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Soil bypass P for enhanced availability in acid soils

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Abstract

Phosphorus (P) is known as one of the essential macroelements for all life, is as important as Nitrogen (N) and Potassium (K) for as a fertilizer component, and unfortunately, is a limited, nonrenewable natural resource, which makes it extremely important to secure efficient utilization as a plant nutrient. When P-fertilizers are applied to soil, strong binding of phosphate can occur, particularly in acid soils. This fixation of fertilizer-P in the soil matrix limits the uptake of fertilizer P by the growing crops, leading to a low recovery of fertilizer P in the harvest. Fixed P is accumulating in soil as more P-fertilizer applied, and this phosphate is so tightly bound to the soil that crops can only take up a minor fraction of it. In this thesis, a conceptual novel P-fertilizer was presented as a hypothetical solution to this P-fixation problem.

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Introduction

At the beginning chapter, there will be a brief review for some literature about the phosphate binding capacity of soils in general, and acid soils in particular. There is evidence that applying very high doses of P-fertilizer could be the only way to get high yields on acid soils that are rich in iron and aluminum. The reason for this is that Al3+ and Fe3+ in such soils are strong sinks for phosphate, effectively competing with the plant roots. Most of the added phosphate is therefore "fixed" by binding to Fe- and Al-oxides. The accumulation of phosphorus in those acid soils is a predictable result then. Moreover, the accumulated "fixed" phosphate is binding so strongly to the soil that crops can only take up a minor fraction of it.

The consequence of this is that high yielding crop production on acid tropical soils require a constant over-fertilization with phosphorous, since the excess P added would continue to be fixed by Al and Fe-rich acid soils, which are P-binding strongly, only continuation of the excessive inputs of phosphates could achieve high crop yields. In theory, the consequence of overfertilization over many years should be that the pool of "fixed" P reach a steady state (equilibrium), i.e. that the P-uptake by crops equals the fertilizer P input. However, no experiments for now have continued long enough to test if it is possible to reach such a P-equilibrium in acid soils. The consequence is that within foreseeable future, intensification of plant production on acid tropical soils will require excessive use of the world's limited phosphate resources.

A hypothetical solution to this P-bonding problem is to compartmentalize fertilizer P in "hot spots" to protect it from the soil matrix, while allowing plant roots to have access. The concept is that this would prevent phosphate from reacting with Al and Fe-oxides, thus remaining plant available for an extended period. This principle was coined as "soil bypass phosphate", SBP. It is hypothesized to sustain high yielding crops on acid tropical soils without adding inordinate doses of P.

Since this is a novel type of phosphors fertilizer, there is a long way from proving the feasibility of the concept to practical industrial production, and a tiered approach is

proposed for this work:

The first step will be to provide "proofs of concept" Which is that phosphate can be compartmentalized in soil in a way that prevents, or at least retards its reaction with a d fixation to the soil matrix, and that these P-compartments can be invaded by plant roots so as to extract the phosphate.

If the first step were proved to work well, then Step 2 would be to run pot experiments in acid soils that rich in Al- and Fe-, in order to quantify the P uptake from SBP, and the availability of residual P for the subsequent crop.

If both two steps were promising, Step 3 would be to start developing practical solutions for industrial production of SBP, such as formulating a suitable matrix and how to produce it.

Phosphorus and fertilizer

Phosphorus (P) is one of the most essential nutrients for all living things. For plants, P is one of three essential macronutrients. P plays an important role in the energy metabolism, photosynthesis, respiration, redox, signal transduction and carbon metabolism, and it is also a crucial component of many key molecules such as nucleic acids, phospholipids, and ATP. Although P is so essential to plants, it only makes up about 0.2% of a plant's dry weight.

The inorganic P in soil is taken up by plants mostly as $H_2PO_4^-$, and remains as inorganic phosphate or is tranformed to phosphate ester or is attached to another

phosphate (Marschner 2012).

Because of the importance that P for plants, it is one of the three commonly added elements in fertilizers. When plants are short of P, they grow slowly, and the leaves become smaller and dark greener or reddish purple (Marschner 2012). Consequently,

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plants cannot grow well without an adequate supply of this nutrient. Just like nitrogen (N), P is also a most frequently limiting macronutrient for plant growth. In order to make crops grow healthily and enhance the agricultural productivity, synthetic N and P fertilizers are inevitably applied.

The world's population was 7.6 billion in 2018 (FAO Resource STAT 2018), 800 million more than 2008 (FAO Resource STAT 2008). The huge population required large amount of food. As a result for increasing food requirements, high yielding crops are needed, and these in turn need high fertilizer input.

According to Lu and Tian (2017), since 1961, the P fertilizer use rate per unit cropland area has trippled. To get higher yield of crop, increasing the amount of fertilizer applied seems to be the easiest way. Since the invention of chemical fertilizer, it plays a significant role in raising global agricultural productivity and helps to ensure food security thereby reduces the number of hungry people. Meanwhile, excessive fertilizer use has brought a series of negative environmental impacts, such as air pollution, water eutrophication and soil acidification (Bouwman et al 2009).

P-binding in soils, plant adaptation to P-limitation:

Phosphate ions have long been known to be strongly bound by alumina- and iron oxides in soils. The kinetics of binding is initiated by a fast adsorption, followed by a slow process following a first order rate, leading to more permanently bound phosphate (Chen et al 1973). The initial fast adsorption is dependent on the presence of competing anions (fluoride), the subsequent first order rate was not (ibid). Further, the formation of strong P binding decreased to zero when pH is increased to 7. Thus, strong binding of P with Al oxides is clearly an acid soil phenomenon (Chen et al 1973). The phenomenon is ascribed to the pK_a value for metal oxides. The pK_a value is known as acid dissociation constant, which indicates the ability of an acid to dissociate hydrogen ions (H^+).. In soil, the valence state of aluminum ions are

trivalent (AL^{3+}), as well as iron ions (Fe^{3+}), which in amount of substance equal to triple hydrogen ions (H^+). It means that these elements are buffering effectively in the acid range. In soils, these metal oxides are present primarily as small crystals (Hinsinger 2001).

The phosphate minerals are most soluble at low pH, while the Al- and Fe-P minerals show the opposite trend, as illustrated in Figure 1 (Hinsinger 2001).
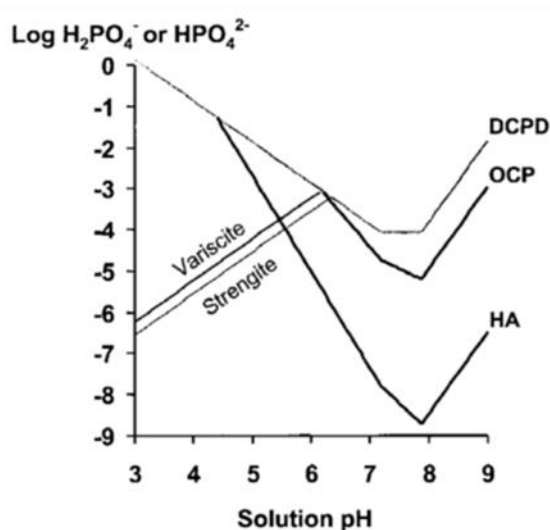
Figure 1. Solubility of different P forms. Where DCPD stands for $CaHPO_4*2H_2O$, OCP stands for $Ca_4H(PO_4)_3*2.5H_2O$, HA stands for $Ca_5(PO_4)_3OH$, variscite stands for $AlPO_4*2H_2O$ and strengite stands for $FePO_4*2H_2O$ (Hinsinger 2001).

Some apparent conflict with the view that Al- and Fe "fixed" is too strongly bound to available readily available: He and Zhu (1998) equilibrated phosphate with a variety of minerals (goethite, montmorillonite, kaolinite, Al-oxide) at pH 7 for 2 months, and tested it's subsequent release/extractability when added to soils. They found that large fractions were effectively released over a 125 days period, and claim that this proves that fixed phosphate in soils is a relatively available P source. The question is whether they really did achieve the formation of "fixed" phosphate during their equilibration at pH 7: according to Chen et al 1973, they should not. Syers et al (2008) also claims evidence that "Al- and Fe-fixed" phosphate is relatively easily available.

The evidence provided, however, appears to be obtained in soils that are unlikely to bind phosphate strongly (Rothamsted soils, and strongly limed feral soils). Similarly, Yost et al (1981) measured the effects of P-fertilization and the residual effects on subsequent crops in a limed P-deficient oxisol. They found that the subsequent corn crop was only able to extract 3.5-7% of the original P-dose. Interestingly, banded P fertilization resulted in substantially higher residual P-availability than broadcast.

The strong binding of P in acid feral soils lead Ehlers et al (2010) to hypothesize that bacterial metabolism could be P-limited in such soils. Their results confirmed a the hypothesis; respiration and microbial growth in response to a dose of organic carbon was indeed enhanced by adding phosphate, but the effect was transient, and the bacteria were apparently able to extract the strongly bound phosphate in the soil within a period of 2-3 days. The result was taken to suggest that microbial P-acquisition spiked by pulses of organic material could effectively release phosphate reservoirs of the soil which would otherwise be inaccessible to plant roots (because plant roots have access to a marginal fraction of the soil volume compared to the ubiquitous soil bacteria).

