 compared to the working mechanism of nano-apatite: desorption instead of dissolution. In addition, the
90 stability of the nanoparticles under conditions of varying pH and Ca concentration was already enhanced
91 in a previous study (Bollyn et al. 2016) to improve mobility in soil.

92 This study was set up to test the fate and the bioavailability of colloidal P in soil. This was studied for both
93 added nanofertilizer-P and for native (colloid) P naturally present in soil. Pot trials and pore water
94 concentrations of P and Fe were used to evaluate the effects. Our hypothesis is that nanofertilizer-P will
95 increase the P uptake in plants compared to the soluble P treatment, since less P is lost by adsorption to the
96 soil minerals. The natural colloidal P concentration was varied by increasing soil pH with either KOH or
97 $\text{Ca}(\text{OH})_2$ as we speculated that colloid P concentrations in pore water can be increased by increasing soil
98 pH, increased organic matter content and decreasing soil solution Ca^{2+} , the three main factors increasing
99 colloidal stability (Bollyn et al. 2016). Hence, the fate and pot trials studies included the effect of KOH
100 and $\text{Ca}(\text{OH})_2$ addition on the fertilizer value of colloidal P, referenced to soluble PO_4 amended treatments.

101 **Materials and Methods**

102 In all experiments, the same type of nanoparticles were used: in-house synthesized nanoparticulate
103 ferrihydrite. Two pot trials were used for testing fertilizer values of colloidal P (*Part I & III*) as
104 nanofertilizer-P or native colloids, all referenced to soluble PO_4 amended treatments, while the colloidal
105 fate studies (*Part II*) were based on pore water isolated by centrifugation. The pore water isolation method
106 was based on the immiscible displacement principle after we found that this method yielded higher
107 recoveries of added small colloids compared to the direct (double chamber) centrifugation method. The

results of the comparison between the centrifugation methods are given in the *Supplementary Information*. The term colloids rather than nanoparticles is used for all analyses since no size measurements were made after soil amendments.

Nano-ferrihydrite synthesis

Small nanoparticulate ferrihydrite particles were synthesized in-house by adaptation of the procedure of Burleson and Penn (2006). Briefly, an equal volume of 0.048 M NaHCO₃ was added to a 0.04 M Fe(NO₃)₃ solution, using a peristaltic pump in 47 min under vigorous magnetic stirring. The subsequent suspension was dialyzed (Spectra/Por 4 dialysis tubing, MWCO 12-14 kDa, Spectrum Laboratories) at 4 °C for five days against demineralized water, with the water being replaced three times per day. Dialyzed solutions were kept in glass bottles at 4 °C, with a final Fe concentration of 650 mg Fe L⁻¹ measured after cold acid digestion in 5 M HNO₃ (final concentration) for 24 h to dissolve colloids. These particles have an average diameter < 10 nm, as measured by dynamic light scattering (DLS, Zetasizer Nano ZS, Malvern Instruments). As a stabilizer, either Suwannee River natural organic matter (NOM, International Humic Substances Society, 2R101N) was used in a mass ratio of Fe:NOM of 1, or hexametaphosphate (Na-HMP, Fluka), since these yielded stable solutions in preliminary tests. In the case of HMP as a stabilizer, 66% P was added as HMP-P and 33% P as PO₄-P. After addition of the stabilizer, P was added as KH₂PO₄. These suspensions were left to equilibrate for about one hour before addition to the soil.

Part I: Bioavailability of nanofertilizer-P

Nanofertilizer-P was supplied as P-loaded HMP-stabilized *n*Fh (P-HMP-*n*Fh) at two P doses and the same dose of P was also supplied as an identical soluble HMP-P mixture. A reference dose-response relationship was set up with KH₂PO₄. These treatments were applied to one soil that was limed with Ca(OH)₂ to avoid Al-toxicity.

One tropical, weathered soil was selected for this experiment, originating from Behenjy, Madagascar (Table 1). The soil was pre-incubated at 10 w/w% for ten days at 25 ± 2 °C. Then, soil pH was adapted to pH 5.5 with 0.5 M Ca(OH)₂ and blanket nutrients were applied to achieve the following concentrations in

133 mg kg⁻¹: 33.3 K (as KCl), 20.8 Mg (as MgSO₄), 27.5 S (as MgSO₄) and 100 N (as NH₄NO₃) and in µg kg⁻¹:
 134 830 Fe (as FeCl₃), 830 Mn (as MnCl₂), 830 Zn (as ZnCl₂), 830 Cu (as CuCl₂), 83 B (as Na₂B₄O₇) and 83
 135 Mo (as Na₂MoO₄). The soil was again incubated for one week at 25 ± 2 °C. One day before planting, P
 136 treatments were applied. Soluble P (KH₂PO₄) was applied at four doses: 0 (control), 150, 250 and 600 mg
 137 P (kg dry soil)⁻¹. For the NP-P treatments, NP suspensions were prepared by mixing *n*Fh, details in
 138 previous section, coated with HMP (as Na-HMP, 66% of total P) and KH₂PO₄ (33% of total P) before
 139 addition to the soil. Two doses were selected: 50 mg P kg⁻¹ at 80 mg Fe kg⁻¹, and 150 mg P kg⁻¹ at 260 mg
 140 Fe kg⁻¹. The same doses of P, with the identical mixture of HMP-P/ KH₂PO₄, were also supplied without
 141 *n*Fh to assess the fertilizer potential of HMP as a non-nanoparticulate form. Finally, soil moisture content
 142 was adjusted to 60% of field capacity (0.21 L kg⁻¹) with demineralized water. Capillary-wetted soil
 143 columns were used with a large height:width ratio. PVC columns (4 cm ø, 25 cm height) were filled with
 144 300 g pretreated soil, filter cloth was used to cover the bottom. Rice seeds (*Oryza sativa*, cv. *Chomrong*
 145 *Dhan*, FOFIFA Madagascar) were germinated for five days in wet paper towels and two seedlings were
 146 planted in soil columns at approximately 1 cm depth. Plants were thinned to one seedling per pot one
 147 week after emergence. Pots were weighed every other day, and water loss was compensated by adding
 148 demineralized water in a cup in which the column was placed. Plants were grown in a growth cabinet
 149 (Weiss Technik) with a 12/12 h day/night cycle, a day/night temperature of 22 °C/20 °C, a photosynthetic
 150 photon flux density of 725 µmol s⁻¹ m⁻² and a relative humidity of 75%. Plants were harvested 20 days
 151 after planting, plant shoots were cut, weighed and oven-dried 60-70 °C. The elemental concentrations in
 152 the shoot were determined with inductively coupled plasma – mass spectrometry (ICP-MS, Agilent 7700x,
 153 Agilent technologies) after hot nitric acid digestion, and shoot P uptake was calculated as shoot P
 154 concentration multiplied by shoot dry weight.

