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The effect of soil amendments on phosphorus mobilization in P saturated soils of Flanders, Belgium

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List of Abbreviations

Summary

Flanders (Belgium) is facing huge water quality issues related to nitrates and phosphates in surface waters caused mainly by agriculture. Because of this, Flemish government has been restricting the use of P Manure by Manure Action Programs since 1996. In relation to phosphorus, the focus in these programs is on P mining by reducing P fertilizer use, because soil P stocks in Flemish soils are huge. As the major part of these P stocks are not (immediately) plant available two possible problems might arise. First of all, P sensitive crops might be dealing with reduced yield and/or delayed harvest. Secondly, P mining might be reduced after a couple of years.

In order to solve these problems, a three-step research strategy was used to select soil amendments (Organic acids, sulphur, Silicates and Phosphates solubilizing bacteria) which could improve the plant availability of P in soils, which have a low or moderate P intensity (0 mg P/kg dry soil $\langle P \cdot \text{CaCl}_2 \rangle$ = 1.5 mg P/kg dry soil) and rather high P quantity (P-AL $>$ 30 mg P/100 g dry soil). Soil sampling on two sites, experimental plot E13.0130 and van Oeckel was done.

Shaking experiment is the first step of this experiment. The first step included the selection of the best amendments and their concentration. This was done by shaking 4 g of soil in 40 ml of 0.5, 1 and 2 mM of organic acids (lactic acid, oxalic acid, citric acid), sodium silicate and sodium sulphate. Out of these tests it could be concluded that 2mM citric acid, 2 mM sodium silicate performed better. Within the organic acids, citric acid produced highest amount of P, 39.05 mg/kg dry soil in E13.0130 and 39.25 mg/dry soil in van Oeckel. Within silicate 2 mM sodium silicate produced 28.62 mg/dry soil and 33.23 mg/dry soil in E13.0130 and Van oeckel respectively. There is no much effect of concentration on P extraction for lactic acid and Na2SO4. From shaking experiment organic acid have negative correlation with pH whereas sodium silicate has significant positive correlation.

In a second step, soils were amended with one of following amendments: citric acid (10 mM/kg dry soil), elemental Sulphur (20 mM/kg dry soil), sodium silicate (10 mM/kg dry soil) and PSB $(126 \times 10^{11} \text{ CFU/kg} \text{ dry soil})$ and incubated in small PE cups at a density of 1.4 kg/dm³ and 50% WFPS at 20° C. After 3, 6, 12, 24 and 48 days P-CaCl₂ and pH-H₂O were determined. From incubation experiment, PSB gave the highest amount of P-CaCl₂ in day 12 ranging from 2.78 to 2.91 mg/kg dry soil for site E13.0130 and 2.99 to 3.06 mg/kg dry for van Oeckel. Similarly, $Na₂SiO₃$ has extracted more P-CaCl₂ in day 24 ranging from 1.87 dry soil to 1.91 mg/kg dry soil in site E13.013 and 1.85 dry soil to 1.96 mg/kg dry soil in site van Oeckel. Negative correlation for P-CaCl² extraction and pH was found for PSB, sulphur and citric acid while for sodium silicate negative correlation was found.

In a final experiment grass was grown in soil supplemented with one of the amendments: citric acid (10mM/kg dry soil), elemental Sulphur (10 mM/kg dry soil), sodium silicate (10 mM/dry soil) and PSB (126 x 10^{11} CFU/kg dry soil). P-CaCl₂, pH-H₂O, dry matter yield and P-uptake by grass were determined 20, 40 and 60 days after germination. On harvested pots P-CaCl₂ decreased with time as plants were up taking them but still with PSB and sodium silicate P- $CaCl₂$ remained higher. Effect of amendments on P-CaCl₂ is higher in all pots throughout the experimental period. PSB produces highest amount of total dry weight of grass 5.98 g in site E13.0130 and 8.32 g in site Van Oeckel. After PSB, sodium silicate produced 4.04 g at site E13.130 and 3.74 g at site Van Oeckel. Similarly, total P-uptake by plants was highest for PSB on both sites followed by sodium silicate. On day 60 total P-uptake for grass grown on PSB is 12.33 mg in site E13.0130 and 22.17 mg in site Van Oeckel. Similarly, total P-uptake for sodium silicate in site E13.0130 is 9.12 mg and in site Van Oeckel is 11.0 mg. So, the three step experiment shows PSB and sodium silicate are best performing. There might be some fertilizer effect on inoculation of PSB but still it's effect is higher. In conclusion, PSB and sodium silicate are found to be effective amendments to increase P availability in acidic sandy soil with a high P status but further pot trials are needed.

Chapter 1 Introduction

Phosphorus (P) is an essential nutrient of life and plays an important role for plants as a building block for cell walls and in the energy management. When the amount of phosphorus available for plants is sub-optimal, this can lead to yield losses in crops ranging from 10% to 15% (Shenoy and Kalagudi, 2005). Therefore, the use of P fertilizers has become essential for agriculture.

The problem arising with this practice is that not all P supplied will stay available for plants. In soils only 0.1 to 1.0 % of total soil P is soluble and plant available (Pierzynski et al., 2005a). This unavailability is due to phosphate fixation which restrains P availability temporarily or permanently in the soil which depends upon chemical fixation reactions. In P-fixation, P is either adsorbed on the soil minerals or it gets precipitated by free Al^{+3} and Fe^{3+} in the soil solution (Havlin et al., 2005).

As a consequence, farmers have been applying P fertilizer at levels much higher than the amount of P removed by the crops. This has led to the accumulation of P in soils. The environmental threshold of P concentration is far less than the agricultural crop demand. According to Pierzynski et al. (2005a), the optimum concentration of P in soil solution for plant growth is >0.2 mgL⁻¹ whereas the threshold concentration of P in surface water to prevent eutrophication should be < 0.03 mgL⁻¹. Due to erosion, run-off and drainage, short-circuiting the buffering capacity of the soil large amounts of P are lost to the environment, causing eutrophication. In Flanders, about 29.4% of agricultural land is at risk of P leaching (VLM, 1997). The estimated share of agriculture in the phosphorus load of surface water in Flanders is 44% (Van Steertegem, 2012). Therefore, agriculture is an important factor in surface water pollution in this region.

To tackle these environmental problems, European Union has formulated the Nitrates Directive and the Water Framework Directive (Amery and Vandecasteele, 2013). The Flemish government has been taking action with the development of Manure Action Programs since 1996 (VLM, 2007). But, even after several years of P nutrient mitigation not enough improvement in water quality has been observed (Jarvie et al., 2013). Legislation measures are restricting farmers more and more on the use of P fertilizers. The maximum P application

standard for Flanders is approximately 5 kg P_2O_5 ha⁻¹y⁻¹ smaller than the amount of P exported by the crop. This to obtain P mining (Amery and Schoumans, 2014).

Further the natural abundance of P is limited. According to (Cooper et al., 2011), 70% of global production of rock phosphate (RP) is currently produced from reserves which will be depleted within 100 years and by 2070 there will be already severe food production deficit because of shortage of P. Therefore, there is an increasing uncertainty about the future of RP supplies in order to ensure the food security (Cooper et al., 2011).

As farmers can use less P fertilizer, due to limited resources and legislation two possible problems might arise in time. First of all, P sensitive crops might be dealing with reduced yield and/or delayed harvest. Secondly, P mining might be reduced after a couple of years. To cope with both of these problems, , it is important to increase the amount of plant available P in soils .. Several studies suggest that the application of soil amendments like organic acids (Gardner and Boundy, 1983; Gang et al., 2012; Strobel, 2001) silicates (Roy, 1969; Smyth & Sanchez, 1980; Carvalho et al., 2000; Sandim et al., 2014), sulphates (Krol et al., 1986; Jaggi et al., 2005; Skwierawska and Zawartka, 2009), and PSB (Illmer and Schinner, 1992; (De Bolle et al., 2013)) can increase the amount of plant available P. In this study we've undertaken a three-step research strategy to select which soil amendments (Organic acids, Sulphates, Silicates and Phosphates solubilizing bacteria) could improve the plant availability of P in soils with low P intensity and high P quantity. So, in particular, this study aims to:

- Select the most promising amendments and concentrations to increase plant available phosphorus (P intensity) in shaking experiments
- Test whether the selected amendments could increase $P-CaCl_2$ when incubated for different time intervals
- Test whether the use of amendments really increased crop yield and P uptake
- Determine which mode of action is responsible for the effect of the amendment

Chapter 2 Literature Review

2.1 Phosphorous

Phosphorus (P) is a crucial nutrient as it is a structural element in nucleic acids (DNA and RNA) and it serves a critical role in cellular regulation, carbon partitioning and energy transfer (ATP). Also P is essential for seed formation, strength of shoots, development of roots and maturation of crops (Stevenson and Cole, 1999). Deficiency of plant available P can cause stunted growth and delayed maturity, which is visually seen as yellowing of the lower leaves in plants. Up to now P deficiency still limits crop yields on more than 40% of the world's arable land (Sylvain and Thomas, 2013).

2.2 Soil phosphorus cycle

The P cycle is given in Figure 1. Unlike nitrogen, there is no atmospheric source of P. The major inputs of the P cycle are fertilizers (inorganic or organic), agricultural wastes, plant residues, and municipal or industrial by-products (Pierzynski et al., 2005a). Outputs are P uptake and losses due to erosion, runoff and leaching which flows to surface water. Soil P is divided into three major forms which are in dynamic equilibrium. In the center of the Figure 1 we can see the P in soil solution which is immediately available for plants. This form of P $(H_2PO_4$, HPO_4^2 can also be defined as P intensity. Sorbed P, secondary P minerals and primary minerals are the occluded forms of P which can be converted to soluble form by dissolution process and are accumulated in insoluble form by sorption process. The occluded form is represented by inorganic P compounds and are resistant to microbial process like mineralization (De Schrijver et al., 2012). Organic P is the another form of P which is dominated by soil biomass, soil organic matter, soluble organic P and decaying plant residues. Organic P is converted to soil solution P by mineralization. Soil solution P can be uptake by plants or loosed by leaching or flow to surface water.

The P concentrations in soil solution is relatively low and varies in the range of $\langle 1 \text{ kgha}^{-1} \rangle$, or <1% of the total quantity of P in the soil (Pierzynski, 1991). Since, P concentration in soil solution is low it should be maintained optimal for optimum plant growth. Because of this P in the soil solution must be replenished several times during the life cycle of a plant to meet its P demand. Hence, effective P management is very important which involves management of the chemical and biological process like *dissolution-precipitation*, *mineralization-immobilization*,

sorption-desorption, and *oxidation- reduction* as shown in fig. 1 (Pierzynski et al., 2005a). Soil P content varies with parent material, texture, and management factors, such as rate and type of P applied and soil cultivation (Sharpley, 1995). In soil, P can be divided into two broad classes as organic P and inorganic P (Pierzynski et al., 2005a). In most soils 50 to 75% of the P is inorganic, although this fraction can vary from 10 to 90% (Sharpley, 1995).

Figure 1 Phosphorous cycle in soil. Source : (Pierzynski et al., 2005a)

2.2.1 Inorganic soil phosphorous

Inorganic P is produced by weathering of different minerals (Pierzynski et al., 2005a) and can be present as hydrous sesquioxides, amorphous, and crystalline Al and Fe compounds in acidic, non-calcareous soils and as Ca compounds in alkaline, calcareous soils (Sharpley, 1995). In unweathered or moderately weathered soils, apatites (Ca phosphates) are the dominant minerals. P is plant available as primary ($PO₄³$) or secondary ($HPO₄²$, $H₂PO⁴$) orthophosphates in the soil solution when it gets dissolved or desorbed from soil minerals or colloids.

The amount of plant available P depends on the soil pH and the activities of Al^{3+} , Fe^{3+} and Ca^{2+} . As shown in fig. (2), at low pH, P is almost completely insoluble since it is fixed to Al^{3+} and Fe^{3+} and at high pH P is strongly held by Ca^{2+} (Tisdale et al., 1985). Similarly, the P is generally highly available at a pH of 6 to 7 (fig.5). Normally, the majority of the orthophosphate is present either as HPO₄⁻² at pH > 7.2 or as H₂PO₄⁻ at pH between 4.0 and 7.2. At the higher pH values soluble complexes like CaHPO⁴ or CaPO4 are present abundantly. But, in most cases these soluble complexes are rapidly converted into orthophosphate by chemical dissociation.

Figure 2 Changes in the form of soil P as affected by the pH for soluble P (alpha = mole fraction of the total P). Source: (Pierzynski et al., 2005a)

2.2.2 Organic soil phosphorus

Organic P forms include relatively labile phospholipids, inositols and fulvic acids, while more resistant forms are comprised of humic acids (Sharpley, 1995). Organic P varies between 3% and 90% of soil P (Pierzynski et al., 2005a), depending upon the nature and management of the soil. High proportions of organic P can be found in soils where significant quantities of organic P are continuously added to soil in plant, animal and microbial detritus (Condron et al., 2005). Microbial decomposition of organic P results in the release of soluble organic P that, with time, is converted into an inorganic form of P (Pierzynski et al., 2005a). Organic P transformations in soil are important in determining the overall biological availability of P, which in turn influences the ecosystem productivity (Condron et al., 2005). Based on the nature of the P bond,

soil organic P is classified into phosphate esters, phosphonates and phosphoric acid anhydrides (Turner et al., 2005). Normally, ortho-P associated with humic compounds through metal bridges is not classified as organic P. The amount, forms and dynamics of organic P in the soil are determined by a combination of biological, chemical and physical conditions (e.g. pH, temperature and soil moisture), together with the history and the intensity of land use and associated levels of P input and removal from the system (Condron et al., 2005; Pierzynski et al., 2005a).

2.3 Major Reactions of Phosphorus in Soils

Figure 3 Scheme of the major forms of P in the soil. Source: (Minor and Stecker, 1993).

P is absorbed by plants, in the orthophosphate (H_2PO^{4-}) and HPO_4^{2-} forms (Pierzynski et al., 2005a). As concentrations of H_2PO_4 ⁻ and HPO_4^{2-} in soils are low, it's important to understand and control the major reactions of P in soils. As shown in fig.2, Soil P chemistry is complicated (Figure 2). Generally, P is added to soil, in the form of soluble fertilizers, but is relatively quickly transformed in an insoluble P form, a process sometimes termed P retrogradation. This insoluble form can again be converted into soluble form. The main processes controlling soil P bioavailability are P sorption and desorption, immobilization and mineralization, dissolution

and precipitation. However, other factors, such as pH , texture, fertilization, $CaCO₃$ and organic carbon may also influence these processes (Bastounopoulou et al., 2011).

2.3.1 P sorption and desorption

P sorption is the capacity of soil to sorb P. Due to the high sorption capacity only a small percentage of P is bioavailable in soil. Adsorption of orthophosphates (PO_4-P) on the surface of Fe (III) and aluminum (hydr)oxides is an important governing process of sorption and desorption (Gustafsson et al., 2012). Orthophosphates can adsorb to the surfaces and edges of hydrous oxides, clay minerals and carbonates by replacing H₂O or OH⁻ (Pierzynski et al., 2005a). Adsorption processes are expected to play a more important role at low pH, because PO4–P adsorption increases with decreasing pH (Goldberg and Sposito, 1984).

Desorption of P mostly occurs through ligand exchange reactions, which means that a decrease in the concentration of P ions in the soil solution, through e.g. plant uptake, and an increase in the concentration of competing anions will both shift the adsorption–desorption equilibrium towards enhanced desorption (Pierzynski et al., 2005a). Desorption signifies the release of P from the solid phase into the solution phase (Stevenson and Cole, 1999).

2.3.2 Immobilization and mineralization

Immobilization is the process of temporarily "tying up" of water soluble P by soil microorganisms which are decomposing plant residues. Immobilized P will be plant unavailable for a while, but will eventually become available again as decomposition proceeds. P mineralization is very important to maintain soil fertility. Nutrients contained in soil organic matter (e.g., phosphorus, nitrogen, and sulfur) are converted to inorganic forms that are available to crops by the process of mineralization. Microorganisms can mineralize organic P inside and outside their cells (Spohn and Kuzyakov, 2013).

Mineralization and immobilization of phosphorus occur simultaneously in the soil. The C:P ratio determines whether there is net mineralization or net immobilization. If C:P ratio is less than 200:1 there will be net mineralization (White, 2013). Net mineralization indicates that there is enough phosphorus in the soil to sustain both plants and microorganisms. When the

C:P ration is between 200:1 and 300:1, immobilization and mineralization rates almost equal (White, 2013). If C:P ratio is greater than 300:1, net immobilization occurs (White, 2013).

2.3.3 Dissolution and Precipitation of P

Figure 4 Soil pH and the solubility of some phosphate minerals. Source: (Lindsay, 1979)

Precipitation is the reverse process of dissolution that leads to the formation of insoluble compounds in soils which usually exceeds its solubility products (Pierzynski et al., 2005b). The availability of P is usually affected by precipitation reactions which is dependent on soil pH. At low pH, P in soil reacts with Fe and Al and form minerals such as strengite and varescite (Figure 4) and make it unavailable for plant uptake. Similarly, at high pH (>8), the precipitation of phosphates reacts with Ca forming a number of Ca-P minerals such as amorphous Ca phosphate, octa-Ca phosphate and apatite (hydroxyapatite) (Gustafsson et al., 2012). Normally, at the pH between 6.0 to 6.5 solubility of P is highest and is available for plants which can also be loosed by surface runoff, percolation or leaching (Pierzynski et al., 2005b) (Figure 4).