According to Hinsinger (2001), it is difficult to differentiate phosphate adsorption and precipitation of P minerals, but both processes contribute to the low mobility of phosphate in soil. The complexity of the mechanisms involved is illustrated by the observation that P in the soil solution increased by pH manipulations in both directions (Hinsinger 2001), which suggests that the phosphate concentration in the soil solution is governed by a plethora of processes. Although the P-binding components in soil are Fe and Al oxides is unarguable, the role of various clays may have been underestimated (Gerard 2016).

Despite the controversies and limited understanding of the mechanisms of P-binding in soil, there is little doubt that P in acid tropical soils is strongly bound, and that the strength of the binding of fertilizer-P increase with time. This notion was put to a test by Linquist et al (1997), by studying the long term fate of P added to a tropical soil with pH 4.8 (first a build-up phase of 2 years with 4 doses of P added to the growing

crops, followed by a residual phase of 2 years with no fertilization to the crops). The P budget showed that at the end of the experiment, 46, 64 and 84% of the added P was retained by the soil for the treatments with low, intermediate and high P doses, respectively. They determined the fractions of P in the soil during the residual phase, and found no indication of a selective decline in any of the P pools (mild versus strong extractions), thus apparently refuting the hypothesis that plants selectively extract P from the easily extractable pools. This could reflect shortcomings of the extraction procedures to differentiate between P-pools, however, and the experiment should have been more long lasting to really put the theory to a test.

Despite the phosphate binding in acid tropical soils, intensive cultivation of food crops with high yields is possible, if limed and provided with ample amounts of phosphate (Sanchez et al. 1982).

Diffusion of phosphate in soils

Phosphate diffuses slowly in soil, which limits the rate of P-acquisition by plant roots, and is the reason why mycorrhiza can enhance the uptake by exploiting more of the soil matrix than the naked roots. The slow diffusion of P is also a reason why packaging of P in granules (bypass P) can help to sustain P in an available form for a longer time than soluble P-fertilizers. The critical factor is then how fast soluble P diffuses out of the "packages".

Several investigators have done empirical determination of the phosphate diffusion rate in soils, using disks pf P-enriched and P-poor soils which are pressed together, and the transport from the P-rich to the P-poor compartment measured over time. To calculate the effective diffusion coefficient, Kaselowsky et al (1990) used the formula:

$$D_e = \frac{m_t^2 \pi}{A^2(C_2 - C_1)^2 t} \qquad (1)$$

Where

D_e is the effective diffusion coefficient, unit: $m^2 s^{-1}$

m_t= the amount of P diffused at time=t

A= cross section area of diffusion unit m^2

C_2 and C_1 = the concentration of P in the fertilized and the unfertilized disk/block

The technique (and the formula) has been used in several investigations, and D_e values are found to depend on soil, bulk density and moisture content:

Kaselowsky et al (1990); silt soil: $6.25*10^{-13}$ $m^2 s^{-1}$

Bhadoria et al (1991a): Loess soil at different bulk densities and moisture content: $0.1-5*10^{-13}$ $m^2 s^{-1}$

Bhadoria et al (1997b); silt soil, measured D_e increased with increasing phosphate concentration: increasing from $2.2*10^{-13}$ $m^2 s^{-1}$ to $13.9*10^{-13}$ $m^2 s^{-1}$ for the P-concentration 380 and 580 mg P kg^{-1} soil, respectively.

Efforts for reducing P-bonding in soil

Liming is effective to neutralize soil acidity, improve plant growth, and activate microorganisms in soil. It may affect phosphate availability through influencing pH in soil, providing Ca and affecting activities of microorganisms in soils and thus the net mineralization or immobilization of soil organic P (Haynes 1982). There are several benefits of liming, but liming costs too much for poor farmers in developing countries; moreover, difficulty of getting, lack of awareness of its importance, inappropriate

usage by farmers and weak extension services reaching the farmers have to be considered as well (Kiplagat et al 2014).

Company like Kingenta provides some solutions, and one of them is a novel fertilizer called polyphosphate-fertilizer. According to McBeath et al (2007), ammonium polyphosphate was proved more efficient than conventional granular P fertilizer, but the disadvantage of this fertilizer is that it has to avoid high temperature and low pH when the fertilizer is stored or used. Another solution is called 'Seed manure simulcasting'. It is a manner of seeding. When sowing the seeds, the fertilizer is put near/beneath the seeds. The young plants have easy access to the concentrated fertilizer in their vicinity, so that the fertilizer would provide enough nutrients for plants growing at the beginning. When the area with fertilizer has a high concentration, soil around the concentrated fertilizer would be saturated, and the available P would keep for longer time before binding by soil. The problem of this seeding manner is that the plants should other have an abundant enough root system, or the roots can proliferate around the concentrated fertilizer point to get enough nutrients for plants growth.

Plant adaptation to patchy distribution of P

Competition experiments suggest that large root systems is not necessarily synonymous with strong competition for P in soils; it appears that plants with a moderate root system can be equally competitive, apparently because they allocate more root growth to P-rich zones and microsites. Thus, there is a tradeoff between the ability to explore large soil volumes and that to exploit P-rich patches within the soil matrix (Casper and Jackson 1997). This is further illustrated by the fact that two component models (convection and diffusion) robustly predict P uptake by some plants species when growing in P-rich soils, but are sometimes completely off target for some plants and in particular in soils with low P content (Hinsinger 2001). There is ample evidence for a P-depletion in the rhizosphere, but interestingly, the opposite

has also been observed, suggesting that plant roots may solubilize soil P at some distance (possibly by excreting organic acids) which are then transported towards the roots with the bulk flow of water, but that a fraction of this is re-adsorbed before reaching the root surface(Hinsinger 2001). It is well known that plant roots can direct their root growth (root hair density, lateral root formation) in response to nutrient rich patches in the soil, and the regulatory system controlling this phenomenon has to some extent been unraveled (Jones and Ljung, 2011, Postma et al 2013). Some plants can grow proteid roots, which are dense clusters of roots formed by lateral root formation (Lambers and Shane 2007). These proteoid roots have been found to exude large amounts of citrate, which may help to solubilize Al and Fe-bound P (citrate ligates Fe and Al, which may be an Al-detoxification reaction). Plant root exudation of a variety of organic acids is upregulated by P-deficiency, thus potentially resulting in more available P. However, this effect has been questioned due to the short half-life of these organic acids in soils (used as C substrates by the soil microorganisms). This raises another question: if large amounts of organic acids are excluded, they will possibly lower the pH on the rhizoplane, but may effectively increase the pH further away from the root due to the alkalinizing effect of microbial degradation of the organic acids.

In theory, an adequate response to P-limitation could be to either lower or increase the pH in the rhizosphere, depending on the soil. However, it is questionable if the plant is "free to choose" between these options: the pH of the rhizosphere is largely governed by the balance between cation and anion uptake, hence plant roots fed with NH_4^+ acidifies their rhizosphere, while plant roots fed with NO_3^- alkalinize the rhizosphere. Numerous experiments have shown that plants growing on NH_4- take up more P than plants fed with NO_3^-. This seems plausible if the soil contains Ca or Mg phosphates, but it is unlikely to be the case for very acidic soils, in which Al and Fe phosphates dominate. There is some evidence for this; in very acidic soils, the P-uptake was retarded by rhizosphere acidification (NH_4^+ uptake) and enhanced by rhizosphere alkalization (NO_3^- uptake). However, conflicting evidence exist as well

(Hinsinger 2001).

The fact that plants can increase their P acquisition by altered root architecture (foraging) and metabolism (exudates, increased rates of P uptake), has inspired plant breeders to include such traits in plant breeding programs so as to provide cultivars with high productivity in low input farming system (developing countries). Although promising, there is little evidence of success in this direction (Richardson et al. 2011)

Can P-hotspots supply enough P to plants?

While it is clear from the foregoing that plants can enhance P-uptake in localized P-rich hot spots in response to P-deficiency aboveground, a crucial question is whether this is efficient enough to sustain high yielding crops. "Split root experiments" shed some light on this question. Borkert and Barber (1983) conducted short term split roots experiments with soybeans to investigate if P-supply to a minor fraction of the roots could provide similar amount of P as when the entire root system is in P-solutions. They found that the P uptake rate per root surface area (in the compartment with P supply) increased with decreasing fractions of the roots in P-containing compartments, but the increase was not sufficient to fully compensate for the lost P-uptake in the P-free compartments. The duration of the experiment was only 6 days, however, which means that   the plants had marginal chances to compensate by increasing root density in the compartments with P. The only thing learned from the experiment is that P uptake is regulated by the need for P in the entire plant . This is of course a very important observation, but longer term experiments would have to be run for the full potential of this regulation to be observed/tested.

Soil Bypass Phosphorous (SBP)

Another solution to secure that fertilizer P is available to plants is "Soil Bypass Phosphorous", SBP, invented and coined by LR Bakken.