155 Pore water extractions using double chamber centrifugation were performed after harvest on the Behenjy
 156 soil samples. This methods was preferred over immiscible displacement, since too little sample volume
 157 was produced with this method in this soil type in preliminary experiments. About 55 g wet soil was

needed to fill a plastic syringe, lined on the bottom with a 0.45 μm membrane filter (mixed cellulose ester, Whatman). The syringe was placed in a centrifuge tube for pore water collection and centrifuged for 15 min at 1800 g. Samples were analyzed with ICP-MS after acidification to 1% HNO_3 . Soils were analyzed for total elemental concentration with ICP-MS after hot aqua regia digestion.

Part II: Fate of colloidal P in pore water

Two tropical P-fixing soils were selected for an assessment of the fate of colloids as affected by soil properties, thereby using ^{57}Fe labeled *nFh* to distinguish added from native colloidal Fe. The two soils were either or not spiked with *nFh* and soil properties (pH, soil solution Ca and OM content) were varied in different combinations: we selected three pH treatments, two Ca treatments, two OM treatments and two types of P-loaded *nFh* (different coatings). The total number of combinations (72) was technically not feasible; therefore, a custom design was modeled in JMP (JMP Pro 12, SAS Institute) to optimize the output with the limited number of runs. In this design, the following capital effects are included: soil type, NP addition, pH, Ca concentration and OM addition. Interactions between the capital effects and a quadratic effect for pH were also included and the I-optimal criterion was selected with 10000 random starts. This led to a restricted factorial design of 25 combinations. All treatment combinations are presented in the *Supplementary Information*.

^{57}Fe -labeled nano-ferrihydrite synthesis

Nanoferrhydrite particles were synthesized by adaptation of the procedure described above, to introduce ^{57}Fe labelling. The 0.04 M $\text{Fe}(\text{NO}_3)_3$ solution was spiked with an enriched ^{57}Fe (metallic ^{57}Fe in HCl, 65% ^{57}Fe) solution, before addition to the NaHCO_3 solution... The isotopic abundance of ^{57}Fe was in the final nanoparticles was thus enriched from 2.12% (natural abundance) to 2.65%, measured after cold acid digestion. The *nFh* suspensions were first mixed with a stabilizer (NOM/HMP), then, ^{33}P -labelled PO_4 (as KH_2PO_4 , Fluka) at a ^{33}P dose of 1.7 MBq kg^{-1} was added. These suspensions were left to equilibrate for

about one hour before addition to the soil. To each soil, an identical nanoparticle dose was added to achieve an increase of 22 mg Fe kg⁻¹, equivalent to 42 mg *n*Fh kg⁻¹, and 2.6 mg P kg⁻¹.

Soil preparation

Two different tropical soils, classified as an Andosol and a Gleysol (WRB, 2007) were selected and named after their village of origin. The topsoil was sampled and selected soil properties are presented in Table 1. The soils had been previously air-dried, sieved at 4 mm and were adjusted to 25% water content for incubation at 20 °C in darkness for one week. The different soils were subjected to different amendments, detailed in *Supplementary Information*. Four days before the start of the experiment (*n*Fh addition), a subset of the soils was mixed with rice straw, as + OM treatment, at 2 g kg⁻¹. In another subset, soil pH was adjusted one day before the experiment by addition of a volume of a 1 M KOH solution required to achieve pH 5.2 and pH 5.8, preliminary tests showed that a stable pH was achieved after one day incubation. At the day of mixing, the equivalent of 50 g of pre-treated dry soil was weighed in plastic pots and the P-coated *n*Fh suspension was added at of 22 mg Fe (kg soil)⁻¹, thereby adding 0.07 mL water g⁻¹ soil. In the treatments with +Ca addition, CaCl₂ was added to obtain 1 mM Ca in the total volume of water added to the air-dry soils to reach field capacity. The soil moisture content in all other treatments was adjusted to field capacity with demineralized water. Soils were incubated for 1 day at 20 °C in darkness before sampling. The unspiked soils were only watered to field capacity, without ³³P or ⁵⁷Fe addition, and were used to determine the background pore water composition.

