Phosphate saturation and phosphate leaching of acidic sandy soils in Flanders: analysis and mitigation options

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Woord vooraf

Hier is het dan, mijn doctoraat. Op de vakgroep werd ik ook wel eens de "paardenmadam" genoemd. Om mijn naam eer aan te doen, en de mensen niet teleur te stellen door niets over de paarden te zeggen doe ik het hier. Een doctoraat kan je in zekere zin vergelijken met de geboorte en het opleren van een paard.

Eerst wordt er verwachtingsvol uitgekeken naar de geboorte van het veulentje. Wel het starten van een doctoraat is eigenlijk vergelijkbaar. Ik heb mijn thesis gedaan bij Prof. De Neve, hij vroeg me of ik interesse had om te doctoreren of assistent te worden. Ik solliciteerde en werd assistent bij Joost en Stefaan, hiermee was het doctoraat "geboren". Het veulentje groeit op en na een tijdje moet het afgericht worden, wat overeen komt met de beginstadia van een doctoraat. Wat is het probleem met fosfor (P) in de bodem? Wat is de omvang ervan? Hoe kan dit het beste aangepakt worden? Eens afgericht wordt er met het paard op wedstrijd gegaan, met positieve en minder positieve uitslagen. Dit was ook het geval bij het "P" onderzoek. Sommige proeven lukten wel en gaven beloftevolle resultaten, andere lukten niet onmiddellijk wat tot de nodige frustraties leidde, maar net zoals bij het trainen van een paard zette ik door en uiteindelijk lukten de proeven wel, we kregen resultaten en artikels werden aanvaard. En dan komt het "moment suprême", de combinatie (paard en ruiter) is klaar om mee te doen aan de grote wedstrijd. Bvb. Jumping Mechelen of het WK jonge paarden binnenrijden en meedoen voor de ereplaatsen, een goed, foutloos parcours afleggen, met spanning naar de barrage gaan en daarna genieten van de geleverde prestatie. Dat is de kers op de taart voor elke ruiter die houdt van zijn sport en die met plezier beoefent. Wel dit boekje is de kers op de taart van mijn doctoraat, de verdediging ervan is de "moment suprême", de barrage.

De realisatie van dit alles is natuurlijk niet iets wat je in je ééntje kan verwezenlijken. Eerst en vooral wil ik Stefaan, en ook Joost bedanken om mij de kans te geven dit te kunnen doen, om me te begeleiden en te sturen. Daarnaast wil ik Stefaan ook bedanken voor het lezen, corrigeren en bijsturen van mijn "schrijfsels". Bij deze wil ik ook mijn juryleden bedanken om dit doctoraat kritisch en deskundig te lezen en te beoordelen.

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Natuurlijk zijn er ook mensen buiten "den unief" die ik wil bedanken voor hun steun. Veerle, bedankt voor de leuke lunches waarbij alles verteld kon worden en er stoom kon afgelaten worden. Veerle, Liesbeth, Tania en Joke bedankt voor jullie steun eerst tijdens de opleiding en dan tijdens het doctoraat. De maandelijkse uitjes waren een leuke ontspanning en hopelijk hebben we nog veel van deze momenten in de toekomst.

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Mama en papa, bedankt dat jullie me de kans gaven om te studeren en te doctoreren. De laatste maanden waren zwaar, wat mijn humeur zeker niet ten goede kwam. Bedankt voor de steun, het vertrouwen en de mogelijkheden die jullie me gaven om dit tot een goed einde te brengen.

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Sara,
24 september 2013

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List of abbreviations

α	The degree of saturation = 0.5
β	Langmuir affinity constant depending on the soil type
ξ	Resistance of drainage
γ	The rate between the total and reversible sorption
φ	conversion factor from g m^{-2} yr^{-1} to kg ha^{-1} yr^{-1}
λ	conversion factor related to the soil solution ratio of the experiment
ρ_b	Bulk density
Al	Aluminium
$\mathrm{Al_{ox}}$	Ammonium-oxalate-oxalic acid extractable Al
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
B	Maximum adsorption fraction
B.	*Bacillus*
BM	Ball milled
c	Average phosphate concentration above the reference depth
C	Carbon
C	P equilibrium concentration in solution
C_x	background concentration of P in groundwater
$C(x,t)$	P concentration as function of time (t) and depth (x) in the soil profile
Ca	Calcium
CFU	Colony-forming units
Cu	Cupper
D	Depth in the soil layer
DOV	Databank Ondergrond Vlaanderen
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EU	European Union
Fe	Iron
$\mathrm{Fe_{ox}}$	Ammonium-oxalate-oxalic acid extractable Fe
FVG	Fosfaat verzadigingsgraad
GWT	Ground water table

h	depth of the drainage system
ICP	Inductive Coupled Plasma
$J(x,t)$	Lateral water flux
k	Langmuir constant
K	Langmuir sorption parameter
k_d	Langmuir desorption parameter
La	Lanthanum
Li	Liereman
MAP	Manure Action Plan
Mg	Magnesium
MHG	Mean highest groundwater height
MLG	Mean lowest groundwater height
Mn	Manganese
NBRIP	National botanical research institute's phosphate growth medium
N	Nitrogen
NP	Net precipitation
Ni	Nickel
OH^-	hydroxide
OM	Organic Matter
P	Phosphorus
P.	*Pseudomonas*
PAM	Polyacrylamide
P_{lac}	Ammonium lactate extractable P
PLEASE	Phosphorus leaching from soils to the environment
P_{ox}	Ammonium-oxalate-oxalic acid extractable P
ppm	Parts per million
PSB	Phosphate solubilising bacteria
PSC	Phosphate sorption capacity
PSD	Phosphate saturation degree
P_w	Water extractable P
Q	amount of adsorbed P
q_{dr}	Magnitude of the drainage flux

Q_m	Adsorption maximum
Q_{ro}	runoff/leaching flux
R	Addition rate
r	Resistance of the drainage system
S_d	Slow P diffusion precipitation reaction into the soil particles
S	Sensitivity of the model
SENTWA	System for the Evaluation of Nutrient Transport to WAter
SWAT	Soil and Water Assessment Tool
Stdev	Standard deviation
t	Reaction time (day^{-1})
USDA	United States Department of Agriculture
VLM	Vlaamse landmaatschappij, Flemish Land Agency
VMM	Vlaamse milieumaatschappij, Flemish environmental agency
WTR	Water Treatment Residual
WFD	Water Framework Directive
WRB	world reference base for soil recourses
x	Model input
XRD	X-ray diffraction
Y	Model output
z	Depth
Ze	Zedelgem
Zw	Zwevezele

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Chapter 1

Introduction

Illustration on p.1:

A river with algal bloom due to eutrophication

Eutrophication can be defined as ‘the enrichment of water by nutrients causing an accelerated growth of algae and higher forms of plant life to produce undesirable effects resulting from anthropogenic enrichment’ (Csatho et al., 2007). The major effect of eutrophication is the enhanced growth of algae and other aquatic plants (Johnston & Dawson, 2005). Other consequences are a decrease in the ecological quality and biodiversity in the aquatic ecosystem, but also a disturbance in the surrounding terrestrial ecosystem. Finally eutrophication also entails the restriction of the use of surface water for recreational ends or for drinking purposes (Smith et al., 2006).

Whilst both nitrogen (N) and phosphorus (P) contribute to eutrophication, several studies have identified that P is the main limiting nutrient in water bodies (Schindler, 2006; Withers & Haygarth, 2007). It seems of little use to control N if P is not controlled first. With the introduction of the EU Water Framework Directive (WFD) (anonymous, 2000) in December 2000, there is now a legislative framework to implement catchment controls over P inputs to EU waters from all sources, including those from agriculture (Withers & Haygarth, 2007). On one hand, eutrophication is mainly caused by the excessive accumulation of nutrients in the soil which eventually are leached into water bodies (i.e. from non-point source). On the other hand, it is also caused by point sources, such as the discharge of untreated municipal wastewaters with high P concentrates (Schindler, 2006). In many countries, such as Belgium, policy measures were taken to tackle point source pollution through waste water treatment. However, these measures were not adequate enough to end eutrophication as the transfer of P from agricultural land to the water bodies remained unsolved (Schindler, 2006) (Figure 1.1).

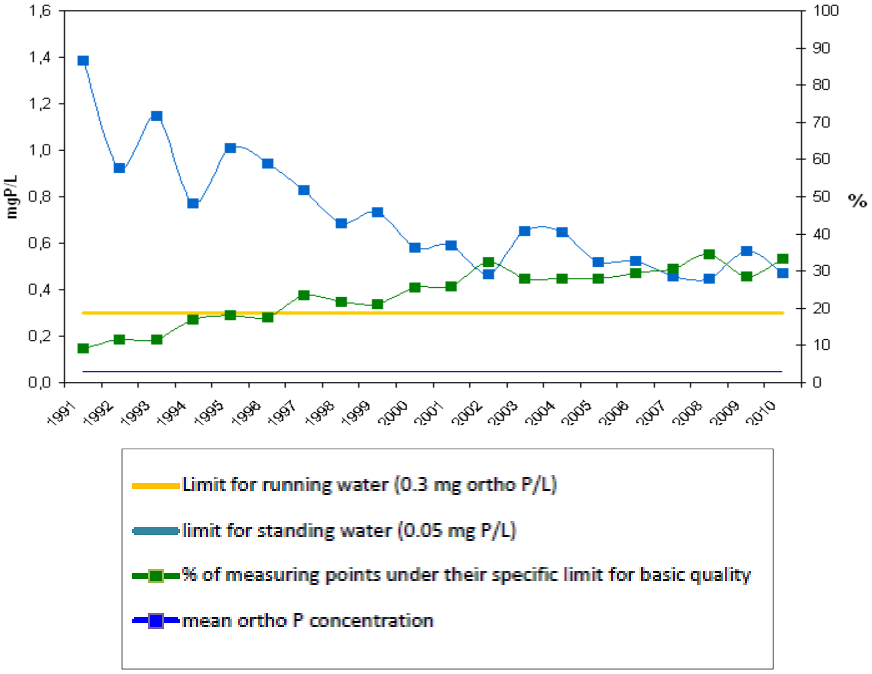

Figure 1.1. Evolution of the orthophosphate concentration and the percentage of measuring points in surface water that meet their specific basic quality norm in Flanders (adapted from (VMM, 2010))

During the last decades, the European Union has been trying to tackle the eutrophication problem by developing new legislation and new planning, monitoring and evaluation instruments, e.g. the Urban Wastewater Directive 91/271/EEC (Anonymous, 1991a), the Nitrates Directive (Anonymous, 1991b) and the Water Framework Directive (WFD) 2000/60/EC (Anonymous, 2000). A study conducted in 10 North West European countries to quantify the change in the concentration of reactive P in rivers revealed that there was a decrease in P concentration in each studied country (Foy, 2007). It also showed that the average mean reactive P in rivers in 2004 was the highest (337 µg P L^{-1}) in Belgium followed by UK (265 µg P L^{-1}) and The Netherlands (124 µg P L^{-1}). Experiments with large water reservoirs have shown that no eutrophication occurs when the phosphorus concentration is reduced to 8-10 µg P L^{-1} even when the N concentration reaches 4-5 mg N L^{-1} (Clasen, 1979).

To estimate the risk that agricultural practices pose in terms of eutrophication different measures of phosphate enrichment of soils have been introduced, e.g. ranking soils according to their P sorption index, the anion exchange resin, Fe-oxide strip and including the concept of degree of phosphate saturation (PSD) (Van der Zee et al., 1990a; Beauchemin & Simard, 1999). Another indicator of P enrichment in the soil is when the amount of available P (ammonium lactate extraction) is above 18 mg P 100 g^{-1} dry soil for cultivated land and above 25 mg P 100 g^{-1} dry soil for pasture. Between 2004-2007, 86% of the cultivated land were above 18 mg P 100 g^{-1} dry soil and 66% of the pastures in Flanders were above 25 mg available P 100 g^{-1} dry soil (Figure 1.2). From 2008 onwards a decrease of around 10% was found for the total amount of agricultural land that was above 18 mg available P 100 g^{-1} dry soil P and for pasture less than 51% was enriched, a decrease of about 15%, what can be seen as a step in the right direction (Overloop et al., 2011). However, it is important to see if the decrease persists after 2010. Another remark that has to be made with the observed decrease is that the limit for P enrichment in the soil (limits fixed by the Pedological Service of Belgium) of the amount of available P is very high, the highest in Europe, where ammonium lactate extraction is used (Jordan-Meille et al., 2012). The limits in Europe are for most countries only half of the limit used in Flanders.

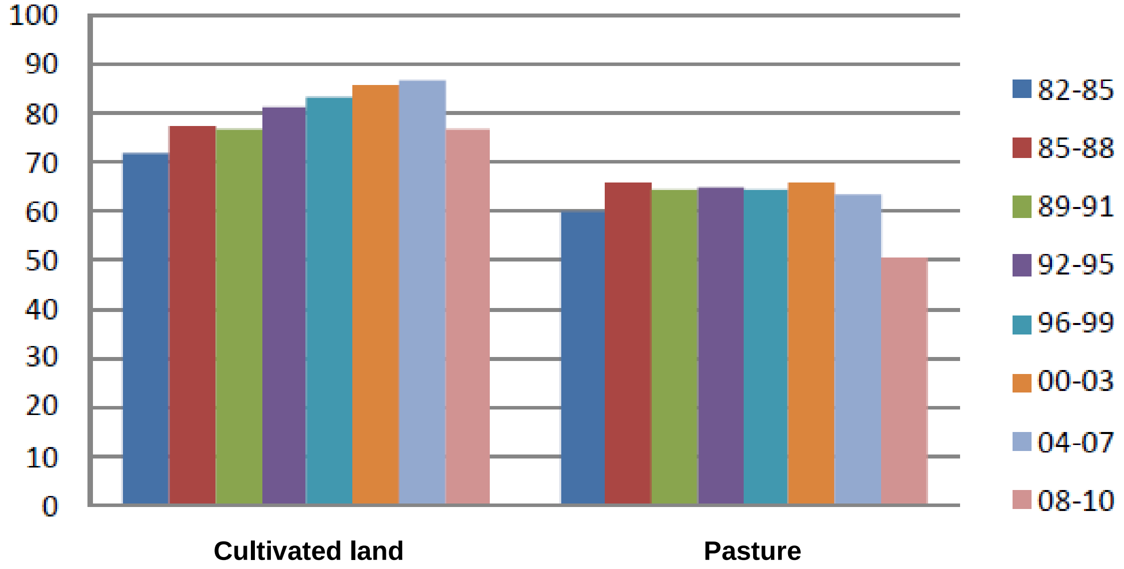

Figure 1.2. Percentage of agricultural soils in Flanders which have been found to be above 18 mg and 25 mg available P 100 g^{-1} dry soil for cultivated land and grassland respectively (Overloop et al., 2011)

The agricultural sector is the biggest contributor to eutrophication when it comes to P enrichment. More research is needed to completely understand the relationships between the added amount of P to the soil, the amount of P that is fixed in the soil and the amount that leaches out. When the inputs and outputs of the P cycle are controlled, P runoff, the P leaching to the groundwater and the eutrophication of the surface water can be tackled.

Soil phosphorus cycle

The P cycle in the soil includes plants and microorganisms (Stevenson & Cole, 1999). In contrast with the N cycle, the P cycle has no important gas phase (Figure 1.3). The major inputs of the P cycle are fertilizers (inorganic or organic), plant residues, agricultural wastes and municipal or industrial by-products (Pierzynski et al., 2005). Outputs from the soil are plant uptake and the loss processes erosion, runoff and leaching. It is crucial to keep these losses as small as possible since they represent a loss of nutrients and have large environmental impacts (eutrophication) (Pierzynski et al., 2005).

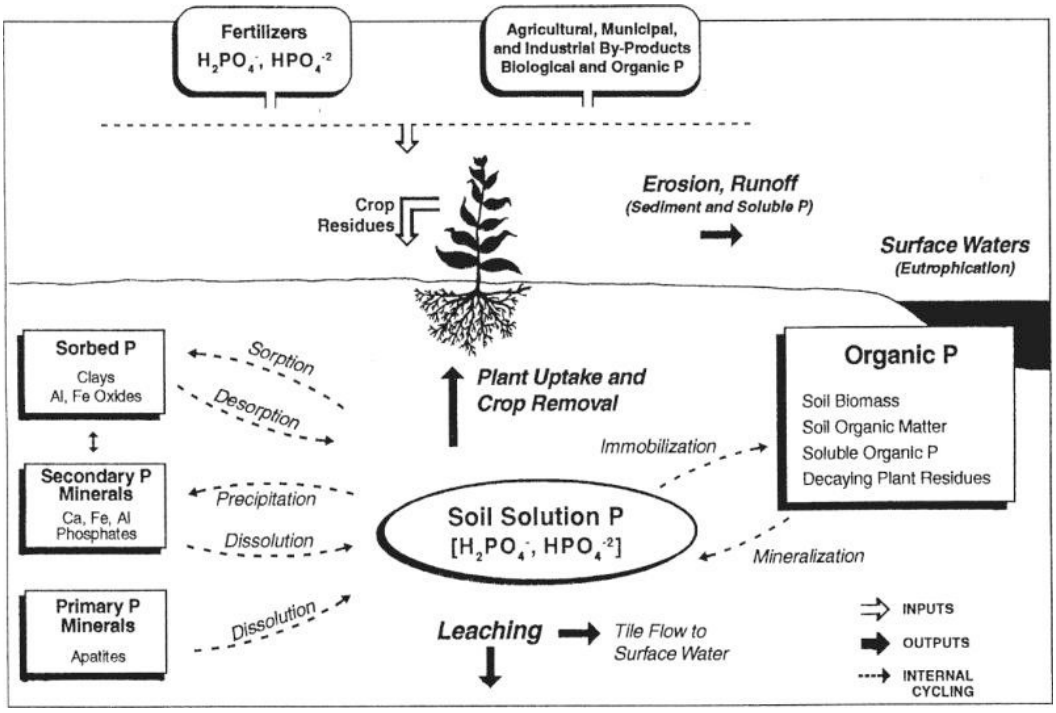

Figure 1.3. Phosphorus cycle in soils (Pierzynski et al. (2005))

P is an essential element for all living organisms as it influences the metabolic activities of living cells. P is also essential as part of the most fungible energy currency of the cell, the adenosine triphosphate, that powers the metabolism and it forms one of the key structural components of all living organisms, the phospholipid cell membrane. The membrane practically defines living organisms by forming the boundary between them and their environment (Vaccari, 2011). Plants deficient in P are stunted in growth and maturity is delayed, which is typically observed with yellowing of the lower leaves. P is needed for seed formation, root development, strength of straw in cereal crops and crop maturity (Stevenson & Cole, 1999).

P in soil undergoes numerous reactions including shifts from inorganic to organic P and back. Soil factors that control the rate of conversion of P between the inorganic and organic forms regulate the short- and long-term fate of P in the environment (Pierzynski et al., 2005). Clearly, it is a challenge to maintain the concentration of P in soil solution in an optimum range for plant growth, i.e. > 0.2 mg ortho-P L^{-1}, while restricting P in surface waters to 0.03 mg ortho-P L^{-1} (Pierzynski & McDowell, 2005). To do this, it is necessary to manage the soil P cycle, and the chemical and biochemical processes involved therein : dissolution – precipitation (mineral equilibria), sorption - desorption, mineralization - immobilization (Pierzynski & McDowell, 2005) (Figure 1.3).

Inorganic soil phosphorus

The fraction of inorganic P in soils varies largely between roughly 10 and 90% of total P, and is primarily present as adsorbed P and solid phases of primary and secondary P (Pierzynski & McDowell, 2005). More than 150 mineral forms of P are known to exist in the lithosphere, varying widely in solubility and thus in their ability to provide bioavailable forms of P. Depending on the development and the time period of weathering of the soil, different minerals will weather and release P into the soil solution (Pierzynski et al., 2005). In unweathered or moderately weathered soils, apatites, i.e. calcium (Ca)-phosphates, are the dominant minerals. In areas of intense weathering, Ca and other basic cations eventually leach out, pH decreases and iron (Fe) and aluminium (Al) dissolve from mineral phases. Precipitates of Fe, Al and P then form and become the main mineral forms of P in highly weathered soils (Pierzynski et al., 2005). As P is dissolved or desorbed from soil minerals and colloids, it enters the soil solution in primary (PO_4^{3-}) or secondary (HPO_4^{2-}, $H_2PO_4^-$) orthophosphates. The relative concentration of these orthophosphates varies as a function of the pH (Figure 1.4). Orthophosphate is present as either HPO_4^{2-} (pH > 7.2) or $H_2PO_4^-$ (pH 4.0 - 7.2) within the normal pH range (4.0 - 9.0) of most soils (Pierzynski & McDowell, 2005).

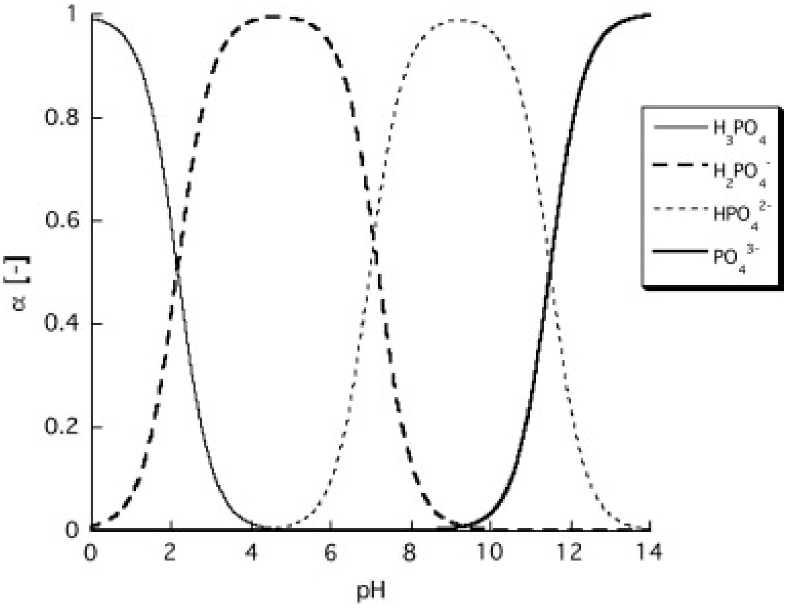

Figure 1.4. Changes in the form of soil P as affected by the pH for soluble P (α = mole fraction of the total P) (Pierzynski et al., 2005)

In alkaline soils, the solid phases most often considered are fluorapatite, hydroxyapatite, tricalcium phosphate, octocalcium phosphate, dicalcium phosphate and dicalcium phosphate dihydrate, listed in order of increasing solubility, while in acidic soils variscite and strengite are likely possibilities.

The amount of P in the soil solution depends on the soil pH and the activities of Al^{3+}, Fe^{3+} and Ca^{2+}. The different P forms have a wide range in solubility depending on the pH. At low pH, P is almost completely insoluble since it is fixed to Al^{3+} and Fe^{3+}. In alkaline soils the P is strongly fixed by Ca^{2+} (Tisdale et al., 1985). The P availability is generally the highest at a pH of 6 to 7 (Figure 1.5).

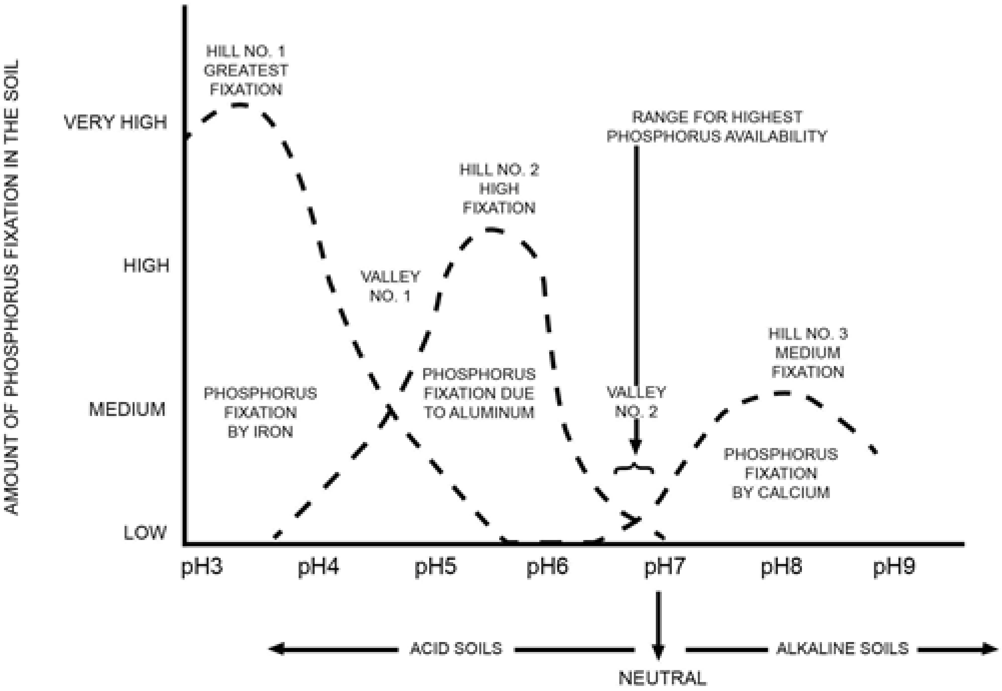

Figure 1.5. Effect of soil pH on the P availability (Stevenson & Cole, 1999)

Organic soil phosphorus

Organic P, i.e. P present as a constituent of organic compounds, ranges somewhere between 3% to 90% of soil P, depending upon the nature and management of the soil (Stevenson & Cole, 1999; Pierzynski & McDowell, 2005). High proportions of organic P can be found in soils where significant quantities of organic P are continually added to soil in plant, animal and microbial detritus (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. It can in general be stated that the organic P content of the soil closely follows the total organic matter content. Microbial decomposition of organic P results in the release of soluble organic P that, with time, is normally converted into a stable inorganic form of P (Pierzynski et al., 2005). 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., 2005). 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). The amount of orthophosphate that is removed by plant or microbial uptake is continually replenished from the solid-phase to sustain plant growth, by a combination of desorption and dissolution of inorganic P and mineralization of organic P (Condron et al., 2005).

Major Reactions of Phosphorus in Soils

Soil P chemistry is exceedingly complicated (Figure 1.6). 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 explains the poor availability of P for plants despite potentially high total concentrations in the soil. The understanding of the P availability in the soil and the major reactions that control the solubility of P are very important in order to investigate the possibilities of P fixation in P saturated soils.

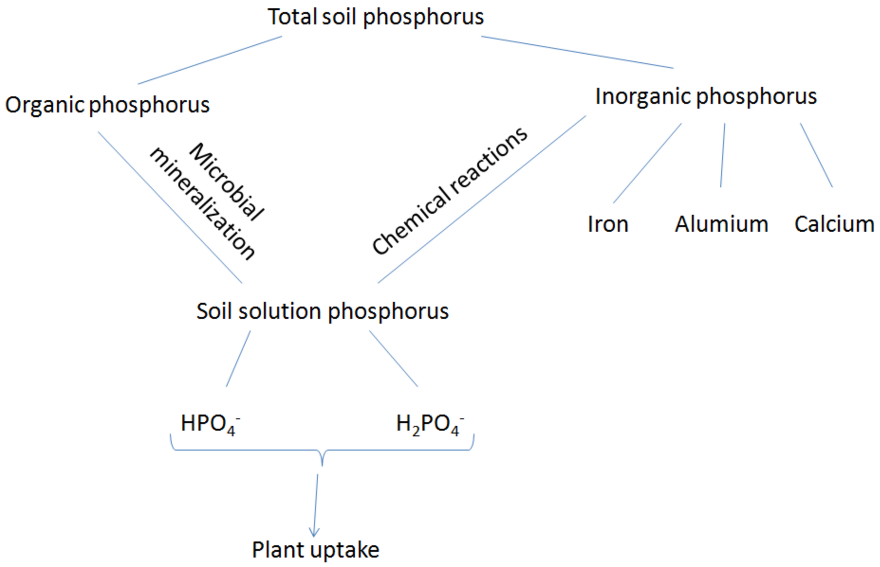

Figure 1.6. Scheme of the major forms of P in the soil (Minor & Stecker, 1993)

P sorption and desorption

Sorption and desorption reactions equilibrate with the soil solution. Orthophosphates can adsorb to the surfaces and edges of hydrous oxides, clay minerals and carbonates by replacing H_2O or OH^- (Pierzynski & McDowell, 2005). The main phosphate sorbing soil surfaces are Al and Fe oxides, hydroxides, and oxyhydroxides (which are collectively referred to as sesquioxides), organic complexes of Al and Fe, edges of silicate clays and calcite (Lair et al., 2009).

Added inorganic P is adsorbed to a variable degree, i.e., weakly (electrostatically) or strongly through covalent bonding (chemisorption) onto variable charge surfaces (Lindsay, 1979). The presence of Al and Fe oxides as small crystals and poorly ordered minerals increases the surface area which also increases P sorption (Parfitt, 1978; Li & Stanforth, 2000). The specific surface area of poorly ordered Fe and Al hydroxides can be as high as 800 m^2 g^{-1}, which is a factor 10 times higher than the specific surface areas of the corresponding crystalline forms (Lookman et al., 1995). Al and Fe oxides have a higher point of zero charge (generally between 7 and 10) which makes them positively charged over the whole pH range usually encountered in soils (Li & Stanforth, 2000).

Desorption refers to the release of P from the solid phase into the solution phase (Stevenson & Cole, 1999). 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 (Hinsinger, 2001; Pierzynski et al., 2005). Higher concentration of competing ligands is needed for effective ligand exchange as phosphate ions have a strong affinity to be adsorbed on the surface of positively charged minerals (Nagaraja et al., 1968; Kirk, 1999).

Precipitation and dissolution of P

Precipitation is the process where insoluble P is formed through the reaction of soluble, ionic P and Ca, Al or Fe. In acidic soils, due to the fact that the availability of hydrated $Fe^{3+}$and Al^{3+} increases, P is precipitated mainly as Al and Fe phosphate minerals (Lindsay, 1979; Lindsay et al., 1989). In neutral or alkaline soils on the other hand, P ions mainly precipitate with Ca^{2+} and to a lesser extent with Mg^{2+} (Lindsay, 1979; Hinsinger, 2001; Welch et al., 2002).

The solubility of these phosphate minerals also varies depending on the pH of the soil and the availability of free metallic cations (Figure 1.7). As pH increases the solubility of Al and Fe phosphates increases due to the fact that Al and Fe precipitate as hydroxides and they are no longer decreasing the P solubility (Lindsay et al., 1989). In contrast, the solubility of calcium phosphate generally decreases with an increase in soil pH up to pH 8 and increases above pH 8 as the Ca^{2+} activity is depressed in calcareous soils (Lindsay, 1979; Welch et al., 2002). Various phosphate minerals coexist in the pH range of 6-6.5 including variscite, strengite and brushite (Lindsay, 1979; Hinsinger, 2001).

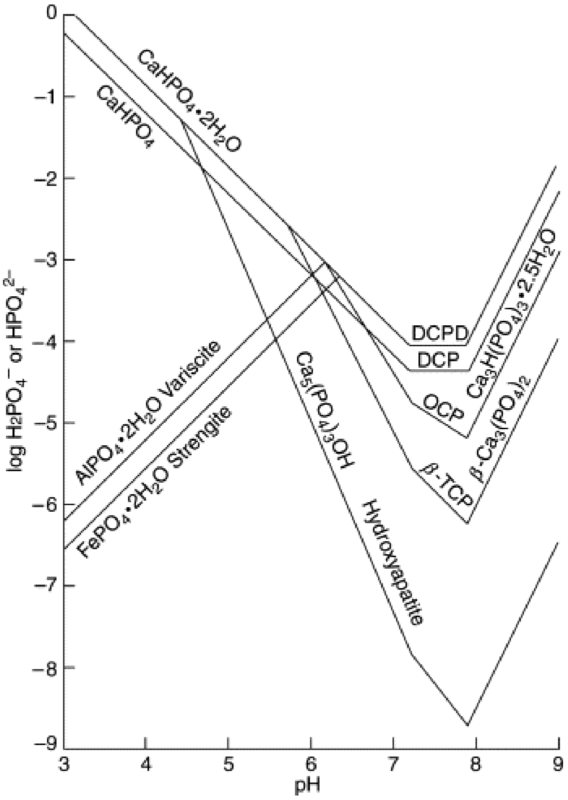

Figure 1.7. Soil pH and the solubility of some phosphate minerals. The concentration of $H_2PO_4^-$ and HPO_4^{2-} is given in moles expressed as log function (Hinsinger, 2001)

Mineralization and immobilization of P

Another important process in the regulation of P availability is mineralization and immobilization of P. The turnover of P via mineralization and immobilization processes is comparable to the N turnover. Mineralization refers to the microbially mediated decomposition of organic compounds, resulting in the release of inorganic forms of nutrients into the soil solution. Immobilization is defined as the conversion of mineral elements by soil microorganisms into biochemical compounds essential for microbial metabolism or into microbial biomass (Pierzynski et al., 2005). The mineralization of organic P in the soil is closely related to the relative amount of C in the organic substrate, which acts as the energy source for the decomposing microorganisms. High C/P ratios provide substantial energy and stimulate microbial growth, thereby consuming all available P. Low C/P ratios can result in an excessive amount of available, soluble P, much more than the needed microbial biomass. This excess is then available for plant uptake, leaching or runoff (Stevenson & Cole, 1999; Pierzynski et al., 2005).

The specific biochemical mechanisms in the conversion of organic to inorganic P in soils occurs through the production of extracellular phosphatase enzymes produced by plant roots, soil microorganisms and mycorrhiza. Enzyme production is believed to be related to the amount of bio available P in soils, increasing when soils become deficient in P, thereby explaining the cyclic pattern of P solubilization (Rodriguez & Fraga, 1999). There are 2 kinds of phosphatase enzymes in the soil that regulate the mineralization of organic P, namely acid phosphatase, which exhibits optimum activity in the pH range 4 to 6 and alkaline phosphatase, with pH optimum of 9 to 11 (Stevenson & Cole, 1999).

Phosphate saturation and P leaching

Phosphate saturation

The risk of P leaching from a soil can be approximated by the phosphate saturation degree (PSD) (Van der Zee et al., 1990a). The PSD relates the sorbed P load of a soil to its phosphate sorption capacity (PSC). As P accumulates in a soil, the difference between the amount of sorbed P and PSC decreases and the soil loses its ability to remove additional P from the soil solution. Ultimately, the build-up of sorbed P relative to PSC augments equilibrium solution P concentrations to the point that P is readily removed in runoff and in leachate (Kleinman et al., 1999).

The determination of the PSD according to Van Der Zee et al. (1990b) is based on the acid ammonium oxalate extraction. In acidic soils, the PSC is dominated by noncrystalline Al and Fe compounds (Freese et al., 1992). The PSD per soil layer of 30 cm, expressed in percentage, is determined as (Van der Zee et al., 1990b):

$$\boldsymbol{PSD = \frac{P_{ox}}{\alpha(Fe_{ox} + Al_{ox})}} \qquad (1.1)$$

Where: P_{ox} = sorbed P fraction extracted with ammonium-oxalate-oxalic acid (mmol P kg^{-1} soil)

$\alpha(Fe_{ox} + Al_{ox})$ = PSC = P sorption capacity (mmol kg^{-1} soil)

α = the degree of saturation = 0.5 (Van der Zee & Van Riemsdijk, 1988)

Fe_{ox} = ammonium-oxalate-oxalic acid extractable Fe (mmol Fe kg^{-1} soil)

Al_{ox} = ammonium-oxalate-oxalic acid extractable Al (mmol Al kg^{-1} soil)

The PSD of a soil profile is determined by calculating the average P_{ox} and the average PSC ($0.5(Al_{ox} + Fe_{ox})$) of the three layers over a depth of 90 cm, or to the groundwater table if it is shallower than 90 cm. α is set at 0.5 and denotes the saturation factor, that is, the sorption strength of ($Fe_{ox} + Al_{ox}$) for P. The value of α was calculated as the ratio between P_{ox} and ($Fe_{ox} + Al_{ox}$) from a set of sandy soil samples pre-saturated with P in a laboratory experiment (Van der Zee, 1988; Koopmans et al., 2004b).

The relation between the PSD of the soil profile and the amount of P which can be leached is described by a Langmuir equation (Van der Zee, 1988):

$$PSD = \frac{\gamma \cdot k \cdot c}{1 + \gamma \cdot k \cdot c} \qquad (1.2)$$

Where γ = the ratio between the total and reversible sorption of P

k = Langmuir constant ($m^3\ mol^{-1}$)

c = average phosphate concentration above the reference depth (mol l^{-1})

Soils with a higher clay content have a higher P sorption capacity (Mozaffari & Sims, 1994; Lookman et al., 1996) while sandy soils have a low sorption capacity (Van Den Bossche et al., 2005). Van der Zee et al. (1990a) formulated the relationship between the P concentration of the soil profile and the concentration of orthophosphate in the groundwater (Figure 1.8). If an orthophosphate concentration of 0.1 mg L^{-1}, at the base of the profile, is postulated as a lower limit for eutrophication, the PSD of the whole soil profile should be less than 24%. This relationship was developed for noncalcareous sandy soils where sorption of orthophosphate is dominated by metal oxides of Fe and Al (Van der Zee & Van Riemsdijk, 1988).

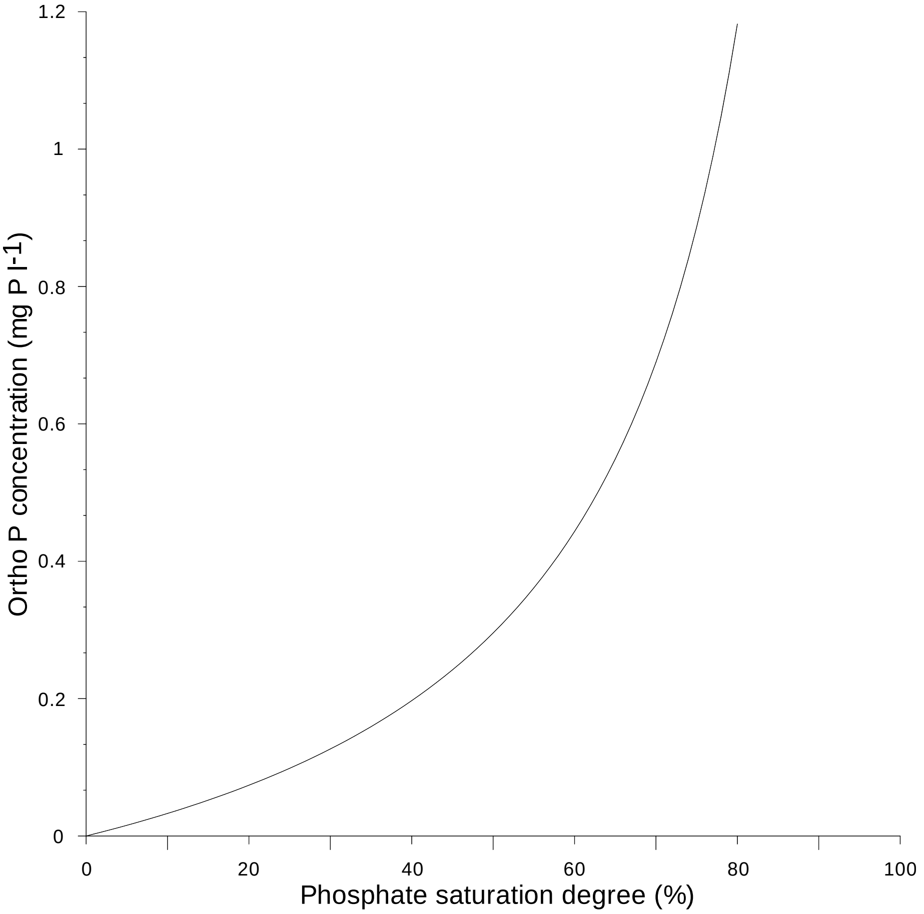

Figure 1.8. Theoretical relation between PSD and ortho-P concentration in the soil solution (Van der Zee, 1988)

Modelling P leaching and transfer from the soil to surface water

Although subsurface P transport is not the predominant loss pathway in most regions, there is increasing evidence of vertical P movement in soils when the P application exceeds the crop P removal over extended periods of time (Radcliffe & Cabrera, 2007; De Bolle et al., 2013a). The increasing P loss from agricultural soils together with a significant decrease in P loss from point sources implies that it is very important to understand the P movement and the amount of P that is moved through a soil (Schoumans & Chardon, 2003).

In many of the large lowland areas of Northern Europe and some parts of North America, P leaching is considered as the dominant process of P losses (Heathwaite et al., 2005; van der Salm et al., 2011). To determine the amount of P leaching from a field three important parameters must be known: the P distribution in the soil profile, the hydrology and the connectivity in the soil profile (Figure 1.9).

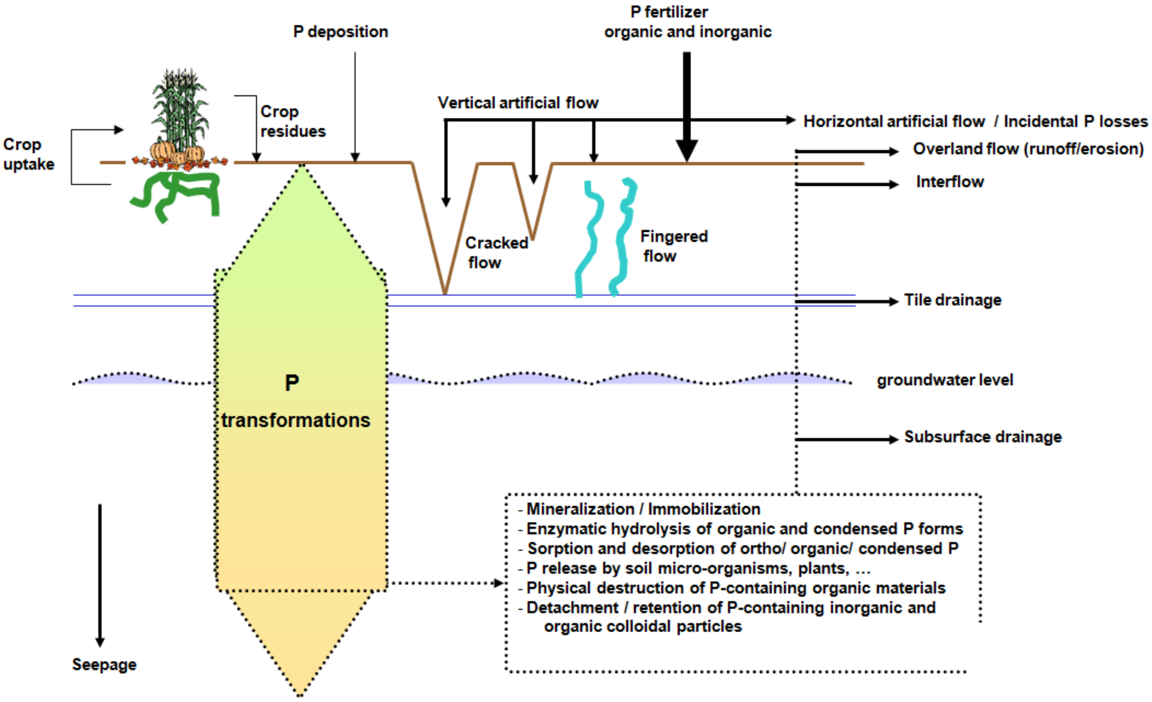

Figure 1.9. Conceptual framework for P transport over and through the soil profile at field scale (Schoumans & Chardon, 2003)

The excessive accumulation of P in the soil buffers the P in the soil solution despite changes in the management. Due to the buffering capacity of the soil, P leaching can continue for many years after initial detection of increased P concentrations, despite changes in management practices (Breeuwsma & Silva, 1992; Schoumans & Groenendijk, 2000). P losses by leaching in sandy soils are strongly affected by the actual distribution of P in the soil profile and the P retention capacity (Schoumans & Groenendijk, 2000; van der Salm et al., 2011). An example of a simple P leaching assessment tool is the PLEASE model (Phosphorus LEAching from Soils to the Enivironment) developed by Schoumans et al. (2013). This model combines information on the actual P distribution in the soil profile with the hydrological pathways. PLEASE is based on the process description of the comprehensive, process based model STONE (Wolf et al., 2005) but is simplified to be used with a limited amount of available data. PLEASE (used in chapter 3) calculates the P leaching losses to the surface water using field characteristics such as the height of the groundwater table, the precipitation surplus, the soil P status and the P sorption capacity of the soil (van der Salm et al., 2011).

Soil P status in Flanders and related legislation

Flemish agriculture is amongst the most productive in Europe, with animal production representing a large proportion (60%) of the total agricultural production value compared to the EU-27 average of 47% (Platteau et al., 2012). The pig production in particular has evolved from small land dependent production to a specialized, large scale and land independent industry in some parts of the region (De Smet et al., 1998). The intensive animal husbandry and in particular the shift towards pig breeding resulting in a massive application of slurry has been mentioned as a main cause for increased P saturation. In Flanders an amount of 69.2 x 10^6 kg P_2O_5 was produced in 2011, of which 39% was produced by cattle, 46% by pigs, 13% by poultry and 2% by other animals (Gielis, 2012). The N/P rate of slurry typically ranges from 2:1 to 6:1, whereas the plant requirements rather range between 7:1 to 11:1. Since slurry application used to be calculated based on the N need of a crop an excess of P was built up (Smith et al., 1998). Mulier et al. (2003) reported that before 2000 the amount of slurry that was added to the soil was largely exceeding the crop N demand, namely an annual N surplus of 66 x 10^6 kg and a P surplus of 15.7 x 10^6 kg, which corresponds to 102.7 kg N ha^{-1} and 24.4 kg P ha^{-1} for all agricultural land in Flanders. However, during the period 2000-2007 the amount of slurry added to the soil decreased, thereby decreasing the surplus for N by 46% and for P by 88%. In the period 2007-2009, the decrease continued for N with 38% and there was even a deficit for P (Overloop et al., 2012).

A first large scale survey, based on the protocol of Van der Zee et al. (1990a), of the P saturation of acidic sandy soils in Flanders was conducted from 1995 – 1997 (VLM, 1997). The results of the survey indicated that an area of 73 km^2 had a PSD of more than 40% with a probability of 95%. According to legislative texts, they were classified as P saturated. An area of 608 km^2 was classified as P critical, having a PSD of more than 30% with a probability of 95%. In 2011 stricter criteria were enforced: a soil was legally classified as P saturated when the PSD was > 35%, with a probability of 95%, soils with a PSD between 25 and 35% were legally classified as “P critical”, whereas soils with a PSD < 25% were categorized as not P saturated (Figure 1.10) (Van Meirvenne et al., 2008; Belgisch staatsblad, 2011).

A study conducted over Flanders categorised an area of 158 km² in West-Flanders, 420 km² in East-Flanders, 46 km² in Antwerp, 56 km² in Limburg and 1 km² in Brabant with PSD above 30% with a probability of 95% (VLM, 1997). De Smet et al. (1996) found that the average PSD in the top soil layer (0-30 cm) was 57% in West-Flanders, a region that is dominated by intensive agricultural production and livestock breeding. As a result of this dramatic situation with respect to P levels, progressively stricter P fertilization legislation has been implemented in order to reduce risks of P losses (Salomez et al., 2009). Similarly high soil test phosphorus and/or total soil P levels have been reported in other livestock production regions in Europe (Breeuwsma & Silva, 1992; Hooda et al., 2001), USA (Pierzynski, 1991) and Canada (Simard et al., 1994).

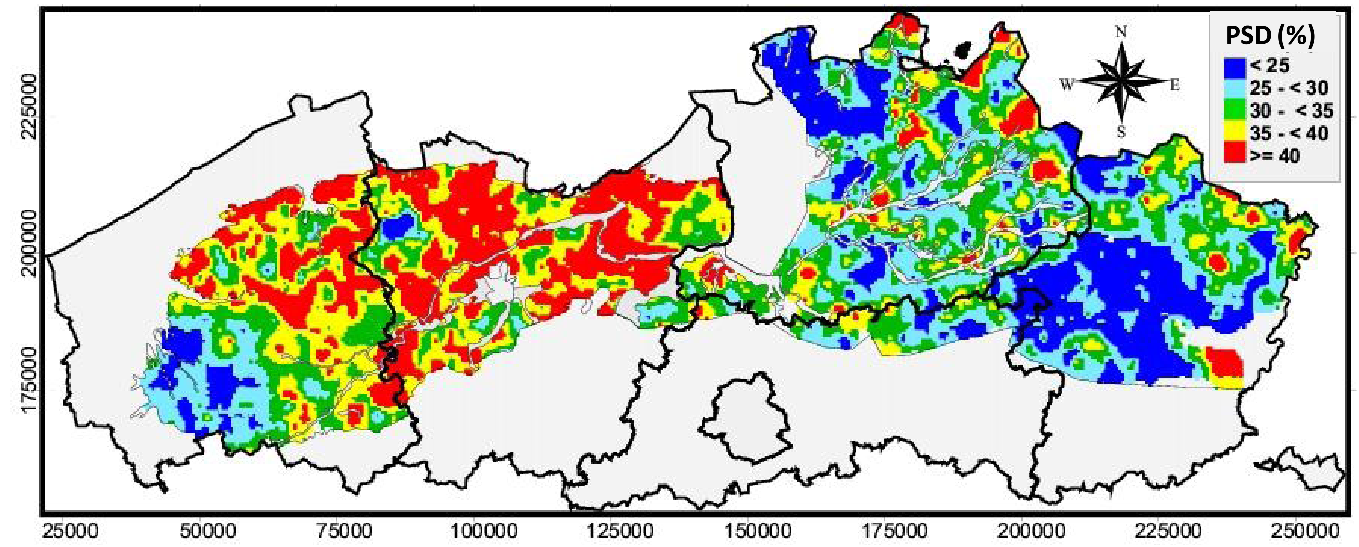

Figure 1.10. Classification of the PSD for soils in Flanders with a probability of 95% (Source Van Meirvenne et al., 2008)

The Nitrates Directive (2000/60/EG) forces the Flemish government to tackle the excessive accumulation and leaching of N into water bodies. To achieve a decrease in nutrient leaching and improve the water quality, several Manure Action Plans (MAP) have been enforced in Flanders since 1991, which also entailed a stricter legislation on the application of P fertilizers in an attempt to gradually reduce risks of P losses (Salomez et al., 2009). In 2007, with the implementation of MAP3, a total P application limit of 100 kg P_2O_5 for grassland and 95 kg P_2O_5 for maize was imposed for fields with a PSD < 40%. However, this is still more than most crops require and hence may result in additional P build-up. More severe P limitations of 40 kg P_2O_5 ha^{-1} are in force only on a very small area (0.84%) of soils with extreme P saturation. The latest MAP, MAP4, which is enforced from January 1st 2011 imposes stricter rules for P application on all agricultural land, which should ultimately result in a general mining of P from 2017 onwards.

Objectives

The European Union aims to improve the water quality in its member states, through a.o. the Nitrates Directive (91/676/EEC) (Anonymous, 1991b) and the Water Framework Directive (2000/60/EG) (anonymous, 2000). Recently, the focus of the European Union is shifting towards the role of P in eutrophication, created by a surplus of P in surface waters. To tackle eutrophication, it is necessary to have strict legislations in the member states in terms of P inputs to surface waters. This study focuses on a number of management options to reduce P leaching from the soil to decrease the environmental pressure. In this way intensive agriculture on soils with a high P content without causing more eutrophication risk could be possible. This study has three main hypotheses (Figure 1.11).

The first hypothesis was “The legislation in place between 1999-2010 has been effective in stabilizing or decreasing the PSD level of agricultural soils”. This hypothesis links to the first two objectives. A first objective was to quantify the evolution of the soil PSD levels in a number of representative agricultural fields in Flanders between two sampling periods separated by almost a decade (Chapter 2). The second objective was to identify critical areas in terms of P leaching in Flanders by using the PLEASE model (i.e. including the hydrological conditions of the field) and to see whether the model can be used for legislative purposes (Chapter 3).

In the critical areas for P leaching mentioned above, the priority should be to prevent (further) leaching of P into the subsoil and the ground- and surface water. Our second hypothesis therefore was “excessive P leaching in P saturated soils can be efficiently reduced by adding amendments with P fixation capacity to the soil”. This hypothesis is connected to the two following objectives. The first objective was to screen the P fixation capacity of a wide variety of potential amendments in a P saturated soil (Chapter 4). The second objective was to quantify the reduction in P leaching of this selection of most efficient amendments on six soils with different P content and PSD (Chapter 4).

Environmental problems in terms of P leaching are significantly reduced if the P can be fixed in the soil. This is only one part of the solution since an answer has to be found to deal with the fixed P. One way is mining of P, but another possibility is to convert the P into a form available to plants by e.g. phosphate solubilizing bacteria (PSB). This leads to the third hypothesis “PSB are able to solubilize P under high P conditions” (Chapter 5), thereby closing the cycle of the excess P in the soil and creating a solution beneficiary both for agriculture and the environment (Chapter 6).

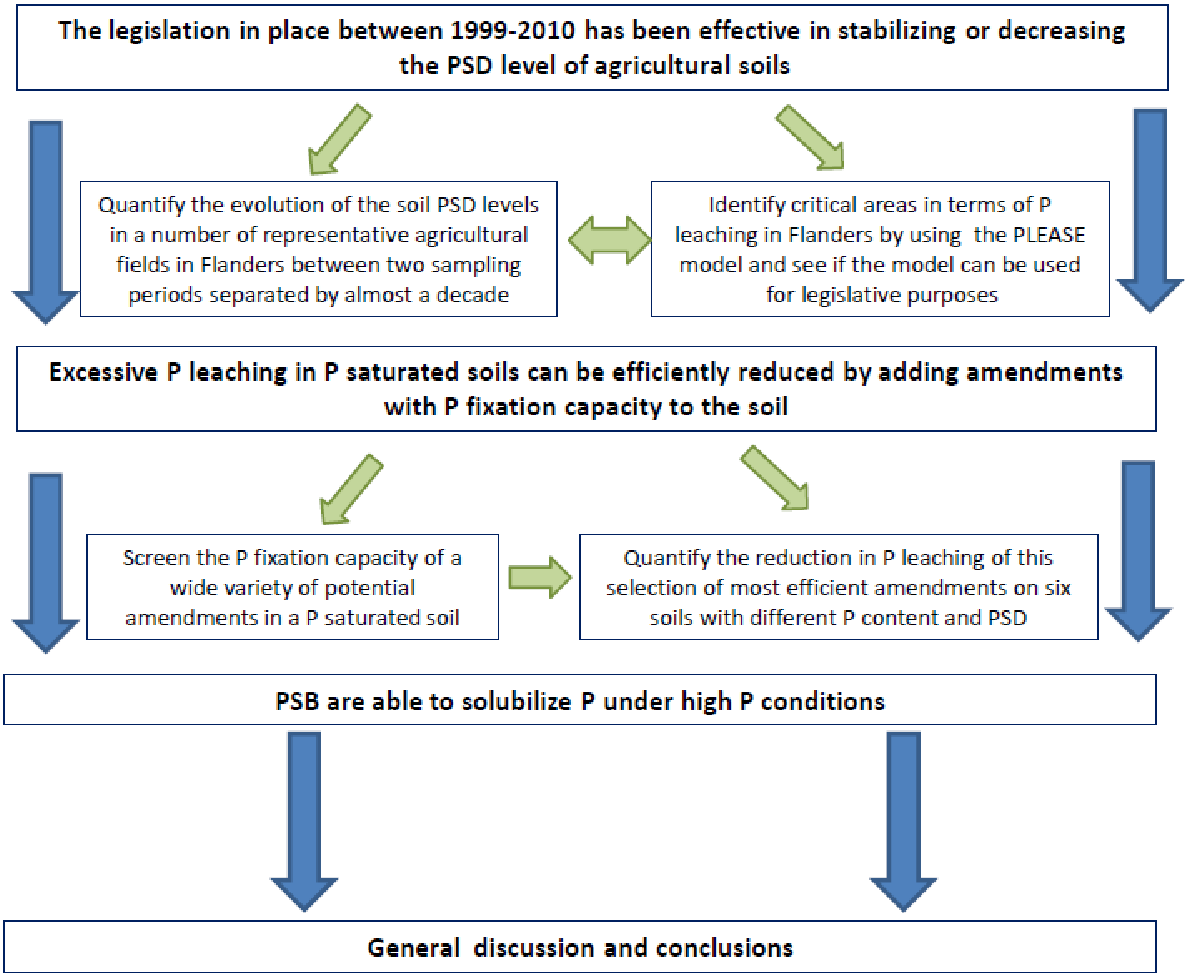

Figure 1.11. Set up of the PhD

Chapter 2

Rapid redistribution of P to deeper soil layers in P saturated acidic sandy soils

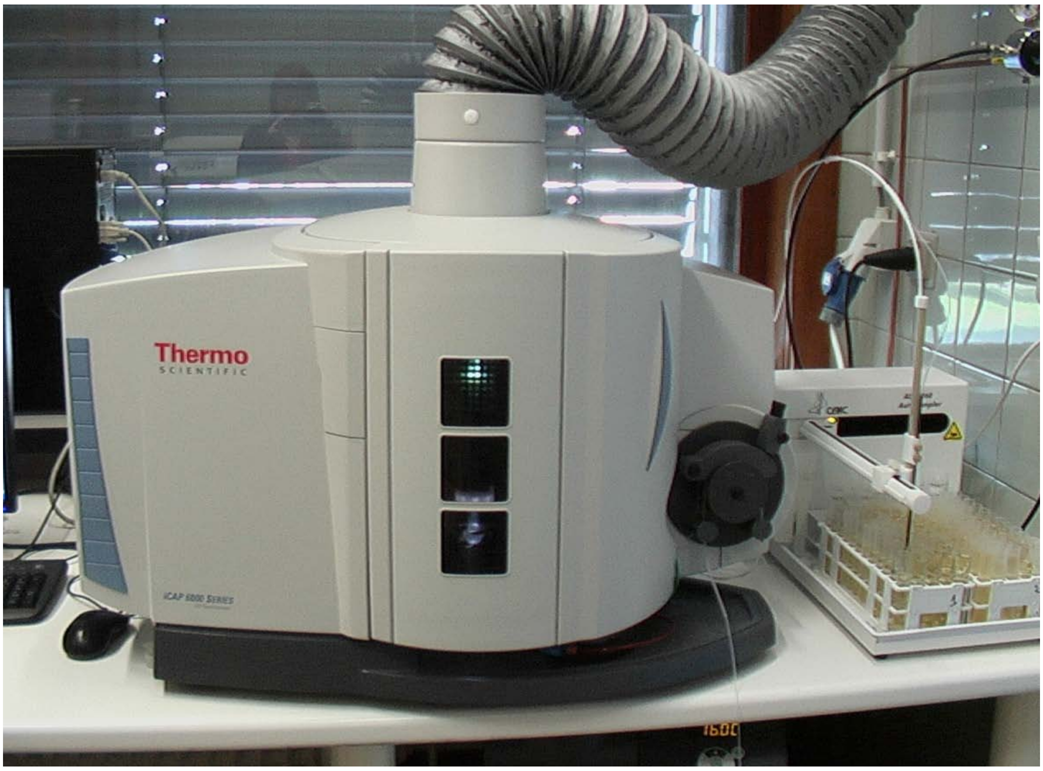

Illustration on p. 21:

ICP, ICAP 6000 series, Thermo scientific, used for the determination of Fe_{ox}, Al_{ox} *and* P_{ox}

This chapter has been published in :

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Abstract

Problems of excessive soil phosphorus (P) levels as a result of intensive agriculture are found in many regions in (Western) Europe, USA, Canada, New Zealand. This may lead to phosphorus leaching in soils with low P binding capacity. However, little is known about the evolution of P saturation degree (PSD) of such soils over time. Between 1995 and 2005 an intensive inventory of the PSD status of acidic sandy soils in Flanders was conducted, the results of which were used to enforce strict rules on P fertilizer inputs on P saturated soils. A new, smaller survey on a selection of these fields was performed during 2009 and 2010. A comparison of the survey results showed that the mean PSD had increased significantly from 46% to 59% over this period. Evidence was found for a strong shift of the PSD from the upper to the lower layers. The PSD level in the top layer (0-30 cm) generally increased significantly ($p<0.01$) from 83% to 91%. The average increase in the PSD level of the 30-60 cm and 60-90 cm layer was even larger, from 33% to 55% and from 14% to 25%, respectively ($p<0.01$). Apparently, the current limits on P fertilizer application have not yet resulted in P mining in these soils and will thus need to be further restricted. The very clear increase in PSD movement in deeper layers shows that these high PSD soils pose a very serious and direct threat to groundwater quality.

