

Running Title: PHOSPHORUS USE EFFICIENCY IN BIO-BASED FERTILIZERS

## Phosphorus Use Efficiency in Bio-based Fertilizers: a Bio-Availability and Fractionation Study

Céline VANEECKHAUTE<sup>1,2,\*</sup>, Joery JANDA<sup>1</sup>, Peter VANROLLEGHEM<sup>2</sup>, Filip TACK<sup>1</sup> and Erik MEERS<sup>1</sup>

<sup>1</sup>Laboratory for Analytical and Applied Ecochemistry, Faculty of Bioscience Engineering, Ghent University, Ghent (Belgium)

<sup>2</sup>modelEAU, Département de génie civil et de génie des eaux, Faculté de Sciences et de Génie, Université Laval, Québec, QC (Canada)

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### ABSTRACT

Although to date some technologies producing bio-based phosphorus (P) fertilizers have been proposed and implemented, the efficient use of the recovered products is still limited due to legislative constraints, lack of insights in their P release with time, and in the corresponding mechanisms. The aim of this paper was to evaluate the fertilizer performance in terms of P release and use efficiency of recovered struvite, iron phosphate (FePO<sub>4</sub>) sludge, digestate, and animal manure as compared to fossil reserve-based mineral triple superphosphate (TSP). First, product physicochemical characteristics and P fractionations in the context of European fertilizer legislation were assessed. Next, a controlled greenhouse experiment was set up to evaluate plant reactions as well as changes in P availability on sandy soils with both high and low P status. P soil fractions were determined in extracts with water (P<sub>w</sub>), ammonium lactate (PAI), CaCl<sub>2</sub> (P-PAE), and in soil solution sampled with Rhizon soil moisture samplers (Prhizon). Based on all results, long-term field trials evaluating the P release effect of struvite and digestate as compared to animal manure and TSP on different soil types with varying P status appear to be worthwhile. These products show promise as sustainable substitutes for conventional P fertilizers and could contribute to a more efficient use of P in agriculture. A refined classification of P application standards/recommendations in terms of soil P status, texture, and fertilizer characteristics, next to the crop P demand, is recommended. Moreover, the additional use of Rhizon samplers for determination of direct available P, including dissolved organic P, is proposed for better understanding and categorization of different P fertilizers in environmental and fertilizer legislations.

**Key Words:** alternative fertilization strategies, digestate, green agriculture, iron phosphate sludge, nutrient recycling, struvite, sustainable resource management

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### INTRODUCTION

The rapid and increasing phosphorus (P) consumption in modern agriculture has raised concerns on both its supply security (Elser and Bennett, 2011; Godfray *et al.*, 2010; Neset and Cordell, 2012; Scholz and Wellmer, 2013) and its impact on the environment (soil P accumulation, leaching, and/or eutrophication) (Kang *et al.*, 2011; Ranatunga *et al.*, 2013; Syers *et al.*, 2008). Consequently, the effective use of soil P and P containing mineral and organic fertilizers, as well as the cradle-to-cradle recycling of P from municipal, agricultural, and other biodegradable waste sources as green renewable fertilizers with high P use efficiency (e.g. slow-release granules), has become highly important (Huang *et al.*, 2012; Ma *et al.*, 2011; Schröder *et al.*, 2011; Syers *et al.*, 2008; Zhang *et al.*, 2013).

Traditional P removal processes from waste(water) streams often involve the addition of iron (Fe) or aluminium (Al) salts, resulting in the production of substantial quantities of Fe/AlPO<sub>4</sub>-sludge

\*Corresponding author. E-mail: [CelineVaneeckhaute@gmail.com](mailto:CelineVaneeckhaute@gmail.com).

(Sano *et al.*, 2012). Alternatively, in the past decades, the controlled precipitation of struvite ( $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ ) through addition of Mg to the waste flow has gained interest as a route for P recovery (Latifian *et al.*, 2012; Ryu *et al.*, 2012; Shu *et al.*, 2006). Moreover, the anaerobic (co-)digestion of animal manure, sludges, organic biological food waste, and/or energy crops has proven to be an effective technology for bio-energy production and release/mineralization of nutrients, which are concentrated in the remaining digestate (Fehrenbach *et al.*, 2008). During a preceding field-scale assessment, it has been observed that the use of a formulated mixture of digestate with its liquid fraction (LF) in agriculture as substitute for animal manure may stimulate P mobilization in the soil, thereby increasing the use efficiency of soil minerals (to be confirmed) (Vaneckhaute *et al.*, 2013, 2014). Especially in P saturated regions (e.g. Flanders, Quebec, Eastern China, Italy, Northern Spain, etc.; MacDonald *et al.*, 2011), the extraction of P from agricultural fields is relevant, for example, to export the recovered P towards P deficient regions, for local reuse (e.g. in the horticultural sector or for plants with high P demand), and/or for industrial purposes. On the other hand, although the use of LF digestate (with high effective nitrogen (N) over P ratio) is interesting in terms of current legislative fertilization standards, its supply of plant available P may be insufficient, depending on the crop P demand and the soil P status. Hence, additional fertilization with a source of bio-available P may be required.

In this context it must be understood that only a small proportion (15--20%) of the total amount of P in the plant (uptake:  $\pm 2.5 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1} \text{ d}^{-1}$ ; EFMA, 2000) is directly provided by the fertilizer applied to that crop. The remainder comes from soil reserves. Hence, there must be adequate reserves of readily available P in the soil (Syers *et al.* 2008). The P status of European soils has been estimated by EFMA (2000). For many countries, some 25% (5--55%) of soils test as very low and low in readily available P. Such soils require significantly more P to be applied than is removed by the crop to increase soil reserves and thus soil fertility. On the other hand, in many countries, some 40% (15--70%) of soils test as high and very high in readily available P. On such soils, when crops are grown that have small, inefficient root systems, but a large daily uptake of P at critical growth stages, it may be necessary to apply more P. On soils with a medium P analysis value, applications need to sustain the P status. This may require a small extra amount of P on top of that removed with the harvested crop (EFMA, 2000).

All the above clearly indicates the relevance of fundamental comparative research on the P release pattern in time of potential sustainable alternatives for chemical P fertilizers produced from natural and exhaustive phosphate rock and/or for animal manure (products with improved P use efficiency). Such an evaluation is essential to determine the agricultural potential of new fertilizers and their responsible application (Erro *et al.*, 2011). Yet, such studies are currently lacking in literature for the above-mentioned bio-based products (struvite, digestate, Fe/AlPO<sub>4</sub>-sludge), although their production and availability is on the rise (Vaneckhaute *et al.*, 2015).

The performance of a fertilizer can be evaluated via i) product fractionation, ii) plant reaction analysis, and/or iii) chemical soil analysis (Dekker and Postma, 2008; Millier and Hooda, 2011; Prummel and Sissingh, 1983; Singh *et al.*, 2005; Sissingh, 1971; van Dam and Ehlert, 2008; Wang *et al.*, 2013). A P fractionation of fertilizers is, in general, based on the P solubility in solvents with different strength and selectivity (e.g. Frossard *et al.*, 2002; He *et al.*, 2004, 2007). With respect to European (EU) legislation, the most important solvents are, ranked from strong to weak: i) mineral acid (MA), ii) neutral ammonium citrate solution (NAC), and iii) water (EC, 2003). Next to the P solubility, the fertilizer performance is usually expressed as bio-availability indices, such as the phosphorus use efficiency (PUE). It can be based on the fresh weight (FW) and dry weight (DW) yield, the growth rate (FW, DW), the P uptake (rate), and the degree and rate in which the P status of the soil changes, as determined by chemical methods (van Dam and Ehlert, 2008). Previous studies have shown that the crop response to P fertilization gives insufficient guidance to determine the fertilizer performance (no correlation), while chemical soil analyses can be conclusive (Árendás and Csathó, 2002; Prummel and Sissingh, 1983; van Dam and Ehlert, 2008). Therefore, most studies evaluating P fertilizers to date are based on soil bioavailability indices. Soil measurements can be divided into P capacity and P intensity of the soil, based on the strength of the extraction method. The P intensity gives an indication of the total amount of inorganic P which is directly available for the plant during a short period of time, while the P capacity gives an indication of the amount of P that may be released in the long term (Dekker and Postma, 2008).