The SBP-idea is to make a P-fertilizer that is immobilized in lumps of fibrous or granular matrix that allows roots to extract P directly, i.e. that the major part of the P "bypass" the soil, hence avoid being fixed. Ideally, the matrix should allow roots to proliferate inside, but the bypass-effect could conceivably also be achieved by dense root growth on the surface of the granules. The matrix should be compact in dry conditions (as a fertilizer commodity), but swell to 2-4 times of its original volume when in contact with soil moisture.

Materials

Soil and fertilization

The soil material used was taken from a spruce forest at Norderås (close the the Norwegian Univerity of Life Sciences), which has a typical podzol profile. The soil for the experiment excavated (approximately 25 cm depth) after removing the humus layer). The forest had never been fertilized. The soil characteristics are: 94% sand, 3% silt, 3% clay, pH (H_2O) 4.5-4.6, 1.29% loss on ignition and 16 mg P-AL kg^{-1}. After drying, the essential nutrients were added. The doses of Nitrogen (N), Potassium (K), Magnesium (Mg), Iron (Fe), Copper (Cu), Manganese (Mn), Boron (B), and Molybdenum (Mo) were 120, 120, 18.5, 10, 6.4, 8.1, 0.27, and 0.26 mg·L^{-1} soil, respectively. The sources of N, P and K were Ca $(NO_3)_2$·$4H_2O$, Ca $(H_2PO_4)_2$ · H_2O, and KCl, respectively. For Mg, Fe, Cu, and Mn, their sulfate salts, and $Na_2B_4O_7$ · $10H_2O$ for B, $(NH_4)_6$ Mo_7O_{24} H_2O for Mo, were used. All the fertilizers were applied in liquid form, and the required amounts fertilizer solutions were thoroughly mixed with the soil of each pot.

Sand for SBP matrix

The material used, called rock sand. It has been crushed from rock and is not rounded shape as it has been along a river. The rock sand was dried in the pretreatment room, and pre-sieved through 0.6 mm sieve. This is to get rid of unnecessary large quantities of dust before wet sieving. After dry sieving are done, wet sieve was performed as planned through 2mm.

Environment

Plants were grown under environmentally controlled conditions with a temperature at 21 °C during day and 16 °C at night with a relative humidity of 55% and 65%, respectively. The photoperiod was set at 16 h during day and 8 h at night with an approximate photon flux of 220 uE/m^2 s. The soil humidity was kept between 60% and 80% of the potential water holding capacity by daily measuring weight of each pot, and adjusted with deionized water throughout the experimental period.

Methods

Soil sampling

An auger with a diameter of about 20 mm was used to collect the soil from pot experiment. At least 9 soil columns in the depth of 0–20 cm were taken from each pot. The top plant litter was removed before the samples were taken. The soil columns were collected in a half-liter cardboard box and mixed well with a trowel.

Soil analysis

The soil samples were analyzed by Norwegian University of Life Science (NMBU).

The soil samples were dried in board paper boxes at 40°C as soon as possible after they were taken from the pots. The temperature was not higher than 40°C for the purpose of reducing the influence of the analysis results.

Soil pH (H_2O)

The pH was measured using the method described in 'methods of soil analysis' (Mc Lean, 1982). Ten cm^3 of dried soil was transferred to a graduated plastic tube with screw top with the help of a cylindrical measure. Twenty-five ml deionized water was added to the tube and the lid was snapped on. NMBU used a ratio of soil to water of 1:5. The mixture was shaken by hand until the soil is well mixed with the water. Then tubes were left at room temperature until the next day. Before measurement at the second day, the sample was shaken once again and left for at least another 15 minutes until most of the soil settled to the bottom.

The pH meter was calibrated with two buffer solutions, pH 4.00 and pH 7.00. The electrode was inserted in the suspension and the pH was measured in the liquid above. The pH was read off when the instrument displayed a stable pH value.

Plant-available phosphorous (P-AL)

The plant-available phosphorous (P-AL) or soluble phosphorus is the amount of the phosphorous that plant could take up from the soil in a short space of time, for example during a growing season. Many different methods are used all around the world. In Norway, Egners AL-method has been used as standard method for around 60 years (Egner, Riehm, and Domingo 1960). Egners AL-method is also known as the

acidic Egner–Riehm–Domingo (ammonium lactate) soil phosphorus (P) test, and all details are described by Egner et al (1960). This AL-method is also used in Sweden, Iceland and partly in Germany and the Netherlands.

For the preparation of extraction solutions (AL-solution), 0.1 mol*L^{-1} ammonium lactate (NH$_4$-lactate) and 0.4 mol*L^{-1} acetic acid with a pH of 3.75 was mixed.

Two grams of soil was transferred to an extraction bottle with capacity of 100 ml with screw top. Forty ml of the AL-solution was added. The bottles were immediately placed on a vibrator after been screwed the top tight and lied lengthways along the line of it. The vibrator was set to shake back and forth at the rate of 125 times per minute for one and half hour precisely at a room temperature.

Total phosphorous (TP)

The total phosphorus (TP) is to measure all the forms of phosphorus in the samples. The form includes orthophosphate, condensed phosphate, and organic phosphate.

Samples were analysed on Agilent Technologies 5110 ICP-OES. Blank samples and control A and B was prepared by the same procedure as the samples. Limit of detection (LOD) was calculated as following: 3 times the standard deviation of the blank samples. Limit of quantification (LOQ) was calculated as following: 10 times the standard deviation of the blank samples.

Phosphorus was measured on wavelength 177.434 nm.

Plant sampling and analysis

The plants were sampled from every pot with roots washed by deionized water.

The plants were put in a paper bag and dried at 40°C for 24 hours, and phosphorous was measured after the samples were prepared with acid digestion by UltraClave. The

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phosphorus was analyzed on Agilent Technologies 5110 ICP-OES and the results reported as g/kg dry sample.

Soil bypass phosphorus fertilizer (SBP)

To make a model-SBP for experimentation, it was decided to make flat disks with soil contact only on one side, in order to simplify the analysis of diffusion between the SBP matrix and the soil (1 dimensional diffusion). Rock sand was chosen as an inert matrix, which was loosely held together by agar. The additional effect of the agar was to minimize transport of phosphate with bulk flow of water within the matrix, which inevitably occurs when roots extracts water.

To make the disks, the rock sand was first amended with phosphate: portions of 0.125 g Ca $(H_2PO_4)_2*xH_2O$ (55% P_2O_5) + 16.875 g of rock sand were placed in plastic tubes with screw top and thoroughly mixed in a rotating mixing machine (Figure 2).

Figure 2. Rotating mixing machine.

Each portion of sand with phosphate was then transferred to 5 cm diam petri dishes lined with gauze, to a depth of 1 cm. The disks were then heated to 50 ^{o}C, and then filled with molten agar (VWR, catalognumber: 20767.298, 20 g agar L^{-1}, 60°C). Preheating of the dishes was to secure that the molten agar filled all the pores before solidifying by cooling.

Figure 3. P-fertilizer disk for Soil Bypass Phosphorous (SBP disk). The rock sand with Ca (H$_2$PO$_4$)$_2$ is placed in a 5 cm diameter petridish lined with gauze, which is then flooded with molten agar. Once the agar is solidified, the disk is airdried, and ready to use. Upon rewetting, the agar swells to 30% of the original volume, securing ~70% air filled macropores.

When the agar had solidified, the gauze was folded over the surface of the disks, which were then airdried air dried at room temperature (Figure 3).

Upon air-drying, the agar shrinks, and upon rewetting it swells to approximately 30 % of the original volume. Thus, the disk will have air-filled macro pores. This is very important because air-filled pores are needed to avoid anoxia when roots invade the matrix.

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Results & Discussion

The first experiment

A preliminary pot experiment was conducted to find suitable experimental conditions for testing SBP with live plants. Ideally, the soil should have low pH to secure strong P-fixation, and marginal P-content to secure P-limited plant growth. The ideal plant would be fast growing, and tolerance to low soil pH. Based on advice from horticulturalists, tomato and cucumbers were chosen as candidate plants. The soil of choice was subsoil from a forest, with pH_{H2O} ~4.5. The plants were not expected to tolerate this low pH, hence we decided to test their growth at different levels of lime. The experiment included different levels of P, while all other nutrients were added in excess, in order to test their response to P: The P-free nutrient solution contained 12 g $Ca(NO_3)$-N L^{-1}, 12 g K_2SO_4-K L^{-1}, 12.5 g $MgSO_4$-Mg L^{-1} + micronutrients (Cu, Mo B, Mo and Zn) was used. To provide P (to different amounts per pot), a phosphate solution was prepared, containing 3 g $Ca(H_2PO_4)_2$-P L^{-1}.

Portions of 3.355 kg soil dryweight were prepared for each pot by thoroughly mixing in in 25 mL of the nutrient solution (all pots), and different amounts of phosphorous solution (0, 5, 15, 25 ml Pot^{-1}) and finely ground lime ($CaCO_3$) (0, 0.5, 2.5 and 5 g pot^{-1}).

For every pot, five seeds were sowed, and four were removed after germinating, leaving the strongest plant intact. The weight of every pot was checked every second day, and water was added according to weight loss to secure 60% of the soil's water holding capacity.