Introduction

In natural ecosystems, phosphorus (P) is commonly a limiting nutrient for plant growth. Therefore it is generally recycled and retained efficiently (Sinaj et al., 2002). Contrary to natural ecosystems, agricultural ecosystems are managed more intensively, often resulting in a nutrient build-up in soils, including a build-up of P. The use of food concentrates and modern technologies enables to maintain high livestock densities in many agricultural areas which implies more manure production, which is eventually returned to the field (Del Campillo et al., 1999). The excessive use of manure together with the use of mineral fertilizers has led to an over application of P fertilizers since the P input exceeds the P output (Neyroud & Lischer, 2003). This imbalance between P inputs and outputs can result over time in excessive P accumulation in the soil and increases the likelihood of P transfer from the soil to ground- and surface water (Haygarth et al., 1998).

Unlike nitrogen, leaching of P has traditionally not been viewed as a major groundwater problem. The leaching of P through the soil by matrix flow has generally been considered to be of little significance as a loss pathway for P, because of the normally strong adsorption of phosphate on the surfaces of reactive minerals in the soil (Indiati, 2000). In many soils, abundant P sorbing oxide components in surface horizons and subsoils keep leachate P levels well below eutrophication thresholds (Elliott et al., 2002).

The susceptibility of P leaching of an acidic sandy soil can be determined by the phosphate saturation degree (PSD) (Van der Zee et al., 1990a). The PSD relates the sorbed P load of a soil to its phosphate sorption capacity (PSC). As P accumulates in a soil, the difference between sorbed P and PSC decreases, thereby causing the soil to lose its ability to remove additional P from the soil solution. Finally, the build-up of sorbed P relative to PSC augments equilibrium solution P concentrations to the point where P is readily removed in runoff and in leachate (Kleinman et al., 1999). Calculation of the PSD is explained in chapter 1. Many soils in Flanders are acidic sandy soils which, in combination with the high to very high soil P fertility, makes them susceptible to P leaching (Van Den Bossche et al., 2005). Repeated excessive P applications in these soils result in a displacement of phosphates towards the deeper soil layers and towards the groundwater table (De Smet et al., 1996).

As a result of this dramatic situation with respect to P levels, stricter legislation for application of P fertilizers has been implemented gradually in an attempt to reduce risks of P losses (Salomez et al., 2009). However, if this stricter legislation is effective in decreasing or at least stabilizing the PSD levels has until now not been investigated. The aim of this study was to compare the PSD as assessed in the recent past (2001-2005) with the current (2010) PSD, thereby investigating whether changes in addition of P fertilization (up to 2009) had an impact on the P status of these soils (both in time and in the P distribution with depth), and in what way the risk for P leaching losses was evolving.

Materials and methods

Land use and P management practices

The sandy to sandy loam soil region of the province West Flanders (Belgium) was chosen as study area. The soils in this region are mainly classified as Eutrudepts or Humudepts according to Soil Taxonomy (Soil Survey Staff, 2010) or Terric Cambisols according to the world reference base for soil recourses (WRB)(WRB, 2006). Between 2001 and 2005 a survey of the PSD was conducted in West Flanders, the results of which will be referred to as sampling1 (Table 2.1) and the corresponding PSD and P_{ox} values from this survey will be referred to as PSD1 and $P_{ox}1$. During 2009 and 2010, 21 of these fields were resampled and analysed again for the PSD, the results of which will be referred to as sampling2. The corresponding PSD and P_{ox} values will be referred to as PSD2 and $P_{ox}2$. All selected fields had remained under agricultural management between both sampling periods, either arable/vegetable farming or pasture (mostly with grazing of livestock).

Table 2.1. General information about the investigated sites

Site	Texture (USDA)	Year of sampling1	Year of sampling2	Municipality	Crops	Dimensions of the field (ha²)
1	Loamy sand	2001	2010	Dentergem	Pasture	1.00
2	Sand	2001	2009	Oostkamp	Pasture	1.50
3	Sand	2001	2010	Wingene	Pasture	1.27
4	Sand	2001	2009	Oostkamp	Pasture	1.44
5	sand	2001	2010	Oostkamp	Pasture	1.60
6	sand	2001	2010	Oostkamp	Pasture	1.03
7	sand	2001	2010	Wingene	Pasture	1.60
8	sand	2001	2009	Oostkamp	Pasture	2.00
9	sand	2001	2010	Wingene	Arable (maize)	0.50
10	sand	2001	2010	Wingene	Arable (maize)	0.40
11	sand	2001	2010	Wingene	Arable (maize)	0.35
12	Loamy sand	2001	2010	Dentergem	Arable (maize)	1.00
13	Loamy sand	2002	2010	Dentergem	Arable (maize)	1.90
14	sand	2002	2009	Oostkamp	Arable (maize/potatoes)	0.32
15	sand	2002	2010	Oostkamp	Pasture	0.75
16	sand	2003	2009	Oostkamp	Arable (maize/potatoes)	0.40
17	sand	2003	2010	Oostkamp	Pasture	1.25
18	sand	2003	2009	Oostkamp	Pasture	1.25
19	sand	2005	2010	Zwevezele	Arable (vegetable)	0.72
20	sand	2005	2010	Zwevezele	Arable (vegetable/maize)	1.31
21	sand	2005	2009	Oostkamp	Pasture	1.00

Soil sampling and analysis

During January and February of 2009 and 2010, the fields were resampled. Each field was sampled to a depth of 90 cm in layers of 30 cm. The sampling was done by randomly taking between 20 and 50 samples with an auger in a cross pattern over the whole field. All auger samples of a 30 cm layer were mixed to yield one composite sample per depth and per field. After further homogenization in the laboratory, the soil samples were air-dried and sieved on a 2 mm mesh sieve.

The soil texture was determined according to the pipette method (Gee & Bauder, 1986). The pH-KCl was measured potentiometrically in a 1:2.5 soil:KCl extract. The P_{ox}, Fe_{ox} and Al_{ox} were determined following an extraction with ammoniumoxalate-oxalic acid (Schwertmann, 1964). Quantification of the P_{ox}, Fe_{ox} and Al_{ox} in the extracts was done by ICP (ICAP 6000 series, Thermo scientific). The PSD was determined following equation 1.1, according to the protocol of Van Der Zee et al. (1990a).

Statistical analysis

Paired sample *t*-tests were used to detect significant differences between the results of sampling 1 and sampling 2 using the statistical software PASW 20 package (SPSS version PASW 20, SPSS Inc., USA).

Results

Evolution of profile average PSD

The PSD1 ranged from 19.3% (for field 1) to 70.2% (for field 21), the PSD2 ranged from 21.7% (again for field 1) to 87.9% (for field 17) (Figure 2.1). Surprisingly, the profile average (0-90 cm) PSD2 was considerably larger than the profile average PSD1 (58.9% and 46.5%, respectively). Pairwise comparison of the PSD per field for all fields with a paired sample *t*-test indeed showed that the PSD2 was significantly ($p<0.01$) larger than the PSD1.

When the time interval of the first sampling was further subdivided into the period 2001-2002 and 2003-2005, we observed significant differences ($p<0.05$) between PSD1 and PSD2 only for the 2001-2002 period, while there was no significant difference for the 2003-2005 period.

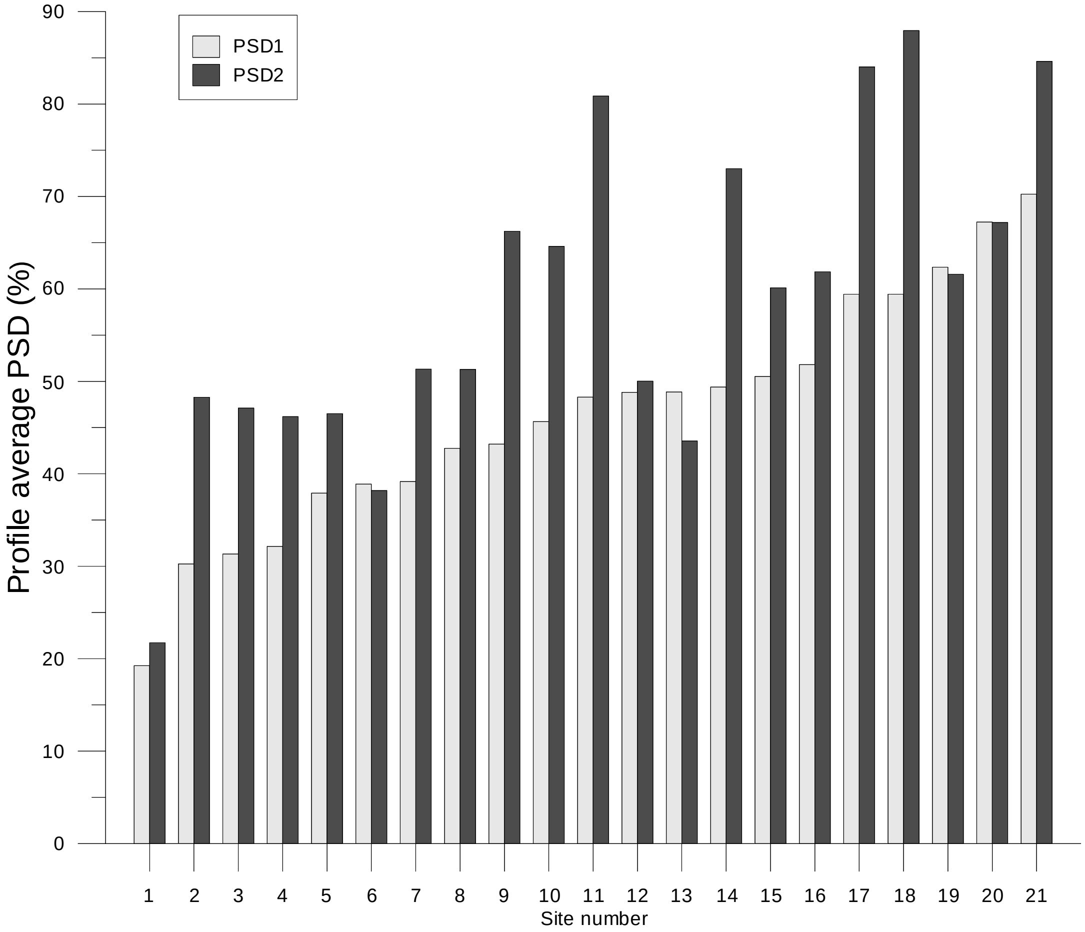

Figure 2.1. The profile (0-90 cm) average phosphate saturation degree (PSD) (%) for both sampling campaigns (sampling 1, between 2001-2005 and sampling 2, between 2009-2010) at 21 sites according to ascending PSD1 value

Evolution of PSD with depth

In the 0 to 30 cm layer PSD1 varied between 25.7% (field 1) and 122% (field 20), and PSD2 varied between 26.3% (field 1) and 119% (field 18) (Figure 2.2), confirming the excessively large PSD values found in this region in the past. A paired *t*-test showed a small but significant difference (p<0.01) in the average PSD between both sampling campaigns. However, a paired *t*-test showed no significant difference between $P_{ox}1$ and $P_{ox}2$ (Figure 2.2). This implies that the significant difference in PSD is not caused by an increase in P_{ox} but by a decrease in PSC, which is explained by a decrease in Fe_{ox} (namely $Fe_{ox}2$ is 6.57% lower in comparison with $Fe_{ox}1$).

The average PSD1 in the 30-60 cm and the 60-90 cm was 33.5% and 14.3% respectively (range 15.4% to 50.6% and 6.13% to 27.6% respectively). The average PSD2 in the 30-60 cm and 60-90 cm layer was 55.2% and 24.7% respectively (range 19.8% to 86.3% and 10.8% to 42.2% respectively) (Figure 2.3 and Figure 2.4). In the 60 to 90 cm layer of field 11 PSD2 was 7 times larger than PSD1. This measurement was considered as an outlier and removed for further calculations. The paired *t*-test showed a significant (p<0.01) increase in PSD in both layers between sampling1 and sampling2. Also $P_{ox}2$ was significantly (p<0.01) larger than $P_{ox}1$ in both layers (Figure 2.3 and Figure 2.4). The increase in PSD in both the 30 to 60 cm and 60 to 90 cm layers was mainly caused by an increase in P_{ox}, and to a lesser extent by a decrease in the PSC (decrease in Fe_{ox}).

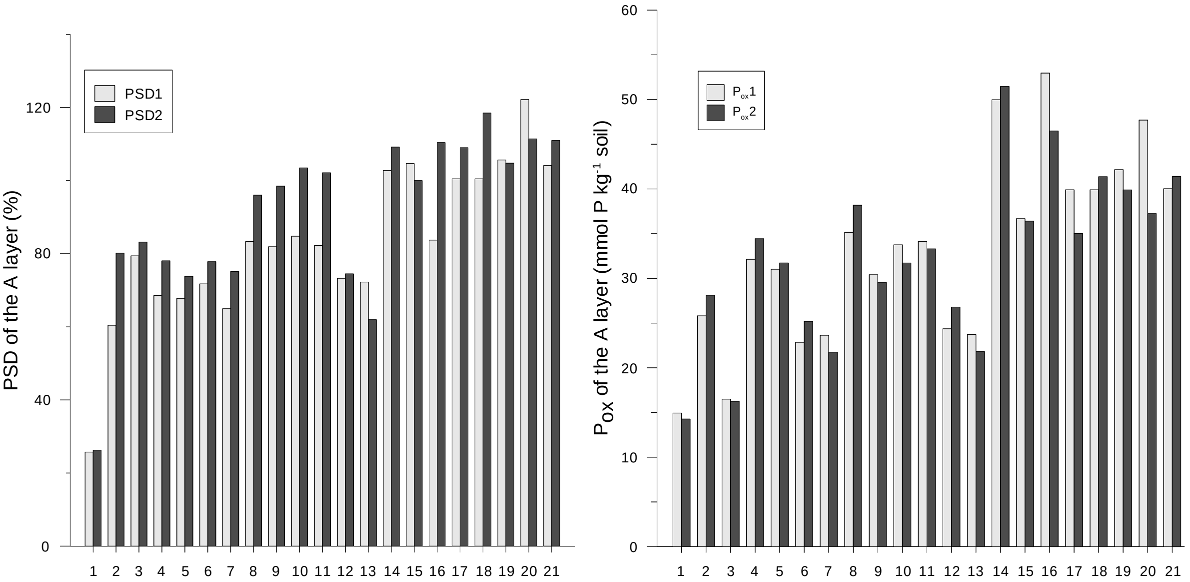

Figure 2.2. The phosphate saturation degree (PSD) (%) and the P_{ox} (mmol P kg^{-1} soil) for both sampling campaigns for the 0-30 cm layer

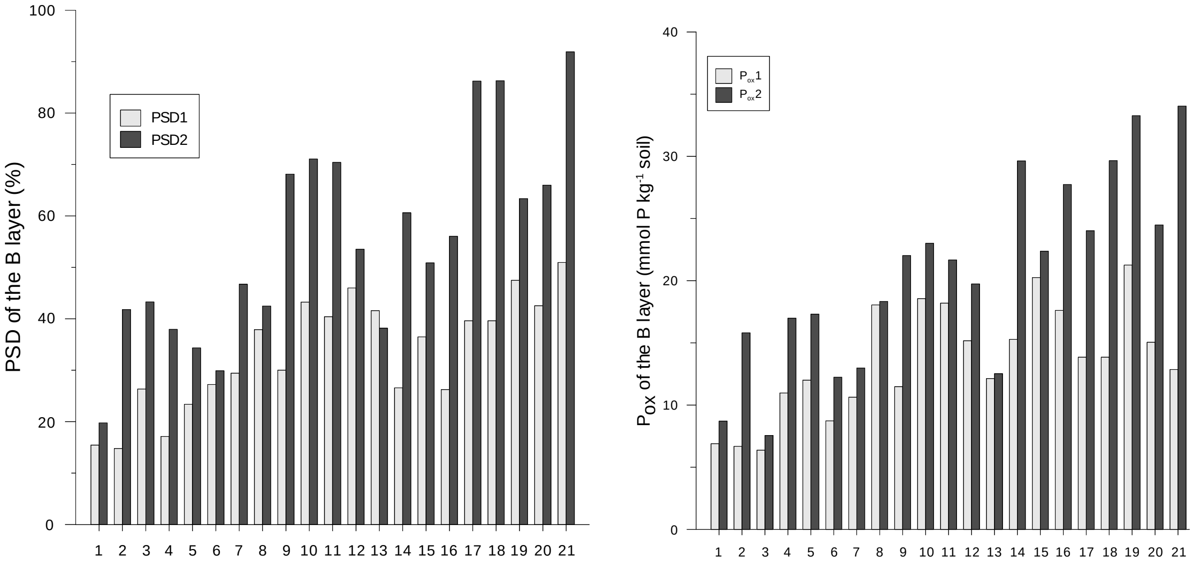

Figure 2.3. The phosphate saturation degree (PSD) (%) and the P_{ox} (mmol P kg^{-1} soil) for both sampling campaigns for the 30-60 cm layer

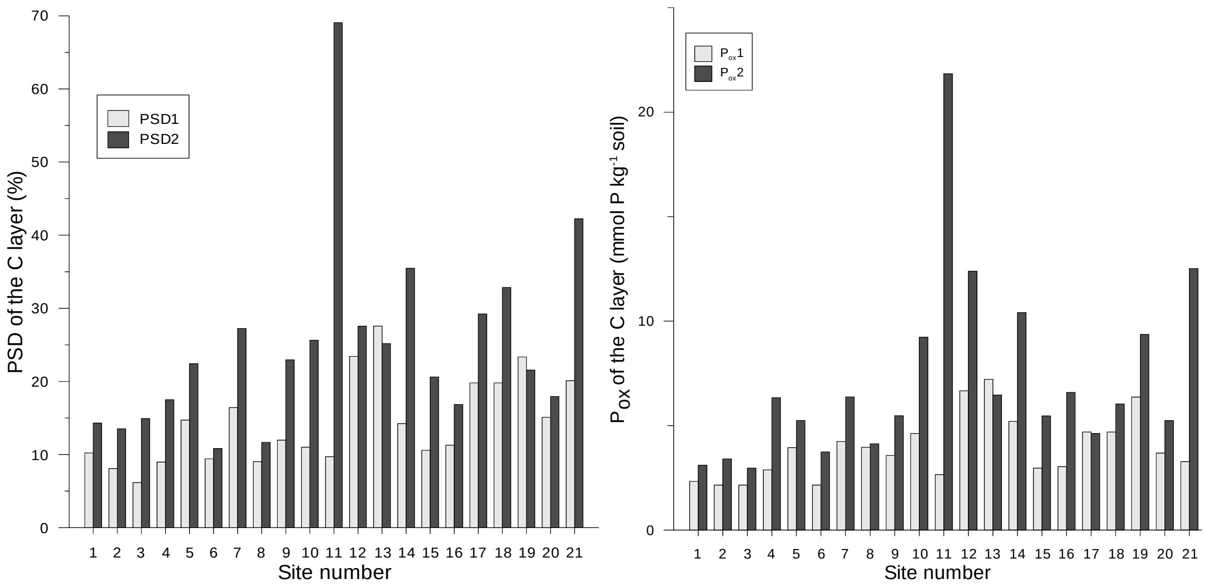

Figure 2.4. The phosphate saturation degree (PSD) (%) and the P_{ox} (mmol P kg^{-1} soil) for both sampling campaigns for the 60-90 cm layer

Discussion

Evolution of the profile average PSD

In general, and taking into account the critical value of 24% for P saturation, the PSD values observed in this study were excessively high. Even with the higher critical value for PSD of 35% used in Flanders, only 4 fields were below this limit in sampling1, and only 1 field in sampling2. The largest increases in PSD were observed for fields where the PSD1 was already very large (i.e. for fields with PSD1 between 43% and 59%). This was not expected since fields with a high PSD1, were already under severe P fertilizer restrictions (located in an area classified as P saturated). The rules for application of P fertilizers pertain to combined organic and inorganic P inputs on soils with PSD > 40% (PSD > 35%, since 01/01/2011), and are based on a nutrient balance approach: a maximum of 40 kg P_2O_5 ha^{-1} can be applied on these soils, which is far below crop P removal for most crops (Table 2.2) (Salomez et al., 2009). Since, the largest increase was found in soils which had already a PSD1 > 35%, this suggests that current restrictions for P fertilizers on these soils are either not sufficient or not implemented effectively. However, an increase in the potential of P leaching can also occur through P redistribution in the soil without having any additional P input. Even in a situation of P mining, there is likely to be more transport of P to deeper layers in soils with a higher PSD. This P ends up in layers with smaller Fe_{ox} and Al_{ox} concentrations, hence lower PSC, resulting in an increase in the potential of P leaching. Due to the current fertilisation rules and the enforcement of even stricter rules in the future in order to reduce eutrophication, common management practices of intensive agriculture on these fields may become impossible.

Table 2.2. Average crop yield and export of phosphorus by a number of important agricultural crops (Salomez et al., 2009)

	D.M. (kg ha^{-1})	‰ P	kg P ha^{-1}	kg P_2O_5 ha^{-1}
Grass	12000	4	48	110
Maize : cobs	8000	2.5	20	46
green parts	8000	1.75	14	32
Sugar beet : beets	15000	1.75	26	59
Leafs	6000	3.25	20	46
Winter Wheat: grain	7500	4	30	69
Straw	6500	1.5	10	23
Potatoes	12000	2.25	27	62

All the fields in this study have been under a comparable (intensive) agricultural management between the 2 sampling events. Notwithstanding the fact that a general increase in PSD was noticed between sampling1 and sampling2, a small decrease in PSD was observed for a limited number of fields (6, 13, 19, 20). Although this decrease between individual fields cannot be demonstrated statistically given the experimental set-up, it suggests that a reduction in P status of these P rich acidic sandy soils should indeed be possible, even under the current intensive agricultural management. The reasons underlying any differences in PSD evolution between individual fields was not within the scope of this study, but are undoubtedly linked to differences in the adoption of correct (P) fertilizer practices between individual farmers.

Evolution of PSD with depth

The profile (0-90 cm) average PSD is used as a measurement of the P leaching risk in the protocol of Van Der Zee et al. (1990a). However, it is also important to consider the distribution of PSD for each soil layer separately. A high PSD in the 0 to 30 cm layer, in combination with low PSD in the 30 to 60 cm and 60 to 90 cm layers is less problematic, since crops will more easily deplete the P available in the topsoil. Therefore accurate assessments of P leaching in a sandy soil must incorporate P status and P sorption capacity of the entire soil profile and not just the surface horizons (Nelson et al., 2005). In the current study only a small (although significant) increase of the PSD in the topsoil was observed. This would suggest that P application was more or less in balance with crop P uptake and no additional P accumulation occurred. However, the PSD in both the 30 to 60 cm and 60 to 90 cm layers increased drastically and significantly. Since P additions occur only in the top layer, the only explanation for the increase of P_{ox} and PSD in the deeper soil layers is a transfer of P from the top layer, which is consistent with the largest increase in PSD in the 30 to 60 cm layer. Since P concentrations in the top layer increased only slightly, but P concentrations in the deeper layers increased drastically, the conclusion is that P fertilization in the period covered was still in excess of crop requirements. These observations have dramatic implications, because it shows that P leaching in these P saturated acidic sandy soils is much faster than previously thought in Flanders, and poses a severe threat to the groundwater even in the short term. Moreover, any P leached below the top layer will be much more difficult to mine by crops and the mining will take more time (Koopmans et al., 2004b). Nelson et al. (2005) found that vertical P movement after excess P additions was observed in P saturated soil horizons, indicating that up to 50% of P leached out was found deeper than 45 cm in a sandy soil. They also found that P leaching in P saturated soils continued even when P additions stopped, thereby demonstrating the need to consider long-term leaching losses when calculating potential environmental impacts of P loss from agricultural soils with low P sorbing capacity (Nelson et al., 2005). The PSD in the top layer is between 80% and 100% which corresponds with an ortho-P concentration of 1 to 15 mg P L^{-1} (Figure 1.8), if the net precipitation surplus is 300 mm y^{-1} a P loss of 3 to 45 kg P ha^{-1} $year^{-1}$ or a loss of 7 to 100 kg P_2O_5 ha^{-1} $year^{-1}$ can be found. This indicates that in these soils the total P loss can even be higher than 50%.

The downward movement of Fe observed here was unexpected because Fe is not seen as a mobile element. However, this is in agreement with the findings of Zhang et al. (2003) and Siemens et al. (2004, 2008). They found that excessive P application altered the charge characteristics of sandy soils, thereby changing the stability of iron oxides in the soil solution and accelerating the transport of colloidal iron oxides out of the soil. Their study showed that the concentration of Fe that moves downward is significantly affected by the rate of phosphate application. Since the investigated soils in our study received an over application of P fertilizers during decades, the downward movement of Fe can be explained by these findings. This downward movement of Fe causes another obstacle in combating the environmental problem of eutrophication.

Conclusions

This study confirms the very large PSD of intensively managed acidic sandy soils in Flanders. It shows that PSD has continued to increase over the past 5-10 years, despite stricter fertilizer legislation. Surprisingly, the strongest increase in PSD is observed in the deeper soil layers (notably 30-60 cm) rather than in the top 30 cm. This can be explained by P redistribution to deeper soil layers where the Fe_{ox} and Al_{ox} concentrations are lower. This shows that P leaching in these acidic sandy soils is probably faster than previously assumed and puts a severe threat to groundwater quality in the near future. Our measurements thus indicate that current P fertilizer restrictions are either not strict enough or, more likely, are not sufficiently implemented in practice. This will probably lead in the near future to even stricter P fertilizer restrictions such as zero-application of P on fields with very high PSD, which may impair soil quality through severe restrictions on organic matter application. As mining by crops is a very slow process, due to hysteresis effects and the non-linear relationship between P_{ox} and the P concentration, it will also be necessary to seek alternative strategies for avoiding further P leaching such as soil amendments that increase the PSC to avoid massive groundwater pollution (see chapter 4).

Chapter 3

Prediction of phosphorus losses in Flanders with the PLEASE model

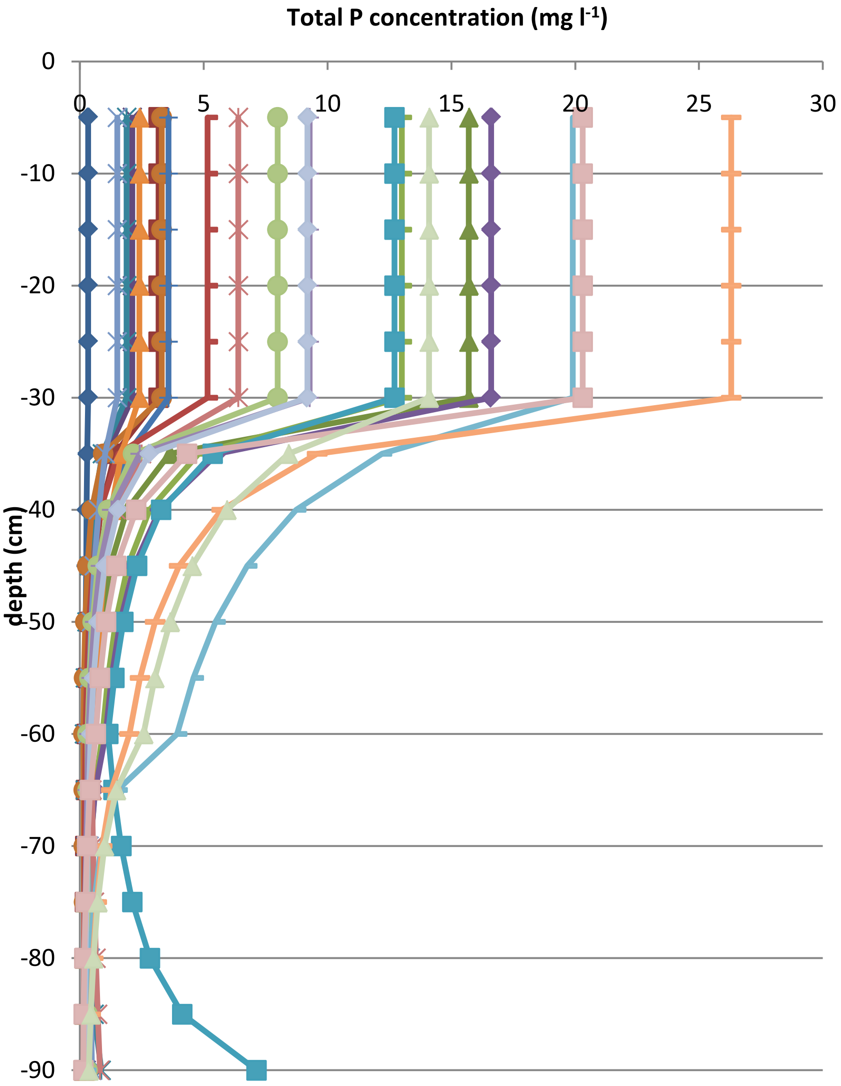

Illustration on p. 35:

Graphical example of a simulated total P flux with PLEASE

A paper with the results of this chapter is in preparation :

Prediction of phosphorus losses in Flanders with the PLEASE model, comparison between actual and potential P losses

Abstract

Up to date all investigations and policies in Flanders in terms of P saturation of agricultural fields have been done according to the PSD concept (Van der Zee et al., 1990a), whereby a soil was legally classified as P saturated when a field had a PSD > 40% and from 2011 onwards a PSD > 35%. The PSD is a good indicator in terms of the P amount in the soil, but is a poor indicator for taking into account the hydrological effects of the soil on the risk of P leaching. A cost effective instrument to evaluate whether the imposed restrictions had the desired result is necessary. In the Netherlands a P leaching assessment tool, namely the PLEASE model has proven to be an efficient instrument in identifying critical fields/areas for P leaching, and is used here on the acidic sandy soils in Flanders.

In this study, the PLEASE model was run on three datasets to investigate the applicability of using the model in Flanders. First it was investigated how many soil data layers where optimal to use. Secondly, a sensitivity analysis of PLEASE was performed. Finally, the classification of P saturated fields was compared with the current classification based on the PSD, thereby evaluating the best technique to identify critical areas in terms of P leaching.

It is most optimal to use four layers in the PLEASE model, since in Flanders the 60-90 cm can contain also high amounts of P which are otherwise not fully included in the model results. The sensitivity analysis indicates that the model has a high sensitivity for P_w and for the net precipitation (NP), therefore it is important to have optimal data for these parameters. Finally, an important difference is found between the classification of the fields in terms of P saturation between PSD and PLEASE, indicating that due consideration of the hydrological conditions plays a crucial role in the assessment of P leaching.

Introduction

In flat areas with shallow groundwater levels, P loss by leaching through soils contributes significantly to the P loads of streams (van der Salm et al., 2011; Schoumans et al., 2013). For a cost-effective implementation of measures to tackle P leaching, practical instruments are needed to identify areas which are most critical (Kronvang et al., 2005).

To assess the risk of P leaching, a simple risk indicator, the phosphate saturation degree (PSD) was developed in the eighties (Van der Zee et al., 1990a; Van der Zee et al., 1990b; Schoumans et al., 2013). The PSD has been used to delineate the potential source areas for increased P leaching. The PSD is an indicator of potential risk but gives no information on actual P loss to streams (Schoumans et al., 2013). A large scale survey in Flanders was done in 1995-1997 to classify acidic sandy soils according to their PSD (Figure 1.10 and also chapter 1 and 2)(VLM, 1997).

To make an estimate of the P loss to surface waters it is necessary to also incorporate the water flows in soil and groundwater. Several approaches and models are used to estimate P loss from land to surface waters, but these methodologies mainly focus on P losses by surface runoff as the contribution of P loss by leaching is difficult to predict (Radcliffe et al., 2009; Schoumans et al., 2013). However, much progress has been made in recent years to deterministically model P leaching (Radcliffe et al., 2009; van der Salm et al., 2011). Such models often require extensive parameterization and have a high demand for data input which are seldom available (Wolf et al., 2005; Schoumans et al., 2009; van der Salm et al., 2011). Practical mitigation planning requires simple tools that assess the risk of P leaching on the basis of readily available data (van der Salm et al., 2011). An example of such a tool is the P index, used e.g. in the United States, Norway,… (Lemunyon & Gilbert, 1993; Bechmann et al., 2005), which ranks fields according to their vulnerability to P losses by various transport processes (Sharpley et al., 2003; van der Salm et al., 2011).

P losses by leaching in sandy soils are strongly affected by actual distribution of P in the soil profile and the P retention capacity (Schoumans & Groenendijk, 2000; van der Salm et al., 2011). P losses by leaching from a field are mainly determined by the P sources (recently applied P and the P content in the soil solution), the rainfall intensity and duration (water resources) and drainage characteristics of the field which influence the water flow within a field (Schoumans et al., 2013). A simple P leaching assessment tool (Phosphorus LEAching from Soils to the Environment, PLEASE) was developed to combine information on the actual P distribution in the soil profile with the hydrological pathways of P movement (van der Salm et al., 2011; Schoumans et al., 2013). PLEASE is based on STONE, a comprehensive, process-based model (Wolf et al., 2005), but is simplified and requires only a limited amount of available data (van der Salm et al., 2011). PLEASE calculates P leaching losses that reach surface waters via lateral groundwater flow using field characteristics such as the height of the groundwater table, the precipitation surplus, the soil P status and the P sorption capacity of the soil (van der Salm et al., 2011).

In Flanders P saturation is assessed with the PSD concept developed by Van der Zee et al. (1990a). This method gives the potential risk for P leaching of a field, thereby only taking the hydrological conditions into account when the GWT is shallower than 90 cm. The PLEASE model includes the hydrological conditions and gives a prediction of the real P loss per field. In this study results of the PLEASE model were compared with results based on the PSD concept to investigate if a field had the same classification in terms of P saturation with both methods and thereby evaluating the importance of including the hydrological conditions in the P leaching risk evaluation. The PLEASE model was chosen because it is easy to use, needs a limited amount of data input parameters and has already proven to work effectively in The Netherlands under similar conditions as in Flanders. Therefore the model was run on three datasets using 21 fields (small dataset) over 105 fields and finally 2683 fields (large independent dataset).

Materials and methods

Model description

PLEASE is a simple static model designed to predict P leaching from the field to surface waters based on the relation between the amount of adsorbed P in soils and the P concentration in soil water as well as the lateral ground water flow from soils to surface waters (van der Salm et al., 2011). The water flow is assumed to be mainly horizontal because the ratio between the field size and the drainage length is much greater than the depth of drainage in the saturated zone (Kirkham, 1958; De Vries, 1975; Schoumans et al., 2013). The P lost from a field to the surface water is determined by the depth of the profile of soluble P concentrations in the soil and the lateral water flow with depth during the year:

$$\boldsymbol{P_{load} = \varphi \int_0^x \int_0^t C(x,t) \cdot J(x,t)\, dt\, dx} \tag{3.1}$$

in which P_{load} is the P loss by leaching from the field to surface water (kg ha^{-1} yr^{-1}), $C(x,t)$ is the P concentration as function of time (t) and depth (x) in the soil profile (g m^{-3}), $J(x,t)$ is lateral water flux (m yr^{-1}), and φ is the conversion factor from g m^{-2} yr^{-1} to kg ha^{-1} yr^{-1}.

Monitoring of the P concentration in the soil solution in the field as a function of depth is time-consuming (Schoumans et al., 2013). As an alternative P concentrations can be calculated based on P accumulation in the soil. The ortho-P concentration in the soil solution is buffered by the amount of P accumulated in soils, which is described by a fast reversible adsorption reaction at the surface of soil particles (Q) and a slow diffusion precipitation reaction into the soil particles (S_d). The P in the S_d pool is strongly bound, compared to the P in the Q pool, and is considered as irreversibly bound P (Van der Zee & Van Riemsdijk, 1986a; Van der Zee & Van Riemsdijk, 1986b; Van der Zee & Van Riemsdijk, 1988; Freese et al., 1995; Barrow, 2008). These studies also showed that the distinction between reversible and irreversible bound P explains the well-known hysteresis effect of phosphate sorption and desorption (Schoumans et al., 2013). The P equilibrium concentration in the soil solution can be calculated by the sorption and desorption characteristics using the Langmuir isotherm, (Schoumans et al., 2013):

$$\boldsymbol{C = \frac{Q}{\mathrm{K}\,(Q_m - Q)}} \tag{3.2}$$

where C is the P equilibrium concentration in solution (mol m^3); Q_m is the adsorption maximum (mmol kg^{-1}); Q is the amount of adsorbed P (mmol kg^{-1}) and K is the Langmuir affinity constant (m^3 mol^{-1}).

For non-calcareous sandy soils Q_m is related to the total amount of oxalate-extractable Al and Fe ($Q_m = \beta\ (Al_{ox} + Fe_{ox})$) (Van der Zee, 1988), with β the Langmuir affinity constant depending on the soil type. The actual amount of reversible P (Q) in soils is in principal determined using the Fe impregnated method (Van der Zee et al., 1987). However, this is a rather complex and time consuming method and since Q is strongly related to the amount of water extractable P (P_w) following formula can be used (Schoumans & Groenendijk, 2000):

$$\boldsymbol{P_w = 137.42\ C_e \left(1 - 0.09722 e^{-K\,k_d\,\beta\,(Al_{ox}+Fe_{ox})\left(1-\frac{K\,C_e}{1+K\,C_e}\right)\,t\,\lambda}\right)} \tag{3.3}$$

in which P_w is the amount of water extractable P (mg P_2O_5 L^{-1}) (Pierzynski, 2000), C_e is the P equilibrium concentration in soil solution (mg L^{-1}), K is the Langmuir affinity constant (m^3 mol^{-1}), k_d is the Langmuir desorption rate (per day), t is the reaction time (day^{-1}) and λ is the conversion factor related to the soil solution ratio of the experiment.

The model is based on the fact that the parameters P_w, Al_{ox} and Fe_{ox} (possibly also P_{ox}) were determined for two soil layers. These soil layers are the plough layer and the layer underneath. The plough layer (D_1 is the boundary line at 30 cm depth, in this study) is assumed to be homogeneous, due to soil tillage, and thus the P concentration is assumed to be constant over the entire layer (Figure 3.1). This implies that the upper limit of the soil layer beneath the plough layer has the same P concentration as the plough layer (Schoumans et al., 2008).

If for the soil layer under the plough layer (between $D_1 - D_2$) the P_w, (P_{ox}), Al_{ox} and Fe_{ox} are determined, the average P concentration can be calculated for this soil layer together with the average amount of phosphate that is easily soluble (Q_2). However it is not plausible that the measured average P concentration is homogenous for the whole depth, since this layer is not tilled. Because P is highly sorbed to soil, a rather sharp front can be expected in soil columns and lysimeters, but, at field scale, the front will become more gradual due to heterogenic flow processes and bioturbation. As a result, it is assumed that the amount of sorbed P (Q) will reduce exponentially with depth (Schoumans et al., 2013) (Figure 3.1). At the bottom of the soil profile, set at 1 m below the lowest ground water height, the adsorbed P is assumed to be in equilibrium with the (natural) background concentration in the shallow aquifer (van der Salm et al., 2011).

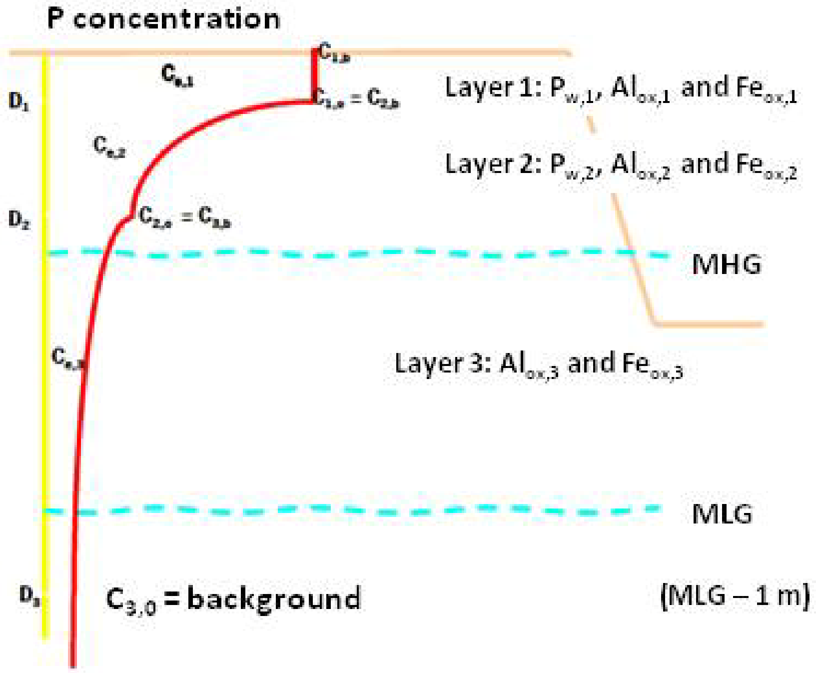

Figure 3.1. Schematic overview of the phosphate concentration profile in function of the depth (Schoumans et al., 2008)

The PLEASE model calculates water fluxes from field to surface water based on data of annual net precipitation surplus, annual water fluxes (upwards or downwards), and long-term upper and lower height of the groundwater table during the year. It is assumed that the sum of the annual net precipitation surplus and upward water fluxes (or minus the downward water fluxes) for the soil layer down to 1 m below the drainage level of the deepest local drainage system will be drained to local trenches and ditches. The distribution of the lateral water fluxes with depth is based on duration curves of groundwater height (Van der Sluijs, 1982). In a groundwater system there are several time scales in which water will reach a certain point, the duration for the nutrients in the groundwater is the same as for the groundwater itself. Therefore depending on the route the groundwater follows it will take days, years or even decades,…. before the nutrients will reach the surface water (Figure 3.2).

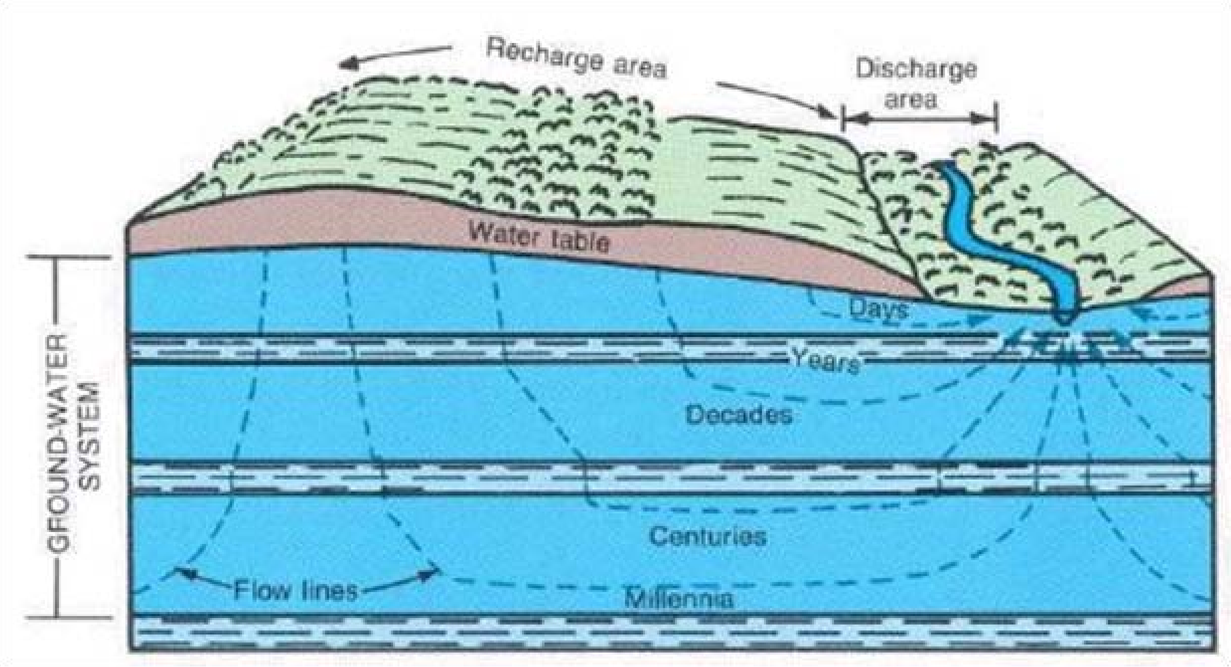

Figure 3.2. Representation of the groundwater system with the different duration heights of the groundwater (anonymous, 2013)

These curves provide information on the period during which a certain groundwater height is exceeded during the year based on observations of the mean highest and mean lowest groundwater height. In PLEASE, a deep (ditches) and a shallow drainage system (trenches) is considered. The magnitude of the drainage flux (q_{dr}) , occurring when the water table is at a certain depth (z), is derived using a linear relation between fluxes, groundwater height, and the depth of the two drainage systems (van der Salm et al., 2011):

$$\boldsymbol{q_{dr}} = \mathbf{10}\left[\frac{\max(0,z-h_{d,1})}{r_1} + \frac{\max(0,z-h_{d,2})}{r_2}\right] \tag{3.4}$$

with $h_{d,1}$ and $h_{d,2}$, the depth of the deep and shallow drainage system (cm below surface), respectively; r_1 and r_2 the corresponding drainage resistance of the shallow and the deeper drainage system, respectively, and 10 is the conversion factor from cm d^{-1} to mm d^{-1}. When data on the drainage levels and resistances are not available, $h_{d,1}$ is assumed to be equal to the mean highest groundwater level, and $h_{d,2}$ is set to the groundwater level that is exceeded 6 months of the year. The drainage resistance (r) can be assessed using the equation given by Ernst (1962) by summing vertical, horizontal, radial and entrance resistance. At the field scale, the radial and entrance resistance represent the major part of the total resistance r and therefore the other terms can be neglected, which results in a linear relationship between the resistance of the drains and their distances. The data in Ernst's study (Ernst, 1978) indicated that the drainage resistances can be iteratively solved by assuming that $r_2 = 1.2\ r_1$ (van der Salm et al., 2011) (Figure 3.3).

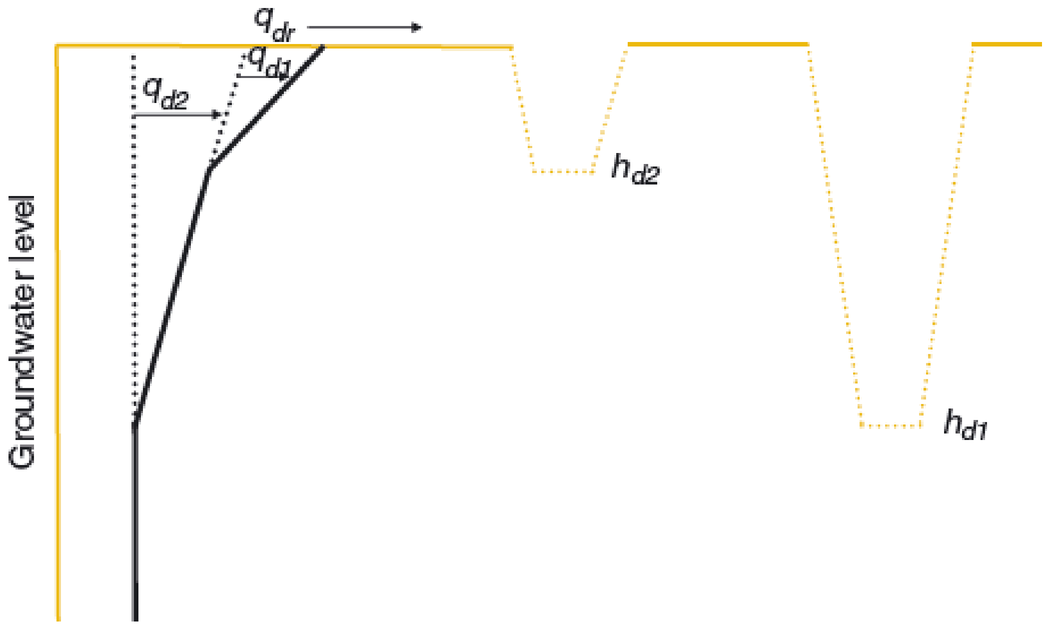

Figure 3.3. Schematic illustration of the drainage flux (q_{dr} in m day^{-1}) as a function of groundwater level and two drainage systems ($h_{d,1}$, depth of the local ditch and $h_{d,2}$ depth of the local trench) (Schoumans et al., 2013)

Van der Salm et al. (2011) stated that the duration curves for groundwater depth are valid for 11 months of the year. In the remaining part of the year, the groundwater depth may exceed the predictions made by the curve. To account for runoff and leaching occurring in very wet conditions, an exponential relationship between runoff/leaching and the mean highest groundwater height (MHG) is used:

$$\boldsymbol{Q_{ro} = 5.0 + 45.0e^{-0.015\,MHG}} \qquad (3.5)$$

where Q_{ro} is the runoff/leaching flux (mm yr^{-1}). The runoff/leaching flux is equally distributed from the surface to the depth of the MHG. According to this function, annual runoff/leaching varies between 5 mm at a deep MHG and 50 mm at a shallow MHG (van der Salm et al., 2011).

Model input

To run PLEASE, data on soil properties, P concentration in deep groundwater, and soil hydrology are necessary (Table 3.1).

Table 3.1. Overview of the soil related and hydrological inputs in the PLEASE model

Soil inputs		Hydrological inputs	
Langmuir sorption parameter	K	Mean highest groundwater height	MHG
Langmuir desorption parameter	k_d	Mean lowest groundwater height	MLG
Maximum adsorption fraction	B	Resistance of shallow drainage system	r_1
Resistance of drainage	ξ	Resistance of deeper drainage system	r_2
Bulk density	ρ_b	Depth of the deep drainage system	$h_{d,1}$
Oxalate extractable Al and Fe	$Al_{ox} + Fe_{ox}$	Depth of the shallow drainage system	$h_{d,2}$
P_w value	P_w	Net precipitation	NP
Background (water) concentration	c_x	Magnitude of the drainage flux	q_{dr}
		Depth of drainage	D

In the actual study the model was run for three or four soil layers (see further). Soil data input comprised measured Al_{ox} and Fe_{ox} values and bulk densities. The P_w was not measured but calculated, this can be done according to equation (3.3). However, this method is very involved, and since Chardon (1994) found a relationship between P_w and P_{ox}, Al_{ox} and Fe_{ox} for acidic sandy soils, following equation is used (Chardon, 1994):

$$\boldsymbol{P_w = 210\left[\frac{P_{ox}}{(Al_{ox}+Fe_{ox})}\right]^{1.43}} \tag{3.6}$$

where P_w is in mg P L^{-1} and P_{ox}, Al_{ox} and Fe_{ox} in mmol kg^{-1}.

The input parameters: the background concentration of P, in the groundwater, as well as the MHG and MLG for each field were determined using the information of DOV-Flanders, obtained by performing a geographical search on the DOV-site per municipality. The groundwater measuring net was chosen as active layer, with the water table measuring net as sub layer. The information from the nearest well, according to the X and Y coordinates, was used. The MHG and the MLG were determined by taking the average value, over eight years, from the four lowest and highest GWT.

PLEASE requires sorption parameters based on Langmuir isotherms. For this, the standard sorption characteristics reported in the protocol of Van der Zee (1988) were used, namely, $K = 35\ m^3\ mol^{-1}$, $k_d = 0.2\ d^{-1}$ and $\beta = 1.67$. The values used for the total drainage flux from the soil to surface water at groundwater level and the net precipitation (NP), depending on the crop and on the groundwater regime (Table 3.2), were based on literature (Table 3.3)(Schoumans et al., 2008). The used groundwater regimes differ from the ones used in the Belgium soil classification. The regimes from literature were chosen because they were connected with values for NP and for magnitude of the drainage flux, making them more optimal to use. The used values originate from the Schuitenbeek, in The Netherlands, because there are no data available for these parameters in Flanders. The Schuitenbeek is an area with almost all sandy soils, comparable to the acidic sandy region of Flanders, with an average precipitation of 750 mm yr^{-1}, compared to 805 mm yr^{-1} (KMI, 2008) in Flanders. The drainage flux can differ from location to location but this problem is overcome by relating the drainage flux to the groundwater regime, which is field specific (Schoumans et al., 2008). So the site specific information about MLG and MHG is related to the drainage flux. The resistance and the depth of the drainage systems were iteratively determined as done in previous research (Schoumans et al., 2008; van der Salm et al., 2011; Schoumans et al., 2013).

Table 3.2. Classification of the groundwater regime in terms MLG and MHG (cm), from light to dark grey, wet over average to dry, respectively (van Bakel et al., 2008)

MLG (cm)	MHG (cm)				
	< 25	25 - 40	40 - 80	80 - 120	≥ 140
< 50	Ia				
50 – 80	IIa				
80 – 120	IIIa	IIIb	IVu		
120 – 180	Vao	Vbo	VIo	VIIo	
≥ 180			VId	VIId	VIIId

Table 3.3. Net precipitation and the magnitude of the drainage flux (mm year^{-1}) per land use and per groundwater regime (Schoumans et al., 2008)

	Net precipitation per land use			Magnitude of the drainage flux
Groundwater regime	**Grass**	**Maize**	**Arable**	
Wet	257	383	348	98
Average	239	326	291	-5
Dry	246	312	289	-174

Comparison of model predictions with measured concentrations and sensitivity analysis

The PLEASE model was run on three datasets consecutively, whereby the P leaching simulated with PLEASE, was compared to the potential P leaching, derived from the PSD and the theoretical relationship between PSD and P concentration in leaching water (figure 1.8). According to Van der Zee et al. (1990b) an orthophosphate concentration of 0.1 mg L^{-1} is equal to a total phosphate concentration of 0.15 mg L^{-1}. At a precipitation surplus of 300 mm the total P loss will thus be 0.45 kg ha^{-1} yr^{-1} (Van der Zee et al., 1990b).

First, the PLEASE model was run on a dataset of 21 fields (location see Table 2.1), the majority of which had a high P content, as discussed in chapter 2. In this first dataset, the model was run for three different situations. In situation 1, we subdivided the (sub)soil in three soil layers using the calculated P_w data up to 60 cm (0-30, 30-60 and 60-(MLG+1m)). In situation 2, the (sub)soil was subdivided in three soil layers using the calculated P_w data up to 90 cm (0-30, 30-90 and 90-(MLG+1m)). For the first layer the P_w of 0-30 cm was used, while for the 30-90 cm layer an average of the P_w of the 30-60 cm and 60-90 cm layers was used. In situation 3, the model was run for four soil layers (0-30 cm, 30-60 cm, 60-90 cm, 90-(MLG+1m)). The difference between situation 1 and situation 2 indicates the effect of the soil sampling depth and the difference between the situation 2 and situation 3 shows the effect of a more detailed vertical representation.

Since this was done for both sampling occasions (sampling1 in 2001-2005 and sampling2 in 2009-2010), an indication is obtained on how sensitive the model is to P changes in the soil over time, which in turn is an indication of the practicability of the use of the model in policy making.

Secondly, the model was run, with four soil layers, on a dataset of 105 fields located in West- and East-Flanders (Figure 3.4). The dataset consists of soil samples taken on the incentive of farmers who were convinced to handle according to good agricultural practices in terms of nutrient management and did not agree with the categorising of their fields in P saturated areas. On these fields following parameters were only measured in the 0-30 cm layer, soil texture, with the pipette-method (Gee & Bauder, 1986) and the pH. For each of the three soil layers (0–30 cm, 30-60 cm and 60-90 cm) the amount of Al_{ox}, Fe_{ox} and P_{ox} was determined with the ammonium-oxalate extraction method (Schwertmann, 1964) and measured via ICP. The P_w was calculated based on the relationship with P_{ox}, Al_{ox} and Fe_{ox} (equation 3.6).