2.4 Measurement of plant available P

Fertilizer recommendation is done to supply essential amount of P required by plants. Fertilizer recommendation is a function of soil P status which is done with chemical extractions. Amount of P in soil is often determined by chemical different chemical extraction methods. A wide range of soil tests have been developed to determine the availability of phosphorus (P) in soils, based on the theoretical concepts of P intensity and P quantity (Van Raij, 1998). As only one test is used for the P estimation often P intensity of P quantity is determined. There are different soil test methods for P extraction, giving in insight in different pools of P present in soil: P total, P-oxalate, P-Al, P-CaL, P-Olsen, Pw and P-CaCl₂ (Van Rotterdam-Los et al., 2013). Though there are different P-test only one test is used for general fertilizer recommendation. According to Van Rotterdam-Los et al. (2013), in the Netherlands two soil tests, one for directly plant available P (P-intensity) and another one for P stock (P-quantity) were recommended for farmers since 1930 but they preferred to use only one test because of cost. For Dutch situation P-AL(ammonium lactate, Egner et al., 1960) and 0.01 P-CaCl2 (Huba et al., 2000) were used to approximate P-quantity and P-intensity respectively (Van Rotterdam-Los et al., 2013). Implementing new concept like the intensity, buffering capacity and quantity concept in fertilizer recommendation can provide more insight into the soil P status and its relationship with crop response to fertilizer (Van Rotterdam-Los et al., 2013).

P-intensity and P-quantity

The P intensity factor is considered to be the activity of orthophosphate in the soil solution, which is immediately available to plant roots (Pypers et al., 2006). But, generally amount of P available in soil solution is lower than crop demand. Therefore, deficient P is replenished form P adsorbed to soil particles. The capacity of soil to resist the change in the concentration of soil available form P is known as buffering capacity (Van Rotterdam-Los et al., 2013). P-Al and P-CaCl² ratio is used to predict the buffering capacity.

Figure 5 gives the visual representation of the soil quantity, buffering capacity, and intensity concept of P. The arrows indicate the buffering and binding process of P that is dependent on Fe, Al and Ca this can be estimated by P-Al/P-CaCl₂ ratio.

Figure 5 Visual representation of the concept of P-intensity (P-CaCl2) and P-capacity (P-Al). Here, P-CaCl2# is P extracted using 0.01 M 1:10 CaCl² in dried soils and P-CaCl2^ is P extracted using 0.01 M 1:10 CaCl² in non-dried soils. Source: (Van Rotterdam-Los et al., 2013)

The soil P that is replenished when soluble P gets absorbed is called P-quantity that can be measured by P-Al. As shown in the figure plant available P can be derived either from Pquantity or direct available nutrients. Total soil stock represents the P that is accumulated in soil in immobilized form like with Fe, Al or Ca.

2.5 Phosphate saturation and losses to the environment

The Phosphate saturation degree (PSD) is a measure of potential P losses by leaching from acidic soils. PSD is a function of the portion of the soil exchangeable sites that are bound/saturated with P (P sorbed) in relation to the number of sites available for P binding capacity (PBC) (Hooda et al., 2000).

The P binding capacity (PBC) of the soil measures the maximum ability to adsorb/precipitate P (Van der Zee, 1990). It is the amount of P that can be bound by soil mainly with Fe and Al.

$PBC = (Fe_{ox} + Al_{ox}) * \alpha$

Al_{OX} and Fe_{OX} are oxalate-extractable Al and Fe and α is an affinity factor. The scaling factor α depends on soil type and experimental conditions (Renneson et al., 2010). The α value of 0.50 has been used for acidic Dutch soil (Van der Zee, 1990).

Concept of PSD was firstly introduced in the Netherlands where PSD of 25% is considered as the threshold of groundwater quality (Breeuwsma et al., 1995). The PSD of soil can be calculated by:

 $PSD = \frac{100P_{ox}}{N(11 - 100P_{ox})}$ $\alpha (Al_{ox} + Fe_{ox})$

Here, Pox is oxalate-extractable P.

PSD is a good indicator of the soil's potential to release P in soluble form and which can get loosed to water sources. PSD is an important soil test method to get an estimate of the risk of P leaching. PSD has already been used as an environmental indicator in some regions/countries all around the world, because it shows a strong relationship with P runoff or P leaching (Renneson et al., 2010). PSD is calculated as the ratio of acid ammonium oxalate P to [Al Fe] (Van der Zee and Van Riemsdijk, 1988).

In the Netherlands, a threshold of 25% was proposed (Breeuwsma et al., 1995) and 40% in Flanders (Chardon and Schoumans, 2007). Researchers like Schoumans and Groenendijk (2000) and Maguire and Sims (2002) have reported that when PSD is < 25% there is low potential for P leaching but if PSD exceed above 25% there is sharp increase in P leaching. Models Schoumans and Groenendijk (2000) have shown that in non-calcareous sandy Dutch soil P losses in subsurface water is higher because of higher PSD.

Generally, P is lost from soil by crop uptake $\&$ removal; runoff $\&$ erosion; and leaching (Mullins, 2009). By the process of erosion both soluble and deposited insoluble form for P is loosed with soil. By the process of runoff mainly dissolved P get away with water. Phosphorus loss by leaching is considered less important than surface runoff because P is held very tightly by soils mainly in P deficit subsoils (Mullins, 2009). But, in case of more build-up of soil P can increase the potential for P loss to surface waters via hydrological pathways such as overland flow and subsurface drainage water which contribute to high P concentration in water

(Sharpley, 1995). In western Europe, P availability in agricultural soils is high due to application of huge amount of the livestock fertilizer (Sylvain and Thomas, 2013). In many soils P concentrations are now above the minimum value that is required for maximum yield (Sylvain and Thomas, 2013). Locally elevated concentrations of P in surface water, and the accumulated P are the long term source of P which cause environmental problem in this region (Chardon and Schoumans, 2007).

Figure 6 Classification of the PSD for soils in Flanders with a probability of 95%. Source: (Van Meirvenne et al., 2008)

Soils in Flanders are dominated by sandy texture. When sandy soil of this region are applied with more P fertilizer and it becomes more susceptible to P leaching (VLM, 1997). It can be seen in Figure 6 that large part of sandy areas for about 4404 km^2 used for agriculture is already P saturated with estimated PSD of more than 35 % (Van Meirvenne et al., 2008).

2.6 Rules and Regulations related to Phosphorus

Nitrate directive (91/676/EEC) and water framework directive (2000/60/ICE) of European Union has restricted the amount of P in surface water to its member nations (Amery and Schoumans, 2014). In Europe, Belgium and The Netherlands have the largest national P surpluses because of intensive animal production in past (Chardon and Schoumans, 2007). The

absence of regulations to prevent the overuse of manure P surplus was very high in Flanders before 1995 because manure directive was started from 1996 (Chardon and Schoumans, 2007). Flanders started legislation based on fertilizer applications allowing to use only the recommended dose (Clercq et al., 2001). There is special regulation for P-saturated areas and they have more restriction on the use of P fertilizer (Amery and Schoumans, 2014). Similarly, farmers are also not allowed to put surplus P and N animal manure. So, the current application standards for Flanders (2014) vary between 65 and 95 kg P_2O_5 /ha/y. The limits depend on the type of crop. Indicative limits for the next Manure Decree (2015-2018) are mentioned (5−10 kg P2O⁵ /ha/y) lower than the standards of 2014 (Amery and Schoumans, 2014). The maximum phosphorus application standards for Flanders are approximately 5 kg P_2O_5 /ha/y smaller than the general phosphorus export by the crop, resulting in a small negative phosphorus input into the soil (Amery and Schoumans, 2014). And, this regulation is same if farmer cultivate one crop per season or multiple crops in a season. So, it is very important to manage P in Flanders. So, all this regulation measures in Flanders are mainly concerned on increasing P mining. Increase in P mining would help to make use of already accumulated P in the soil which can help to reduce P losses to the environment.

Stricter P legislation in Flanders has restricted the amount of P used by farmers. This might have led over time in low P intensity and high P quantity. But, the main aim of P fertilizer restriction is to ultimately result in P mining (De Bolle et al., 2013). But, with usual crop rotation it takes several decades as P mining efficiency decrease with time (Sharma et al., 2007). So, with time P quantity can't replenish P intensity fast enough. This in result, can cause a reduced P mining.

2.7 Need for soil amendments to increase plant available P

P fertilizers are derived from phosphate-rich rocks which are located in a few places on Earth and are finite (Schoumans et al., 2015). Sign of scarcity of phosphate rock was shown up since 2008 after significant rise in the price by more than 900% (Heckenmüller et al., 2014). In oneway P scarcity is increasing and in other way there is already deposited P in the soil of Flanders but it may become less for plants under mining scenario. Legislations measure in Flanders on restricted use of P fertilizer has risk of reducing P mining. Reduced P mining ultimately lead to reduced amount of plant available P in soil which might ultimately cause impact on crop yield and its quality. So, for efficient long term P mining soil amendments is useful in order not to limit crop P uptake (De Bolle et al., 2013).

2.7.1 Effect of Organic acid amendments

Jones (1998) has reported the binding capacities of oxalic and citric acids on Fe and Al sites are the most efficient agents to mobilize soil P. Formic, acetic, citric, oxalic and malic acids are simple organic acids produced by plants and exuded by plant roots and commonly found in the soils (Strobel, 2001). According to Khademi et al. (2010) root exuded organic acids and anions may be a significant P acquisition mechanism operating in soils. Study of Gardner and Boundy (1983) have reported citrate exuded by root of *Lupinus albus* L., have improved P acquisition by the plant by freeing up fixed P in soil.

According to Earl et al. (1979), organic acids caused dissolution of the soil components and thereby decreased P adsorption. Dissolution of soil components such as Fe and A1 oxides is caused by chelation of metal ions with organic acids, and the ability to chelate depends on the stability constant of the organic acids for metal ions. Bolan et al. (1994) reported that organic acids increase the availability of P in soils mainly through both decreased adsorption of P and increased solubilization of P compounds.

Plants can mobilize organic and inorganic forms of phosphorus (P) in soils by exudation of lowmolecular-weight organic acids and anions (Gang et al., 2012). According to Gang et al. (2012), organic acids are believed to be involved in several chemical reactions like sorption/desorption, and precipitation/dissolution of the poorly soluble P in the soils. Gang et al. (2012) reported the effectiveness of organic acids to mobilize P occurred in the order of tricarboxylic (tricarboxylate) > dicarboxylic (dicarboxylate) > monocarboxylic acid (monocarboxylate). Wei et al. (2009) investigated the effects of four major organic acids (citric, oxalic, maleic, and formic acids) on the available P determination with soil extract with soil and amendment solution ratio of (1:10) and found that 2 mM oxalic acid and 3 mM citric acid extracted higher amounts of P from soil when compared to other concentration.

2.7.2 Effect of Silicate amendments

The higher P availability with silicates can be attributed to the sum of two effects: the alkalizing power of Si and competition for the same site between Si and P (Sandim et al., 2014). The reaction of phosphate ion adsorption to soil colloids is directly related to the reaction conditions of soil, since with a rise of pH the solubility of iron and aluminum phosphates is increased with reduction of phosphate anion adsorption to the soil solid phase (Sandim et al., 2014).

Silicate increase the soil P availability because the silicate anion occupies sites of phosphate anion adsorption and saturates sites where P could be adsorbed (Sandim et al., 2014). The higher availability of P in the soil and for the plant uptake is because anions of silicates exert competition with P for the same adsorption sites (Smyth and Sanchez, 1980). According to Carvalho et al. (2000), use of Ca silicate (CaSiO₃) and/or Mg silicate (MgSiO₃) increases competition for the same adsorption site between Si and P which reduces P fixation.

Roy et al. (1971), who studied Si application prior to phosphate fertilization found increased P availability but they reported this was more influenced by pH increase than by competition for the same adsorption site between Si and P. In an experiment carried out by Castro et al. (2013) in acidic soil in Congo, they use slag (silicate of calcium and magnesium) in a rate that can reach the base saturation of soil up to 70%. After six months they found that the Si slag considerably increased the P level in soil. Castro et al. (2013) reported adsorption sites for phosphate are saturated or blocked by the silicate anion, which can improve P fertilization efficiency. In another experiment done by Pulz et al. (2008), used the Ca and Mg silicate concentration that raise the base saturation up to 60% in medium textured oxisol and they found greater availability of P and Si in soil and greater absorption of Si and P by the plants. So, the effect of silicates in plant available P can be either by completion for the binding sites or can also be by simple pH effect.

2.7.3 Effects of elemental S and sulphates amendments in P availability

Elemental Sulphur (S) and sulphate amendments increase P availability by different mode of action. Sulphates have mainly an effect of competition of binding sites whereas elemental S has both an effect of pH and competition for binding sites. Hence, elemental S can make P available in soluble form in soil by two mechanisms. The effect produced by elementary S depends on

the rate of its oxygenation in soil and its dose (Germida and Janzen, 1993). This was also proved by Skwierawska and Zawartka (2009) because they found concentration of available P in soil increased during third year of their experiment only in the field fertilized with elemental S.

There are different interpretations of influence of Sulphur on the availability of P in soil. According to Jaggi et al. (2005) the change in soil pH causes mineralization of P into inorganic forms as well as liberation of Al and Fe ions, which reacts with sulphates and bind only few phosphate ions. They also reported addition of elemental S improves the availability of P in cultivated soils, irrespective of the initial soil pH. Krol et al. (1986) and Jaggi et al. (2005) have reported that the content of available phosphorus increases in the soil under the influence of sulphur. According to Gądor and Motowicka-Terelak (1986), presence of free sulphur acid in soils creates favorable condition for the release of P from compounds and make them soluble. (Motowicka-Terelak and Terelak, 1998) demonstrated that sulphur, by binding aluminum sulphate, reduced phosphorus fixation in soil, while excessive amounts of sulphates may result in incomplete utilization of phosphorus supplied with fertilizers, as they inhibit the growth of crops.

In an experiment conducted by (Skwierawska and Zawartka, 2009) in acid brown, heavy loamy sand soil with pH (KCl)= 5.30, in Poland; they applied 40, 80 and 120 kgha⁻¹ in the sulphate form and as elemental Sulphur. During their experiment they found that application of 120 kg⋅ha⁻¹ of S-SO₄ caused significant increase in the content of available phosphorus in soil in the layers at 0-40 and 40-80 cm depth. They also reported the effect of sulphur on mobilization of phosphorus depends on the form of sulphur and duration of experiment.

2.7.4 Effect of Phosphate Solubilizing Bacteria (PSBs)

In addition to fertilization and enzymatic decomposition of organic compounds, microbial Pmobilization is another possible way to increase plant-available P (Illmer and Schinner, 1995). A number of microorganisms, including bacteria, have the capability of solubilizing mineral phosphates, there by affecting P cycle both in natural and agricultural ecosystems (Vazquez et al., 2000). Authors like (Parks et al., 1990); Yadav and Singh (1991) have attributed Psolubilization by PSB is caused due to the release of organic acids. Principal mechanism for mineral phosphate solubilization involves production of organic acids, and acid phosphatases in mineralization of organic phosphorous in soil (De Bolle et al., 2013). Illmer and Schinner

(1995) have found that the probable reason for solubilization is by the release of protons during respiration or $NH⁴⁺$ assimilation.

PSB in soil varies from 0.5 to 50% of total respective population (Vazquez et al., 2000). Among PSBs, *Pseudomans*, *Bacillus*, and *Rhizobium* species are dominating in soil environment (De Bolle et al., 2013). Trivedi and Sa (2008) reported *Pseudomonas* species solubilize P under a range of temperature condition. *Pseudomonas putida* is the most intensively studied species of genus Pseudomonas (Manna et al., 2001).

Phosphate solubilizing bacteria (PSB) transform unavailable P into plant available forms, and could thus proof to be very useful even in P saturated soils under severe fertilization restrictions, namely to increase the P mining efficiency (De Bolle et al., 2013). *Pseudomonas putida* and *Bacillus brevis* perform best as PSB in high P conditions where the P is fixed with Al or Fe, which is the case for the acid sandy soils in Flanders (De Bolle et al., 2013). Strains from genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Aspergillus* and *Cephalosporium* are among the phosphate solubilizers.

Amendments	Mechanisms	Source	
Organic Acids	accelerate mineralization of	Gang et al. (2012)	
(lactic acid, oxalic acid, citric	stable P pools; chelation of	Khademi et al. (2010)	
acid)	metal ions with organic acids;	Wei et al. (2009)	
	change in pH	Earl et al. (1979)	
Silicates	completion binding sites;	Sandim et al. (2014)	
(Na_2SiO_3)	acid correction	Castro and Crusciol (2013)	
		Pulz et al. (2008)	
		Smyth and Sanchez (1980)	
Sulphates/S	effect of competition of	Skwierawska and Zawartka	
	binding sites $(SO42)$; effect	(2009)	
	of pH and competition for	Jaggi et al. (2005)	
	binding sites (S)	Lamers et al. (1998)	
		Koerselman et al. (1993)	
PSBs	production of organic acids	Igual et al. (2001)	
(Pseudomonas, Bacillus,	acid phosphatases and	Yadav and Singh (1991)	
Rhizobium, Aspergillus)	enzyme which mineralizes Parks et al. (1990)		
	organic P in soil.	De Bolle et al. (2013)	

Table 1 Summary of P solubilizing amendments

Chapter 3 Material and Methods

3.1 Study Area and Soil Sampling

Study area is Flanders, Belgium. Soil sampling was done in two sites; experimental plot E13.0130 with geographical coordinate of 50° 56' 38.14" north and 3° 31' 13.98" east and Van Oeckel with geographical coordinate 51° 17' 2.19" north and 4° 57' 52.13" east. These both sites are acidic sandy soils known for their rather high P quantity and moderate to low P intensity. The plough layer (0-30 cm) was sampled randomly by following a cross pattern over the field. Sampled soils were air dried and sieved with 2 mm mesh sized sieve.

3.2 Physical and Chemical Characterization of the Soils

3.2.1 Texture

Soil texture was determined using the pipette method (Gee and Dani, 2002). 20 g of sieved airdried soil sample was put into a 2 L beaker. Dispersion of the clay fraction was done by removing the cementing materials like CaCO₃ and organic matter. The fine fractions (silt and clay) were separated from the sand fraction by wet sieving on a 50 micrometer sieve. The clay \langle ($\langle 2\mu$ m) and silt fraction (2-50 μ m) were separated by pipetting with a Robinson-Köhn pipette after sedimentation at a constant temperature and fixed settling, according to Stokes' law. All fractions were weighed after drying at 105 °C, and the results were expressed as a percentage. Based on these percentages and the texture triangle, the texture of each of the soils was determined.