After a period of 30 days, significant differences were observed in plant growth before they were harvested (Figure 4). After plants were harvested, soil and plant samples were analyzed and results are shown in Table 1.

Figure 4. Significant differences in plant growth. Tomato plants were shown in left figure and cucumber plants were shown in right figure

Table 1. Treatment for every pot, soil pH after harvesting, dryweight, P-uptake and P-recovery of plants. The P-uptake from fertilizer added is the P-uptake of plant from soil in the pot that added P fertilizer minus P-uptake of plant from soil in the pot that did not add P fertilizer at the same lime dose.

Pot No.	plant	Lime g*pot^{-1}	P added mg*pot^{-1}	Soil pH	Plant dryweight g	P content of plant g*kg^{-1}	P-uptake from soil mg	P-uptake from fertilizer added mg	P-recovery %
1	tomato	5	0	6.6	0.21	0.81	0.17		
2		5	15	6.3	0.22	0.81	0.18	0.01	0.07
3		5	45	6.4	0.24	0.94	0.23	0.06	0.13
4		5	75	6.4	0.33	1.00	0.33	0.16	0.21
5		2.5	75	5.8	0.19	0.94	0.18		
6		0.5	75	4.8	0.06	0.76	0.05		
7		0	0	4.5	0.16	0.80	0.13		
8	cucumber	5	0	6.4	1.80	1.20	2.16		
9		5	15	6.6	3.84	1.20	4.61	2.45	16.33
10		5	45	6.6	8.42	1.30	10.95	8.79	19.53

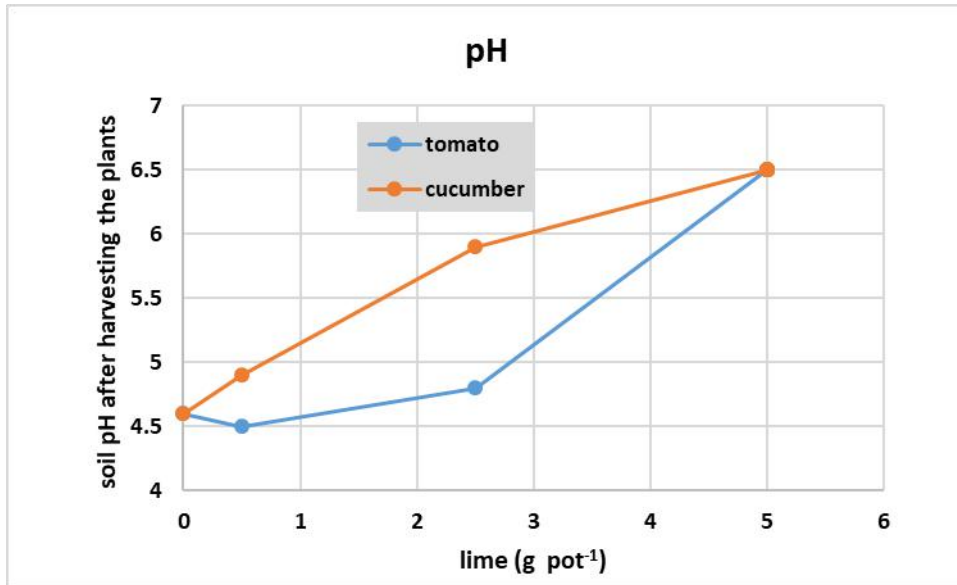| 11		5	75	6.5	10.05	1.40	14.07	11.91	15.88
12		2.5	75	5.9	8.28	2.10	17.39		
13		0.5	75	4.9	0.41	1.30	0.53		
14		0	0	4.6	0.24	0.99	0.24		

Figure 5. Soil pH after plants were harvested in different lime level.
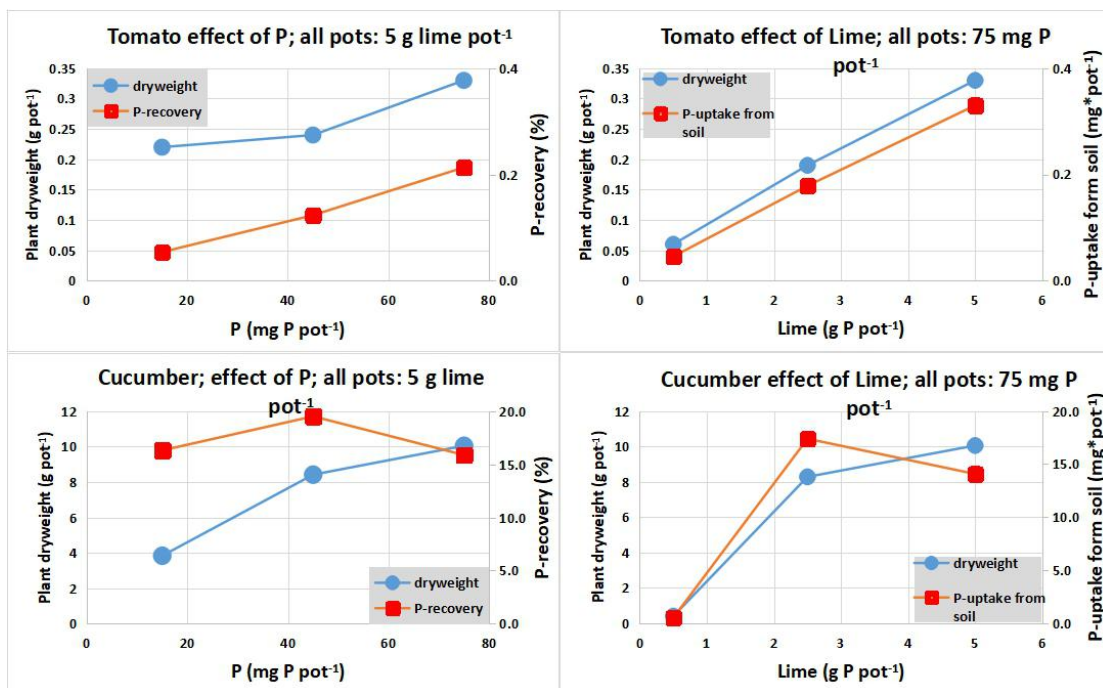

Figure 6. Measured plant biomass and P-uptake of tomatoes and cucumbers. Two left panels show the measured dryweight and calculated P-uptake with the same lime dose but different P-fertilizer dose. Two right panels show dryweight and P-uptake with same P-fertilizer dose but different lime dose.

Soil pH measured after the plants were harvested was clearly affected by plant growth,

both at the low and the intermediate level of lime: in the cucumber plots, the pH was much higher than in the tomato pots (Figure 5). The tentative explanation would be that cucumber plants took more of the phosphate from soil than the tomato plants (the uptake of PO_4^{3-} alkalinize the rhizosphere, Marschner et al, 1986).

The results (Figure 6) clearly demonstrated that tomato plants failed to grow at low pH, and even at the highest pH level, the tomato plants produce marginal biomass compared to cucumber. Cucumber was therefore chosen as a test plant for further experiments.

The lower right figure shows cucumber plants respond positively to increasing P at 2.5 g*pot^{-1} lime does (pH 5.9), and grows reasonably well at low pH. In consideration of the pH values used here are the endpoint pH, which was much higher than the initial pH at the intermediate lime dose, the results suggest that cucumber is more suitable for this experiment than tomatoes.

Soil pH changing trends experiment

More plant experiments were planned than could be realized within the present study, and these were intended to be run with improved control of the soil pH. As a preliminary work for these experiment, a liming experiment with higher resolution than the foregoing was conducted, to enable a more precise control of soil pH. Seven pots were prepared with 30 mg P pot^{-1} in all, and with increasing amounts of (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0g lime pot^{-1}). In addition, one pot was filled with soil without lime or P. After lime and P-fertilizer were added, on the second day the first sample was taken from every pot to measure the soil pH. Then, on the 3rd, 7th, 14th, 28th and 42nd day, taking samples from every pot to measure the soil pH. Results are shown in Table 2.

Table 2. Soil pH changing in different lime dose after certain days. For every pot, 3.355 kg soil was filled in. These pots were without plants.