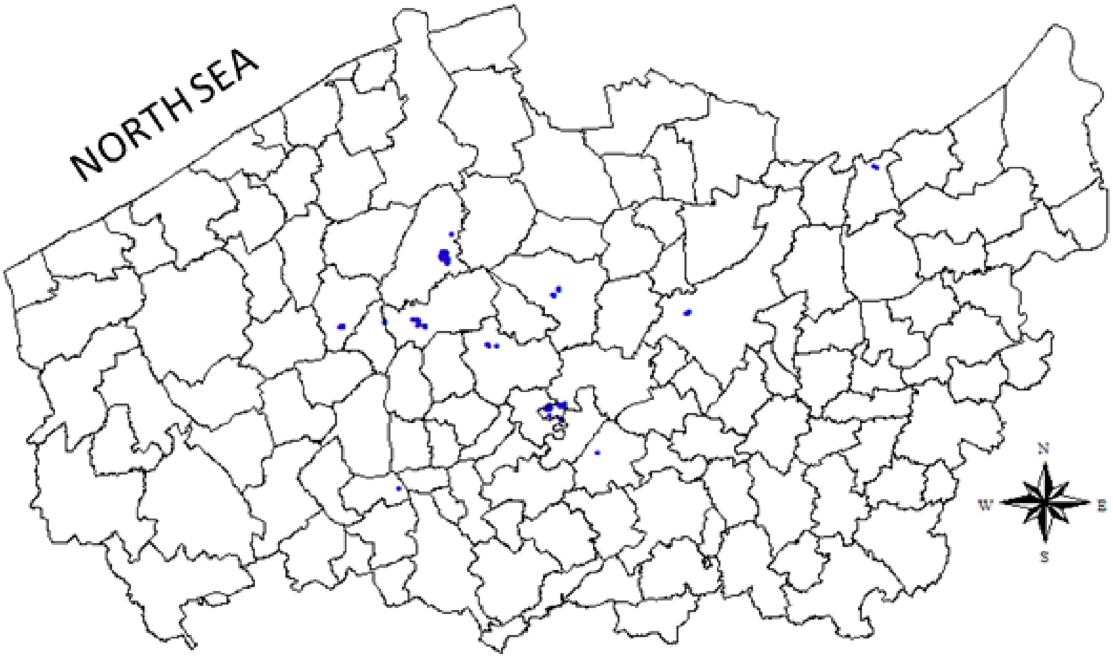

Figure 3.4. Geographical situation of the 105 fields used in the second data set (located in West and East Flanders)

Again for all fields the simulated P leaching was compared to the potential risk of P leaching obtained from the PSD. This data set was also used to perform a model sensitivity analysis, which quantifies the sensitivity of the model output to systematic changes in the model parameters. As such, the main sources of prediction uncertainty can be identified and the uncertainty in the model prediction can be decreased by increasing the quality of the most sensitive model parameter(s). In this study, the objective of the sensitivity analysis is to identify which model parameters have a significant impact on the prediction of P leaching. A local method for sensitivity analysis was chosen in order to investigate the model sensitivity for a specific scenario, *i.e.* at a fixed set of parameter values from the parameter domain. The sensitivity *S* of the model output *y* to model parameter *x* is calculated as the partial derivative of *y* to *x*, evaluated at one particular value of *x* using the finite difference approximation:

$$\boldsymbol{S} = \frac{\partial y/y}{\partial x/x} = \frac{\left(y(x+\Delta x)-y(x)\right)/y(x)}{\Delta x/x} \tag{3.7}$$

Following De Pauw and Vanrolleghem (2006) the model inputs were disturbed with 1% of the initial value, so that $\Delta x/x$ has a constant value of 0.01. The change in model output (input) is expressed relative to the undisturbed model output (input) which allows to compare the sensitivities among the different model parameters. Although we are aware that the sensitivity analysis was done changing one parameter at a time, and thus disregarding possible interactions between the different model parameters (Saltelli & Annoni, 2010), this approach was chosen because of the ease of implementation (no modifications to the PLEASE model are required) and the ease of interpretation.

Finally, the model was run on the large dataset (2683 data points (Figure 3.5)) that was used to make a P saturation map of Flanders (Figure 1.11). Based on the results obtained by the PLEASE model a P leaching sensitivity map of Flanders was made and compared with a map derived from the PSD.

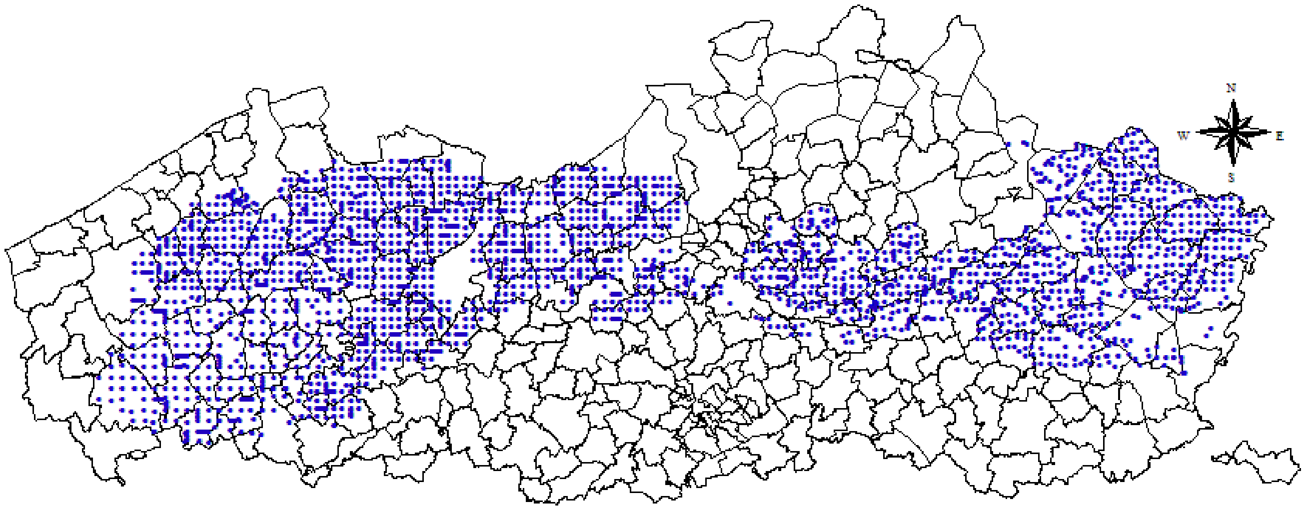

Figure 3.5. Geographical situation of the 2683 fields used in the second data set (located in Flanders)

Results

Data set of 21 fields: influence of number of layers and sampling dates

The results were in the same order of magnitude when the model was run for the three different situations, whereby the number of layers in terms of P_w input was tested (Figure 3.6). However, a higher total P flux was found when the model was run with four layers (situation 3) since the P content in the 60-90 cm layer was taken into account. The PSD and the PLEASE model are two different ways to determine whether a field is at risk for P leaching. According to the relationship in Figure 1.8, a PSD of 24% is equivalent to 0.45 kg P ha^{-1} yr^{-1} (Van der Zee et al., 1990a). The limit value for a field, in terms of P saturation is a (simulated) P flux of 0.45 kg ha^{-1} yr^{-1}. The P flux, as simulated by PLEASE for the data of sampling1, was beneath this value for fields 2, 12 and 13 in situation 1, for fields 1, 2, 12 and 13 in situation 2 and for field 12 in situation 3 (Figure 3.7). For sampling2, the simulated flux of field 13 was below the limit for all three situations (Figure 3.8). In situation 2 and situation 3 field 12 was also below the limit. Field 11 was characterized by a large P content in the 60-90 cm layer at sampling2 compared to sampling1 and this lead to a high simulated P flux of 3.41 kg ha^{-1} yr^{-1} in situation 3 which was not taken into account in situation 1 (2.15 kg ha^{-1} yr^{-1}) and only partly in situation 2 (namely 2.58 kg ha^{-1} yr^{-1}).

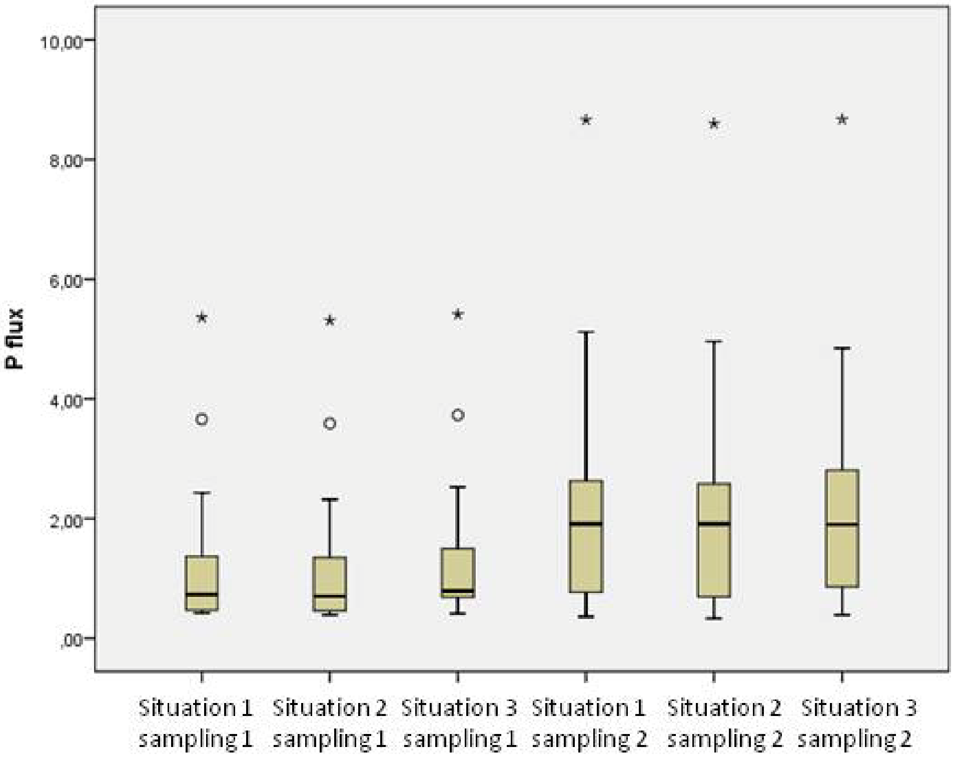

Figure 3.6. Box plots of the different situations at both sampling dates

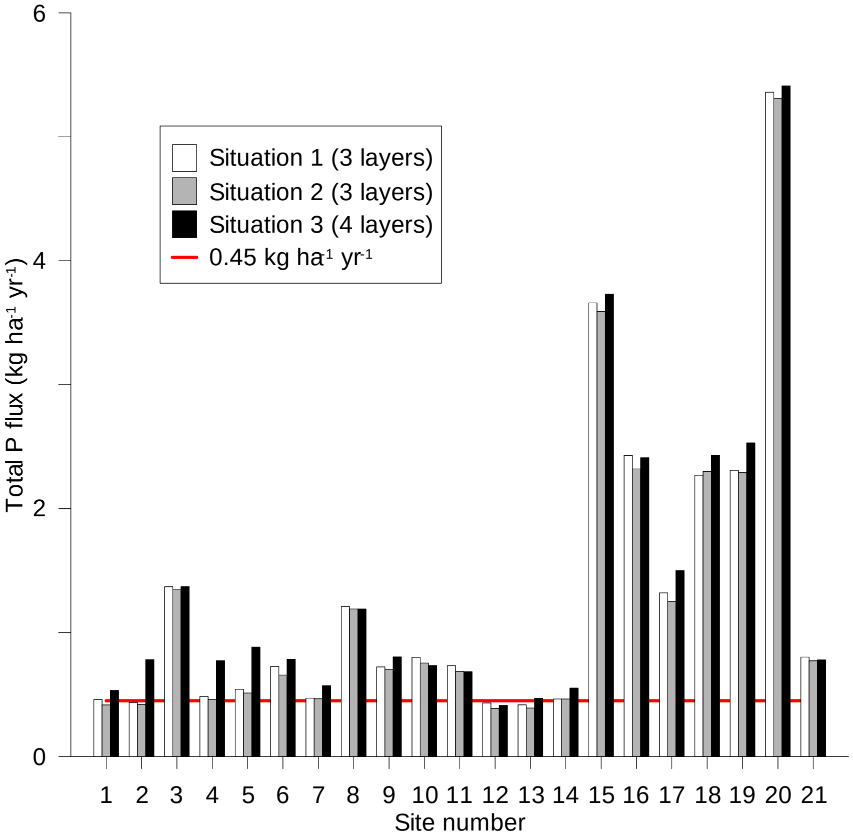

Figure 3.7. The simulated PLEASE total P flux values (kg ha^{-1} yr^{-1}) for 21 fields at sampling1 for the three different situations

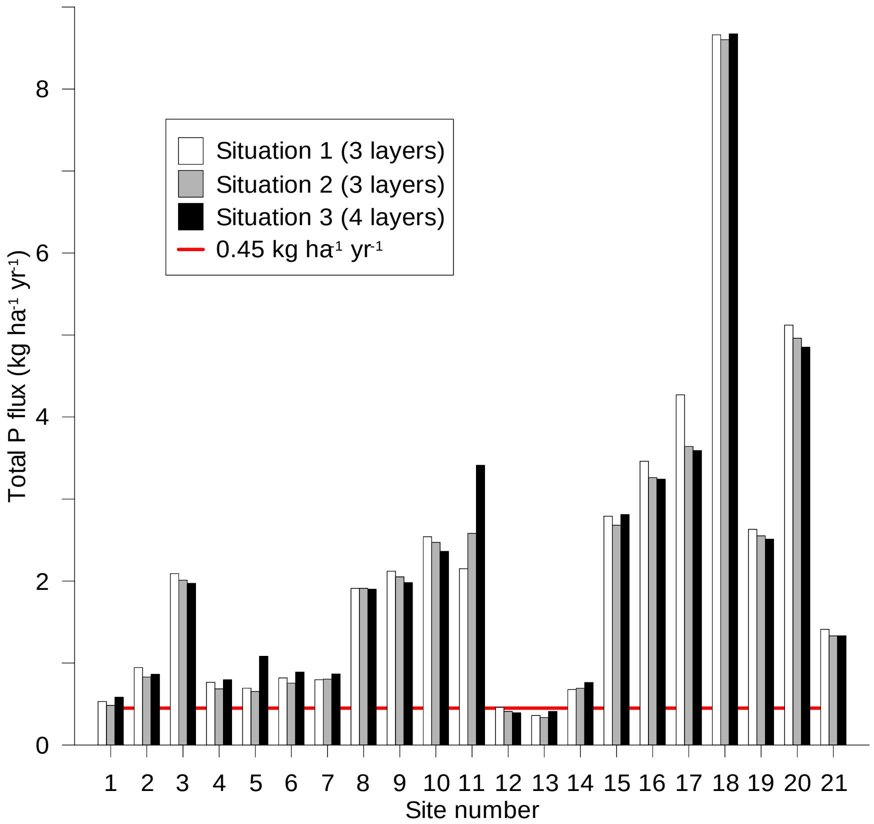

Figure 3.8. The simulated PLEASE total P flux values (kg ha^{-1} yr^{-1}) for 21 fields at sampling2 for the three different situations

If both methods give the same indication of P saturation all data points should be close to or on the theoretical relation (Figure 3.9). In 90.5% (sampling1) and in 85.7% (sampling2) of the cases both methods gave the same classification of the fields (Table 3.4). However field 1, at both sampling dates, was classified as not P saturated with the assessment of the PSD (a PSD of 19.25 at sampling1 and 21.73 at sampling2) so, not at risk of P leaching, but according to the PLEASE model this field was categorized at risk of P leaching. Field 1 had as characteristics a rather low P content but a high GWT (Figure 3.10). Field 12 and field 13 were classified as P saturated only at sampling2 and thereby at risk of P leaching, but according to PLEASE they had a total P flux lower than 0.45 kg ha^{-1} yr^{-1}. The simulated P losses for fields 12 and 13 were for both sampling dates very close to 0.45 kg ha^{-1} yr^{-1}. They had a high P content in the upper soil layer, with a rapid decline in depth, and a low GWT (Figure 3.10). Similar to the increase over time of the PSD values, an increase was found when the total P flux (results of PLEASE model) was compared between both sampling dates for all fields, except for fields 12, 13, 19 and 20 (Table 3.5).

Table 3.4. Classification of the 21 fields according to the potential P leaching (PSD) and the actual P leaching (PLEASE model, four data layers) at both sampling dates

Class	PSD (%)	Total P flux (kg ha^{-1} yr^{-1})	Sampling1 Percentage (%)	Sampling1 Field	Sampling2 Percentage (%)	Sampling2 Field
I	≤ 25	≤ 0.45	0		0	
II	≤ 25	> 0.45	4.76	1	4.76	1
III	> 25	≤ 0.45	4.76	12	9.52	12, 13
IV	> 25	> 0.45	90.48	All except 1, 12	85.72	All except 1, 12, 13

Table 3.5. Modelled total P flux, with the PLEASE model, for both sampling campaigns (in kg ha^{-1} yr^{-1})

Field	Sampling1	Sampling2	Field	Sampling1	Sampling2
1	0.533	0.583	12	0.412	0.391
2	0.781	0.861	13	0.47	0.408
3	1.37	1.97	14	0.552	0.761
4	0.773	0.794	15	3.73	2.81
5	0.883	1.08	16	2.41	3.24
6	0.785	0.889	17	1.5	3.59
7	0.571	0.865	18	2.43	8.67
8	1.19	1.9	19	2.53	2.51
9	0.803	1.98	20	5.41	4.85
10	0.734	2.36	21	0.779	1.33
11	0.683	3.41			

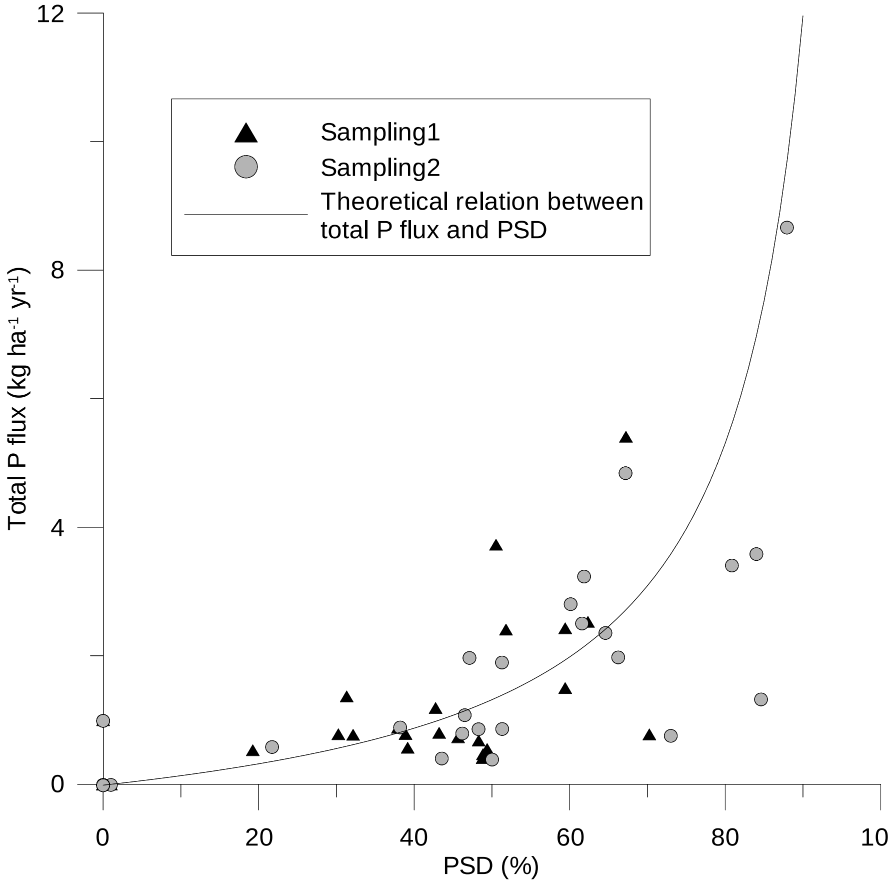

Figure 3.9. Scatter plot of the simulated total P flux, with PLEASE (four data layers), and the measured PSD for 21 fields in Flanders on both sampling dates

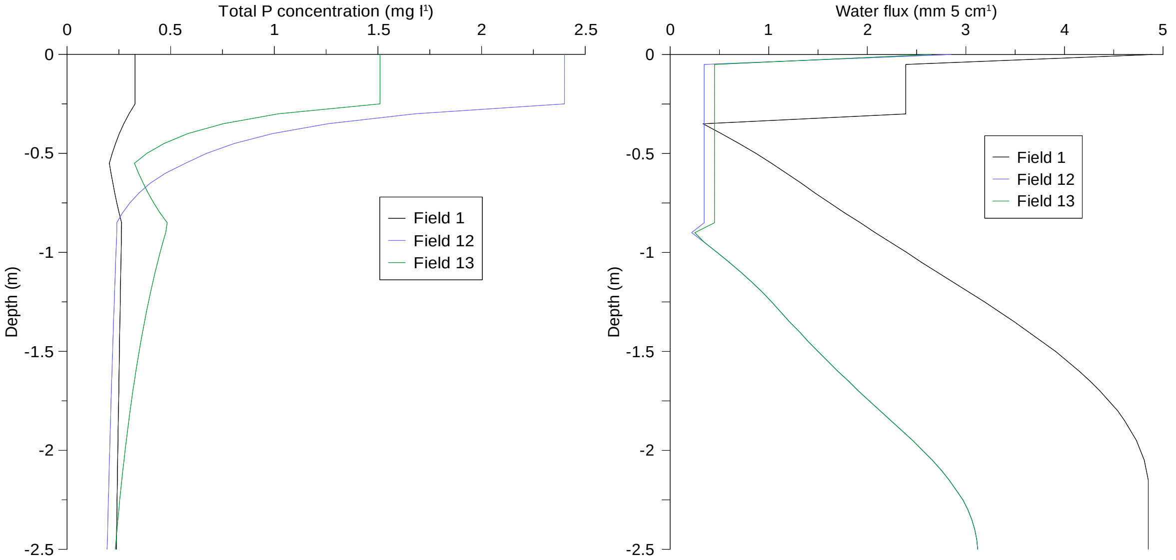

Figure 3.10. Representation of the total P concentration (mg l^{-1}) and the water flux (mm 5 cm^{-1}) within the soil profile for field 1, 12 and 13 at sampling 2

Data set of 105 fields: sensitivity analysis of the input parameters in PLEASE

A sensitivity analysis was done on the input parameters, used for Flanders, in the PLEASE model. This is important to have an idea on which input parameters are most essential in Flanders to have an optimal result of the model results. For four of the investigated inputs, K, ξ, C_x and MLG, the model is rather insensitive ($S < 0.5$), for P_w and net precipitation (NP) S is greater than 1, while for the other six investigated inputs S is between 0.5 and 1 (Figure 3.11). If S is greater than 1 a change of 1% in the model input gives an effect on the average model response of more than 1%, which is considered as a very high model sensitivity.

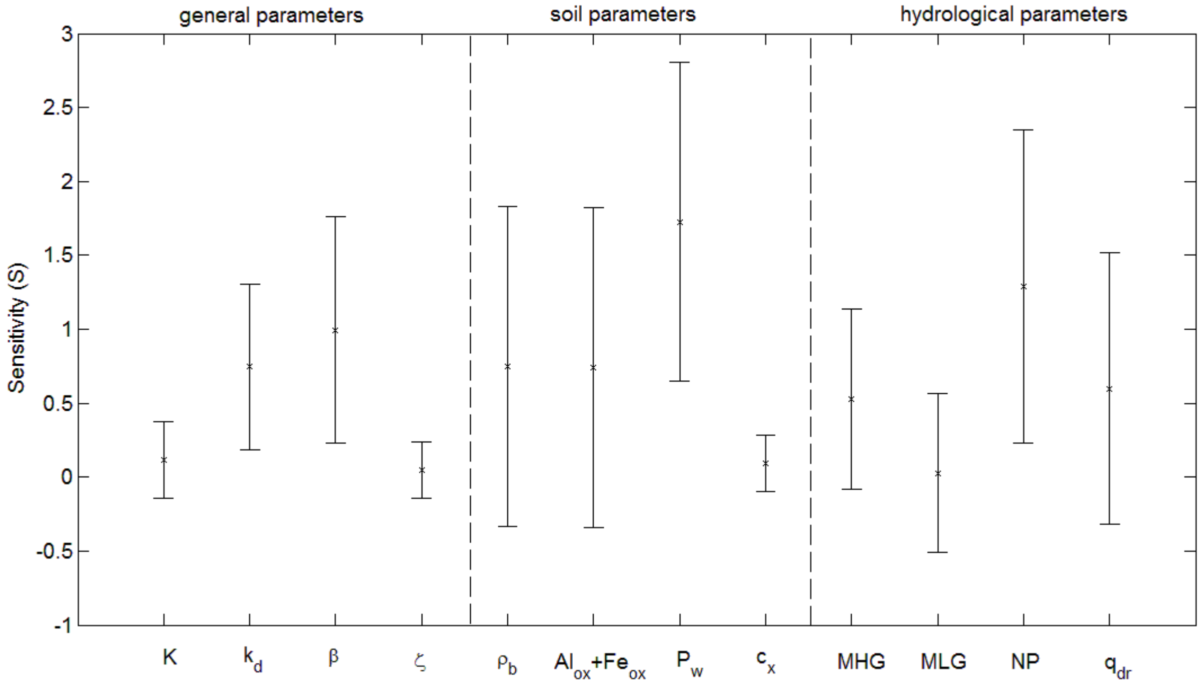

Figure 3.11. Sensitivity of the simulated P flux according to the general, soil and hydrological inputs; the average and standard deviation is determined on 105 PLEASE simulations

The different inputs were divided in three classes based on the average and standard deviation on S for 105 data points (Figure 3.12). Class I contains the inputs that are least crucial since they have a low sensitivity and a low dispersion of S, namely C_x, ξ, K and MLG. Class II are the inputs with an average effect on the sensitivity S and an average to high dispersion of S, namely k_d, MHG, q_{dr}, β, ρ_b and Al_{ox}+Fe_{ox}. Class III contains the inputs with the highest effect and the highest dispersion of the S, namely NP and P_w.

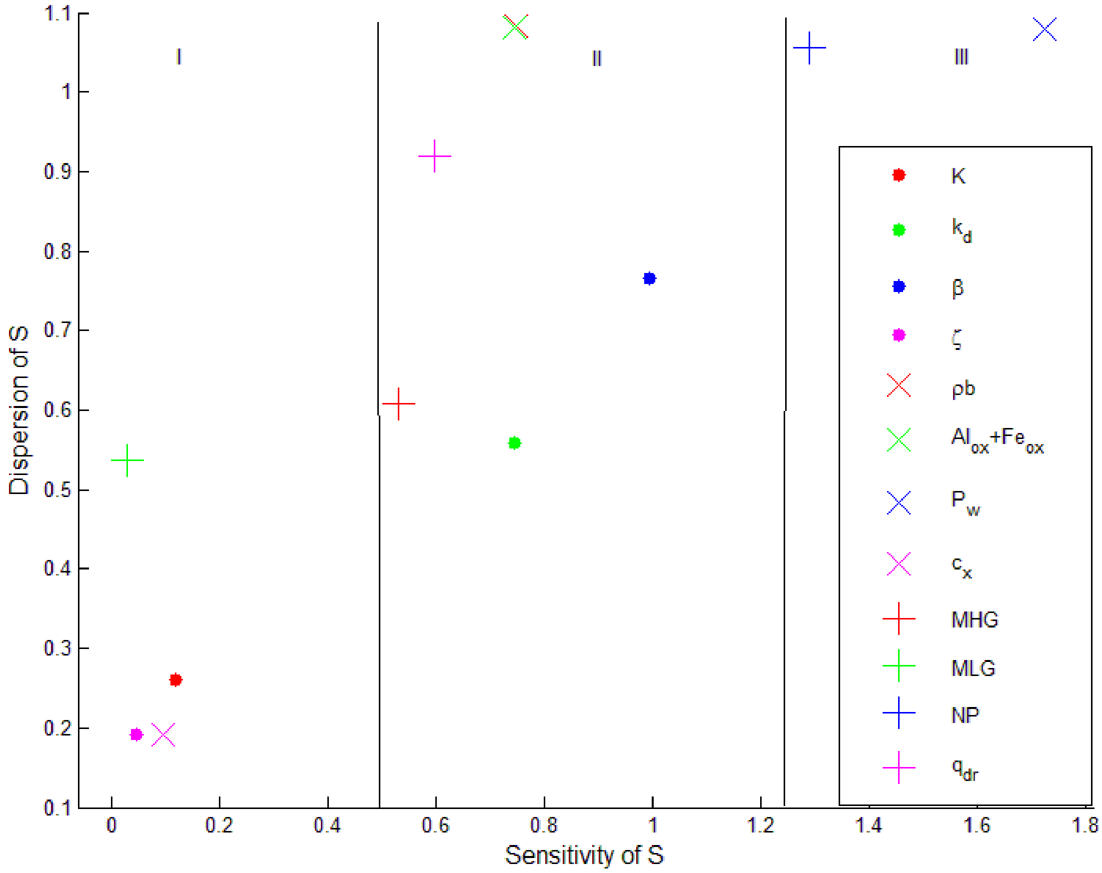

Figure 3.12. Scatter plot between the average sensitivity and the standard deviation on the sensitivity, for 105 data points. De model inputs were divided into three classes that reflect the importance of the inputs from the model

Data set of 105 fields: comparison actual and potential P loss

The results of the second data set (105 data points) show that for a number of fields the simulated P flux and the measured PSD follow the theoretical relationship between PLEASE and PSD (Figure 3.13). However, also a significant number of data points do not follow this relationship and have a lower actual P leaching risk compared with the potential P leaching risk (these points are beneath the theoretical relationship). On the other hand some fields result in a higher actual P leaching risk compared with the potential P leaching risk (located above the theoretical relationship in figure 3.12). The comparison of the simulated actual P loss (PLEASE) and the potential P loss (PSD) made per field, indicated that in 60% of the cases the same classification (namely 12.4% not P saturated and 47.6% P saturated) was found. On the other hand, 38.1% of the fields were categorized as P saturated, but had a lower P flux than 0.45 kg ha^{-1} yr^{-1}. In only 1.9% of the cases a field classified as not P saturated with the PSD method had a higher P flux than 0.45 kg ha^{-1} yr^{-1} (Table 3.6).

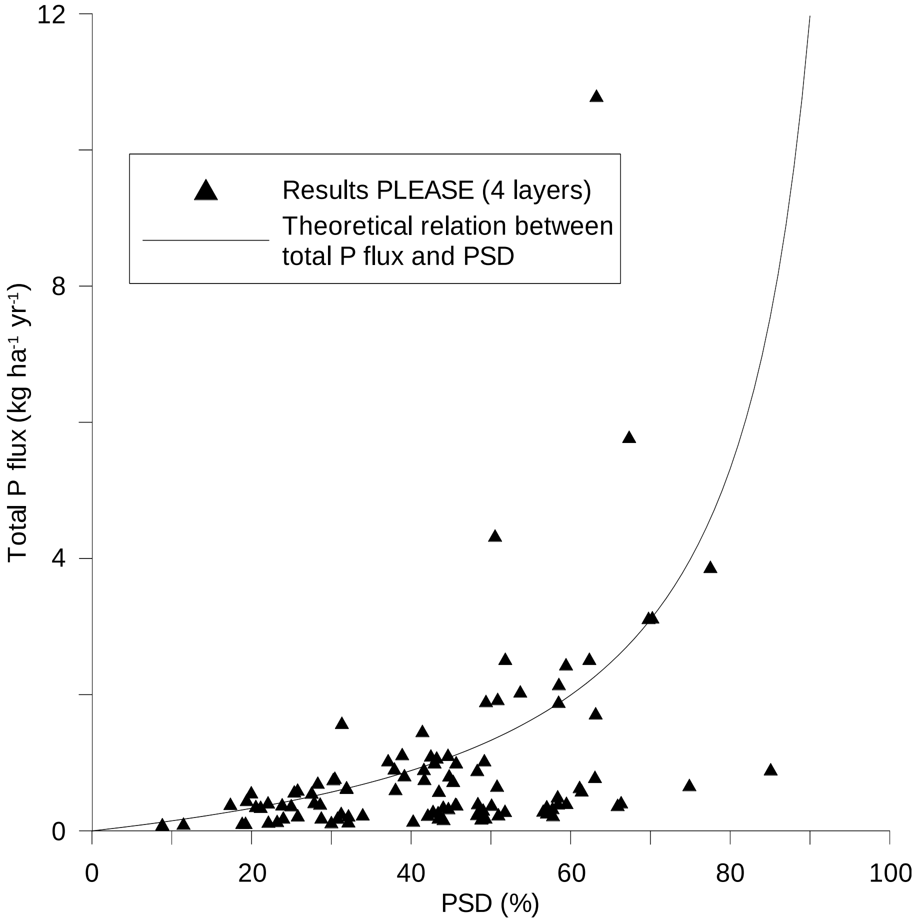

Figure 3.13. Scatter plot of the simulated total P flux and the measured PSD for the second data set (105 fields located in West and East-Flanders) with the PLEASE model (4 data layers)

Table 3.6. Classification of the 105 fields according to the potential P leaching to groundwater (PSD) and the actual P leaching to surface water (PLEASE model)

Class	PSD (%)	Total P flux (kg ha^{-1} yr^{-1})	Percentage (%)
I	≤ 25	≤ 0.45	12.4
II	≤ 25	> 0.45	1.9
III	> 25	≤ 0.45	38.1
IV	> 25	> 0.45	47.6

Regional data set of the acidic sandy soils in Flanders (2683 points)

The NP in the model PLEASE is dependent on the land use. Since collecting the land use of all fields in the acidic sandy region would be a very complex and time consuming exercise, the model was run for three simplified scenarios (each with their NP value), assuming that the entire area was under arable, maize or grassland. The results of the actual and potential P loss were compared for the acidic sandy region in Flanders (2683 data points, Figure 3.14) for these three scenarios, yielding the same classification in terms of P saturation in 67.4% (arable), 67.8% (maize) and 67.4% (grass) of the cases (Table 3.7).

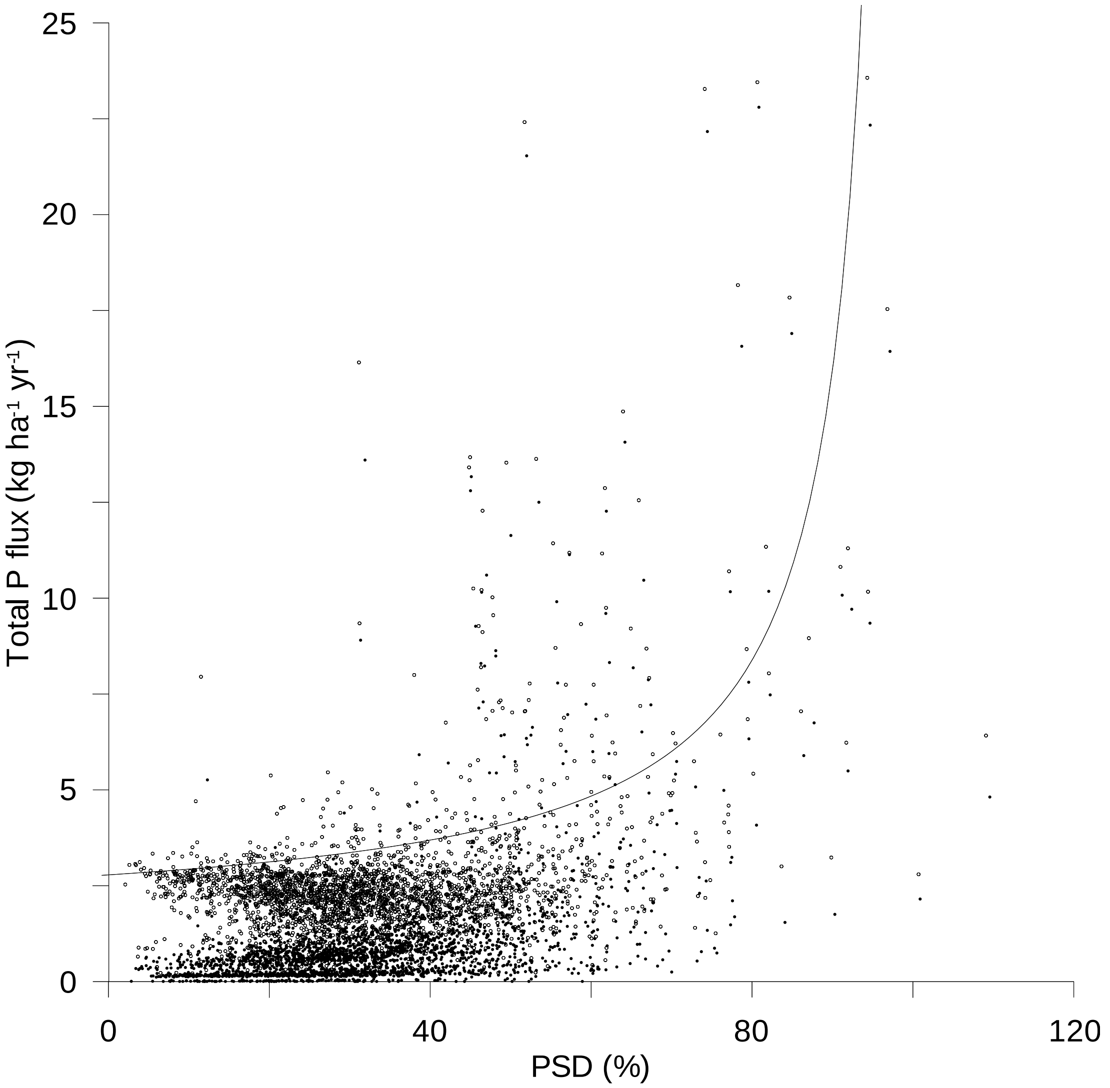

Figure 3.14. Scatter plot of the simulated average total P flux and the measured PSD for 2683 fields in the acidic sandy region of Flanders, with the PLEASE model (4 data layers)

Table 3.7. Classification of the 2683 fields according to the potential P leaching to groundwater (PSD) and the actual P leaching to surface water (PLEASE model)

Class	PSD (%)	Total P flux (kg ha^{-1} yr^{-1})	Percentage if arable (%)	Percentage if maize(%)	Percentage if grass (%)
I	≤ 25	≤ 0.45	17.75	16.37	20.21
II	≤ 25	> 0.45	14.5	15.88	12.04
III	> 25	≤ 0.45	18.08	16.29	20.54
IV	> 25	> 0.45	49.67	51.46	47.21

Since the differences between the simulated total P flux considering the different land use scenarios were rather small, the average of the simulated total P flux for these scenarios was calculated (Figure 3.15). The PLEASE model, predicting the actual P loss, classified 47% of the fields as P saturated for the acid sandy region of Flanders, 17% was classified as P critical and 36% without problems in terms of P saturation. The classification based on the PSD (potential P loss) indicated 38% as P saturated (PSD > 35%), 30% as P critical (25% < PSD < 35%) and 32% as no problems in terms of P saturation (PSD < 25%), (Figure 3.16). The difference between the classification done by the PLEASE model and by PSD can be find in Figure 3.17.

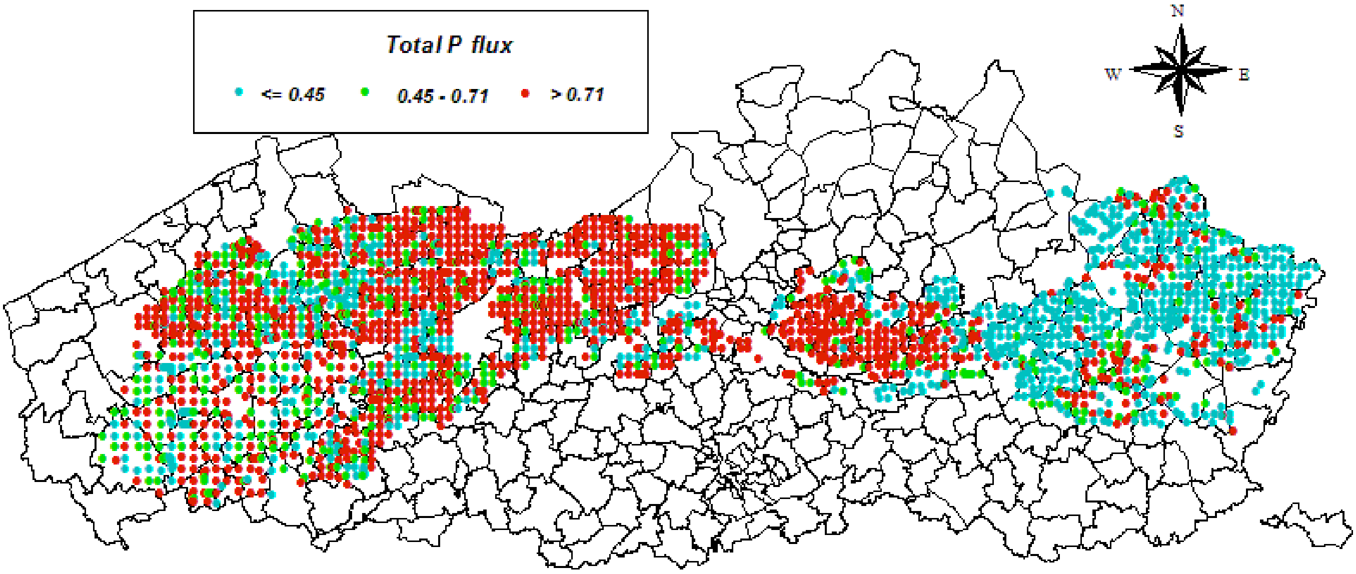

Figure 3.15. Classification of the fields in the acidic sandy region of Flanders according to the simulated total P flux (kg ha^{-1} yr^{-1}) by PLEASE

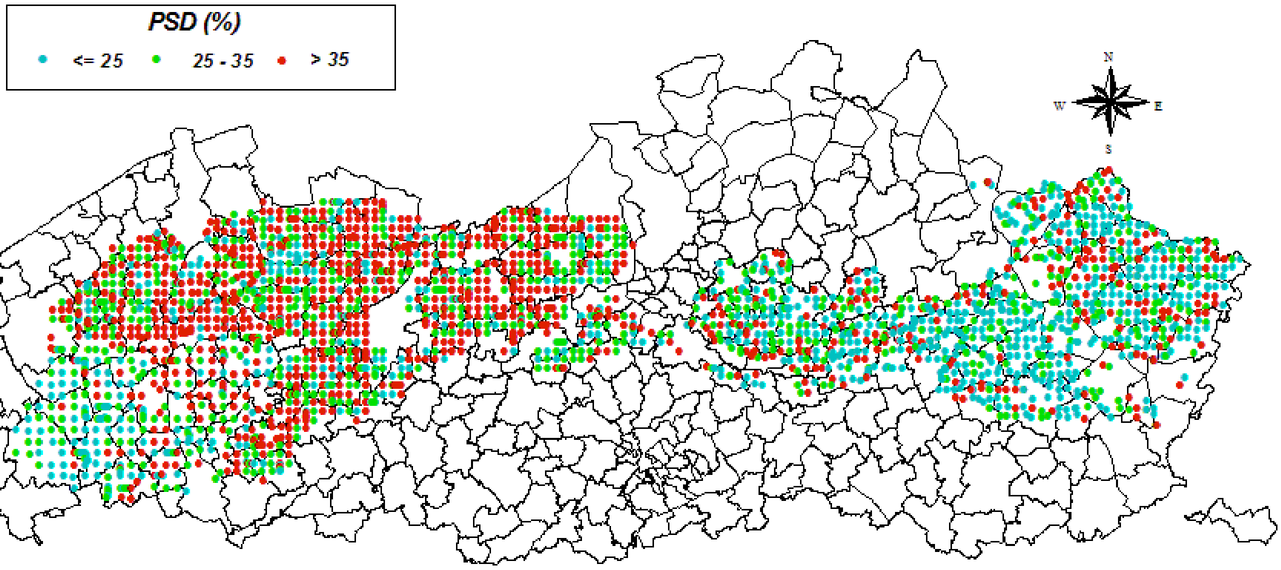

Figure 3.16. Classification of the fields in the acidic sandy region of Flanders according to the PSD (%)

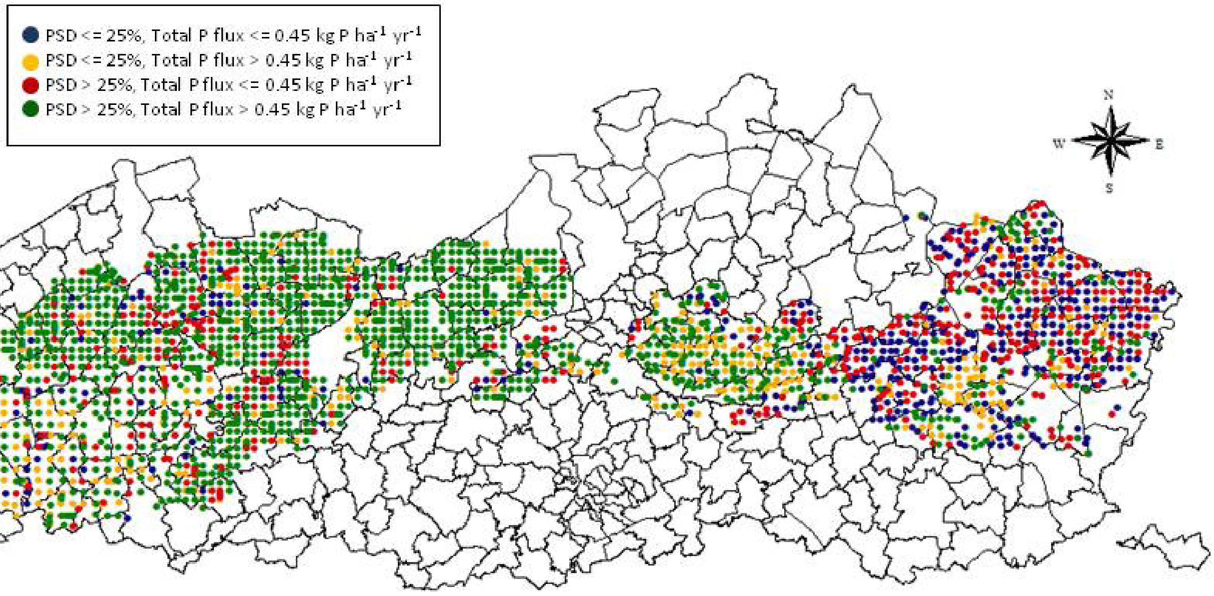

Figure 3.17. Difference in classification of the fields in the acidic sandy region of Flanders according to the simulated total P flux by the PLEASE model and the PSD

Discussion

Data set of 21 fields: influence of number of layers and sampling dates

Schoumans et al. (2013) stated that PLEASE is a simple model to assess the annual contribution of non point P loss by leaching from agricultural land to surface water. In the standard simulations of the model, one soil layer was used beneath 50 cm and background P concentrations were used to calculate the decline in P concentrations in the soil between 50 cm and a depth of 1 m below the lowest groundwater table (Schoumans et al., 2008; Dupas & Van der Salm, 2010). This application is comparable to situation 1 which is the standard simulation, namely using 3 soil layers. In The Netherlands, the plough layer is commonly considered to be 20 cm whereas in Flanders this is 30 cm. For situation 1 is assumed that the penetration depth of P in the soil is not deeper than 60 cm (50 cm in the Netherlands (Schoumans, 2004)). However, the measured P_{ox} indicates for all 21 investigated soils that P penetrates deeper than 60 cm in Flanders.

Generally, the differences between the situations in simulated P flux are rather small. However, using 4 data layers as input for the PLEASE model, situation 3, instead of 3 data layers, situation 1 and 2, had for some fields a rather important influence on the simulated total P flux. Since not only the amount of P_w but also the amount of Al_{ox} and Fe_{ox} of the 60-90 cm layer were used and due to the fact that some fields, in Flanders, have high P or Al, Fe concentrations in this layer it is desirable to use the results from the PLEASE model with the 4 layer data input. Therefore it is advisable to run the PLEASE model with four soil data layers, situation 3, so that no information in terms of P is neglected.

Since the PSD is also an indicator of P leaching, which is used as a decision tool in terms of P saturation in Flanders, both techniques, PSD and PLEASE, should give the same recommendation for the same site if the effect of the hydrological conditions of a field are negligible. When the results from both indicators were compared, generally the same trend was seen per field and over time (Figure 3.7 and Figure 3.9). However, some important differences were noticed. Field 12 and 13 which had a PSD higher than 25% and were classified as P saturated were, according to PLEASE, not P saturated. The low simulated total

P flux can be explained by the fact that these fields have a low GWT (Figure 3.10). This proves that including the hydrological factors more specifically gave a better estimation of the potential P loss per field (Schoumans et al., 2013). The method of Van der Zee et al. (1990a) calculates an average P over the whole soil profile (until 90 cm or until the MLG if deeper than 90cm), so a potential P loss in the soil is calculated and not the actual P loss. This is also proven for field 1, with a PSD ≤ 25%, which is at risk of P leaching due to the high GWT.

Data set of 105 fields: sensitivity analysis of the input parameters in PLEASE

The sensitivity analysis indicates to what extent the simulated P_{load} is sensitive to small changes in the model inputs. This makes it possible to identify the inputs that have the greatest impact on the model predictions and enables the user to reduce the uncertainty of the model simulations by reducing the uncertainty of the most sensitive inputs and collecting the data as efficiently as possible (Saltelli et al., 2000; Frey & Patil, 2002). With an additional soil survey or by using more detailed literature data it is possible to have a more accurate assessment of these input parameters. In this study the sensitivity of PLEASE is investigated for several general, soil related and hydrological input parameters. NP and P_w are the parameters that gave the highest sensitivity and the highest dispersion of the *S*, therefore it is important that their data input is most accurate to obtain an optimal result in predicting the actual P leaching.

The general model inputs K (= 35 $m^3\ mol^{-1}$) and ξ (= 0.25 $d\ m^{-1}$) have a small impact on the sensitivity of the model, k_d and β, however, have a considerable higher impact. The soil texture influences the sorption kinetics, a correct determination of the soil texture per field is therefore necessary. The general inputs are currently not given per data point but arbitrary for the model, a value for K and β per data point could be an improvement. Our findings are partly in agreement with the findings of Schoumans et al. (2013), who found that an increase in β and K reduces P losses. They found that the influence of K is strong at values up to 15 $m^3\ mol^{-1}$. At higher values, the impact is relatively small with a minimum at 25 $m^3\ mol^{-1}$. For soils with a high soil P status a higher K value leads to a much higher P concentration at the measured P_w values (Schoumans et al., 2013).

Of the soil related inputs C_x had the lowest sensitivity (S = 0.15) with low variability between the fields. This indicates that the P concentration in the groundwater has a minimum effect on the P leaching of a field and a simple assessment is sufficient, which is in agreement with Schoumans et al. (2013). As expected, the higher the soil P status the more important the P_w values for the prediction of the actual P loss. Since P_w has a very high impact on the results of the model it is important to have the most accurate inputs measured for each field. Since in Flanders P_w is not seen as a standard parameter it is advisable to further fine-tune the relationship between P_w and Al_{ox}, Fe_{ox}, P_{ox}, or to include it as a standard parameter in soil analysis.

Most of the investigated hydrological input parameters had a low to average ($0 < S < 0.6$) sensitivity on the prediction of P loss. MLG seems to be the least important hydrological input parameter in terms of sensitivity of the model. Therefore it is sufficient to determine the MLG and even the MHG (S = 0.5) by taking the average value, over eight years, from the four lowest and four highest GWT found in the groundwater table measuring net. The NP seems to be very important in the calculation of the P loss of a field, which is a problem since it is difficult to have crop dependable NP values per field. However when the model's sensitivity for NP is investigated more in detail it was found that for a higher P flux the sensitivity for NP decreased drastically. This is logical since NP is only important for the determination of the H_2O flux and a high P flux is only found when the P concentration in the soil solution is (already) high. It was also found that the effect of the H_2O flux on P loss is important at soil depths below 50 cm, while in superficial soil layers (< 50 cm) the H_2O flux had almost no effect on the P loss (Schoumans et al., 2013).

Comparison of the simulated actual P loss and the potential P loss for the second data set (105 fields) and for the acidic sandy region of Flanders

In 60% of the cases (in the second data set) and in 67% of the cases (large data set) both methods had the same classification of a field in terms of P leaching. Since the P saturation limit has implications in terms of the agricultural management of the fields it is important to have the most optimal decision method. For the acidic sandy region, between 16% and 20% of the fields were categorized as P saturated based on the PSD but according to the PLEASE model simulations, these fields were not classified as P saturated. This implies, as also described in literature, that it is important to take the hydrological aspects of P leaching into account. Namely when a field is characterized by a high groundwater table and there is direct contact between the P in the soil solution and the groundwater table, the risk of increased eutrophication becomes inevitable, even with a low PSD status. The opposite is also possible, namely having a high PSD status but a very low groundwater table which decreases the risk of connectivity between the P in the soil solution and the groundwater thereby decreasing the risk of eutrophication. Similar conclusions have been drawn for the assessment of P losses by surface runoff and erosion (Haygarth & Sharpley, 2000).

With the PLEASE method a considerably higher number of fields were classified as P saturated (9%) in comparison with the PSD method, but a considerable lower number of fields was classified as P critical. When looking more closely at both maps it is noticed that the hydrological conditions of the field play a role in the P leaching risk (Figure 3.15 and Figure 3.16). On a local scale significant differences between the actual and potential P loss are found e.g. a higher P leaching risk was found with PLEASE for the provinces Antwerp and Flemish Brabant. Since it is already proven that PLEASE works good in sandy soils (van der Salm et al., 2011) it is important to consider using PLEASE as a tool for future adaptation of the legislation. Today in Flanders a soil is classified as being P saturated by the concept of the PSD. This is a reliable method when looking at the P content in the soil, however as stated before it does not take the hydrological conditions of the soil fully into account, which can be significantly different from area to area and even from soil to soil.

Conclusions

The PLEASE model requires several general, soil specific and hydrological input parameters. All these parameters have an uncertainty factor which has an effect on the uncertainty of the outcome of the simulated P loss of a field. To have the most accurate prediction of the P loss it is important to minimize the uncertainty of the parameters. The sensitivity analysis showed that the prediction of the P loss is most sensitive to P_w, soil specific input, and NP, hydrological input, which means that great differences in the model can be obtained with small changes to these inputs. For P_w more accurate inputs can be obtained relatively easy since it can be measured on the field. A high accuracy needs to be aimed for NP as these data are not available in Flanders for the moment.

In this study the PLEASE model was tested on several scales ending with the whole acidic sandy region of Flanders, to predict the risk of P leaching. A comparison between the actual and potential P loss indicated that only in 67% of the cases the same classification was found. When the differences in local hydrology are not taken into account it is possible that mitigation measures are applied at the wrong fields because not all soils with a high PSD will lead to a high P loss. On the other hand, fields with a low PSD could have a relative high P loss due to hydrological conditions. Therefore it is advisable also to include the hydrological conditions and the PLEASE model seems to be a promising method for this.

Chapter 4

The use of soil amendments to increase the P sorption capacity of acidic sandy soils

Illustration on p. 67:

Products added to the soil. From left to right, first row: olivine, biotite, zeolite and gypsum and the second row: bauxite, dried Fe-sludge, Phoslock® and Sachtofer.

A publication of this chapter is in preparation :

Minerals and wastewater treatment products effectively increase P sorption capacity in acidic sandy soil (2013). Science of the total environment

Abstract

A way of trying to tackle P leaching is by adding products to the soil that increase its P sorption capacity. In this study four types of products, namely mineral salts ($CaCO_3$, $CaCl_2$, $CaCO_3$+$CaCl_2$, $AlCl_3$, $AlSO_4$, $FeCl_3$ and $FeSO_4$), primary and soil minerals (olivine, biotite, zeolite, gypsum), bauxite, an industrial by-product (dried Fe sludge) and specially designed products (Phoslock® and Sachtofer) were tested for their ability to fix P in an acidic sandy soil.

All amendments reduced the availability of P in the tested phosphate saturated soil. The Al salts were effective only at low addition rates. Grinding (ball milling) of the amendments did not result in an increase of the P fixation capacity of the amendments. The P fixation efficiency of olivine, biotite or zeolite was significantly greater when they were first chemically pre-treated (HCl 1h). This resulted in a P fixation capacity of 70% for olivine, 74% for biotite and 79% for zeolite at an addition rate of 1.5%. Dried Fe sludge and the Fe salts had the best P fixation capacities, even at low addition rates. Dried Fe sludge with an addition rate of 0.1% had a P fixation capacity of 45%. For $FeCl_3$ and $FeSO_4$ in an addition rate of 0.1% a reduction in $P_{inorganic}$ of 50% and 70% was found respectively. Sachtofer and Phoslock® gave also promising results in P fixation capacity.

The most promising products out of the screening experiment were tested in a P fixation experiment. The mineral salts ($CaCO_3$, $AlCl_3$, $FeCl_3$ and $FeSO_4$), primary and soil minerals (olivine, biotite, zeolite, gypsum), bauxite, an industrial by-product (dried Fe-sludge) and specially designed products (Phoslock® and Sachtofer) were chosen. The products were tested on their ability to fix P during 8 successive rain periods and their effect on different P contents in the soil.

The results showed that the P level of the soil plays an important role in the P fixation efficiency of the added products. Generally it was found that out of the selected minerals pre-treated olivine and biotite had the highest P fixation capacity. When comparing the results of the industrial by-product and the specially designed products it was found that dried Fe sludge had the highest P fixation capacity.

Introduction

Phosphorus (P) losses from heavily fertilized agricultural soils cause severe environmental problems in Flanders, the northern part of Belgium (De Bolle et al., 2013a). Elevated P concentrations in surface waters contribute to the deterioration of surface water quality (Agyin-Birikorang et al., 2007). In the EU, especially in Belgium, The Netherlands and Denmark, many of the acidic sandy soils have a high PSD and are subjected to strict P fertilization restrictions, what ultimately should result in P depletion or P mining.

However, it is felt that, with current crop rotations, it will take many decades to bring P levels back to environmentally safe levels, because P mining efficiency decreases rapidly with time (Callahan et al., 2002; Sharma et al., 2007). For example, Watson et al. (2007) found no change in Olsen P after 5 years with no P application on grazed grassland soil. These authors estimated that only 8.4 kg P ha^{-1} yr^{-1} was removed, mainly via animal live-weight gain (Watson et al., 2007). A similar experiment, zero P application for 5 years on non-grazed grassland, in the Netherlands resulted in a rapid decline in the weakly bound P concentrations in the soil solution in the upper 5 cm of the soil, but the effect was less pronounced at lower depths, and therefore the overall effect on P leaching to surface water may be expected to be small (Koopmans et al., 2004b; van der Salm et al., 2009). A declining trend in P off-take was also noticed, indicating that mining efficiency decreases significantly over time as a result of decreasing P concentrations in the grass and a decline in dry matter production (van der Salm et al., 2009). Therefore accompanying or alternative measures will be needed to further minimize P leaching from saturated soils in the short term (Brauer et al., 2005; Chardon et al., 2012).

One possible alternative management option would be to increase the P fixation capacity of the upper soil layers with addition of materials rich in Al, Fe and/or Ca, because Ca-rich materials tend to remove P by Ca phosphate precipitation, whereas Al- and Fe-rich materials can precipitate and absorb P (ligand exchange)(Callahan et al., 2002; Chardon et al., 2012; Stoner et al., 2012).