3.2.2 Organic carbon

The organic carbon content was determined using the method of Walkley and Black (1934). 1 g of air-dried soil was taken and put into an Erlenmeyer flask (500 ml). Next 10 ml $K_2Cr_2O_7$ (1 N) and 20 ml concentrated H2SO⁴ were added and shaken smoothly under a fume hood. Then, the solution was left for 30 minutes. After 30 minutes 150 ml H2O was added. Before the titration 10 ml conc. H3PO⁴ followed by four drops of ferroine indicator were added. The solution was titrated with $1 \text{ N } F \in SO_4$ till the color changed from green to reddish brown. By knowing the amount of oxidizing agent to oxidize the organic carbon, soil carbon was determined.

3.2.3 CaCO³ content

CaCO³ present was measured by titration using the method of Gee and Dani (2002). 1 g of soil was put into a 250 ml Erlenmeyer. After adding 25 ml H₂SO₄ (0.5 N) the solution was diluted with distilled water to a volume of 150 ml and placed on a hot plate at 80 °C for 1 hour. After cooling, 0.5 ml of an indicator was added. The solution was titrated with 0.5 N NaOH until the color changes from red to green. Based on the quantity of $H₂SO₄$ consumed, the amount of CaCO₃ present was calculated.

3.2.4 pH-KCl

The soil pH was measured potentiometrically in 1:2.5 ratio (soil: KCl extract). Soil samples were stirred thoroughly with a glass rod. Then the suspended samples were kept at room temperature for 10 minutes. Samples were stirred again with a glass rod before measuring with a pre-calibrated pH meter (Thermo Orion Model 420+).

3.3 P-status of soils

3.3.1 P intensity

P intensity is the P that is directly plant available. This can be determined by extraction with P-CaCl₂

P-CaCl² was determined by shaking 5g of air dried and sieved soil for 2 hours in 50ml of 0.01M CaCl² solution. Afterwards the P concentration in the extract was determined using the method of Murphy and Riley (1962).

3.3.2 P quantity (P-AL)

P-quantity is a measure of P of the current P stock which can replenish soluble P when taken up by the plants. This quantity is determined by P-AL of the soil.

P quantity was determined based on the method of Egnér et al., (1960). 5g of air dried and sieved soil was shaken for 4 hours in a 100 ml ammonium lactate solution. After having filtered the samples over 589/3 whatman filter P content of the extract was determined using ICP-OES (ICAP 6000 series; Thermo Scientific).

3.3.3 Phosphorus saturation degree

The Phosphate saturation degree (PSD) is a function of the portion of the soil exchangeable sites that are bound with P (P sorbed) in relation to the number of sites available for P binding capacity (PBC) (Hooda et al., 2000). PSD is a good indicator of the soil's potential to release P in soluble form and which can get loosed to water sources.

 $PSD = [P_{OX}/(Fe_{OX} + Al_{OX}) \times 0.5]) \times 100$

 $PBC = (Fe_{OX} + Al_{OX}) X 0.5$ PBC is expressed in mmol/kg.

P, Fe and Al oxalate determination (P_{OX}) was done by following the method of Egnér et al., (1960). 5 g air-dried and sieved soil was shaken in 100 ml ammonium oxalate solution for 2 hours. After shaking and filtering P, Fe and Al content of the samples was determined using ICP-OES).

3.4 Shaking experiment

Shaking experiment was done to identify the most promising amendment and their concentration to increase the P-intensity of soil. During this experiment, 4 grams of each soil type (E13.0130 and Van Oeckel) with 40 ml of a solution of Citric Acid, Lactic Acid, Oxalic Acid, Sodium Silicate and Sodium Sulphate at a concentration of 0.5 mM, 1 mM and 2 mM were shaken for 24 hours at 100 RPM in a shaker. After shaking, samples were filtered using 589/3 ashless Whatman filter paper and the filtrate was collected in a 100 ml dark polyethylene bottle. Finally, the P content and the pH of the extract were determined.

3.4.1 Amount of P in extract

P content in the extract of the solution from shaking experiment was determined colorimetric by the method of Murphy and Riley (1962).

3.4.2 pH of extract

In a 50 ml beaker, 10 ml of the extract from shaking experiment was put and pH was measured with a pre-calibrated pH meter (Thermo Orion Model 420+).

3.5 Incubation Experiment

Incubation experiment was done to see the effect of amendment on P-intensity under more realistic conditions and to verify the result of shaking experiment. On the basis of the results from the shaking experiment, the most promising combinations of additive and concentration were selected. Soil sample from sites E13.0130 and van Oeckel amended with four different amendments; citric acid (10 mmol/kg dry soil), sodium silicate (10 mmol/kg dry soil), elemental sulphur (20 mmol/kg dry soil), and PSB (*Pseudomonas putida*) (126 x 10^{11} CFU/kg dry soil) and incubated for 3, 6, 12, 24 and 48 days. Concentration of citric acid, sodium silicate and elemental Sulphur are analogous to 1mM citric acid, 1 mM sodium silicate and 2 mM sulphur in the shaking experiment and concentration of PSB is chosen according to De Bolle et al. (2013). Incubation was done at 18 °C at 50% water-filled pore space (WFPS) and density 1.4 kg/dm³. After each incubation period, P-CaCl₂ and pH-H₂O were determined.

3.5.1 P-CaCl²

P-CaCl₂ was determined by shaking incubated soil with 0.01 M CaCl₂ solution in the ratio 1:10. Afterwards the P concentration of the samples was determined using the method of Murphy and Riley (1962).

3.5.2 pH-H2O

In a 100 ml beaker, 50 ml of distilled water was added to the beaker making soil: water ratio (1:5) and stirred with a glass rod. The soil suspension was left for 18 hours, and again the sample was stirred before measuring the pH. Then, the pH was measured with a pre-calibrated pH meter (Thermo Orion Model 420+).

3.6 Greenhouse experiment

Greenhouse experiment was conducted to check the effect of amendments on P intensity, P mining and crop yield. Soils from the site E13.130 and Van Oeckel were supplemented with citric acid (10 mmol/kg dry soil), sodium silicate (10 mmol/kg dry soil), elemental sulphur (20 mmol/kg dry soil), and PSB (*Pseudomonas putida*) (126 x 10^{11} CFU/kg dry soil) and filled in a grey plastic pots at 50% water-filled pore space (WFPS) and density 1.4 kg/dm³. For control soil was treated with normal tap water. Concentration of amendments and soil samples were

same as in incubation experiment. 0.25 g tetraplod Italian ryegrass was sown in each pot. Day temperature and night temperature in the greenhouse were maintained at 20 and 10°C respectively. Normal tap water was applied as an irrigation source. Harvesting was done 20, 40 and 60 days after germination. At each harvesting period $P\n-CaCl₂$ of the soils in each pot, dry matter yield (aboveground and below-ground) and P-uptake by grass in each pot was determined.

3.6.1 P-CaCl²

P-CaCl2 content of soils in each pot was determined by shaking an equivalent amount of 5 g dry soil in 50 ml 0.01M CaCl₂ for two hours in the mechanical shaker at the speed of 100 RPM. The P content of the extract was determined colorimetric (method of Murphy & Riley, 1962)

3.6.2 pH-H2O

In a 100 ml beaker, 50 ml of distilled water was added to the beaker making soil: water ratio (1:5) and stirred with a glass rod. The soil suspension was left for 18 hours, and again the sample was stirred before measuring the pH. Then, the pH was measured with a pre-calibrated pH meter (Thermo Orion Model 420+).

3.6.3 Determination of dry matter yield

After 20, 40 and 60-day grass was harvested. Above ground and below ground biomass were collected by flushing all soil from each pot on a sieve with a 1 mm mesh size. collected biomass was dried at 50 °C for 24 hours in a hot air oven and the above ground, below ground and the total biomass weight were determined.

3.6.4 P content of biomass

The biomass was grinded in a mill. Replicates were bulked to have a large enough sample. One gram of each grinded sample was transferred to a crucible. Crucibles were placed in the muffle furnace for 4 hours 30 min at 580 °C temperature. After cooling the ashes were transferred to beakers with 20-30 ml 1 N HNO₃ and heated on a hot plate for 1 hour. After one-hour digestion, the digested samples were filtered with 589/3 filter paper and collected in a 100 ml conical flask. 1 N HNO_3 was added up to 100 ml . Then the P content was measured with ICP-OES

(ICAP 6000 series; Thermo Scientific). After measuring total P content in the dry matter P uptake by grass was also calculated by following formula:

Total P uptake (mg P) = P content (mg/g dry matter) x dry matter yield (g)

3.7 Statistical Analysis

For all statistical analysis the computer package SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp) was used. In shaking experiment Analysis of variance (ANOVA) was carried out in order to see the interaction between the soil, amendments and concentration. From ANOVA effect of concentrations on P extraction was seen. Tukey HSD post-hoc analysis was done to see the difference of mean with different concentration for different amendment. Similarly, in order to check effect of pH on Pextraction Pearson's correlation test was done.

For incubation experiment ANOVA was carried out in order to see the interaction between the soil, amendments and days of incubation. From ANOVA effect of amendments on $P\text{-}CaCl₂$ extraction was seen. Tukey HSD post hock analysis was done to see the difference of mean with different amendments on period of incubation. Similarly, in order to check effect of pH on P-CaCl₂ extraction Pearson's correlation test was done.

For greenhouse experiment ANOVA was carried out in order to see the interaction between the soil, amendments and harvesting days on P-CaCl₂ extraction from harvested pots. Similarly, ANOVA also done for the analysis effect of amendments on biomass weight and P-uptake form soil. Tukey HSD post hock analysis was done to see the difference of mean with different amendments on P-CaCl₂ extracted from the harvested pots, biomass weight of grass and Puptake. Pearson's correlation calculation was done to check to see if there is pH effect on P-CaCl₂ extraction.

Levene's test was used to evaluate the assumption of homogeneity of variances for all the data and the differences were considered statistically significant when P<0.05.

Chapter 4 Results

4.1 General soil properties

In Table 2 the general characteristics of soils from E13.0130 and Van Oeckel are given. As soil E13.0130 consists of 87 % Sand, 7 % silt and 3 % clay; it can be classified as a sandy soil (according to the USDA classification). Soil coming from Van Oeckel consists of 87% of sand, 10.1% of silt and 2.9% of clay can also be classified as sandy soil. Further, both soils are acidic $(pH-KCl = 5.26$ and 5.30), which is typical for the Flemish region. Organic carbon content is 1.91% and 2.19% for the site E13.0130 and Van Oeckel respectively. Soils from both sites had almost no free CaCO₃.

Table 2 General properties of the soils

Sites	USDA text. class	% Clay $(0-2\mu m)$	% Silt $(2-50 \mu m)$	% Sand $(50-200 \mu m)$	pH (KCI)	% Org C	$%$ CaCO ₃
E13.0130	Sand			87	5.26	1.91	0.00
Van Oeckel	Sand	2.9	10.1	87	5.30	2.19	0.00

P status of the soils used in the study

P-intensity of site E13.0130 was 1.07 mg P/kg dry soil (Table 3) which is moderate and Van Oeckel was 0.51 mg/kg dry soil which is a rather low value. P-AL of the site E13.130 was 19.61 mg P/100 g whereas for the site Van Oeckel it was 21.25 mg P/100 dry soil which are both moderate to high values. PSD is an environmental indicator to estimate the potential of P leaching. PSD in both sites was higher than 40% but this value was measured for the upper 30 cm only while normally this is measured for the zone 0-90 cm.

Table 3 P status of the sampled soil

4.2 Shaking experiment

4.2.1 Amount of P-extracted

Error Bars: +/- 2 SD

Figure 7 Amount of P extracted from soil after shaking with amendments (citric acid lactic acid, oxalic acid, sodium silicate and sodium sulphate) at concentrations (0.5, 1 & 2 mM), Calcium chloride (0.01 M) is control.

General trend on amounts of P extracted from soils after shaking experiment showed all the amendments had released more P in comparison to control i.e. 0.01 M CaCl₂ (Figure 7). For all three concentrations in both sites, oxalic acid, citric acid and sodium silicate had produced higher amount of P while sodium sulphate and lactic acid could extract least. At site E13.0130 citric acid extracted highest amount of P which ranges 8.71 to 38.62 mg/kg dry soil. Similarly, at site Van Oeckel also citric acid extracted highest amount of P ranging from 8.40 to 39.25

mg/kg dry soil whereas, sodium silicate had released P ranging from 16.59 to 33.23 mg/kg dry soil.

There was effect of concentration on amount of P extraction. There was increasing trend of concentration on amount of P-extraction for all amendments. But, the effect of concentration was more strong for oxalic acid, citric acid and sodium silicate whereas least effect was observed for lactic acid and sodium sulphate. In both sites for 0.5 mM and 1 mM concentrations, sodium silicate had extracted highest amount of P whereas for 2 mM concentration citric acid had extracted most. 2 mM citric acid had extracted highest amount of P for both sites which was 39.05 mg/kg dry soil for E13.0130 and 39.25 mg/kg dry soil for site Van Oeckel.

Three-way ANOVA was done to check the effect of the factors concentration, amendment and site. The three-way interaction was not significant ($p = 0.619$) and could be removed. Then, the two way interactions between soil and amendment; amendment and concentration; and soil and concentration were checked. All the two-way interactions between sites, amendments and concentration were found to be statistically significant. Therefore, data were splitted according to sites, and the interaction of amendments and concentration was tested. Statistically significant interaction ($p = 0.000$) between amendments and concentration for site E13.0130, were found. Therefore, all data were splitted according to amendment and concentration for each sites.

The ANOVA analysis of P (mg/kg dry soil) and concentration showed for E13.0130, lactic acid did not have significant effect. Similarly, for site van Oeckel concentration of Na₂SO₄ had no significant effect. Except these two there was significant interaction between P (mg/kg dry soil) and concentrations for all amendments for both sites.

Tukey HSD analysis of amount of P extracted by each amendment at 0.5, 1 and 2 mM concentrations at site E13.0130 showed oxalic acid, citric acid, sodium silicate had effect of concentration on amount of P extraction. For oxalic acid, citric acid and sodium silicate; increase in concentration had increased amount of P extracted. For $Na₂SO₄$ effect of concentration was seen only for 1 mM and 2 mM. For citric acid, 2 mM could produce 30.34 mg/kg dry soil higher than its concentration at 0.5 mM.

In site Van Oeckel, for all amendments there was positive mean differences of P extraction with increasing concentration. But oxalic acid at 1 mM concentration produced 2.80 mg less P than at 0.5 mM concentration. For sodium silicate mean difference of P for concentration 0.5 mM and 1 mM was 16.64 mg/kg dry soil.

The ANOVA analysis of amendments and amount of P (mg/kg dry soil) for both sites showed there was significant effect of amendments on P (mg/kg dry soil) extraction on both sites. Multiple mean comparisons of different amendments (0.5, 1 and 2 mM) and amount of extracted P (mg/kg dry soil) at site E13.0130 showed all amendments for all three concentration had positive mean difference with control (0.01 M CaCl₂) and sodium silicate and citric acid had highest significant mean difference. Lactic acid and sodium sulphate had positive mean difference with control but were not significant. For concentration 0.5 mM and 1 mM sodium silicate produced higher amount of P in comparison to other amendments. But, for 2 mM citric acid exceeded all other amendments and produced highest amount of P. 0.5 mM and 1 mM sodium silicate had produced 11.24 mg, and 16.81 mg more P than control. Similarly, 2 mM citric acid had produced 38.09 mg more P than control. Lactic acid and sodium sulphate could extract only 1.47 mg and 1.89 mg respectively higher than control at 2 mM concertation.

Multiple mean comparisons of different amendments (0.5, 1 and 2 mM) and amount of extracted P (mg/kg dry soil) at Van Oeckel showed all amendments for all three concentration had positive mean difference with control (0.01 M CaCl₂). Sodium silicate and citric acid had highest significant mean difference. 0.5 mM and 1 mM sodium silicate had produced 15.32 mg, and 23.08 mg more P than control. Similarly, 2 mM citric acid had produced 38.50 mg more P than control.

4.2.2 Effect of pH on amount of P extracted

pH-CaCl² of both soils showed both soils were really acidic. Effect of pH on amount of P extracted was checked to know whether the increase in P extracted was related to pH or also other modes of action are taking place (e.g. competition for binding sites). At both sites, sodium sulphate was more acidifying while carboxylic acids (lactic acid, oxalic acid and citric acid) had less acidifying effect (Table 4). Similarly, sodium silicate had slightly alkalizing effect. Increase in concentration had increased pH for extracts from sodium silicate and decreased for other amendments. For citric acid in both sites, increase in concentration from 1 mM to 2 mM had sharply decreased its pH.

Table 4 Mean pH of extract solution with citric acid, lactic acid, oxalic acid, sodium silicate and sodium sulphate at concentrations of 0.5, 1 and 2 mM. Here 0.01 M CaCl2 has been used as control.

			mean pH of extract					
			lactic	oxalic	citric acid	sodium	sodium	control (0.01M
			acid	acid		silicate	sulphate	$CaCl2$)
concentration .5			5.56	6,04	5,38	7,28	4,80	4.83
	1,0	E13.0130	5.35	6,48	6,79	7,37	4,75	4.98
	2,0		5.24	4,67	3,88	7,50	4,62	4.52
5. concentration			5.58	6,08	5,63	7,19	4,78	4.36
	1,0	van Oeckel	5.65	6,75	6,69	7,37	4,69	4.98
	2,0		5.17	5,26	4,73	7,40	4,61	4.54

Pearson's correlation between amount of extracted P (mg P/kg dry soil) and the pH showed, for organic acids P extracted increases with decrease in pH. Similarly, for sodium silicate increasing pH caused increase in P content. But, for sodium sulphate change in P-extract could not be attributed to pH effect. Among the organic acid, lactic acid had strong negative correlation $(r = -0.96)$ with pH but the pH range was rather small as seen in the scatter plot (Figure 8). Sodium silicate showed positive correlation $(r = 0.72)$. Similarly, in Van Oeckel, among organic acids, oxalic acid ($r = -0.83$) and citric acid ($r = -0.69$) had significant negative correlation. There was no significant correlation for lactic acid ($r = 0.25$) but the range of pH change was narrow as seen in the scatter plot. Sodium silicate showed significant positive correlation ($r = 0.74$). In both sites, sodium sulphate didn't have correlation between the amount of P extracted and pH but the pH changes was not that big. This mean for sodium sulphate there was no pH effect but competition for sorption sites.