Pot No.	Lime g*pot^-1	Lime g*kg^-1	P added mg*pot^-1	Days after liming					
				1	3	7	14	28	42
				Soil pH					
1	0	0	0	4.65	4.73	4.91	4.93	5.01	4.94
2	0	0	30	4.67	4.78	4.97	4.89	4.98	4.97
3	0.5	0.15	30	4.87	5.01	5.17	5.25	5.28	5.26
4	1.0	0.30	30	5.06	5.41	5.67	5.50	5.48	5.46
5	1.5	0.45	30	5.57	5.74	5.77	5.68	5.67	5.64
6	2.0	0.60	30	5.78	5.76	5.91	5.97	5.96	5.82
7	2.5	0.75	30	6.18	6.19	6.12	6.16	6.11	6.05
8	3.0	0.89	30	6.37	6.36	6.22	6.23	6.24	6.10
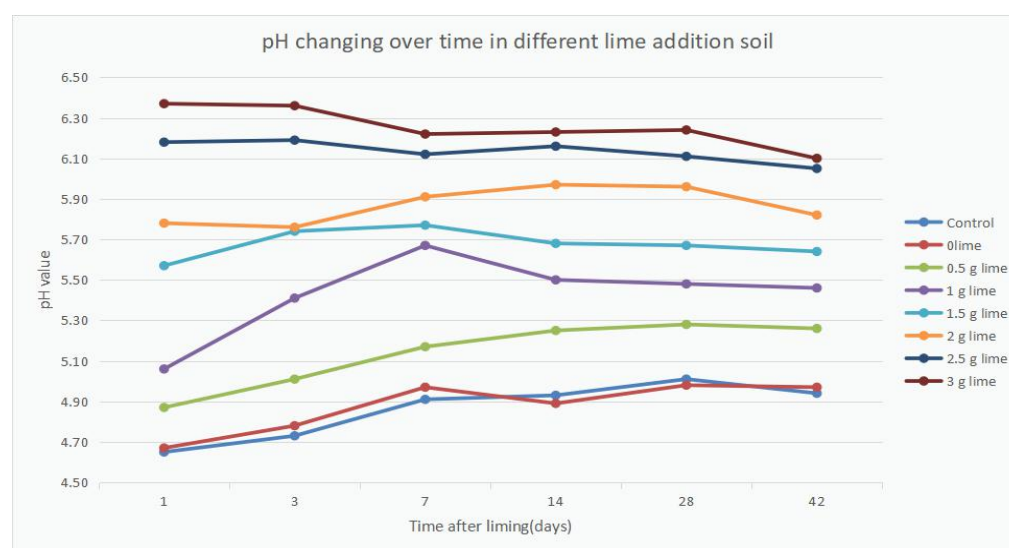
Figure 7. Measured pH in soils as affected by liming. Different doses of lime was incorporated into the pots.

Figure 7 shows that the changing trends of initial soil (No.1 pot, 0 g lime and 0 g P per pot) and soil only added P (No.2 pot, 0 g lime and 30 mg P per pot) are almost the

same. Which means whether P was added or not, little or even no influence on pH changing would appear in this type of sandy soil. As the lime dose was increasing, soil pH was also increasing, and all the trends of pH changing were similar. The two upper lines which represented lime doses $2.5g*pot^{-1}$ and $3.0g*pot^{-1}$ (No.7 and No.8 pot) show that when the lime addition is more than $2g*pot^{-1}$, soil pH would higher than 6, which is proper for plant growth. Meanwhile, Figure 7 shows when lime addition is between 1.5 to $2.0g*pot^{-1}$(No.5 and No.6 pot), the differences of pH between beginning and the end were only around 0.1, and that is to say, the soil pH is stable.

"Buried disk" pot experiment

The success criteria for SBP are 1) the compartments retain the phosphate when in contact with soil and 2) that the P in the compartments remains easily extractable, i.e. that the diffusion of iron and aluminium oxides into the compartment is too slow to cause fixation. To test this, a "buried disk" experiment was conducted.

Sixteen pots with soil (3.355 kg per pot, as in the previous experiment) were prepared for for the "buried disk" experiment; eight pots without lime, and eight 2.5 g lime per pot (same lime treatment as in the previous experiment). Seven of the eight pots of each treatment were without P added to the soil, while two remaining received 30 mg P per pot. A SBP-disk was buried in each pot, and at intervals throughout a period of 42 days, disks were excavated and analyzed for total phosphate (TP) and ammonium lactate extractable P (P_{AL}). The results are illustrated in Figure 8. TP and P_{AL} both declined with time, but there was considerable experimental noise. The reason for the variable result could be that the disks contained different amounts of $Ca(H_2PO_4)_2$ initially due to insufficient solubility.
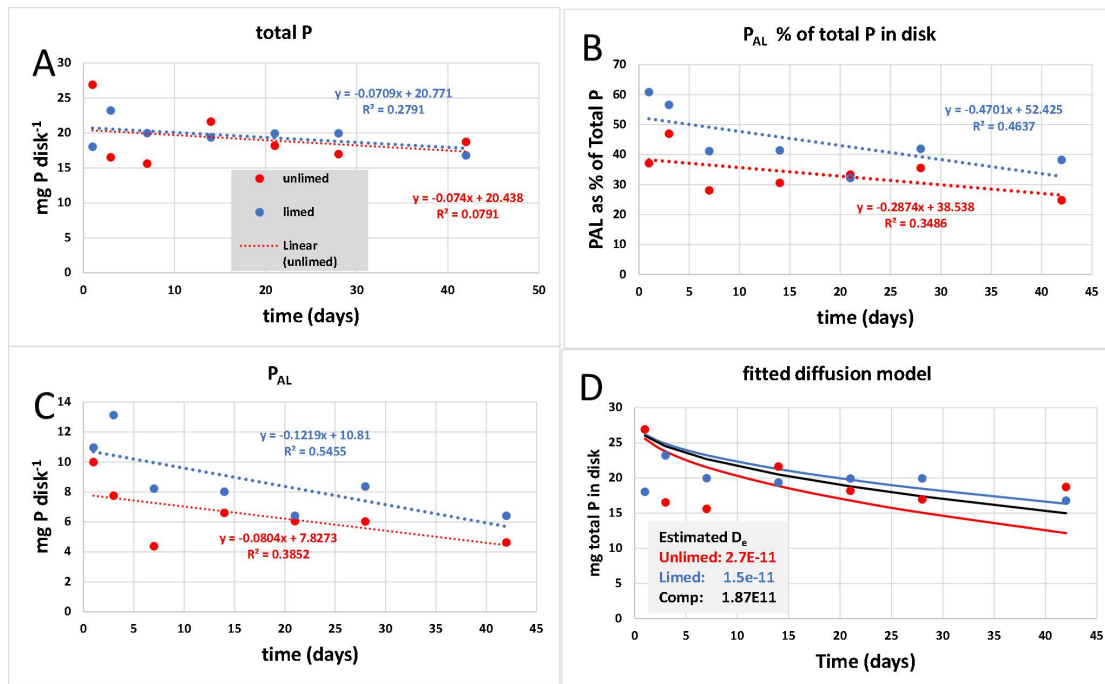
Figure 8. Measured P in disks buried in soil. Two left figure shows the measured total P (TP) and ammonium lactate extractable P (P$_{AL}$) throughout the experiment with SBP-disks buried in limed (blue color) and unlimed soil (red color), with linear regressions. The regressions coefficients were not significantly different from zero for TP, but for P$_{AL}$ (p= 0.025 for pooled data, i.e. both limed and unlimed)). The upper right figure shows the P$_{AL}$ as % of TP, also with linear regressions (same color code). As for P$_{AL}$, the regression coefficient was significant only for pooled data (p=0.05). The lower right figure (D) shows TP together with the diffusion function fitted to the measured data by least square, thus estimating the diffusion coefficient D$_e$ (m^{2}s^{-1}). This estimation was done separately for limed and unlimed treatment and for the entire dataset, and the results are shown in the panel ("Comp= estimate based on the entire dataset). The estimation is explained in the text below.

The experiments with disks in soil was designed to estimate the rate of phosphate loss by diffusion from a SBP (Soil Bypass P fertilizer) into the soil volume, and also to assess the rate of P-fixation by reactions with iron and aluminum oxides in SBP. This is illustrated in the figure 9.
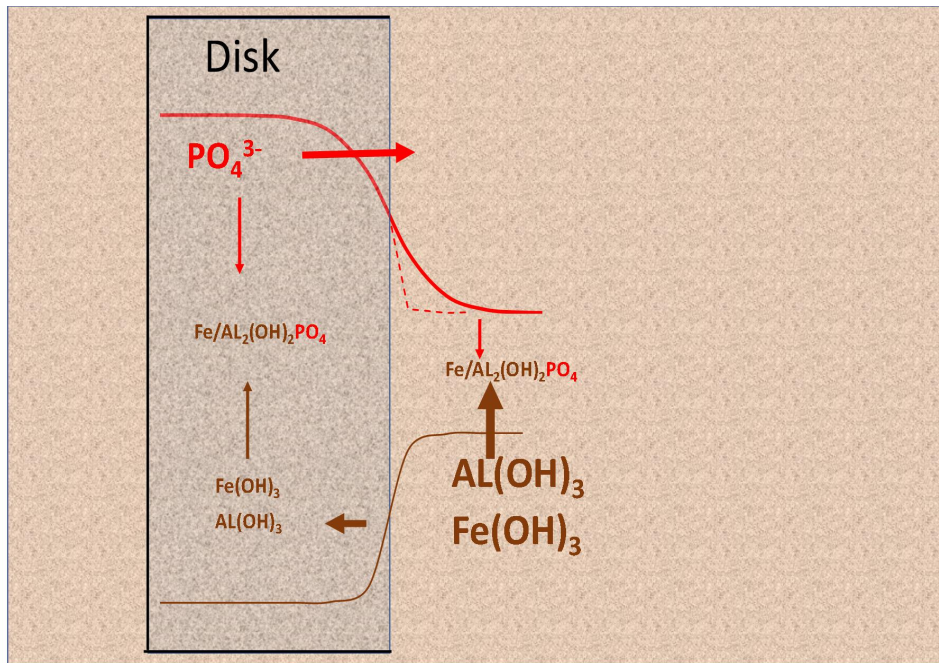
Figure 9. Concept figure for reactions and transport between disks and the surrounding soil. The figure Illustrates the phenomena that reduce the availability of phosphate in SBP, as tested by placing a 5 cm diam Petridish with SBP (=washed sand + Ca (H₂PO₄)₂ glued together by agar) in an acid soil with high concentration of Fe/Al- oxides. Through the to contact zone (only one side of the disk), phosphate ions will diffuse into the soil and become fixed by reactions with Fe/Al- oxides.   However, Fe/Al- oxides will also diffuse into SBP, and react with phosphate. The concentration gradients for PO_4^{3-} and Fe/Al- oxides after some time of contact (and diffusion) is illustrated, as predicted by diffusion alone. The dashed line for PO_4^{3-} is more realistic, since free PO_4^{3-} reacts with Fe/Al- oxides in the soil matrix.