In some countries, e.g. the Netherlands, Switzerland, and Norway, fertilizer recommendations are based on the P status of the soil, measured as PAI and Pw number. It corresponds to an extraction with ammonium lactate and water, respectively (Ehlert *et al.*, 2006; Singh *et al.*, 2005; Sissingh, 1971). The PAI number is a measure of the P capacity of the soil, whereas the Pw number reflects a combination of the soil P capacity and intensity. In the latest decade, also the PAE method (Plant

Available Elements) has received increased attention. It concerns a multi-element extraction with 0.01 M calcium chloride ( $\text{CaCl}_2$ ), and hence provides a simple alternative for the many extraction procedures that are currently used for single nutrients (Ehlert *et al.*, 2006; Houba *et al.*, 2000; van Erp *et al.*, 1998). With respect to P (P-PAE), this measurement gives an indication of the P intensity (Houba *et al.*, 2000). An important limitation of all these standard methods is that root formation, soil compaction, and mineralization of organic matter is not or not sufficiently accounted for (Amoakwah *et al.*, 2013; Ehlert *et al.*, 2006; Soine, 2009). Underestimations have been observed in literature, especially for the determination of direct available P (Amoakwah *et al.*, 2013; Sánchez-Alcalá *et al.*, 2014). Alternatively, the use of Rhizon soil moisture samplers (SMS) allows assessing the total amount of P in the actual soil solution (Rhizon), including dissolved organic and inorganic forms (Eijkelkamp, 2003). Besides the ease of sampling, Rhizon SMS for direct extraction of soil moisture also overcomes disadvantages related to traditional sampling using ceramic cups, such as the exchange of (divalent) cations and P (Grossmann and Udluft, 1991). Moreover, in contrast to the above standard methods, the use of Rhizon samplers is not destructive, less laborious and time consuming, and most importantly, it does not change the composition of the soil solution in the process of extracting it (Amoakwah *et al.*, 2013; Sánchez-Alcalá *et al.*, 2014).

The first aim of this study is to evaluate the fertilizer performance of bio-based recovered products (struvite,  $\text{FePO}_4$ -sludge, digestate from co-digestion) and pig manure as compared to fossil reserve-based mineral fertilizer, triple superphosphate (TSP,  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ ). After product physicochemical analysis and P fractionation (in the framework of EU legislation), a controlled greenhouse experiment was set up in order to: i) evaluate the PUE based on plant reactions and changes in the chemical soil P bio-availability status during the most critical main growing period, and ii) confirm and further study under precise conditions some nutrient release mechanisms previously observed under practical field conditions (see above; Vaneeckhaute *et al.*, 2013, 2014, 2015). A second aim is to overcome the limitations of standard soil extraction methods by using Rhizon SMS to determine the P delivery in the short term. A controlled greenhouse experiment was preferred for this purpose above a field trial so as to minimize potential soil disturbances, e.g. of hydraulic levels, to which the various extraction methods are sensitive (Eijkelkamp, 2003). Based on the results, practical implications are discussed and recommendations in terms of legislative revisions and associated further field research are provided. As such, this paper gives valuable information to guide further efforts to optimize P supply and minimize accumulation and eutrophication risks, aiming at a more responsible and efficient use of P in agriculture.

## MATERIAL AND METHODS

### *Product characterization and phosphorus fractionation*

The DW content was determined as residual weight after 72 h drying at 80 °C in an oven (EU 170, Jouan s.a, Saint Herblain, FR). Organic carbon (OC) was determined after incineration of the dry samples during 4 h at 550 °C in a muffle furnace (Nabertherm, Lilientahl, DE). The loss of ignition (= weight loss after incineration) was divided by a conversion factor of 1.72 to calculate OC, hence assuming that organic matter contains 58% OC (Van Ranst *et al.*, 1999). Electrical conductivity (EC) and pH were determined potentiometrically using a WTW-LF537 (Wissenschaftlich Technischen Werkstätten, Weilheim, DE) electrode and an Orion-520A (Orion Research, Boston, USA) pH-meter, respectively. The solid samples were first equilibrated for 1 h in deionized water at a 5:1 liquid to dry sample ratio and subsequently filtered (MN 640 m, Macherey-Nagel, DE). Total N content was determined using a Kjeltex system 1002 distilling unit (Gerhardt Vapodest, Königswinter, DE) after digestion of the sample in a sulphuric-salicylic acid mixture. The captured ammonia ( $\text{NH}_3$ ) in the distillate was then titrated with 0.01 mol  $\text{L}^{-1}$  hydrogen chloride (HCl) in the presence of a methyl red bromocresol green mixed indicator (Van Ranst *et al.*, 1999). Ammonium ( $\text{NH}_4$ ) was determined using the Kjeltex-1002 distilling unit after addition of magnesium oxide (MgO) to the sample and subsequent titration (Van Ranst *et al.*, 1999). The amount of effective N for organic fertilizers was calculated from the analysis of total N and  $\text{NH}_4$ -N based on the official formula used for the determination of fertilizer N recommendations (Inagro, Beitem, BE, personal communication 2012): Effective N = ( $N_{\text{tot}} - \text{NH}_4\text{-N}$ ) X 0.475 + ( $\text{NH}_4\text{-N}$  X 0.8). It states that 80% of the  $\text{NH}_4$ -N is plant available. On top of that, 47.5% of the remaining N, i.e. nitrates and organic N, becomes plant available in the short term. Total P was determined using the colorimetric method of Scheel (1936; Van Ranst *et al.*, 1999) after wet digestion of the liquid samples using nitric acid ( $\text{HNO}_3$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). The absorbance at 700 nm of samples and standards was determined using a Jenway 6400 spectrophotometer (Barloworld Scientific T/As Jenway, Felsted, UK). Calcium (Ca),

magnesium (Mg), and potassium (K) were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Vista MPX, Palo Alto, USA) (Van Ranst *et al.*, 1999) after wet digestion in HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>. The determination of the fraction of P soluble in water, mineral acid (= mixture of HNO<sub>3</sub> and sulfuric acid, H<sub>2</sub>SO<sub>4</sub>), and neutral ammonium citrate ((NH<sub>4</sub>)<sub>2</sub>C<sub>6</sub>H<sub>6</sub>O<sub>7</sub>) was determined as described in EC (2003).

#### Greenhouse experimental set-up

Soils used in the greenhouse experiment were: i) a nutrient-rich sandy soil with high P status (Pw > 55 mg P<sub>2</sub>O<sub>5</sub> L<sup>-1</sup> soil; Alterra, 2012) from Ranst, Belgium (pH = 5.0; EC(1:5) = 111 μS cm<sup>-1</sup>; bulk density = 1.262 kg L<sup>-1</sup>; oxalate extractable Fe/Al: Fe<sub>ox</sub> = 34 mmol kg<sup>-1</sup> soil, Al<sub>ox</sub> = 66 mmol kg<sup>-1</sup> soil), and ii) a nutrient-poor, P deficient (Pw < 36 mg P<sub>2</sub>O<sub>5</sub> L<sup>-1</sup> soil; Alterra, 2012) laboratory-grade Rheinsand (pH = 7.9; EC(1:5) = 67 μS cm<sup>-1</sup>; bulk density = 1.612 kg L<sup>-1</sup>). Methods used for soil physicochemical analysis are described below. Although Rheinsand is rarely used for agricultural production, tests on this soil may provide additional information on the fertilizer effect itself, i.e. the absolute amount of available P effectively provided by the fertilizers only. Indeed, on P saturated soils (as is often the case in Flanders), differences in the P delivery by the fertilizers themselves may be difficult to detect due to large background concentrations. Moreover, the comparison between the high P and low P soil may provide information on the indirect P liberation from the soil complex as a result of fertilizer application. Hence, comparison with the Rheinsand soil was thought to be useful.