Figure 8 Scatter plot showing the relationship between pH and amount of P (mg/kg dry soil) extracted in sites E13.130 and Van Oeckel

4.3 Incubation experiment

4.3.1 P-CaCl² extraction

Figure 9 Evolution of P intensity (P-CaCl2) in soil incubated with citric acid (10 mmol/kg dry soil), sodium silicate (10 mmol/kg dry soil), elemental sulphur (20 mmol/kg dry soil), and PSB $(126 \times 10^{11} \text{ CFU/kg}$ dry soil) compared to control (incubated with demineralized water) for 3, *6, 12, 24 and 48 days.*

General trend of P-CaCl₂ of soils incubated with amendments after 3, 6, 12, 24 and 48 days showed, P-CaCl₂ had increased initially and then decreased with time of incubation (Figure 9). P-CaCl₂ measured was higher for PSB and sodium silicate for all incubation time. P-CaCl₂ for PSB showed very high peak on day 12 for both sites and dropped down suddenly on 24 days. Citric acid had increased P-CaCl² during early days of incubation and then decreased. For both sites, sodium silicate had released higher amount of P-CaCl₂ for long duration of incubation.

Elemental sulphur had worked slowly in comparison to other amendments. Similarly, all the amendments had released more P-CaCl₂ than control in both sites.

At site E13.0130 the effects of citric acid, and elemental sulphur were rather small, while Na₂SiO₃ and PSB gave a rather high increase in P-CaCl₂. PSB had released highest amount of P-CaCl₂ ranging from 0.98 mg/kg dry soil to 2.91 mg/kg dry soil. Na₂SiO₃ produced higher P- $CaCl₂$ (241.91 mg/kg dry soil) on day 12. P-CaCl₂ extraction for the treatment elemental S varied from 0.59 to 0.68 mg/kg dry soil which was least among all other amendments.

At site van Oeckel also effects of citric acid, and elemental sulphur were quite small, while Na₂SiO₃ and PSB gave a rather high increase in P-CaCl₂. PSB had released highest amount of P-CaCl² ranging from 1.25 mg/kg dry soil to 3.03 mg/kg dry soil. Like on site E13.0130, PSB had peaked P-CaCl₂ by 3.06 mg/kg dry soil. After 24 days of incubation P-CaCl₂ extraction started to decrease for all amendments.

Three-way ANOVA showed statistically significant interaction ($p = 0.000$) between the factors site, amendment and days of incubation on P intensity. Therefore, data were splitted according to sites, and the interaction of amendments and days of incubation was tested. Statistically significant interaction between amendments and days of incubation for both sites with, ($p =$ 0.000) for site E13.0130 and van Oeckel with, $(p = 0.000)$ were found. Therefore, all data were splitted according to days of incubation for both sites. The ANOVA analysis of P (mg/kg dry soil) and amendments on days of incubation for each sites showed that there was statistically significant effect of amendments on P-CaCl₂ obtained.

For E13.0130, Na₂SiO₃ had significant highest mean difference on P-CaCl₂ extraction in comparison with other amendments during all period of incubation but on day 12 PSB exceeded Na₂SiO₃ by 1.06 mg P/kg dry soil. After Na₂SiO₃, PSB had higher significant mean difference with amendments ranging from 0.30 mg to 2.09 mg P/kg dry soil.

For the van Oeckel site PSB had significant highest mean difference on P-CaCl₂ ranging from 0.05 mg to 1.90 mg/kg dry soil in comparison with other amendments during all period of incubation except on day 3 when citric acid exceeds higher than 0.84 mg P/kg dry soil. Like on site E13.0130 all the amendments had produced more P-CaCl₂ in comparison to control.

4.3.2 Effect of pH on P-CaCl² extracted

Effect of pH on amount of P CaCl₂ was checked to know whether the change in P-CaCl₂ was an effect of pH or other modes of action are taking place. For E13.030 pH-H2O when incubated with citric acid was almost similar to control while for $Na₂SiO₃$ was slightly alkaline which ranges from 7.04 to 7.16. For citric acid and $Na₂SiO₃$ pH was not changing and was almost similar for all time periods while for PSB and sulphur it was changing. Among four amendments elemental sulphur had more acidifying effect but it was seen after 12 days of incubation.

Table 5 pH-H2O of soil incubated with amendments for 3, 6, 12, 24 and 48 days for site E13.0130

				Citric Acid	Control	Na ₂ SiO ₃	PSB	Sulphur
days of incubation	3	рH	Mean	6,16	6,58	7,16	7,24	6,39
	6	рH	Mean	6,36	6,59	7,11	7,12	6,10
	12	рH	Mean	6,41	6,56	7,04	6,04	5,46
	24	pН	Mean	6,73	6,51	7,14	5,63	4,67
	48	рH	Mean	6,78	6,47	7,05	5,70	4,62

Like for soil from E13.0130, pH-H₂O for soil incubated with citric acid was almost similar to control in van Oeckel also. Similarly, for treatment $Na₂SiO₃$ pH ranges from 7.05 to 6.86. For PSB pH was decreasing from 7.17 at day 3 to pH = 5.68 on day 48. In this site also sulphur starts to show its more acidifying effect from day 12.

Table 6 pH-H2O of soil incubated with amendments for 3, 6, 12, 24 and 48 days for site van Oeckel

				Citric Acid	Control	Na ₂ SiO ₃	PSB	Sulphur
days of incubation	3	рH	Mean	6,04	6,81	7,05	7,17	6,48
	6	рH	Mean	6,16	6,58	7,02	6,97	6,20
	12	рH	Mean	6,28	6.40	6,82	5,83	5,34
	24	рH	Mean	6,67	6.48	6,95	5,66	4,84
	48	рH	Mean	6,71	6,59	6,86	5,68	4,69

The correlation coefficient showed there was significant positive correlation for sodium silicate and significant negative correlation for citric acid (Table 7).

At site E13.130, there was significant positive correlation between P and pH for Na_2SiO_3 (r = 0.72). Whereas citric acid had negative correlation ($r = -0.58$) with pH which means P-CaCl₂ increase with decrease in pH. There was strong negative correlation for control but from the scatter plot (Figure 10) very small pH range (6.40 to 6.81) was seen.

At site van Oeckel also there was significant positive correlation between P-CaCl₂ extracted and pH for Na₂SiO₃ ($r = 0.72$). Whereas citric acid had strong negative correlation ($r = -0.94$) with pH which means P-CaCl₂ increase with decrease in pH. There was no statically significant correlation between P-CaCl₂ and pH-H₂O for PSB and sulphur.

Table 7 Correlation analysis of extracted P-CaCl² (mg/kg dry soil) after incubation with the pH of the extracted solution at different sites

sites			Amendments		
	PSB	Na ₂ SiO ₃	Elemental S	Citric acid	control
E13.0130	-0.03	$0.72**$	-0.08	$-0.58*$	$-0.90**$
Van Oeckel	0.10	$0.69**$	-0.36	$-0.94**$	0.31

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

4.4 Greenhouse experiment

4.4.1 P-CaCl²

Error Bars: +/- 2 SD

Figure 10 P intensity (P-CaCl2) of soils mixed with citric acid (10 mmol/kg dry soil), sodium silicate (10 mmol/kg dry soil), elemental sulphur (20 mmol/kg dry soil), and PSB (126 x 10¹¹ CFU/kg dry soil) in the pot after growing grass for 20, 40 and 60 days.

Pots treated with PSB and sodium silicate measured higher amount of P-CaCl₂ for all harvesting time (Figure 10). P-CaCl₂ increased until day 20 and then decreased until last harvesting time but remained higher than control.

At site E13.130 P-CaCl₂ decreased with time as P had been taken up by the grass. P-CaCl₂ for pot treated with citric acid remain higher until day 60. PSB, citric acid and $Na₂SiO₃$ had shown an initial increase in P-CaCl₂. Pot treated with PSB measured highest P-CaCl₂ (1.28 mg/kg dry soil) on day 20 which steadily decreases up to $(0.57 \text{ mg/kg} \text{ dry soil})$ on day 60. P-CaCl₂ for

sulphur remain higher on day 40 and 60. On day 20 sulphur had P-CaCl₂ similar as control (0.79 mg/kg dry soil) but it rose steadily on day 40 (0.93 mg/kg dry soil) and day 60 (1.05 mg/kg dry soil). Citric acid produced higher P-CaCl₂ on day 40. Effect of amendments on P-CaCl² was higher than control for all time.

For site Van Oeckel also first P -CaCl₂ for all amendments increases until day 20, and then decreases but remains higher than the control. Effect of sulphur increases up to day 20 then stays constant until day 40 and then starts to decrease. On day 20, PSB had more P-CaCl₂ (2.54) mg/kg dry soil) but on day 40 and 60 Na₂SiO₃ produce highest amount (1.77 mg/kg dry soil and 0.98 mg/kg dry soil respectively). On day 60, sulphur and citric acid produces same amount of P-CaCl₂ (0.75 mg/kg dry soil).

Three-way ANOVA was done to check the effect of the factors sites, amendments, and harvest time on P-CaCl₂ extraction. With, $(p = 0.000)$ we could conclude that the three-way interaction was statistically significant. Therefore, data were splitted according to sites, and the interaction of amendments on harvesting days after germination were tested. Statistically significant interaction between amendments and harvesting days for both sites with, $(p = 0.000)$ for site E13.0130 and van Oeckel with, $(p = 0.000)$ were found. Therefore, all data were splitted according to harvesting days for both sites. The ANOVA analysis of $P\text{-}CaCl₂$ (mg/kg dry soil) and amendments on days of harvesting for each sites show that there was statistically significant effect of amendments and P-CaCl₂ obtained.

On day 20, Tukey HSD analysis of $P\text{-}CaCl₂$ (mg P/ kg dry soil) of the soils treated with PSB had significant highest mean difference on P-CaCl₂ in comparison with other amendments. On day 40 citric acid had significant highest mean difference on P-CaCl₂ in comparison with other amendments. Similarly, at day 60 sulphur had significant highest mean difference on P-CaCl² in comparison with other amendments. Multiple comparison of means clearly showed that all the amendments are making more P available for plant uptake than control throughout the incubation period.

Like in site E13.0130, PSB had significant highest mean difference on P-CaCl₂ in comparison with other amendments. But in 40 days harvesting time $Na₂SiO₃$ had significant highest mean difference on P-CaCl₂ extraction in comparison with other amendments. Multiple comparison

of means clearly showed that P-CaCl₂ is significantly positive in comparison to control for all the amendments.

4.4.2 Effect of pH-H2O on P-CaCl² extracted

For both sites, elemental sulphur was acidifying whereas citric acid and PSB had similar pH as control. Similarly, sodium silicate was slightly alkalizing. There was no much change in pH with time.

At site E13.0130, pH-H2O for citric acid pH doesn't change compared to the control while for elemental sulphur pH ranges between 4.46 to 4.92 (Table 8). Similarly, for PSB pH was slightly acidifying ranging from 5.96 to 6.41 and for sodium silicate pH was slightly alkalizing which was between 7.15 to 7.33.

Table 8 Mean pH-H2O in extracts of soil E13.0130 after harvesting grass grown in soil treated with different amendments for 20, 40 and 60 days.

					рH		
	$sites = E13.0130$		sodium elemental				
			citric acid	control	sulphur	PSB	silicate
days after	20	Mean	6,53	6,59	4,92	5,96	7,19
germination	40	Mean	6,45	6,66	4,46	6,41	7,15
	60	Mean	6,53	6,54	4,49	6,78	7,33

Table 9 shows at site Van Oeckel citric acid doesn't changes pH compared to control. Similarly, elemental sulphur had acidifying effect but it was more acidic with increase in growing time. This could be because we had applied elemental sulphur in powder form which takes time to oxidize. pH-H2O for soil treated with PSB was slightly acidic (6.09 to 6.79) whereas sodium silicate had slightly alkalizing effect (7.12 to 7.24).

					рH		
	sites = Van Oeckel		sodium elemental				
			citric acid	control	sulphur	PSB	silicate
days after	20	Mean	6,37	6,59	5,06	6,09	7,19
germination	40	Mean	6,36	6,57	4,98	6,30	7,12
	60	Mean	6,50	6,53	4,94	6,79	7,24

Table 9 Mean pH-H2O in extracts of soil Van Oeckel after harvesting grass grown in soil treated with different amendments for 20, 40 and 60 days.

Citric acid and elemental sulphur had negative correlation with pH in both sites (Table 10). In site E13.0130, there was statistically significant strong negative correlation PSB $(r = -0.99)$ for citric acid. Similarly, for Na₂SiO₃ there was positive correlation ($r = 0.72$). No correlation was observed for PSB.

In site Van Oeckel there was negative correlation for citric acid ($r = -0.89$). Unlike in site E13.130, Sodium silicate was negatively correlated $(r = -0.67)$ in Van Oeckel. Similarly, elemental S had no significant correlation. Control at site van Oeckel showed significant positive correlation but from the scatter plot it could be seen that change in pH range was very small.

Table 10 Pearsons's Correlation analysis of extracted P-CaCl² (mg/kg dry soil) after incubation with the pH of the extracted solution at both sites

sites	Amendments							
	Citric acid	control	Elemental S	PSB	sodium silicate			
E13.0130	$-0.99**$	0.54	$-0.81**$	$-0.68*$	$0.72**$			
Van Oeckel	$-0.89**$	$0.67*$	-0.45	-0.57	$-0.67*$			

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Scatter plots in figure 12 show the relation of P -CaCl₂ and pH -H₂O on 20, 40 and 60 days after germination in soils from site E13.130 and Van Oeckel treated with amendments.

Figure 12 Relation of extracted P-CaCl² and pH-H2O after harvesting on 20, 40 and 60 days after germination in soils soil from E13.130 and Van Oeckel treated with amendments, citric acid (10 mmol/kg dry soil), sodium silicate (10 mmol/kg dry soil), elemental sulphur (20 mmol/kg dry soil and PSB (126 x 10¹¹ CFU/kg dry soil).

4.4.3 Determination of dry matter

Figure 11 Dry biomass (above ground and below ground and total) weight (g) of grass grown in two types of soil treated with amendments, citric acid (10 mmol/kg dry soil), sodium silicate (10 mmol/kg dry soil), elemental sulphur (20 mmol/kg dry soil), and PSB (126 x 10¹¹ CFU/kg dry soil) and harvested in 20, 40 and 60 days after germination.

Above ground biomass

At E13.130 dry weight of biomass was higher for pots treated with PSB and sodium silicate and lowest for citric acid (Figure 11). On day 20, all pots produced almost similar above ground biomass but for citric acid it was even lower than control as there was very few germinations of seeds. PSB, sodium silicate and sulphur produced more dry matter than control on day 40 and 60. For all the time dry weight for pot treated with citric acid remained below control. For sodium silicate above ground dry matter yield was 1.78 g on day 60 which was almost twice as control (0.98 g). On day 60 sulphur had similar yield as control (0.90 g).

At Van Oeckel also PSB, sodium silicate and sulphur produced more above ground biomass than control whereas citric acid remained lowest for all the time. In this case also with citric acid germination was not so successful. On day 20 all the pots with different amendments produced similar amount of biomass but on day 40 and 60 yield was much higher for PSB and sodium silicate. On day 60, PSB and sodium silicate produced 3.71 g while citric acid could produce only 0.91g which was even lower than control (0.92 g).

Three-way ANOVA was done to check the three-way interaction between sites, amendments, and harvesting day with above ground dry biomass yield and the interaction was found significant ($p = 0.000$). Then, the interaction was splitted according to harvesting days, and the interaction of above ground biomass weight and amendments was tested. The ANOVA analysis of above ground dry biomass weight and amendments on days of harvesting showed that at site E13.0130 on day 40 and 60 there was significant interaction of amendments and dry biomass weight but on site Van Oeckel there was significant interaction for all harvesting days.

For site E13.0130, on day 20 no significant mean difference on aboveground dry matter yield was found for all amendments. But for harvesting day 40 and 60, PSB had significant positive mean difference followed by sodium silicate. Citric acid had significant negative mean difference with other amendments on day 40 and 60 suggesting its lowest yield. On day 60 PSB and sodium silicate could produce 2.11 g and 0.80 g more above ground dry mass than control.

For site Van Oeckel, on day 20 only PSB had significant positive mean difference with control. On day 40 and 60 sodium silicate and PSB had exceeded the yield of control. On day 60 PSB and sodium silicate could produce 2.78 g and 0.67 g more above ground dry mass than control.

Below ground biomass

At site E13.0130, PSB and sodium silicate produced much higher amount of below ground biomass while sulphur produced almost similar and citric acid remained lower than control. On day 20 PSB produced 0.24 g but on day 40 and 60 it produced 2.33 g and 2. 88 g respectively.

At site Van Oeckel PSB, sodium silicate and sulphur had produced higher biomass than control while citric acid was lower all the time. Like at site E13.0130 on day 20 yield was almost similar for all amendments except for citric acid which was much lower. On day 60 also PSB produced highest amount (4.61 g) followed by sodium silicate (2.14 g). Similarly, on day 60, sulphur could produce 1.45 g while control only 1.28 g.

Three-way ANOVA was done to check the three-way interaction between sites, amendments, and harvesting day with below ground dry biomass yield and significant ($p = 0.000$) interaction was found. Then, the data was splitted according to harvesting days, and the interaction of below ground biomass weight and amendments was tested. ANOVA analysis of below ground dry biomass weight and amendments on days of harvesting showed that at site E13.0130 on day 40 and 60 there was significant interaction of amendments and dry biomass weight but on site Van Oeckel there was significant interaction for all harvesting days.