To estimate the effective diffusion coefficient, D_e , we used equation (2), taken from Kaselowsky et al 1990.

$$D_e = \frac{M_t^2 \pi}{A^2 \Delta C^2 t} \qquad (2)$$

Where

- M_t is the amount of P transported from the disk to the soil (=loss of TP) in time t

- A is the cross section area of P diffusion (i.e. the cross area of the disk = 0.0021 m²)

- ΔC is the initial difference in P concentration between the disk and the soil (mg m^{-3}). For this, we used P_{AL}-concentration in the soil, rather than TP, because P_{AL} would be at least partially mobile, while most of the TP was assumed to be immobilized in the soil matrix. P_{AL} in the soil was 0.28 mg P kg^{-1} soil dry weight (standard error = 0.06, n=12) while TP was 376 mg P kg^{-1} soil dry weight (standard error = 9, n=12). AP-concentration of 0.28 mg P kg^{-1} is equivalent to 420 mg P m^{-3} soil bulk density=1.5 kg L^{-1}. In the discs the P concentration was 30 mg in a volume of 18 ml =$1.67*10^6$ mg P m^{-3}. Thus $\Delta C=1.67*10^6$ mg P m^{-3}.

The relationship between the net loss of P_{AL} and TP in the disks suggest some fixation of P: the net loss of total P was only 0.07 mg P disk^{-1} day^{-1} (regression, Figure 8), while the net loss of P_{AL} was 0.12 mg P disk^{-1} day^{-1}. The difference is not statistically significant, however (overlapping confidential intervals), while the regression of P_{AL}/TP was (p=0.05). This extra loss of P_{AL} could be due to sorption to or reaction with Fe/Al oxides on the surface of the sand, possibly enhanced by diffusion of Fe/Al oxides from the soil into the disk, which would then react with phosphate and precipitate on the soil particles. Given the lack of significance, the data provide no evidence for P-fixation within the disk, but the trend corroborate the hypothesis.

To estimate D_e based on the data for TP throughout, we solved equation (2) for M as a function of time t (equation 3), and estimated De by fitting the predicted M_t to measured M_t using the Generalized Reduced Gradient Solver in Excel.

$$M_t = \left[\frac{D_e A^2 \Delta C^2 t}{\pi} \right]^{05} \qquad (3)$$

The estimated D_e was ~20*10^{-12} m²s^{-1} (27*10^{-12} for unlimed soil, 15*10^{-12} for limed soil, and 19*10^{-12} for the whole dataset, Fig 8D), which is higher than that measured in soils, although there is considerable varitation:

- Kaselowsky et al (1990) found D_e for silt soil to be 0.625*10^{-12} m²s^{-1}.

- Bhadoria et al (1991a): Loess soil at different bulk densities and moisture content: D_e ranged from 0.01*10^{-12} m²s^{-1} (dry soil) to 5*10^{-12} m²s^{-1} (wet soil). The effect of bulk density on De was less consistent than that of moisture content.

- Bhadoria et al (1991b); silt soil, measured D_e increased with increasing phosphate concentration: increasing from 2.2*10^{-13} m²s^{-1} to 13.9*10^{-13} m²s^{-1} for the P-concentration range 380-580 mg P kg^{-1} soil. This probably reflects that the relative concentration of the most mobile fraction of P (free PO_4^{3-} relative to total P) increase with increasing dose of P. In our disks, the P concentration was 1000 mg kg^{-1} sand, which is approximately twice as much as the highest dose tested by Bhadiora et al (1991b).

One alternative approach to estimate D_e would be to disregard the results for the first 7 days, and estimate both initial P and De, using the Generalized Reduced Gradient Solver in Excel and the measured P from 14 to 42 days. The implicit assumption is that a fraction of P is lost relatively rapidly during the first 14 days, and that subsequent loss is much slower, either due to accumulation of P and/or a depletion of the sorption capacity in the soil at the surface of the disk. For this purpose, the data for limed and unlimed soil were pooled, and the result is shown in Figure 10:
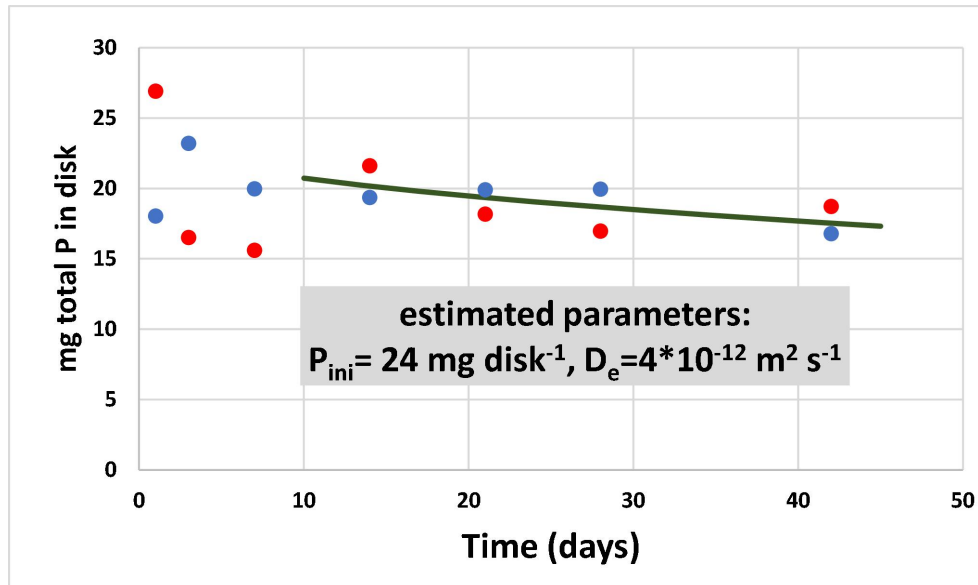
Figure 10. Estimation of diffusion coefficient (D_e) for the slow loss of P after 14 days. The pooled data (limed and un-limed soil) for the period 14-42 days were used to estimate both D_e and the initial P at time 0 (P_{ini}), using the Generalized Reduced Gradient Solver in Excel). The fitted model and the estimated parameters are shown in the panel. The estimated P_{ini}=24 implies that ~6 mg P was lost from the disks during the first 14 days by a relatively fast reaction, while the subsequent loss was very slow, i.e. with a D_e=$2*10^{-12}$ m^2s^{-1}.