TSP was collected at Triferto, Ghent, struvite at the NuReSys water treatment plant of Clarebout Potatoes, Nieuwkerke-Heuvelland, and FePO<sub>4</sub>-sludge at the piggery of Innova Manure, Ichtegem, all in Belgium. Animal manure was sampled at the piggery of Ivaco, Gistel, Belgium, and digestate was sampled at the biogas plant SAP Eneco Energy, Houthulst, Belgium. The latter concerns a full-scale mesophilic (37 °C) anaerobic co-digestion plant (capacity: 60,000 ton y<sup>-1</sup>, 2.83 MW<sub>el</sub>) with an input feed consisting of animal manure (30%), energy maize (30%), and organic biological waste supplied by the food industry (40%). Two replicate samples of each waste stream were collected in polyethylene sampling bottles (10 L) and transported within 1 h to the laboratory for physicochemical analysis, carried in cooler boxes filled with ice (± 4 °C). In the laboratory, the replicate samples were stored cool (1-5 °C) and kept separated for separate analysis after homogenization of each particular sample. The product characteristics can be found in Table I. The obtained data were used to calculate the maximum allowable product dosage for the different cultivation scenarios in compliance with the Flemish manure decree (MAP4, 2011).

TABLE I Product physicochemical characterization (average ± standard deviation, n = 2)

Parameter	TSP <sup>a)</sup>	Struvite	FePO <sub>4</sub> -sludge	Pig manure	Digestate
pH	2.6	8.4	4.6	7.7	8.6
Conductivity (mS cm <sup>-1</sup> )	29	547	15	35	37
DW <sup>b)</sup> (%)	100	100	2.0 ± 0.0	6.2 ± 0.1	9.8 ± 0.0
OC <sup>c)</sup> (% on DW)	1.6 ± 0.0	29 ± 0	25 ± 0	37 ± 1	34 ± 1
Total P <sub>2</sub> O <sub>5</sub> (g kg <sup>-1</sup> DW)	430 ± 5	293 ± 3	26 ± 1	53 ± 0	29 ± 0
Total N (g kg <sup>-1</sup> DW)	0.49 ± 0.03	52 ± 2	55 ± 0	105 ± 0	67 ± 0
NH <sub>4</sub> -N (g kg <sup>-1</sup> DW)	0.23 ± 0.06	28 ± 1	13 ± 0	74 ± 2	39 ± 0
Effective N (g kg <sup>-1</sup> DW)	0.31 ± 0.04	34 ± 1	30 ± 1	74 ± 2	45 ± 0
Total K <sub>2</sub> O (g kg <sup>-1</sup> DW)	1.9 ± 0.3	11 ± 0	116 ± 5	74 ± 6	58 ± 0
Effective N / P <sub>2</sub> O <sub>5</sub> / K <sub>2</sub> O	0.00072/1/0.0044	0.12/1/0.038	1.1/1/4.5	1.4/1/1.4	1.5/1/2.0
Total Ca (g kg <sup>-1</sup> DW)	138 ± 1	0.58 ± 0.00	9.5 ± 0.0	29 ± 0	26 ± 0
Total Mg (g kg <sup>-1</sup> DW)	2.1 ± 0.0	87 ± 1	5.0 ± 0.0	14 ± 0	6.1 ± 0.0

<sup>a)</sup>TSP = Triple Superphosphate; <sup>b)</sup>DW = Dry weight; <sup>c)</sup>OC = Organic carbon.

Plastic containers (height: 14 cm, diameter: 13 cm) were filled with 1 kg of soil and the soil moisture solution was brought to field capacity (23% for sand, 19% for Rheinsand). After two days of equilibration (March 16 2012), an equivalent product dose of 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was applied to all containers (Table II). This refers to the maximum allowable yearly amount of P application to a sandy soil in Flanders with the purpose of maize cultivation (MAP4, 2011).

TABLE II Product and macronutrient dosage to soil by bio-based fertilizer application (standardized to 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>). Differences in N, K, Ca, and Mg application were corrected by adding the appropriate amount of a 1 M NH<sub>4</sub>NO<sub>3</sub>, K<sub>2</sub>SO<sub>4</sub>, CaSO<sub>4</sub>·2H<sub>2</sub>O, and/or MgSO<sub>4</sub>·7H<sub>2</sub>O solution

Fertilizer type	Product	Total N	Effective N	Total P <sub>2</sub> O <sub>5</sub>	Total K <sub>2</sub> O	Total Ca	Total Mg	OC <sup>b)</sup>
	t DW <sup>a)</sup> ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>
TSP <sup>c)</sup>	0.19	0.093	0.059	80	0.36	26	0.40	3.0
Struvite	0.27	14	9.2	80	3.0	0.16	24	78
FePO <sub>4</sub> -sludge	3.08	169	92	80	357	29	15	770
Pig manure	1.51	159	112	80	112	44	21	559
Digestate	2.76	185	124	80	160	72	17	938

<sup>a)</sup>DW = Dry weight; <sup>b)</sup>OC = Organic carbon; <sup>c)</sup>TSP = Triple superphosphate.

Simultaneously, a control treatment without P fertilization was set up. Differences in N, K, Ca, and Mg application between the scenarios were corrected by adding the appropriate amount of a 1 M ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), potassium sulfate (K<sub>2</sub>SO<sub>4</sub>), calcium sulfate (CaSO<sub>4</sub>·2H<sub>2</sub>O), and magnesium sulfate (MgSO<sub>4</sub>·7H<sub>2</sub>O) solution up to the fertilizer recommendation levels of 135 kg effective N ha<sup>-1</sup>, 250 kg K<sub>2</sub>O ha<sup>-1</sup>, 100 kg CaO ha<sup>-1</sup>, and 50 kg MgO ha<sup>-1</sup>, respectively, and without exceeding the field capacity. Soils were homogenized and soil moisture content was again brought to field capacity with deionized water. Each treatment was repeated four times, resulting in a total of 48 containers (5 amendments and 1 control, 2 soil types, 4 replications).

After four days of equilibration (March 21 2012), seven energy maize seeds of the species Atletico (breeder: KWS, Belgium; Food and Agricultural Organisation (FAO) ripeness index: 280; P demand: high) were sown in each container at a depth of 2 cm. The containers were covered with perforated plastics in order to reduce evapotranspiration. When the plants reached the height of the plastic, the plastics were removed, and the plants were thinned out to five plants per container. In each container a Rhizon SMS (MOM 10 cm male luer, PE/PVC tubing, 9 mL vacuette; Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) was inserted diagonally from the topsoil through the soil column. A greenhouse bench at ± 20 °C was divided into four blocks representing the four replications, and in each block 12 containers were randomly placed.

The plants were lightened with Brite-Grow bio growing lamps (LUX 1500, 36 W) 50 cm above the plants in a day-night cycle (6 AM till 8 PM). The soils were weighed daily and the soil moisture content was adjusted to field capacity each time. After one week, leakage of soil solution was visible in two containers: one struvite and one control treatment, both on the sandy soil. These two containers were removed from the experiment.

Homogeneous soil samples (10 g) were taken for analysis of PAI, Pw, and P-PAE by means of a soil auger the first two weeks and the last two weeks of the experiment. Rhizon soil moisture extracts were sampled weekly during the experiment and the P concentration in the soil solution as well as the pH were analyzed each time. Furthermore, the length of the plants was measured weekly. After five weeks of growth, the plants were harvested, their yield was determined, and plant samples were taken for physicochemical analysis. The soils were maintained on the greenhouse bench and were moisturized every week up to field capacity. Finally, PAI, P-PAE, and Pw in the soils were measured again after six months.

#### *Plant and soil analysis*

The DW content of the biomass was determined as residual weight after one week drying at 65 °C. Macronutrients (N, P, K, Ca, Mg) in the biomass were determined following the same methodology as described for the product analysis above.