Various P sorbing materials have been proposed, and these can be classified in four main categories : (1) simple mineral salts, divided into 3 subcategories, based on their ability to fix P in different soil types (Gupta & Abrol, 1990) (Table 4.1), (2) primary and soil minerals or their synthetic analogs (e.g., ironhydroxides, allophane, and wollastonite), (3) industrial waste materials or by-products (e.g., steel slag and blast furnace slag), and (4) specially designed products (e.g., expanded clay aggregates or lanthanum modified clays) (Buda et al., 2012; Chardon et al., 2012), whereby category 1 was proposed for soil and categories 2 till 4 were proposed for water.

Table 4.1. Simple mineral salts divided in three subcategories according to their P fixation capacity in the soil (Gupta & Abrol, 1990)

Categories	Example amendments	P fixation capacity
Acid or acid forms of salts	$FeSO_4$, $(Fe_2SO_4)_3$ $Al(OH)_3$, FeS_2,	high in acidic sandy soil
Soluble salts	$CaCl_2$	in both acidic & alkaline soil
Sparingly soluble salts	$CaCO_3$, $CaSO_4$	high in alkaline soil

Buda et al. (2012) gave an overview of a number of P sorbing materials that provide a metal cation to react with dissolved P in water, to create an insoluble compound by sorption processes, including adsorption, precipitation or both (Table 4.2).

Table 4.2.Types of P sorbing materials, amendments, with the main reactive compound and examples of application (Buda et al., 2012)

Material	Compounds	Example applications
Natural materials		
Minerals (e.g. iron oxide, shale, limestone)	Fe, Al, Ca	Filter for wastewater, agricultural runoff
Polymineralic soils sands	Fe, Al, Ca	Constructed wetland
Synthetic filtration products		
Expanded calcinated clay aggregates	Ca	Constructed wetlands
Lanthanum modified clay (Phoslock)	La	Trap P in sediments
Synthetic analogs of natural minerals (e.g. zeolite)	Al	Wastewater treatment
By-products from industrial activities		
Blast furnace steel slag	Ca, Al	Golf course runoff
Red mud	Fe, Al, Ca	Septic tank effluent
Fly ash	Al, Fe	Constructed wetlands
Melter slag + basic slag	Ca	Drainage backfill, filter sock
Flue gas desulfurization gypsum	Ca	Agricultural drainage water
Drinking water treatment residuals	Fe, Al	Immobilizing P in soils
Oxygenation products of anaerobic groundwater	Fe	Tile drain envelope

The fixation of P in soil by using amendments is much less intensively studied than the use of such amendments to fix P in water. The P fixation in water is a remedial measure to stabilize or improve the water quality. The products used for this type of P fixation are e.g. limestone, zeolite, red mud, Phoslock®, gypsum, iron oxide and Sachtofer (Wild et al., 1996; Haghseresht et al., 2009; Gibbs et al., 2011; Buda et al., 2012; Chardon et al., 2012). The effect of zeolites, a highly porous aluminosilicate mineral originating from volcanic rocks, on P fixation was pointed out by Jardin and Pöpel (1994). They concluded that a main part of Al contained in zeolites might be available for P precipitation during anaerobic digestion (Wild et al., 1996). In another study a 30% P removal was found when stormwater, from urban areas, was filtered with zeolite (Wium-Andersen et al., 2012). In the same study also the P removal capacity of olivine, a magnesium iron silicate and one of the most common minerals on earth, was investigated and was found to be higher than 90%. Olivine treatment to soil additionally results in C sequestration. On a geological time scale, the weathering of basic silicate rocks and the subsequent precipitation of Ca and Mg carbonates is the main process controlling CO_2 concentration in the atmosphere (ten Berge et al., 2012). Addition of ground olivine to soil increases the reaction rate exponentially, resulting in very stable C sequestration on decadal time scales (ten Berge et al., 2012).

Water treatment residuals (WTR) (high in amorphous Fe or Al hydroxides) are usually disposed of in landfills and can therefore be obtained at minimal or no costs from drinking water treatment facilities (Agyin-Birikorang et al., 2007). Short-term laboratory studies have demonstrated the efficacy of WTR in reducing soluble P concentrations in runoff and leachate water from areas amended with animal manure (Elliott et al., 2002; Elliott et al., 2005). Agyin-Birikorang et al. (2007) found a reduction in water soluble P of more than 60% as compared to the control when an aluminium based WTR was added to a soil with a history of heavy manure application.

Because the above mentioned amendments are highly efficient in removing P from waste water it was hypothesized that they would also efficiently reduce the P concentrations in the soil solution. To this end a large number of potentially useful amendments were screened for their ability to reduce P concentrations in a soil and salt solution slurry, at various addition rates and pre-treatments. The amendments that appeared most promising from the above screening experiments were further tested for their ability to reduce P leaching from a number of soils during a batch leaching experiment under controlled conditions in the laboratory.

Materials and methods

Screening experiment

General analysis of the soil and the amendments

An acidic sandy soil with a PSD of 67.2% (0-90 cm), located in Zwevezele (site 20 chapter 2), West Flanders, Belgium, and further referred to as Zw, was selected from an agricultural field with a crop rotation of vegetables and maize. The soil was sampled, on August 19th 2010, by randomly taking around 200 samples with an auger in a random pattern over the whole field to a depth of 30 cm and mixing them to yield one composite sample for the whole field. After further homogenization in the laboratory, the soil samples were air-dried and sieved on a 2 mm mesh sieve. The soil texture was classified as sand (Soil Survey Staff, 2010) containing 87.5% sand, 9.9% silt and 2.6% clay. The soil had a pH of 5.56 and an organic carbon content of 1.1%, with a PSD and P_{lac} of respectively 109% and 689 mg kg^{-1} in the top layer. An adjusted P fractionation method of Hedley (Hedley et al., 1982; De Schrijver et al., 2012) was performed on the top soil layer (Table 4.3).

Table 4.3. The different P fractions in the top layer of Zw

P fraction	total P (mg kg^{-1} soil)	$P_{inorganic}$ (mg kg^{-1} soil)	$P_{organic}$ (mg kg^{-1} soil)
Readily soluble and exchangeable P	87 ± 4.3	12 ± 0.9	74
Desorbable P	221 ± 13.8	12 ± 1.2	209
Fe/Al oxide-associated P	696 ± 51.2	44 ± 0.7	652
Ca associated P	250 ± 9.7	38 ± 1.6	213
Residual P	163	163	0

Following amendments from the four main categories mentioned above were tested for their P fixation potential: the mineral salts $CaCO_3$, $CaCl_2$, $CaCO_3$+$CaCl_2$, $AlCl_3$, $AlSO_4$, $FeCl_3$ and $FeSO_4$, the minerals (olivine, biotite, zeolite and gypsum), bauxite, the industrial by-product dried Fe sludge and the specially designed products Phoslock® and Sachtofer.

Phoslock® is a lanthanum-modified bentonite clay which has recently been developed (Meis et al., 2012). Lanthanum (La) is a rare earth element that is relatively abundant in the earth's crust in comparison with other rare earth elements (Ross et al., 2008). Lanthanum can be incorporated into the structure of high exchange capacity minerals such as bentonite by taking advantage of the cation exchange capacity of clay minerals. During the preparation of Phoslock®, La-ions are exchanged with surface adsorbed exchangeable cations. The rare earth element in the clay structure can react with the phosphate anion in the water body (Ross et al., 2008).

Except for the mineral salts, a total elemental analysis was done. The samples were pre-ignited at 850°C for 30 min in platinum crucibles, mixed thoroughly with 2 g of lithium metaborate powder in the platinum crucible and fused for 15 min at 950°C in a preheated muffle furnace. The crucible and flux that was formed were allowed to cool down and the flux was dissolved in a 4% nitric acid solution. Elemental analysis was then performed by inductively coupled plasma optical emission spectrometry. X-ray diffraction patterns of powder samples were collected on a Philips X'PERT SYSTEM with a PW 3710 based diffractometer, equipped with a Cu tube anode, a secondary graphite beam monochromator, a proportional xenon filled detector, and a 35 position multiple sample changer. The incident beam was automatically collimated. The irradiated length was 12 mm. The secondary beam side comprised a 0.1 mm receiving slit, a soller slit, and a 1° anti-scanner slit. The tube was operated at 40 kV and 30 mA, and the XRD data were collected in a theta, 2-theta geometry from 3.00' onwards, at a step of 0.020° 2-theta, and a count time of 1 second per step.

Pre-treatment of the amendments

To increase the reaction rate (or weathering) of the amendments, they were also pre-treated physically (ball milled, BM) and/or chemically. Olivine, biotite, zeolite, bauxite and Phoslock® were ball milled. Olivine, biotite and zeolite were further pre-treated as follows: the ball milled minerals were mixed with HCl (37%, 1/3 ratio) and shaken on a rotational shaker for 1h, 2.5h, 5h or 24h, respectively. Finally, the pre-treated minerals were dried at 105°C in the oven.

Screening experiment set up

The P fixation capacity of the amendments was tested by adding varying amounts to 5 g of dried control soil in triplicate (Table 4.4). The mixture was put in small plastic containers, 20 ml of KCl (0.01M) was added, the mixture was shaken for 40 h on a horizontal shaker, and was filtered through Whatman 589/3 filters.

Table 4.4. Addition rates of the various soil amendments

Addition rates of the mineral salts			Addition rates of the other products		
Rate	**Amount added (g [5 g soil]$^{-1}$)**	**% amendment to soil**	**Rate**	**Amount added (g [5 g soil]$^{-1}$)**	**% amendment to soil**
Blank	0	0	**Blank**	0	0
R1	0.005	0.1	**R1**	0.005	0.1
R2	0.025	0.5	**R2**	0.025	0.5
R3a	0.05	1.0	**R3**	0.075	1.5
R4	0.125	2.5	**R4**	0.125	2.5
R5	0.25	5	**R5**	0.25	5

The inorganic P ($P_{inorganic}$) in the filtrate was measured with the method of Murphy and Riley (Murphy & Riley, 1962) by putting 5 ml of filtrate and 4 ml of reagent in a volumetric flask of 50 ml and diluting it with distilled water. After 30 min the mixture was transferred in a cuvet and P was measured spectrophotometerically at 882 nm (Varian, cary 50). P concentrations in the solution were given in mg P L^{-1} extraction solution. Finally, the pH of the filtrate was measured with a pH meter (Thermo, orion model 420).

Fixation experiment

General soil analysis

Six acidic sandy soils with a range in P content were selected. Three soils were located in Liereman, Old-Turnhout, Antwerp, further referred to as Li, and three soils were located in West-Flanders (two in Zedelgem, further referred to as Ze, and one in Zwevezele, further referred to as Zw). The soils were sampled in December 2011 by randomly taking between 20 and 50 samples with an auger in a cross pattern over the whole field to a depth of 30 cm. All augerings were mixed to yield one composite sample per field. After further homogenization in the laboratory, the soil samples were air-dried and sieved on a 2 mm mesh sieve. All soils, except Zw, were under grassland at the time of sampling. Due to experimental failure the adjusted P fraction method of Hedley could not be executed for the soils of Li and Ze.

Fixation experiment set up

The amendments and pre-treatments that appeared most promising from the screening experiment (see section: results screening experiment) were further used in the fixation experiment (Table 4.5) on these six soils, except for the mineral salts which were only tested on Zw.

Table 4.5. The tested products with their addition rates (addition rates : see screening experiment)

Mineral salts	Addition rate	Minerals and Bauxite	Addition rate	Industrial Byproduct	Addition rate	Specially designed products	Addition rate
$FeCl_3$	R1	Olivine	R3	Dried	R1	Phoslock®	R2
	R4	Olivine BM+1h HCl	R3	Fe sludge	R2	Phoslock®	R3
$FeSO_4$	R1	Biotite	R3		R3	Sachtofer	R2
	R2	Biotite BM+1h HCl	R3			Sachtofer	R3
$AlCl_3$	R1	Zeolite	R3				
$CaCO_3$	R2	Zeolite BM+1h HCl	R3				
		Gypsum	R2				
		Bauxite	R2				

To this end the amendments were thoroughly mixed with 80 g of pre-incubated soil (volumetric moisture content of 23.5%) and put into a PVC column (height 10 cm, inner diameter 4.6 cm, Figure 4.1). The bottom of the tubes was closed with a polyester sieve (250 µm MW) and a paper filter (a Whatman filter, 589/3) on top of the sieve in order to prevent soil loss during the fixation experiment. The experiment was performed in triplicate for each amendment per soil and also three unamended control samples were included.

Figure 4.1. Set up of the fixation experiment

In total eight leachate events (on eight consecutive days) were done, per event, 80 ml of $CaCl_2$ (0.005 M) was added to each column. All leachates were analysed for pH and $P_{inorganic}$.

Statistical analysis

The results gathered from the screening and the fixation experiment were statistically analyzed using the statistical software PASW 20 package (SPSS version PASW 20, SPSS Inc., USA). The decrease in $P_{inorganic}$ of the leachates of the amended treatments was statistically (analysis of variance [ANOVA]) analysed and also compared with the unamended control. The P fixation capacity of the amendments was statistically analyzed with a one way ANOVA (with a *t*-test).

Results screening experiment

Total elemental characterization of the products

When treating the soil it is important to know the exact composition of the added products. Generally, the results of the elemental analysis were consistent with the expected values from the various products (Table 4.6).

Table 4.6. Total elemental analysis of the selected minerals, industrial by-product and specially designed products (in % or in mg kg^{-1})

%	olivine	biotite	zeolite	gypsum	bauxite	dried Fe sludge	Phoslock®	Sachtofer
SiO_2	42.0	38.6	67.2	0.2	7.0	5.3	55.3	0.6
Al_2O_3	0.4	8.7	12.4	0.2	23.1	0.3	13.5	0.8
Fe_2O_3	7.9	8.7	1.4	0.1	41.1	62.5	3.5	13.6
MnO	0.1	0.1	0.0	0.0	0.1	0.9	0.0	0.5
MgO	48.1	19.1	0.8	0.0	0.3	0.2	2.7	2.1
CaO	0.2	9.4	3.1	29.1	10.4	6.8	1.9	25.3
K_2O	0.0	7.5	2.7	0.0	0.1	0.1	0.6	0.1
TiO_2	0.0	0.3	0.2	0.0	4.2	0.0	0.4	3.1
P_2O_5	0.0	0.4	0.0	0.8	0.1	4.2	0.1	0.0
mg kg^{-1}								
Cd	<15	<15	<15	<15	<15	<15	<15	<15
Cu	7	9	<5	9	52	21	8	5
La	115	28	32	324	134	25	36553	15
Ni	3053	8	7	<5	826	123	14	71
Pb	14	<10	23	<10	187	115	53	31
S	212	180	<100	169470	181	1064	<100	133173
Zn	59	72	30	<5	75	281	72	221

The used olivine can be classified as fosterite, Fe (7.9% Fe_2O_3) is the dominant P fixating element and it also contains an important amount of MgO (48.1%) what could be important in terms of C sequestration. However, olivine contains a rather high undesirable amount of Ni, which may pose an environmental risk. Biotite contains the three (main) P fixating elements in the same rate, Al (8.7% Al_2O_3), Fe (8.7% Fe_2O_3) and Ca (9.4% CaO), together with a considerable amount of MgO (19.1%). Zeolite and gypsum have one dominant P fixating element, namely Al (12.4% Al_2O_3) and Ca (29.1% CaO) respectively. Gypsum also contains a high amount of sulphur and a noticeable amount of La. In bauxite the three (main) P fixating elements, Al (23.1% Al_2O_3), Fe (41.1% Fe_2O_3) and Ca (10.4% CaO), are found with a domination of Fe, but also a noticeable amount of unwanted Ni and lead (Pb).

As expected Fe (62.5%) is the dominant P fixating element in dried Fe sludge and also a noticeable amount of sulphur is found. A potential drawback of dried Fe sludge as P fixating product is the presence of 4.2% of P_2O_5 and also a noticeable amount of unwanted Pb and Ni. For Phoslock® the mean P fixating element was La with in secondary order Al (13.5% Al_2O_3) and Fe (3.5% Fe_2O_3), for Sachtofer it was Ca (25.3% CaO) and Fe (13.6% Fe_2O_3). La is the main reactive compound for P fixation in Phoslock® which is also found in a higher amount in gypsum. With the addition of Sachtofer also an amount of sulphur is added to the soil.

P fixation potential of the amendments

The $P_{inorganic}$ concentration in the extraction solution of the control soil was 1.19 mg P L^{-1} (Figure 4.2). The lowest addition rates of the mineral salts (R1) resulted in a significant decrease in $P_{inorganic}$ compared to the control but higher rates of the Ca salts did not result in an additional P fixation. Addition of higher amounts of Al salts (R3a or R4) increased the $P_{inorganic}$ concentrations to levels much higher than the control soil. In the Fe salts an almost total P fixation was found from R2 onwards.

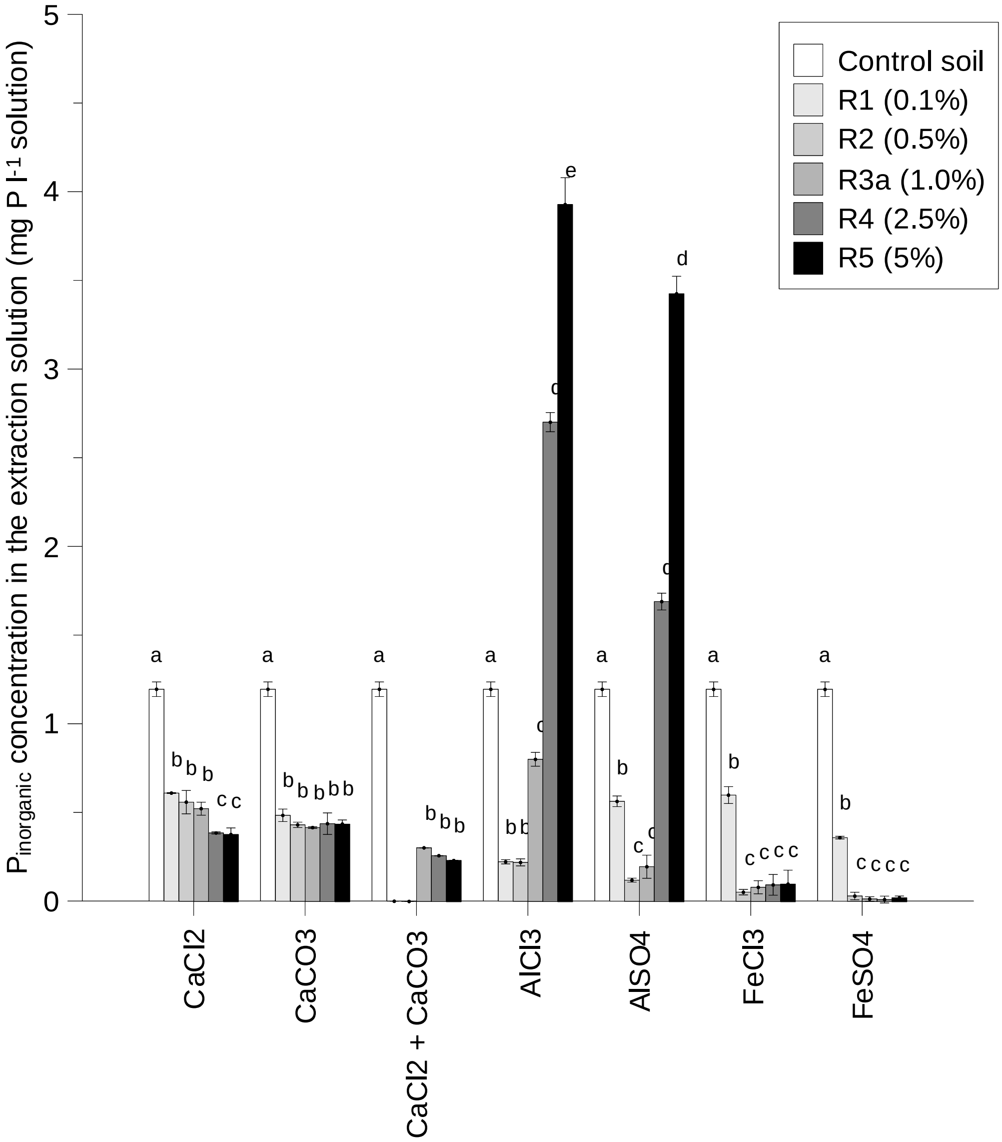

Figure 4.2. P$_{inorganic}$ concentration in the extraction solution (mg P L^{-1} solution) found after addition of the mineral salts at different addition rates (different letters mean significant differences between addition rates of the same product)

A small P fixation effect was observed when adding non-pre-treated olivine or ball milled (BM) olivine at R5 (Figure 4.3). However, from R1 onwards the chemically pre-treated olivine significantly decreased P$_{inorganic}$ compared to the control. At the highest addition rates almost complete P fixation was achieved. The olivine BM + 1h HCl had the most significant P fixation capacity.

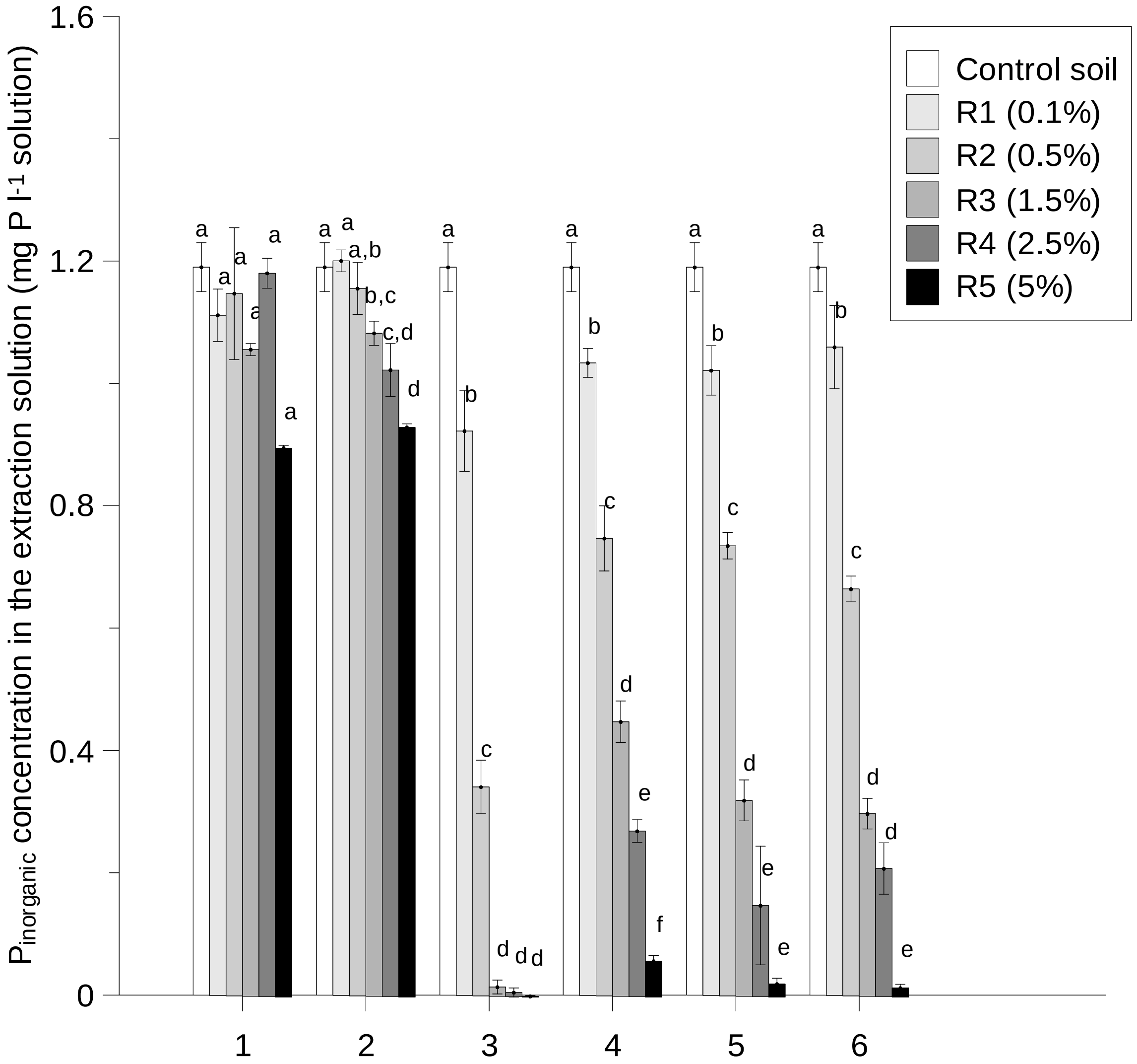

Figure 4.3. $P_{inorganic}$ concentration in the extraction solution (mg P L^{-1} solution) found with the different olivine additions at 5 different rates. Namely, olivine (1), olivine BM (2), olivine BM + 1h HCl (3), olivine BM + 2.5h HCl (4), olivine BM + 5h HCl (5) and olivine BM + 24h HCl (6)

The addition of non-pre-treated biotite and ball milled biotite gave a decrease in P from R2 onwards (Figure 4.4), contrary to non-pre-treated zeolite or ball milled zeolite (Figure 4.5). A high P fixation capacity was observed with the addition of chemical pre-treated biotite and zeolite. As of R1 a significant decrease in $P_{inorganic}$ was noticed and from R2 onwards more than 65% of the $P_{inorganic}$ was fixed in the soil. For both minerals the pre-treatment with 1h HCl had already a significant effect.

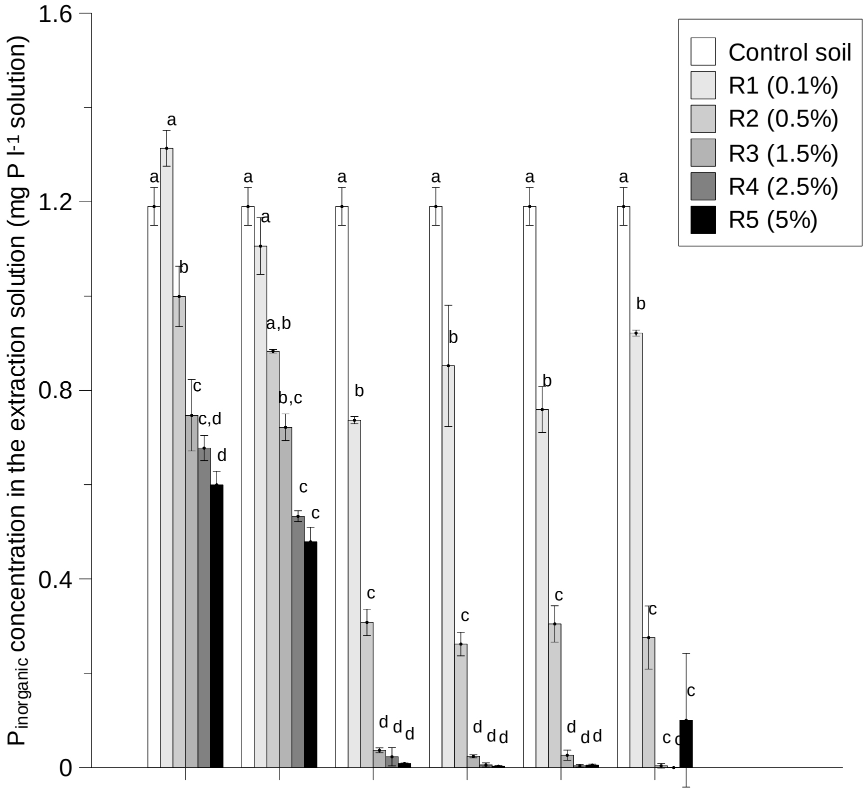

Figure 4.4. P$_{inorganic}$ concentration in the extraction solution (mg P L^{-1} solution) found with the different additions of biotite at 5 different rates. Namely, biotite (1), biotite BM (2), biotite BM + 1h HCl (3), biotite BM + 2.5h HCl (4), biotite BM + 5h HCl (5) and biotite BM + 24h HCl (6)

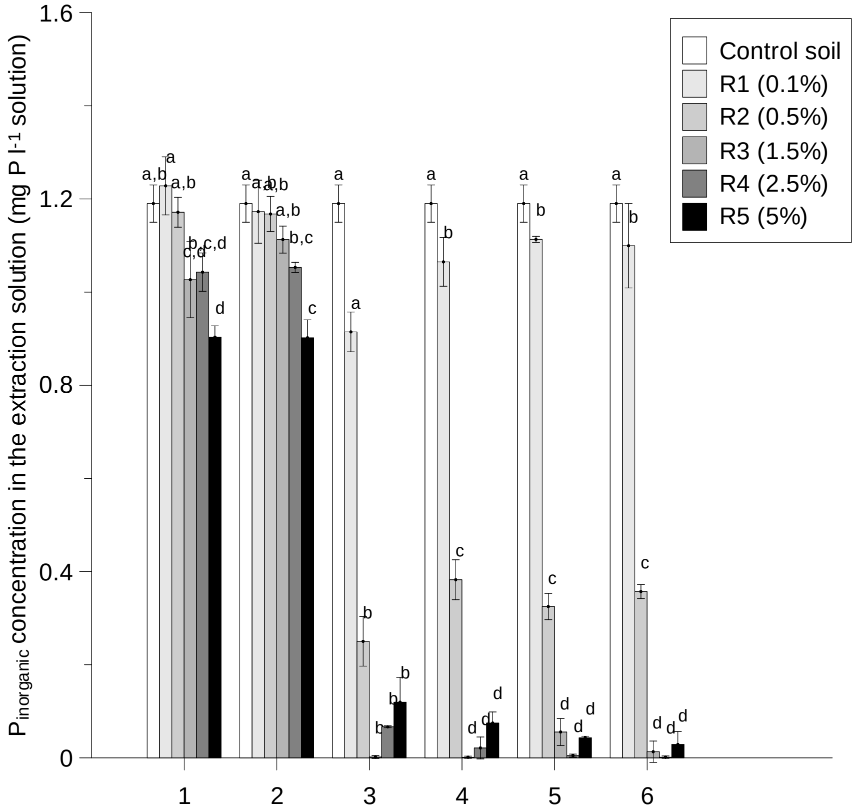

Figure 4.5. P$_{inorganic}$ concentration in the extraction solution (mg P L^{-1} solution) with the different additions of zeolite at 5 different rates. Namely, zeolite (1), zeolite BM (2), zeolite BM + 1h HCl (3), zeolite BM + 2.5h HCl (4), zeolite BM + 5h HCl (5) and zeolite BM + 24h HCl (6)

Addition of gypsum R1 immediately gave a small decrease in $P_{inorganic}$ compared to the control, however from R2 onwards, higher addition rates had no additional significant effect on P fixation (Figure 4.6). The addition of non-pre-treated bauxite or ball milled bauxite resulted in an immediate significant effect on the P fixation compared with the control. However, higher addition rates did not give a further decrease in $P_{inorganic}$. The physical pre-treatment of bauxite had no or a negative effect on the P fixation capacity in comparison with the non-pre-treated bauxite. The lowest addition rate of dried Fe-sludge (R1) caused already a significant decrease in the $P_{inorganic}$ concentration compared to the control, from R3 onwards all P was fixed. The addition of Phoslock® (R2 onwards) gave a significant decrease in $P_{inorganic}$ compared to the control. Physical pre-treatment of Phoslock® was inefficient for P fixation in comparison with non-pre-treated Phoslock®. The lowest addition rates of Sachtofer already gave a significant decrease in $P_{inorganic}$, but the highest rates did not result in complete fixation.

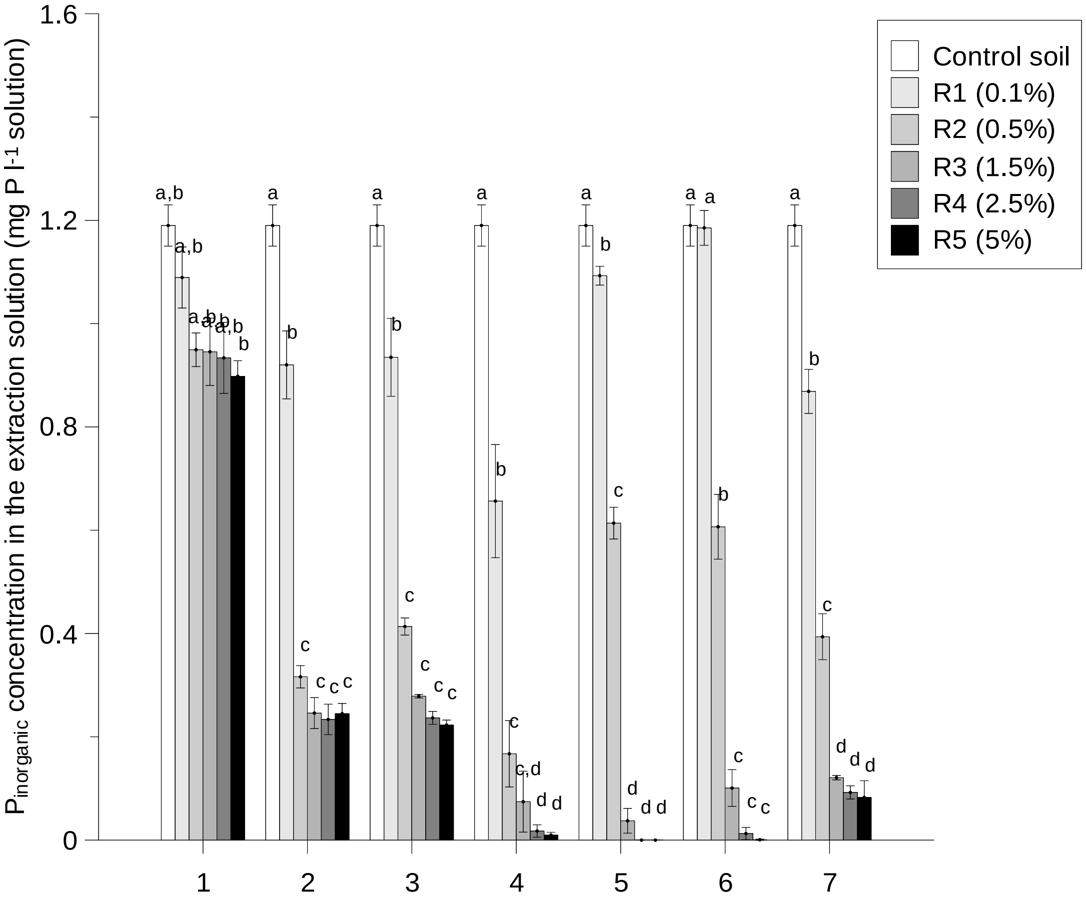

Figure 4.6. $P_{inorganic}$ concentration in the extraction solution (mg P L^{-1} solution) found with addition of gypsum (1), bauxite (2), bauxite BM (3), dried Fe sludge (4), Phoslock® (5), Phoslock® BM (6) and Sachtofer (7) in 5 different rates

Results fixation experiment

General soil properties

According to USDA, all six soils were sandy (Table 4.7). The soils can be classified according to their PSD and their P_{ox} content. Li3 had the lowest PSD in the top layer, namely 21.5%, followed by Li1 (55.1%), Li2 (64.7%) and Ze2 (70.3%). The soils with the highest PSD were Ze1 (98.2%) and Zw (109%). All soils did not contain $CaCO_3$ and were acidic with a pH-KCl between 4 and 5.6.

Table 4.7. General soil parameters of the top layer (0-30 cm) of the investigated soils (value ± stdev)

Soil parameter	Zw	Ze1	Ze2	Li1	Li2	Li3
Sand (%)	87.5	83.1	90.4	92.7	93.2	93
Silt (%)	9.9	12.8	6.8	5.4	5	5.8
Clay (%)	2.6	4.1	2.8	2	1.8	1.2
OC (%)	1.1	1.00	1.68	1.08	1.44	2.62
$CaCO_3$ (%)	0.00	0.00	0.00	0.00	0.00	0.00
pH KCl	5.56	5.07	4.46	4.52	4.48	4.08
PSD top layer (%)	109	98.2	70.5	55.1	64.7	21.5
P_{ox} (mmol kg^{-1} soil)[a]	68.7 ± 0.32	31.7 ± 0.3	21.3 ± 0.3	8.98 ± 0.5	15.7 ± 0.8	5.38 ± 0.5
P_{lac} (mg P kg^{-1})*	68.9 ± 1.6	528 ± 2.9	360 ± 2.1	147 ± 0.9	297 ± 5.7	65.3 ± 8.4
Total P (mg kg^{-1} soil)	1417 ± 105	1117 ± 34	735 ± 7.1	438 ± 37	635 ± 1.9	395 ± 24
Ca (mg kg^{-1} soil)*	1610 ± 270	1235 ± 79	870 ± 22	452 ± 3.7	557 ± 6.5	626 ± 61
K (mg kg^{-1} soil)*	69.3 ± 1.90	109 ± 6.9	68.3 ± 3.6	21.5 ± 0.2	31.4 ± 9.5	22.2 ± 0.04
Na (mg kg^{-1} soil)*	20.0 ± 3.85	21.1 ± 4.4	38.3 ± 1.8	9.99 ± 0.5	12.8 ± 2.4	12.3 ± 0.5
Mg (mg kg^{-1} soil)	184 ± 1.01	70.7 ± 2.7	35.8 ± 2.8	38.2 ± 0.2	39.6 ± 1.5	20.2 ± 1.3
Al_{ox} (mmol kg^{-1} soil)[a]	56.5 ± 0.49	28.9 ± 0.6	43.5 ± 0.8	26.2 ± 0.4	43.4 ± 0.9	41.6 ± 0.9
Fe_{ox} (mmol kg^{-1} soil)[a]	70.0 ± 0.72	35.7 ± 0.4	16.7 ± 0.3	6.40 ± 0.6	5.09 ± 0.3	8.47 ± 0.1

*found with ammoniumlactate extraction method, [a]found with ammoniumoxalate-oxalic acid extraction method

P fixation efficiency of the simple salts

The cumulative P lost by leaching from the unamended Zw soil was 20.3 ± 1.1 mg $P_{inorganic}$ kg^{-1}. All salt treatments resulted in a significant ($p < 0.05$) decrease in cumulative amount of $P_{inorganic}$ leaching (Figure 4.7). $FeCl_3$ R1 had the smallest P fixation efficiency compared to the control, namely 18.7%, followed by $FeSO_4$ R1 (31.3%) and $CaCO_3$ R2 (31.6%). The $AlCl_3$ R1 treatment resulted in a P fixation efficiency of 51.0% in comparison to the control. Almost all $P_{inorganic}$ was fixed in the $FeSO_4$ R2 (91.2%) or $FeCl_3$ R4 (96.5%) treatments.

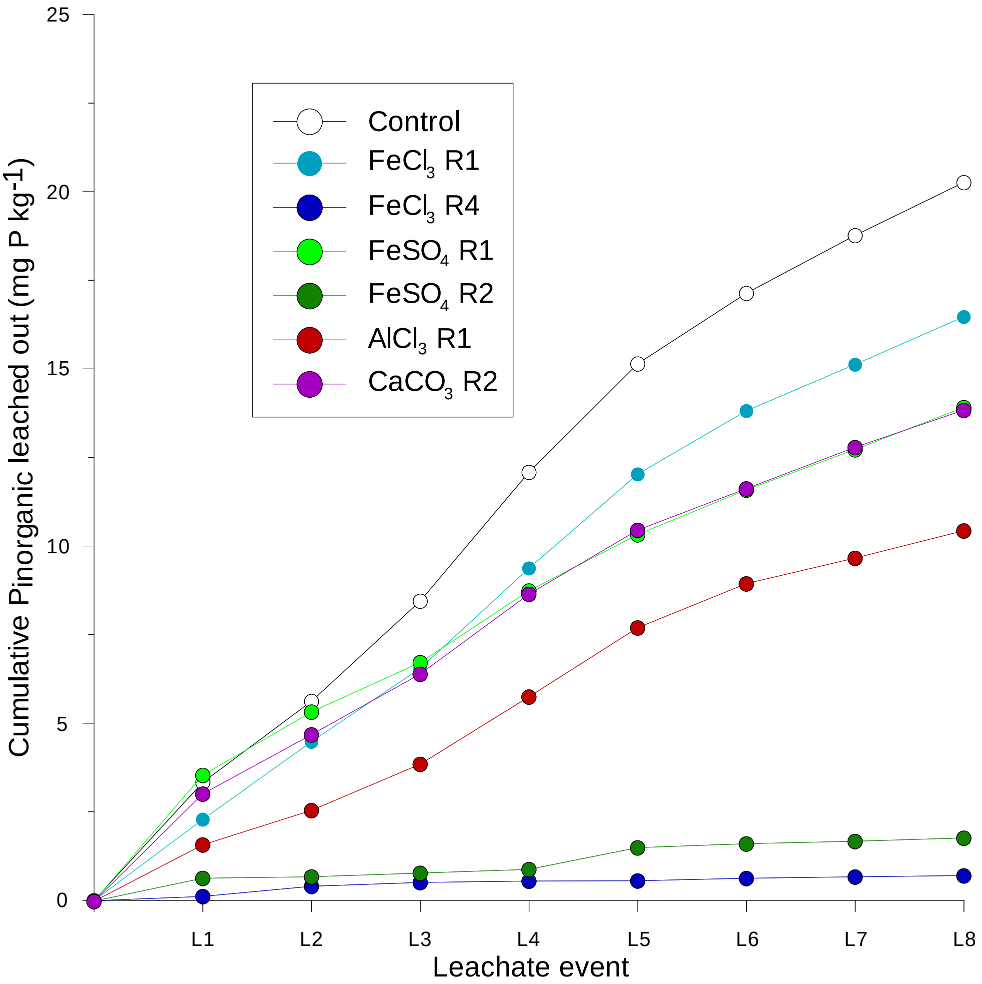

Figure 4.7. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Zw over the eight leachate events with addition of simple salts (stdev for the total leachate and per leachate event can be found in appendix I)

To have an optimal P fixation when treating the soil with the amendments, it is important to know following parameters, the total amount of P leaching and the incubation time of the products before P is efficiently fixed (Figure 4.7 and appendix I). At each leachate event, the control soil had the highest $P_{inorganic}$ loss. All treatments of Zw, except $FeCl_3$ R4 and $FeSO_4$ R2, followed more or less the same pattern per leachate event as the control. The highest $P_{inorganic}$ loss was found with the fourth leachate event for the control soil as well as for all treated samples. From the fifth leachate event onwards a topping off in the cumulative curve was found. $FeCl_3$ R4 and $FeSO_4$ R2 treatment had a different leaching pattern than the control sample, since almost all P was fixed. During the second leachate event the $FeCl_3$ R4 treatment gave the highest $P_{inorganic}$ loss, whereas during the other leachate events almost no $P_{inorganic}$ was lost. With the $FeSO_4$ R2 treatment almost all $P_{inorganic}$ was fixed, only a small loss was noticed during the second and the fifth leachate event.

P fixation efficiency of the minerals, bauxite, industrial by-product and specially designed products

As expected the cumulative $P_{inorganic}$ losses of the control samples were different for the six soils (Figure 4.8 to Figure 4.19). The highest cumulative loss of $P_{inorganic}$, 13.3 ± 1.9 mg P kg^{-1} in the control sample, was found for Zw, the soil with the highest PSD, followed by Ze1 (6.61 ± 1.2 mg P kg^{-1}) and Li2 (2.26 ± 0.3 mg P kg^{-1}). A cumulative $P_{inorganic}$ loss of less than 1 mg P kg^{-1}, was found for the control samples of Ze2, Li1 and Li3, namely 0.83 ± 0.28 mg P kg^{1}, 0.36 ± 0.17 mg P kg^{-1} and 0.06 ± 0.01 mg P kg^{-1} respectively.

The dynamics of the $P_{inorganic}$ loss as a function of time found for the amended samples was comparable to the controls, except for Li3. In all soils, the gypsum R2 treatment resulted in higher $P_{inorganic}$ losses compared to the controls. The zeolite R3 in Zw, Ze2, Li1 and olivine R3 in Ze2 resulted in a higher amount of $P_{inorganic}$ loss over the entire experiment in comparison with the control. On the contrary, the addition of the chemically pre-treated minerals resulted in the highest P fixation of all tested minerals in all soils.

The Fe-sludge R2 or R3 and Phoslock® R3 treatments, resulted in the highest P fixation of the industrial by-product and the specially designed products in all soils. A comparable amount of $P_{inorganic}$ was fixed in the Sachtofer or bauxite treatments.

In Li3, all treatments resulted in a cumulative $P_{inorganic}$ loss smaller than 0.25 mg P kg^{-1}, with very large standard deviations. Therefore no significant differences were found between the control and the amended samples (Figure 4.18 and Figure 4.19).

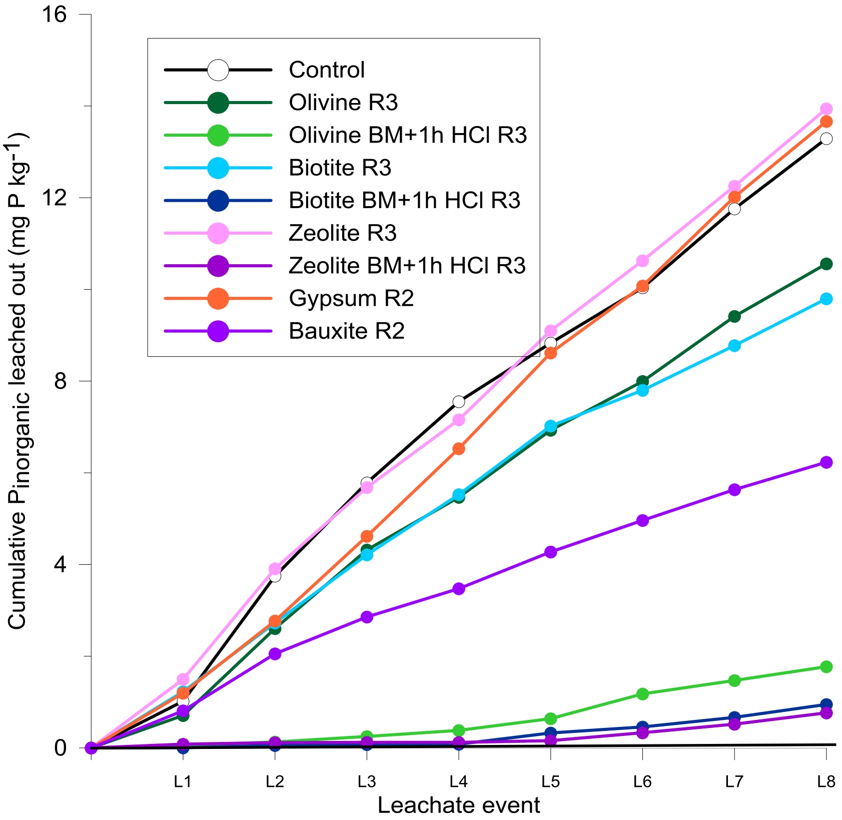

Figure 4.8. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Zw over the eight leachate events with addition of minerals and bauxite (stdev for the total leachate and per leachate event can be found in appendix I)

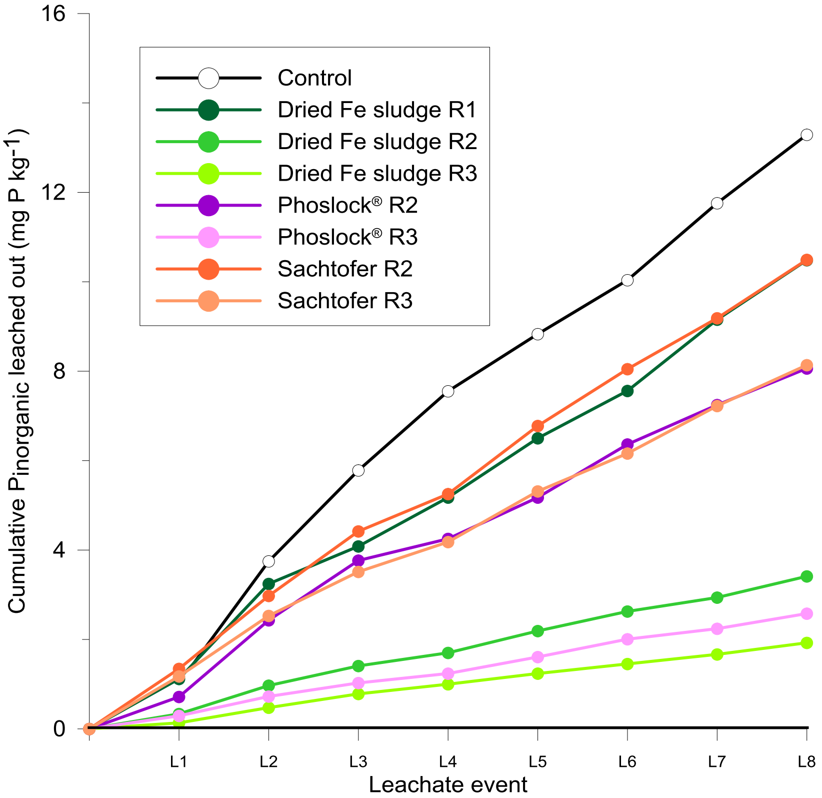

Figure 4.9. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Zw over the eight leachate events with addition of industrial by-product and specially designed products (stdev for the total leachate and per leachate event can be found in appendix I)

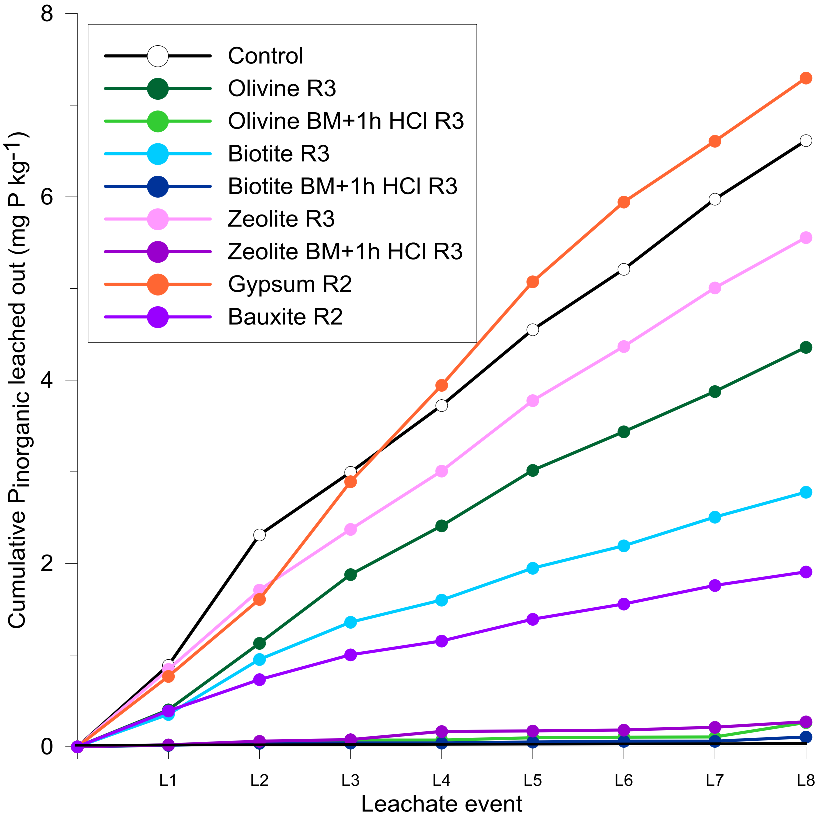

Figure 4.10. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Ze1 over the eight leachate events with addition of minerals and bauxite (stdev for the total leachate and per leachate event can be found in appendix I)

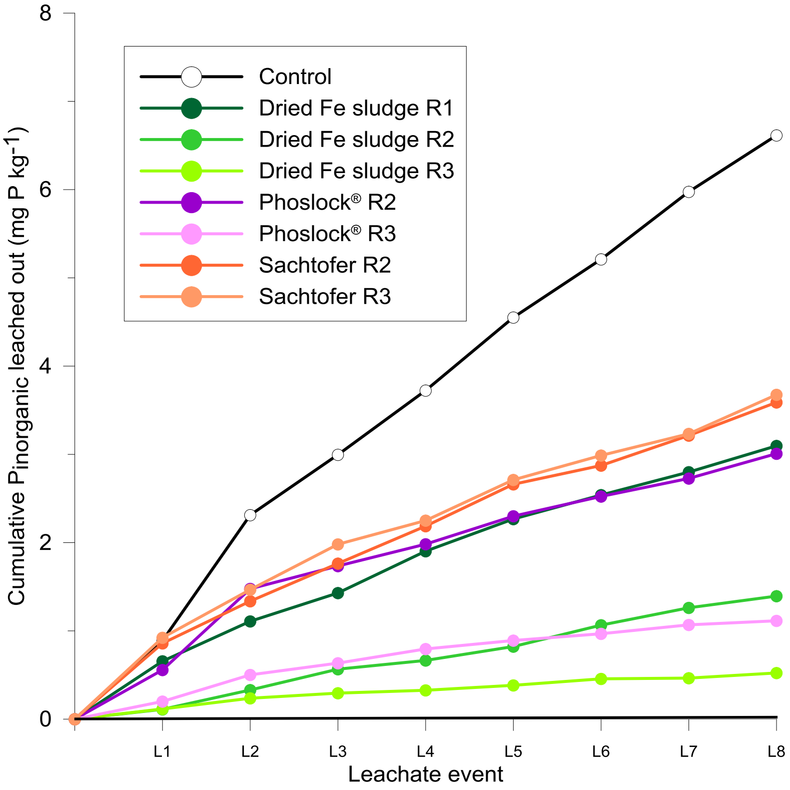

Figure 4.11. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Ze1 over the eight leachate events with addition of industrial by-product and specially designed products (stdev for the total leachate and per leachate event can be found in appendix I)

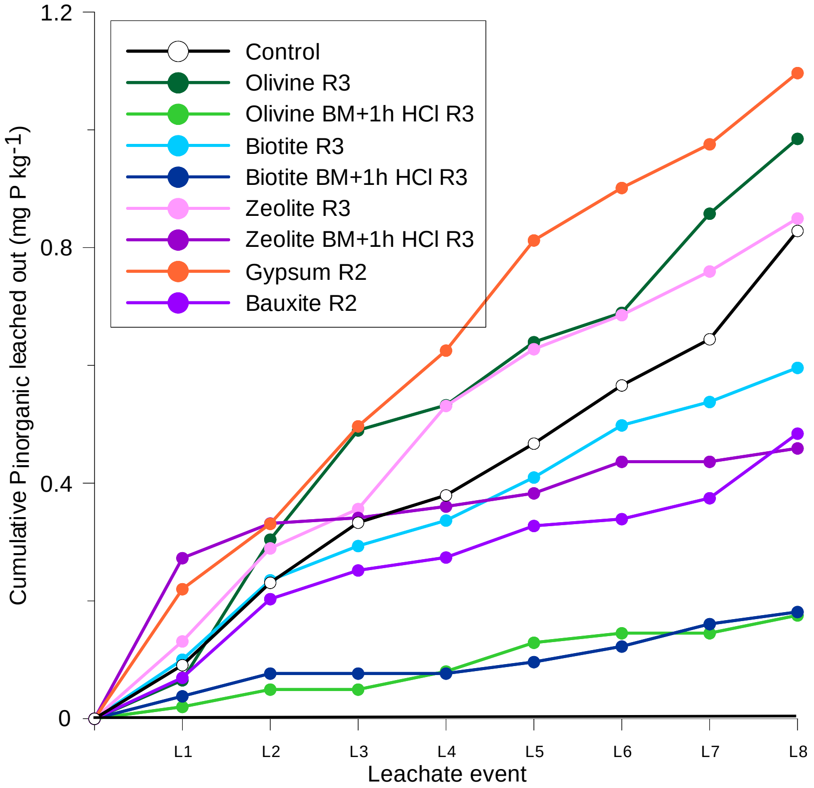

Figure 4.12. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Ze2 over the eight leachate events with addition of minerals and bauxite (stdev for the total leachate and per leachate event can be found in appendix I)

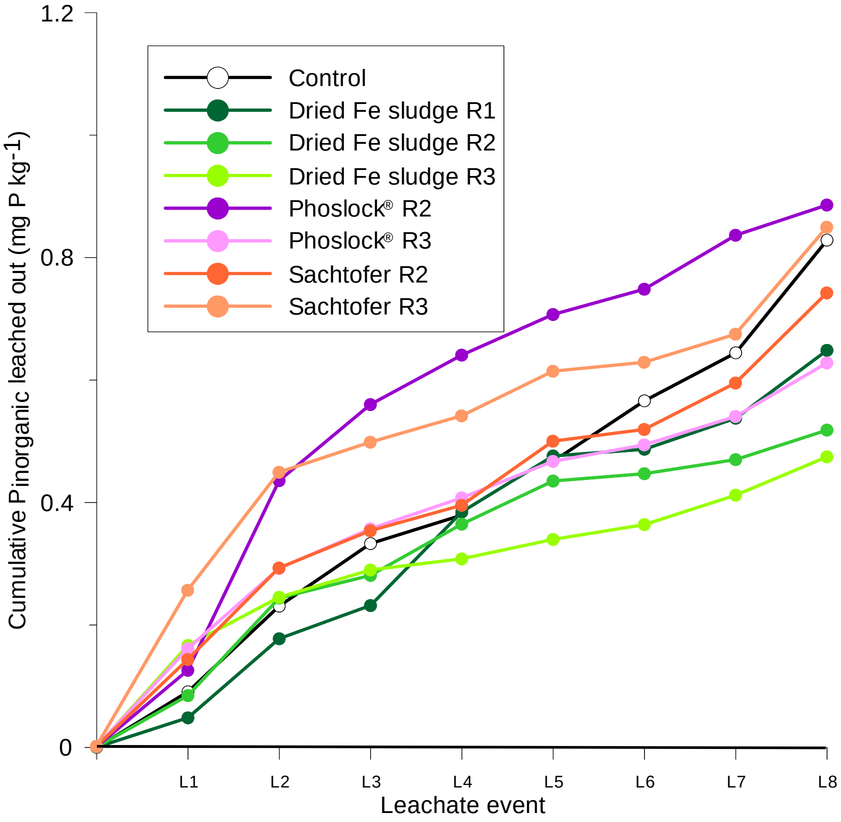

Figure 4.13. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Ze2 over the eight leachate events with addition of industrial by-product and specially designed products (stdev for the total leachate and per leachate event can be found in appendix I)