Soil analysis

The pore waters were collected with immiscible displacement centrifugation (Gollany et al. 1997) by putting 20 g soil into a 30 mL centrifuge tube, adding 3 mL of Fluorinert FC-40 (Sigma-Aldrich, ρ = 1.85 g cm⁻³) as the solvent, and mixing this thoroughly with the soil. Samples were centrifuged at 15 000 g (max. speed, Heraeus, Multifuge X3R) for one hour. About 3 mL of pore water was recovered for the Betafo and Soavinimerina soil (Soavi). We suspect the displacement by the solvent was hindered by strong water retention by the soil, which was already apparent by the difficult mixing, causing only small

pore water volumes to be recovered. The solution was filtered over a pre-rinsed 0.45 μm filter. Filtered suspensions were subsequently digested cold overnight with 5M HNO_3 (final concentration) before analysis by ICP-MS and liquid scintillation counting (LSC, Perkin Elmer Tri-Carb 2800TR). Both ^{56}Fe and ^{57}Fe were measured in He-mode using ^{72}Ge as internal standard. For LSC, 1 mL of digested sample was added to 5 mL milli-Q and 10 mL of Ultima Gold AB, measurements were corrected for background, decay, and quenching. Filtered but undigested samples were analyzed for total organic carbon (TOC) content by catalytic combustion after acidification to pH 2 and O_2 purging (Analytic Jena Multi NC 2100).

Part III: Bioavailability of nanofertilizer-P and native colloids

A full factorial design was set up with two soils, three pH treatments (natural pH or amended with $\text{KOH}/\text{Ca}(\text{OH})_2$) and three P treatments (no P, nanofertilizer-P or soluble P) to address both the effect of nanofertilizer-P under different pH conditions and the effect of natural colloidal P. All treatments in both pot trials were set up in triplicate.

Two tropical, weathered soils were selected for this pot trial: Behenjy and Soavi (Table 1). Both soils are highly P deficient as revealed by earlier field trials and corroborated by the pot trials performed here. Soils were pre-incubated at 10 w/w% for one week at 25 ± 2 $^\circ\text{C}$. One day before planting, N was added at 100 mg N kg^{-1} (as KNO_3). On a subset of soils, pH was adjusted to 5.5 ± 0.1 with either 1 M KOH or 0.5 M $\text{Ca}(\text{OH})_2$. Fertilizer P was added at 150 mg P kg^{-1} either as soluble KH_2PO_4 or as P-HMP-coated *nFh* (only in Behenjy), a control treatment without P addition was also included ('no P'). Nanoparticle suspensions were similar as in *Pot trial I*, yielding a final dose of 300 mg Fe kg^{-1} . Finally, soil moisture content was adjusted to 60% field capacity with demineralized water (0.21 L kg^{-1} for Behenjy and 0.29 L kg^{-1} for Soavi). The same set-up and analyses was used as in *Part I*, but four seeds were planted per column and thinned to one seedling per pot one week after emergence. Plants were harvested 25 days after planting and analyzed as described above. Pore water extractions using double chamber centrifugation were performed after harvest on the Behenjy soil samples, identical to Part I.

Statistical Analyses

The plant responses to P fertilizer dose and type of fertilizer in Part I were analyzed with a regression model with doses in a quadratic function and testing if the type of fertilizer affects the linear part (slope) of the response curve to the doses (JMP Pro 12, SAS Institute). The significance of the fertilizer type on the slope was tested on three different responses: yield, P concentration or P uptake. Details are given in the Supplementary Information The pore water elemental composition; i.e., Fe, Al, P and ^{33}P , of the different treatments in Part II were related to soil factors, i.e., soil treatments and soil properties with stepwise linear regression (JMP Pro 12, SAS Institute). The soil factors include the soil type (nominal), soil pH, soil solution Ca concentration, the OM addition (nominal) and the NP addition (no, HMP- and NOM-stabilized) and type (HMP- or NOM-stabilized). Forward modelling was used, whereby in each step the term with the smallest p-value is entered. Significance of treatment effects was evaluated with multiple linear regression using standard least squares in JMP (JMP Pro 12, SAS Institute). All pairwise comparison using Tukey HSD test was performed to test significant difference between means over different treatments (JMP Pro 12).

Results

Part I: Bioavailability of nanofertilizer-P

Plant growth clearly responded to the KH_2PO_4 dose and the same was true for shoot P content and shoot P uptake (Table 2). Three different P sources were supplied, each at two/three total soil P doses: KH_2PO_4 , NP-P (as P-HMP-*n*Fh) or P applied as a soluble mixture of HMP/ KH_2PO_4 with the HMP/ KH_2PO_4 ratio identical to that of the NP-P. The NP fertilizer effect was tested at two doses with only one dose having corresponding P doses in treatments with other P sources. Therefore, we performed a regression analysis to generally test relative fertilizer effects of the NP relative to the soluble forms (details in Supplementary Information). Overall, there was no significant effect of P sources on shoot dry weight at equivalent total P dose (Table 2). Regression analysis showed that shoot P uptake and shoot P concentration were significantly lower in the NP-P treatment compared to the soluble P treatment. No difference was found

in any of the plant-related measurements between KH_2PO_4 and HMP/ KH_2PO_4 or between NP-P and HMP/ KH_2PO_4 . Pore water extraction was performed on Behenjy soil samples with double chamber centrifugation, since immiscible displacement centrifugation showed difficulties in extracting ample volume in this soil type. Phosphorus addition increased pore water P concentration, independent of the P source; only at 600 mg P kg^{-1} , the concentrations for optimal P growth (0.2 mg L^{-1}) were reached (Figure 1). The NP treatments did not increase Fe in the pore water.