Soil pH and EC were determined using the same procedure as described for the products. Field capacities were determined in accordance with the Compendium for Sampling and Analysis provided in the Flemish waste and soil remediation decree (CSA, 2012). For the determination of PAI, 2.5 g of soil was mixed with 50 mL of ammonium lactate solution (pH 3.75), shaken for 4 h and filtered until colorless using a white ribbon filter (MN 640 m, Macherey-Nagel, Düren, DE; CSA, 2012). For the determination of Pw, 4 g of soil and 240 mL of distilled water were mixed in a 250 mL flask, shaken for 1 h and filtered (white ribbon) until colorless (EL&I, 2009; Sissingh, 1971). For P-PAE, 1 g of dry soil was mixed with 25 mL 0.01 M CaCl<sub>2</sub> in a 40 mL centrifuge tube, shaken for 1 h, centrifuged during 10 min at 4,000 rpm (Heraeus megafuge 1.0, Kendro Laboratory Products, Hanau, DE), and filtered (white ribbon) (Van Ranst *et al.*, 1999). Note that the P-PAE number is generally expressed as mg P kg<sup>-1</sup> soil, whereas the Pw and PAI numbers are officially expressed as mg P<sub>2</sub>O<sub>5</sub> L<sup>-1</sup> soil and mg

P<sub>2</sub>O<sub>5</sub> 100 g<sup>-1</sup> soil, respectively. The P content in the filtered extraction solutions and Rhizon SMS extracts was then determined using the colorimetric method of Scheel (see above). Finally, in acidic sandy soils, P ions are expected to react with Fe and Al ions to form poorly soluble compounds (Hillel, 2008). Hence, an extraction of the soil with ammonium oxalate ((NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub>) and oxalic acid (H<sub>2</sub>C<sub>2</sub>O<sub>4</sub>) was also performed in order to determine the active forms of Fe and Al separately. The procedure described in CSA (2012) was used for this purpose.

#### *Phosphorus use efficiency (PUE)*

Average PUE's (%) of the bio-based fertilizers were calculated based on the plant reaction and the soil status using the following equation:

$$PUE(\text{parameter})_{\text{biofertilizer}} = \frac{(\text{parameter}_{\text{biofertilizer}} - \text{parameter}_{\text{control}})}{(\text{parameter}_{\text{reference}} - \text{parameter}_{\text{control}})} \times 100$$

where 'bio-fertilizer' refers to the bio-based fertilizers under study, 'control' to the blank treatment, 'reference' to the TSP treatment, and where 'parameter' can refer to:

- the plant P uptake, the plant FW and DW yield: PUE(uptake), PUE(FWyield), and PUE(DWyield). Here, the PUE refers to the percentage of P in the bio-based fertilizers that has the same effectiveness as the reference fossil reserve-based mineral P fertilizer, TSP;
- the PAI, P<sub>w</sub>, P-PAE, and the P concentration in the soil solution extracted with Rhizon SMS: PUE(PAI), PUE(P<sub>w</sub>), PUE(PAE), and PUE(Prhizon). Here, the PUE refers to the increment in soil P status by application of the bio-based fertilizers as compared to the increment by application of TSP.

#### *Statistical analysis*

Statistical analyses were conducted using SAS 9.3. A one-way ANOVA model was used to determine the effect of the fertilizer type (i.e. the independent variable, between-groups factor) on the different plant and soil parameters (i.e. the dependent variable) per measurement. Furthermore, a two-way mixed ANOVA model was used to determine whether any change in plant and soil parameters (i.e. the dependent variable) was the result of the interaction between the type of treatment (i.e. the between-group factor) and time (in weeks, i.e. the within-group factor). As the interaction term between time and treatment was never significant at the 5% significance level ( $\alpha = 0.05$ ), it was eliminated from the model. Follow-up tests were performed to determine whether the mean value for each plant and soil parameter was significantly different in time, and whether the average of these parameters over the whole experimental period was significantly different between the treatments. The condition of normality was checked using the Kolmogorov Smirnov test and QQ-plots, whereas equality of variances was checked with the Levene test. When homoscedascity was found, significance of effects was tested by use of an F-test and post-hoc pair-wise comparisons were conducted using Tukey's honestly significant difference (HSD) test ( $\alpha = 0.05$ ). When no homoscedascity was found, a Welch F-test combined with a post-hoc Games-Howell test was used ( $\alpha = 0.05$ ). When the condition of normality was not fulfilled, the non-parametric Kruskal-Wallis test was applied instead of the one-way ANOVA. For convenience of discussion, significant parameter correlations were determined using the Pearson correlation coefficient, r.

## RESULTS

### *Product characterization and phosphorus fractionation*

First, it must be noticed that TSP and struvite were dry, granular products, while the other products were liquids. For recognition of new P fertilizers in the framework of EU fertilizer legislations, the amount of P soluble in water and ammonium citrate, next to the total amount of P must be demonstrated. Moreover, the amount of P soluble in mineral acid must be higher than 2% (EC, 2003). The extracted P fractions for the different products under study are shown in Table III.

TABLE III Phosphorus (P) fractionation: total P, P soluble in water, neutral ammonium citrate (NAC), and mineral acid (MA) (mean  $\pm$  standard deviation; n = 2)

Parameter	TSP <sup>a)</sup>	Struvite	FePO <sub>4</sub> -sludge	Pig manure	Digestate
Total P <sub>2</sub> O <sub>5</sub> (g kg <sup>-1</sup> DW <sup>b)</sup> )	430 $\pm$ 5	293 $\pm$ 3	26 $\pm$ 1	53 $\pm$ 0	30 $\pm$ 0
P <sub>2</sub> O <sub>5</sub> extractable in water (g kg <sup>-1</sup> DW)	413 $\pm$ 1	5.0 $\pm$ 0.0	1.0 $\pm$ 0.0	45 $\pm$ 2	23 $\pm$ 0
P <sub>2</sub> O <sub>5</sub> extractable in NAC <sup>c)</sup> (g kg <sup>-1</sup> DW)	410 $\pm$ 1	282 $\pm$ 3	25 $\pm$ 1	48 $\pm$ 0	28 $\pm$ 0
P <sub>2</sub> O <sub>5</sub> extractable in MA <sup>d)</sup> (g kg <sup>-1</sup> DW)	398 $\pm$ 1	288 $\pm$ 5	23 $\pm$ 0	52 $\pm$ 0	30 $\pm$ 0

a) Triple superphosphate; b) Dry weight; c) Neutral ammonium citrate; d) Mineral acid.

The relative solubility of P in neutral ammonium citrate was high for all fertilizers (91--100% of total P), similar as the solubility in mineral acid (92--100%). The amount of P soluble in water was low for struvite (1.7% of total P) and FePO<sub>4</sub>-sludge (3.9%) as compared to TSP (96%), while their relative amount of P soluble in mineral acid was in the same line as the reference. Digestate had approximately the same P solubility's in the different extraction reagents as animal manure (79--100% of total P). Compared to TSP, the P solubility of both products in water was lower, while it was higher in mineral acid. Furthermore, the pH of TSP and FePO<sub>4</sub>-sludge was low (2.6--4.6), while for struvite and digestate it was alkaline (8.4--8.6) (Table I). The pH of pig manure was quasi neutral. Finally, all bio-based fertilizers under study added significantly more organic carbon (OC) to the soil as compared to TSP (Table II).

### Plant reaction

#### Biomass yield and phosphorus uptake

On the sandy soil at the harvest all treatments showed a significantly higher FW biomass yield (g FW container<sup>-1</sup> or kg<sup>-1</sup> soil; Table IV), DW biomass yield (g DW kg<sup>-1</sup> soil; Table IV), and length (cm; Fig. 1) as compared to the reference TSP. Conversely, the DW content (%) and P content (mg P kg<sup>-1</sup> plant DW) of the biomass were significantly higher for the TSP treatment. However, the absolute P uptake per container (mg P container<sup>-1</sup> or kg<sup>-1</sup> soil) was only significantly higher for TSP as compared to the control (Table IV).

#### Fig. 1

Fig. 1 Plant length (cm) as a function of time (d) for the different treatments on sand (A) and on Rheinsand (B) (mean, error bars:  $\pm$  1 standard deviation; n = 4). p-values refer to statistical analysis using one-way ANOVA. When a significant difference was observed ( $P < 0.05$ ), post-hoc pair-wise comparisons were added using small letters.

On Rheinsand, no significant differences were observed in the biomass length (Fig. 1) and DW yield (Table IV). The DW content was significantly lower for TSP and FePO<sub>4</sub>-sludge as compared to the control and digestate, while FePO<sub>4</sub>-sludge had a significantly higher FW yield than the control, manure, and digestate. The use of TSP, manure, and digestate resulted in a significantly higher P content (g kg<sup>-1</sup> plant DW) and absolute P uptake (mg P container<sup>-1</sup> or kg<sup>-1</sup> soil) as compared to the control and FePO<sub>4</sub>-sludge. Moreover, the plant P uptake at the harvest was significantly lower for struvite as compared to TSP on Rheinsand.