The mean comparisons of below ground biomass weight for site E13.0130 showed that on day 20 no significant mean difference was found for all amendments. On day 40 PSB and sodium silicate had significant positive mean difference with control. On day 60 PSB, sodium silicate and sulphur could produce 1.37 g and 0.74 g and 0.07 g more below ground dry mass than control.

The mean comparisons of below ground biomass weight for Van Oeckel showed, on day 20 only PSB had significant positive mean difference with control. On day 40 and 60 sodium silicate and PSB had exceeded the yield of control. On day 60 PSB and sodium silicate could produce 3.33 g and 0.86 g more above ground dry mass than control.

Total biomass

For all time treatment PSB had higher total biomass followed by sodium silicate (Figure 12). At site E13.0130, for 20 days after germination not much difference on biomass yield was observed but starting from 40 days after germination differences were more. Citric acid led to poor germination resulting least total biomass for all harvesting time. On day 20 total biomass weight was almost similar for all amendments except citric acid. On day 60, total plant dry weight for PSB (5.98 g) and sodium silicate (4.04 g) had increased significantly in comparison to control (2.49 g) .

At site Van Oeckel PSB had produced more total dry weight for all harvesting time. On day 20 sodium silicate produced similar total dry weight as control but on day 40 and 60 yield increased significantly. Total dry matter yield for sulphur remained slightly higher than control on 40 and 60. Total biomass produced by PSB on day 40 (5.25 g) and 60 (8.32 g) are much higher than other amendments. On day 40 and 60 sodium silicate produced 3.20 g and 3.74 g total dry weight respectively while sulphur could produce 1.92 g and 2.47 g respectively.

Three-way ANOVA was done to check the three-way interaction between sites, amendments, and harvesting day with total dry biomass yield and three-way interaction was found statistically significant ($p = 0.000$). Then, the interaction was splitted according to harvesting days, and the interaction of total dry biomass weight and amendments was tested. ANOVA analysis of total dry biomass weight and amendments on days of harvesting showed that at site E13.0130 on day 40 and 60 there was significant interaction of amendments and dry biomass weight but on site Van Oeckel there was significant interaction for all harvesting days.

The mean comparisons of total biomass weight with different amendments for different harvesting days for site E13.0130 showed, on day 20 no significant mean difference on total dry matter yield was found for all amendments. On day 40 PSB and sodium silicate had significant positive mean difference with control. On day 60 PSB, sodium silicate could produce 3.48 g and 1.54 g more below ground dry mass than control.

The mean comparisons of total biomass weight at site Van Oeckel showed, on day 29, only PSB had significant positive mean difference with control. While on day 40 and 60, PSB, Sodium silicate and sulphur had exceeded control. Citric acid had remained below control all the time. On day 60 PSB, sodium silicate and sulphur could produce 6.11 g and 1.53 g and 0.26 g more total dry biomass than control.

4.4.4 Determination of P uptake

Error Bars: +/- 2 SD

Figure 12 P uptake (mg/pot) by above ground, below ground and total plant parts of grass grown in two soil types treated with amendments, citric acid (10 mmol/kg dry soil), sodium silicate (10 mmol/kg dry soil), elemental sulphur (20 mmol/kg dry soil), and PSB (126 x 10¹¹ CFU/kg dry soil) and harvested in 20, 40 and 60 days after germination.

P uptake (Above ground)

P uptake by grass on pot treated with PSB and $Na₂SiO₃$ was initially slightly higher than other amendments but later on increased considerably (Figure 12). At site, E13.130 P uptake per pot was higher for PSB and sodium silicate and lowest for citric acid. Pot with sulphur had higher P than control on day 40 only. Pot with citric acid had lower P than control for all harvesting time. On 20 day pots treated with sodium silicate took more P (2.39 mg) than other amendments. At Van Oeckel, pot with sulphur had more P on above ground plant parts than control on day 40 and 60.

Three-way ANOVA was done to check the three-way interaction between sites, amendments, and harvesting day with P-uptake by above ground biomass and three-way interaction was found significant ($p = 0.000$). Then, the interaction was splitted according to harvesting days, and the interaction of P-uptake and amendments was tested. ANOVA analysis of P-uptake by above ground biomass and amendments on days of harvesting showed that at site E13.0130 on day 40 and 60 there was significant interaction of amendments and P-uptake while on site Van Oeckel there was significant interaction for all harvesting days.

The mean comparisons of P-uptake by above ground plant parts on site E13.0130 showed that on day 20 there was no significant mean differences on P-uptake for any amendments though sodium silicate had positive mean difference with all amendments. On harvesting day 40 PSB had significant positive mean difference with other amendments followed by sodium silicate. On day 40, P uptake with PSB was 5.42 mg higher than that of control.

The mean comparisons of P-uptake by above ground at site Van Oeckel showed PSB had significant positive mean differences on P-uptake with all other amendments. Sodium silicate had positive mean difference on P-uptake with all amendments except PSB. P-uptake for citric acid was even lower than that of control.

P uptake (Below ground)

At site E13.0130, P content in below ground plant parts was almost similar for all amendments on day 20 (Figure 12). On day 40 and 60 PSB, sodium silicate had more P content in below ground plant parts than control. P uptake was similar for pot with PSB and sodium silicate in day 40 and 60. For PSB, P content on below ground plant part was 0.65 mg on day 20 while it raised to 5.25 mg on day 40 and decreased slightly lower to 5.05 mg P per pot on day 60.

At site Van Oeckel on day 20 P uptake by below ground plant parts were almost similar like in E13.0130 but on day 40 and 60 P uptake by plants grown with PSB was much higher. On day 40 PSB had highest P content of 9.88 mg per pot while for sodium silicate it was 5.30 mg/pot and for sulphur it was 3.0 mg/pot.

Three-way ANOVA was done to check the three-way interaction between sites, amendments, and harvesting day with P-uptake by below ground biomass and three-way interaction was found significant ($p = 0.000$). Then, the interaction was splitted according to harvesting days,

and the interaction of P-uptake by below ground biomass and amendments was tested. ANOVA analysis of P-uptake by below ground biomass and amendments on days of harvesting showed that at site E13.0130 on day 40 and 60 there was significant interaction of amendments and Puptake while on site Van Oeckel there was significant interaction for all harvesting days.

The mean comparisons of P-uptake by below ground biomass for site E13.0130 showed on day 20 there was no significant mean differences on P-uptake for any amendments though control had positive mean difference with all amendments. On harvesting day 40 PSB had significant positive mean difference with other amendments followed by sodium silicate. P uptake by belowground biomass on pot with PSB was 1.71 mg higher than that of control.

The mean comparisons of P-uptake by below ground plant parts at site Van Oeckel also showed, PSB had significant positive mean differences on P-uptake with all other amendments. Sodium silicate had positive mean difference on P-uptake with all amendments except PSB for all harvesting days. Elemental sulphur had positive mean difference on P-uptake with citric acid and control but differences are not statistically significant.

Total P uptake

Total P uptake for pot with PSB was significantly higher in both sites. After PSB total P was highest for sodium silicate. Pot with sulphur had almost similar P content as control while citric acid had significantly lower P uptake. In the shaking experiment and incubation experiment citric acid was performing well but in dry matter yield and P uptake citric acid is not favorable. The reason behind this could be the acidic nature of citric acid.

At site E13.0130 on day 20 total P uptake was almost similar for all pots but on day 40 and 60 it was higher for PSB and sodium silicate. On 60 days P-uptake on pots with PSB and sodium silicate had increased significantly in compared to other amendments and control. On 60-day average total P content for PSB, sodium silicate and sulphur was 12.61 mg, 9.10 mg and 5.74 mg respectively.

At site Van Oeckel also total P uptake on day 20 was almost similar but it increased much more for PSB. Like in site E13.130, pots with PSB and sodium silicate had more total P than other amendments. PSB had twice more total P content than sodium silicate on day 40 and 60. Pot treated with sulphur had more total P uptake per pot in day 40 and 60. Total P content for pot with PSB and sodium silicate on day 40 was 17.20 and 9.02 mg per pot. Similarly, on day 60 total P uptake for pot with PSB was 22.17 mg per pot and for sodium silicate was 11.00 mg per pot.

Three-way ANOVA was done to check the three-way interaction between sites, amendments, and harvesting day with total P uptake and three-way interaction was found significant ($p =$ 0.000). Then, the interaction was splitted according to harvesting days, and the interaction of total P-uptake and amendments was tested. ANOVA analysis of total P-uptake by the grass and amendments on days of harvesting showed that at site E13.0130 on day 40 and 60 there was significant interaction of amendments and P-uptake while on site Van Oeckel there was significant interaction for all harvesting days.

The mean comparisons of P-uptake with different amendments for different harvesting time for site E13.0130 showed, on day 20 there was no significant mean differences on P-uptake for any amendments though sodium silicate had positive mean difference with all amendments. On harvesting day 40 PSB had significant positive mean difference with other amendments followed by sodium silicate. P uptake with PSB was 7.13 mg higher than that of control on day 40. Similarly, on day 40 P uptake with sodium silicate was 2.44 mg higher than control. On day 60 also P-uptake with PSB was higher in comparison to other amendments.

The mean comparisons of total P-uptake by grass at site Van Oeckel showed PSB had significant positive mean differences on P-uptake with all other amendments. Sodium silicate had positive mean difference on P-uptake with all amendments except PSB. Similarly, elemental sulphur had positive mean difference on P-uptake with citric acid and control but differences were not statistically significant.

Chapter 5 Discussion

Shaking experiment

Shaking experiment was done to select most promising amendments and concentration to increase plant available P. Both soils used for shaking experiment are acid sandy soil with no free CaCO3. PSD in both sites were higher than 40% though this value was measured for the upper 30 cm only. According to Van der Zee (1990) risk of P leaching starts at a PSD of 25% in acidic sandy soils. Hence the risk of P leaching from the upper 30 cm to deeper soil layers in our studied sites is high. Similarly, both soil had rather high to moderate P-quantity but Pintensity was lower. The low P-intensity $(P-CaCl₂)$ is due to the fact that much of the P was in unavailable forms and could be slowly available to the crop during the growing season or the next crop because of the residual effect. But for optimum plant growth P is essential (Van Rotterdam-Los et al., 2013). As Flanders legislation has set limits to the amount of P-fertilizer applied to field it is necessary to increase P-intensity of the soil.

P availability enhanced by amendments

P extraction on shaking experiment by amendments for all concentration remain higher. Among the amendments used lactic acid and sodium sulphate was found to be less effective than other amendments. Results from shaking experiment clearly shows that addition of organic acid has increased amount of P in extraction. According to Bolan et al. (1994), organic acids increase the availability of P in soils mainly through both decreased adsorption of P and increased solubilization of P compounds. Result shows the effectiveness of organic acids in amount of P-extraction followed the order lactic acid < oxalic acid < citric acid which is consistent with the previous study done by Bolan et al. (1994) and Gang et al. (2012). Mechanism of increase in P by organic acid is by mineralization of stable pools of P. Bolan et al. (1994) have reported that organic anions have chelation mechanism with the metal ions, such as Fe and A1, and thereby release anions bound to the metal ions and liberates phosphate in soluble form. In our experiment 2mM Citric acid has extracted highest amount of P i.e. around 39mg/kg dry soil in both sites. Wei et al. (2009) also have found 2 mM citric acid extracted higher amounts of P from soil when compared to other concentration.

Silicate is more efficient in correcting acidity due to its high solubility (Alcarde and Rodella, 2003). Castro et al. (2013) also have reported silicate is more efficient for P availability and reducing toxic aluminum. Increase in concentration of silicates have extracted increasing amount of P. Roy et al. (1971) have also reported that extractable P increased with increasing rates of silicate applied to the soil. Results shows in both sites for 0.5 mM and 1 mM concentrations, sodium silicate has extracted highest amount of P. Silicate has extracted highest amount of P in shaking experiment on both sites. Similarly, we also found increasing concentration for sodium silicate has increased the P-extraction is highest for 2 mM.

Sulphate increases the amount of P extraction by competition for the binding sites. In our experiment effect of sodium sulphate on P extraction was not that higher than other amendments. This could be because the soil was already acidic. Motowicka-Terelak and Terelak (1998) have demonstrated that sulphur reduce phosphorus fixation in soil by binding aluminum by sulphate. While comparing with other amendments sodium sulphate has produced less amount of P. 2 mM of sodium sulphate could produce only 2.41 mg/kg dry soil in site E13.0130 and 2.88 mg/kg dry soil for site Van Oeckel.

Most promising amendments chosen for shaking experiment were 1 mM citric acid, 1 mM sodium silicate and 2 mM elemental sulphur. Though the P-extraction was highest for 2 mM citric acid, increase in concentration form 1 mM to 2 mM has sharply decreased its pH. So, 1 mM citric acid was chosen for incubation experiment.

Effect of pH on amount of P extraction

There are different arguments about the pH dependence of phosphate solubilization because studies have reported different results from no effect or an increase or a decrease in solution phase of phosphate with decreasing pH, depending on the soil and pH range investigated (Jones et al., 2003).

Result shows at both sites, sodium sulphate is more acidifying while organic acids have less acidifying effect. Similarly, sodium silicate has slightly alkalizing effect. Negative Pearson's correlation has been found for pH and amount of P extracted with organic acids. Geelhoed et al. (1999) have also reported increase in P in solution with decrease in pH. Organic anions, the conjugate bases of organic acids, may play an important role in improving the availability of soil phosphate (Geelhoed et al., 1999). Positive significant correlation has found for amount of P extracted and pH for sodium silicate. Since the soil samples were acidic, alkalizing effect of sodium silicate might have effect on P availability. Roy et al. (1971) have reported that silicate decreased P sorption more effectively at low soil pH than at high soil pH. They have found that P sorption was greater at pH 5.5 than at 6.2 which was similar to our soil. This agree with most concept of P availability in soils.

Incubation experiment

Incubation experiment was done to test whether the selected amendments could increase P-CaCl² when incubated for different time intervals. Our results clearly demonstrate that addition of citric acid, elemental sulphur, PSB and sodium silicate significantly increased the amount of P-CaCl2 extraction in comparison to control. Generally, the effectiveness was highest for PSB and sodium silicate.

PSB releases organic acid and organic acids helps to enhances P-CaCl₂ in soil. Studies by Gang et al. (2012) indicated that organic acids accelerates the weathering of stable P pool, which would be significant for increasing P-intensity in soil. In our study on day 12, PSB has suddenly peaked P-CaCl₂ extraction. Similar result was also observed by De Bolle et al. (2013). Main reason behind this could be bacteria becoming more active during that time and they might have released more phosphates enzyme which helped in the weathering of stable P pool.

Na₂SiO₃ produced higher P-CaCl₂ after PSBs in the incubation experiment. For the acid sandy soil sodium silicate is effective in two ways. One is the competition for the binding site and another is alkalizing effect. According to Sandim et al. (2014), silicate increase the soil P availability because the silicate anion occupies sites of phosphate anion adsorption and saturates sites where P could be adsorbed. The higher competition for the same adsorption site between Si and P could reduce the P fixation. Similarly, the alkalizing power of Si also helps in solubilizing P because in acidic soil P in soil reacts with Fe and Al and make them insoluble but when pH increases phosphates are released from them.

After PSB and sodium silicate, citric acid extract higher amount of P-CaCl₂. For citric acid more effect has been observed on the initial stage of incubation. Result shows, on 3rd day of incubation (2.10 mg/Kg dry soil) which goes on decreasing afterwards and on day 12 P--CaCl_2 measured is even lower than control. According to Jones et al. (2003) addition of citric acid to soil release protons which can be involved in dissolution, complexation, hydroxylation and exchange reactions with the soil's solution and solid phases which release phosphates in soil solution.

In the shaking experiment sulphate was used but in incubation experiment and greenhouse experiment we decided to use elemental sulphur. As sulphur has both pH effect and competition for binding sites elemental sulphur was chosen. Elemental Sulphur has extracted more P-CaCl₂ in incubation experiment. Jaggi et al. (2005) have reported that the change in soil pH causes mineralization of P into inorganic forms as well as liberation of Al and Fe ions, which reacts with sulphates and bind only few phosphate ions. They also reported addition of elemental sulphur improves the availability of P in cultivated soils, irrespective of the initial soil pH. Results of Skwierawska and Zawartka (2009) also shows that elemental sulphur has pH effect on P availability in addition to binding effect. But, during incubation experiment, elemental Sulphur has worked slowly in comparison to other amendments. The reason behind this could be that the elemental sulphur was used in powder form and it might have taken time to oxidized. From incubation experiment citric acid, sodium silicate, elemental sulphur are found to be better performing.

Greenhouse experiment

In order to access the amount of P release by the soil as a result of P removal (P uptake by the crop) P-CaCl₂ is measured. P-CaCl₂ decreases to the end of the experiment it is because P- $CaCl₂$ is taken up by grass. PSB, Citric acid and Na₂SiO₃ show an initial increase in P-CaCl₂ indicating these amendments can make more P-CaCl₂ available. Pots treated with PSB and sodium silicate measured higher amount of P-CaCl₂ for all harvesting time. In site Van Oeckel there is initial increase in $P\text{-}CaCl₂$. That could be because of some mineralization, then $P\text{-}CaCl₂$ is normal as it is taken up by plants. For all amendments $P-CaCl₂$ remains higher than control which indicates that P-intensity has increased in pots by application of amendments. P-CaCl₂ for pot treated with citric acid remain higher until day 60 because very few seeds have been germinated in pots treated with citric. Effect of amendments on P-CaCl₂ is higher than control for all time. This proves that amendments are working better than control for all harvesting time. In case of Sulphur it has higher effect on day 40 and 60 for both soils because sulphur was used in powder form and it took time to oxidize which is similar result as seen in incubation experiment.