Part II: Fate of colloidal P in pore water

The addition of KOH to the soils had an overarching effect on the pore water composition obtained (see below), therefore data are discussed first for the treatments with no KOH addition, i.e., for soils at natural soil pH. The background pore water in these treatments contained P concentrations below the limit of detection for ICP-MS ($< 120 \mu\text{g L}^{-1}$, including 40-fold dilution; data not shown), which confirmed the P deficient status of these soils. Linear regression revealed that soil type explained most of the Fe or Al concentrations. The Fe concentrations in the pore water in *nFh*-spiked soils spiked were never significantly larger than those in the unspiked soils (*Supplementary Information, Figure S1*) whereas an increase with 45 mg Fe L^{-1} is predicted if all added Fe remains suspended, suggesting that all added particles are immobilized. The addition of the ^{57}Fe -labeled *nFh* did also not increase the $^{57}\text{Fe}/^{56}\text{Fe}$ signature compared to the background.

The pore water turned black with increasing KOH dose, indicating the release of colloids associated with OM. Increasing KOH-doses resulting in higher soil pH, increased total Fe from 2 mg Fe L^{-1} to 170 mg Fe L^{-1} , total P from 0.1 mg total P L^{-1} to over 5 mg P L^{-1} and the DOC from 100 to 3000 mg C L^{-1} (Figure 2), all well above values that can be found naturally in solutions or leachates of weathered soils. Stepwise multiple linear regression revealed that solution pH, soil type and Ca mainly explain the Fe, Al, P and ^{33}P concentrations (Table 3). Again, spiking of *nFh* had no statistical effect on the total Fe concentrations in solution, i.e., the addition of *nFh* did not enhance soil solution colloid concentrations above the natural background. We speculated that the $^{57}\text{Fe}/^{56}\text{Fe}$ ratio allowed tracing the added colloids, however, the

$^{57}\text{Fe}/^{56}\text{Fe}$ signature in suspended colloids was within the natural background range for most treatments, indicating an exchange of the added NPs with the large pool of native colloids in soil. The recovery of ^{33}P , as a proxy for spiked *nFh* recovery, was highest in the Soavi soil, with a maximum recovery of 10% of the initial ^{33}P dose, and increased with increasing pH (Figure 2). The ^{33}P recovery was unaffected by the type of *nFh* dosing, i.e., either HMP- or NOM-coated (details not shown). Also other elements, often associated with the natural colloidal fraction, i.e., TOC, P, Fe and Al, were strongly correlated with pH, (Figure 2). In general, the linear fit was almost perfect between measured elements and pH in Betafo soil with R^2 values between 0.83 and 0.98. For Soavi, the linear trends were still present but with a larger variation. Concomitant with TOC, also other metals are released, e.g., Pb, (Klitzke et al. 2008) which was also observed in our experiment, however, largest concentration increases were found for Fe, P and Al. Strong correlations between colloidal Fe, TOC and P were also observed in both soils (Figure 3). The Ca concentration measured in the pore water had a strong explanatory value for the Fe concentration (Table 3). Background Ca concentrations were very low, only 0.05 mM in the Betafo soil, and 0.06 mM in the Soavi soil. The Ca concentration in the +Ca watering solution was 1 mM, but the measured Ca concentration in the pore water was well below that. In the Betafo soil, the highest measured Ca concentration was 0.12 mM Ca, while this was 0.74 mM in the Soavi soil, likely as a result of ion exchange reactions in soil. The solution Fe in soil Betafo was unrelated to solution Ca concentration (Figure 3), which may be related to the low Ca concentration range. Quite surprisingly, there is a strong but *positive* relation between the Ca concentration and the Fe concentration in the soil collected at Soavi. This contrasts any other finding in previous work on the role of Ca on colloid stability. Against our hypothesis, OM addition had no effect on Fe concentration in the pore water.

Part III: Bioavailability of nanofertilizer-P and native colloids

This trial consisted of factorial combinations of two soils, three liming treatments (no, KOH or $\text{Ca}(\text{OH})_2$), and three P treatments (no, KH_2PO_4 and NP-P), excluding the NP treatment in one soil. Post-harvest analysis of plant and soil showed that one treatment was not set up as intended: in the $\text{Ca}(\text{OH})_2$ -amended

Behenjoy soil with 150 mg P kg⁻¹ as soluble P, the P dose was accidentally not added, thereby yielding one missing reference treatment for that soil. Plant growth was almost absent on all KOH-amended soils that was set up to stimulate the natural colloid concentration (Table 4). Germination was slow and growth was limited, with very small roots, while this was not the case in unlimed soil or the soils limed with Ca(OH)₂. In the unlimed soils, there was no difference in shoot dry weight, shoot P uptake and P content between the sources of P in soil Behenjoy (Tukey HSD, $p > 0.05$), confirming the results in limed soil of *Part I*. Both liming type and P addition had a significant effect on shoot dry weight, shoot P uptake and shoot P content. Liming with Ca(OH)₂ had a positive effect on shoot dry weight in the Behenjoy soil. In the Soavinimerina soil, the effect of P addition on shoot dry weight was the most important factor, whereas Ca(OH)₂ had no such effect (data not shown).

The pore water composition of Behenjoy soil, sampled after the pot trial, was also determined. In unlimed soils, the addition of NP-P or soluble P both increased the total P to about 25 µg P L⁻¹ (Figure 1), but only one out of three replicates was detectable for each of the treatments. This shows that P addition increased the P concentration in the pore water independent of the P source, however, P deficient concentrations are still present, i.e., concentrations below 0.2 mg P L⁻¹. Several diluted samples were below ICP-MS detection limit (3 µg L⁻¹), meaning that the pore water contained less than 15 – 30 µg P L⁻¹, depending on the dilution. The highest P concentrations were found in the KOH-amended soils, confirming in the results of *Part II*. However, in these soils there was no effect of either P treatment on P concentrations compared to the no-P control. The Fe concentration increased with KOH-amendment, but no significant effect was found due to *n*Fh addition (Figure 1).