#### Phosphorus use efficiency

The PUE(FWyield) and PUE(DWyield) on the sandy soil were mostly negative as the yield of the reference TSP was lower than the control (Table IV). Among the bio-based products, the best average PUE's based on crop yield were observed for FePO<sub>4</sub>-sludge and digestate, the latter simultaneously showing the highest PUE(uptake). Also on Rheinsand, the PUE(FWyield) and PUE(DWyield) were the highest for FePO<sub>4</sub>-sludge, however its PUE(uptake) was the lowest. The PUE(uptake) for manure and digestate were the highest on Rheinsand, yet their PUE(FWyield) and PUE(DWyield) were negative as the yields were slightly lower than the control.

TABLE IV Biomass yield and phosphorus uptake (average  $\pm$  standard deviation,  $n = 4$ ), as well as average phosphorus use efficiencies (PUE) for the different treatments on sand and Rheinsand. p-values and small letters refer to statistical analysis using one-way ANOVA and post-hoc pair-wise comparisons

Sand	Yield	PUE (FWyield <sup>a</sup> )	DW <sup>b</sup> )	Yield	PUE(DWyield <sup>b</sup> )	P uptake	PUE(uptake)
	g FW <sup>a</sup> kg <sup>-1</sup>	%	%	g DW <sup>b</sup> kg <sup>-1</sup>	%	mg kg <sup>-1</sup>	%
	$P < 0.0001$	-	$P < 0.0001$	$P = 0.0002$	-	$P = 0.012$	-
Control	26 $\pm$ 2a	0	19 $\pm$ 0ab	4.7 $\pm$ 0.3a	0	32 $\pm$ 2b	0
TSP <sup>c</sup>	20 $\pm$ 2b	100	20 $\pm$ 2a	4.0 $\pm$ 0.2b	100	39 $\pm$ 3a	100
Struvite	27 $\pm$ 0a	-21 <sup>d</sup>	17 $\pm$ 0bc	4.7 $\pm$ 0.1a	9.6 <sup>de</sup>	34 $\pm$ 2ab	22
FePO <sub>4</sub> -sludge	29 $\pm$ 1a	-68 <sup>d</sup>	17 $\pm$ 0c	4.9 $\pm$ 0.1a	-16 <sup>d</sup>	33 $\pm$ 3ab	16
Pig manure	28 $\pm$ 1a	-46 <sup>d</sup>	17 $\pm$ 0bc	4.8 $\pm$ 0.2a	-9 <sup>d</sup>	35 $\pm$ 2ab	37
Digestate	29 $\pm$ 1a	-67 <sup>d</sup>	17 $\pm$ 1c	4.8 $\pm$ 0.2a	-15 <sup>d</sup>	37 $\pm$ 2ab	80
Rheinsand	Yield	PUE (FWyield <sup>a</sup> )	DW <sup>b</sup> )	Yield	PUE(DWyield <sup>b</sup> )	P uptake	PUE(uptake)
	g FW <sup>a</sup> kg <sup>-1</sup>	%	%	g DW <sup>b</sup> kg <sup>-1</sup>	%	mg kg <sup>-1</sup>	%
	$P = 0.003$	-	$P < 0.0001$	$P = 0.2$	-	$P < 0.0001$	-
Control	15 $\pm$ 1b	0	26 $\pm$ 0a	3.9 $\pm$ 0.1a	0	5.4 $\pm$ 0.7c	0
TSP <sup>c</sup>	16 $\pm$ 1ab	100	25 $\pm$ 0b	4.0 $\pm$ 0.8a	100	9.2 $\pm$ 0.6a	100
Struvite	16 $\pm$ 2ab	75	25 $\pm$ 1ab	3.9 $\pm$ 0.3a	67	7.0 $\pm$ 1.5bc	42
FePO <sub>4</sub> -sludge	17 $\pm$ 0a	159	24 $\pm$ 0b	4.1 $\pm$ 0.1a	233	5.6 $\pm$ 0.9c	3.3
Pig manure	15 $\pm$ 1b	-8.9 <sup>e</sup>	26 $\pm$ 1ab	3.8 $\pm$ 0.1a	-67 <sup>e</sup>	8.4 $\pm$ 0.8ab	80
Digestate	14 $\pm$ 1b	-45 <sup>e</sup>	27 $\pm$ 1a	3.8 $\pm$ 0.1a	-100 <sup>e</sup>	7.8 $\pm$ 0.5ab	63

<sup>a</sup>FW = Fresh weight; <sup>b</sup>DW = Dry weight; <sup>c</sup>TSP = Triple superphosphate; <sup>d</sup>PUE shows the opposite sign as results for the reference TSP are lower than the control; <sup>e</sup>Result for the bio-based fertilizer is lower than the control.

### Soil bioavailability indices

#### P-PAE, PAI and Pw number

Bioavailability curves and corresponding statistics per measurement of P-PAE, PAI and Pw are presented in Figure 2.

#### Fig. 2

Fig. 2 P-PAE (A; mg P kg<sup>-1</sup> soil), Pw (B; mg P<sub>2</sub>O<sub>5</sub> L<sup>-1</sup> soil), PAI on sand (C; mg P<sub>2</sub>O<sub>5</sub> 100 g<sup>-1</sup> soil) and PAI on Rheinsand (D; mg P<sub>2</sub>O<sub>5</sub> 100 g<sup>-1</sup> soil) as a function of time (wk) after sowing for the different treatments (mean, error bars:  $\pm$  1 standard deviation;  $n = 4$ ). p-values and small letters refer to statistical analysis using one-way ANOVA and post-hoc pair-wise comparisons ( $\alpha = 0.05$ ).

First, it must be remarked that P-PAE and Pw could only be detected on the sandy soil as the values on Rheinsand were lower than the detection limit of both the available spectrophotometer (0.66 mg P L<sup>-1</sup>; Jenway 6400, Barloworld Scientific T/As, Felsted, UK) and the continuous flow analyzer (0.05 mg P L<sup>-1</sup>; AA3, BRAN+LUEBBE, Norderstedt, DE).

Over the whole experimental period, the mean P-PAE (mg P kg<sup>-1</sup> soil) was significantly higher ( $P < 0.0001$ ) for TSP as compared to the other treatments and the control, as well as for struvite compared to the control, digestate, and FePO<sub>4</sub>-sludge (Fig. 2A). The effect of FePO<sub>4</sub>-sludge on the P-PAE number was in average significantly lower ( $P < 0.0001$ ) than that of all other treatments. The two-way ANOVA for P-PAE indicated a significant ( $P < 0.0001$ ) decrease for all treatments from week 2 to 4 and week 4 to 5. The mean Pw over time (mg P<sub>2</sub>O<sub>5</sub> L<sup>-1</sup> soil) for TSP, digestate, and struvite was significantly higher ( $P < 0.0001$ ) than for the control and FePO<sub>4</sub>-sludge (Fig. 2B). A significant decrease ( $P = 0.0021$ ) was observed in week 2 for all treatments. Overall, the mean PAI (mg P<sub>2</sub>O<sub>5</sub> 100 g<sup>-1</sup> soil) in time on the sandy soil was significantly higher ( $P < 0.0001$ ) for TSP than for all other treatments (Fig. 2C), while on Rheinsand this effect was only significant ( $P = 0.030$ ) compared to FePO<sub>4</sub>-sludge (Fig. 2D). Both on sand and Rheinsand, no significant changes in time were found based on the weekly average PAI ( $P > 0.1$ ). After six months, no more significant effect of the treatment on the P-PAE ( $P = 0.15$ ) and PAI number ( $P = 0.10$ ) was observed, whereas the control showed a significantly higher ( $P = 0.0069$ ) Pw number than struvite, manure, and FePO<sub>4</sub>-



sludge.

### *pH and phosphorus content in the soil solution (Prhizon)*

The pH and P content in the soil solution extracted with rhizon SMS are presented in Figure 3.