Effect of pH-H2O on P-CaCl² extracted

Amendments are found to have different pH effects. In both sites, elemental sulphur is acidifying whereas citric acid and PSB has similar pH as control. The use of sodium silicate has also benefit in increasing pH in addition to increasing P-CaCl₂. Similarly, by using PSB as an amendment has not changed the pH of the soil. For sulphur change in pH has been observed with the time of incubation. This could be because of use of powder sulphur which might have taken time to oxidize. Similarly, the use of sulphur has further decreased the pH of the soil. So, we have to be careful while using elemental Sulphur because it is further adding acidity to the soil which is already acidic.

Determination of dry matter

Like in other results, PSB and sodium silicate produced much higher amount of both above and below ground biomass in both sites. The positive effect of increase in biomass on pot treated with PSB and sodium silicate can be attributed mainly to an increase in the availability of P to plants. Since, PSB and sodium silicate change the pH in opposite directions as compared to the control the amount to dry matter production can attributed to the pH effect caused by the amendments. PSB used in our experiment was grown in a Caesin soya bean digest medium. It contained nutrients like pancreatic digestion of casein (17 g/L) , enzymatic digest of soya bean (3 g/L), dipotassium hydrogen phosphate (2.5 g/L). So, the effect of PSB on the dry matter yield can also be attributed to some of the nutrients effect.

On day 20 all amendments produced similar amount of below ground biomass except citric acid which produced much lower. As growing period was small on day 20 all treatment could have performed similar. But, on day 40 and 60 PSB have produced higher amount of dry matter in comparison to other amendments. Similarly, Citric acid has significant negative mean difference with other amendments on day 40 and 60 suggesting its lowest yield. Citric acid has extracted more P both in shaking experiment and incubation experiment. But, it's performance on seed germination has been found really poor. Since, the sampled soil is acidic sandy soil pH effect might have cause Al toxicity which could have affected on seed germination.

In case of sulphur dry matter yield is not so high as compared to the control. As, the sulphur has added further acidity to soil it might have led to less dry matter yield. Motowicka-Terelak and Terelak (1998) demonstrated that sulphur, by binding aluminum sulphate, reduced phosphorus fixation in soil, while excessive amounts of sulphates may result in incomplete utilization of phosphorus, as they inhibit the growth of crops. So, in acidic soil sulphur might have growth inhibiting effects on crops.

P uptake by grass

Dry matter production on both above and below ground was found significantly influenced by treatments. P uptake by grass is directly related with the amount of P available on soil for plants. According to Mullins (2009), P concentrations in plant typically range from 0.1 to 0.5% on a dry weight which is similar to our findings. Results shows addition of sodium silicate and PSB increased P uptake in the grass during all three different harvesting period. Research by Roy et al. (1971) have also reported the effect of silicate has increased P nutrition in sugarcane plants. Similarly, Pulz et al. (2008) found higher P availability in soil in potato plants after silicate application. The reason for increase in P level because of sodium silicate could be due to release of P in soil by the competition for binding sites. Castro et al. (2013) have also reported that the P level of leaves were increased by silicate application and could be associated to competition between silicate $(H_3SiO_4^-)$ and phosphate $(H_2PO_4^-)$ for same absorption sites (Castro et al., 2013).

P content in grass is lowest for citric acid which is different than expected. Citric acid has increased the amount of P-CaCl₂ but it doesn't have good germination effect on grass. This effect could be because of acidic nature of citric acid. As the soil is already acidic, use of citric acid has further lowered the pH of soil affecting on germination as well as P uptake. Similar, effect is seen for elemental sulphur also though the effect is not that bad for sulphur as in the case of citric acid. For sulphur P uptake has been found to be higher on 40 days. This fact confirms that sulphur makes P available later than other amendments. Sodium silicate shows its effect from the beginning as P-uptake proving that sodium silicate has early action on P availability. Similarly, for PSB P uptake has remained higher in all growing period. PSB was grown in the nutrient medium and there could be some additional nutrient effect of growing medium on P-uptake.

Chapter 6 Conclusion and recommendations

Flanders legislation has restricted P fertilization on acid sandy soils with high PSD which ultimately should result in P mining. A way to bring phosphate saturated soils back to environmental safe P level is by P mining through plants. In this context soil amendments could be very useful for increasing mining efficiency over time.

Shaking experiment was done to select the most promising amendments and concentrations to increase plant available phosphorus (P intensity). P extracted by amendments at all concentration after shaking experiment remained higher than control but P extracted by lactic acid and sodium sulphate is considerably lower. P-extraction is highest for 2 mM citric acid, and sodium silicate.

Incubation experiment was done to selected amendments that increase $P-CaCl₂$ when incubated for different time intervals. During incubation experiment PSB gave higher amount of P-CaCl₂ on day 12 and sodium silicate produced highest amount of $P-CaCl₂$ on day 24. Elemental sulphur showed its effect slowly and citric acid didn't perform well. PSB and sodium silicate remain higher during all the incubation time.

Greenhouse experiment was done to test if the use of amendments really increased crop yield and P uptake. In greenhouse experiment PSB and sodium silicate was found to be best performing. For citric acid germination was very poor. PSB yield highest amount of dry matter and also the P-uptake was higher. Similarly, sodium silicate also significantly increased the dry matter yield and P-uptake was also higher for plants treated with PSB.

During this three step experiment mode of action of amendment was also studied. Mode of action for organic acid could be competition for binding sites. For sodium silicate both pH effect and competition for binding site could be responsible for liberating phosphates. Similarly, for PSB main mode of action could be because of release of organic acid and phosphatase enzyme. No, pH effect has been observed for PSB. For sulphur both competition for binding sites and pH effect is possible.

In conclusion, PSB and sodium silicate can be effective in acid sandy soil of Flanders for increasing P-intensity and increasing P-mining in soil. But, still more potting experiment for different crops is recommended. PSB grown only with physiological water can give real understanding on PSB effect but for this experiment we used Casein soya bean digest medium so this might have nutrients effect on dry matter yield and P uptake as well. Hence, we recommend to use PSB grown on physiological water in further potting experiment. In future this study could be replicated with different crops and also in agricultural farm to have a better understanding of the effect of amendments on P mobilization and availability to plants.

References

- Alcarde, J., and Rodella, A. (2003). Quality and legislation of fertilizers and correctives. *Curi, N.; Marques, JJ*, 291-334.
- Amery, F., and Schoumans, O. (2014). "Agricultural phosphorus legislation in Europe," Institute for Agricultural and Fisheries Research (ILVO).
- Amery, F., and Vandecasteele, B. (2013). The phosphorus cycle in North-West European agricultural soils. *In* "NUTRIHORT: Nutrient management, nutrient legislation and innovative techniques in intensive horticulture" (B. V. Karoline D'Haene, Raf De Vis, Sara Crappé, Danny Callens, Els Mechant, Georges Hofman, Stefaan De Neve, ed.), pp. 124-129, Ghent, Belgium.
- Bastounopoulou, M., Gasparatos, D., Haidouti, C., and Massas, I. (2011). Chemical fractionation and sorption of phosphorus in greek inceptisols. *Journal of Agricultural Science and Tehnology* **1**, 33-38.
- Bolan, N. S., Naidu, R., Mahimairaja, S., and Baskaran, S. (1994). Influence of low-molecularweight organic acids on the solubilization of phosphates. *Biology and Fertility of Soils* **18**, 311-319.
- Breeuwsma, A., Reijerink, J., and Schoumans, O. (1995). Impact of manure on accumulation and leaching of phosphate in areas of intensive livestock farming.
- Carvalho, R., Furtini Neto, A., Curi, N., Fernandes, L., and Oliveira Junior, A. (2000). Dessorção de fósforo por silício em solos cultivados com eucalipto. *Revista Brasileira de Ciência do Solo* **24**, 69-74.
- Castro, G., Spadotti, A., Crusciol, C., and Alexandre, C. (2013). Yield and mineral nutrition of soybean, maize, and Congo signal grass as affected by limestone and slag. *Pesquisa Agropecuária Brasileira* **48**, 673-681.
- Castro, G. S. A., and Crusciol, C. A. C. (2013). Yield and mineral nutrition of soybean, maize, and Congo signal grass as affected by limestone and slag. *Pesquisa Agropecuária Brasileira* **48**, 673-681.
- Chardon, W. J., and Schoumans, O. F. (2007). Soil texture effects on the transport of phosphorus from agricultural land in river deltas of Northern Belgium, The Netherlands and North-West Germany. *Soil Use and Management* **23**, 16-24.
- Clercq, P., Gertsis, A., Hofman, G., Jarvis, S., Neeteson, J., and Sinabell, F. (2001). Nutrient management legislation in European countries.
- Condron, L. M., Turner, B. L., Cade-Menun, B. J., Sims, J., and Sharpley, A. (2005). Chemistry and dynamics of soil organic phosphorus. *Phosphorus: Agriculture and the environment*, 87-121.
- Cooper, J., Lombardi, R., Boardman, D., and Carliell-Marquet, C. (2011). The future distribution and production of global phosphate rock reserves. *Resources, Conservation and Recycling* **57**, 78-86.
- De Bolle, S., Gebremikael, M. T., Maervoet, V., and De Neve, S. (2013). Performance of phosphate-solubilizing bacteria in soil under high phosphorus conditions. *Biology and fertility of soils* **49**, 705-714.
- De Schrijver, A., Vesterdal, L., Hansen, K., De Frenne, P., Augusto, L., Achat, D. L., Staelens, J., Baeten, L., De Keersmaeker, L., and De Neve, S. (2012). Four decades of postagricultural forest development have caused major redistributions of soil phosphorus fractions. *Oecologia* **169**, 221-234.
- Earl, K., Syers, J., and McLaughlin, J. (1979). Origin of the effects of citrate, tartrate, and acetate on phosphate sorption by soils and synthetic gels. *Soil Science Society of America Journal* **43**, 674-678.
- Gądor, J., and Motowicka-Terelak, T. (1986). Wpływ zasiarczenia gleb na ich właœciwoœci oraz na plonowanie roœlin w doœwiadczeniu lizymetrycznym. *Cz. II. Wpływ zasiarczania gleb siarką elementarną na plonowanie i skład chemiczny roœlin uprawnych. Pam. Puł* **88**, 25-37.
- Gang, X., Hongbo, S., Rongfu, X., Nie, Y., Pei, Y., Sun, Z., and Blackwell, M. (2012). The role of root-released organic acids and anions in phosphorus transformations in a sandy loam soil from Yantai, China. *Afr J Microbiol Res* **6**, 674-679.
- Gardner, W., and Boundy, K. (1983). The acquisition of phosphorus by Lupinus albus L. IV. The effect of interplanting wheat and white lupin on the growth and mineral composition of the two species. *Plant and Soil*, 391-402.
- Gee, G. W., and Dani, O. (2002). "Particle-size analysis," SSSA, Madison, WI.
- Geelhoed, J., Van Riemsdijk, W., and Findenegg, G. (1999). Simulation of the effect of citrate exudation from roots on the plant availability of phosphate adsorbed on goethite. *European Journal of Soil Science* **50**, 379-390.
- Germida, J., and Janzen, H. (1993). Factors affecting the oxidation of elemental sulfur in soils. *Fertilizer research* **35**, 101-114.
- Goldberg, S., and Sposito, G. (1984). A Chemical Model of Phosphate Adsorption by Soils: I. Reference Oxide Minerals1. *Soil Science Society of America Journal* **48**, 772-778.
- Gustafsson, J. P., Mwamila, L. B., and Kergoat, K. (2012). The pH dependence of phosphate sorption and desorption in Swedish agricultural soils. *Geoderma* **189–190**, 304-311.
- Havlin, J. L., Beaton, J. D., Tisdale, S. L., and Nelson, W. L. (2005). "Soil fertility and fertilizers: An introduction to nutrient management," Pearson Prentice Hall Upper Saddle River, NJ.
- Heckenmüller, M., Narita, D., and Klepper, G. (2014). "Global availability of phosphorus and its implications for global food supply: An economic overview." Kiel Working Paper.
- Hooda, P., Rendell, A., Edwards, A., Withers, P., Aitken, M., and Truesdale, V. (2000). Relating soil phosphorus indices to potential phosphorus release to water. *Journal of Environmental Quality* **29**, 1166-1171.
- Igual, J. M., Valverde, A., Cervantes, E., and Velázquez, E. (2001). Phosphate-solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. *Agronomie* **21**, 561-568.
- Illmer, P., and Schinner, F. (1995). Solubilization of inorganic calcium phosphates— Solubilization mechanisms. *Soil Biology and Biochemistry* **27**, 257-263.
- Jaggi, R., Aulakh, M., and Sharma, R. (2005). Impacts of elemental S applied under various temperature and moisture regimes on pH and available P in acidic, neutral and alkaline soils. *Biology and Fertility of Soils* **41**, 52-58.
- Jarvie, H. P., Sharpley, A. N., Spears, B., Buda, A. R., May, L., and Kleinman, P. J. A. (2013). Water Quality Remediation Faces Unprecedented Challenges from "Legacy Phosphorus". *Environmental Science & Technology* **47**, 8997-8998.
- Jones, D. (1998). Organic acids in the rhizosphere a critical review. *Plant and Soil* **205**, 25- 44.
- Jones, D., Dennis, P., Owen, A., and Van Hees, P. (2003). Organic acid behavior in soils– misconceptions and knowledge gaps. *Plant and Soil* **248**, 31-41.
- Khademi, Z., Jones, D. L., Malakouti, M. J., and Asadi, F. (2010). Organic acids differ in enhancing phosphorus uptake by Triticum aestivum L.—effects of rhizosphere concentration and counterion. *Plant and Soil* **334**, 151-159.
- Koerselman, W., Van Kerkhoven, M. B., and Verhoeven, J. T. (1993). Release of inorganic N, P and K in peat soils; effect of temperature, water chemistry and water level. *Biogeochemistry* **20**, 63-81.
- Krol, M., Kobus, J., and Maziarczyk, B. (1986). Biological and chemical changes occurring in different soils under the influence of elementary sulphur application. *Pam. Pu³* **88**, 39- 55.
- Lamers, L. P., Tomassen, H. B., and Roelofs, J. G. (1998). Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. *Environmental Science & Technology* **32**, 199- 205.
- Lindsay, W. L. (1979). "Chemical equilibria in soils," John Wiley and Sons Ltd.
- Maguire, R., and Sims, J. (2002). Measuring agronomic and environmental soil phosphorus saturation and predicting phosphorus leaching with Mehlich 3. *Soil Science Society of America Journal* **66**, 2033-2039.
- Manna, M. C., Ghosh, P. K., Ghosh, B. N., and Singh, K. N. (2001). Comparative effectiveness of phosphate-enriched compost and single superphosphate on yield, uptake of nutrients and soil quality under soybean–wheat rotation. *The Journal of Agricultural Science* **137**, 45-54.
- Motowicka-Terelak, T., and Terelak, H. (1998). "Siarka w glebach Polski: stan i zagrożenie," Państwowa Inspekcja Ochrony Środowiska.
- Mullins, G. L. (2009). "Phosphorus, agriculture & the environment," Department of Crop and Soil Environmental Sciences, Virginia State University, Virginia.
- Murphy, J., and Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**, 31-36.
- Parks, E. J., Olson, G. J., Brinckman, F. E., and Baldi, F. (1990). Characterization by high performance liquid chromatography (HPLC) of the solubilization of phosphorus in iron ore by a fungus. *Journal of industrial microbiology* **5**, 183-189.
- Pierzynski, G. M. (1991). The chemistry and mineralogy of phosphorus in excessively fertilized soils. *Critical Reviews in Environmental Science and Technology* **21**, 265- 295.
- Pierzynski, G. M., McDowell, R. W., Sims, J., and Sharpley, A. (2005a). Chemistry, cycling, and potential movement of inorganic phosphorus in soils. *Phosphorus: Agriculture and the environment*, 53-86.
- Pierzynski, G. M., Vance, G. F., and Sims, J. T. (2005b). "Soils and environmental quality," CRC press.
- Pulz, A. L., Crusciol, C. A. C., Lemos, L. B., and Soratto, R. P. (2008). Influência de silicato e calcário na nutrição, produtividade e qualidade da batata sob deficiência hídrica. *Revista Brasileira de Ciência do Solo* **32**, 1651-1659.
- Pypers, P., Delrue, J., Diels, J., Smolders, E., and Merckx, R. (2006). Phosphorus intensity determines short-term P uptake by pigeon pea (Cajanus cajan L.) grown in soils with differing P buffering capacity. *Plant and soil* **284**, 217-227.
- Renneson, M., Vandenberghe, C., Marcoen, J. M., Bock, L., and Colinet, G. (2010). Study of the relevance of phosphorus saturation in Walloon Region (Southern Belgium). *In* "Proceedings of the 19th World Congress of Soil Science; Soil Solutions for a changing World". IUSS.
- Roy, A. C., Ali, M. Y., Fox, R. L., and Silva, J. A. (1971). Influence of calcium silicate on phosphate solubility and availability in Hawaiian latosols. *In* "Symposium on soil fertility and evaluation", pp. 756-765.
- Sandim, A. d. S., Büll, L. T., Furim, A. R., Lima, G. d. S., and Garcia, J. L. N. (2014). Phosphorus availability in oxidic soils treated with lime and silicate applications. *Revista Brasileira de Ciência do Solo* **38**, 1215-1222.
- Schoumans, O., and Groenendijk, P. (2000). Modeling soil phosphorus levels and phosphorus leaching from agricultural land in the Netherlands. *Journal of Environmental Quality* **29**, 111-116.
- Schoumans, O. F., Bouraoui, F., Kabbe, C., Oenema, O., and van Dijk, K. C. (2015). Phosphorus management in Europe in a changing world. *Ambio* **44**, 180-192.
- Sharma, N. C., Starnes, D. L., and Sahi, S. V. (2007). Phytoextraction of excess soil phosphorus. *Environmental Pollution* **146**, 120-127.
- Sharpley, A. N. (1995). Soil phosphorus dynamics: agronomic and environmental impacts. *Ecological Engineering* **5**, 261-279.
- Shenoy, V. V., and Kalagudi, G. M. (2005). Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnology Advances* **23**, 501-513.
- Skwierawska, M., and Zawartka, L. (2009). Effect of different rates and forms of sulphur on content of available phosphorus in soil. *Journal of Elementology* **14**.
- Smyth, T. J., and Sanchez, P. A. (1980). Effects of lime, silicate, and phosphorus applications to an Oxisol on phosphorus sorption and ion retention. *Soil Science Society of America Journal* **44**, 500-505.
- Spohn, M., and Kuzyakov, Y. (2013). Phosphorus mineralization can be driven by microbial need for carbon. *Soil Biology and Biochemistry* **61**, 69-75.
- Stevenson, F. J., and Cole, M. A. (1999). "Cycles of soils: carbon, nitrogen, phosphorus, sulfur, micronutrients," John Wiley & Sons.
- Strobel, B. W. (2001). Influence of vegetation on low-molecular-weight carboxylic acids in soil solution—a review. *Geoderma* **99**, 169-198.
- Sylvain, P., and Thomas, N. (2013). Why do we have to increase P use efficiency and recycling in cropping systems? *In* "NUTRIHORT: Nutrient management, innovative techniques and nutrient legislation in intensive horticulture for an improved water quality" (B. V. Karoline D'Haene, Raf De Vis, Sara Crappé, Danny Callens, Els Mechant, Georges Hofman, Stefaan De Neve, ed.), pp. 111-115, Ghent, Belgium.
- Tisdale, S. L., Nelson, W. L., and Beaton, J. D. (1985). "Soil fertility and fertilizers," Collier Macmillan Publishers.
- Trivedi, P., and Sa, T. (2008). Pseudomonas corrugata (NRRL B-30409) mutants increased phosphate solubilization, organic acid production, and plant growth at lower temperatures. *Current microbiology* **56**, 140-144.
- Turner, B. L., Cade-Menun, B. J., Condron, L. M., and Newman, S. (2005). Extraction of soil organic phosphorus. *Talanta* **66**, 294-306.
- Van der Zee, S. E., and Van Riemsdijk, W. H. (1988). Model for long-term phosphate reaction kinetics in soil. *Journal of Environmental Quality* **17**, 35-41.
- Van der Zee, S. E. A. T. M., Van Riemsdijk, W.H. & De Haan, F.A.M. (1990). "The protocol of phosphate saturated soils Part I," Faculty of agriculture, Wageningen, The Netherlands.
- Van Meirvenne, M., Tariku, M., De Neve, S., Hofman, G., Salomez, J., and De Bolle, S. (2008). Afbakening van de fosfaatverzadigde gebieden in Vlaanderen op basis van een kritische fosfaatverzadigingsgraad van 35%. *Ghent University, Ghent*.
- Van Raij, B. (1998). Bioavailable tests: alternatives to standard soil extractions. *Communications in Soil Science & Plant Analysis* **29**, 1553-1570.
- Van Rotterdam-Los, D., Reijneveld, A., van Oostrum, M., Abbink, G., and Bussink, W. (2013). A more turstworthy P recommendation by implementing the intesnity, buffering capacity, quantity concent into agricultural practice. *In* "NUTRIHORT: Nutrient management, innovative techniques and nutrient legislation in intensive horticulture for an improved water quality. " (B. V. Karoline D'Haene, Raf De Vis, Sara Crappé, Danny Callens, Els Mechant, Georges Hofman, Stefaan De Neve, ed.), pp. 136-141, Ghent, Belgium.
- Van Steertegem, M. (2012). MIRA indicatorrapport 2011. *Milieurapport Vlaanderen, Vlaamse Milieumaatschappij, Aalst, Belgium*.
- Vazquez, P., Holguín, G., Puente, M., Lopez-Cortes, A., and Bashan, Y. (2000). Phosphatesolubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils* **30**, 460-468.
- VLM (1997). Phosphate saturation of sandy acid soil in Flanders. *Vlaamse Landmaatschappij, Brussels*.
- VLM (2007). Afbakening van de fosfaatverzadigde gebieden in Vlaanderen op basis van een kritische fosfaatverzadigingsgraad van 35%. *Vlaamse Land Maatschappij*.
- Walkley, A., and Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science* **37**, 29-38.
- Wei, L. L., Chen, C. R., and Xu, Z. H. (2009). The effect of low-molecular-weight organic acids and inorganic phosphorus concentration on the determination of soil phosphorus by the molybdenum blue reaction. *Biology and Fertility of Soils* **45**, 775-779.
- White, R. E. (2013). "Principles and practice of soil science: the soil as a natural resource," John Wiley & Sons.
- Yadav, K., and Singh, T. (1991). Phosphorus solubilization of microbial isolate from a calcifluvent. *Journal of the Indian Society of Soil Science* **39**, 89-93.