Discussion

The objective of this study was to test the fate and the bioavailability of colloidal P in soil, both for supplied nanofertilizer-P and for native (colloid) P naturally present in soil. Following successful trials in nutrient solution (Bollyn et al. 2017), we now aimed to assess the fertilizer potential of P-loaded FeOx colloids in a highly weathered soil (Part I). This pot trial confirmed the strong P deficiency in the Behenjoy

soil. Pore water P concentration P in the unamended Behenjy soil were below detection limit ($< 15 - 30 \mu\text{g L}^{-1}$), while adequate P pore water concentration is between $150 - 1500 \mu\text{g P L}^{-1}$, depending on plant species. No differences in growth or P uptake were found between application of equal P dose as soluble P or NP-P in the Behenjy soil. The addition of P-HMP-*n*Fh did not increase the P concentration in the pore water compared to the treatment with soluble P added, as we had hypothesized. As a consequence, there was no increased fertilizer efficiency in the use of P-HMP-*n*Fh compared to soluble P. In contrast, the nanoparticles probably only served as an extra P binding surface, as is reflected by the slightly lower P content in plants grown on P-HMP-*n*Fh amended soil compared to the soluble P. The source of soluble PO_4 , either only KH_2PO_4 or as a mixture of KH_2PO_4 and HMP, did not impact growth or P uptake. This is supported by previous results, where HMP in solution proved to almost completely (80%) bioavailable compared to KH_2PO_4 (Bollyn et al. 2017). The presence of soil-borne microorganisms likely speeds up the hydrolysis, making HMP as bioavailable as KH_2PO_4 , a result that was previously found for other polyphosphates (Dick 1985; McBeath et al. 2009).

To better understand the critical soil parameters that affect the stability of added P-loaded nanoparticles and native colloids, a fate study was set up in Part II examining the pore water composition under varying conditions. At natural soil pH, both the total Fe concentration and the $^{57}\text{Fe}/^{56}\text{Fe}$ -signature were not raised above the background values by the addition of *n*Fh. The $\text{Fe}_{\text{oxalate}}$ concentrations of the soils are $6000 - 7000 \text{ mg Fe kg}^{-1}$ illustrating that, overall, the added Fe dose of the *n*Fh (22 mg Fe kg^{-1}) is small compared to the native stock. Most likely, the native and added Fe colloids are exchanged and the total Fe colloid concentration is more affected by other properties than by the mere addition of synthesized colloids. This means that, at natural soil pH, the recovery of P-loaded *n*Fh in solution is poor and that these will unlikely contribute to the bioavailable P pool.

In contrast, in the KOH-amended soils a dramatic mobilization of natural colloids to the pore water was observed. Such a mobilization has been noted before, often in studies focusing on the risk of metal leaching in contaminated soils (Kaplan et al. 1996; Klitzke et al. 2008). This is mainly related to the

increase in the negatively surface potential that enhances repulsion with the soil minerals, thereby stabilizing the colloids (Kaplan et al. 1997; Kretzschmar and Sticher 1997). As a consequence, colloids are stable at larger ionic strength as more electrolytes are needed to collapse the electric double layer (EDL) (Kretzschmar and Sticher 1997). The unexpectedly large colloid concentration in the KOH-amended soils calls for attention. Traditional soil liming with CaO or CaCO₃ was avoided here to uncouple the stabilizing pH effect from the destabilizing Ca effect, i.e., the pH effect noted here due to KOH should not be mixed with the pH effect in traditional Ca-based soil liming tests. The KOH addition increases the pH and increases the CEC, thereby even allowing solution Ca²⁺ activities to decrease by binding to the newly formed anionic charges. As a result, the natural organic colloids, along with associated Fe- and Al-containing minerals, are dispersed, yielding the dark color of the solution. Strong correlations between colloidal Fe, Al, TOC and P concentration were previously observed in several soils (Ilg et al. 2005; Pokrovsky et al. 2005; Montalvo et al. 2015a). In Ferralsols, the clay fraction is dominated by Al/Fe-oxyhydroxides, while in Andosols aluminosilicates and humic-metal-phosphorus complexes (HMEP) govern this fraction. Our data also points to either colloidal Fe/Al (oxyhydr)oxides coated with organic matter and P, or HMEP. The latter has been identified mainly in Podzols and Andosols (Gerke 2010), thus could be the predominant form in the Betafo soil. However, since we did not perform any size fractionation or even dialysis/ultrafiltration to determine truly dissolved concentrations, this can only be speculated. It is safe to assume that virtually all measured Fe is colloidal, ionic Fe³⁺ or their inorganic complexes are marginally small (< 0.03 mg Fe L⁻¹) at the pH values considered.

The KOH-induced mobilization of colloidal P in these soils is excessive: P deficient tropical soils typically contain <0.02 mg P L⁻¹, the deficiency threshold being about 0.2 mg P L⁻¹. Here, soil solution P concentration exceeded 5 mg P L⁻¹ through KOH addition. In addition, the concentrations of organic C are very large: soil solution concentration in peat soils are reported to vary between only 50-70 mg C L⁻¹ while we measured values up to 50 times higher (Figure 2). Therefore, it is likely that part of the P is

present as organic P, given these high concentrations of organic C. Also in other studies, organic P is known to associate mainly with small colloids ($< 0.45 \mu\text{m}$) (Shand et al. 2000; Missong et al. 2018).