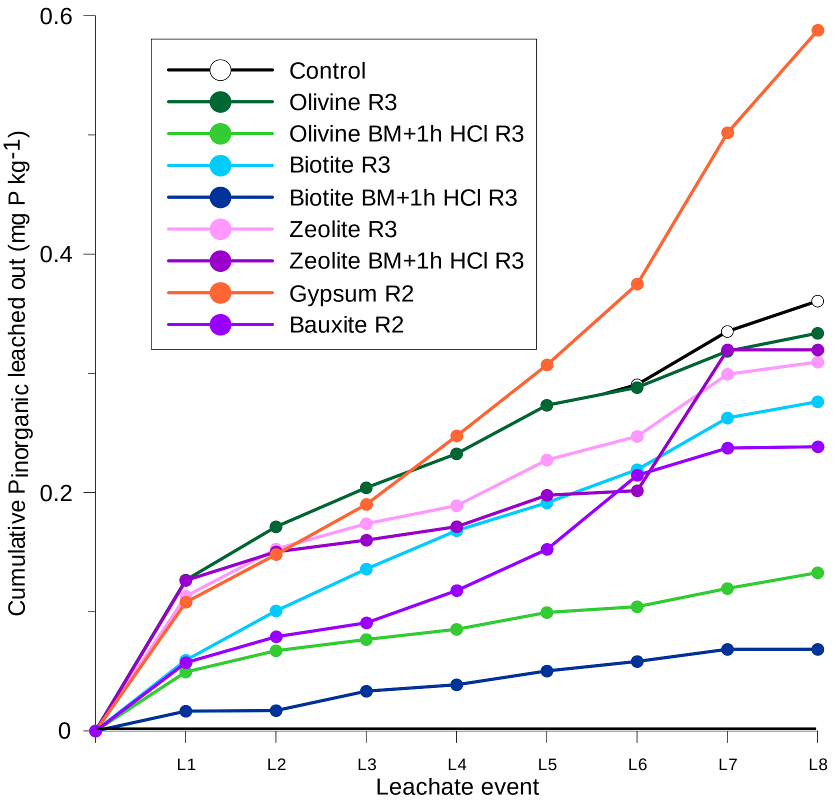

Figure 4.14. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Li1 over the eight leachate events with addition of minerals and bauxite (stdev for the total leachate and per leachate event can be found in appendix I)

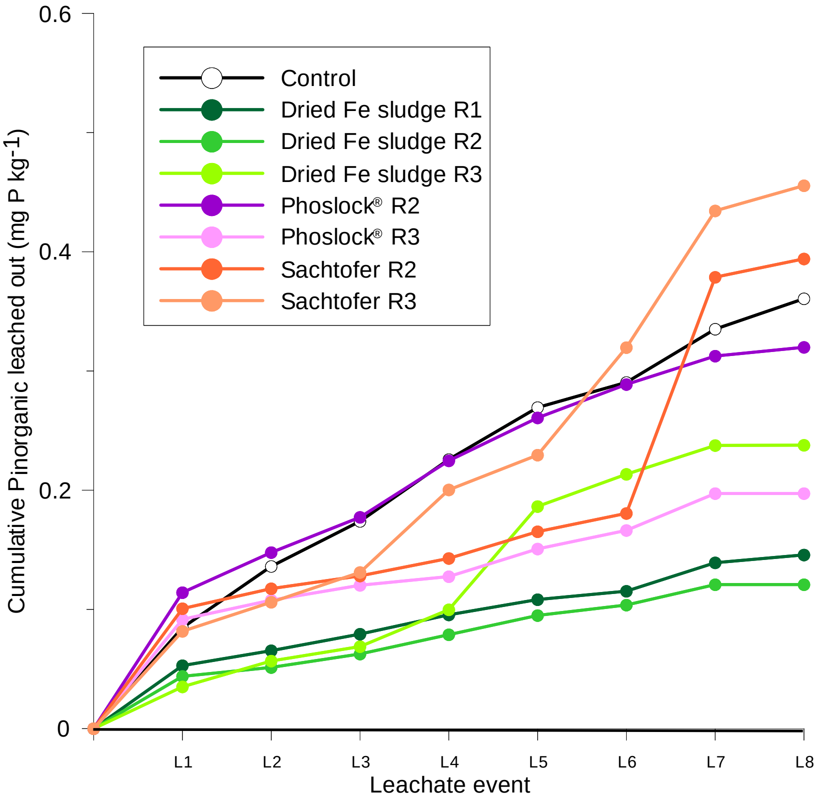

Figure 4.15. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Li1 over the eight leachate events with addition of industrial by-product and specially designed products (stdev for the total leachate and per leachate event can be found in appendix I)

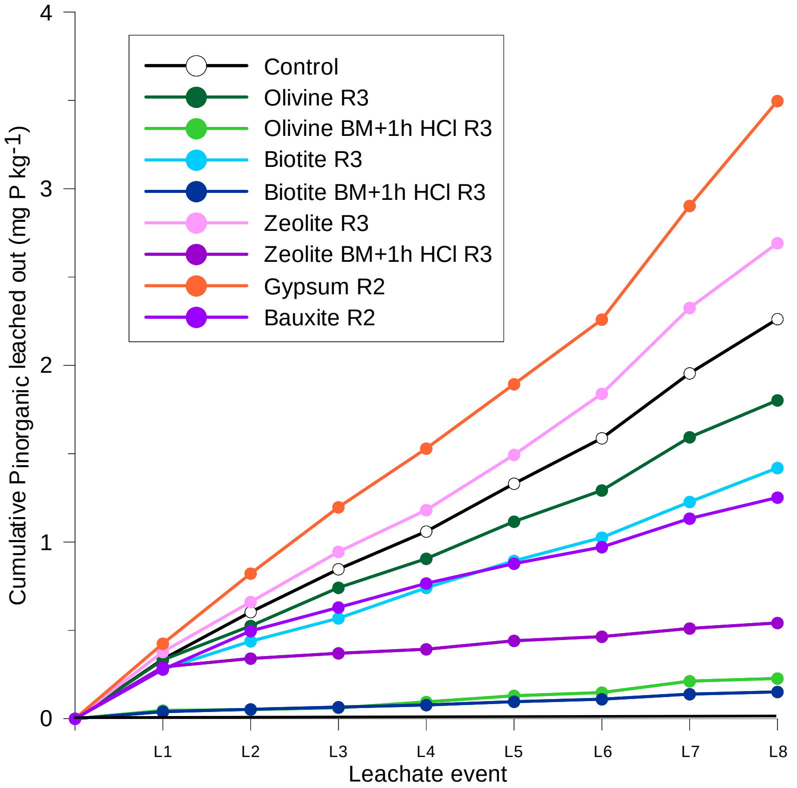

Figure 4.16. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Li2 over the eight leachate events with addition of minerals and bauxite (stdev for the total leachate and per leachate event can be found in appendix I)

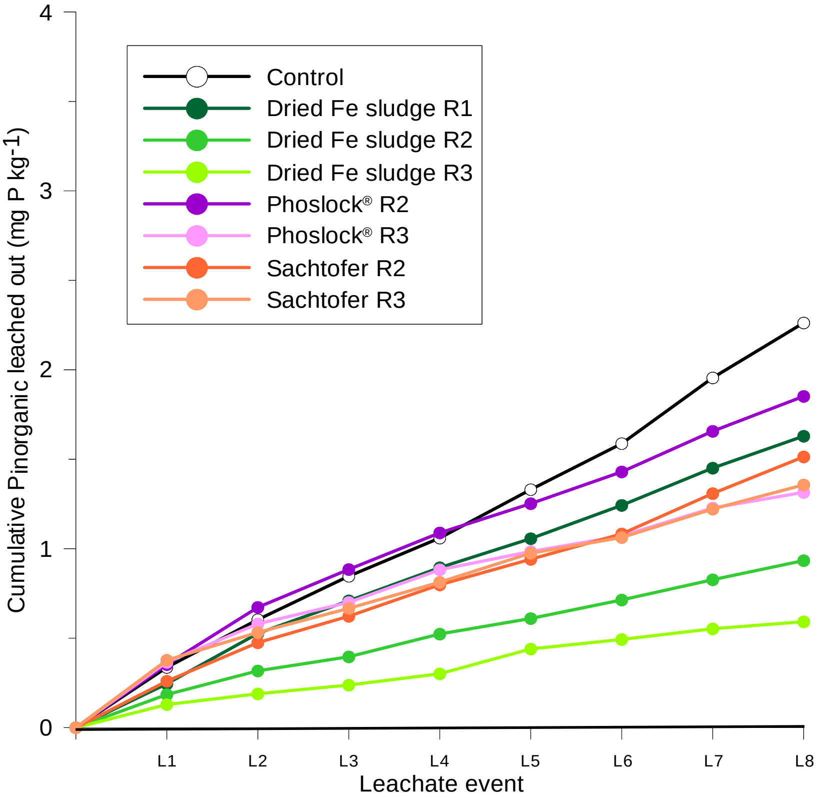

Figure 4.17. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Li2 over the eight leachate events with addition of industrial by-product and specially designed products (stdev for the total leachate and per leachate event can be found in appendix I)

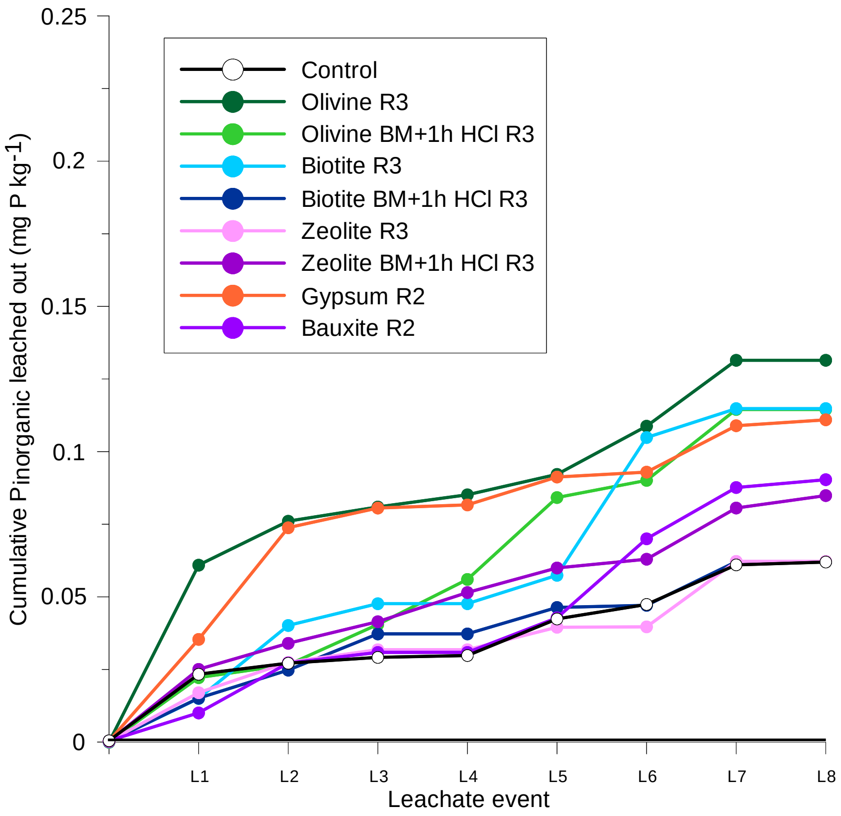

Figure 4.18. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Li3 over the eight leachate events with addition of minerals and bauxite (stdev for the total leachate and per leachate event can be found in appendix I)

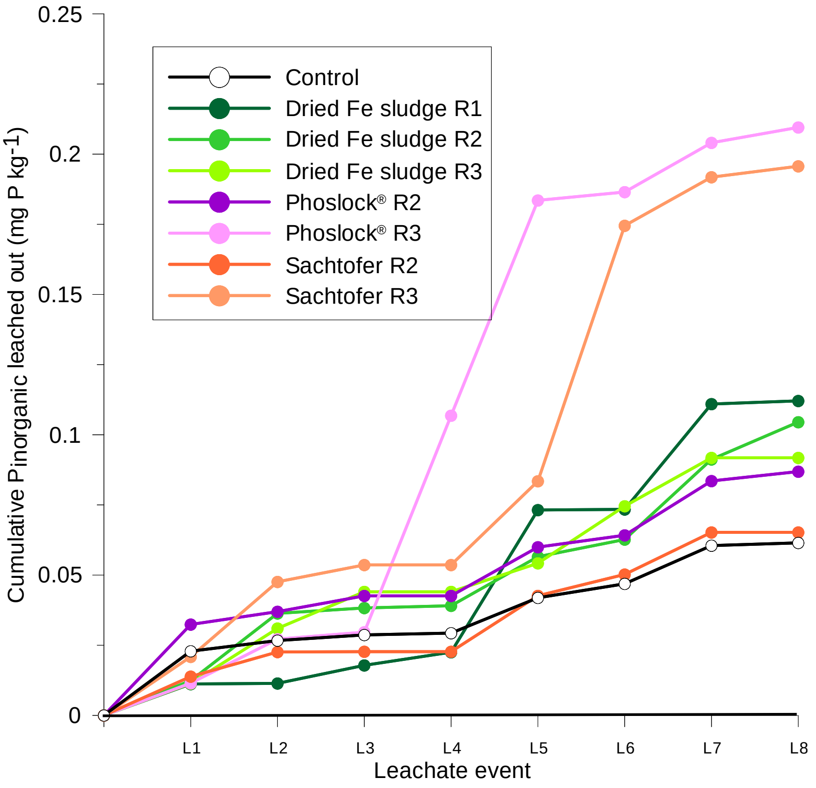

Figure 4.19. Cumulative amount of $P_{inorganic}$ (mg P kg^{-1}) leached out of Li3 over the eight leachate events with addition of industrial by-product and specially designed products (stdev for the total leachate and per leachate event can be found in appendix I)

Discussion

The P build up in the acidic sandy soils with a PSD such as in soil Zw (PSD in the top layer of 109%), poses a potential risk of P leaching and contamination of the ground- and surface water. Reducing the P solubility and increasing the P retention capacity in the soil are seen as the best management practices to reduce P leaching risks (O'Connor et al., 2005).

Screening experiment

Addition of mineral salts

All seven salts reduced the $P_{inorganic}$ concentration in the extraction solution, however, their potential varied greatly. The Al salts had a high P fixation capacity at low rates, e.g. 81% P fixation for $AlCl_3$ R1. However, when these products were added at higher rates, (from R3a onwards) a significant increase in $P_{inorganic}$ concentration in the extraction solution was found. The decrease in pH when the products were added in higher rates could be an explanation for this effect, since it has been reported that the optimum pH for P fixation in a soil by Al salts is between 5.6 and 7.7 (Ann et al., 2000). The Fe salts significantly reduced the $P_{inorganic}$ concentration in the extraction solution, namely 50% with $FeCl_3$ R1 and 96% with R2; with $FeSO_4$ a reduction of 70% with R1 and 97.5% with R2. The Fe salts (from addition rate R2 onwards) were more effective in P fixation than the Al salts which is in agreement with other findings (Ann et al., 2000). This can be explained by the stronger affinity of Fe^{3+} for PO_4^{3-} and a stronger hydrolyzing power than Al^{3+} (Hsu, 1976; Ann et al., 2000). The Ca salts resulted in a significant $P_{inorganic}$ reduction in the extraction solution, however an increase in addition rate did not further increase the P fixation capacity. Likewise, Jenkins et al. (1971) reported that, in water, increased carbonate concentrations may also cause slower Ca phosphate crystal growth rates by substitution of orthophosphate ions with the carbonate ions in the solid form of Ca phosphate compounds. However, the P precipitation with Ca ions is kinetically much faster than Ca carbonate precipitation (Ann et al., 2000). At higher addition rates the mixture of $CaCO_3$ and $CaCl_2$ had a higher P fixation capacity, which is in agreement with other findings (Yang et al., 2007). The P fixation capacity together with the increase in pH obtained by mixing simple salts with the soil is promising for application on acidic sandy soils.

Addition of olivine, biotite or zeolite

The addition of olivine or zeolite to the soil led to a rather small decrease of the $P_{inorganic}$ concentration in the extraction in comparison with the control. On the contrary, zeolite R1 and biotite R1 addition resulted in an increase of the $P_{inorganic}$ concentration in the extraction solution which is in agreement with the findings of an experiment with P removal from water (Wium-Andersen et al., 2012). These authors reported an increase in soluble P when water was treated with zeolite. However, they found that olivine had the highest P sorption capacity in water, which is in contradiction with our results for olivine additions to the soil, this can be explained due to the large composition variety of olivine and probably the olivine used in our experiment and in the reported experiment had another element composition. Chemical pre-treatment of these minerals with HCl for 1h proved to be highly efficient since all $P_{inorganic}$ was fixed from R3 onwards. The reason that chemically pre-treated minerals were able to fix P more efficiently is due to the fact that pre-treatment is an enhanced way of mineral weathering, which results in a strong increase in sorption sites for P. Wild et al. (1996) found that, in sludge, some phosphates may be adsorbed to Al centers on the zeolite surface, but to make Al available for precipitation reactions, the zeolites have to be hydrolyzed (i.e. degraded). This degradation is induced by an uptake of protons, followed by a change of Al coordination from tetrahedral to octahedral (Wild et al., 1996). Two effects favor the hydrolysis of aluminosilicates: a drastic pH decrease (< 4) or the presence of chelating ligands like citrate or acetate (Burgess et al., 1994). Wild et al. (1996) proved that the precipitation processes are strongly influenced by pH. This is in agreement with our results on P fixation in the soil, namely a decrease in pH gave an increase in the P fixation capacity. In another study, P removal from stormwater rich in P was 30% when filtered with zeolite (Wium-Andersen et al., 2012). The same study found a P removal capacity for olivine of more than 90%.

Addition of gypsum, bauxite, dried Fe sludge, Phoslock® or Sachtofer

A small decreasing trend in $P_{inorganic}$ concentration in the extraction solution was observed with gypsum R1, the additional fixation effect of higher addition rates was negligible. This is only partly in agreement with the findings of O'connor et al. (2005). They also found that a higher addition rate (2%) of gypsum did not increase the P fixation capacity of the soil. However, they found a more significant decrease in P of almost 40% when gypsum was added at a rate of 0.05% and a decrease of more than 60% at an addition rate of 0.5%, compared to a reduction of only 20% in our results. Rechcigl et al. (2000) reported that gypsum and lime amendments had no effect on the P concentrations in runoff. Anderson et al. (1995) found that small gypsum rates (0.1 g kg^{-1}) were able to fix P in the soil. However, if the high pH values revert to the natural low soil pH values as low as 4.5, the solubility of Ca-P compounds is predicted to increase and P would be released (O'Connor et al., 2005). An explanation for the findings of O'connor et al. (2005) is that the P solubility in a soil depends on the soil pH. Soils with a high pH typically limit P solubility via precipitation of various Ca-P compounds (O'Connor et al., 2005). Gypsum and lime products have been widely used to increase the pH on agricultural soils and these products have also been used to reduce P loss in heavily manure-impacted soils (Callahan et al., 2002; Cox et al., 2005; Watts & Torbert, 2009). The addition of gypsum to the soil is responsible for a significant increase of the pH, which is levelling off from R3 onwards. This is in agreement with the findings of Callahan et al. (2002), who also found an increase in pH after addition of gypsum to a soil with a starting pH of 5.5. It was stated that also the impurities in gypsum could affect the pH more rapidly than gypsum itself (Callahan et al., 2002).

Bauxite R1 gave a direct effect of P fixation, however, from R2 onwards the beneficial P fixation effect was rather small. This is in agreement with other experiments where bauxite is found to be an efficient amendment in terms of P fixation (McDowell & Nash, 2012). The pH increase after bauxite addition is also in agreement with other experiments (McDowell & Nash, 2012). However, addition of ball milled bauxite caused a significant decrease in pH of the filtrate. Since ball milled bauxite simulates an increase in the normal weathering rate of the mineral in the soil, it is important to be aware that bauxite could have a long term effect on the soil pH through weathering of the mineral, chemically as well as physically. The physical pre-treatment probably releases an increasing amount of available Al, which leads to an overall decrease in pH.

The dried Fe sludge, which is originating from the waste water treatment plant, gave the best results in terms of P fixation capacity. Already with the smallest addition rate of Fe sludge a significant decrease in $P_{inorganic}$ was found and from R3 onwards almost all P was fixed. This $P_{inorganic}$ fixation capacity is higher than reported by O'connor et al. (2005), who found a P fixation of 87% at an addition rate of 5%, (which was obtained here at an addition rate of only 0.5%). The addition of dried Fe sludge resulted in a significant increase in pH from R3 onwards, what could be seen as a secondary positive effect.

The products that are designed for P fixation in water bodies, namely Phoslock® and Sachtofer also gave promising results. At 1.5% addition rate more than 90% fixation efficiency was obtained with Sachtofer and almost 100% with Phoslock®. Under anoxic conditions (not the case in this research) it is reported that P bound by Phoslock® is retained, by forming a highly stable mineral known as rhabdophane ($LaPO_4.nH20$), and that Phoslock® is effective over pH 5-9 (Ross et al., 2008). From Phoslock® R3 onwards a significant increase in pH is observed. Reports indicated that the P adsorption capacities of Phoslock® is pH dependent, although this pH effect is not unambiguous (Haghseresht et al., 2009; Gibbs et al., 2011). Gibbs et al. (2011) measured an increase in P adsorption capacity with increasing pH. Another study indicated that the extent of P removal of Phoslock® decreased rapidly as the pH of water increased from 7 to 9, which could be attributed to the formation of hydroxyl species of the lanthanum ions thereby decreasing the number of P binding sites on the Phoslock® surface (Ross et al., 2008; Haghseresht et al., 2009).

Fixation experiment

P fixation efficiency by simple salts

All salts had a P fixation capacity, but this capacity varied with the type of salt. The fact that soil amendments vary greatly in their effectiveness in reducing P leaching in sandy soils is also reported in the literature (Yang et al., 2007).

Fe salts were most efficient and $FeSO_4$ addition resulted in a significant higher P fixation efficiency, even at a lower addition rate, in comparison with $FeCl_3$. However, when the P fixation efficiency between the Fe salts and $AlCl_3$ was compared, it should be considered that a higher addition rate was used for the Fe salts. For the lowest addition rate (R1) $AlCl_3$ was more efficient in P fixation than the Fe salts. $AlCl_3$ was not investigated at higher addition rates in the fixation experiment because the screening experiment had demonstrated a higher P release with increasing addition rates. The smaller P fixation capacity of $CaCO_3$ was in agreement with other reports (Ann et al., 2000). Ann et al. (2000) found that the most efficient P fixation salt was $FeCl_3$ and the effective addition amount required to minimize P release was 1-2 g kg^{-1} $FeCl_3$ or 7 g kg^{-1} $CaCO_3$, which is a (much) lower addition amount than was required in our results.

When the $P_{inorganic}$ loss is investigated per leachate event all amendments followed the same trend and this trend was also observed before in literature. As an example Yang et al. (2007) conducted a leaching experiment over 32 days with eight leaching events of 250 ml of deionized water. They found an increase in the concentrations of reactive P in the leachate fo the second leaching event followed by a significant decrease in P loss during the third leaching event, afterwards the P loss remained practically unchanged. Our results followed a similar but prolonged trend.

P fixation efficiency by minerals, bauxite, industrial by-product and specially designed products in different soils

The six investigated soils can be ranked by their decreasing P content: Zw, Ze1, Ze2, Li2, Li1 and finally Li3. The PSD or the P content of a soil was not always a good indicator for the amount of P that will leach from a soil, as apparent from P leaching rates in the control soils. Ze2 had a higher PSD and P content compared to Li2, but more $P_{inorganic}$ was lost during the experiment in Li2 (2.26 ± 0.2 mg P kg^{-1}) compared to Ze2 (0.83 ± 0.3 mg P kg^{-1}). This can be explained by a higher amount of desorbable P fraction in Li2 and a lower amount of Fe/Al oxide-associated P in comparison with Ze2. The Fe_{ox} content found is also significantly lower for Li2 (5.09 ± 0.3 mg Fe_{ox} kg^{-1}) compared to Ze2 (16.7 ± 0.3 mg Fe_{ox} kg^{-1}). This indicates that P fixed on Fe is a rather stable P form in the soil, which decreases the risk of P leaching.

The P fixation efficiency of the added products indicates, as expected, that there is no straight forward answer to the question : “which product will fix most P in the soil?”. The results show that the soil type and its P content play an important role in the P fixation efficiency of the added products.

The gypsum R2 treatment gave a higher cumulative P loss, in all six soils, compared to the control sample, which is in agreement with the findings of Summers et al. (1996), who found a slight increase in P leaching on a sandy soil after addition of gypsum. This was attributed to the amount of P in the gypsum (0.15%) or to the displacement and competition between sulphate and phosphate ions for binding sites. Another study concluded that gypsum addition may enhance P movement through the subsoil probably via macropores (Cox et al., 2005). A possible explanation of the lack of P fixation by gypsum in these soils could be the fact that they were acidic and sandy, where previously gypsum amendments were shown to be efficient on clay and clay loam soils (Ekholm et al., 2012; Uusitalo et al., 2012b). Gypsum addition increases the pH of acidic soils, thereby solubilizing more P fixed to Al or Fe than could be bound by the added Ca, and consequently results in an increase in soluble P. In almost all studied soils the zeolite treatment had none or a negative effect on the P fixation capacity but to a lesser extent than gypsum. The effect of zeolite addition to the soil thus corresponded to its effect in water, i.e. generally a poor P sorption capacity (Wium-Andersen et al., 2012).

The chemically pre-treated minerals were most effective in terms of P fixation capacity in soils that were rich or very rich in P. They were able to fix almost all $P_{inorganic}$ during the eight consecutive leaching events. However, at the end of the leaching experiment a small increase in $P_{inorganic}$ loss was observerd in comparison with the control, which could indicate that the P fixation effect of the pre-treated minerals is time dependent. Dried Fe sludge R3 and Phoslock® R3 were also able to fix almost all $P_{inorganic}$. Surprisingly increasing rate of dried Fe sludge did not increase the P fixation capacity in Ze2. For the soils with a normal to high amount of P, chemically pre-treated biotite and dried Fe sludge were able to fix most of $P_{inorganic}$ during the leaching experiment. An important drawback to the addition of dried Fe sludge is the presence of 4.2% of P_2O_5. This means that per ha 79.8 kg P with R1 to 1142 kg P with R3 was added with dried Fe sludge. Although the results show that the additional P in dried Fe sludge is unavailable and also fixed in the soil together with fixation of P in the soil it is unwanted to add P on soils that have already problems in terms of P saturation.

In the soil with the lowest PSD, namely Li3, no or a negative effect in P fixation was observed and no significant differences between the products were found. As a consequence, in a soil with a low P status it is advisory not to add any of the investigated products. If one of these products would be added to e.g. increase the pH, it is important to realise that there will be a $P_{inorganic}$ increase. This indicates that adding a product without having an idea of the P status of the soil is not the right approach.

CONCLUSIONS

The use of soil amendments to realize an immediate, temporary, fixation of P in acidic sandy soils is a mostly novel management strategy, that has never been investigated for mitigating P leaching losses from soils. At the same time the fixed P is not lost but stored in the soil and could be used by the plants in a later stadium.

The screening experiment confirmed that all the tested amendments were able to reduce the availability of P in the phosphate saturated soil of Zwevezele. The physical pre-treatment of the amendments (by ball milling), did not increase the P fixation efficiency, and for ball milled bauxite even a small decrease in P fixation efficiency was observed. On the other hand, chemically pre-treated olivine, biotite or zeolite were significantly more efficient in P fixation in comparison with their untreated amendments. A point of attention when adding the chemically pre-treated minerals to the soil is the drop in pH of the filtrate that was found. The most promising amendments in terms of P fixation with their optimal addition rates were then tested in a leaching experiment on six acidic sandy soils with different P contents to really investigate their P fixation potential. Chemically pre-treated minerals together with dried Fe-sludge and Phoslock® R3 are the most efficient products in terms of P fixation.

The fact that the P content of the soil plays an important role in P fixation efficiency of the products, has to be taken into account when products are recommended in terms of best management practices. Further research under field conditions is necessary to completely understand the P fixation efficiency of the products and to make recommendations on which product is most optimum to use in a soil with a known P content.

Chapter 5

Performance of phosphate-solubilizing bacteria in soil under high P conditions

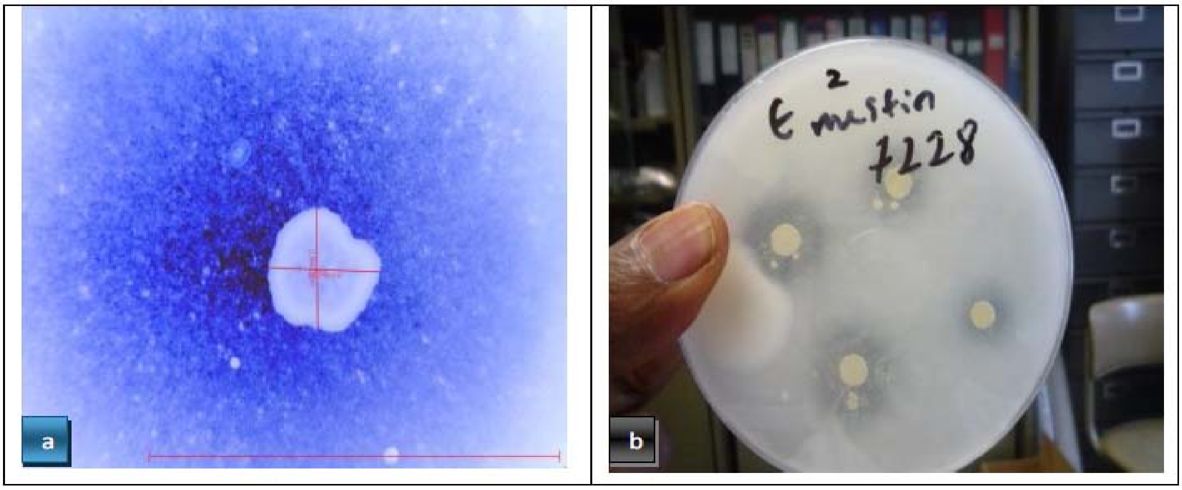

Illustration on p. 101:

The colony diameter for P. corrugata on Al-P medium (a) and on Ca-P medium (b)

This chapter has been published in :

De Bolle S., Gebremikael M.T., Maervoet V. & De Neve S. Performance of phosphate-solubilizing bacteria in soil under high phosphorus conditions. Biology and Fertility of Soils, 2013, 49, 705-714.

Abstract

A way to bring phosphate saturated soils back to an environmentally safe P level is by P mining through plants. Phosphate solubilising bacteria (PSB) could be very useful to increase the mining efficiency over time. The goal of this research was to investigate the adaptation and performance of PSB in conditions of high total P content in soil. In the first experiment, the P solubilizing capacity of five PSB species (three *Bacillus* spp. and two *Pseudomonas* spp.) were tested under fully controlled conditions on several growth media with different forms of insoluble phosphate ($FePO_4$, $AlPO_4$ or $(Ca)_3(PO_4)_2$) added at different rates. In contradiction with the normally used technique of halo determination, the colony growth (i.e. colony diameter) after 14 days of inoculation demonstrated that all five bacteria species were able to proliferate and solubilize P on each of the tested growth media. In the second experiment the same bacteria species were inoculated in pure quartz sand amended with a nutrient solution and P was added separately in an insoluble form, as Fe-P, Al-P or Ca-P. The extractable ammonium lactate P ranged from 3.2 to 6.9 and 29.0 to 40.7 mg P kg^{-1} sand for the insoluble Al-P and Fe-P treatments, respectively. *Pseudomonas putida* and *Bacillus brevis* performed best as PSB at high P concentration where the P is fixed with Al or Fe. In the third experiment *Pseudomonas putida* and *Bacillus brevis* were inoculated in an acidic sandy P saturated soil for 4 weeks. The inoculation of the PSB gave promising results in solubilising P.

Introduction

Many agricultural soils have accumulated large P reserves as a result of excess P fertilization over the years (Fernández et al., 2007; Bolster et al., 2012). Problems of excessive P levels are found in many countries with industrialized agriculture (Djodjic et al., 2004; Ketterings et al., 2005; Ajmone-Marsan et al., 2006; Uusitalo et al., 2007; Reijneveld et al., 2010). In Belgium, about 80% of arable lands and 40% of grasslands are considered fairly high to very high in soil P (Reijneveld et al., 2010). A lot of these fields are located in the acidic sandy region. Traditionally, P losses by erosion have been viewed as the main or even the sole source of P losses to natural waters (Kleinman & Sharpley, 2003; Volf et al., 2007). However, P leaching plays an important role in acidic sandy soils with high P levels (Van Den Bossche et al., 2005) (see chapter 2). In the EU, many of these acidic sandy soils with high PSD are subjected to strict P fertilization restrictions, which ultimately should result in P mining. However, it is felt that with current crop rotations, it takes several decades, because P mining efficiency decreases rapidly with time (Sharma et al., 2007). Ideally, efficient long term P mining from agricultural soils would thus require methods that keep P levels (temporarily) high enough in order not to limit crop P uptake.

One such way could be to increase P availability by the addition of phosphate solubilising bacteria (PSB) to the soil. A number of soil microorganisms, including bacteria, have the capability of solubilising mineral phosphates, thereby affecting the P cycle both in natural and agricultural ecosystems (Vazquez et al., 2000). In soil, PSB constitute from 0.5 up to 50% and P solubilising fungi from 0.05 to 0.1% of the total respective populations (Vazquez et al., 2000; Gyaneshwar et al., 2002). Generally, the PSB outnumber the P solubilizing fungi by 2 to 150 fold (Kucey, 1983).

Especially the *Pseudomonas, Bacillus* and *Rhizobium* species are widely distributed in the soil environment (Martin & Travers, 1989; Wang et al., 2001; Thakuria et al., 2009). *Pseudomonas* species were reported to solubilize P under a range of temperature conditions (Trivedi & Sa, 2008) and the most intensively studied species of this genus are *P. putida* (Manna et al. 2001; Villegas & Fortin 2002), *P. corrugata* (Pandey & Palni, 1998), *P. aeruginosa* (Musarrat et al., 2000), *P. stutzeri* (Vazquez et al., 2000) and *P. fluorescens*

(Deubel et al., 2000). In particular P. putida has been reported as an efficient PSB (Kuiper et al., 2002; Rosas et al., 2006). Within the genus *Bacillus*, *B. brevis*, *B. cereus*, *B. circulans*, *B. polymyxa*, *B. thuringiensis* and *B. megaterium* species were all reported to solubilise P (de Freitas et al., 1997; Turan *et al.*, 2007). Within the *Rhizobium* species the research has mainly focussed on *R. leguminosarum* (Rodriguez & Fraga, 1999) and *R. phaseoli* (Thakuria et al., 2009).

Addition of PSB to a soil to increase the P availability to agricultural crops has been widely investigated (Gyaneshwar et al., 2002). To the best of our knowledge, all studies on PSB have focused on the solubilization of P in artificial growth media or in soils which were low in total P. Nearly all these studies were concerned with P solubilization from Ca-phosphate sources (Vessey, 2003; Arcand & Schneider, 2006). Only some studies were found which looked into P solubilization from $AlPO_4$ by *Pseudomonas* sp. (Illmer et al., 1995; Puente et al., 2004; Henri et al., 2008), and these were done at low $AlPO_4$ levels.

To date, no studies have explored the potential of using PSB to increase P uptake in acidic soils rich in total P. However, such studies may prove very useful in these soils high in P where the objective is P mining, since the mining efficiency is known to decrease drastically with time if no P fertilizer is added. The point of interest of this study was the solubilization of Al-P and Fe-P in soils with a high total P content, and this as compared to Ca-P solubilization. The aim of this study was three-fold; to investigate (1) whether the PSB were tolerant and able to grow effectively in environments with high total insoluble P concentrations, thereby investigating if the evaluating procedure through halo determination is useful for all P forms; (2) whether PSB could solubilise the unavailable P in Al- and Fe-phosphates when added to a pure quartz sand substrate; and (3) whether PSB were able to solubilize P in acidic sandy soils high in total P to assess their potential for increasing P mining efficiency.

Materials and methods

Bacteria selection

Pseudomonas, *Bacillus* and *Rhizobium* spp. are found to be the most effective PSB (Rodriguez & Fraga, 1999; Rodriguez et al., 2006; Richardson & Simpson, 2011). Based on previous studies (de Freitas et al., 1997; Kuiper et al., 2002) five bacteria were selected, namely *B. brevis* (ATCC 8246), *B. polymyxa* (ATCC 842), *B. thuringiensis* (ATCC 10792), *P. corrugata* (ATCC 29736) and *P. putida* (ATCC 12633). The bacteria were obtained from DSMZ (Germany) and cultured in nutrient broth (OXOID LTD., England) under shaking at 30°C for the *Bacillus* species and at 25°C for the *Pseudomonas* species.

This study was carried out in a three stage approach. In a first stage PSB growth was monitored on media where an insoluble P source was applied as Al-P, Fe-P or Ca-P. In a second stage, the P solubilising capacity of the PSB was tested in an experiment using sand as a substrate, thus creating more realistic conditions, but still allowing to have maximum control over P dynamics, which is more difficult to achieve in a real soil environment. In a third stage the PSB were tested in real acidic sandy soil with a high total P concentration.

Growth media experiment

The growth media were based on the national botanical research institute's phosphate growth medium (Nautiyal, 1999) but with some modifications. Each growth media was composed of: glucose, 10.0 g L^{-1}; $MgCl_2.6H_2O$, 5 g L^{-1}; $MgSO_4.7H_2O$, 0.25 g L^{-1}; KCl, 0.2 g L^{-1}, plus variable amounts of N and insoluble P sources (Table 5.1) together with a sufficient amount of bacteriological agar (agar no.1, OXOID LTD., Hampshire, England). The pH was adjusted to 5.0 for the growth media with insoluble Al- or Fe-P sources and to 7.5 for the growth media with insoluble Ca-P source (Table 5.1), so as to achieve similar pH conditions as found in soils with these respective P sources.

Table 5.1 The amount (in g L^{-1}) of the insoluble P, N in each growth media and the pH of the different growth media

	Fe-P			Al-P			Ca-P	
P amount	**Low**	**Low**	**High**	**Low**	**Low**	**High**	**Low**	**High**
N amount	**Low**	**High**	**Low**	**Low**	**High**	**Low**	**Low**	**Low**
$FePO_4$	5.0	5.0	15.0	-	-	-	-	-
$AlPO_4$	-	-	-	5.0	5.0	15.0	-	-
$Ca_3(PO_4)_2$	-	-	-	-	-	-	2.5	15.0
$(NH_4)SO_4$	0.1	0.5	0.1	0.1	0.5	0.1	0.1	0.1
Agar	10.0	10.0	15.0	15.0	15.0	20.0	10.0	10.0
pH	5.0	5.0	5.0	5.0	5.0	5.0	7.5	7.5

The growth media were sterilized using an autoclave (SANOclav, Bad Überkingen-Hausen, Germany) at 121°C for 25 min and then transferred aseptically into sterilized Petri plates. Per plate, a bacterial strain was stabbed in quadruplicate on the plate using sterile toothpicks. For each growth medium and each bacterial strain, four replicate plates were used. The plates were incubated at 25°C for 14 days. The colony growth and, if possible, the halo zone were measured at the 3rd, 7th and the 14th day of the inoculation using a microscope (OPTIKA stereo microscope, x6.7 magnification, Italy).

Experiment on sand medium

Quartz sand (0.05 - 0.5 mm diameter) was washed, consecutively with 0.5% NaOH, distilled water and 5% HCl in a 1:1 sand/solution ratio under shaking for 1 h, in order to remove all nutrients. Finally, the sand was washed with demineralised water until the electric conductivity was smaller than 3 µs, and then oven-dried (105°C).

Firstly, the insoluble P sources were thoroughly mixed with 40 g of sand and brought into polypropylene tubes (diameter of 3.4 cm, height of 6.7 cm). The amount of insoluble P added to the sand (500 mg P kg^{-1}) was based on the typical P content of acidic sandy soils in Flanders, namely 500-1000 mg P_{ox} kg^{-1}. Secondly, the bacteria were mixed with the nutrient solution (Table 5.2) and this solution was added to the sand, as a bacterial inoculum of 2.2 x 10^8 colony-forming units (CFU) g^{-1} of sand, based on the population size of PSB in soils as found by Hu et al. (2009).

The nutrient solution, without P, was prepared with sucrose as main C source. The micronutrient solution, the Fe and Al solution were prepared separately (Table 5.2). For the treatments with insoluble Fe-P as P source, 1 litre of main nutrient solution was prepared by adding respectively 5 ml micronutrient solution and 1 ml Al solution. For the treatments with Al-P source the same composition was used but 1ml Al solution was replaced by 1ml Fe solution. For treatments with Ca-P as P source, 1 ml Al and 1ml Fe solutions were added to the main solution together with the 5ml micronutrient solution. Per tube, 6.3 ml of the nutrient solution was added. Per treatment and per extraction day, there were three replications. Finally, all tubes were covered with perforated parafilm to reduce water loss but still allowing sufficient oxygen supply and stored in a closed incubator at 20°C for 10 days. Samples were taken at the 5th and the 10th day of incubation. Immediately after sampling, the samples were dried in the oven for 2 h at 60°C to stop all biological activity.

Table 5.2. The composition (in mg L^{-1}) of the nutrient solutions added to the sand

Main nutrient solution		Micronutrient solution	
Nutrient	**Amount (mg L^{-1})**	**Nutrient**	**Amount (mg L^{-1})**
$CaCl_2.2H_2O$	1175.31	$MnSO_4.H_2O$	310.00
K_2SO_4	348.40	$ZnSO_4.7H_2O$	90.00
$MgSO_4.7H_2O$	492.62	$CoCl_2.6H_2O$	0.80
$CuSO_4.5H_2O$	249.55		
KNO_3	404.41	**Fe & Al solution**	
H_3BO_3	309.06	**Element**	**Amount (mg L^{-1})**
Na_2CO_3	211.98		
Ammonium acetate	332.20	$Al_2SO_4.8H_2O$	119.00
Sucrose	14850	Fe	27.92
$H_{24}Mo_7N_6O_{24}$	0.18		

Experiment with acidic sandy soil

Two acidic sandy soils (further referred to as soil1 and soil2), located in Oostkamp, Belgium, with a total P content of 1078 mg kg^{-1} and 1267 mg kg^{-1} were selected. The soil texture of both soils was sand (USDA classification) with a composition of 87.1% sand, 10.0% silt and 2.90% clay for soil 1 and 87.9% sand, 9.0% silt and 3.10% clay for soil 2. In the top layer (0-30 cm) a PSD of 77.8% and 106.1% for soil1 and soil2 was found, respectively. For both soils there was no free $CaCO_3$ and the organic matter content was 4.5 and 3.6% respectively. The pH was 5.1 and 4.3 for soil1 and soil2, respectively. In this study *B. brevis* and *P. putida* were inoculated separately and in combination (dual inoculation) in the same concentration as in the sand experiment. The PSB, in an inoculum of 2.2 x 10^8 CFU g^{-1}, were mixed with 80 g of pre-incubated soil. Each tube was then covered with the parafilm and holes were made to create aerobic conditions. There were three replicates per treatment per sampling date. The samples were randomly stored in a closed incubator at 20°C for 5 days.

Measurements

The water extractable P (P_w), the ammonium lactate extractable P (P_{lac}) (Egnér et al., 1960) and the pH were measured, both in the quartz sand (after 10 days, pH on the 5th and on the 10th day) and in the soil (at week 2 and week 4). The P_w, which is considered as the available P pool, was measured as reported by Self-Davis et al. (2009). Two grams of dry sand/soil and 20 ml of distilled water were put in a centrifuge tube and shaken for 1 h on a rotational shaker. The soil slurries were then centrifuged at 3220xg followed by filtration (Whatman ashless filter, 589/3). The P_w in the filtrate was determined colorimetrically at 882 nm according to Murphy & Riley (1962).

The P_{lac}, which is considered the plant available P pool in these soils (Van Den Bossche et al., 2005) was measured at both sampling occasions, by extracting the soil with ammonium lactate (extraction ratio 1:20, (Otabbong et al., 2009)) in dark polyethylene bottles that were shaken for 4 h on a rotational shaker. The P concentration in the filtered extract was measured colorimetrically at 700 nm (spectrophotometer, Varian, cary 50) according to Scheel (1936). The pH was measured potentiometrically in a 1:2.5 soil/KCl extract (pH-KCl).

Calculation and statistical analysis

The results gathered from the three experiments were statistically analysed using the statistical software PASW 20 package (SPSS version PASW 20, SPSS Inc., USA). The growth diameters of the PSB were statistically compared (ANOVA) between the different P sources as well as between the different PSB. In the sand experiment, a comparison between the different P sources as well as between the PSB were carried out. The results were statistically analysed with a paired-sample *t*-test. For the sand and the acidic sandy soil experiment a one-way ANOVA (with a *t*-test) was done for the pH, the P_{lac} and the P_w. The results were also compared between dates with a paired-sample *t*-test. Pearson correlation coefficients were calculated between the pH, P_w and P_{lac} for the soil experiment for each PSB and for both soils.

Results

Growth media experiment

The colony growth diameters, after 14 days, indicated that all five PSB were able to grow on each of the respective growth media, with varying soluble N and insoluble P concentrations (Figure 5.1 to Figure 5.3). For all growth media, the colony growth diameter was found to be in the same order of magnitude, varying between 2 and 8 mm.

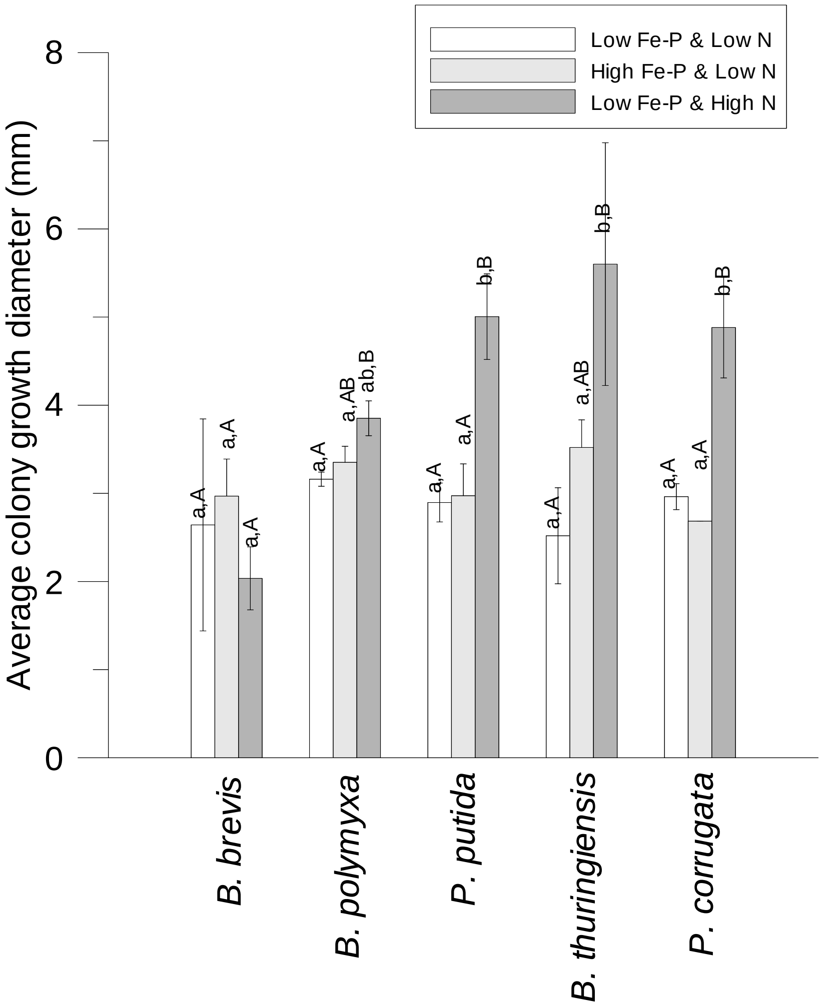

Figure 5.1. The average colony growth diameter (in mm) at the end of the incubation (14 days at 25°C) for the Fe-P treatments (p<0.05, significance between PSB is marked in *lowercase letters* and significance between treatments is marked in *uppercase letters*)

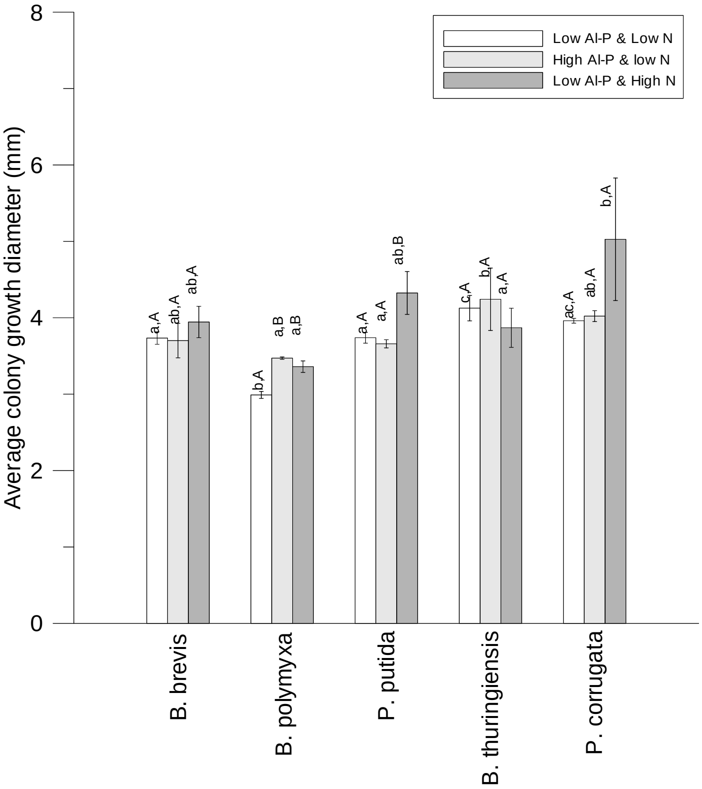

Figure 5.2. The average colony growth diameter (in mm) at the end of the incubation (14 days at 25°C) for the Al-P treatments ($p<0.05$, significance between PSB is marked in *lowercase letters* and significance between treatments is marked in *uppercase letters*)

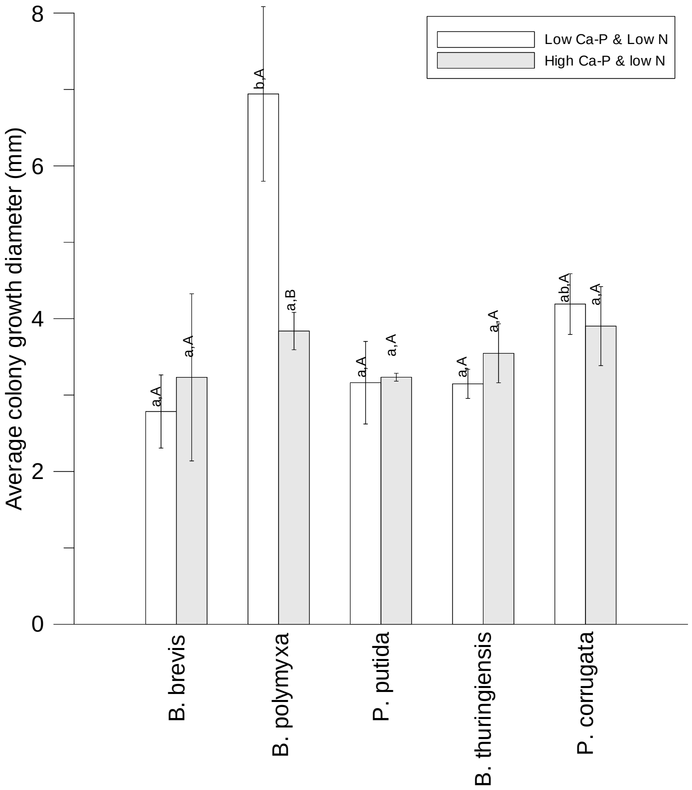

Figure 5.3. The average colony growth diameter (in mm) at the end of the incubation (14 days at 25°C) for the Ca-P treatments ($p<0.05$, significance between PSB is marked in *lowercase letters* and significance between treatments is marked in *uppercase letters*)

The colony diameter for all PSB, with exception of *P. corrugata,* tended to increase with increasing insoluble Fe-P, from 5 to 15 g L^{-1}, but this was not statistically significant ($p>0.05$). All PSB, except *B. brevis,* showed a significant increase in the colony growth diameter with an increasing N concentration, from 0.1 to 0.5 g $NH_4(SO_4)$ L^{-1}, at a constant insoluble Fe-P concentration of 5 g L^{-1}.

The colony growth diameter for all PSB tended to be in the same order of magnitude, irrespective of the insoluble Al-P concentration. For *B. polymyxa*, however, a significant increase ($p<0.05$) was found in the colony growth diameter with increasing Al-P concentration. An increasing growth diameter of all PSB, except *B. thuringiensis*, was found when the N concentration increased, for *B. polymyxa* and *P. putida* even a significant increase was found. However, the effect of increasing P concentration was greater for the Fe-P treatment (max. increase of 3.08 mm) than for the Al-P treatment (max. increase of 1.06 mm).

An increase in Ca-P concentration reduced the colony growth diameter significantly for *B. polymyxa* (from 6.94 mm to 3.84 mm, Figure 5.3).

Sand experiment

The solubilization effect was dependent on the type of P compounds and the type of PSB added to the sand (Table 5.3). Large differences were found between the P solubility in the controls of the treatments. In the Al-P treatment, only *B. brevis* showed a trend of P solubilization, namely, a P_w value of 4.2 mg P kg^{-1} compared with 3.2 mg P kg^{-1} for the control treatment. The P_w value found with *B. thuringiensis*-, *P. putida*- and *P. corrugata*-inoculated samples decreased significantly ($p<0.05$) in comparison with the control treatment. All PSB were able to solubilise P when P was under the form of Fe-P. The P_w values for PSB inoculated samples varied from 0.7 to 1.6 mg P kg^{-1} sand in comparison with 0.6 mg kg^{-1} sand for the control treatment. The samples inoculated with *B. brevis*, *P. putida* and *P. corrugata* resulted in a significant ($p<0.05$) P solubilization effect for the Fe-P treatment in comparison with the control. A higher P_w value was observed for *P. putida* and

B. thuringiensis for the Ca-P treatment in comparison with the control. However, only *P. putida* (44.8 mg P kg^{-1} sand) was significantly different ($p<0.05$) to the control (35.4 mg P kg^{-1} sand), thereby proving to be an effective PSB in solubilising P in a high Ca-P environment.

Table 5.3. Mean P_w values (mg kg^{-1} sand ± standard deviation) measured after 10 days of incubation for the Al-P, Fe-P and Ca-P treatments in the sand experiment

Treatment	Al-P	Fe-P	Ca-P
Control	3.2 ± 1.03	0.6 ± 0.22	35.4 ± 6.22
B. brevis	4.2 ± 1.72	1.3 ± 0.75*	30.7 ± 0.88
B. polymyxa	2.6 ± 0.60	1.1 ± 0.77	33.6 ± 2.4
P. putida	0.6 ± 0.16**	1.5 ± 0.14*	44.8 ± 3.10*
B. thuringiensis	0.6 ± 0.15**	0.7 ± 0.11	39.9 ± 7.95
P. corrugata	0.6 ± 0.17**	1.6 ± 0.52*	34.6 ± 3.15

*$p<0.05$, significantly higher than the control; **$p<0.05$, significantly lower than the control

When the P was under the form of Al-P, the P_{lac} values for the tubes inoculated with PSB ranged between 2.9 and 6.9 mg P kg^{-1} measured on the tenth day (Table 5.4). Significantly higher P_{lac} values were found for *B. brevis*, *P. putida* and *P. corrugata* in comparison with the P_{lac} value of the control. For the Fe-P treatment the results for P_{lac} for the PSB inoculated samples varied between 38.8 and 40.7 mg P kg^{-1} measured on the tenth day. When comparing these results with the results for the control sample, no significant effects were found with PSB inoculation for the Fe-P treatment. PSB inoculation in the insoluble Ca-P treatments resulted in P_{lac} values between 220.7 and 228.8 mg P kg^{-1} on the tenth day. Within the Ca-P treatments, significantly higher P_{lac} values than the control were found for *P. putida*, *B. thuringiensis* and *P. corrugata*.

Table 5.4. Mean P_{LAC} (mg kg^{-1} sand ± standard deviation) measured on the tenth incubation day for the Al-P, Fe-P and Ca-P treatment

	Al-P	Fe-P	Ca-P
	10th day	10th day	10th day
Control	5.8 ± 1.1	39.9 ± 2.8	220.6 ± 2.2
B. brevis	6.9 ± 1.6	39.0 ± 2.1	220.7 ± 3.4
B. polymyxa	2.9 ± 0.1	39.9 ± 2.6	223.6 ± 2.6
P. putida	6.8 ± 0.8	39.3 ± 3.2	228.2 ± 2.3
B. thuringiensis	3.7 ± 0.2	40.7 ± 2.3	228.8 ± 6.0
P. corrugata	4.9 ± 0.4	38.8 ± 1.6	228.3 ± 5.9

The largest differences in pH, both between sampling dates and between the control and PSB treatments, were observed in the Al-P treatments (Figure 5.4). In the Fe-P treatments much smaller differences in pH were found between the control and the PSB treatments. However, for *P. putida* and *P. corrugata*, which were the most effective PSB, a decrease in the pH was found compared to the control. No significant decreases in pH were observed for the PSB inoculated Ca-P treatments compared to the control, although a decreasing trend was noticed for *B. brevis*, *P. putida* and *P. corrugata*.