P reactions within the disks

In the previous experiment, a decline in P_{AL} over time in the buried disks could be the result of P-fixation to iron and Al-oxides on the surface of the sand particles, in addition to the loss of P to the surrounding soil. However, it could also be ascribed to P-fixation in the disks themselves. To assess the kinetics of P-binding with the disks themselves, disks were prepared but not buried in soil, and monitored total P and P_{AL} over a period of 42 days. The result shows that immediately after making the disks, 55 % of the added P could not be extracted with Al, suggesting a fast reaction with the material initially, but no further loss of easily extractable P (= P_{AL}). This proves that reactions with the material in the disks could not account for the observed decline in P_{AL} in the buried disks (Figure 8).
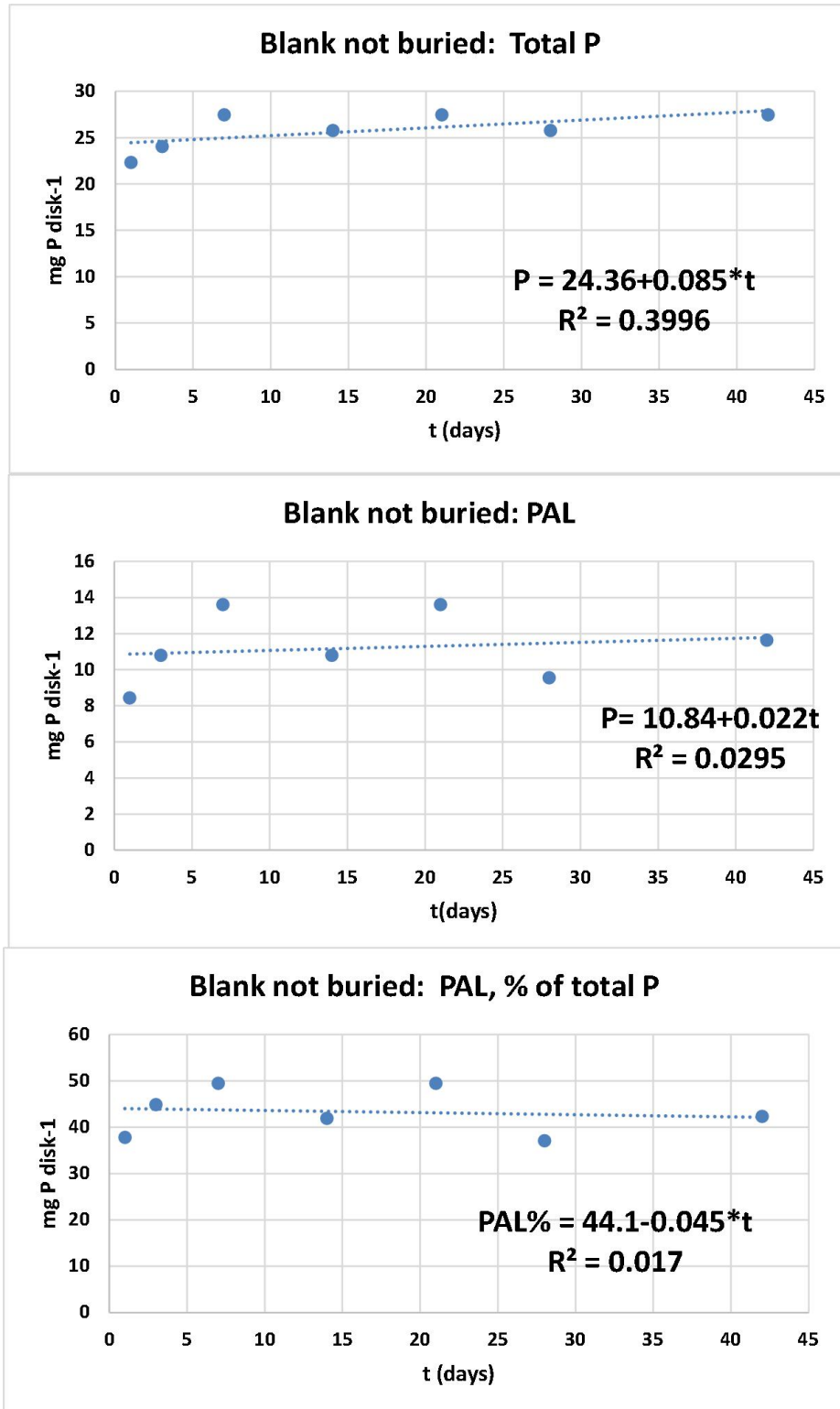
Figure 11 Phosporous in disks which were not buried in soil. The panels show measured total P (Top panel), P_{AL} (mid panel), and P_{AL} as % of total P, all plotted against time after preparation, and with linear regression functions. For all variables, the regression was not significant, i.e. the regression coefficient was not significantly different from 0 (see table 3). The results show that $P_{AL} \sim 45\%$ of total P from the very start, and with no significant decline with time.

Table 3 Regression coefficient estimates, confidence limits and significance for results shown in figure 11.

Regression coefficients and their 95% confidence limits				
	estimate	min	max	p-value
P_{AL}	0.022	-0.12	0.17	0.71
Ptotal	0.085	-0.035	0.2	0.13
PAL% of Ptot	-0.045	-0.43	0.34	0.77

Extrapolation to spheres.

The observed diffusion of phosphate from the discs to the soil allowed us to estimate the diffusion coefficient for P (from the disk to the soil) in one dimension. This can be extrapolated to spherical SBP, using the equations for heat loss from spherical bodies, developed by Carslaw and Jaeger (1986). This was calculated stepwise (in Excel) for period of 150 days:

$$m = \frac{r}{(D*t)^{0.5}} \tag{4}$$

$$C_n = e^{\left(-\frac{(n*m)^2}{\pi^{0.5}}\right)} - n*m*ERFC(n*m) \tag{5}$$

$$S = \sum_{n=1}^{6} C_n \tag{6}$$

$$U = \left(\frac{1-\sqrt{D*t}}{r}\right) * \left(\frac{6}{\sqrt{\pi}} + 12*S\right) + 3*D*t/r^2 \tag{7}$$

where D is the diffusion coefficient in cm^2 sec, r is the radius of the sphere (cm), t is the time (s), n is an integer (1-6), ERFC is Gauss error function, and U is the residual P in the sphere (fraction of the initial).

The diffusion coefficients estimated by the measured loss from the buried disks were expressed in m^2s^{-1}, which was converted to cm^2s^{-1} (10^{-12} m^2s^{-1} =10^{-8} cm^2s^{-1}).

U estimates the fraction of residual P in sphere, assuming unlimited capacity for P-fixation in the surrounding medium, which is probably not the case. Thus (1-U) estimates the maximum possible loss of P from the sphere. The exercise is useful for

approximating the maximum possible P loss from spherical SBP with different diameters, which is shown in Figures 12 and 13. Figure 12 shows the predicted loss, assuming the high diffusion coefficients estimated by the entire dataset, i.e De= $2*10^{-11}$ m^2s^{-1} = $2*10^{-7}$ cm^2s^{-1} (Figure 8). Figure 13 shows the predicted loss if assuming the 10 times lower diffusion coefficient estimated by the slow decline in disk-P during the period 14-42 days, i.e. De = $2*10^{-12}$ m^2s^{-1} = $2*10^{-8}$ cm^2s^{-1} (Figure 10).

The calculated losses from spheres probably represent maximum estimates for at least two reasons. One is that the regression coefficient for total P against time (linear regression, Figure 1) was in fact not statistically significant, and the true transport coefficient could have been much low than that estimated. Secondly, the P transport rate coefficient appeared to decline with time in the buried disk experiment (see Figure 10), which is indeed plausible: ~50% of the P in the disks could not be extracted with ammonium lactate (Figures 8 and 11), which implies that 50% of the P was effectively immobilized in the disc matrix. Since the mobile P in the disk would diffuse faster than the immobile fraction, a gradual depletion of mobile P in the contact zone would take place. The calculated transport coefficients were probably higher than the true transport coefficient for the immobile fraction of P in the disks ($P_{total} - P_{AL}$), but lower than the transport coefficient for the most mobile fraction of the P in the disks.

One should also keep in mind that the calculation of the loss from spheres is based on the assumption of an unlimited capacity of the soil to fix P (the calculation assumes that the concentration of mobile P at the surface of the spheres is close to 0). This would only hold for a limited amount of time, and the gradual increase of mobile P in the soil near the surface of the spheres would gradually retard the rate of loss.