The impact of two other soil properties, Ca and OM, was also studied, but results were not according to our hypotheses. We predicted that increasing OM-content would enhance colloidal stability by blocking positively charged sites on soil, while increasing Ca-concentration would cause flocculation of colloids leading to aggregation. In Betafo, no effect of Ca was found, while in the pore water of Soavi a positive effect between Ca and Fe concentration was found, contrary to the destabilizing effect of Ca that is frequently reported. It is speculated that the measured Ca is likely part of the suspended colloids. Indeed, the KOH-induced mobilization of TOC up to 2 g C L^{-1} renders sufficient colloidal CEC to enhance colloidal Ca. For example, assuming that the CEC of organic colloids is about $4 \text{ mmol}_c (\text{g C})^{-1}$, this allows the mobilization of 4 mmol Ca L^{-1} . This also means that Ca speciation (determination of ionic Ca^{2+}) is required to identify the reverse trend of Ca noted here in the KOH-amended soils. No stabilizing effect of OM was found, as we hypothesized. The dried rice straw that was used contains a lot of recalcitrant organic components, such as lignin and (hemi)celluloses, and has a slow decomposition rate. Therefore, it is likely that the four day incubation period was insufficient to mineralize adequate amounts of rice straw to have a positive effect on colloid stability by blocking positively charged soil mineral sites. For organic manure, a positive effect on colloidal P concentrations has been reported, which was linked to a tendency for solute transport for organic or organically-bound P (Makris et al. 2006; VandeVoort et al. 2013). A longer incubation period, or a more labile OM, would be advised to confirm this effect in the future.

The final part was built on the promising results found by KOH addition in the fate experiment of Part II. The effect of pH change by KOH or $\text{Ca}(\text{OH})_2$ on the fertilizer potential of P-loaded FeOx colloids was assessed in a pot trial. Similar to the results in Part I no differences in growth or P uptake were found between application of equal P dose as soluble P or NP-P in the unlimed Behenjy soil,. Increasing pH with KOH was expected to lead to a large contribution of natural colloids to the bioavailable P pool since it was found to improve colloid mobility in soil in *Part II*. It was unsure if these highly KOH-dosed soils would

affect plant growth since contrasting effects are reported in literature. Most studies examining the effect of high doses of K^+ /KOH on soil quality emerged from the land application of liquid waste and wastewater. Conclusions range from high K^+ application having a similar negative effect on infiltration and soil hydraulic conductivity as Na^+ , to observations that it has similar beneficial effects as Ca^{2+} (Arienzo et al. 2009). This was attributed to differences in soil mineralogy, especially clay mineralogy. In soils dominated with kaolinitic or illitic clays, K^+ has similar negative effects as Na^+ on soil structural stability, whereas in soils containing mainly smectites, K^+ had a similar effect as Ca^{2+} that flocculates the colloids (Arienzo et al. 2009). Reduced hydraulic conductivity in KOH-amended soils results from dissolution of SOM and dispersing clay particles, caused by the extension of the EDL due to high monovalent cation concentration, which then block macropores (Lieferrig and McLay 1996; Nyamangara et al. 2007). Plant growth on KOH-amended soils in our experiment was extremely poor, so it is likely that the soil structure was damaged due to the high KOH dose applied. Hence, this suggests that magnifying colloidal concentrations to improve P mobility has a potential inherent negative effect on soil structure.

The negative result that was obtained in this final bioassay on soil, demands a thorough re-examination of our initial concept, which was based on two observations: (i) in weathered soils, almost 100% of P in soil solution is colloidal P, and (ii) the truly dissolved PO_4 -P concentration measured in pore water of weathered soils are only between 0.5 and 20 $\mu g P L^{-1}$, while adequate P concentrations needed to sustain plant growth are above 200 $\mu g P L^{-1}$ (Maertens et al. 2004; Montalvo et al. 2015a). Colloidal P in soil solution is thus expected to contribute to P uptake at low truly dissolved P concentration, since plant growth cannot be sustained at these low truly dissolved P concentration. This was confirmed in solution in our previous work (Bollyn et al. 2017), but positive results were not achieved here in soil. Therefore, we will theoretically review the possible augmentation of P bioavailability with colloidal P. As a first step, the diffusion of nanoparticles/colloids is compared to the diffusion of PO_4 . The diffusion coefficient in solution (D_i) of a particle is determined by its size through the Stokes-Einstein equation. A 1 nm-sized NP will have a D_i two times smaller than PO_4 , while 10 nm-sized NP will have a D_i already 20 times smaller

than PO_4 . The P flux to the root will be proportionately reduced (Figure 4a). In a porous media, D_i has to be converted to the effective diffusion coefficient by taking into account the tortuosity and volumetric water content of the medium, and, more importantly, the soil buffer power (b). For PO_4 , b is proportionately related to the K_d of the soil. For colloidal species, the value of b is unsure, but likely the buffer power of nanoparticles is lower than that of PO_4 . In a well-buffered system, the bulk concentration is achieved closer to the root surface compared to a poorly-buffered system (Figure 4b). In the case of nanoparticles, the low diffusion coefficient is thus likely accompanied by a poor buffering. Conversely, a higher P concentration in the bulk solution is likely to be achieved for NP-P, due to the lower reactivity of P bound to nanoparticles compared to ionic P, resulting in a high P concentration near the roots, possibly canceling out the negative effect of low buffer power.