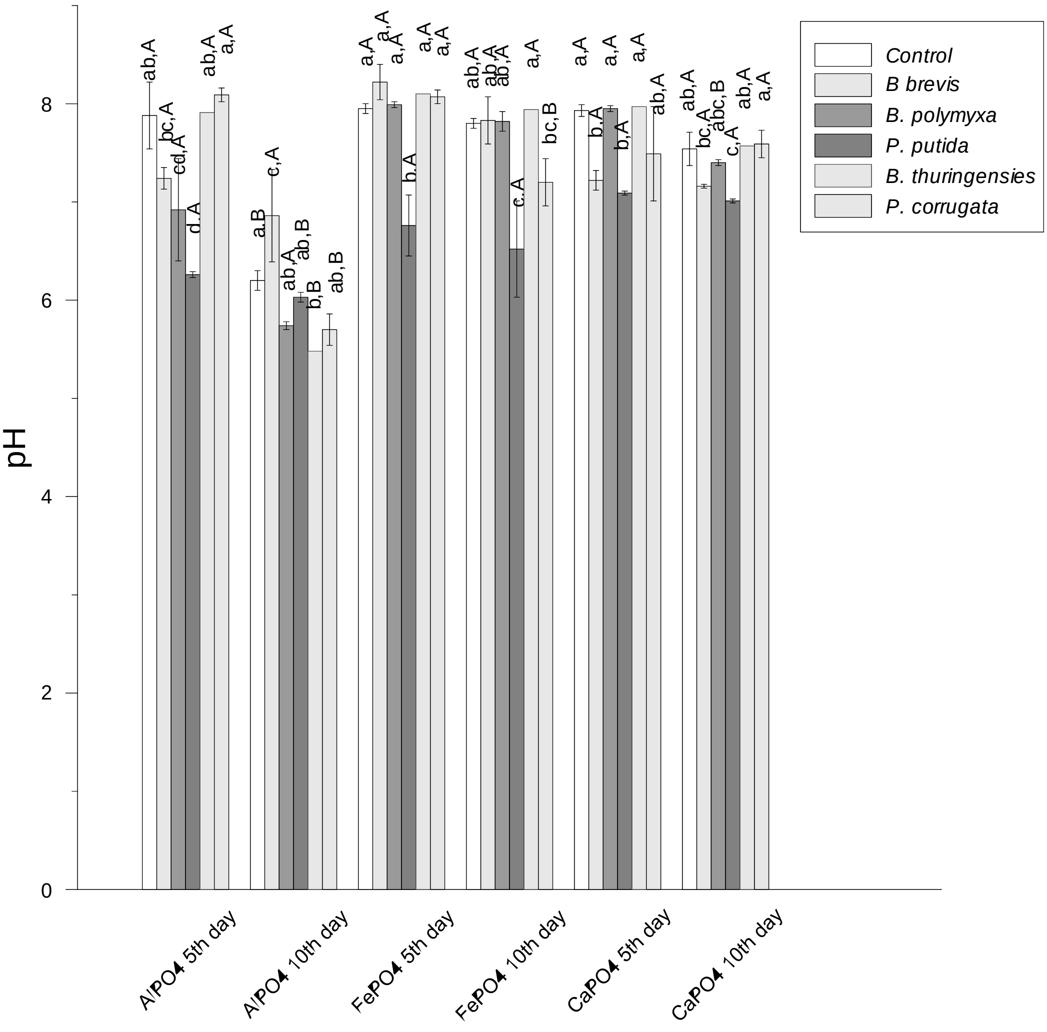

Figure 5.4. Changes in pH-KCl measured on the fifth and tenth incubation day in a sand medium for the control and the inoculated samples for the Al-P, Fe-P and Ca-P treatment ($p<0.05$, significance between the control and PSB is marked in *lowercase letters* and significance between days in treatments is marked in *uppercase letters*)

Soil experiment

The P_w values ranged between 18.1 and 19.9 mg P kg^{-1} for soil1 and between 33.8 and 38.1 mg P kg^{-1} for soil2 (Table 5.5). Significantly higher P_w values than the control for soil1 (after week 2) were found with the single and dual inoculations of *B. brevis* and *P. putida*. We observed a small but significant decrease in pH in week 4 in all PSB inoculated soils (Table 5.5). The P_{lac} ranged between 351.3 and 385.4 mg P kg^{-1} for soil1 and between 460.2 and 533.0 mg P kg^{-1} for Soil2 (Table 5.5). Significantly higher P_{lac} values were found for the inoculation with *B. brevis* than in the control for both soils. For soil2, a significantly higher P_{lac} value was found with *P. putida*.

Table 5.5. P_{LAC},P_W and pH (mean ± stdev) at the end of incubation week 2 and 4 for soil1 and soil2

	treatment	**P_{lac} (mg kg^{-1} soil)**		**P_W (mg kg^{-1} soil)**		**pH-KCl**	
		Week 2	**Week 4**	**Week 2**	**Week 4**	**Week 2**	**Week 4**
Soil1	**Control**	370.4 ± 8.3	365.8 ± 7.3	18.1 ± 0.6	19.9 ± 0.5	5.01 ± 0.03	5.04 ± 0.02
	B. brevis	384.4 ± 5.9*	360.1 ± 4.3	19.3 ± 0.2*	19.6 ± 0.3	5.03 ± 0.01	4.99 ± 0.01*
	P. putida	371.4 ± 2.3	359.4 ± 8.9	19.2 ± 0.7*	19.9 ± 0.3	4.96 ± 0.04	4.93 ± 0.01*[a]
	B. brevis + P. putida	385.4 ± 3.2*	351.0 ± 5.1	19.8 ± 0.5*	19.6 ± 0.2	5.00 ± 0.01	4.94 ± 0.01*
Soil2	**Control**	466.1 ± 9.2	463.3 ± 7.6	35.3 ± 1.4	38.1 ± 0.3	4.23 ± 0.08	4.14 ± 0.01
	B. brevis	477.0 ± 0.3*	472.2 ± 2.7*	35.4 ± 0.5	36.5 ± 0.6	4.15 ± 0.01*	4.09 ± 0.01*
	P. putida	480.7 ± 6.3*	479.8 ± 6.1**	35.7 ± 0.2	37.5 ± 0.8	4.15 ± 0.02	4.07 ± 0.01*
	B. brevis + P. putida	533.0 ± 4.6**	460.2 ± 3.4	33.8 ± 2.2	36.2 ± 0.7	4.17 ± 0.03	4.09± 0.01*

*p<0.05,**p<0.01; significantly higher than the control, [a]significantly different between two sampling dates

Discussion

Growth media experiment

Several methods have been used for screening the efficiency of PSB to solubilise P, but, almost all of these used low amounts of the insoluble P source in solid or liquid media. Additionally, all these studies used Ca-P as the sole source of P at a pH of 7 (Nautiyal, 1999; Mehta & Nautiyal, 2001; Rosas et al., 2006). In one study the solubilising efficiency of *Burkholderia* spp. was investigated on $AlPO_4$ (Delvasto et al., 2008) and in another study, the toxicity of Al to *Bacillus megaterium* was investigated (Davis et al., 1971). Here, we assessed the performance of PSB on insoluble Fe-P and Al-P and thus in media of acidic nature. Moreover, we used total added P concentrations that were much larger than in previous studies, because we wanted to mimic conditions in soils with high levels of phosphate saturation.

Traditionally, the clearing/halo zone around the colony is used as an indicator for P solubilization (Mehta & Nautiyal, 2001). However, it has been reported that many fungi or PSB that did not produce any halo zone on agar plates (Delvasto et al., 2008; Collavino et al., 2010), were able to solubilize insoluble inorganic phosphates in liquid medium (Leyval & Berthelin, 1989; Mehta & Nautiyal, 2001). Because the evaluation of P solubilization with the plate assay by halo zone is not always conclusive and because Malboobi et al. (2009) found that the active growth of bacteria corrolates well with P solubilization, the colony growth diameter of the PSB should be viewed merely as a first indication of the P solubilization potential, thereby proving that the halo zone is not the best indicator for P solubilization and more interest is given on the effect the treatment had on the colony growth diameter. An increase in the insoluble P concentration tended to have no negative effects on the colony growth diameters in the Fe-P and Al-P treatments. For the Ca-P treatment, a significant decrease in colony growth was found for *B. polymyxa*, suggesting that this species is not an effective PSB under high Ca-P conditions. *B. brevis*, *P. putida* and *B. thuringiensis* had higher colony growth diameters under high Ca-P conditions, showing that they were able to adapt and solubilise P in high Ca-P environments. Problems of P saturation and P leaching are mostly found in acidic sandy soils with high amounts of P associated with Al and Fe compounds. If PSB are to be effective in increasing P mining efficiency in such soils, it is important that they are able to solubilise Al-P and Fe-P and that they continue to be effective under conditions of high total P. Our results show that PSB were equally efficient in solubilising Al-P and Fe-P as in solubilising Ca-P. *B. polymyxa* performed even better under high insoluble Al-P and Fe-P treatments than under high insoluble Ca-P treatments. The ability of these PSB to grow and solubilise P under the specific conditions as imposed in this experiment is an important finding with respect to increasing mining efficiency in P saturated soils.

The increase in colony diameter following an increase in N concentration as observed for most of the PSB, indicates that environments with high N content are more conducive to proliferation of these PSB, which contradicts the findings of Nautiyal (1999). Exceptions to this were *B. brevis* in a Fe-P environment and *B. thuringiensis* in the Al-P environment. The improved performance of the PSB under higher N conditions would be a clear advantage for using them in P saturated soils. Indeed, such soils mostly have an overall high chemical fertility, including high mineral N availability as a result of e.g. mineralization.

Sand experiment

The sand medium provides an intermediary situation between the growth media (more realistic than growth media) and natural soil (sand medium allows more control over the experiment). The analysis of P_w provides a close approximation of the actual P concentration in the soil solution (i.e. directly plant available P), without creating an acidic environment and thereby avoiding an overestimation of the P solubilization for the insoluble Ca-P treatments. The P_w values for the Ca-P treatments were one order of magnitude higher than the P_w values for the Al-P and Fe-P treatments, which can be explained by the differences in solubility products of these P sources. The solubility product of Fe-P is 5 times smaller than that of Al-P and 60 times smaller than that of Ca-P. *B. brevis*, *P. corrugata* and *P. putida* prove to be the most effective PSB in solubilization of fixed P to Al or Fe out of the P_w results. The most effective PSB here are consistent with the ones that were most efficient in the growth medium experiment.

The inoculation with PSB did not always increase P_{lac} values as compared to the uninoculated control samples. This could be explained by a temporal P immobilization by the PSB, which would imply that the P is solubilised, yet not in the soil solution. The fact that *B. polymyxa* and *B. thuringiensis* are solubilising less P in the Al treatment could be explained by Al toxicity for these PSB, which is in agreement with the results of an Al toxicity test done on *Bacillus* sp. (Davis et al., 1971). This indicates that *Pseudomonas* sp. are able to survive in media with high Al amounts, which is in agreement with the findings of Illmer & Shinner (1999). PSB are efficient for P mining in high total P conditions. Almost always an increase in P_{lac} was found over time indicating the continuity of the P solubilization efficiency of the PSB. *P. putida*, *P. corrugata* and *B. brevis* proved to be the most efficient PSB for all insoluble

P treatments which is in agreement with results from other experiments using Ca-P (Manna et *al.*, 2001; Kuiper et al., 2002; Villegas & Fortin, 2002). These PSB were most efficient for both experiments, the growth medium and the sand experiment, thereby confirming that the colony growth diameter of the PSB is an indicator for testing the P solubilization efficiency of the PSB.

A decrease in the pH after inoculation with PSB has been considered as one of the mechanisms by which the PSB transform the insoluble Ca-P into a plant available P form (Illmer et al., 1995; Nautiyal, 1999; Gyaneshwar et al., 2002). It has been found that *P. putida* solubilises P by lowering the pH as a result of organic acid production (Vyas & Gulati, 2009). This was also confirmed with a significant decrease in pH for the samples with inoculation of *P. putida* for Al-P and for the Ca-P treatments. However, no correlation was found between the pH and the P solubilization for the Fe-P treatments. The absence of correlation in the case of Fe-P might indicate that other mechanisms such as chelation and/or ligand exchange were more important than a decrease in pH (Whitelaw, 2000; Gyaneshwar et al., 2002).

Soil experiment

An increasing trend in plant available P (P_{lac}) was found for both of the acidic sandy soils with PSB inoculation compared to the control, except after week 4 for soil1, but the effects were more pronounced in soil2 which had a higher total P content. This confirms that PSB are able to solubilise P in soils rich in total P. The relatively higher values of P_{lac} for soil2 inoculated with *P. putida* compared to the control can be explained by the better adaption of *P. putida* to lower pH conditions (Villegas & Fortin, 2002).

The dual inoculation of *P. putida* and *B. brevis* was more efficient in P solubilization than the control and mostly more efficient than separate inoculation. Our results are in agreement with other research, where dual inoculations (mainly using PSB and fungi or N fixing bacteria) are reported to perform better and have a higher P availability than found for separate inoculation (Kim et al., 1998; Rosas et al., 2006).

The efficiency of the PSB to solubilize P, was found for both methods P_w and P_{lac}. This proves that the PSB can be used for P mining since P_w is an indication of the easily available

P in the soil and P_{lac} indicates P availability for the plant, two indicators which are important for P mining of soils.

PSB are known for lowering the pH of the inoculated media by producing organic acids (Rodriguez & Fraga, 1999; Arcand & Schneider, 2006), which was also found in our results. However, the differences in the soil experiment were smaller than in the sand experiment because of the buffering capacity of the soil. The absence of a significant correlation effect between lowering of pH and increase in P_{lac} indicates the possibility that the PSB solubilise P in these acidic sandy soils in a different way. According Arcand & Schneider (2006) and Vyas & Gulati (2009) PSB also solubilize P through chelation or ligand exchange as a result of organic acid production.

Conclusions

The present study examined the P solubilization efficiency of PSB under high insoluble P conditions. In a growth media experiment all five tested PSB species were able to grow on all the different growth media, i.e. the tested PSB were able to adapt to high insoluble P conditions. When the same PSB were inoculated in sand, to create more realistic conditions, they proved again to be able to solubilise P in high P conditions. Addition of *B. brevis*, *P. putida* and *P. corrugata* resulted in significantly higher available P concentrations than in the control. Especially *B. brevis* and *P. putida* exhibited the most promising capacities to solubilise P in high insoluble P conditions. This was especially true in the Al-P and Fe-P treatments, which indicates their ability to solubilise P in acidic sandy soils with a high total P content. Further research is needed to evaluate the effect of PSB in bigger pot and field experiments.

Chapter 6

General discussion and conclusions

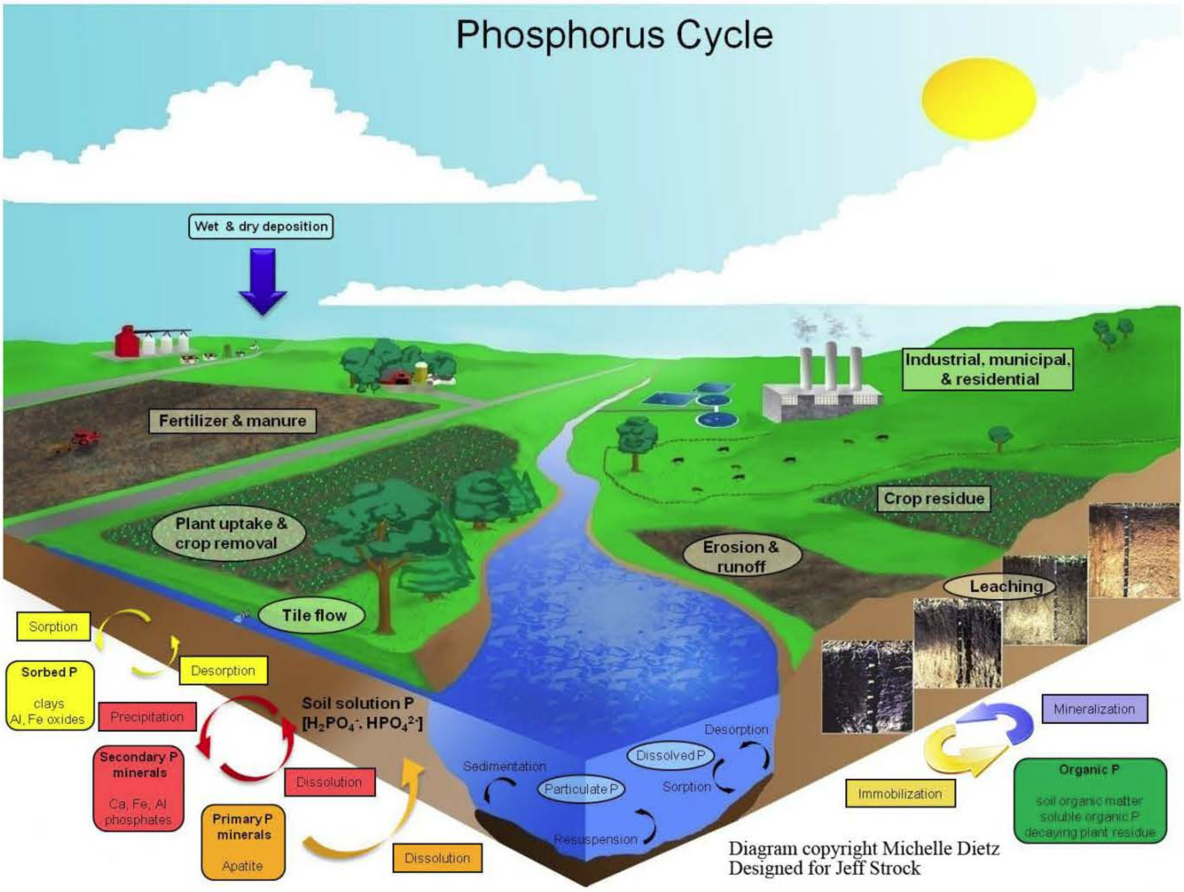

Illustration on p. 121:

Important inputs and outputs in the P cycle

Introduction

During the last decades eutrophication of surface and groundwater has become a focus point for the European Union, and the European member states have to tackle eutrophication and counteract the consequences of eutrophication through the implementation of a number of Directives (Haygarth et al., 1998; Foy, 2007). As elaborated in the introduction (Chapter 1) it is known that phosphorus (P) plays a crucial role in eutrophication, which can be caused by point and non-point sources. Flanders has already taken efficient measures to tackle the point source pollution, however a lot of problems remain with the non-point sources such as the transfer of P from agricultural land to the water bodies. Until the early 1990s, there has been very little research conducted on P transfer from agricultural land. As the potential linkages between agricultural intensification, increased P concentrations in land runoff and eutrophication became widely appreciated (Withers & Haygarth, 2007), an extensive survey was done from 1995-1997 (in Flanders) to quantify the accumulation and the (potential) loss of P. For acidic, light textured soils the relationship between the P saturation of the profile and the concentration of orthophosphate at groundwater level was given by Van der Zee et al. (1990a). If an orthophosphate concentration of 0.1 mg L^{-1} at the base of the profile is postulated as a lower limit for eutrophication, the P saturation of the whole soil profile should be less than 24%.

The evolution of the ortho-P concentration in large water bodies and in small catchments in agricultural areas (MAP measuring points) shows a decrease in the large water bodies from 1999 until now (Figure 6.1), mainly as the result of a reduction in the P loads point sources e.g. waste water treatment. However, since 2000, hardly any change is noticed in the ortho-P concentration in the small catchments and since 2003 these values have been consistently higher than the concentrations found in the large water bodies. This indicates that diffuse P losses from agricultural land have become more important than P input from other sources such as industry and households (Overloop et al., 2012). Clearly urgent management measures are needed in order to improve these excessive P concentrations.

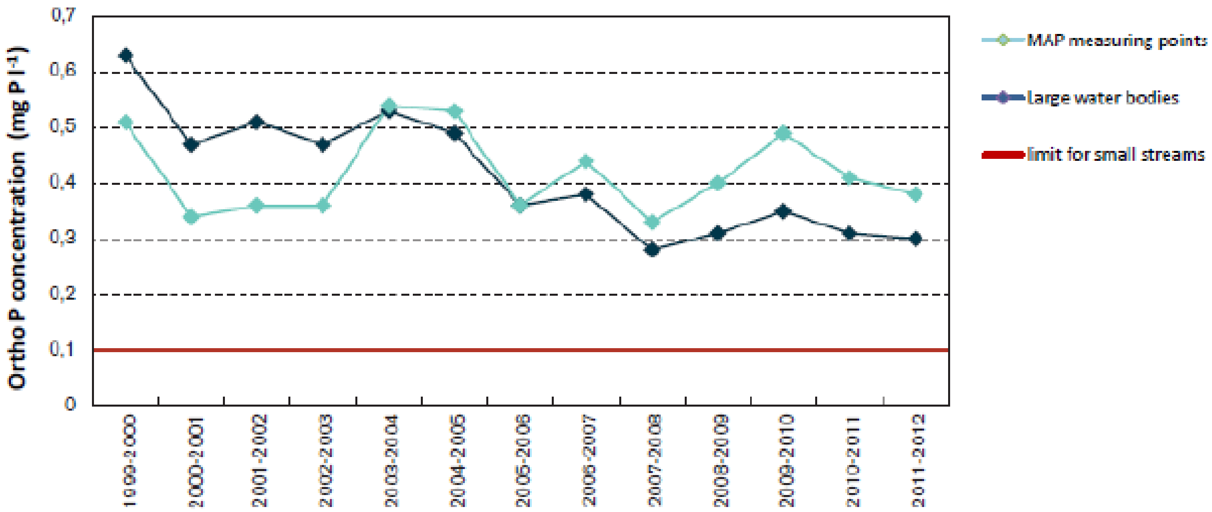

Figure 6.1. Evolution of the ortho-P concentration in the large water bodies and the MAP measuring points (Overloop et al., 2012)

In this study, we have focused on two main aspects of P leaching problems. The first aspect is the recent evolution of P saturation in a selection of acidic sandy soils in Flanders, and the calculation of P losses from acidic sandy soils as an alternative for potential P leaching losses (as given by the PSD). The second aspect is the potential of a number of novel mitigation options aimed at i) reducing further leaching of P from the surface layers and ii) increasing the removal rate of P by the use of PSB. One crucial point that was not investigated here was the efficiency of specific crop rotations in removing P from soil by normal crop P uptake. This point will also be discussed here to some extent. We are not aware of additional mitigation options available at this moment (apart from specific measures that can be taken on drained fields) that could help in permanently reducing P leaching risks from these soils. The two main aspects of this study will be brought together and critically discussed here, including the general potential of biological P mining in general.

Has the legislation in place between 1999-2010 been effective in stabilizing or decreasing the PSD level of agricultural soils?

A first objective was to investigate and quantify the present P status and the evolution of PSD in agricultural soils in Flanders. A large scale survey of P saturation of acidic sandy soils was done from 1995-1997 (VLM, 1997). Based on these results a classification was made of the P saturated soils in Flanders with a soil being legally P saturated with a PSD > 40% (leads to an orthophosphate concentration of 0.2 mg l^{-1} at the base of the soil profile). The soil was classified as P critical with a PSD > 30% and soils were classified as not P critical and not P saturated with a PSD < 30%. In 2009 some data of the same survey were reanalysed and stricter limits in terms of P saturated soils were defined (namely a soil was considered as legally P saturated when the PSD > 35% and as P critical when the PSD > 25%). During 2009-2010 a new, limited sampling campaign was performed on 21 fields to re-evaluate the PSD (chapter 2). This allowed to evaluate whether the legislation in place between 1999-2010 had been effective in stabilizing or reducing the problem of P saturation of these soils. It also allowed to investigate if the PSD values, that have been inventorized more than 15 years ago, can still be considered representative for the current situation. A significant change in PSD over this period could have important implications for policies aimed at reducing diffuse P losses and protecting the water quality.

The results of Chapter 2 indicated that the PSD of the limited selection of fields did not improve between both sampling campaigns, on the contrary, rather an increase was observed. Moreover, it was found that the process of P leaching through soil is probably occurring at a much higher pace than initially thought. The leaching of P to deeper soil layers has implications for the P uptake by plants, because roots are generally more abundant in surface soil layers, more root surface area is exposed to available soil nutrients and the highest amount of P is taken up there (Koopmans et al., 2004b). If P is leached to layers which are unreachable for roots the only option is by fixing P on P fixation elements such as Al and Fe. The presence of these elements, generally, also decreases with depth, which makes the potential to withhold the leached P highly unlikely. This implies that P will ultimately reach the ground- and surface water and will intensify the eutrophication.

With these conclusions in mind the question arises whether the decrease of available P as reported by Overloop et al. (2011) (Figure 1.2) is really a step in the right direction in terms of P saturation, or rather that this decrease is a result of P leaching to deeper soil layers. The fact that ortho-P concentrations in surface waters are not improving seems to confirm this latter hypothesis (Figure 6.1).

The second objective was to identify the critical areas in terms of P leaching by using the PLEASE model. As stated above the P saturation status of a field in Flanders, like in several other countries, is determined based on the PSD. However, the PSD merely gives a potential risk of P loss, but does not give actual leaching since e.g. the hydrological conditions are not fully considered. A possible way of calculating the real P leaching risk is by modelling the P loss taking both the soil and the hydrological conditions into account. However, a lot of the current P models either ignore P leaching or treat it in a simplistic manner (Radcliffe & Cabrera, 2007). For example ANSWERS-2000, WEND-P and EveFlow do not model P leaching. SWAT (Soil and Water Assessment Tool) uses a field-capacity water balance approach to estimate vertical flux of water within the soil profile, whereby the concentration of P in leaching water is determined by the labile P concentration in the top soil layer only (Radcliffe & Cabrera, 2007; Schoumans et al., 2009). On the other hand, a comprehensive process oriented simulation model, like STONE, needs a large number of input parameters, which is not cost effective and makes it unpractical to be used on a large scale (Schoumans et al., 2009; Schoumans et al., 2013).

At this moment, in Flanders, the nutrient input loads (N and P) in the surface waters are calculated using the SENTWA model. The SENTWA model (system for the evaluation of nutrient transport to water) is a semi-empiric, deductive emission model to quantify the nutrient loads to surface waters, including the spatial variation in the nutrient loads and concentrations. The contributing sources and their dependence on rainfall and catchment characteristics are implemented in a conceptual way. One of the biggest weaknesses in the current SENTWA model, however, is the poor implementation of existing knowledge on N and P transformation processes making the model quite inelastic to changes in the management. Therefore it is not the most optimal tool for policy making (Peeters *et al.*, 2009). Presently a new nutrient emission model based on Arcgis software, Arc-NEMO, is being developed in Flanders. This model will try to calculate the water, N and P balance of the soil in agricultural areas and the transport of N and P of these areas to surface waters.

The PLEASE model, a simple mechanistic model, calculates a P concentration based on the P status of the soil and a lateral water flux profile, which is based on the net precipitation surplus, information of the mean highest and mean lowest groundwater depth (Schoumans et al., 2013). Next to the important advantage of needing only a rather limited data input, the model was proven already to perform well on (acidic) sandy soils in The Netherlands. Therefore in this study the PLEASE model was used on a regional scale in Flanders. With the modelled actual P loss a map of P saturation classification was made for the acidic sandy region and compared with the current map of P saturation classification according to the PSD (Chapter 3). By using the PLEASE model, the relative percentage of fields categorized as P critical decreased with 13%, but the relative percentage of P saturated fields increased with 9%. There were 4% more fields classified as being not P saturated or P critical. The hydrological conditions have an important effect on P leaching, e.g. fields having a low or medium PSD but being located in areas with a high groundwater table are sometimes more susceptible to P leaching than fields with a high PSD and a low groundwater table. These differences indicate that the way of determining P saturation is important in terms of policy making. Therefore the most optimal legislation should be based on a combination of P content in the soil (PSD) and on the hydrological conditions (PLEASE model). To obtain the most optimal results with the PLEASE model it is advisable to adapt it to the specific local conditions and to include P_w determination in the standard analysis of a field together with the PSD determination because it is a crucial model input. To have the best results possible it is necessary to invest in easy accessible data that can be used as input parameters of the PLEASE model.

From the above results we conclude that the legislation in place between 1999-2009 has not been efficient in stabilizing or decreasing the PSD levels. Using only the limit of P saturation, namely PSD > 40% (1999-2011) and PSD > 35% (2011-current), as a decision parameter for fertilization rules is not adequate enough. A further restriction of the P fertilization amounts should also include other parameters, such as the hydrological conditions.

Can excessive P leaching in P saturated soils be reduced efficiently with amendments with P fixation capacity?

Since the European Union counts on a significant amelioration of the P concentration in the surface water it is important to take measures that produce a quick and adequate response. The quickest effect on the water quality can be expected when a decrease or a standstill in P input to the surface water is obtained. This could be realised through increasing the P fixation capacity of soils, i.e. creating a barrier which prevents P leaching to deeper soil layers. In this study we screened several mineral salts, primary and secondary minerals, Fe-sludge and specially designed products for their potential to fix P in the soil. The Fe salts showed to be most efficient, next to chemically pre-treated minerals together with dried Fe-sludge and Phoslock® R3 in terms of P fixation (chapter 4).

The addition of Fe and Al salts is commonly used in water treatment, but also the other products investigated in this study have been used mainly for P fixation in water (Buda et al., 2012; Meis et al., 2012; Uusitalo et al., 2012a; Wium-Andersen et al., 2012). Yet until now little research was done on their P fixation capacity in soils (O'Connor et al., 2005; Agyin-Birikorang et al., 2007). Before implementation on a large scale some questions need to be answered. First it is important to assess the large scale availability of these products. Olivine is accessible for mining at many locations on various continents (ten Berge et al., 2012). Within Europe, huge reserves are accessible in Norway, Sweden, Spain, Italy, Austria, Greece, Cyprus and Turkey (Schuiling & Krijgsman, 2006). Huge beds of zeolite-rich sediments, formed by the alteration of volcanic ash in lake and marine waters are located in the western United States, Turkey, Eastern Europe,.... These beds were found to contain as much as 95% of a single zeolite and can easily be mined by surface methods (Mumpton, 1999). Next to the wide availability and easy exploitation of olivine and zeolite these minerals are already commercially available as liming product (olivine) or as catalyst and fodder additive (zeolite). However, when using these minerals for P fixation in the soil an additional cost of chemical pre-treatment may have to be considered (in this study they were pre-treated with 1M HCl for 1h). Before putting this into practice on a large scale additional research is necessary to optimize this pre-treatment, thereby investigating the possibility to reuse the HCl and/or use other, less corrosive acids.

The specially designed products are also already commercially available but their price may be prohibitive. Sachtofer costs 100 euro ton^{-1}, amounting to 2200 euro per ha and 6200 euro per ha for addition rates R2 (0.5%) and R3 (1.5%), respectively. Phoslock® is extremely expensive with a price between 2500 and 3500 euro ton^{-1} and thus excluded from use for this reason. The Fe sludge used in this study is a by-product of a waste water treatment plant where the Fe sludge is currently transported to a biogas plant. If it would become marketable in the future it could be an economically interesting product, representing a win-win situation for the waste water industry and the agricultural sector. However, Fe sludge contains a substantial amount of P (Chapter 4). The results showed that Fe sludge is able to fix the soluble P in the soil without any doubt which means that the added P is in a non available form. But this would be at odds with the perspective of bringing the soil to an environmentally safe P level. Therefore it can be expected that the application of Fe sludge will be problematic within the current nutrient legislation. On the other hand, it has to be considered that in this fixation experiment the Fe sludge of only one waste water treatment plant was investigated, and that possibly Fe sludge produced in other waste water plants may have different P concentrations. Indeed, in other studies where Fe sludge was used concentrations of 2.6 g P kg^{-1} (Chardon et al., 2012) and 3.12 g P kg^{-1} (O'Connor et al., 2005) were found, which is higher than the 1.7 mg P kg^{-1} in our study.

Fe sludge or Fe coated sand can still have an important role in tackling the eutrophication caused by P in directly removing P in drainage water. The removal of particulate P in drainage water can occur in constructed wetlands, yet an effective constructed wetland requires a size that can be problematic in regions with intensive agriculture and high land prices (Chardon et al., 2012). Hence, new measures are needed to provide immediate water quality protection while occupying little agricultural land (Groenenberg et al., 2013). A way to do this is to envelope the pipe drains with Fe coated sand. Results in The Netherlands showed that enveloping a pipe drain with Fe coated sand resulted in an average dissolved reactive P removal of 94%, resulting in effluent concentrations that were below 0.15 mg total P L^{-1} (Groenenberg et al., 2013). This technique could also be interesting to investigate as a useful mitigation option in Flanders.

The use of olivine also provides an opportunity for C sequestration. Olivine forms a complete solid solution series between the Mg end member, forsterite and the Fe end member, fayalite. Olivine will react with carbon dioxide (CO_2) and form other phases, including carbonate minerals (Olsson et al., 2012), resulting in C sequestration on a geological time scale. The pathway for mineral sequestration follows two main steps: first, cations must be liberated from the crystal structure of an appropriate silicate mineral and then, they must combine with CO_2 (Olsson et al., 2012). Other research pointed out that enhancing the weathering by grinding the minerals increases the process of C sequestration (ten Berge et al., 2012). These findings together with the findings of this study make olivine a particularly attractive product to treat acidic sandy soils with high P content. Not only would it tackle the P saturation problem of the fields but it would lead to durable C sequestration thereby making it possible for agriculture to realize an important reduction with respect to greenhouse gasses emissions.

The results of this study are promising but further research is necessary. When treating the soil with the above mentioned products it is important to consider that they may contain pollutants. For example, when Phoslock® would be added to the soil and it is already added to lakes in order to reduce P loads, high amounts of La are also added, however the effects of La on the environment, both on short and on long term, have not yet been investigated. Research has also to be performed on the effect of substantial Ni additions to the soil, since a relatively fast release of bio available Ni from olivine into the food chain and the wider environment could set limits to permissible olivine doses. Ten berghe et al. (2012) found an increase of Ni concentration in grass (from 0.53 to 0.69 mg Ni kg^{-1}), already at a dose of 1630 kg olivine ha^{-1}, which implies that, when olivine is used in agricultural systems, the Ni accumulation in the soil and plants must be well monitored and remain within certain limits (ten Berge et al., 2012). Therefore long term field studies are required to fully grasp the possible impact olivine has on the environment.

As a conclusion it can be stated that indeed it is possible to efficiently reduce the P leaching in P saturated soils by adding amendments with P fixation capacity. This is a good short term mitigation option since the P leaching to deeper soil layers is reduced drastically. However, on the long term excessive amounts of P remain in the soil and there is actually no indication on how long the P fixation capacity of the products will last. Therefore this should be combined with definite mitigation options that efficiently extract the excessive P amounts from soil. An option is through biological P mining.

Are PSB able to solubilize P under high P conditions?

A way to remove the excessive amount of P from the soil is by phytoextraction or P mining. P mining of the soil by plants, which includes harvesting P taken up from the soil by a crop grown without external P application, has been seen as a possible management strategy for P enriched soils (Koopmans et al., 2004b). It is known that the plant P uptake is strongly influenced by rhizosphere solution conditions and that root activity can modify the chemistry of the rhizosphere thereby altering P availability and uptake (Wang et al., 2004). Upon P removal from the soil solution by plant uptake, a fast initial desorption reaction for P adsorbed to surface sites of Al- and Fe-(hydr)oxides is expected (Van der Zee et al., 1987). Desorption of P bound inside Al- and Fe-(hydr)oxides followed by diffusion of P to the outer layers of the aggregates, can replenish the decrease of P adsorbed on surface sites (Barrow, 1983). Because intra-aggregate diffusion is slow, this P becomes available again only in the long term (Koopmans et al., 2004a). Next to the slow replenishment another obstacle for P mining by plants has to be considered. Namely, the maximal desorption rate will not be reached for soil aggregates that are not in direct contact with the roots, due to the transport limitations in the soil matrix. This may cause the build up of a P concentration gradient toward the root, limiting further P desorption (Jungk & Claassen, 1997; Koopmans et al., 2004a). Therefore the achieved P uptake rates are low for common row crops and forage grasses (Whitehead, 2000). Van der Salm et al. (2009) found a decreasing trend in the P exported over time with a P mining experiment from a grassland on a sandy soil with a high P content (Table 6.1). This indicates that the mining efficiency drastically deceases over time due to a decline in the dry matter production of the grass. They also found that the P off-take of the plots with a surplus of 0 kg P ha^{-1} yr^{-1} was almost similar to the P off-take on the mining plots. The average P surplus of the surplus plots was generally higher than expected during their experiment, which indicated that no P mining was done on the fields that still received P (Table 6.2). Furthermore Van der Salm et al. (2009) also found only a reduction of soil P in the top soil layer (0-0.05 m) which indicates that the effects of P mining on P leaching to groundwater will be small.

Table 6.1. Amount of P exported in kg P ha^{-1} yr^{-1} (also given in P_2O_5) with grass from a sandy soil based on the results of a mining experiment performed by van der Salm et al. (2009)

	Dry matter content (Mg ha^{-1} yr$^{-1)}$	P content (g kg^{-1})	P Exported (kg ha^{-1} yr^{-1})	P_2O_5 Exported (kg ha^{-1} yr^{-1})
2002	14.7	2.80	41.2	94.3
2003	10.6	2.90	30.7	70.4
2004	11.5	3.10	35.7	81.7
2005	14.6	3.00	43.8	100
2006	7.80	3.00	23.4	53.6

Table 6.2. Average P off take by grass and P surplus (2002-2006) at the mining plots and the P surplus plots for a loamy sand soil and a sandy soil (van der Salm et al., 2009)

	P off take (kg P ha^{-1} yr^{-1})		P surplus (kg P ha^{-1} yr^{-1})	
	Loamy sand soil	**Sandy soil**	**Loamy sand soil**	**Sandy soil**
Mining	35	35	-32	-35
P0	31	33	5	4
P9	32	37	13	12

*P0 means a P surplus of 0 kg P ha^{-1} yr^{-1} and P9 means a P surplus of 9 kg P ha^{-1} yr^{-1}

Therefore it is felt that the current cropping systems will require a long period, several decades, to reduce the high P concentrations to an environmentally safe level, and further research is needed to increase P mining efficiency (Koopmans et al., 2004b; Sharma et al., 2007).

A possible option to increase P mining efficiency, leads to the third hypothesis of this study, namely “are phosphate solubilizing bacteria (PSB) able to solubilize P under high P conditions?”. As stated in Chapter 5 until now PSB are only used on tropical or calcareous Mediterranean soils with a low P content (Gyaneshwar et al., 2002; Sundara et al., 2002; Welch et al., 2002; Wan & Wong, 2004). Under such conditions it was found that the PSB were able to solubilize P making it plant available.

No research has been done on the P solubilizing efficiency of PSB under acidic conditions with high P contents, which are mostly fixed on Al and Fe (the conditions found in the acidic sandy region of Flanders). The results of chapter 5 showed that the tested PSB were able to adapt to high insoluble P conditions and to solubilize P. Significantly higher available P concentrations than in the control were found with the addition of *B. brevis*, *P. putida* and *P. corrugata*. Especially *B. brevis* and *P. putida* exhibited the most promising capacities to solubilise P in high insoluble P conditions where the P is fixed with Al and Fe, which indicates their usability in Flanders.

The PSB have the potential to accelerate the P mining process. As discussed above a decrease in extracted P is found over time with P mining because of the slow replenishment of the adsorbed P by diffusion of P. The PSB have their own mechanisms to solubilize P such as the production of organic acid and the release of H^+ ions for solubilization of inorganic (mineral) P and the release of enzymes that mineralize organic P. These PSB are commonly found in the rhizosphere (Kim et al., 1998). Therefore, further research is necessary on these solubilization processes and the working mechanisms of enzymes such as phytase to completely understand their working and to have accurate values on the improvement of crop P uptake with the interaction of PSB in the soil and considering the climatologic conditions in Flanders.

Further research to evaluate the effect of PSB in bigger pot experiments and under more realistic field conditions has to be done. In collaboration with Hogent already one bigger pot experiment (using 1.5 kg soil) where PSB (a mixture of *B. brevis*, *P. putida* and *P. corrugata*) were inoculated on 3 soils with a range of total P content and cropped with grass (*Lolium perenne*) was set up. Preliminary results indicate an increase in the P availability to the growing crop, demonstrating the potential of PSB inoculation for increasing P depletion rates by grass in soils high in P. In the discussed experiments, only a limited number of PSB were tested, which were, according to literature, the most optimal PSB. Since the data gathered from the literature were based on results found on other soil (types) and in other climatic conditions it could be possible that other PSB perform equally good or even better under the circumstances of Flanders. Through isolation of bacteria from native soils and testing them on their P solubilizing capacity it could even be possible to work with bacteria that are better adapted to the local circumstances and realise an even higher optimization of the P and N uptake by plants.

The solubilization of P by PSB can be the key to the solution to keep allowing intensive agriculture on the P saturated soils. To reach the environmental standards set by the European Union, the P fertilization rules will become more strict, even zero P application in some areas is a possible outcome. In these areas PSB could provide the necessary P for achieving the desired crop yields and at the same time decrease the P content in the soil.

However, to optimize the biological P mining, it is important to know the accurate P amounts which have to be brought on the agricultural fields on one hand and the amount which is exported from the fields on the other hand (Figure 6.2). The difference between the inputs and outputs is the surplus in the soil P balance and can be seen as a measure for potential pollution of the environment (Lenders et al., 2011). The soil balance is only as precise as the data used in the P balance. Especially the data of the P export by plants or by crop rotations, the plant P uptake, is the most crucial element in the soil P balance. A small estimation error in the plant P uptake leads to a large error of the P balance. These data are at this moment not or only limited available in Flanders. Therefore the data of the USDA national nutrient database are used in the soil balances for Flanders (Lenders et al., 2011). However, compared to the US the soil conditions, e.g. historical P saturation and agricultural practices, are different in Flanders. Therefore, the negative P balance of 0.5 million kg P found for the agriculture in Flanders in 2009 can be questioned. These factors demonstrate the need for more accurate data on all inputs, above all crop P uptake and removal, to obtain a more reliable P balance.

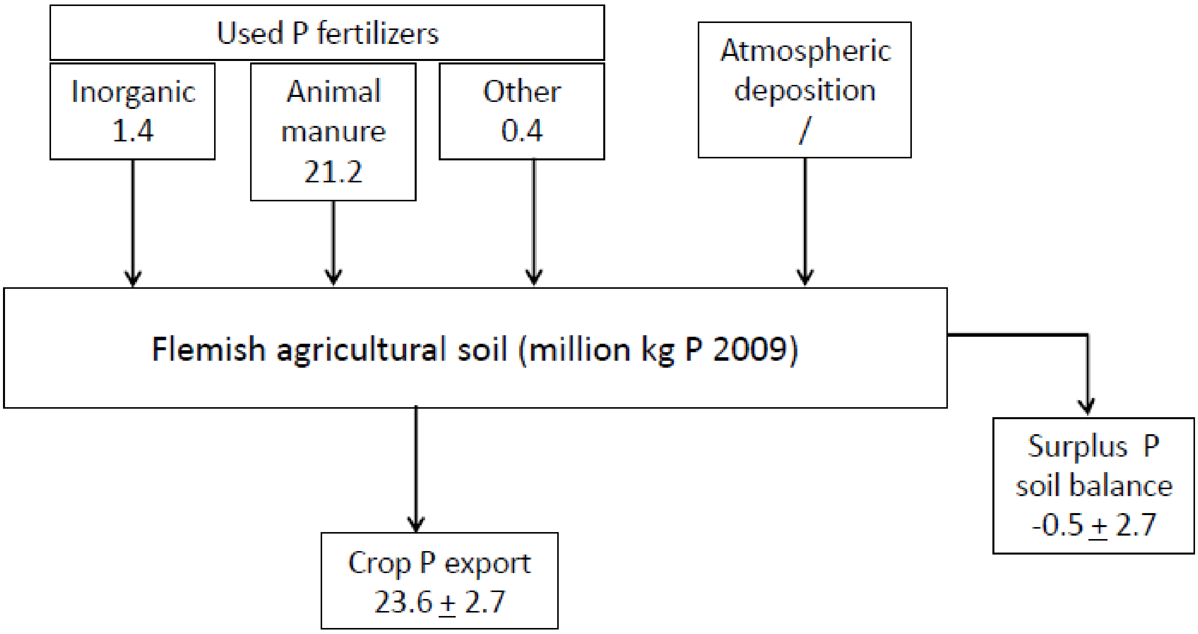

Figure 6.2. Schematic overview of the soil balances in Flemish agriculture in million kg P in 2009 (adapted from Lenders et al. (2011))

Since there are still a lot of problems in terms of P leaching (Figure 6.1, chapter 2 and 3) the allowed amount of P fertilizer has to decrease. Moreover, a recent study indicated that a general reduction of fertilization is feasible since a (much) higher amount of P is advised to the farmers in Flanders than in 22 other countries with a comparable high amount of plant available P in the soil (Jordan-Meille et al., 2012). Stricter maximum allowable P fertilization amounts have been implemented in the new Manure Action Plan 4 (MAP4) (Table 6.3). In not P saturated areas a gradual decrease in the maximum P amount which can be given is stipulated. This will result in a small P mining in 2017-2018, since the allowed amount of P fertilization is estimated to be lower than the P removal by crops. For P saturated soils (PSD > 35%) the maximum P_2O_5 application standard is 40 kg P_2O_5 ha^{-1}. Contrary to the N application standards (based on a parcel approach), the maximum P_2O_5 application standards are at farm level basis, giving the farmer more flexibility.

Table 6.3. Proposal for maximum allowable amounts of P_2O_5 application in the entire territory of Flanders, except for the 4.400 ha in areas with a phosphate saturation degree (FVG) of more than 35% with a 95% degree of certainty where a maximum application standard of 40 kg P_2O_5 ha^{-1} is proposed for all crops; and the respective estimated P_2O_5 mining of the soil (Belgische Staatsblad, 2011)

Year / Crop	Crop export (kg P_2O_5 ha^{-1})	Proposal maximum allowable amounts of P_2O_5 application (kg P_2O_5 ha^{-1})			
		2011-2012	2013-2014	2015-2016[1]	2017-2018[1]
Grassland mowing (+ sod)	97	95	95	95	90
Grassland (Mowing + grazing)	91	90	90	90	90
1 cut of grass* + maize	108	95	95	95	90
Silage maize	83	80	80	75	70
Corn maize	79	80	80	75	70
Winter wheat (Triticale)	82	75	75	70	70
Winter barley + other cereal crops	74	75	70	70	70
Fodder beets	60	75	65	55	55
Sugar beets	59	75	65	55	55
Potatoes (except early potatoes)	58	75	65	55	55
Vegetables, crops with low N-demand, leguminous crops and other crops	no data	75	65	55	55
P_2O_5 mining of the soil (kg P_2O_5)		**519.557**	**1.223.347**	**2.991.307** **No P mining**	**4.357.972** **P mining**

* Rye, used as fodder crop (field removal), can be an alternative for 1 cut of grass

° This is the crop export of 2009. The crop export will be recalculated in 2014 on the average yield anno 2014.

[1] Not yet decided

To conclude, the results of this research indicate that there are still (large) problems in terms of P saturation in the acidic sandy region in Flanders. Lowering the P fertilization alone will not solve the problem on a short or even medium term, since the P saturation is a historical problem. This research however has given two alternatives that can help reaching the European standards allowing at the same time to continue intensive agriculture on these soils. First by fixing P in the soil to stop P leaching to deeper, less plant accessible, soil layers, then by making the previously fixed P plant available by using PSB, thereby mining the soil and providing in the crops' P needs. This type of management practices would help to create a more closed P cycle in agriculture, dealing with the historical problem and finally improving the image of the agricultural sector by tackling various environmental issues.

Ultimately it has to be noticed that this study is only performed for the acidic sandy region because P leaching problems are primarily found on these soils. Other not acidic or not sandy soils were not investigated since it was assumed that there are no problems of possible P leaching to ground- and surface water. However, until now no results exist on P leaching research in heavy textured or calcareous soils. Since intensive agriculture is also done in these areas it is advisable to investigate the possibility of P leaching in these soils and, if needed, the possible measures that can be taken to solve these issues. Only then, the eutrophication problem of P leaching can fully be dealt with.

Summary

The last decades the European Union is trying to tackle the problem of eutrophication. Eutrophication can be defined as the enrichment of water by nutrients causing an accelerated growth of algae and other aquatic plants, thereby decreasing the ecological quality and biodiversity of the aquatic system. Enrichment of nutrients can be interpreted as an increase in the nitrogen (N) and phosphorus (P) content of the water bodies. A lot of research has been done about the N mechanisms and N leaching from soil to ground- and surface water, but up to date little is known about the P mechanisms and P leaching from soils. This partly because, until some years ago, P was thought to be fixed in the soil. However, through repeatedly adding excessive amounts of P fertilizers the soil became P saturated entailing the risk of P leaching to deeper soil layers.

This problem is especially an issue for acidic sandy soils and since a lot of intensive agriculture is done on these soils in Flanders, the P problem is prominent here. A large scale survey was conducted from 1995 to 1997 to determine their P saturation according to the protocol of Van der Zee. These results were used as the base for all legislation in terms of P fertilization. This legislation became more severe in 2009 namely a soil was legally classified as P saturated when it had a PSD > 35% (> 40% in 1999), and as P critical if the PSD > 25% (> 30% in 1999). The first part of this study investigated whether the legislation in place is effective in stabilizing or decreasing the PSD in Flanders. Therefore, in 2009-2010, a small sampling campaign was done on 21 selected fields to investigate the evolution in their PSD. The results showed a general increase of the PSD instead of an equal or a lower PSD when compared to the ones of almost a decade ago. Moreover a downward P movement was found, indicating that P, similar to N, is also leaching to deeper soil layers, but over a longer time period. Both findings led to the conclusion that current legislation or methodology of determination of P saturated areas is insufficient to deal with the P leaching problem.

Since P is leaching at a rapid paste and therefore reaching deeper soil layers much faster than expected, the hydrological conditions, such as the height of the groundwater table are an important factor. However, these are not sufficiently incorporated in the P saturation determination by the PSD concept. Moreover it is also preferable that the determination can be done with a minimal number of data inputs. Therefore the PLEASE model, a simple P leaching assessment tool, that proved already to be working efficiently on (acidic) sandy soils in The Netherlands was used. After some modifications to optimize the model for the Flemish circumstances, a sensitivity analysis was done. It revealed that P_w and the net precipitation (NP) were the input parameters that caused the most uncertainty in the model. Therefore they should be acquired in the most optimum manner. Finally, when comparing the classification of P saturated fields based on the results of the PLEASE model with the current classification based on the PSD some important changes on local scale were found. The risk of P leaching caused by hydrological conditions, which are for the moment not enough considered in Flanders, can be substantial and should be taken up in the process of making new restrictions and rules.

The analysis of the P leaching problem is important but also the search for mitigation options to deal with it is crucial. This is done in the second part of this study. Primarily, it can be considered to add P fixating products to the acidic sandy soils to increase the P binding capacity of the soil, thereby decreasing or stopping the P leaching to ground- and surface water. Results indicated that Fe salts, chemically pre-treated minerals, dried Fe sludge from the waste water treatment and Phoslock® were able to fix the P most efficiently in several soils with a range of P concentrations. The P fixation experiment showed that the PSD of a soil is not always the best indicator for the P fixation efficiency of the products. These results are promising in tackling the P problem in a short time, since these products allow to store the P in the upper soil layers through fixing it there.

This is already a step forward in terms of tackling the eutrophication caused by P but is no long term solution, since an excessive amount of P is still located in the soil. This fact together with the still increasing prices for P fertilizers has led to the third part of this research and the second mitigation option, namely making P more plant available by adding phosphate solubilizing bacteria (PSB) to the soil. These PSB are already successfully used in tropical, calcareous soil with a low amount of available P but with the presence of phosphate rock. In this research 5 PSB of the *Bacillus* and *Pseudomonas* species were tested on several growth media with high insoluble P contents fixed to Al and Fe and these PSB all proved to be able to adapt to these circumstances and to solubilize P. Afterwards they were tested in a sand medium and on acidic sandy soils with a high P content. The results indicated that out of the tested PSB the *Pseudomonas putida* and *Bacillus brevis* performed best in high P concentrations, indicating their potential to be used in the acidic sandy soils of Flanders.

When both mitigation options are used together, namely first fixing the P in the top soil layer and afterwards using this fixed P as fertilizer for the crops by making it plant available it would create a long term solution for the P saturated soils. These soils would stop leaching P on a short term and would be mined on the long term, also allowing to avoid using additional P fertilizers and therefore making it a profitable alternative for agriculture and beneficial for the environment.

Samenvatting

De Europese Gemeenschap probeert gedurende de laatste tientallen jaren het eutrofiëringprobleem aan te pakken. Eutrofiëring kan gedefinieerd worden als de aanrijking van water door nutriënten die bijgevolg een versterkte groei van algen en andere waterplanten veroorzaken waardoor de ecologische kwaliteit en de biodiversiteit van het water vermindert. Aanrijking door nutriënten kan gezien worden als een toename van de hoeveelheid stikstof (N) en fosfor (P) in het water. Er werd reeds veel onderzoek verricht naar de N mechanismen in en de N uitspoeling uit de bodem naar grond- en oppervlakte water, maar tot op vandaag is er weinig geweten over de P mechanismen in en de P doorslag van de bodem. Dit wordt gedeeltelijk veroorzaakt door het feit dat, tot een paar jaar terug, er van uitgegaan werd dat P door de bodem werd vastgehouden. Maar door het herhaaldelijk toedienen van hoge hoeveelheden P meststoffen is de bodem P verzadigd wat een potentieel risico op P uitspoeling naar diepere bodemlagen betekent.

Dit probleem uit zich vooral op zuur zandige bodems en gezien in Vlaanderen deze gronden veelvuldig gebruikt worden voor landbouw, is het probleem hier prominent aanwezig. In de periode 1995-1997 werd een grootschalig onderzoek, gebaseerd op het protocol van Van der Zee, uitgevoerd om de P verzadiging van de zuur zandige regio in Vlaanderen in kaart te brengen. Deze resultaten werden gebruikt als basis voor het opstellen van de wetgeving inzake P bemesting. Deze regelgeving werd strikter in 2009 waarbij een bodem wettelijk als P verzadigd werd beschouwd indien de gemeten fosfaat verzadigingsgraad (FVG) > 35% (> 40% in 1999), en als P kritisch indien de FVG > 25% (> 30% in 1999). Het eerste gedeelte van deze studie onderzocht of de huidige wetgeving effectief is inzake stabilisering of vermindering van de FVG in Vlaanderen. Om de evolutie van de FVG te onderzoeken, werden in 2009-2010 op beperkte schaal, op 21 vooraf geselecteerde velden, opnieuw stalen genomen. Na vergelijking van deze resultaten met deze van een tiental jaar geleden werd over het algemeen een toename van de FVG vastgesteld in plaats van de verwachte, gelijke of lagere waarde. Bovendien werd een neerwaartse P beweging gevonden, hetgeen erop wijst dat P, net zoals N, naar dieper gelegen bodemlagen uitspoelt, maar over een langere periode. Beide vaststellingen leiden tot de conclusie dat de actuele wetgeving of de methode om de P verzadigde gebieden te bepalen niet voldoen om het P uitspoelingprobleem op te lossen.

Vermits P uitspoelt in een snel tempo en daardoor de diepere grondlagen sneller dan verwacht bereikt worden, zijn de hydrologische condities, zoals de diepte van de grondwatertafel, een belangrijke factor. Nochtans worden deze gegevens bij het bepalen van de P verzadiging met de FVG niet voldoende opgenomen. Bovendien is het belangrijk dat de bepaling kan gebeuren met een beperkte invoer van data. Daarvoor werd gebruik gemaakt van het PLEASE model, een simpel instrument om P uitspoeling vast te stellen. Dit model werd in Nederland reeds getest op zuur zandige bodems en heeft daar zijn doeltreffendheid bewezen. Het model werd aangepast en geoptimaliseerd naar de Vlaamse omstandigheden, waarna werd een gevoeligheidsanalyse werd uitgevoerd. Deze analyse onthulde dat de invoer van de parameters wateroplosbare P (P_w) en het netto neerslagoverschot (NP) de meeste onzekerheid in het model veroorzaakte. Daarom is het belangrijk dat deze parameters op een optimale manier bepaald worden. Tenslotte, wanneer de resultaten van de classificatie van P verzadigde velden, gebaseerd op het PLEASE model, en de huidige classificatie, gebaseerd op de FVG, met elkaar vergeleken werden, werden op locale schaal enkele belangrijke verschillen vastgesteld. Het risico op P uitspoeling veroorzaakt door de hydrologische omstandigheden kan substantieel zijn. Hiermee wordt in Vlaanderen op dit ogenblik te weinig rekening gehouden, terwijl dit zou moeten meegenomen worden bij de opmaak van nieuwe beperkingen of regels.

De analyse van het P uitspoelingprobleem is belangrijk, maar evenzeer dient gezocht te worden naar mogelijkheden die het kunnen oplossen, namelijk mitigatie opties. Daarover gaat het tweede deel van deze studie. Ten eerste kan overwogen worden om P vastleggingproducten aan zuur zandige bodems toe te voegen om zo de P fixatie capaciteit te verhogen, waarbij de P uitspoeling naar het grond- en oppervlakte water verminderd of gestopt wordt. Resultaten hebben aangegeven dat Fe zouten, chemisch voorbehandelde mineralen, gedroogd Fe slib afkomstig van waterzuiveringstations en Phoslock® het meest efficiënt waren om P te fixeren (vast te houden) in meerdere bodems met een verscheidenheid aan P concentraties. Het P fixatie experiment toonde aan dat de FVG van een bodem niet altijd de beste indicator is voor het bepalen van de P vastleggingefficiëntie van de producten. Deze resultaten zijn veelbelovend voor het aanpakken van het P probleem op korte termijn, vermits deze producten toelaten de P in de bovenste bodemlagen op te slaan door ze daar te binden.

Dit is al een stap voorwaarts om de eutrofiëring veroorzaakt door P aan te pakken maar dit is geen lange termijn oplossing vermits er teveel P aanwezig blijft in de bodem. Dit gegeven, samen met de steeds duurder wordende P meststoffen, was de aanleiding voor het derde gedeelte van deze studie en de tweede mitigatie optie, namelijk P beschikbaar maken in de bodem door toevoeging van fosfaat oplossende bacteriën (PSB). Deze PSB werden reeds met succes gebruikt in tropische, kalkhoudende bodems met een lage hoeveelheid beschikbare P, maar met de aanwezigheid van fosfaatgesteenten. In dit onderzoek werden 5 PSB van de *Bacillus* and *Pseudomonas* soort getest op verschillende voedingsbodems met een hoge onbeschikbare P hoeveelheid die gebonden is op Al en Fe. Alle PSB hebben bewezen zich aan deze omstandigheden te kunnen aanpassen en waren in staat P op te lossen. Daarna werden ze getest in puur zand en in een zuur zandige bodem met een hoog P gehalte. De resultaten gaven dat aan van de geteste PSB, *Pseudomonas putida* en *Bacillus brevis* het best functioneerden bij hoge P concentraties. Hierbij werd hun potentieel om gebruikt te worden in zuur zandige bodems in Vlaanderen aangetoond.

Wanneer beide mitigatie opties samen toegepast worden, namelijk eerst P vasthouden (binden) in de bovenste bodemlaag en daarna deze opgeslagen P opneembaar maken voor de planten en zo gebruiken als meststof voor de gewassen, zou dit een oplossing op lange termijn kunnen betekenen voor de P verzadigde bodems. Op korte termijn zou de P uitspoeling tegengehouden worden en zou voor een P uitmijning van de bodem op lange termijn gezorgd worden. Bovendien zou het gebruik van bijkomende P meststoffen vermeden worden, waardoor het enerzijds een winstgevend (voordelig) alternatief zou zijn voor de landbouw en anderzijds heilzaam (nuttig) zou zijn voor het milieu.