#### **Fig. 3**

Fig. 3 pH and P<sub>2</sub>O<sub>5</sub> concentration (mg L<sup>-1</sup>) in the soil solution (Prhizon) as a function of time (wk) after sowing on sand (A,C) and Rheinsand (B,D) (mean, error bars: +/- 1 standard deviation; n = 4). p-values and small letters refer to statistical analysis using one-way ANOVA and post-hoc pair-wise comparisons ( $\alpha = 0.05$ ). Detection limit: 0.05 mg L<sup>-1</sup>.

On sand, the average pH over time was significantly lower ( $P < 0.0001$ ) for pig manure as compared to all other treatments, as well as for TSP compared to struvite, FePO<sub>4</sub>-sludge, the control, and digestate (Fig. 3A). Conversely, the average Prhizon (mg P<sub>2</sub>O<sub>5</sub> L<sup>-1</sup>) over time was significantly higher ( $P < 0.0001$ ) for manure as compared to struvite, the control, and FePO<sub>4</sub>-sludge (Fig. 3C). The latest showed significantly lower Prhizon values ( $P < 0.0001$ ) than the other treatments and the control, while digestate showed a significantly higher ( $P < 0.0001$ ) average pH than all other treatments, both on sand and Rheinsand (Fig 3A,B).

### *Phosphorus use efficiency*

The average PUE based on the various soil analyses is presented in Figure 4 as a function of time. On the sandy soil, all fertilizers presented a lower PUE(PAE) and PUE(PAI) than the reference TSP during the whole experimental period (Fig. 4A,C). Struvite showed the highest PUE(PAE), while the P-PAE number for FePO<sub>4</sub>-sludge was even lower than the control. PUE(Pw) increased in time for struvite and digestate relative to TSP (Fig. 4B). For FePO<sub>4</sub>-sludge, it was negative and decreasing. PUE(Prhizon) was very high (up to > 100%) for pig manure on both sand and Rheinsand (Fig. 4E,F). On sand, the curve for struvite showed a similar pattern as for pig manure up to week 3 (Fig. 4E). However, on Rheinsand the values for struvite were always lower as compared to the reference and pig manure (Fig. 4F).

#### **Fig. 4**

Fig. 4 Average phosphorus use efficiency (PUE) as a function of time (wk) after sowing for the different treatments relative to the reference TSP (PUE = 100%), based on P-PAE (A), Pw (B), PAI sand (C), PAI Rheinsand (D), Prhizon sand (E), and Prhizon Rheinsand (F).

## DISCUSSION

### *Effect of bio-based fertilizer on biomass yield and phosphorus uptake*

The P use efficiency based on the plant reaction can be expressed in terms of the plant yield and P uptake. However, as the plant yield is mainly influenced by the N effect, as well as multiple other factors, such as micronutrient availability, the effect of P is hard to detect, especially for complex organic P fertilizers. Therefore, in literature the PUE based on the plant reaction is mostly calculated in terms of P uptake, if it is calculated at all (van Dam and Ehlert, 2008; Mohanty *et al.*, 2006). Indeed, some authors showed that there is no correlation between the crop response and the P supply (Árendás and Csathó, 2002; Prummel and Sissingh, 1983; van Dam and Ehlert, 2008).

Also in the present study, no relevant effect of the fertilizer type on the biomass yield was observed. For the acidic sandy soil, this is not surprising due to the high P status (Pw control > 55 mg P<sub>2</sub>O<sub>5</sub> L<sup>-1</sup> soil) of Flemish soils, i.e. no response to P in terms of plant growth is expected. Though, an awkward observation was that on this high-P sandy soil, the use of the reference TSP resulted in lower yields and lengths as compared to all other treatments under study (Table IV; Fig. 1). A similar effect was observed in the study of, for example, van Dam and Ehlert (2008), Liu *et al.* (2011), Meena *et al.* (2007), Mohanty *et al.* (2006), and Uddin *et al.* (2012). These authors attributed this phenomenon to the fact that most of the P contained in TSP is water-soluble (96% in this study) and therefore partly captured by the substantial amount of Fe and Al oxides in acidic sandy soils (Fe<sub>ox</sub> = 34 mmol kg<sup>-1</sup>, Al<sub>ox</sub> = 66 mmol kg<sup>-1</sup> in this study). By means of a literature review, van Dam and Ehlert (2008) showed that the relative efficiency in terms of plant yield for animal manure as compared to TSP can

vary between 30 and 378% (140% in this study). The higher values were, indeed, related to conditions that hinder the operation of the readily soluble P fertilizer reference, such as phosphate fixation by Fe and Al compounds and precipitation with Ca compounds. On Rheinsand, which had a low P level (Pw control < 36 mg P<sub>2</sub>O<sub>5</sub> L<sup>-1</sup> soil), the highest FW biomass yields were obtained for TSP and FePO<sub>4</sub>-sludge (Table IV). Hence, the above problem did not (or less) occur in this case.

As mentioned above, a more relevant comparison of the fertilizer effect may be made based on the plant P uptake. The P uptake was the highest for TSP both on sand and Rheinsand. Yet, on the P-rich soil all amendments could cover the crop P demand (no significant difference with TSP), while on the P-poor soil FePO<sub>4</sub>-sludge and struvite showed a significantly lower P uptake as compared to TSP. This indicates that the initial soil P status plays an important role in determining the plant P availability and uptake. The use of pig manure and digestate resulted in a plant P uptake comparable to TSP on the P deficient soil, indicating that the absolute fertilizer effect in terms of direct available P was similar. The application of FePO<sub>4</sub>-sludge resulted in the lowest P uptake (Table IV), indicating that the P in FePO<sub>4</sub>-sludge is most fixed. This can be attributed to the stronger binding capacity of Fe and P as compared to Ca/Mg and P (Hillel, 2008; Zumdahl, 2005). In general, it can be stated that the fertilizers with the highest P solubility in water (i.e. TSP, manure, and digestate) resulted in the highest plant P uptake.

### *Effect of bio-based fertilizer on soil phosphorus availability*

#### *Struvite*

The P solubility of struvite in water was much lower as compared to the reference TSP, whereas the solubility in neutral ammonium citrate and mineral acid was relatively high (Table III), in line with literature findings (Barak and Stafford, 2006; Bridger *et al.*, 1962). These measurements indicate that struvite has slow-release properties. This was confirmed by the bio-availability curve for Prhizon on P deficient Rheinsand (Fig. 4F), which showed an increase in direct available soluble P from ± 0% to ± 75% as compared to TSP in approximately two weeks time. Moreover, it is in line with the slow-release properties of this product for NH<sub>4</sub>-N found in literature (Latifian *et al.*, 2012; Ryu *et al.*, 2012; Shu *et al.*, 2006; WERF, 2010).

In spite of these findings, struvite demonstrated the highest efficiency (relative to TSP) among the bio-based fertilizers in terms of direct available P on the P-rich sandy soil (Fig. 4A,E). This was confirmed by the significant correlation between the P-PAE for struvite and TSP on sand ( $r = 0.63$ ,  $P = 0.030$ ). The high PUE(PAE) and PUE(Prhizon) on sand may be attributed to the higher amount of NH<sub>4</sub>-N relative to P<sub>2</sub>O<sub>5</sub> in struvite (Table I). In the study of Bridger *et al.* (1962) on struvite and other metal ammonium phosphates, P release appeared to be largely the result of microbial nitrification of the ammonium constituent rather than simple dissolution. The uptake of NH<sub>4</sub><sup>+</sup> by the roots as well as the nitrification of NH<sub>4</sub><sup>+</sup> into nitrate (NO<sub>3</sub><sup>-</sup>) are acidifying processes, which can increase soil P mobilization and uptake in the rhizosphere (Bridger *et al.*, 1962; Diwani *et al.*, 2007). Indeed, during the first three weeks of growth on struvite-amended soils, the pH in the soil solution was the lowest (Fig. 3A), while the amount of direct available P was the highest (Fig. 3C). Note that a similar effect on soil P bio-availability was found when applying bio-based recovered ammonium sulfate (AmS) from acidic air scrubbing during the field trials performed by Vaneeckhaute *et al.* (2015). Other contributing factors to the extra soil P liberation could be the presence of Mg in struvite (Gonzalez-Ponce *et al.*, 2009; Ryu *et al.*, 2012) and/or its high salt content (Hartzell *et al.*, 2010).