Colloidal P is, at best, 50 % bioavailable relative to PO_4 ions in solution (Bolln et al. 2017). This implies that colloidal P in solution has to be present at, at least, two times larger concentration than soluble P to achieve the bioavailable P concentration. Taking into account the lower bioavailability and the smaller diffusion coefficient, 1 nm-sized NP-P has to be present at 0.8 mg P L^{-1} , and 10-nm sized NP-P at 8 mg P L^{-1} in the pore water to be as bioavailable as truly dissolved $\text{PO}_4\text{-P}$ (0.2 mg P L^{-1}). In our pot trials, plants supplied with an equal P dose either as P_i or NP-P (10 nm) did show similar yields, in contrast to these calculations. The low reactivity of NP-P to soil minerals is expected to counteract the lower bioavailability and diffusivity, however, it is difficult to predict the extent of this effect. However, we speculate that for the large 10 nm particles, the effects of D_i will dominate. Such high colloid concentrations in solution would be required that the negative effect on soil structure is likely to occur as was noticed in the KOH-amended soils. This leads us to conclude that, although native colloids contribute to plants in P deficient conditions, unrealistically large concentrations of colloidal P in solution are needed to compensate for their lower diffusivity and lower bioavailability compared to soluble P. Only very small nanoparticles (1 nm) might still be useful as a basis for the development of P nanofertilizers. The same is true for organic P

455 colloids which release P by phosphatases in the rhizosphere, but this process is based on another
456 mechanism that was not assessed here.

457 In conclusion, the addition of P-loaded mineral colloids or the mobilization of native colloids could not
458 increase P uptake in plants above that of an equivalent dose of soluble P. Potentially, smaller nanoparticles
459 (1 nm) might be tested and the same is true for the additional of small labile organic colloids.

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Figures

Fig 1 Pore water composition of Behenji soil from *Part I* (a-b) and *Part III* (c-d). Phosphorus was applied in different doses in *Part I* (a-b) using either KH₂PO₄ (‘Soluble P’), P-HMP coated nano-ferrihydrite (‘NP-P’), or HMP/KH₂PO₄ in the same ratio as in the NP-P treatment. In *Part III* (c-d), the soil was either kept at natural pH (‘no liming’), or amended to pH 5.5 with KOH or Ca(OH)₂. P addition was either absent (‘no P’) or present at 150 mg P kg⁻¹ using soluble P or NP-P, in the Ca(OH)₂ the soluble P treatment is not present. The dashed horizontal line illustrates the limiting dissolved P concentration in solution for adequate plant growth (200 μg P L⁻¹). Vertical bars represent standard deviation (n = 3)

Fig 2 Fate of ^{33}P loaded nano-Ferrihydrite ($n\text{Fh}$) in soil solution: relationships between soil pH and soil solution concentration of Fe, Al, P, organic carbon (TOC), and recovery of ^{33}P in soil solution compared to initial applied dose (%) for $n\text{Fh}$ -spiked Betafo and Soavinimerina (Soavi) soils. The soil pH increased upon KOH addition to soil. Full line represents linear fit with R^2 values given per soil, grey area represents 95% confidence intervals.

Fig 3 Elemental associations in pore water, all data are total elemental concentrations in 0.45 μm filtered and digested solution collected from P deficient soils obtained from Betafo (x) and Soavi (o). The TOC is the total organic carbon. The variable composition of the pore water is dominated by the effect of KOH addition to soil, not by addition of P loaded nano-Ferrihydrite addition. The KOH addition increases the pH and lowers the concentrations of polyvalent cations that lead to extreme mobilization of native colloids

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605 **Fig 4** (left) Calculated diffusive flux of P in a steady-state system with P supplied in solution as inorganic
 606 P (P_i, solid line), or nanoparticulate P with a diameter of 1 nm (dashed line) or 10 nm (dotted line). The
 607 diffusive flux is 2 times lower in the case of P supplied as 1 nm NPs compared to P_i, and even 20 times
 608 lower in the case of P supplied as 10 nm NPs compared to P_i (right). The concentration in solution relative
 609 to the bulk concentration increases with distance from the root surface. In a system which has a buffer
 610 power of 100, the slope is steeper compared to a system with a buffer power of 10. In two systems at an
 611 equal P dose (mg P kg⁻¹) but with P supplied as ionic P (P_i) or NP-P, both the buffer power and the D_i will
 612 be different. If only b is taken into account, the bulk solution concentration is achieved closer to the roots
 613 in a well-buffered system (b = 100) compared to a poorly-buffered system (b = 10). However, a larger P
 614 concentration in the bulk solution (mg P L⁻¹) can be achieved with NP-P compared to ionic P at an equal
 615 dose (mg P kg⁻¹) due to a lower reactivity towards the soil minerals, which is indicated by the dotted line.

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619 **Tables**

620 Table 1: Selected characteristics of topsoil (0-20 cm).

	Behenjy	Betafo	Soavinimerina (Soavi)
<i>Site characteristics</i>			
Country of origin	Madagascar	Madagascar	Madagascar
<i>Topsoil properties</i>			
pH (CaCl ₂) ^a	4.0	4.1	4.3
C %	2.9	6.4	2.6
CEC ^b cmol _c kg ⁻¹	1.8	4.5	2.7
P _{oxalate} ^d mg P kg ⁻¹	30	290	70
Al _{oxalate} ^d mg Al kg ⁻¹	2180	9590	620
Fe _{oxalate} ^d mg Fe kg ⁻¹	1290	7330	5990
Mn _{oxalate} ^d mg Mn kg ⁻¹	20	1300	20
Soil Type ^e	Ferralsol	Andosol	Gleysol

621 ^a pH determined in a 1:5 0.01M CaCl₂ suspension; ^b CEC determined at soil pH by Cohex method; ^d Ammonium622 oxalate extractable P, Al, Fe and Mn (Schwertmann, 1964); ^eWRB (2007)

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Table 2: *Part I*: Shoot dry weight (mg), shoot P uptake ($\mu\text{g P shoot}^{-1}$), and shoot P content of rice grown in Behenji soil at three different P sources: P added as soluble P, as NP-P (P-HMP-*n*Fh colloids), or as HMP/ KH_2PO_4 in the same ratio as applied with the nanoparticles. Each P source was applied in two (NP-P and HMP/ KH_2PO_4 : 50 and 150 mg P kg^{-1}) or three different doses (soluble P: 150 – 250 – 600 mg P kg^{-1}).