Nevertheless, the exercise is useful in showing that the radius of SBP is critical. The loss decrease with increasing radius, but not linearly: much is achieved by increasing the radius from 0.3 to 2 cm.
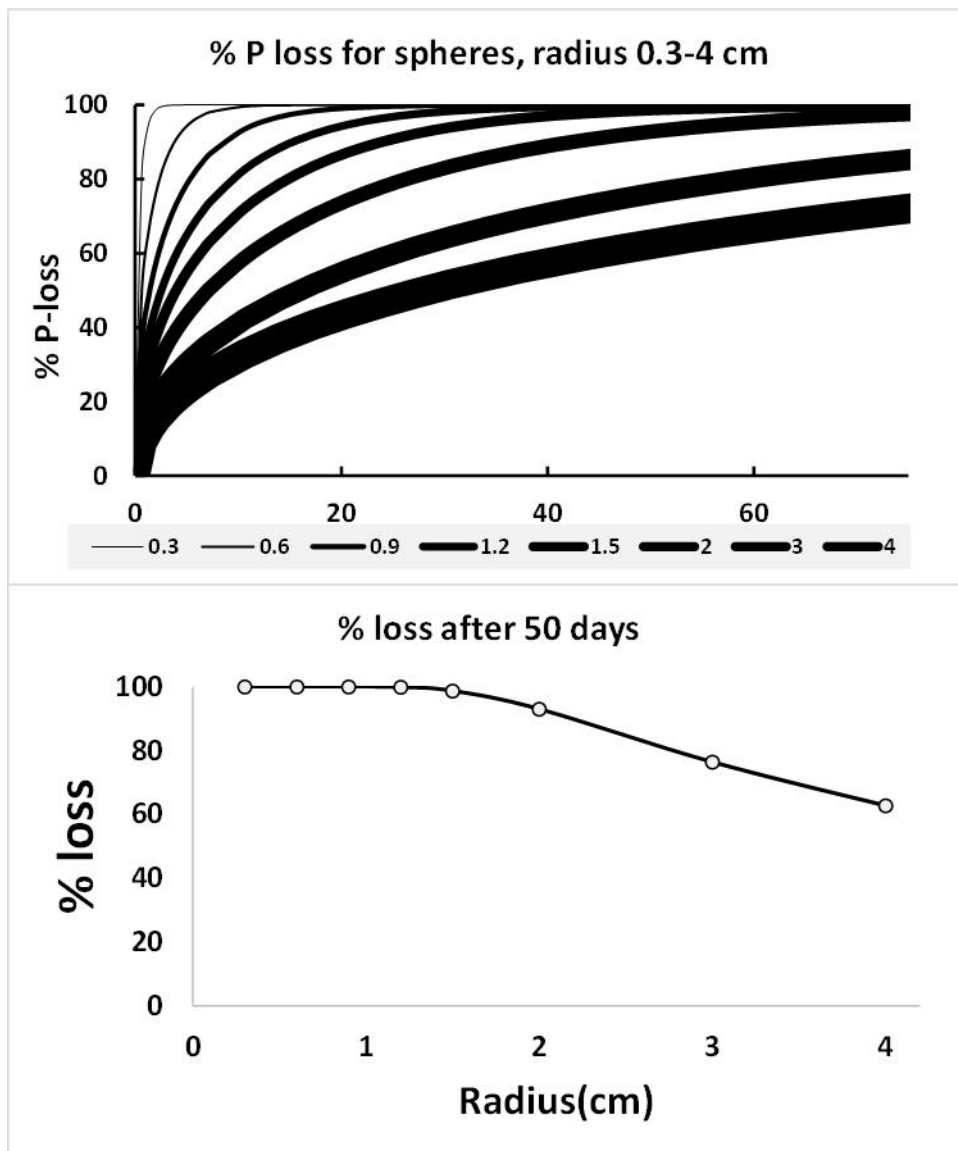
Figure 12. Estimated maximum P loss from spherical SBP for De=2*10⁻¹¹ m²s⁻¹. The panel shows the estimated loss of P from spheres of radius 0.3-4 cm, using the high diffusion coefficient determined by the buried disk experiment where all data were included, i.1. De= 19*10⁻¹² m²s⁻¹ (Fig 8), and equations 4-7. The top panel shows loss plotted against time for different diameters, bottom panel shows the % loss after 50 days for different radius.
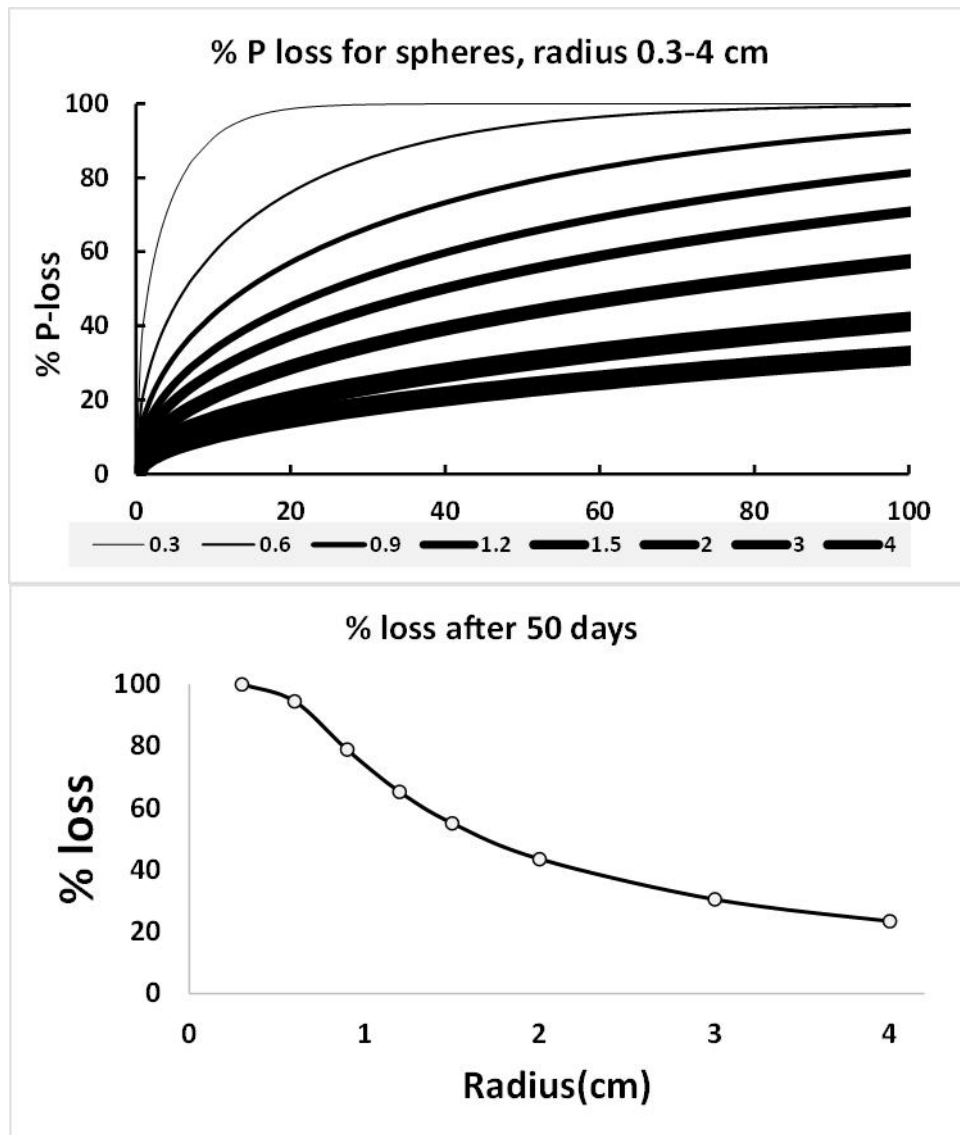
Figure 13. Estimated maximum P loss from spherical SBP for De=2*10^{-12} m^{2}s^{-1}. The panel shows the estimated loss of P from spheres of radius 0.3-4 cm, using the high diffusion coefficient determined by the buried disk experiment including only the data for the period 14-42 days (Figure 10), i.e. De= 2*10^{-12} m^{2}s^{-1}, and equations 4-7. The top panel shows loss plotted against time for different diameters, bottom panel shows the % loss after 50 days for different radius.

Final discussion and conclusions

The aim of this thesis was to explore the possibility of producing a P- fertilizer which remains plant available for a long time in acid soil, so as to secure effective utilization of the fertilizer P by crops.

To provide some proof of concept, a plant bioassay system was needed; i.e. a pot experiment in which the crop plant is P-limited by growing in a soil with low concentrations of P and not the least, with a strong capacity to bind P by reactions with Al and Fe oxides. For this purpose, tomato and cucumber were tried out as to their ability to grow in an acid forest soil, and whether the experiment could be designed so as to secure P-limited growth. The task proved far from trivial: tomato plants grew very poorly in the soil at any pH level tested, while cucumber showed a minimum lime dose of 2.5 g lime per pot (Figure 6), resulting in soil pH increasing from 4.7 to 6 (Table 2, figure 5). This (cucumber and with 2.5 g lime per pot) is an operational window, although it would be desirable to lower the dose of lime to secure strong P-binding in the soil. Unfortunately, time did not allow this, beyond testing the soil pH in response to a range of lime doses from 0 to 2.5 g per pot (Table 2).

The criteria for model SBP matrix were

A) a matrix with air-filled macropores when moist (in soil), to secure aerobic conditions
B) a matrix allowing penetration by roots
C) a matrix with minimal loss of P by diffusion to the surrounding soil, and minimal diffusion of Al and Fe-oxides from the soil into the matrix

To fulfill criteria A and B, the SBP-matix was made of relatively coarse sand, glued together with agar which left open pores in the matrix after drying and rewetting because the agar swells to only 30 % of its original volume after rewetting. Since the agar is rather soft glue, roots would be able to penetrate and expand within the matrix. For obvious reasons, agar will not be the glue in a future design of SBP, and neither

will sand: agar is too expensive and sand is too heavy. The ideal material for SBP would rather be a fibrous material that could be compacted, and which swells upon rewetting. Criterium C needed experimental testing, however, and this was the task of the final experiment. In principle, SBP should be spherical because spheres have the smallest specific surface area compared to other shapes, and the chance that Fe and AL in the soil binding to phosphorus in SBP would be reduced. However, the experiments for testing criterium C (loss of P to the soil) were done with disks (SBP-disks), rather than spheres, for simplifying the determination of the effective diffusion coefficient.

The experiments with SBP-discs enabled an assessment of the effective diffusion of P (D_e) from the disks to the surrounding soil, albeit with considerable uncertainty. Given this uncertainty (the D_e-estimates ranged from $2*10^{-12}$ to $2*10^{-11}$ m^2s^{-1}), the estimated loss of P from spherical SBP covered a wide range. The exercise also allowed an exploration of the effect of the size of the SBP-spheres, which is crucial. For SBP-particles smaller than 1 cm in diameter the loss of P was substantial.

The calculated P-loss from spheres suggest that more efforts should be made to find a less soluble form of P than used in the present experiments, in order to secure marginal loss of P within the time frame of a cropping season, even at moderately sized SBP-spheres.

Any practical implementation would need to be SBP with matrix with less weight than sand, low price (agar is very expensive), and preferentially with a fibrous material that would allow compression and reswelling upon moisture absorption (when in the soil).

Despite the shortcomings of the model SBP produced for these investigations, it should be good enough to provide proof of concept by experiments with P-limited cucumber plants, measuring their growth (and P-uptake) in response traditional P fertilization with the fertilization with SBP as spheres >2 cm in diameter.

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