At the end of the growing season, PUE(PAI) and especially PUE(Pw) increased (Fig. 3B,C,D), indicating that struvite addition increased the soil P reserves, mainly the readily available pool, for release in the longer term. As the plant P uptake was significantly lower for struvite than for TSP on Rheinsand after five weeks of growth, it is likely that the release and plant uptake of P directly provided by struvite application was not yet completed at the moment of harvest. On the high-P sandy soil, no significant difference in plant P uptake between struvite and TSP was found, indicating that the amount of P liberated from the soil was sufficient to support the crop demand. This difference in soil P uptake on P-rich sand and P-poor Rheinsand confirms again the short-term soil P mobilization provoked by struvite application (see above). Further research on the P delivery and plant P uptake (and the corresponding mechanisms) in the longer term on different soil types with varying P status is advised for this product.

## Iron phosphate sludge

Iron phosphate ( $\text{FePO}_4$ ) sludge showed not useful as starter fertilizer for crop growth, as its P solubility in water was very low (Table III), as were all soil bio-availability indices. In agreement to Hahn *et al.* (2002) and Nieminen *et al.* (2011), the solubility in neutral ammonium citrate was 100%. Accordingly, the efficiency of this product to supply direct available P was low, and the lowest of all fertilizers under study. Yet, the P capacity over time was slightly increasing, indicating that the addition of  $\text{FePO}_4$ -sludge slowly increased the amount of P that can be released in the longer term. Hence, the product may have slow release properties. This phenomenon was also reflected in the highly significant correlation for PAI on sand between struvite and  $\text{FePO}_4$ -sludge ( $r = 0.86$ ,  $P < 0.0001$ ). Nevertheless, as the P-PAE and Prhizon were even lower than the control, the use of this product for agricultural crop production is discouraged, especially on P-rich soils, so as to avoid further soil P accumulation. On the other hand, the product's ability to fixate P is of increasing interest for forestry on drained peat- and wetlands to reduce P leaching and increase P adsorption (Larsen *et al.*, 1959; Nieminen *et al.*, 2003, 2011; Scheffer and Kuntze, 1999; Silfverberg and Hartman, 1999). A long study period will, however, be required because of the slow development of active root/mycorrhiza associations that may be necessary for significant P release (Nieminen *et al.*, 2003, 2011). As such, the duration of the growth response after P fertilization is expected to be over 30 years.

## Digestate and animal manure

The efficiency of digestate in supplying direct available P was slightly increasing during the greenhouse experiment (Fig. 4A), indicating that P from digestate was released slower than from the reference TSP. The product had a relatively high P solubility in water, though lower than TSP, while its solubility in mineral acid was 100% (Table III). The PUE(Pw) was therefore high (Fig. 4B). Pig manure released direct available P somewhat faster than digestate, as the PUE(PAE) was higher after one week, but equal after four weeks (Fig. 4A). In addition, its P solubility in water was slightly higher as compared to digestate, while the solubility in mineral acid was slightly lower (Table III). This is in line with the observed bio-availability indices: P-PAE (Fig. 2A) and Prhizon (Fig. 3C,D) were higher for pig manure than for digestate, whereas Pw was slightly lower (Fig. 2B).

All these results correspond to observations made during a preceding field-scale assessment (Vaneckhaute *et al.*, 2013, 2014, 2015) and to literature data (Huang *et al.*, 2012; Möller and Müller, 2012; Güngör *et al.*, 2007; Güngör and Karthikeyan, 2008), indicating that anaerobic (co-)digestion of animal manure reduces the fraction of direct available inorganic P in the soil solution, whereas it increases the fraction of readily available soil P that can be released in the short term. This phenomenon would be caused by the enhanced formation and precipitation of calcium phosphate, magnesium phosphate, and/or struvite through mineralization of N, P, and Mg during (co-)digestion in combination with a substantial increase of the manure pH (Hjorth *et al.*, 2010; Le Corre *et al.*, 2009; Möller and Müller, 2012). As a comprehensive example, Güngör *et al.* (2007) showed that 43% of the mineral P species in dairy manure were struvite and 57% more weakly bounded dicalcium phosphate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ), whereas 78% struvite and 22% hydroxylapatite ( $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ ) were detected in the digested manure sample. Noteworthy in this perspective is that the correlation for both P-PAE and the pH in the soil solution was highly significant between struvite and digestate, with  $r = 0.90$  ( $P < 0.0001$ ) and  $r = 0.85$  ( $P < 0.0001$ ) for the P-PAE and pH, respectively. Moreover, during previous field trials performed by Vaneckhaute *et al.* (2015) a significant correlation was found between the Ca, Mg, and P use efficiency when applying digestates or its liquid fraction. Consequently, the conversion of animal manure through anaerobic (co-)digestion and the subsequent use of digestate on agricultural fields may offer a solution to control water soluble P in soils, meanwhile supplying sufficient P to support plant growth, similar as was observed during the previous field trials (Vaneckhaute *et al.*, 2015).

Another interesting finding is that the P intensity of the soil, measured as P-PAE, was lower for digestate and pig manure than for TSP (Fig. 7.4A), while Prhizon was higher, especially for pig manure (Fig. 3C,D). It is likely that this extra amount of soluble P for the organic fertilizers, digestate and pig manure, was attributed to the release of organic  $\text{P}_2\text{O}_5$  in the soil solution (Huang *et al.*, 2012; Roboredo, 2012), which cannot (or not completely) be measured with the PAE method. Indeed, the P-PAE number was significantly correlated for the mineral fertilizers, struvite and TSP ( $r = 0.63$ ,  $P < 0.0001$ ), but no significant correlation was found between the P-PAE measurements for the other products. On the other hand, on Rheinsand, the correlation of P in the soil solution (Prhizon), which

includes dissolved organic forms, between TSP and pig manure ( $r = 0.76$ ,  $P < 0.0001$ ), as well as TSP and digestate ( $r = 0.73$ ,  $P < 0.0001$ ), was significant, although only a relatively weak correlation was found between TSP and struvite ( $r = 0.59$ ;  $P = 0.010$ ). Huang *et al.* (2012) emphasized that this organic dissolved P fraction in soils also plays a role in plant P utilization. Hence, measurements carried out in the context of fertilizer recommendations and legislative standards should be able to detect both inorganic and organic P fractions.

As the average PUE(Prhizon) was much higher for pig manure than for TSP, both on sand and Rheinsand (Fig. 4E,F), and since pig manure is a liquid fertilizer, application of this product might cause a higher risk of leaching in the field, especially on soils low in Fe and Al (cfr. Kang *et al.*, 2011; Yang *et al.*, 2012). Since also the efficiency in terms of P uptake and yield on sand was slightly higher (Table IV) and the soil pH significantly higher for the digestate treatment than for pig manure (Fig. 3), treating manure by anaerobic (co-)digestion before field application appears again as an interesting option from an environmental point of view. Meanwhile renewable energy can be produced. Although it is likely that similar results will be obtained for each digestion process fed with animal manure, it should be noted that the present results are based on digestate from co-digestion of pig manure (30%) with organic biological waste from the food industry (40%) and energy maize (30%).

Finally, an interesting point is that all bio-based fertilizers under study, especially digestate, added significantly more organic carbon (OC) to the soil as compared to TSP (Table II). Application of these products could therefore also contribute to the struggle against OC depletion in many agricultural soils worldwide.

### *Practical implications and recommendations*

In the wastewater and manure processing industry, Fe- and Al-salts are often used for P removal during solid-liquid separation so as to improve coagulation/flocculation practices and achieve water discharge levels. However, this paper indicates that the resulting  $\text{FePO}_4$ -sludge is not valuable for reuse as a fertilizer in terms of P release for crop growth. An increased accumulation of P in the soil is expected when using the product for agricultural purposes. Its use may be interesting on drained soils, though this remains to be evidenced. Similar results will likely be obtained for  $\text{AlPO}_4$ -sludge because of the comparable P binding properties of trivalent Fe and Al. However, the fertilizer effect of  $\text{AlPO}_4$ -sludge in terms of P bio-availability remains to be confirmed.