Annex

ANOVA analysis of shaking experiment

Tests of Between-Subjects Effects

a. R Squared = ,993 (Adjusted R Squared = ,989)

Tests of Between-Subjects Effects

a. R Squared = ,992 (Adjusted R Squared = ,989)

Tests of Between-Subjects Effects^a

a. sites = E13.0130

b. R Squared = ,991 (Adjusted R Squared = ,987)

Tests of Between-Subjects Effects^a Dependent Variable: P (mg/kg soil)

a. sites = Van Oeckel

b. R Squared = ,994 (Adjusted R Squared = ,991)

Tests of Between-Subjects Effects^a

 $a.$ sites = E13.0130, amendments = calcium chloride

b. R Squared = ,886 (Adjusted R Squared = ,847)

Tests of Between-Subjects Effects^a

a. sites = E13.0130, amendments = citric acid

b. R Squared = ,982 (Adjusted R Squared = ,976)

Tests of Between-Subjects Effects^a

 $a.$ sites = E13.0130, amendments = lactic acid

b. R Squared = ,431 (Adjusted R Squared = ,241)

Tests of Between-Subjects Effects^a

 $a.$ sites = E13.0130, amendments = oxalic acid

b. R Squared = ,965 (Adjusted R Squared = ,953)

Tests of Between-Subjects Effects^a

a. sites = $E13.0130$, amendments = sodium silicate

b. R Squared = ,981 (Adjusted R Squared = ,974)

Tests of Between-Subjects Effects^a

 $a.$ sites = E13.0130, amendments = sodium sulphate

b. R Squared = ,761 (Adjusted R Squared = ,681)

Tests of Between-Subjects Effects^a

a. sites = Van Oeckel, amendments = calcium chloride

b. R Squared = ,878 (Adjusted R Squared = ,838)

a. sites = Van Oeckel, amendments = citric acid

b. R Squared = ,989 (Adjusted R Squared = ,986)

Tests of Between-Subjects Effects^a

a. sites = Van Oeckel, amendments = lactic acid

b. R Squared = ,764 (Adjusted R Squared = ,686)

Tests of Between-Subjects Effects^a

a. sites = Van Oeckel, amendments = oxalic acid

b. R Squared = ,963 (Adjusted R Squared = ,950)

Tests of Between-Subjects Effects^a

a. sites = Van Oeckel, amendments = sodium silicate

b. R Squared = ,972 (Adjusted R Squared = ,963)

a. sites = Van Oeckel, amendments = sodium sulphate

b. R Squared = ,103 (Adjusted R Squared = -,196)

Post hoc analysis of amount of P (mg p/kg dry soil) and concentration for each sites

Dependent Variable: P (mg/kg soil) Tukey HSD

Multiple Comparisons^a

Based on observed means.

The error term is Mean Square(Error) = $4,721$.

*. The mean difference is significant at the 0,05 level.

a. sites = E13.0130, amendments = citric acid

Multiple Comparisons^a

Based on observed means.

The error term is Mean Square(Error) = ,285.

a. sites = E13.0130, amendments = lactic acid

Multiple Comparisons^a

Dependent Variable: P (mg/kg soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $3,270$.

*. The mean difference is significant at the 0,05 level.

a. sites = E13.0130, amendments = oxalic acid

Multiple Comparisons^a

 $-1,8392$

7,7464

-8,5885

19,2885

14,4957

Based on observed means.

The error term is Mean Square(Error) = $1,390$.

*. The mean difference is significant at the 0,05 level.

a. sites = $E13.0130$, amendments = sodium silicate

Multiple Comparisons^a

Dependent Variable: P (mg/kg soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $,067$.

*. The mean difference is significant at the 0,05 level.

 $a.$ sites = E13.0130, amendments = sodium sulphate

Multiple Comparisons^a

Dependent Variable: P (mg/kg soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $2,879$.

*. The mean difference is significant at the 0,05 level.

a. sites = Van Oeckel, amendments = citric acid

Multiple Comparisons^a

Dependent Variable: P (mg/kg soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $,090$.

*. The mean difference is significant at the 0,05 level.

a. sites = Van Oeckel, amendments = lactic acid

Multiple Comparisons^a

Dependent Variable: P (mg/kg soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = 2,436.

*. The mean difference is significant at the 0,05 level.

a. sites = Van Oeckel, amendments = oxalic acid

Multiple Comparisons^a

Dependent Variable: P (mg/kg soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $2,001$.

*. The mean difference is significant at the 0,05 level.

a. sites = Van Oeckel, amendments = sodium silicate

Multiple Comparisons^a

Based on observed means.

The error term is Mean Square(Error) = $,059$.

a. sites = Van Oeckel, amendments = sodium sulphate

ANOVA analysis for data splitted according to sites and concentration

a. site = E13.0130, concentration = $,5$

b. R Squared = ,919 (Adjusted R Squared = ,885)

a. site = E13.0130, concentration = 1,0

b. R Squared = ,949 (Adjusted R Squared = ,928)

Tests of Between-Subjects Effects^a

a. site = $E13.0130$, concentration = $2,0$

b. R Squared = ,982 (Adjusted R Squared = ,974)

Tests of Between-Subjects Effects^a

a. site = Van Oeckel, concentration = 5

b. R Squared = ,963 (Adjusted R Squared = ,948)

Tests of Between-Subjects Effects^a Dependent Variable: Amount of P (mg P/kg dry soil)

a. site = Van Oeckel, concentration = 1,0

b. R Squared = ,974 (Adjusted R Squared = ,963)

Tests of Between-Subjects Effects^a Dependent Variable: Amount of P (mg P/kg dry soil)

a. site = Van Oeckel, concentration = 2,0

b. R Squared = ,987 (Adjusted R Squared = ,982)

Post Hoc analysis

Multiple Comparisons^a

Dependent Variable: Amount of P (mg P/kg dry soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $2,981$.

*. The mean difference is significant at the 0,05 level.

a. site = $E13.0130$, concentration = $,5$

Multiple Comparisons^a

Dependent Variable: Amount of P (mg P/kg dry soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $3,384$.

*. The mean difference is significant at the 0,05 level.

a. site = E13.0130, concentration = $1,0$

Multiple Comparisons^a

Dependent Variable: Amount of P (mg P/kg dry soil) Tukey HSD

The error term is Mean Square(Error) = 6,205.

*. The mean difference is significant at the 0,05 level.

a. site = E13.0130, concentration = 2,0

Multiple Comparisons^a

Dependent Variable: Amount of P (mg P/kg dry soil) Tukey HSD

The error term is Mean Square(Error) = 1,719.

*. The mean difference is significant at the 0,05 level.

a. site = Van Oeckel, concentration = ,5

Multiple Comparisons^a

Dependent Variable: Amount of P (mg P/kg dry soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = 2,715.

*. The mean difference is significant at the 0,05 level.

a. site = Van Oeckel, concentration = $1,0$

Multiple Comparisons^a Dependent Variable: Amount of P (mg P/kg dry soil) Tukey HSD

		Mean			95% Confidence Interval	
(I) amendment	(J) amendment	Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
calcium chloride	citric acid	$-38,5054$ [*]	1,76204	,000	$-44,4239$	$-32,5868$
	lactic acid	$-1,2843$	1,76204	,974	$-7,2029$	4,6343
	oxalic acid	$-18,9823$ [*]	1,76204	,000	$-24,9008$	$-13,0637$
	sodium silicate	$-32,4873$ [*]	1,76204	,000	$-38,4059$	$-26,5688$
	sodium sulphate	$-2,1934$	1,76204	,808	$-8,1120$	3,7252
citric acid	calcium chloride	38,5054	1,76204	,000	32,5868	44,4239
	lactic acid	37,2211	1,76204	,000	31,3025	43,1396
	oxalic acid	19,5231	1,76204	,000	13,6045	25,4417
	sodium silicate	6,0180 [*]	1,76204	,045	,0995	11,9366
	sodium sulphate	36,3120*	1,76204	,000	30,3934	42,2305
lactic acid	calcium chloride	1,2843	1,76204	,974	$-4,6343$	7,2029
	citric acid	$-37,2211$ [*]	1,76204	,000	$-43,1396$	$-31,3025$
	oxalic acid	$-17,6980$ [*]	1,76204	,000	$-23,6166$	$-11,7794$
	sodium silicate	$-31,2030$	1,76204	,000	$-37,1216$	$-25,2845$
	sodium sulphate	-0.9091	1,76204	,994	$-6,8277$	5,0095
oxalic acid	calcium chloride	18,9823	1,76204	,000	13,0637	24,9008
	citric acid	$-19,5231$ [*]	1,76204	,000	$-25,4417$	$-13,6045$
	lactic acid	17,6980*	1,76204	,000	11,7794	23,6166
	sodium silicate	$-13,5050$ [*]	1,76204	,000	$-19,4236$	$-7,5865$
	sodium sulphate	16,7889	1,76204	,000	10,8703	22,7074
sodium silicate	calcium chloride	32,4873	1,76204	,000	26,5688	38,4059
	citric acid	$-6,0180$ [*]	1,76204	,045	$-11,9366$	-0.0995
	lactic acid	31,2030	1,76204	,000	25,2845	37,1216
	oxalic acid	13,5050 [*]	1,76204	,000	7,5865	19,4236
	sodium sulphate	30,2939	1,76204	,000	24,3754	36,2125
sodium sulphate	calcium chloride	2,1934	1,76204	,808	$-3,7252$	8,1120
	citric acid	$-36,3120$	1,76204	,000	$-42,2305$	$-30,3934$
	lactic acid	,9091	1,76204	,994	$-5,0095$	6,8277
	oxalic acid	$-16,7889$ [*]	1,76204	,000	$-22,7074$	$-10,8703$
	sodium silicate	-30.2939 [*]	1,76204	,000	$-36,2125$	-24.3754

The error term is Mean Square(Error) = 4,657.

*. The mean difference is significant at the 0,05 level.

a. site = Van Oeckel, concentration = 2,0

Annex II: Incubation experiment

Tests of Between-Subjects Effects

a. R Squared = ,993 (Adjusted R Squared = ,989)

Tests of Between-Subjects Effects^a

 $a.$ site = E13.130

b. R Squared = ,994 (Adjusted R Squared = ,991)

Tests of Between-Subjects Effects^a

a. site = Van Oeckel

b. R Squared = ,990 (Adjusted R Squared = ,986)

a. site = E13, days of incubation = 3

b. R Squared = ,971 (Adjusted R Squared = ,959)

Tests of Between-Subjects Effects^a

a. site = $E13$, days of incubation = 6

b. R Squared = ,994 (Adjusted R Squared = ,991)

Tests of Between-Subjects Effects^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil)

a. site = E13, days of incubation = 12

b. R Squared = ,998 (Adjusted R Squared = ,997)

Tests of Between-Subjects Effects^a

a. site = $E13$, days of incubation = 24

b. R Squared = ,995 (Adjusted R Squared = ,993)

a. site = $E13$, days of incubation = 48

b. R Squared = ,981 (Adjusted R Squared = ,973)

Tests of Between-Subjects Effects^a

a. site = Van Oeckel, days of incubation = 3

b. R Squared = ,993 (Adjusted R Squared = ,990)

Tests of Between-Subjects Effects^a

 $a.$ site = Van Oeckel, days of incubation = 6

b. R Squared = ,960 (Adjusted R Squared = ,944)

Corrected Total 1,548 14

Tests of Between-Subjects Effects^a

a. site = Van Oeckel, days of incubation = 12

b. R Squared = ,997 (Adjusted R Squared = ,996)

a. site = Van Oeckel, days of incubation = 24

b. R Squared = ,977 (Adjusted R Squared = ,968)

Tests of Between-Subjects Effects^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil)

a. site = Van Oeckel, days of incubation = 48

b. R Squared = ,993 (Adjusted R Squared = ,990)

Post-hoc analysis for amendments on different days of incubation

Multiple Comparisons^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $,003$.

*. The mean difference is significant at the ,05 level.

a. site = E13, days of incubation = 3

Multiple Comparisons^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,002.

*. The mean difference is significant at the ,05 level.

a. site = E13, days of incubation = 6

Multiple Comparisons^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil) Tukey HSD

The error term is Mean Square(Error) = ,003.

*. The mean difference is significant at the ,05 level.

a. site = E13, days of incubation = 12

Multiple Comparisons^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,002.

*. The mean difference is significant at the ,05 level.

a. site $=$ E13, days of incubation $=$ 24

Multiple Comparisons^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil) Tukey HSD

The error term is Mean Square(Error) = $,005$.

*. The mean difference is significant at the ,05 level.

a. site = $E13$, days of incubation = 48

Multiple Comparisons^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,003.

*. The mean difference is significant at the ,05 level.

a. site = Van Oeckel, days of incubation = 3

Multiple Comparisons^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil) Tukey HSD

The error term is Mean Square(Error) = $,006$.

*. The mean difference is significant at the ,05 level.

a. site = Van Oeckel, days of incubation = 6

Multiple Comparisons^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $,002$.