References

Agyin-Birikorang, S., O'Connor, G. A., Jacobs, L. W., Makris, K. C. & Brinton, S. R. 2007. Long-term phosphorus immobilization by a drinking water treatment residual. *Journal of Environmental Quality,* **36,** 316-323.

Ajmone-Marsan, F., Cote, D. & Simard, R. R. 2006. Phosphorus transformations under reduction in long-term manured soils. *Plant and Soil,* **282,** 239-250.

Anderson, D. L., Tuovinen, O. H., Faber, A. & Ostrokowski, I. 1995. Use of soil amendments to reduce soluble phosphorus in dairy soils. *Ecological Engineering,* **5,** 229-246.

Ann, Y., Reddy, K. R. & Delfino, J. J. 2000. Influence of chemical amendments on phosphorus immobilization in soils from a constructed wetland. *Ecological Engineering,* **14,** 157-167.

Anonymous 1991a. Council Directive 91/271/EEC of 21 May 1991 concerning the collection, treatment and discharge of urban waste and the treatment and discharge of waste water from certain industrial sectors. *European Official journal of legislation,* **L 135,** 40-52.

Anonymous 1991b. Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. *European Official journal of legislation,* **L 375,** 0001-0008.

Anonymous 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *European Official journal of legislation,* **L 327,** 1-73.

Anonymous 2013. http://www.tankonyvtar.hu/hu/tartalom/tamop425/0032_vizgazdalkodas/ch01s02.html. In. (ed s.n.).

Arcand, M. M. & Schneider, K. D. 2006. Plant- and microbial-based mechanisms to improve the agronomic effectiveness of phosphate rock: a review. *Anais Da Academia Brasileira De Ciencias,* **78,** 791-807.

Barrow, N. J. 1983. A mechanistic model for describing the sorption and desorption of phosphate by soil. *Journal of Soil Science,* **34,** 733-750.

Barrow, N. J. 2008. The description of sorption curves. *European Journal of Soil Science,* **59,** 900-910.

Beauchemin, S. & Simard, R. R. 1999. Soil phosphorus saturation degree: Review of some indices and their suitability for P management in Quebec, Canada. *Canadian Journal of Soil Science,* **79,** 615-625.

Bechmann, M., Krogstad, T. & Sharpley, A. 2005. A phosphorus index for Norway. *Acta Agriculturea Scandinavica Section B-Soil and Plant,* **55,** 205-213.

Belgisch Staatsblad, B. 2011. Decreet van 22 december 2006 houdende de bescherming van water tegen de verontreiniging door nitraten uit agrarische bronnen. In., Belgium, pp. 103.

Bolster, C. H., Vadas, P. A., Sharpley, A. N. & Lory, J. A. 2012. Using a Phosphorus Loss Model to Evaluate and Improve Phosphorus Indices. *Journal of Environmental Quality,* **41,** 1758-1766.

Brauer, D., Aiken, G. E., Pote, D. H., Livingston, S. J., Norton, L. D., Way, T. R. & Edwards, J. H. 2005. Amendment effects on soil test phosphorus. *Journal of Environmental Quality,* **34,** 1682-1686.

Breeuwsma, A. & Silva, S. 1992. Phosphorus fertilisation and environmental effects in the Netherlands and the Po region (Italy). In., DLO The Winand Staring Centre, Wageningen, The Netherlands.

Buda, A. R., Koopmans, G. F., Bryant, R. B. & Chardon, W. J. 2012. Emerging Technologies for Removing Nonpoint Phosphorus from Surface Water and Groundwater: Introduction. *Journal of Environmental Quality,* **41,** 621-627.

Burgess, J., Drasdo, D. N. & Moon, E. 1994. Effects of citrate, mandelate and pH on the release of aluminum aluminosilicates. *Toxical and Environmental Chemistry,* **41,** 101-107.

Callahan, M. P., Kleinman, P. J. A., Sharpley, A. N. & Stout, W. L. 2002. Assessing the efficacy of alternative phosphorus sorbing soil amendments. *Soil Science,* **167,** 539-547.

Chardon, W. J. 1994. relationschip between phosphorus availability and phosphorus saturation index. In., AB-DLO, Haren, The Netherlands, pp. 19.

Chardon, W. J., Groenenberg, J. E., Temminghoff, E. J. M. & Koopmans, G. F. 2012. Use of Reactive Materials to Bind Phosphorus. *Journal of Environmental Quality,* **41,** 636-646.

Clasen, J. 1979. Aim of Phosphorus Removal at the Main Tributary of Wahnbach Reservoir Regarding Oligotrophication. *Zeitschrift Fur Wasser Und Abwasser Forschung-Journal for Water and Wastewater Research,* **12,** 110-122.

Collavino, M. M., Sansberro, P. A., Mroginski, L. A. & Aguilar, O. M. 2010. Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. *Biology and Fertility of Soils,* **46,** 727-738.

Condron, L. M., Turner, B. L. & Cade-Menun, B. J. 2005. Chemistry and Dynamics of Soil Organic Phosphorus. *Phosphorus: Agriculture and the Environment***,** 87-121.

Cox, J. W., Varcoe, J., Chittleborough, D. J. & van Leeuwen, J. 2005. Using gypsum to reduce phosphorus in runoff from subcatchments in South Australia. *Journal of Environmental Quality,* **34,** 2118-2128.

Csatho, P., Sisak, I., Radimszky, L., Lushaj, S., Spiegel, H., Nikolova, M. T., Nikolov, N., Cermak, P., Klir, J., Astover, A., Karklins, A., Lazauskas, S., Kopinski, J., Hera, C., Dumitru, E., Manojlovic, M., Bogdanovic, D., Torma, S., Leskosek, M. & Khristenko, A. 2007. Agriculture as a source of phosphorus causing eutrophication in Central and Eastern Europe. *Soil Use and Management,* **23,** 36-56.

Davis, W. B., McCauley, M. J. & Byers, B. R. 1971. Iron requirements and aluminum sensitivity of an hydroxamic acid-requiring strain of *Bacillus-megaterium*. *Journal of Bacteriology,* **105,** 589-594.

De Bolle, S., De Neve, S. & Hofman, G. 2013a. Rapid redistribution of P to deeper soil layers in P saturated acid sandy soils. *Soil Use and Management,* **29,** 76-82.

De Bolle, S., Gebremikael, M. T., Maervoet, V. & De Neve, S. 2013b. Performance of phosphate-solubilizing bacteria in soil under high phosphorus conditions. *Biology and Fertility of Soils,* **49,** 705-714.

de Freitas, J. R., Banerjee, M. R. & Germida, J. J. 1997. Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). *Biology and Fertility of Soils,* **24,** 358-364.

De Pauw, D. J. W. & Vanrolleghem, P. A. 2006. Practical aspects of sensitivity function approximation for dynamic models. *Mathematical and Computer Modelling of Dynamical Systems,* **12,** 395-414.

De Schrijver, A., Vesterdal, L., Hansen, K., De Frenne, P., Augusto, L., Achat, D. L., Staelens, J., Baeten, L., De Keersmaeker, L., De Neve, S. & Verheyen, K. 2012. Four decades of post-agricultural forest development have caused major redistributions of soil phosphorus fractions. *Oecologia,* **169,** 221-234.

De Smet, J., Hofman, G., Vanderdeelen, J., Van Meirvenne, M. & Baert, L. 1996. Phosphate enrichment in the sandy loam soils of West-Flanders, Belgium. *Fertilizer Research,* **43,** 209-215.

De Smet, J., Vanderdeelen, J. & Hofman, G. 1998. Effect of soil properties on the kinetics of phosphate release. *Communications in Soil Science and Plant Analysis,* **29,** 2135-2147.

De Vries, J. J. 1975. Some calculations methods for the determination of travel times of groundwater. *AQUA-VU, series A***,** 3-15.

Del Campillo, M. C., Van der Zee, S. E. A. T. M. & Torrent, J. 1999. Modelling long-term phosphorus leaching and changes in phosphorus fertility in excessively fertilized acid sandy soils. *European Journal of Soil Science,* **50,** 391-399.

Delvasto, P., Valverde, A., Ballester, A., Munoz, J. A., Gonzalez, F., Blazquez, M. L., Igual, J. M. & Garcia-Balboa, C. 2008. Diversity and activity of phosphate bioleaching bacteria from a high-phosphorus iron ore. *Hydrometallurgy,* **92,** 124-129.

Deubel, A., Gransee, A. & Merbach, W. 2000. Transformation of organic rhizodepositions by rhizosphere bacteria and its influence on the availability of tertiary calcium phosphate. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde,* **163,** 387-392.

Djodjic, F., Borling, K. & Bergstrom, L. 2004. Phosphorus leaching in relation to soil type and soil phosphorus content. *Journal of Environmental Quality,* **33,** 678-684.

Dupas, R. & Van der Salm, C. 2010. Validation of the model PLEASE at site scale. In., Alterra Wageningen UR, Wageningen.

Egnér, H., Riehm, H. & Domingo, W. R. 1960. Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor - and Kaliumbestimmung. *Kungl. Lantbrukshoegsk.,* **26,** 199-215.

Ekholm, P., Valkama, P., Jaakkola, E., Kiirikki, M., Lahti, K. & Pietola, L. 2012. Gypsum amendment of soils reduces phosphorus losses in an agricultural catchment. *Agricultural and Food Science,* **21,** 279-291.

Elliott, H. A., Brandt, R. C. & O'Connor, G. A. 2005. Runoff phosphorus losses from surface-applied biosolids. *Journal of Environmental Quality,* **34,** 1632-1639.

Elliott, H. A., O'Connor, G. A. & Brinton, S. 2002. Phosphorus leaching from biosolids-amended sandy soils. *Journal of Environmental Quality,* **31,** 681-689.

Ernst, L. F. 1962. Grondwaterstromingen in de verzadigde zone en hun berekeningen bij aanwezigheid van horizontale evenwijdige open leidingen. In: *Pudoc.* Utrecht University, Wageningen, pp. 189.

Ernst, L. F. 1978. Drainage of undulating sandy soils with high groundwater tables. 1. Drainage formula based on a constat hydraulic head ratio. *Journal of Hydrology,* **39,** 1-30.

Fernández, L. A., Zalba, P., Gómez, M. A. & Sagardoy, A. 2007. Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. *Biology and Fertility of Soils,* **43,** 805-809.

Foy, R. H. 2007. Variation in the reactive phosphorus concentrations in rivers of northwest Europe with respect to their potential to cause eutrophication. *Soil Use and Management,* **23,** 195-204.

Freese, D., Van der Zee, S. E. A. T. M. & Van Riemsdijk, W. H. 1992. Comparison of different models for phosphate sorption as a function of the iron and aluminum-oxides of soils. *Journal of soil science,* **43,** 729-738.

Freese, D., Van Riemsdijk, W. H. & Van der Zee, S. E. A. T. M. 1995. Modeling phosphate-sorption kinetics in acid soils. *European Journal of Soil Science,* **46,** 239-245.

Frey, H. C. & Patil, S. R. 2002. Identification and review of sensitivity analysis methods. *Risk Analysis,* **22,** 553-578.

Gee, G. W. & Bauder, J. W. 1986. Particle-size analysis. In: *Methods of soil analysis, Part I, Physical and mineralogical methods.* (ed A. Klute), American Society of Agronomy/Soil Science Society of America, Madison, USA, pp. 283-411.

Gibbs, M. M., Hickey, C. W. & Oezkundakci, D. 2011. Sustainability assessment and comparison of efficacy of four P-inactivation agents for managing internal phosphorus loads in lakes: sediment incubations. *Hydrobiologia,* **658,** 253-275.

Gielis, R. 2012. Voortgangsrapport mestbank 2012 over de mestproblematiek in Vlaanderen. In., VLM, Vlaamse Land Maatschappij, pp. 168.

Groenenberg, J. E., Chardon, W. J. & Koopmans, G. F. 2013. Reducing Phosphorus Loading of Surface Water Using Iron-Coated Sand. *Journal of Environmental Quality,* **42,** 250-259.

Gupta, R. K. & Abrol, I. P. 1990. Salt-affected soils : their reclemation and management for crop production. *Advances in Soil Science,* **11,** 223-288.

Gyaneshwar, P., Kumar, G. N., Parekh, L. J. & Poole, P. S. 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant and Soil,* **245,** 83-93.

Haghseresht, F., Wang, S. & Do, D. D. 2009. A novel lanthanum-modified bentonite, Phoslock, for phosphate removal from wastewaters. *Applied Clay Science,* **46,** 369-375.

Haygarth, P. M., Chapman, P. J., Jarvis, S. C. & Smith, R. V. 1998. Phosphorus budgets for two contrasting grassland farming systems in the UK. *Soil Use and Management,* **14,** 160-167.

Haygarth, P. M. & Sharpley, A. N. 2000. Terminology for phosphorus transfer. *Journal of Environmental Quality,* **29,** 10-15.

Heathwaite, A. L., Dils, R. M., Liu, S., Carvalho, L., Brazier, R. E., Pope, L., Hughes, M., Phillips, G. & May, L. 2005. A tiered risk-based approach for predicting diffuse and point source phosphorus losses in agricultural areas. *Science of the total environment,* **344,** 225-239.

Hedley, M. J., Stewart, J. W. B. & Chauhan, B. S. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and laboratory incubations. *Soil Science Society of America Journal,* **46,** 970-976.

Henri, F., Laurette, N. N., Annette, D., John, Q., Wolfgang, M., Francois-Xavier, E. & Dieudonne, N. 2008. Solubilization of inorganic phosphates and plant growth promotion by strains of *Pseudomonas fluorescens* isolated from acidic soils of Cameroon. *African journal of microbiology research,* **2,** 171-178.

Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil,* **237,** 173-195.

Hooda, P. S., Truesdale, V. W., Edwards, A. C., Withers, P. J. A., Aitken, M. N., Miller, A. & Rendell, A. R. 2001. Manuring and fertilization effects on phosphorus accumulation in soils and potential environmental implications. *Advances in Environmental Research,* **5,** 13-21.

Hsu, P. H. 1976. Comparison of iron(III) and aluminum in precipitation of phosphate from solution. *Water Research,* **10,** 903-907.

Hu, J., Lin, X., Wang, J., Chu, H., Yin, H., Yin, R. & zhang, J. 2009. Popoulation size and specific potential of P-mineralizing and -solubilizing bacteria under long-term P-deficiency fertilization in a sandy loam soil. *Pedobiologia,* **53,** 49-58.

Illmer, P., Barbato, A. & Schinner, F. 1995. Solubilization of hardly-soluble $AlPO_4$ with P-solublizing microorganisms *Soil Biology & Biochemistry,* **27,** 265-270.

Illmer, P. & Schinner, F. 1999. influence of nutrient solution on Al-tolerance of *Pseudomonas* sp. . *FEMS Microbiology letters,* **170,** 187-190.

Indiati, R. 2000. Addition of phosphorus to soils with low to medium phosphorus retention capacities. I. Effect on soil phosphorus sorption properties. *Communications in Soil Science and Plant Analysis,* **31,** 1179-1194.

Jardin, N. & Pöpel, H. J. 1994. Phosphate fixation in sludges from enhanced biological P-removal during stabilization. In: *Chemical water and wastewater treatment III.* eds R. Klute & H. H. Hahn), Springer, Berlin, Heidelberg.

Jenkins, D., Ferguson, J. F. & Menar, A. B. 1971. Chemical processes for phosphate removal. *Water Research,* **5,** 369-389.

Johnston, A. E. & Dawson, C. J. 2005. Phosphorus in agriculture and the relation to water quality. In., report to Agricultural Industries Confederation, pp. 72.

Jordan-Meille, L., Rubæk, G. H., Ehlert, P. A. I., Genot, V., Hofman, G., Goulding, K., Recknagel, J., Provolo, G. & Barraclough, P. 2012. An overview of fertilizer-P recommendations in Europe: soil testing, calibration and fertilizer recommendations. *Soil Use and Management,* **28,** 419-435.

Jungk, A. & Claassen, N. 1997. Ion diffusion in the soil-root system. In: *Advances in Agronomy, Vol 61.* (ed D. L. Sparks), pp. 53-110.

Ketterings, Q. M., Kahabka, J. E. & Reid, W. S. 2005. Trends in phosphorus fertility of New York agricultural land. *Journal of Soil and Water Conservation,* **60,** 10-20.

Kim, K. Y., Jordan, D. & McDonald, G. A. 1998. *Enterobacter agglomerans*, phosphate solubilizing bacteria, and microbial activity in soil: effect of carbon sources. *Soil Biology & Biochemistry,* **30,** 995-1003.

Kirk, G. J. D. 1999. A model of phosphate solubilization by organic anion excretion from plant roots. *European Journal of Soil Science,* **50,** 369-378.

Kirkham, D. 1958. Seepage of steady rainfall through soil into drains. *Transactions - American Geophysical Union,* **39,** 892-908.

Kleinman, P. J. A., Bryant, R. B. & Reid, W. S. 1999. Development of pedotransfer functions to quantify phosphorus saturation of agricultural soils. *Journal of Environmental Quality,* **28,** 2026-2030.

Kleinman, P. J. A. & Sharpley, A. N. 2003. Effect of broadcast manure on runoff phosphorus concentrations over successive rainfall events. *Journal of Environmental Quality,* **32,** 1072-1081.

KMI 2008. Statistieken (in Dutch and French) http://www.meteo.be/nederlands/pages/Klimatologisch/century/statistieken.html. In. (ed s.n.).

Koopmans, G. F., Chardon, W. J., de Willigen, P. & Van Riemsdijk, W. H. 2004a. Phosphorus desorption dynamics in soil and the link to a dynamic concept of bioavailability. *Journal of Environmental Quality,* **33,** 1393-1402.

Koopmans, G. F., Chardon, W. J., Ehlert, P. A. I., Dolfing, J., Suurs, R. A. A., Oenema, O. & Van Riemsdijk, W. H. 2004b. Phosphorus availability for plant uptake in a phosphorus-enriched noncalcareous sandy soil. *Journal of Environmental Quality,* **33,** 965-975.

Kronvang, B., Bechmann, M., Lundekvam, H., Behrendt, H., Rubaek, G. H., Schoumans, O. F., Syversen, N., Andersen, H. E. & Hoffmann, C. C. 2005. Phosphorus losses from agricultural areas in river basins: Effects and uncertainties of targeted mitigation measures. *Journal of Environmental Quality,* **34,** 2129-2144.

Kucey, R. M. N. 1983. Phosphate-solubilizing bacteria and fungi in various cultivated and virgin alberta soils. *Canadian Journal of Soil Science,* **63,** 671-678.

Kuiper, I., Kravchenko, L. V., Bloemberg, G. V. & Lugtenberg, B. J. J. 2002. *Pseudomonas putida* strain PCL1444, selected for efficient root colonization and naphtalene degradation, effectively utilizes root exudate components. *Molecular Plant-Microbe Interactions,* **15,** 734-741.

Lair, G. J., Zehetner, F., Khan, Z. H. & Gerzabek, M. H. 2009. Phosphorus sorption-desorption in alluvial soils of a young weathering sequence at the Danube River. *Geoderma,* **149,** 39-44.

Lemunyon, J. L. & Gilbert, R. G. 1993. The concept and need for a phosphorus assessment tool. *Journal of Production Agriculture,* **6,** 483-496.

Lenders, S., Oeyen, A., d'hooghe, J. & Overloop, S. 2011. Bodembalans van de Vlaamse landbouw, cijfers voor 2007-2009. In., DLV-AMS en VMM rapport, pp. 45.

Leyval, C. & Berthelin, J. 1989. Interactions between *laccaria-laccata*, agrobacterium-radiobacter and beech roots - influence on P, K, Mg, and Fe mobilization from minderals and plant-growth. *Plant and Soil,* **117,** 103-110.

Li & Stanforth, R. 2000. Distinguishing adsorption and surface precipitation of phosphate on goethite (α-FeOOH). *Journal of Colloidal Interface Science*, 12-21.

Lindsay, W. L. (ed) 1979. *Chemical equilibria in soils* John Wiley & Sons, New York, USA.

Lindsay, W. L., Vlek, P. L. G. & Chien, S. H. 1989. Phosphate minerals. In: *In Minerals in soil environment second editon.* eds J. B. Dixon & S. B. Weed), Soil science society of America, Madison, WI, USA, pp. 1089-1130.

Lookman, R., Freese, D., Merckx, R., Vlassak, K. & Van Riemsdijk, W. H. 1995. Long-term kinetics of phosphate release from soil. *Environmental Science and Technology,* **29,** 1569-1575.

Lookman, R., Jansen, K., Merckx, R. & Vlassak, K. 1996. Relationship between soil properties and phosphate saturation parameters - A transect study in northern Belgium. *Geoderma,* **69,** 265-274.

Malboobi, M. A., Owlia, P., Bebhabani, M., Sarokhani, E., Moradi, S., Yakhcali, B., Deljou, A. & Heravi, K. M. 2009. Solubilization of organic and inorganic phosphates by three highly efficient soil bacterial isolates. *World Journal of Microbiol biotechnology,* **25,** 1471-1477.

Manna, M. C., Ghosh, P. K., Ghosh, B. N. & 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. *Journal of Agricultural Science,* **137,** 45-54.

Martin, P. A. W. & Travers, R. S. 1989. Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. *Applied and Environmental Microbiology,* **55,** 2437-2442.

McDowell, R. W. & Nash, D. 2012. A Review of the Cost-Effectiveness and Suitability of Mitigation Strategies to Prevent Phosphorus Loss from Dairy Farms in New Zealand and Australia. *Journal of Environmental Quality,* **41,** 680-693.

Mehta, S. & Nautiyal, C. S. 2001. An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Current Microbiology,* **43,** 51-56.

Meis, S., Spears, B. M., Maberly, S. C., O'Malley, M. B. & Perkins, R. G. 2012. Sediment amendment with Phoslock (R) in Clatto Reservoir (Dundee, UK): Investigating changes in sediment elemental composition and phosphorus fractionation. *journal of Environmental management,* **93,** 185-193.

Minor, H.C. & Stecker, J. 1993. Phosphorus in Missouri soils. http://extension.missouri.edu/p/G9180#reactions

Mozaffari, M. & Sims, J. T. 1994. Phosphorus availability and sorption in an atlantic coastal-plain watershed dominated by animal-based agriculture. *Soil Science,* **157,** 97-107.

Mulier, A., Hofman, G., Baecke, E., Carlier, L., De Brabander, D., De Groote, G., De Wilde, R., Fiems, L., Janssens, G., Van Cleemput, O., Van Herck, A., Van Huylenbroeck, G. & Verbruggen, I. 2003. A methodology for the calculation of farm level nitrogen and phosphorus balances in Flemish agriculture. *European Journal of Agronomy,* **20,** 45-51.

Mumpton, F. A. 1999. La roca magica: Uses of natural zeolites in agriculture and industry. *Proceedings of the National Academy of Sciences,* **96,** 3463-3470.

Murphy, J. & Riley, J. P. 1962. A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta,* **26,** 31-36.

Musarrat, J., Bano, N. & Rao, R. A. K. 2000. Isolation and characterization of 2,4-dichlorophenoxyacetic acid-catabolizing bacteria and their biodegradation efficiency in soil. *World Journal of Microbiology & Biotechnology,* **16,** 495-497.

Nagaraja, S., Posner, A. M. & Quirk, J. P. 1968. Desorption of phosphate from kaolinite by citrate and bicarbonate. *soil science society of America Proceedings,* **32,** 507-&.

Nautiyal, C. S. 1999. an efficient mircobiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology letters,* **170,** 265-170.

Nelson, N. O., Parsons, J. E. & Mikkelsen, R. L. 2005. Field-scale evaluation of phosphorus leaching in acid sandy soils receiving swine waste. *Journal of Environmental Quality,* **34,** 2024-2035.

Neyroud, J. A. & Lischer, P. 2003. Do different methods used to estimate soil phosphorus availability across Europe give comparable results? *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde,* **166,** 422-431.

O'Connor, G. A., Brinton, S. & Silveira, M. L. 2005. Evaluation and selection of soil amendments for field testing to reduce P losses. *Soil and Crop Science Society of Florida Proceedings,* **64,** 22-34.

Olsson, J., Bovet, N., Makovicky, E., Bechgaard, K., Balogh, Z. & Stipp, S. L. S. 2012. Olivine reactivity with CO_2 and H_2O on a microscale: Implications for carbon sequestration. *Geochimica Et Cosmochimica Acta,* **77,** 86-97.

Otabbong, E., Borling, K., Katterer, T. & Mattsson, L. 2009. Compatibility of the ammonium lactate (AL) and sodium bicarbonate (Olsen) methods for determining available phosphorus in Swedish soils. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science,* **59,** 373-378.

Overloop, S., Bossuyt, M., Claeys, D., Wustenberghs, H., D'hooghe, J., Elsen, A. & Eppinger, R. 2011. Milieurapport Vlaanderen, Achtergronddocument 2011 Vermesting. In. (ed s.n.), Vlaamse milieumaatschappij, pp. 113.

Overloop, S., Bossuyt, M., Claeys, D., Wustenberghs, H., D'hooghe, J., Elsen, A. & Eppinger, R. 2012. Milieu- en natuurrapport (MIRA) Vlaanderen, indicatorraport 2012, achtergronddocument 2012 vermesting. In. (ed s.n.), Vlaamse Milieumaatschappij, pp. 162.

Pandey, A. & Palni, L. M. S. 1998. Isolation of *Pseudomonas Corrugata* from Sikkim Himalaya. *World Journal of Microbiol biology and biotechnology,* **14,** 411-413.

Parfitt, R. L. 1978. Anion sorption by soils and soil materials. *Advances in Agronomy,* **30,** 1-50.

Peeters, B., D'heygere, T., Huysmans, T., Ronse, Y. & Dieltjens, I. 2009. modellering waterkwaliteitsscenario's wetenschappelijk rapport thema 'kwaliteit van het oppervlaktewater'. In., vmm, mechelen.

Pierzynski, G. M. 1991. The chemistry and mineralogy of phosphorus in excessively fertilized soils. *Critical Reviews in Environmental Control,* **21,** 265-295.

Pierzynski, G. M. (ed) 2000. *Methods of phosphorus analysis for soils sediments, residuals and waters*.

Pierzynski, G. M. & McDowell, R. W. 2005. Chemistry, Cycling, and Potential Movement of Inorganic Phosphorus in Soils. *Phosphorus: Agriculture and the Environment***,** 53-86.

Pierzynski, G. M., Sims, J. T. & Vance, G. F. 2005. *Soils and environmental quality,* Lewis Publishers, Boca Raton, FL.

Platteau, J., Van Gijseghem, D., Van Bogaert, T. & Maertens, E. 2012. Landbouwrapport 2012 (in dutch). In. (ed s.n.), Departement Landbouw en Visserij, Brussel, pp. 423.

Puente, M. E., Bashan, Y., Li, C. Y. & Lebsky, V. K. 2004. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. *Plant Biology,* **6,** 629-642.

Radcliffe, D. E. & Cabrera, M. L. 2007. *Modeling Phosphorus in the Environment,* CRC PressINC.

Radcliffe, D. E., Freer, J. & Schoumans, O. 2009. Diffuse Phosphorus Models in the United States and Europe: Their Usages, Scales, and Uncertainties. *Journal of Environmental Quality,* **38,** 1956-1967.

Rechcigl, J. E., Bottcher, A. B., Littell, R. C., Alcordo, I. S. & Boruvka, L. 2000. Evaluating limestone and gypsum for reducing P leaching and runoff form beef cattle forage. In: *Annual progress report (March 1999-Januari 2000).* South Florida Water Management District, West Palm Beach.

Reijneveld, J. A., Ehlert, P. A. I., Termorshuizen, A. J. & Oenema, O. 2010. Changes in the soil phosphorus status of agricultural land in the Netherlands during the 20th century. *Soil Use and Management,* **26,** 399-411.

Richardson, A. E. & Simpson, R. J. 2011. Soil Microorganisms Mediating Phosphorus Availability. *Plant Physiology,* **156,** 989-996.

Rodriguez, H. & Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances,* **17,** 319-339.

Rodriguez, H., Fraga, R., Gonzalez, T. & Bashan, Y. 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil,* **287,** 15-21.

Rosas, S. B., Andrés, J. A. & Rovera, M., Correa, N.S. 2006. Phosphate-solublizing *Pseudomonas putida* can influence the rhizobia-legume symbiosis. *Soil Biology & Biochemistry,* **38,** 3502-3505.

Ross, G., Haghseresht, F. & Cloete, T. E. 2008. The effect of pH and anoxia on the performance of Phoslock (R), a phosphorus binding clay. *Harmful Algae,* **7,** 545-550.

Salomez, J., De Bolle, S., Sleutel, S., De Neve, S. & Hofman, G. 2009. Nutrient legislation in Flanders (Belgium). In: *18th symposium of the international scientific centre of fertilizers: More sustainability in agriculture : new fertilizers and fertilization management.* eds P. Sequi, D. Ferri, E. Rea, F. Montemurro, A. V. Vonella & F. Fornaro), "fertilitas Agrorum", Rome, Italy, pp. 546-551.

Saltelli, A. & Annoni, P. 2010. How to avoid a perfunctory sensitivity analysis. *Environmental Modelling & Software,* **25,** 1508-1517.

Saltelli, A., Tarantola, S. & Campolongo, F. 2000. Sensitivity analysis as an ingredient of modeling. *Statistical Science,* **15,** 377-395.

Scheel, K. C. 1936. Colorimetric determination of phosphoric acid in fertilizers with the Aulfrich photometer. *Zeitschrift Fur Analytische Chemie,* **105,** 256-259.

Schindler, D. W. 2006. Recent advances in the understanding and management of eutrophication. *Limnology and oceanography,* **5,** 356-363.

Schoumans, O. F. 2004. Inventarisatie van de fosfaatverzadiging van landbouwgronden in Nederland. In., Alterra, Wageningen, pp. 50.

Schoumans, O. F. & Chardon, W. J. 2003. Risk assessment methodologies for predicting phosphorus losses. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde,* **166,** 403-408.

Schoumans, O. F. & 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., Groenendijk, P., van der Salm, C. & Pleijter, M. 2008. Methodiek voor het karakteriseren van fosfaatlekkende gronden; PLEASE: technische beschrijving. In., Alterra, Wageningen, pp. 76.

Schoumans, O. F., Silgram, M., Groenendijk, P., Bouraoui, F., Andersen, H. E., Kronvang, B., Behrendt, H., Arheimer, B., Johnsson, H., Panagopoulos, Y., Mimikou, M., Lo Porto, A., Reisser, H., Le Gall, G., Barr, A. & Anthony, S. G. 2009. Description of nine nutrient loss models: capabilities and suitability based on their characteristics. *Journal of Environmental Monitoring,* **11,** 506-514.

Schoumans, O. F., Van der Salm, C. & Groenendijk, P. 2013. PLEASE: a simple model to determine P losses by leaching. *Soil Use and Management,* **29,** 138-146.

Schuiling, R. D. & Krijgsman, P. 2006. Enhanced weathering: An effective and cheap tool to sequester CO2. *Climatic Change,* **74,** 349-354.

Schwertmann, U. 1964. Differenzierung des Eisenoxide des Bodensdurch Extraktion mit Ammoniumoxalat-lösung. *Zeitschrft Pflanzenerzährung, Düngung und Bodenkunde,* **105,** 194-202.

Self-Davis, M. L., Moore, P. A. & Joern, B. C. 2009. Water- or Dilute Salt-Extractable Phosphorus. In: *Soil In Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters second edition.* eds J. L. Kovar & G. M. Pierzynski), Tech University, Virginia, pp. 131.

Sharma, N. C., Starnes, D. L. & Sahi, S. V. 2007. Phytoextraction of excess soil phosphorus. *Environmental Pollution,* **146,** 120-127.

Sharpley, A. N., Weld, J. L., Beegle, D. B., Kleinman, P. J. A., Gburek, W. J., Moore, P. A. & Mullins, G. 2003. Development of phosphorus indices for nutrient management planning strategies in the United States. *Journal of Soil and Water Conservation,* **58,** 137-152.

Siemens, J., Ilg, K., Lang, F. & Kaupenjohann, M. 2004. Adsorption controls mobilization of colloids and leaching of dissolved phosphorus. *European Journal of Soil Science,* **55,** 253-263.

Siemens, J., Ilg, K., Pagel, H. & Kaupenjohann, M. 2008. Is Colloid-Facilitated Phosphorus Leaching Triggered by Phosphorus Accumulation in Sandy Soils? *Journal of Environmental Quality,* **37,** 2100-2107.

Simard, R. R., Cluis, D., Gangbazo, G. & Pesant, A. R. 1994. Phosphorus sorption and desorption indexes in soil. *Communications in Soil Science and Plant Analysis,* **25,** 1483-1494.

Sinaj, S., Stamm, C., Toor, G. S., Condron, L. M., Hendry, T., Di, H. J., Cameron, K. C. & Frossard, E. 2002. Phosphorus exhangeability and leaching losses from two grassland soils. *Journal of Environmental Quality,* **31,** 319-330.

Smith, K. A., Chalmers, A. G., Chambers, B. J. & Christie, P. 1998. Organic manure phosphorus accumulation, mobility and management. *Soil Use and Management,* **14,** 154-159.

Smith, V. H., Joye, S. B. & Howarth, R. W. 2006. Eutrophication of freshwater and marine ecosystems. *Limnology and oceanography,* **51,** 351-355.

Soil Survey Staff 2010. *Keys to Soil Taxonomy,* USDA-Natural Resources Conservation Service, Washington, DC.

Stevenson, F. J. & Cole, M. A. 1999. *Cycles of Soil : Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients,* Wiley & Sons, New York.

Stoner, D., Penn, C., McGrath, J. & Warren, J. 2012. Phosphorus Removal with By-Products in a Flow-Through Setting. *Journal of Environmental Quality,* **41,** 654-663.

Summers, R. N., Smirk, D. D. & Karafilis, D. 1996. Phosphorus retention and leachates from sandy soil amended with bauxite residue (red mud). *Australian Journal of Soil Research,* **34,** 555-567.

Sundara, B., Natarajan, V. & Hari, K. 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. *field crop research,* **77,** 43-49.

ten Berge, H. F. M., van der Meer, H. G., Steenhuizen, J. W., Goedhart, P. W., Knops, P. & Verhagen, J. 2012. Olivine Weathering in Soil, and Its Effects on Growth and Nutrient Uptake in Ryegrass (*Lolium perenne* L.): A Pot Experiment. *Plos One,* **7**.

Thakuria, D., Talukdar, N. C., Goswami, C., Hazarika, S., Kalita, M. C. & Bending, G. D. 2009. Evaluation of rice-legume-rice cropping system on grain yield, nutrient uptake, nitrogen fixation, and chemical, physical, and biological properties of soil. *Biology and Fertility of Soils,* **45,** 237-251.

Tisdale, S. L., Nelson, W. L. & Beaton, J. D. (eds) 1985. *Soil fertility and fertilizers,* Macmillam, New York.

Trivedi, P. & Sa, T. M. 2008. *Pseudomonas corrugata* (NRRL B-30409) mutants increased phosphate solubilization, organic acid production, and plant growth at lower temperatures. *Current Microbiology,* **56,** 140-144.

Turan, M., Ataoglu, N. & Sahin, F. 2007. Effects of *Bacillus* FS-3 on growth of tomato (*Lycopersicon esculentum L.*) plants and availability of phosphorus in soil. *Plant Soil and Environment,* **53,** 58-64.

Turner, B. L., Cade-Menun, B. J., Condron, L. M. & Newman, S. 2005. Extraction of soil organic phosphorus. *Talanta,* **66,** 294-306.

Uusitalo, R., Ekholm, P., Lehtoranta, J., Klimeski, A., Konstari, O., Lehtonen, R. & Turtola, E. 2012a. Ca-Fe oxide granules as potential phosphate barrier material for critical source areas: a laboratory study of P retention and release. *Agricultural and Food Science,* **21,** 224-236.

Uusitalo, R., Turtola, E., Gronroos, J., Kivistoe, J., Mantylahti, V., Turtola, A., Lemola, R. & Sato, T. 2007. Finnish trends in phosphorus balances and soil test phosphorus. *Agricultural and Food Science,* **16,** 301-316.

Uusitalo, R., Ylivainio, K., Hyvaluoma, J., Rasa, K., Kaseva, J., Nylund, P., Pietola, L. & Turtola, E. 2012b. The effects of gypsum on the transfer of phosphorus and other nutrients through clay soil monoliths. *Agricultural and Food Science,* **21,** 260-278.

Vaccari, D. A. 2011. Chemosphere Phosphorus Cycle Issue – Introduction. *Chemosphere,* **84,** 735-736.

van Bakel, P. J. T., Massop, H. T. L., Kroes, J. G., Hoogewoud, J., Pastoors, R. & Kroon, T. 2008. Updating the hydrology component in STONE 2.3; Adjusting boundary conditions and parameters, linking NAGROM and SWAP, and plausibility test. In., Statutory Research Tasks Unitfor Nature and the Environment, Wageningen, pp. 108.

Van Den Bossche, A., De Neve, S. & Hofman, G. 2005. Soil phosphorus status of organic farming in Flanders: an overview and comparison with the conventional management. *Soil Use and Management,* **21,** 415-421.

van der Salm, C., Chardon, W. J., Koopmans, G. F., van Middelkoop, J. C. & Ehlert, P. A. I. 2009. Phytoextraction of Phosphorus-Enriched Grassland Soils. *Journal of Environmental Quality,* **38,** 751-761.

van der Salm, C., Dupas, R., Grant, R., Heckrath, G., Iversen, B. V., Kronvang, B., Levi, C., Rubaek, G. H. & Schoumans, O. F. 2011. Predicting Phosphorus Losses with the PLEASE Model on a Local Scale in Denmark and the Netherlands. *Journal of Environmental Quality,* **40,** 1617-1626.

Van der Sluijs, P. 1982. De grondwatertrap als karakteristiek van het grondwaterstandsverloop. *H2O,* **15,** 42-46.

Van der Zee, S. E. A. T. M. 1988. Transport of reactive contaminants in herterogeneous soil systems. In., Agricultural university, Wageningen.

Van der Zee, S., Fokkink, L. G. J. & Van Riemsdijk, W. H. 1987. a new technique for assement of reversibly adsorbed phosphate. *Soil Science Society of America Journal,* **51,** 599-604.

Van der Zee, S. E. A. T. M. & Van Riemsdijk, W. H. 1986a. Sorption kinetics and transport of phosphate in sandy soil *Geoderma,* **38,** 293-309.

Van der Zee, S. E. A. T. M. & Van Riemsdijk, W. H. 1986b. Transport of Phosphate in a heterogeneous field. *Transport in Porous Media,* **1,** 339-359.

Van der Zee, S. E. A. T. M. & 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. 1990a. Het protokol fosfaatverzadigde gronden. Deel I : Toelichting. (in dutch). In: *Vakgroep bodemkunde en plantenvoeding.* landbouwuniversiteit Wageningen, the Netherlands.

Van der Zee, S. E. A. T. M., Van Riemsdijk, W. H. & De Haan, F. A. M. 1990b. Het protokol fosfaatverzadigde gronden. Deel II : Technische uitwerking (in dutch). In: *Vakgroep bodemkunde en plantenvoeding.* landbouwuniversiteit Wageningen, the Netherlands.

Van Meirvenne, M., Tariku, M., De Neve, S., Hofman, G., Salomez, J. & De Bolle, S. 2008. Afbakening van de fosfaatverzadigde gebieden in Vlaanderen op basis van een kritische fosfaatverzadigingsgraad van 35%. In. (ed s.n.), Ghent University, Ghent, pp. 62.

Vazquez, P., Holguin, G., Puente, M. E., Lopez-Cortes, A. & Bashan, Y. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils,* **30,** 460-468.

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil,* **255,** 571-586.

Villegas, J. & Fortin, J. A. 2002. Phosphorus solubilization and pH changes as a result of the interactions between soil bacteria and arbuscular mycorrhizal fungi on a medium containing NO_3- as nitrogen source. *Canadian Journal of Botany-Revue Canadienne De Botanique,* **80,** 571-576.

VLM 1997. Phosphate saturation in sandy soils in Flanders. In. (ed F. L. Agency), VLM Meise [In Dutch].

VMM 2010. Jaarrapport Water 2010. In., Vlaamse milieumaatschappij, pp. 78.

Volf, C. A., Ontkean, G. R., Bennett, D. R., Chanasyk, D. S. & Miller, J. J. 2007. Phosphorus losses in simulated rainfall runoff from manured soils of Alberta. *Journal of Environmental Quality,* **36,** 730-741.

Vyas, P. & Gulati, A. 2009. Organic acid production in vitro and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. *BMC Microbiology,* **9**.

Wan, J. H. C. & Wong, M. H. 2004. Effects of earthworm activity and P-solubilizing bacteria on P availability in soil. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde,* **167,** 209-213.

Wang, C., Ramette, A., Punjasamarnwong, P., Zala, M., Natsch, A. & Défago, G. 2001. Cosmopolitan distribution of phlD-containing dicotyledonous crop-associated biocontrol *pseudomonas* of worldwide origin. *FEMS Microbiology Ecology,* **37,** 105-116.

Wang, Z. Y., Kelly, J. M. & Kovar, J. L. 2004. In situ dynamics of phosphorus in the rhizosphere solution of five species. *Journal of Environmental Quality,* **33,** 1387-1392.

Watson, C. J., Smith, R. V. & Matthews, D. I. 2007. Increase in phosphorus losses from grassland in response to Olsen-P accumulation. *Journal of Environmental Quality,* **36,** 1452-1460.

Watts, D. B. & Torbert, H. A. 2009. Impact of gypsum applied to grass buffer strips on reducing soluble P in surface water runoff. *Journal of Environmental Quality,* **38,** 1511-1517.

Welch, S. A., Taunton, A. E. & Banfield, J. F. 2002. Effect of microorganisms and microbial metabolites on apatite dissolution. *Geomicrobiology Journal,* **19,** 343-367.

Whitehead, D. C. 2000. *Nutrient elements in grasslands: soil-plant-animal relationship,* CABI Publishers, New York.

Whitelaw, M. A. 2000. Growth promotion of plants inoculated with phosphate-solubilizing fungi. *Advances in Agronomy,* **69,** 99-151.

Wild, D., Kisliakova, A. & Siegrist, H. 1996. P-fixation by Mg, Ca and zeolite A during stabilization of excess sludge from enhanced biological P-removal. *Water Science and Technology,* **34,** 391-398.

Withers, P. J. A. & Haygarth, P. M. 2007. Agriculture, phosphorus and eutrophication: a European perspective. *Soil Use and Management,* **23,** 1-4.

Wium-Andersen, T., Nielsen, A. H., Hvitved-Jacobsen, T., Kristensen, N. K., Brix, H., Arias, C. & Vollertsen, J. 2012. Sorption Media for Stormwater Treatment-A Laboratory Evaluation of Five Low-Cost Media for Their Ability to Remove Metals and Phosphorus from Artificial Stormwater. *Water Environment Research,* **84,** 605-616.

Wolf, J., Beusen, A. H. W., Groenendijk, P., Kroon, T., Rötter, R. & Van Zeijts, H. 2005. The integrated modeling system STONE for calculation nutrient emissions from agriculture in the Netherlands. *Environmental modeling software,* **18,** 597-617.

WRB 2006. World reference base for soil resources 2006. World Soil Resources Reports No. 103. In., FAO, Rome.

Yang, J., He, Z., Yang, Y., Stoffella, P. J., Yang, X. E., Banks, D. J. & Mishra, S. 2007. Use of admendments to reduce leaching loss op phosphorus and other nutrients from a sandy soil in Florida. *environmental science and pollution research,* **14,** 266-269.

Zhang, M. K., He, Z. L., Calvert, D. V. & Stoffella, P. J. 2003. Colloidal iron oxide transport in sandy soil induced by excessive phosphorus application. *Soil Science,* **168,** 617-626.

Appendix

Appendix I

Fixation experiment

In appendix I the bar chart with standard deviation and the results of the statistical analysis are given. This was done for the simple salt treatments to Zw and for the minerals, dried Fe sludge and specially designed products with addition rates given in Table 4.5 to all six soils. The graphs are ordered by field, in the same way as in Chapter 4, first the total $P_{inorganic}$ loss followed by the $P_{inorganic}$ loss per leachate event.

P fixation efficiency of the simple salts

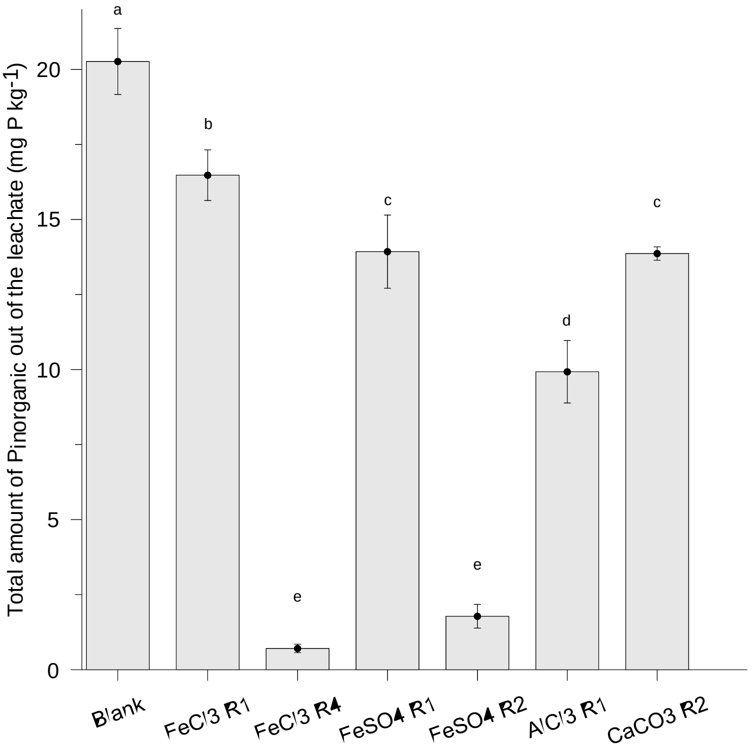

Figure I.1. Total $P_{inorganic}$ (mg P kg^{-1}) leached out of Zw over the eight leachate events with addition of simple salts ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments)

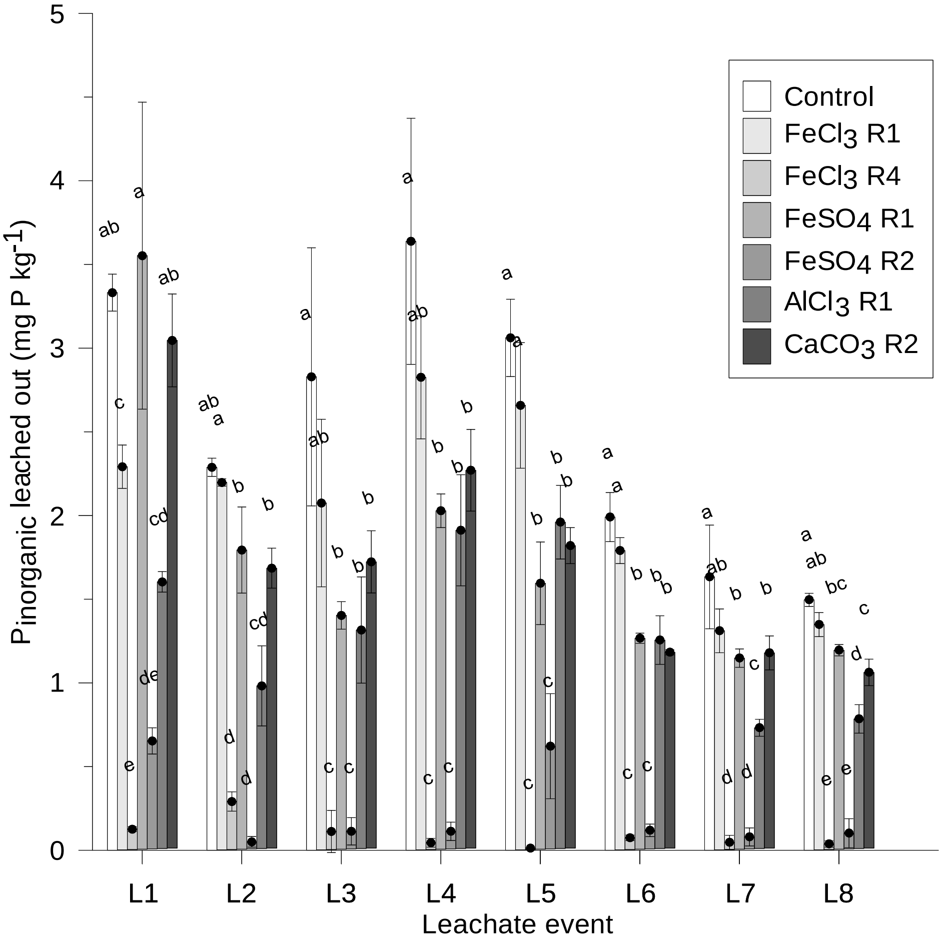

Figure I.2. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event with addition of simple salts to Zw ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

P fixation efficiency of the minerals, bauxite, industrial by-product and specially designed products for Zw

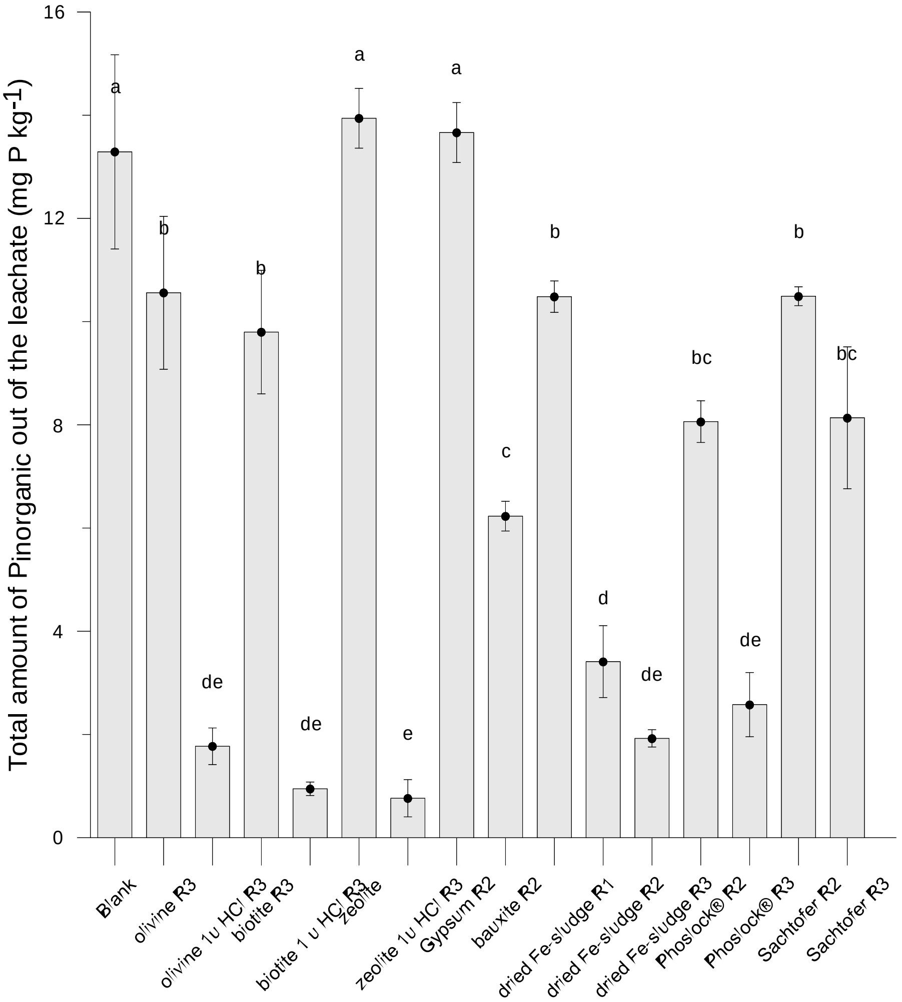

Figure I.3. Total $P_{inorganic}$ (mg P kg^{-1}) leached out of Zw over the eight leachate events with addition of minerals, bauxite, an industrial by product or specially designed products($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments)

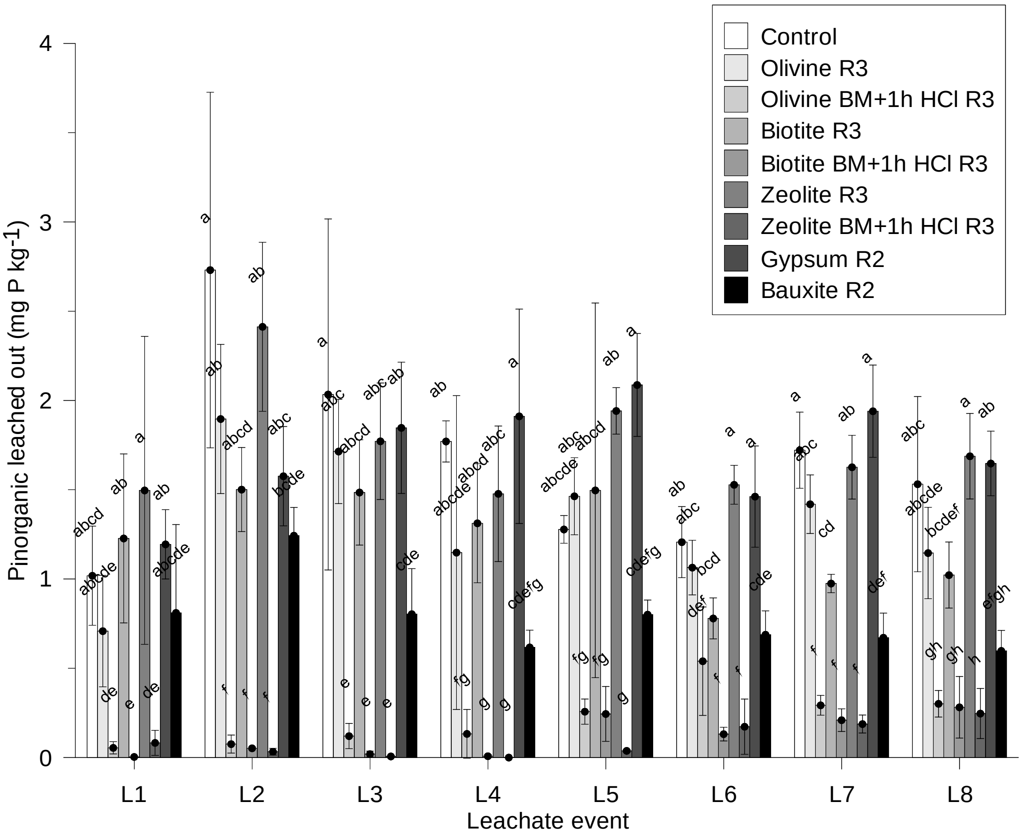

Figure I.4. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event with addition of the minerals and bauxite to Zw (p<0.05, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

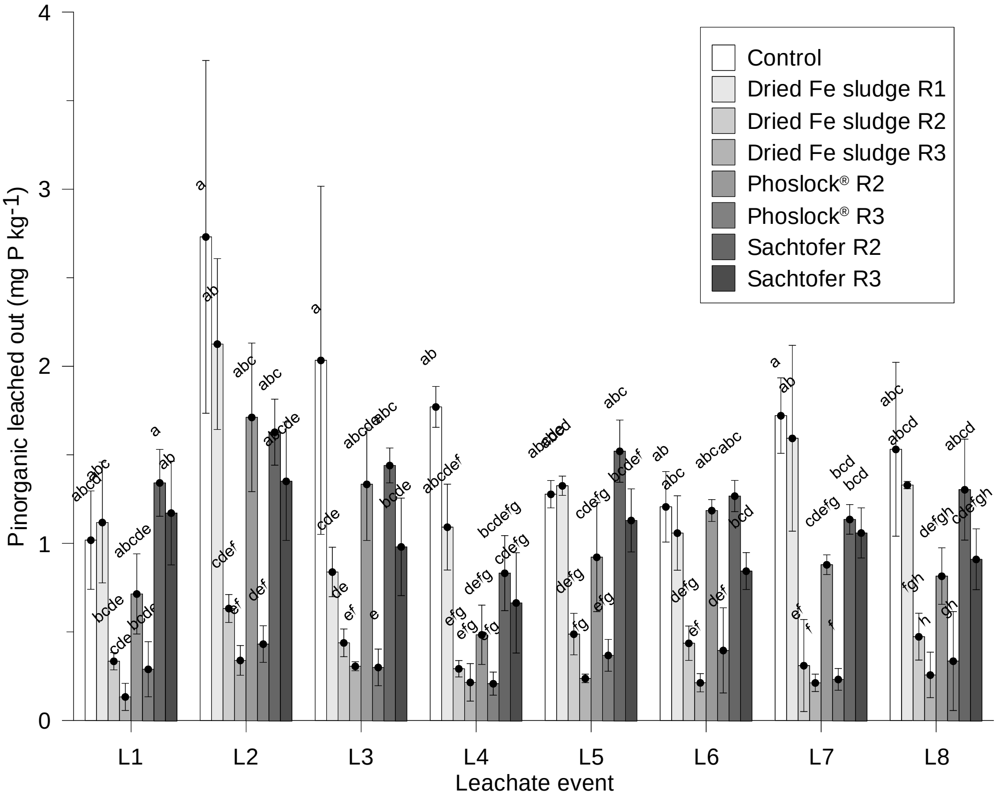

Figure I.5. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event of Zw with addition of the by product and specially designed products (p<0.05, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