	P dose (mg P kg^{-1})				
	0	50	150	250	600
<i>source of P</i>	<i>Shoot dry weight (mg)</i>				
KH_2PO_4	27 (5)		207 (64) ^a	266 (47)	241 (42)
NP-P		77 (3)	170 (77) ^a		
HMP/ KH_2PO_4		69 (10)	209 (41) ^a		
	<i>Shoot P uptake ($\mu\text{g P shoot}^{-1}$)</i>				
KH_2PO_4	16 (3)		772 (252) ^a	1301 (214)	1995 (587)
NP-P		113 (13)	455 (254) ^a		
HMP/ KH_2PO_4		105 (11)	680 (164) ^a		
	<i>Shoot P content (mg P kg^{-1})</i>				
KH_2PO_4	610 (10)		3720 (190) ^a	4890 (50)	8160 (1280)
NP-P		1460 (120)	2600 (310) ^b		
HMP/ KH_2PO_4		1550 (360)	3250 (400) ^{ab}		

Letters represent pairwise difference between P sources at the given P dose. Mean values followed by the same letter are not statistically different at $p < 0.05$. Values in parentheses are standard deviations of mean ($n = 3$)

Table 3: The coefficient of determination (R^2) in stepwise regression on ^{33}P recovery (%), total dissolved P (TDP) (mg P L^{-1}), Al (mg L^{-1}) and Fe (mg L^{-1}) concentration in the pore water. Single effects include the measured pH and the measured Ca concentration in the pore water as continuous variables, and addition of OM and addition of NP as categorical variables. Forward modelling was used, whereby the term with smallest p-value is entered first. R^2 values are given for the total model, including previous steps.

	³³ P recovery (%)		TDP (mg L ⁻¹)		Al (mg L ⁻¹)		Fe (mg L ⁻¹)	
	Effect	R ²	Effect	R ²	Effect	R ²	Effect	R ²
Step 1	pH	0.51	pH	0.73	pH	0.71	Ca	0.83
Step 2	Soil type	0.65	Soil type	0.78	Ca	0.88	pH	0.93
Step 3	Ca	0.67	Ca	0.82	Soil type	0.90	Soil type	0.95
Step 4	OM	n.s.	OM	n.s.	OM	n.s.	OM	n.s.
Step 5	NP type	n.s.	NP type	n.s.	NP addition	n.s.	NP addition	n.s.

n.s.: not significant

641 Table 4: *Part III*: Shoot dry weight (mg), shoot P uptake ($\mu\text{g P shoot}^{-1}$), and shoot P content (mg P kg^{-1}) of
642 rice grown in Behenjy (top) and Soavinimerina (bottom) soil at three different pH treatments: no liming,
643 liming with KOH, and liming with Ca(OH)_2 ; and two or three different P treatments: no added P, and P
644 added at 150 mg P kg^{-1} as KH_2PO_4 or NP-P.

BEHENJY			
	no liming	KOH	Ca(OH)_2
<i>pH</i>	4.1 ± 0.1	5.5 ± 0.1	5.5 ± 0.1
<i>Source of P</i>	<i>Shoot dry weight (mg)</i>		
no P	39 (9)	5*	52 (7)
KH_2PO_4	85 (14)	3 (2)	<i>n.d.</i>
NP-P	88 (13)	5 (1)	281 (34)
	<i>Shoot P uptake ($\mu\text{g P shoot}^{-1}$)</i>		
no P	28 (7)	14 (2)	16 (8)
KH_2PO_4	344 (69)	10 (6)	<i>n.d.</i>
NP-P	330 (40)	12 (5)	936 (36)
	<i>Shoot P content (mg P kg^{-1})</i>		
no P	720 (60)	1740 (1560)	320 (170)
KH_2PO_4	4060 (240)	4100 (2090)	<i>n.d.</i>
NP-P	3760 (130)	2510 (300)	3350 (300)

SOAVINIMERINA			
	no liming	KOH	Ca(OH)_2
<i>pH</i>	4.4 ± 0.1	5.5 ± 0.1	5.5 ± 0.1
<i>Source of P</i>	<i>Shoot dry weight (mg)</i>		
no P	59 (13)	15 (11)	61 (13)
KH_2PO_4	401 (45)	18 (6)	345 (20)
	<i>Shoot P uptake ($\mu\text{g P shoot}^{-1}$)</i>		
no P	41 (11)	20 (4)	47 (10)
KH_2PO_4	914 (174)	32 (8)	706 (143)
	<i>Shoot P content (mg P kg^{-1})</i>		
no P	690 (40)	1550 (840)	770 (3)
KH_2PO_4	2280 (25)	1840 (280)	2040 (330)

645 Values in parentheses are standard deviations of the mean ($n = 3$). *n.d.*: not determined. *: only one replicate
646 survived, no standard deviation possible.