*. The mean difference is significant at the ,05 level.

a. site = Van Oeckel, days of incubation = 12

Tukey HSD (I) amendment (J) amendment Mean Difference $(I-J)$ Std. Error Sig. 95% Confidence Interval Lower Bound | Upper Bound Citric Acid Control -,2235 ,08653 ,147 -,5082 ,0613 Na2SiO3 -1,0725^{*} ,08653 ,000 -1,3572 ,7877 PSB -1,5259^{*} ,08653 ,000 -1,8107 -1,2411 Sulphur -,9292* 08653 ,000 -1,2140 -,6445
08653 ,147 -,0613 ,5082 Control Citric Acid ,2235 ,08653 ,147 -,0613 ,5082 Na2SiO3 -,8490^{*}
PSB -1.3024^{*} ,08653 ,000 ,000 ,08653
,08653 ,000 -1,5872 -1,0176 $-1,3024$ * $-1,5872$

Multiple Comparisons^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil)

The error term is Mean Square(Error) = ,011.

*. The mean difference is significant at the ,05 level.

a. site = Van Oeckel, days of incubation = 24

Multiple Comparisons^a

Dependent Variable: P-CaCl2 (mg P/kg dry soil) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,002.

*. The mean difference is significant at the ,05 level.

a. site = Van Oeckel, days of incubation = 48

Annex III: Greenhouse experiment

ANOVA of P-CaCl²

Tests of Between-Subjects Effects

a. R Squared = $,982$ (Adjusted R Squared = $,973$)

Tests of Between-Subjects Effects^a

Dependent Variable: P mg/kg of soil

a. sites = E13.0130

b. R Squared = ,951 (Adjusted R Squared = ,928)

Tests of Between-Subjects Effects^a

a. sites = Van Oeckel

b. R Squared = ,987 (Adjusted R Squared = ,980)

 $a.$ sites = E13.0130, days after germination = 20

b. R Squared = $,930$ (Adjusted R Squared = $,902$)

Tests of Between-Subjects Effects^a

a. sites = $E13.0130$, days after germination = 40

b. R Squared = $,870$ (Adjusted R Squared = $,818$)

Tests of Between-Subjects Effects^a

a. sites = $E13.0130$, days after germination = 60

b. R Squared = $,949$ (Adjusted R Squared = $,928$)

 $a.$ sites = Van Oeckel, days after germination = 20

b. R Squared = ,982 (Adjusted R Squared = ,975)

Tests of Between-Subjects Effects^a

a. sites = Van Oeckel, days after germination = 40

b. R Squared = ,887 (Adjusted R Squared = ,842)

Tests of Between-Subjects Effects^a

 $a.$ sites = Van Oeckel, days after germination = 60

b. R Squared = $,939$ (Adjusted R Squared = $,915$)

Post hoc for P-CaCl² with different amendments for different time

Multiple Comparisons^a

Dependent Variable: P mg/kg of soil

Tukey HSD

The error term is Mean Square(Error) = $,004$.

*. The mean difference is significant at the 0,05 level.

a. sites = $E13.0130$, days after germination = 20

Multiple Comparisons^a

Dependent Variable: P mg/kg of soil Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,007.

*. The mean difference is significant at the 0,05 level.

a. sites = E13.0130, days after germination = 40

Multiple Comparisons^a

Dependent Variable: P mg/kg of soil Tukey HSD

The error term is Mean Square(Error) = ,005.

*. The mean difference is significant at the 0,05 level.

a. sites = E13.0130, days after germination = 60

Multiple Comparisons^a

Dependent Variable: P mg/kg of soil Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,006.

*. The mean difference is significant at the 0,05 level.

a. sites = Van Oeckel, days after germination = 20

Multiple Comparisons^a

The error term is Mean Square(Error) = ,013.

*. The mean difference is significant at the 0,05 level.

a. sites = Van Oeckel, days after germination = 40

Multiple Comparisons^a

Dependent Variable: P mg/kg of soil Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,002.

*. The mean difference is significant at the 0,05 level.

a. sites = Van Oeckel, days after germination = 60

ANOVA above ground biomass dry weight (g)

Tests of Between-Subjects Effects^a

a. plant part = Above ground

b. R Squared = ,990 (Adjusted R Squared = ,985)

Tests of Between-Subjects Effects^a

a. plant part = Above ground, sites = $E13.0130$, days after germination = 20

b. R Squared = ,453 (Adjusted R Squared = ,234)

Multiple Comparisons^a

The error term is Mean Square(Error) = $.004$.

a. plant part = Above ground, sites = $E13.0130$, days after germination = 20

Tests of Between-Subjects Effects^a

a. plant part = Above ground, sites = $E13.0130$, days after germination = 40

b. R Squared = ,985 (Adjusted R Squared = ,979)

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,014.

*. The mean difference is significant at the 0,05 level.

a. plant part = Above ground, sites = $E13.0130$, days after germination = 40

a. plant part = Above ground, sites = E13.0130, days after germination = 60

b. R Squared = ,978 (Adjusted R Squared = ,969)

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,026.

*. The mean difference is significant at the 0,05 level.

a. plant part = Above ground, sites = $E13.0130$, days after germination = 60

Tests of Between-Subjects Effects^a

a. plant part = Above ground, sites = Van Oeckel, days after germination = 20

b. R Squared = ,774 (Adjusted R Squared = ,684)

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,002.

*. The mean difference is significant at the 0,05 level.

a. plant part = Above ground, sites = Van Oeckel, days after germination = 20

Tests of Between-Subjects Effects^a

 $a.$ plant part = Above ground, sites = Van Oeckel, days after germination = 40

b. R Squared = ,974 (Adjusted R Squared = ,964)

Multiple Comparisons^a

The error term is Mean Square(Error) = ,009.

*. The mean difference is significant at the 0,05 level.

a. plant part = Above ground, sites = Van Oeckel, days after germination = 40

Tests of Between-Subjects Effects^a

a. plant part = Above ground, sites = Van Oeckel, days after germination = 60

b. R Squared = ,992 (Adjusted R Squared = ,989)

Multiple Comparisons^a

The error term is Mean Square(Error) = $,013$.

*. The mean difference is significant at the 0,05 level.

a. plant part = Above ground, sites = Van Oeckel, days after germination = 60

ANOVA below ground biomass dry weight (g)

Tests of Between-Subjects Effects^a

 $a.$ plant part = below ground

b. R Squared = ,973 (Adjusted R Squared = ,960)

Tests of Between-Subjects Effects^a

a. plant part = below ground, sites = E13.0130, days after germination = 20

b. R Squared = ,486 (Adjusted R Squared = ,281)

Multiple Comparisons^a

The error term is Mean Square(Error) = ,004.

a. plant part = below ground, sites = $E13.0130$, days after germination = 20

Tests of Between-Subjects Effects^a

a. plant part = below ground, sites = E13.0130, days after germination = 40

b. R Squared = ,870 (Adjusted R Squared = ,819)

Multiple Comparisons^a

The error term is Mean Square(Error) = ,071.

*. The mean difference is significant at the 0,05 level.

a. plant part = below ground, sites = E13.0130, days after germination = 40

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,030.

*. The mean difference is significant at the 0,05 level.

a. plant part = below ground, sites = $E13.0130$, days after germination = 60

Tests of Between-Subjects Effects^a

a. plant part = below ground, sites = Van Oeckel, days after germination = 20

b. R Squared = ,693 (Adjusted R Squared = ,570)

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,002.

*. The mean difference is significant at the 0,05 level.

a. plant part = below ground, sites = Van Oeckel, days after germination = 20

Tests of Between-Subjects Effects^a

 $a.$ plant part = below ground, sites = Van Oeckel, days after germination = 40

b. R Squared = ,951 (Adjusted R Squared = ,931)

Multiple Comparisons^a

The error term is Mean Square(Error) = ,091.

*. The mean difference is significant at the 0,05 level.

a. plant part = below ground, sites = Van Oeckel, days after germination = 40

Tests of Between-Subjects Effects^a

 \overline{a} . plant part = below ground, sites = Van Oeckel, days after germination = 60

b. R Squared = ,963 (Adjusted R Squared = ,948)

Multiple Comparisons^a

The error term is Mean Square(Error) = ,109.

*. The mean difference is significant at the 0,05 level.

a. plant part = below ground, sites = Van Oeckel, days after germination = 60

ANOVA for total biomass dry weight (g)

Tests of Between-Subjects Effects^a

 $a.$ plant part = total

b. R Squared = ,986 (Adjusted R Squared = ,980)

Tests of Between-Subjects Effects^a

a. plant part = total, sites = $E13.0130$, days after germination = 20

b. R Squared = ,509 (Adjusted R Squared = ,312)

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g) Tukey HSD

The error term is Mean Square(Error) = $,015$.

a. plant part = total, sites = $E13.0130$, days after germination = 20

Tests of Between-Subjects Effects^a

a. plant part = total, sites = E13.0130, days after germination = 40

b. R Squared = ,955 (Adjusted R Squared = ,936)

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g) Tukey HSD

The error term is Mean Square(Error) = $,116$.

*. The mean difference is significant at the 0,05 level.

a. plant part = total, sites = E 13.0130, days after germination = 40

Tests of Between-Subjects Effects^a

a. plant part = total, sites = $E13.0130$, days after germination = 60

b. R Squared = ,981 (Adjusted R Squared = ,973)

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g)

Based on observed means.

The error term is Mean Square(Error) = $,066$.

*. The mean difference is significant at the 0,05 level.

a. plant part = total, sites = $E13.0130$, days after germination = 60

Tests of Between-Subjects Effects^a

 $a.$ plant part = total, sites = Van Oeckel, days after germination = 20

b. R Squared = ,790 (Adjusted R Squared = ,706)

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $,007$.

*. The mean difference is significant at the 0,05 level.

a. plant part = total, sites = \bar{V} an Oeckel, days after germination = 20

Tests of Between-Subjects Effects^a

a. plant part = total, sites = Van Oeckel, days after germination = 40

b. R Squared = ,965 (Adjusted R Squared = ,952)

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,129.

*. The mean difference is significant at the 0,05 level.

a. plant part = total, sites = Van Oeckel, days after germination = 40

Tests of Between-Subjects Effects^a

a. plant part = total, sites = Van Oeckel, days after germination = 60

b. R Squared = ,986 (Adjusted R Squared = ,980)

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g) Tukey HSD

The error term is Mean Square(Error) = ,129.

*. The mean difference is significant at the 0,05 level.

a. plant part = total, sites = \overline{V} an Oeckel, days after germination = 60

ANOVA of above ground biomass P (mg) content

Tests of Between-Subjects Effects^a

a. plant part = Above ground

b. R Squared = ,983 (Adjusted R Squared = ,976)

a. plant part = Above ground, sites = $E13.0130$, days after germination = 20

b. R Squared = ,461 (Adjusted R Squared = ,246)

Multiple Comparisons^a

Dependent Variable: P content (mg)/pot Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,138.

a. plant part = Above ground, sites = E13.0130, days after germination = 20

Tests of Between-Subjects Effects^a

 $a.$ plant part = Above ground, sites = E13.0130, days after germination = 40

b. R Squared = ,969 (Adjusted R Squared = ,956)

Multiple Comparisons^a

The error term is Mean Square(Error) = ,228.

*. The mean difference is significant at the 0,05 level.

a. plant part = Above ground, sites = $E13.0130$, days after germination = 40

Tests of Between-Subjects Effects^a

a. plant part = Above ground, sites = $E13.0130$, days after germination = 60

b. R Squared = ,972 (Adjusted R Squared = ,960)

Multiple Comparisons^a

The error term is Mean Square(Error) = ,151.

*. The mean difference is significant at the 0,05 level.

a. plant part = Above ground, sites = $E13.0130$, days after germination = 60

Tests of Between-Subjects Effects^a

a. plant part = Above ground, sites = Van Oeckel, days after germination = 20

b. R Squared = ,806 (Adjusted R Squared = ,729)

Multiple Comparisons^a

Dependent Variable: P content (mg)/pot Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,074.

*. The mean difference is significant at the 0,05 level.

a. plant part = Above ground, sites = Van Oeckel, days after germination = 20

Tests of Between-Subjects Effects^a

a. plant part = Above ground, sites = Van Oeckel, days after germination = 40

b. R Squared = ,975 (Adjusted R Squared = ,965)

Multiple Comparisons^a

Dependent Variable: P content (mg)/pot Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,194.

*. The mean difference is significant at the 0,05 level.

a. plant part = Above ground, sites = Van Oeckel, days after germination = 40

Tests of Between-Subjects Effects^a

a. plant part = Above ground, sites = Van Oeckel, days after germination = 60

b. R Squared = ,994 (Adjusted R Squared = ,991)

Multiple Comparisons^a

Dependent Variable: P content (mg)/pot Tukey HSD

Based on observed means.

Dependent Variable: P co

The error term is Mean Square(Error) = ,119.

*. The mean difference is significant at the 0,05 level.

a. plant part = Above ground, sites = Van Oeckel, days after germination = 60

ANOVA for Below Ground Biomass P (mg) content

Tests of Between-Subjects Effects^a

a. plant part = below ground

b. R Squared = ,973 (Adjusted R Squared = ,960)

Tests of Between-Subjects Effects^a

 $a.$ plant part = total, sites = E13.0130, days after germination = 20

b. R Squared = ,509 (Adjusted R Squared = ,312)

Multiple Comparisons^a

Dependent Variable: dry biomass wt (g) Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,015.

a. plant part = total, sites = $E13.0130$, days after germination = 20

Tests of Between-Subjects Effects^a

a. plant part = below ground, sites = E13.0130, days after germination = 40

b. R Squared = ,887 (Adjusted R Squared = ,842)

Multiple Comparisons^a

Dependent Variable: P content (mg)/pot Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $,348$.

*. The mean difference is significant at the 0,05 level.

a. plant part = below ground, sites = $E13.0130$, days after germination = 40

Tests of Between-Subjects Effects^a

 $a.$ plant part = below ground, sites = E13.0130, days after germination = 60

b. R Squared = ,947 (Adjusted R Squared = ,925)

Multiple Comparisons^a

The error term is Mean Square(Error) = ,110.

*. The mean difference is significant at the 0,05 level.

a. plant part = below ground, sites = E13.0130, days after germination = 60

Tests of Between-Subjects Effects^a

a. plant part = below ground, sites = Van Oeckel, days after germination = 20

b. R Squared = ,783 (Adjusted R Squared = ,696)

Multiple Comparisons^a

The error term is Mean Square(Error) = ,023.

*. The mean difference is significant at the 0,05 level.

a. plant part = below ground, sites = Van Oeckel, days after germination = 20

Tests of Between-Subjects Effects^a

a. plant part = below ground, sites = Van Oeckel, days after germination = 40

b. R Squared = ,956 (Adjusted R Squared = ,938)

Multiple Comparisons^a

Dependent Variable: P content (mg)/pot Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $,582$.

*. The mean difference is significant at the 0,05 level.

a. plant part = below ground, sites = Van Oeckel, days after germination = 40

Tests of Between-Subjects Effects^a

 $a.$ plant part = below ground, sites = Van Oeckel, days after germination = 60

b. R Squared = ,956 (Adjusted R Squared = ,938)

Multiple Comparisons^a

Based on observed means.

The error term is Mean Square(Error) = $,674$.

*. The mean difference is significant at the 0,05 level.

a. plant part = below ground, sites = Van Oeckel, days after germination = 60

ANOVA for total biomass (P content)

Tests of Between-Subjects Effects^a

 $a.$ plant part = total

b. R Squared = ,984 (Adjusted R Squared = ,977)

Tests of Between-Subjects Effects^a

a. plant part = total, sites = $E13.0130$, days after germination = 20

b. R Squared = $,535$ (Adjusted R Squared = $,349$)

Multiple Comparisons^a

The error term is Mean Square(Error) = ,225.

a. plant part = total, sites = $E13.0130$, days after germination = 20

Tests of Between-Subjects Effects^a

a. plant part = total, sites = $E13.0130$, days after germination = 40

b. R Squared = ,953 (Adjusted R Squared = ,934)

Multiple Comparisons^a

Dependent Variable: P content (mg)/pot Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = $,830$.

*. The mean difference is significant at the 0,05 level.

a. plant part = total, sites = $E13.0130$, days after germination = 40

a. plant part = total, sites = E13.0130, days after germination = 60

b. R Squared = ,976 (Adjusted R Squared = ,967)

Multiple Comparisons^a

Dependent Variable: P content (mg)/pot Tukey HSD

Based on observed means.

The error term is Mean Square(Error) = ,303.

*. The mean difference is significant at the 0,05 level.

a. plant part = total, sites = E 13.0130, days after germination = 60

Dependent Variable: P content (mg)/pot					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6,960 ^b	4	1.740	12.723	,001
Intercept	62,759		62,759	458,938	,000
Amendements	6,960	4	1,740	12.723	,001
Error	1,367	10	,137		
Total	71,086	15			
Corrected Total	8,327	14			

Tests of Between-Subjects Effects^a

 $a.$ plant part = total, sites = Van Oeckel, days after germination = 20

b. R Squared = ,836 (Adjusted R Squared = ,770)

Multiple Comparisons^a

Dependent Variable: P content (mg)/pot

Based on observed means.

The error term is Mean Square(Error) = ,137.

*. The mean difference is significant at the 0,05 level.

a. plant part = total, sites = Van Oeckel, days after germination = 20

Tests of Between-Subjects Effects^a Dependent Variable: Source Type III Sum of df Mean Square F Sig. Corrected Model 392,185^b 4 98,046 85,988 ,000
Intercept 918,692 1 918,692 805,704 ,000 Intercept 918,692 1 918,692 805,704 ,000 Amendements 392,185 4 98,046 85,988 ,000
Error 11.402 10 11.40 Error 11,402 10 1,140 Total 1322,279 15 Corrected Total $403,587$ 14

 $a.$ plant part = total, sites = Van Oeckel, days after germination = 40

b. R Squared = ,972 (Adjusted R Squared = ,960)

Multiple Comparisons^a

The error term is Mean Square(Error) = $1,140$.

*. The mean difference is significant at the 0,05 level.

a. plant part = total, sites = Van Oeckel, days after germination = 40

Tests of Between-Subjects Effects^a

a. plant part = total, sites = Van Oeckel, days after germination = 60

b. R Squared = ,986 (Adjusted R Squared = ,981)

Multiple Comparisons^a

The error term is Mean Square(Error) = ,851.

*. The mean difference is significant at the 0,05 level.

a. plant part = total, sites = Van Oeckel, days after germination = 60