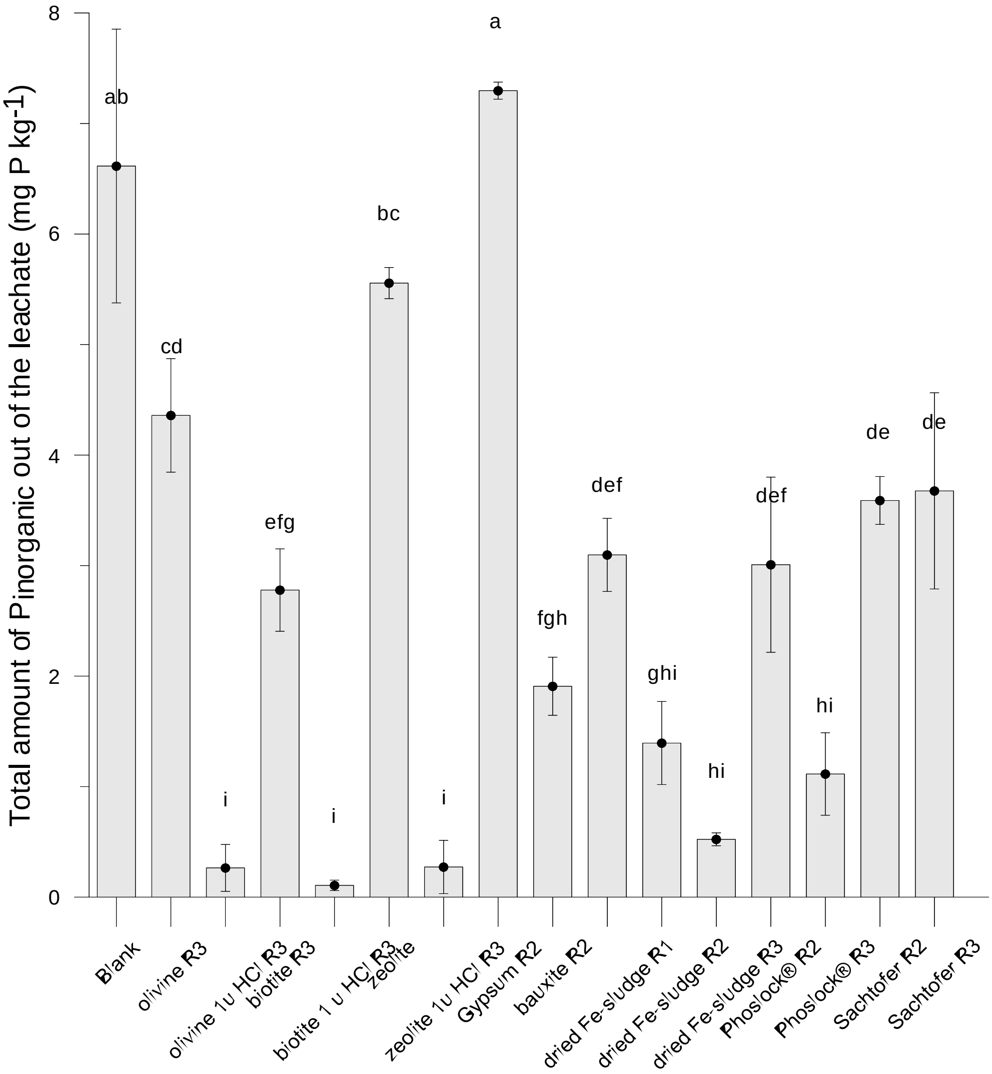

Figure I.6. Total $P_{inorganic}$ (mg P kg^{-1}) leached out of Ze2 over the eight leachate events with addition of minerals, bauxite, an industrial by product or specially designed products ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments)

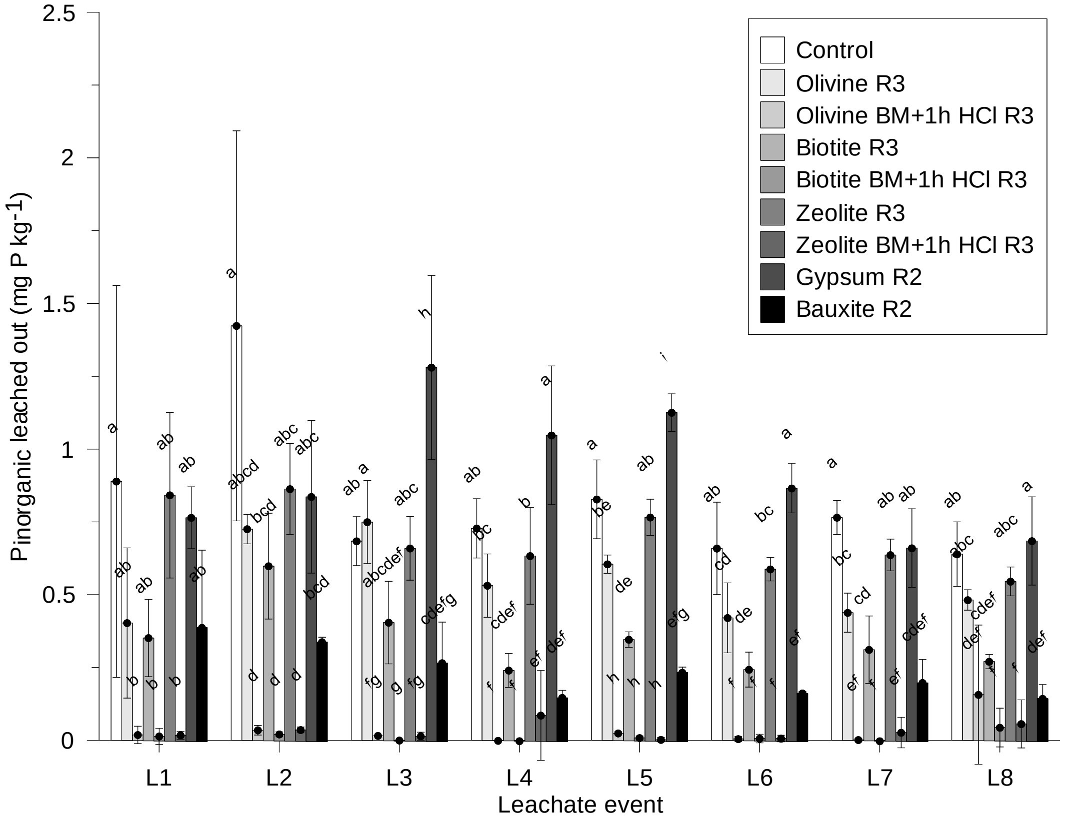

Figure I.7. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event with addition of the minerals and bauxite to Ze1 ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

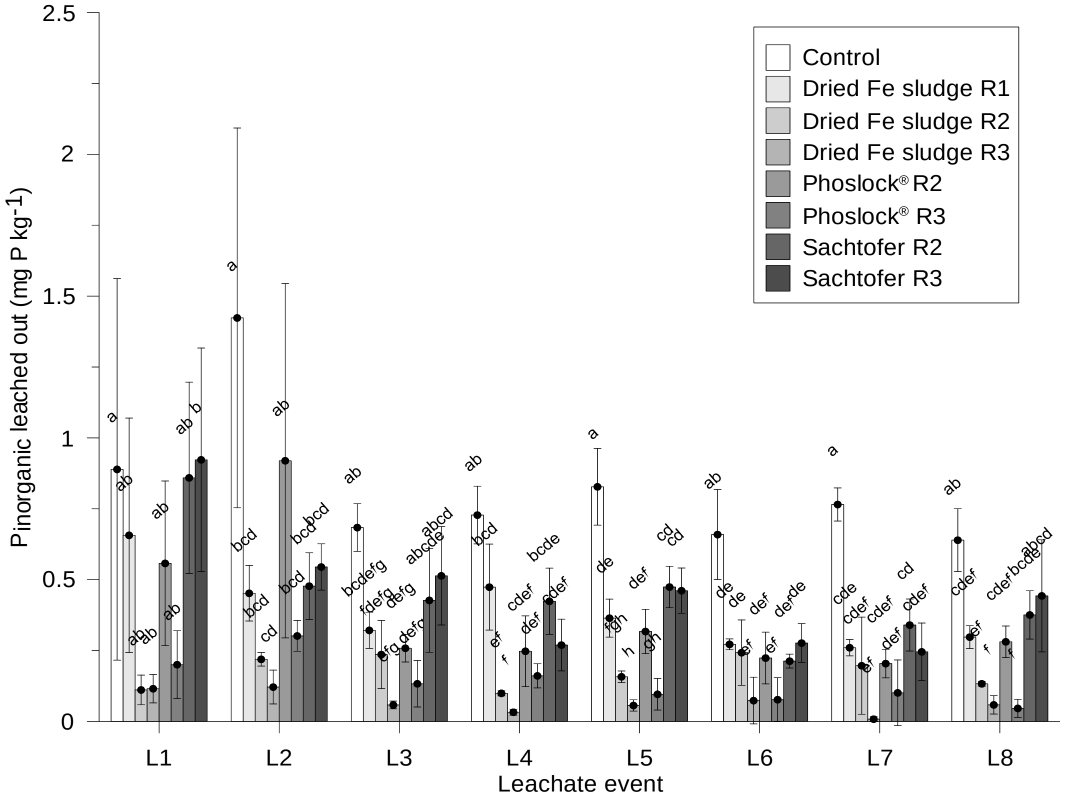

Figure I.8. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event of Ze1 with addition of the by product and specially designed products ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

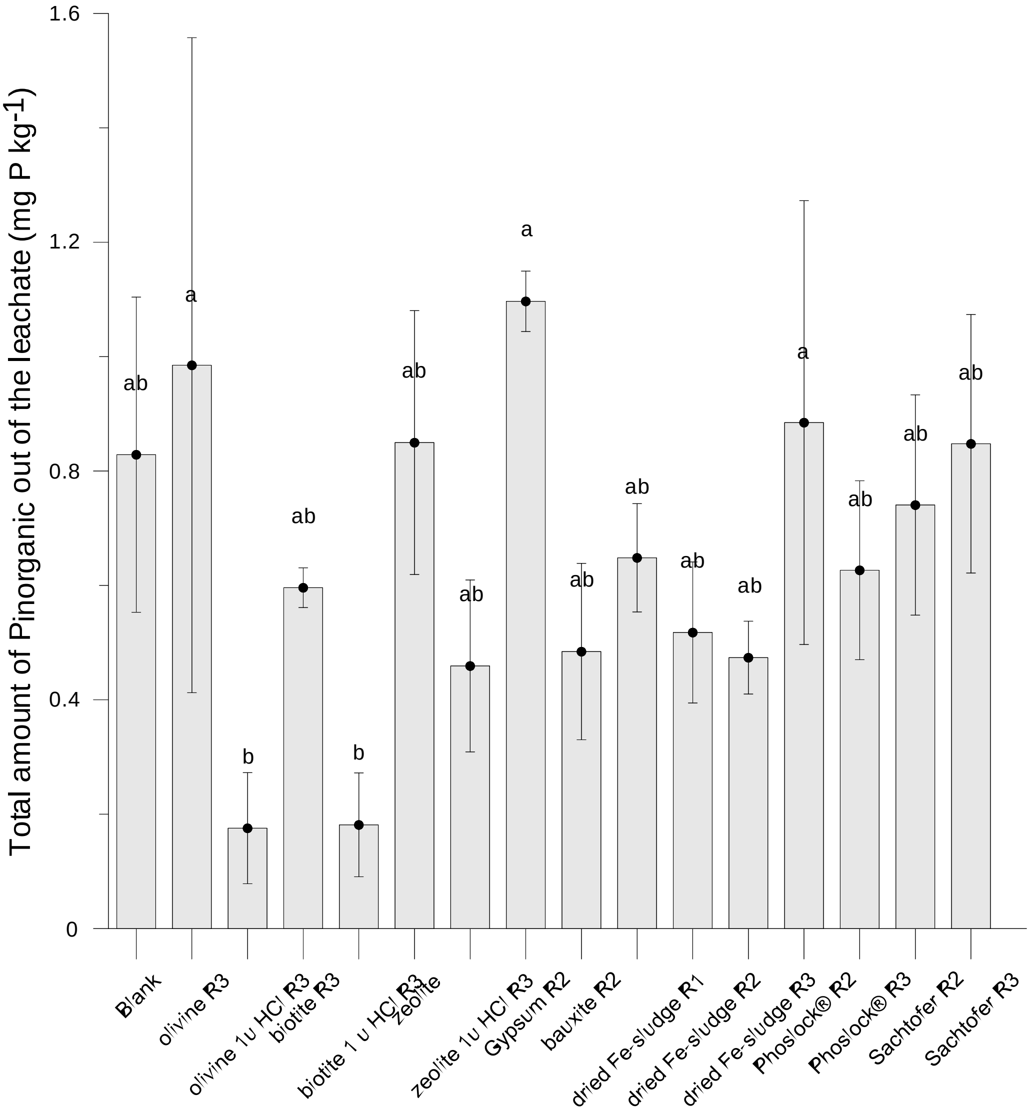

Figure I.9. Total amount of $P_{inorganic}$ (mg P kg^{-1}) that has been leached out of Z2 with addition of minerals, bauxite, industrial by product or specially designed products ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments)

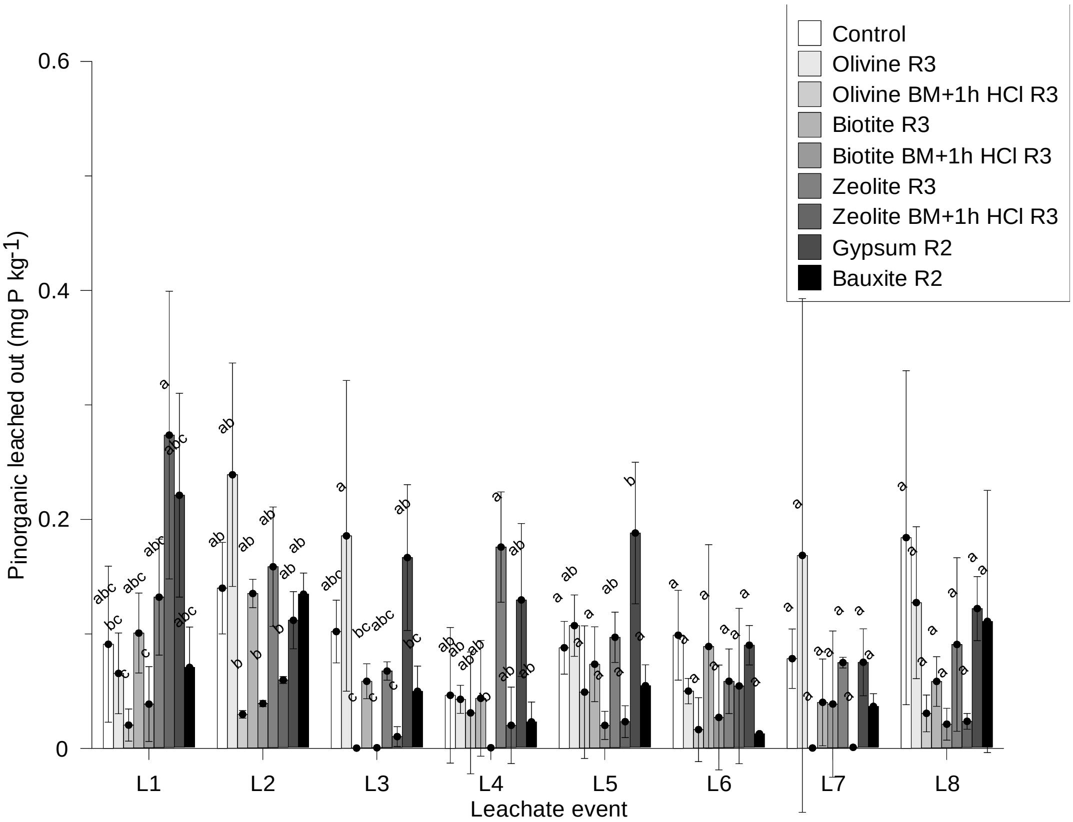

Figure I.10. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event with addition of the minerals and bauxite to Ze2 ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

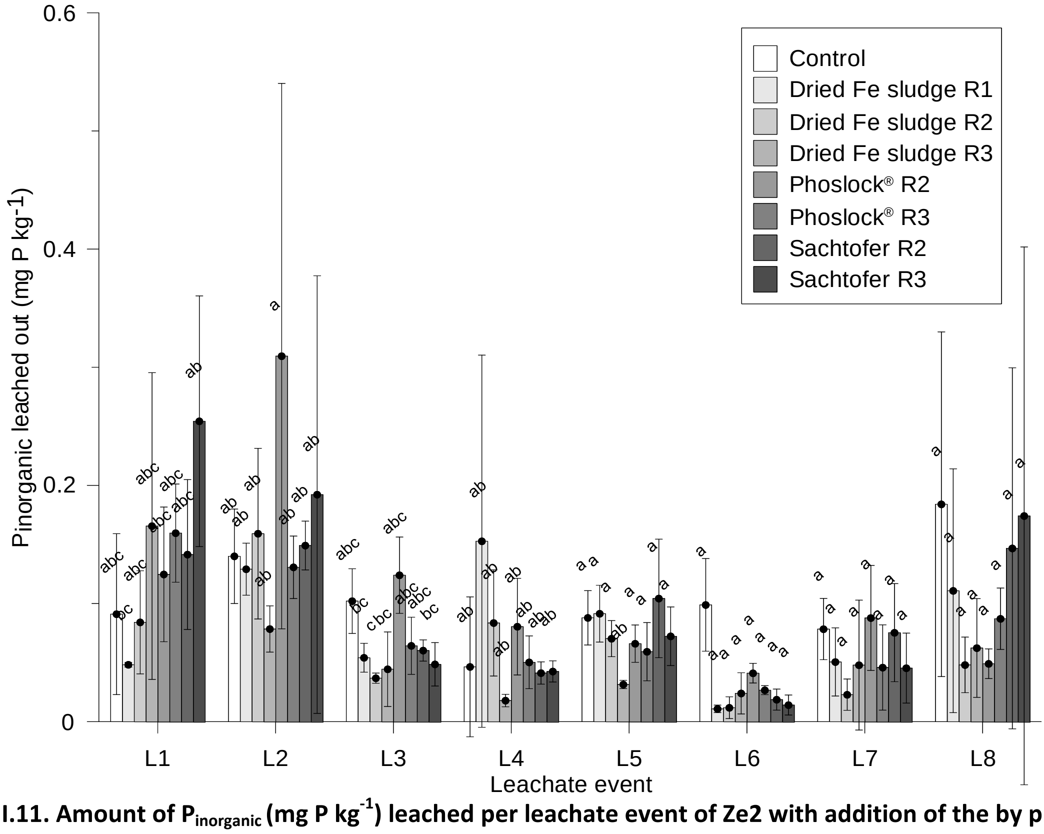

Figure I.11. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event of Ze2 with addition of the by product and specially designed products ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

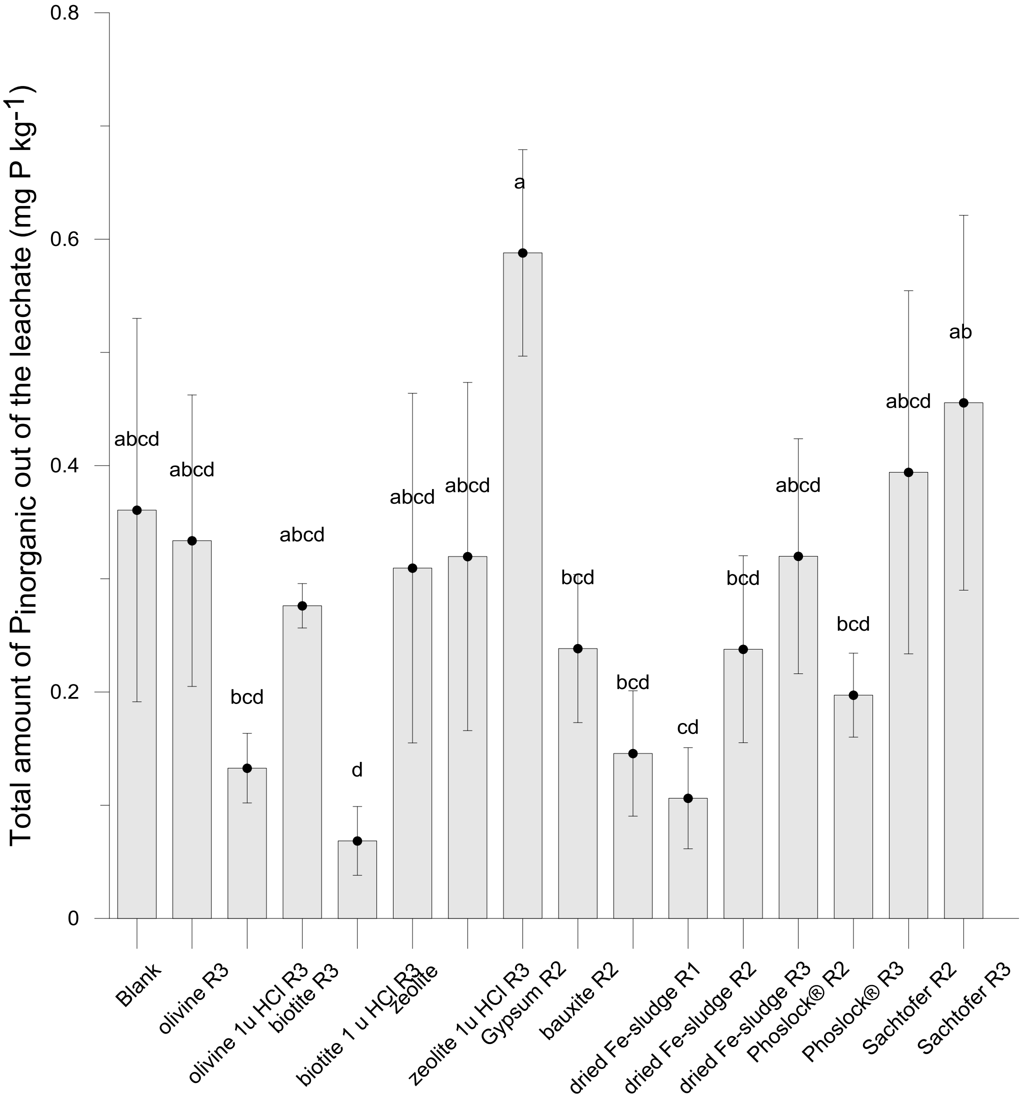

Figure I.12. Total $P_{inorganic}$ (mg P kg^{-1}) leached out of Li1 over the eight leachate events with addition of minerals, bauxite, an industrial by product or specially designed products ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments)

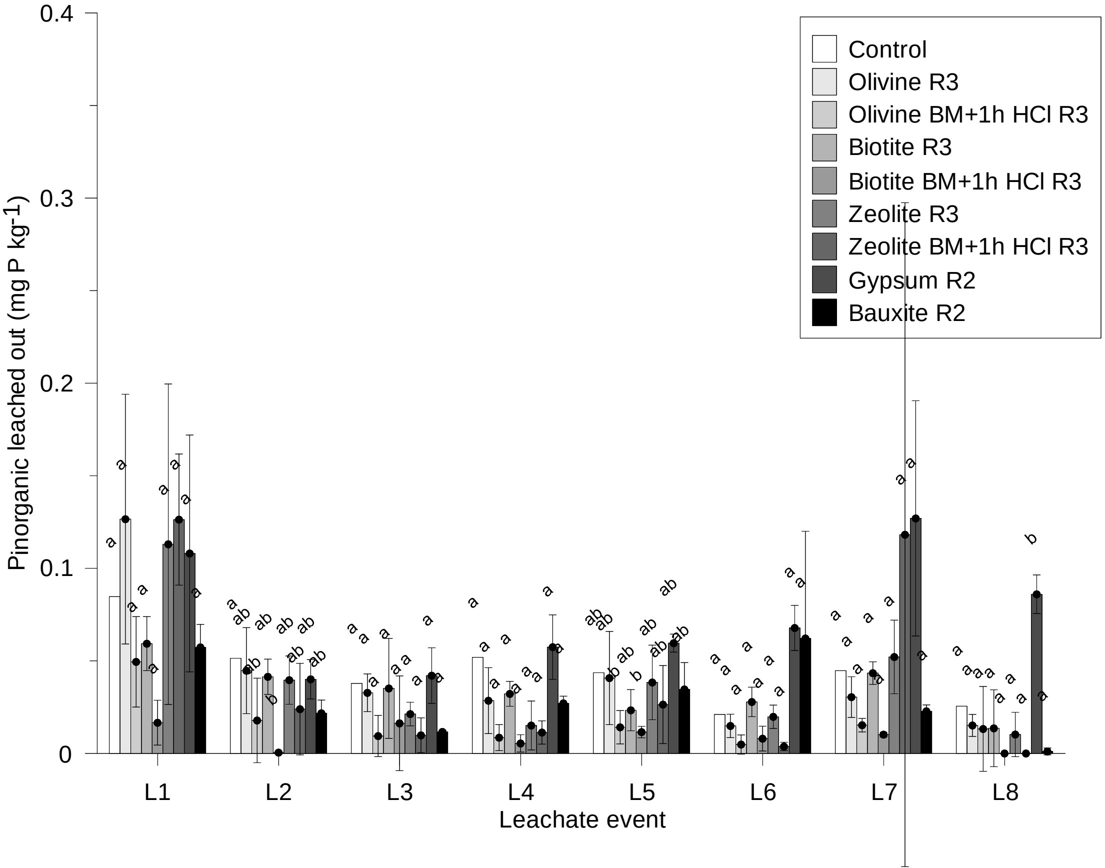

Figure I.13. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event with addition of the minerals and bauxite to Li1 (p<0.05, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

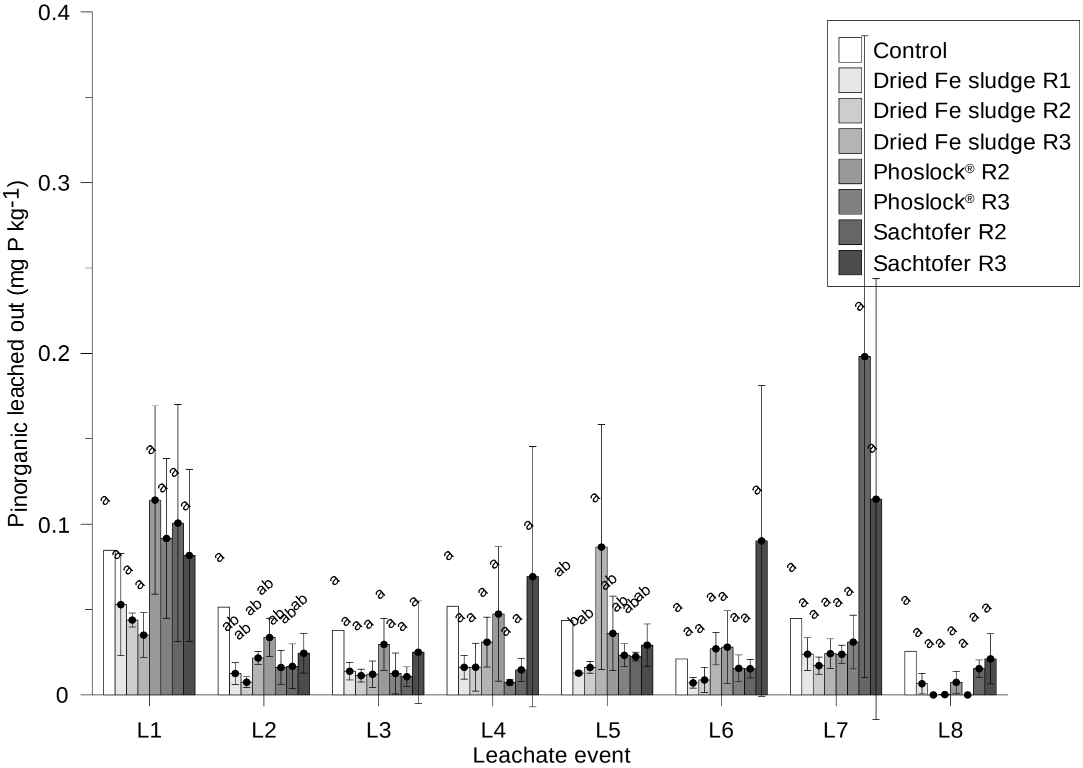

Figure I.14. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event of Li1 with addition of the by product and specially designed products (p<0.05, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

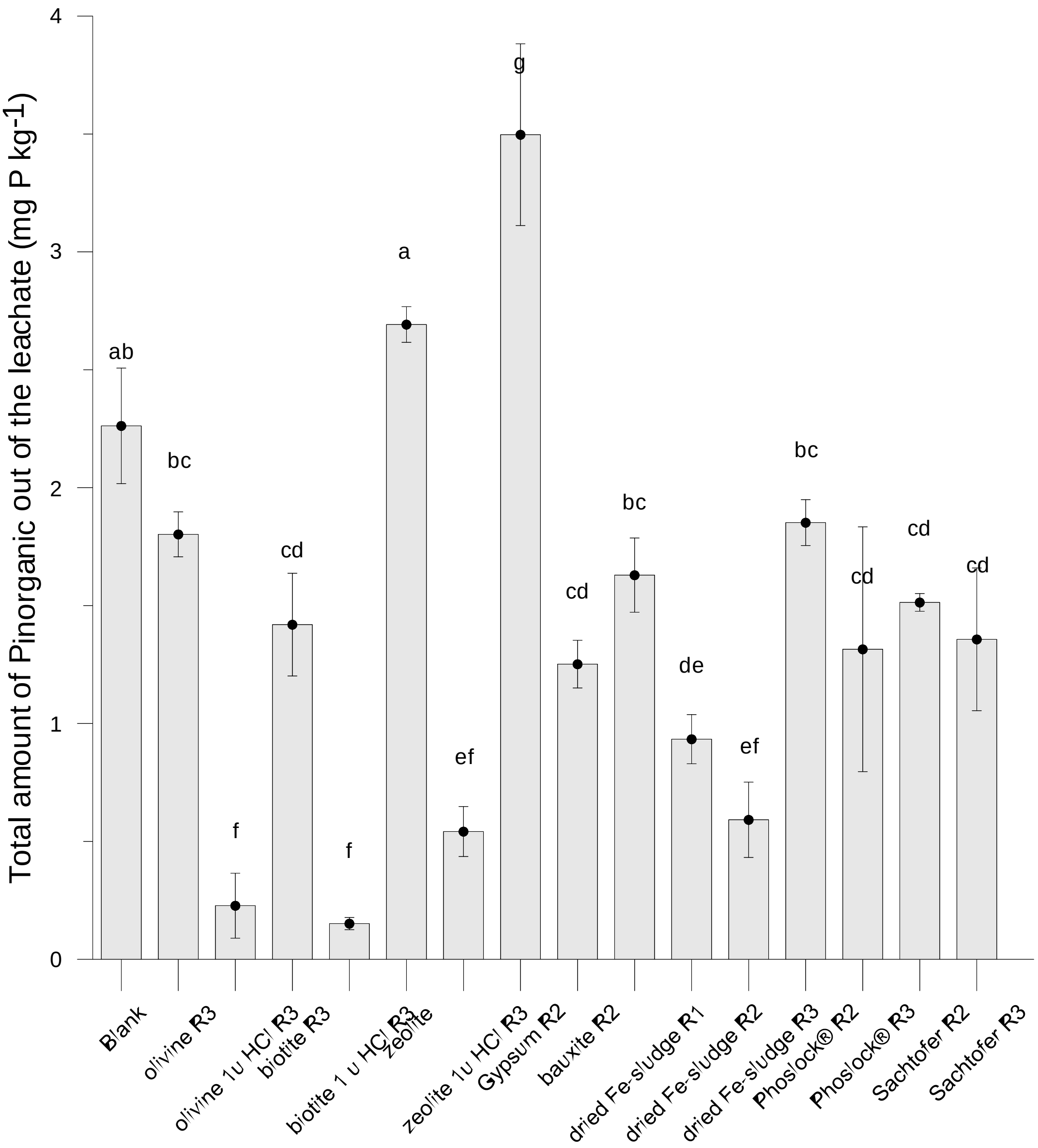

Figure I.15. Total $P_{inorganic}$ (mg P kg^{-1}) leached out of Li2 over the eight leachate events with addition of minerals, bauxite, an industrial by product or specially designed products ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments)

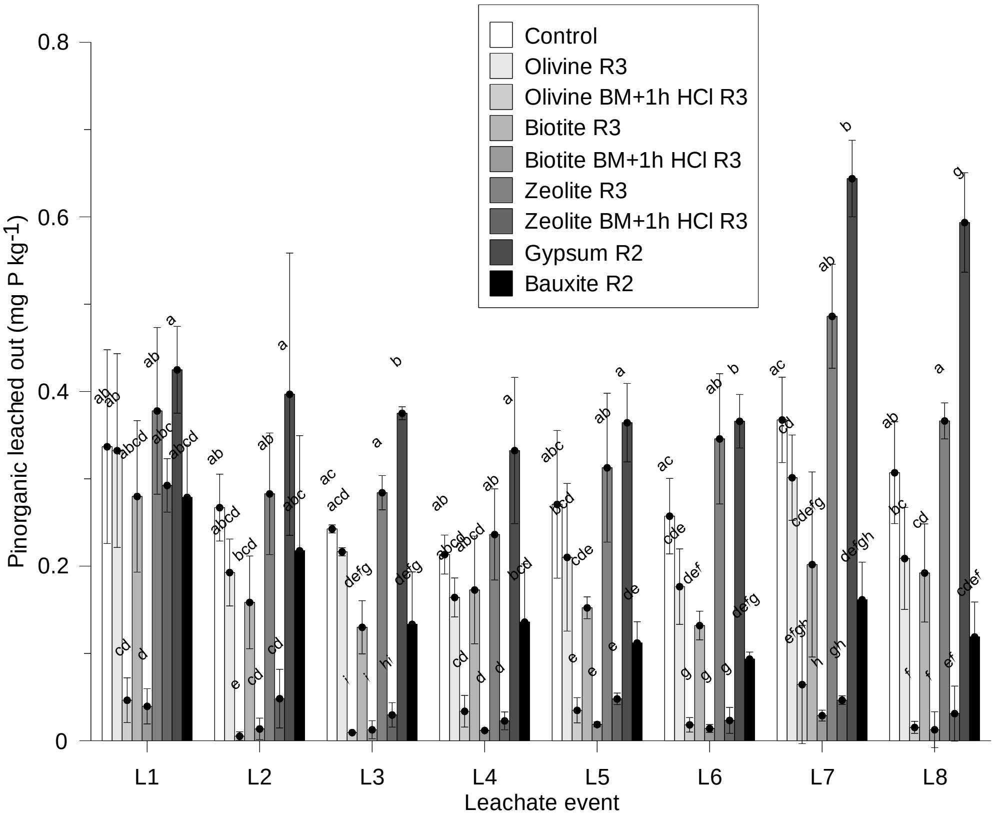

Figure I.16. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event with addition of the minerals and bauxite to Li2 ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

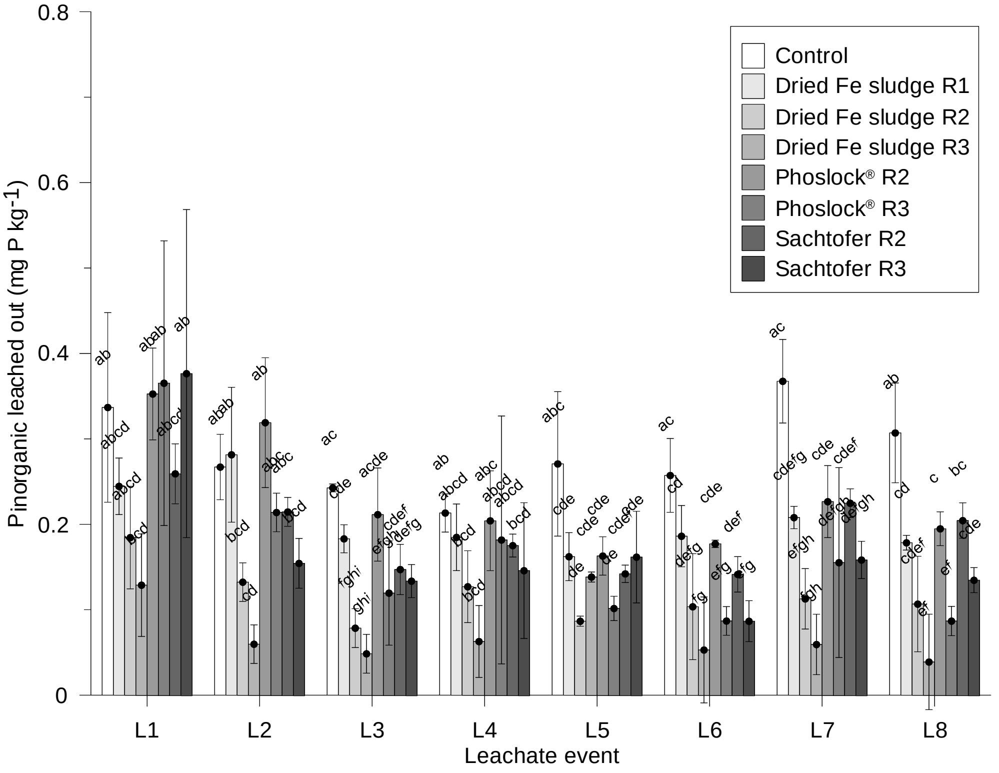

Figure I.17. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event of Li2 with addition of the by product and specially designed products ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

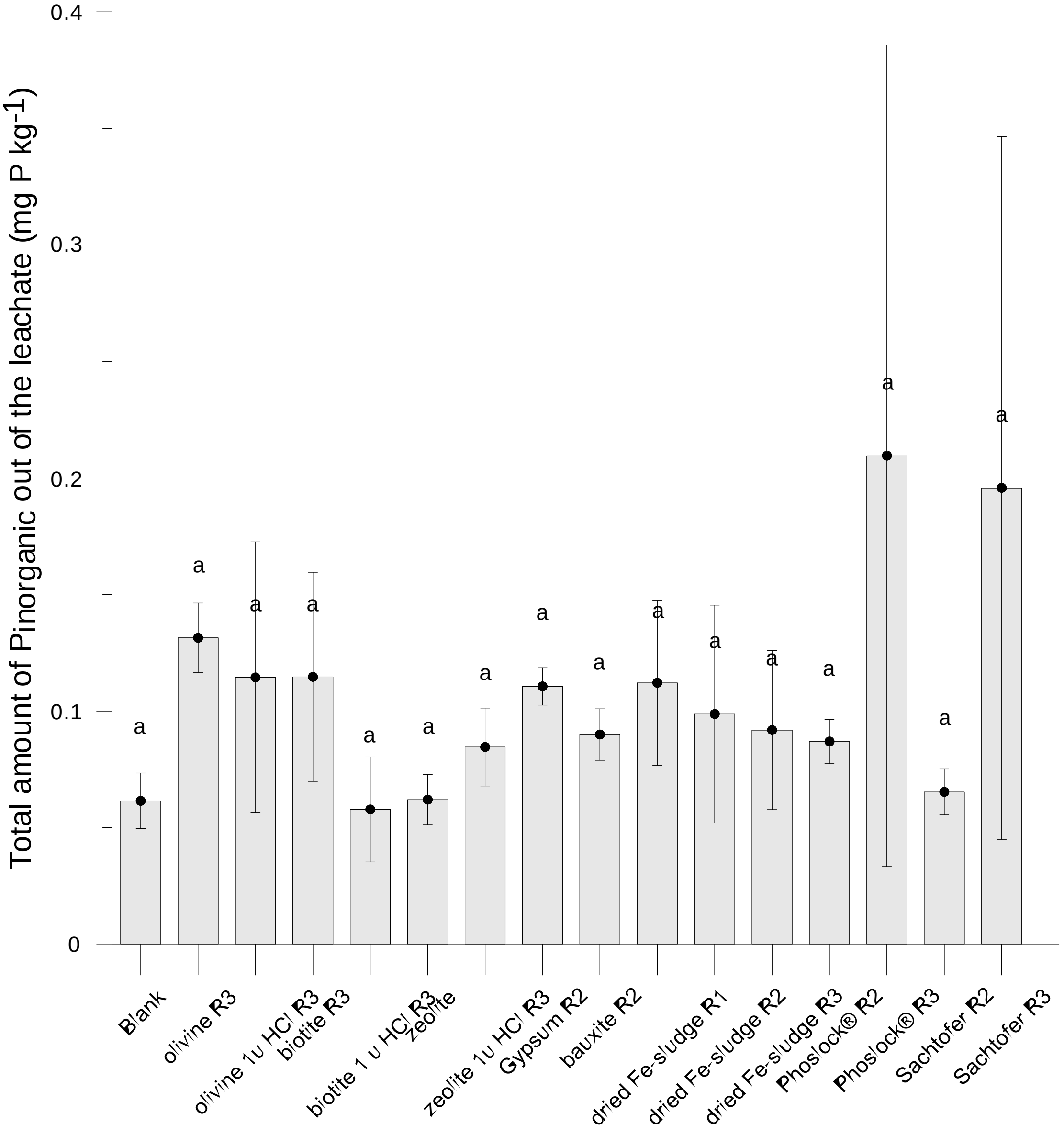

Figure I.18. Total $P_{inorganic}$ (mg P kg^{-1}) leached out of Li3 over the eight leachate events with addition of minerals, bauxite, an industrial by product or specially designed products ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments)

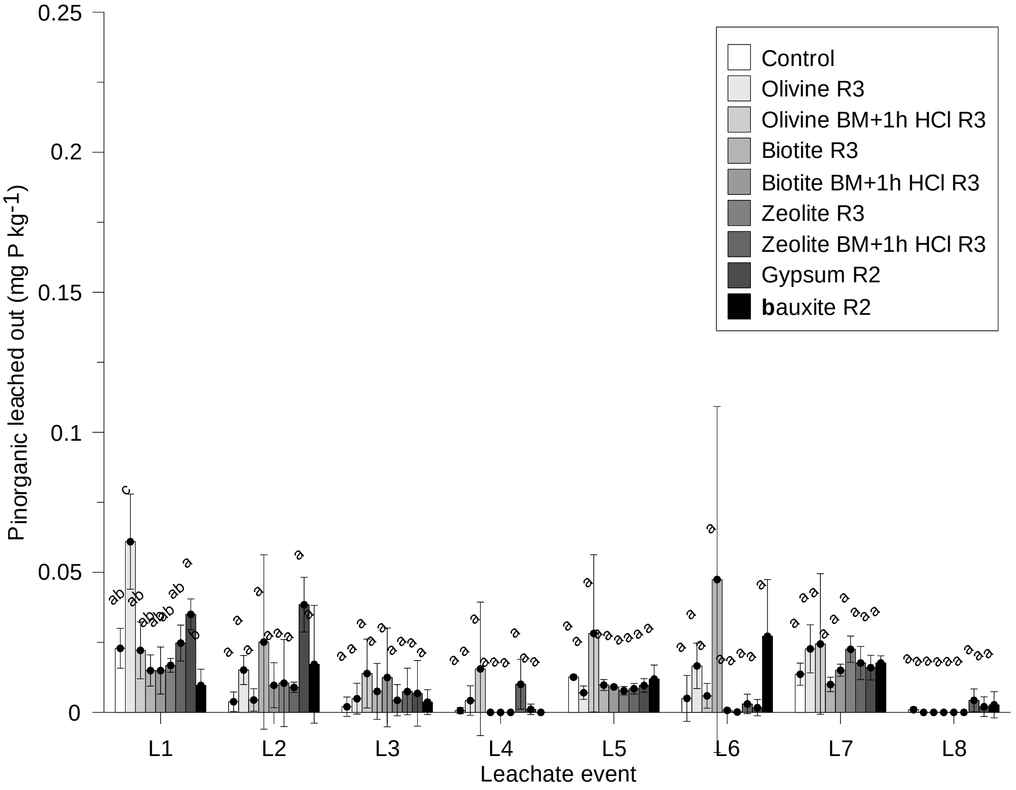

Figure I.19. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event with addition of the minerals and bauxite to Li3 ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

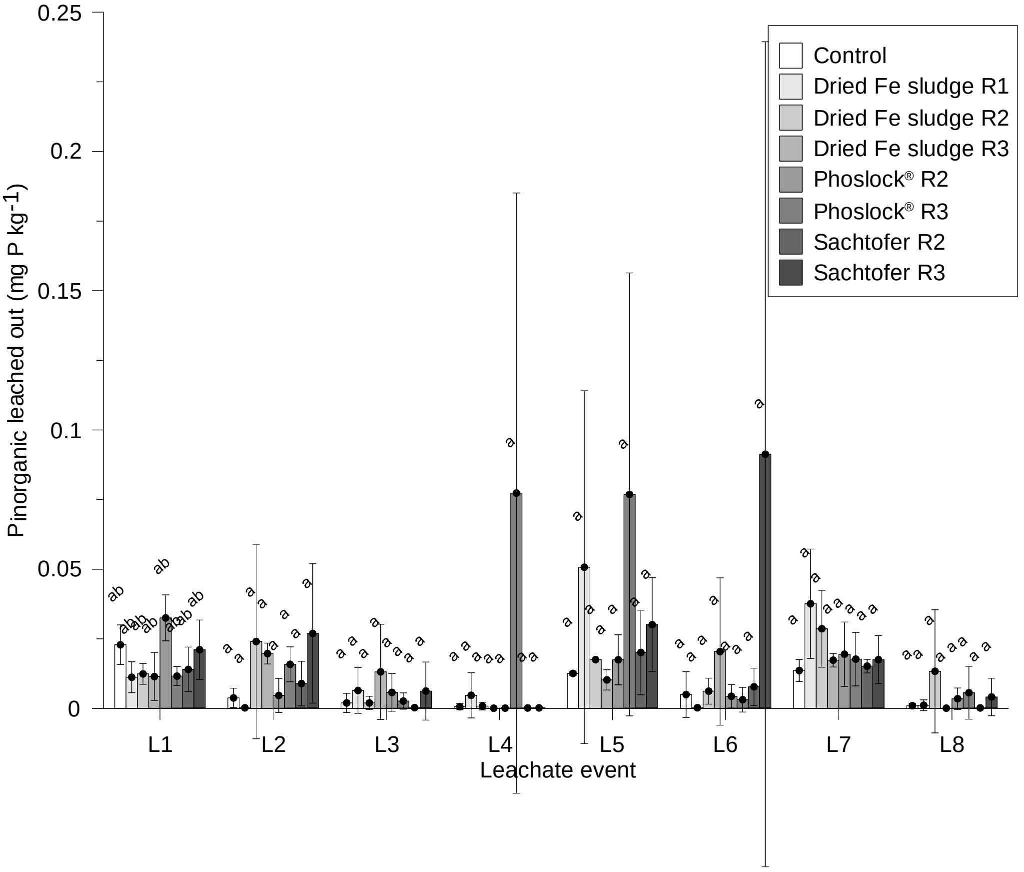

Figure I.20. Amount of $P_{inorganic}$ (mg P kg^{-1}) leached per leachate event of Li3 with addition of the by product and specially designed products ($p<0.05$, significance of amount of $P_{inorganic}$ loss between treatments per leachate event)

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1995 – 2001 Sint Gertrudis college, Wetteren

Secondary education, Sciences-Mathematics

2001 – 2007 Ghent University, Faculty of Bioscience Engineering

Bio-engineer in Land and Forest Management, option Soil and Water

Thesis subject : N-losses from crop residues in organic and conventional cropping systems (promotors Prof. dr.ir. S. De Neve, Ir. Van Den Bossche Annemie)

2009 – 2012 Instituut voor permanente vorming

Environmental coordinator, type A

PROFESSIONAL EXPERIENCE

2007-2013 Department of soil management, Ghent University, Research assistant with Prof. Dr. ir. S. De Neve scientific promotor.

SCIENTIFIC PUBLICATIONS

Publications in international Journals with peer review (A1)

De Bolle S., De Neve S. & Hofman G. 2013. Rapid redistribution of P to deeper soil layers in P saturated acid sandy soils. *Soil Use and Management,* 29**,** 76-82.

De Bolle S., Gebremikael M.T., Maervoet V. & De Neve S. 2013. Performance of phosphate-solubilizing bacteria in soil under high phosphorus conditions. *Biology and Fertility of Soils*, *49, 705-714.*

Baitilwake, M.A., De Bolle, S., Salomez, J., Mrema, J.P. & De Neve, S. 2012. Effect of organic fertilizers on nitrate accumulation in vegetables and mineral nitrogen in tropical soils of Morogoro, Tanzania. *Experimental Agriculture, 48, 111-126.*

Baitilwake, M.A., De Bolle, S., Salomez, J., Mrema, J.P. & De Neve, S. 2011. Effects of manure nitrogen on vegetables' yield and nitrogen efficiency in Tanzania. *International Journal of Plant Production*, 5, 417-420.

Verspecht, A., Vandermeulen, V., De Bolle S., Moeskops, B., Vermang, J., Van Den Bossche, A., Van Huylenbroek, G. & De Neve, S. 2011. Integrated policy approach to mitigate soil erosion in West Flanders. *Land Degradation & Development*, 22, 84-96.

Van den Bossche A, De Bolle S, De Neve S, Hofman G. 2009. Effect of tillage intensity of different crop residues in a temperate climate. *Soil Tillage & Research,* 103, 316-324.

Sleutel, S., Moeskops, B., Huybrechts, W., Vandenbossche, A., Salomez, J., De Bolle, S., Buchan, D. & De Neve, S. 2008. Modeling soil moisture effects on net nitrogen mineralization in loamy wetland soils. *Wetlands*, 28, 724-734.

Other Publications

De Bolle S. & De Neve, S. 2013. Minerals and wastewater treatment products effectively increase P sorption capacity in acidic sandy soils. NEV2013, International Workshop on Nitrogen, Environment and Vegetables, April 15-17, Torino, Italy, p. 125

De Bolle S. & De Neve, S. 2012. Fixation of phosphorus by adding amendments to P saturated soils in Flanders. Eurosoil, Soil Science for the Benefit of Mankind and Environment, July 2-6, Bari, Italy, p. 697

De Bolle S., Van der Salm, C., Schoumans, O.F. & De Neve, S. 2012. Predicting phosphorus losses with the model PLEASE in the acid sandy region of Flanders. Eurosoil, Soil Science for the Benefit of Mankind and Environment, July 2-6, Bari, Italy, p. 2463

De Bolle S., Van der Salm, C., Schoumans, O.F. & De Neve, S. 2012. Predicting phosphorus losses with the model PLEASE in the acid sandy region of Flanders. Day of Young Soil Scientists, February 22, Brussels, Belgium, p. 26

De Bolle S., Van der Salm, C., Schoumans, O.F. & De Neve, S. 2011. Predicting phosphorus losses with the model PLEASE in the acid sandy region of Flanders, October 12-14, Keszthely, Hungary, p. 23

De Bolle, S., Mesfin, G.T. & Neve, S. 2011. Investigation of the P solubilizing effect of phosphate solubilizing bacteria in high P conditions. International symposium of Interactions of Soil Minerals with Organic Components and Microorganisms: Soil Interfaces in an changing world, June 26-July 1, Montpellier, France, p. 94

De Bolle S., Mesfin, T.G. & De Neve, S. 2011. Can phosphate solubilising bacteria be of use on phosphate saturated soils? Day of Young Soil Scientists, February 21, Brussels, Belgium, p. 23

De Bolle S. & De Neve, S. 2010. Evolution in time of the phosphate saturation level in generally and changes within the depth layers on a field. In book of abstract of IPW6, International Phosphorus Workshop, September, Seville, Spain, p. 30

De Bolle, S., Mesfin, T. G. & De Neve, S. 2010. Can Phosphate Solubilising Bacteria be of use on phosphate saturated soils? Workshop WG 2 & WG 3 COST 869, Jokionen, Finland, p. 40

De Bolle, S. & De Neve, S. 2009. P-forms and their behaviour in Flemish soils. Symposium on applied biological sciences, November 6, Leuven, Belgium, p. 8

Salomez, J., De Neve, S., De Bolle, S., Van den Bossche, A., Sleutel, S. & Hofman, G. 2009. Impact of nutrients on the environment and nutrient legislation. Earth, Bioresources: Social, Biological, Food and Energy challenges, 50 – 56.

Morais, P., De Neve, S, De Bolle, S. & Cordovil, C. 2009. Nitrous oxide emissions from soils amended with different types of exogenous organic materials. 16th Nitrogen Workshop, 181 – 182.

Ameloot, N., De Neve, S. & De Bolle, S. 2009. N_2O emissions from soil with different biochar amendments. Proceedings of the North American Biochar conference, Boulder, USA.

De Bolle, S., Salomez, J., Van Hoof, K., De Neve, S. & Hofman, G. 2009. River basin management plan for the River Scheldt in Flanders. Workshop WG 3 COST 869, May 18-19, Wageningen, The Netherlands, p. 38

De Bolle, S., Salomez, J., De Neve, S. & Hofman, G. 2009. P-forms and their behaviour in Flemish soils. Day of Young Soil Scientists, February 25, Brussels, Belgium, p.29

Salomez J., De Bolle S., Bries J., De Neve S. & Hofman G. 2008. Potassium and magnesium in manures and organic by products. Proceedings No 619, International Fertilizer Society, York, UK, 1-23.

De Bolle, S., Salomez, J., De Neve, S. & Hofman, G. 2008. The Manure decree as large scale measure to reduce nutrient loads in Flanders. Workshop WG 4 COST 869, May 18-22, Waidhofen an der Ybbs, Austria

Van Meirvenne M., Tariku M., De Neve S., Hofman G., Salomez J. & De Bolle S. 2008. Afbakening van de fosfaatverzadigde gebieden in Vlaanderen op basis van een kritische fosfaatverzadigingsgraad van 35%. Onderzoeksopdracht VLM/Mestbank/TWOL2006/MB2006/5, eindrapport. Vlaamse Landmaatschappij-VLM, Mestbank. 58 pp.

POSTER AND ORAL PRESENTATIONS

De Bolle S. & De Neve, S. 2013. Minerals and wastewater treatment products effectively increase P sorption capacity in acidic sandy soils. NEV2013, International Workshop on Nitrogen, Environment and Vegetables, April 15-17, Torino, Italy. *Poster presentation*

De Bolle, S. Van Craenenbroeck, K. & De Neve, S. 2013. Minerals and wastewater treatment products effectively increase P sorption capacity in acidic sandy soils. Scientific European P workshop, February 6-7, Wageningen, The Netherlands. *Poster presentation*

De Bolle S. & De Neve, S. 2012. Fixation of Phosphorus by adding amendments to P saturated soils in Flanders. Eurosoil, Soil Science for the Benefit of Mankind and Environment, July 2-6, Bari, Italy. *Oral presentation*

De Bolle S., Van der Salm, C., Schoumans, O.F. & De Neve, S. 2012. Predicting phosphorus losses with the model PLEASE in the acid sandy region of Flanders. Eurosoil, Soil Science for the Benefit of Mankind and Environment, July 2-6, Bari, Italy. *Poster presentation*

De Bolle S., Van der Salm, C., Schoumans, O.F. & De Neve, S. 2012. Predicting phosphorus losses with the model PLEASE in the acid sandy region of Flanders. Day of Young Soil Scientists, February 22, Brussels, Belgium. *Poster presentation*

De Bolle S., Van der Salm, C., Schoumans, O.F. & De Neve, S. 2011. Predicting phosphorus losses with the model PLEASE in the acid sandy region of Flanders. October 12-14, Keszthely, Hungary. *Poster presentation*

De Bolle, S., Mesfin, G.T. & Neve, S. 2011. Investigation of the P solubilizing effect of phosphate solubilizing bacteria in high P conditions. International symposium of Interactions of Soil Minerals with Organic Components and Microorganisms: Soil Interfaces in an changing World, June 26-July 1, Montpellier, France. *Oral presentation*

De Bolle S., Mesfin, T.G. & De Neve, S. 2011. Can phosphate solubilising bacteria be of use on phosphate saturated soils? Day of Young Soil Scientists, February 21, Brussels, Belgium. *Poster presentation*

De Bolle S. & De Neve, S. 2010. COST 869: A European research approach on tackling the N&P eutrophication and leaching problems to water bodies. ISEI7 - 7th International Conference on Ecological Informatics, December 13-16, Ghent, Belgium. *Oral presentation*

De Bolle S. & De Neve, S. 2010. Evolution in time of the phosphate saturation level in generally and changes within the depth layers on a field. In Book of Abstract of IPW6, International Phosphorus Workshop, September 27- October 1, Seville, Spain. *Oral presentation*

De Bolle, S., Mesfin, T. G. & De Neve, S. 2010. Can phosphate solubilising bacteria be of use on phosphate saturated soils? Workshop WG 2 & WG 3 COST 869, Jokionen, Finland. *Poster presentation*

De Bolle, S. & De Neve, S. 2009. P-forms and their behaviour in Flemish soils. Symposium on applied biological sciences, November 6, Leuven, Belgium. *Poster presentation*

De Bolle, S., Salomez, J., Van Hoof, K., De Neve, S. & Hofman, G. 2009. River basin management plan for the River Scheldt in Flanders. Workshop WG 3 COST 869, May 18-19, Wageningen, The Netherlands. *Poster presentation*

De Bolle, S., Salomez, J., De Neve, S. & Hofman, G. 2009. P-forms and their behaviour in Flemish soils. Day of Young Soil Scientists, February 25, Brussels, Belgium. *Poster presentation*

De Bolle, S., Salomez, J., De Neve, S. & Hofman, G. 2008. The Manure decree as large scale measure to reduce nutrient loads in Flanders. Workshop WG 4 COST 869, May 18-22, Waidhofen an der Ybbs, Austria. *Oral presentation*

Attendance at conferences

European Sustainable Phosphorus Conference. 2013, March 6-7, Brussels, Belgium.

Nitrate conference. 2012, May 10-11, Copenhagen, Denmark.

Bioforum “bio, bodem en bemesting”. 2011, March 16, Kruishoutem, Belgium.

VCM “een nieuwe markt voor mest”. 2011, March 16, Gent, Belgium.

GPS – opbrengst schatten. June 1, 2010, Dronten, The Netherlands.

Closing congress Striver. 2009, May 28-29, Brussels, Belgium.

VCM “wat rest na mest?”. 2010, January 14, Bruges, Belgium.

IPW5 (International Phosphorus Workshop) Diffuse Phosphorus Loss Risk Assessment, Mitigation Options and Ecological Effects in River Basins. 2007, September 3-7, Silkeborg, Denmark.

Student guidance

Guidance of practical exercises

Earth sciences (1st bachelor B. Sc.)

Soil science (3rd bachelor B. Sc.)

Nutrient management (1st master B. Sc.)

Soil Fertility (Physical Land Resources)

Environmental Soil Science (Environmental sanitation)

Guidance of excursions

Earth sciences

Soil science

Organic farming

Tutor of thesis

Ranises, M.B., PLR thesis (2012-2013). Phosphorus leaching from non-acidic or non-sandy soils: Evaluating the P Saturation Protocol.

Van Craenenbroeck K., Applied Biological Sciences thesis (2012-2013). Effect van toedienen van silicaten op fosfaatverzadigde landbouwbodems.

Loosvelt, L., thesis Environmental coordinator, type A (2011-2012). Gebruik van het model PLEASE bij de identificatie van risicogebieden voor fosfor uitspoeling in Vlaanderen.

Fatouma, O.S., PLR thesis (2010-2011). Fixation of phosphorus by adding amendments to phosphate saturated soils in Flanders.

Braems B., Applied Biological Sciences thesis (2010-2011). Effecten van wortelexudaten op de fosforbeschikbaarheid in fosfaatverzadigde bodems.

Tsegaye Mesfin, G., PLR thesis (2009-2010). effects of phosphate solubilising bacteria for increasing phosphorus availability in intensive agricultural soils in Flanders.