In the transition towards a more efficient use of nutrients in agriculture, alternative P recovery and/or release techniques are recommended instead of the traditional methods for P removal. The present paper demonstrates that manure treatment via anaerobic (co-)digestion (with other bio-degradable wastes) and/or struvite precipitation may deliver sustainable substitutes (digestate, struvite) for chemical P fertilizers and/or animal manure in agriculture. Moreover, the application of struvite may increase the liberation of P from the soil complex in high-P soils. Field-scale assessments using these bio-based products on soils with different P status are suggested to evaluate the P release and uptake in the long term, and to provide sufficient information for the establishment of responsible fertilizer application recommendations.

Although these new fertilizers are already produced and available today (quantities depend on the region, i.e. legislations, nutrient excesses, etc.), marketing of these products also depends on the economic viability of the nutrient recovery/release technique in question and the economic competitiveness of the products as compared to commonly used fertilizers (Vaneekhaute *et al.*, 2015). Herewith another important bottleneck arises: in many regions all derivatives produced from animal manure are currently still categorized as 'animal manure' and/or 'waste' in environmental and/or fertilizer legislation and can therefore not or only sparingly be returned to agricultural land (FAO, 2004; Lemmens *et al.*, 2007; WCC, 2015). Hence, the need exists for greater differentiation between soils, crops, and fertilizer types in the recommendations given on P fertilizer requirements (EC, 2003), as well as in fertilizer legislations. For example, in the Flemish fertilizer regulation (MAP4, 2011), currently only one standard for total P application as function of the crop type counts. In contrast to N standards, no distinction is made between P application from animal manure, alternative organic fertilizers, or mineral fertilizers. Moreover, the standard is currently independent of the soil P status and soil texture. Only for P saturated sandy soils one stricter norm exists, but even here no further classification is imposed.

Nevertheless, in the present study, important differences in P solubility and bio-availability for various P fertilizers and different soil P statuses were observed. Hence, a more refined legislative framework in terms of P application is advised. For this purpose, a combination of measurements of the soil chemical P status, texture, and fertilizer properties (mainly P fractionation,  $\text{NH}_4\text{:P}$ -ratio, and P-binding compounds as Fe, Al, Ca, and Mg), in addition to the crop demand for P, is recommended.

Regarding the aim to reduce P leaching and run-off, the most important parameter to evaluate is direct available P. As the P-PAE method does not (sufficiently) account for the release of dissolved organic P, measurements with Rhizon SMS are proposed as a valuable complementary method to provide the fundamental information for better categorization of different P fertilizers in environmental and fertilizer legislations. Bio-availability indices based on the crop yield are generally less conclusive, hence their use is less advisable.

Besides pot experiments, the use of Rhizon SMS in field trials is possible, but the samplers have to be inserted in the soil from trenches. Trenches disturb, however, the hydraulic properties of the soil. Less mobile elements may be sampled correctly from trenches, but concentrations of mobile nutrients may differ from those in undisturbed soil due to differing soil water conditions (Eijkelkamp, 2003). An alternative for trenches are manholes, e.g. augered with an Edelman auger. Further research on the soil disturbing impact of the various sampling methods at field-scale compared to results obtained from controlled greenhouse experiments is recommended.

Finally, an important note is that, due to legislative constraints, the current practice of digestate processing in P saturated regions mostly involves a solid-liquid separation step (Vaneekhaute *et al.*, 2015). The purpose is basically to concentrate the organic matter, P, Ca, and Mg in a thick fraction, which can then be pasteurized and exported (to P-poor regions). As such, the P recovery potential as concentrated and pure struvite from the liquid fraction is limited, although (local) recovery of this mineral fertilizer may be interesting and relevant, e.g. for horticultural purposes or for crops with high (bio-available) P demand. Moreover, through export, the valuable and effective organic carbon is eliminated from the local agricultural cycle (Vaneekhaute *et al.*, 2015), while organic carbon depletion in many soils worldwide has become an alarming issue. This leads to the suggestion to stimulate the release of P in the liquid fraction for subsequent mineral (and pure) P recovery as struvite. As such, thick fractions with a more interesting (i.e. higher) C:P-ratio for local reuse as soil conditioner can be recovered, and soil organic carbon could be maintained. Pre-treatment methods to improve the release of P in the liquid fraction during solid-liquid separation are therefore gaining importance in P saturated regions. This will be aspect of future research.

## CONCLUSIONS AND PERSPECTIVES

Greenhouse experimental results indicate that some P-containing recovered bio-based products can be used as sustainable substitute for chemical P fertilizers and/or animal manure in agriculture. Struvite provided a high P availability for the plant in the beginning of the growing season, as well as a stock for delayed slow release. The product seems to stimulate the liberation of P from the soil complex, thereby providing sufficient direct plant available P to support plant growth on P-rich sandy soils. The addition of FePO<sub>4</sub>-sludge proved not useful in terms of short-term P release. Its use as a fertilizer for agricultural crop production should be discouraged, especially on P-rich soils. Hence, from an agronomic point of view, the implementation of struvite recovery in waste(water) treatment facilities seems more valuable than traditional practices of P removal using Fe-salts. Moreover, the P use efficiency in animal manure could be improved via anaerobic (co-)digestion and application of the resulting digestate for crop production. As added benefits, negative environmental impacts of untreated animal manure are avoided, renewable energy is produced, important amounts of organic carbon are added to the soil, and the soil pH is maintained.

Furthermore, this study confirmed previous literature findings that chemical soil analyses are more conclusive than the plant reaction in terms of P fertilizer performance. The additional use of Rhizon soil moisture samplers for determination of total direct available P is proposed for better understanding and categorization of different inorganic and organic P fertilizers in environmental and fertilizer legislations. This may contribute to an improved differentiation between soils, crops, and fertilizer types in the recommendations and standards given on P fertilizer requirements. Indeed, a classification of P application standards in terms of the soil P status, texture, and fertilizer properties, next to the crop P demand, is recommended. Based on the results of the presented greenhouse experiment, field-scale validation of recovered struvite and digestates as compared to animal manure and chemical P fertilizers seems worthwhile. Particular attention should be given to the soil bio-availability indices, including Rhizon, on various soil types with different texture and P status. This should further help to refine the P fertilizer legislations and associated recommendations.

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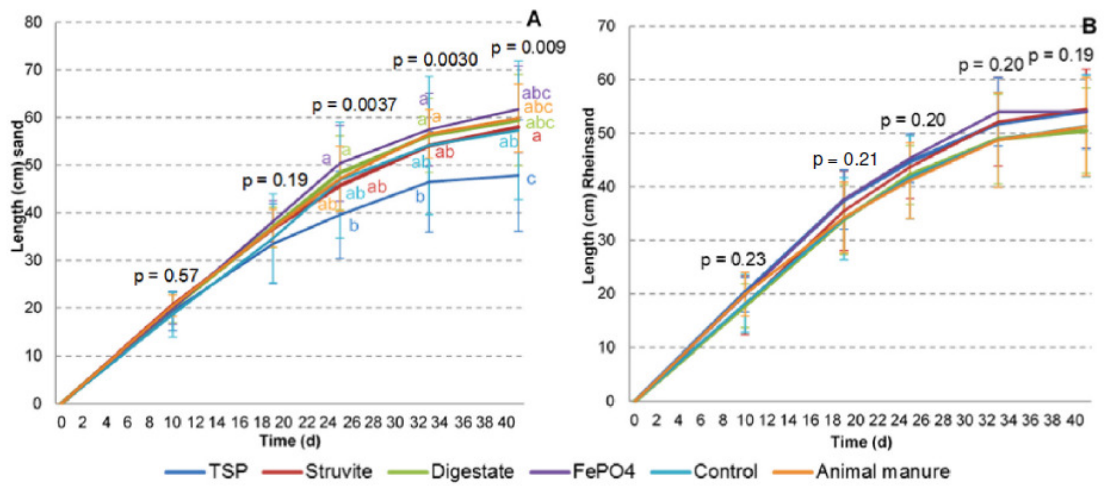


Fig. 1

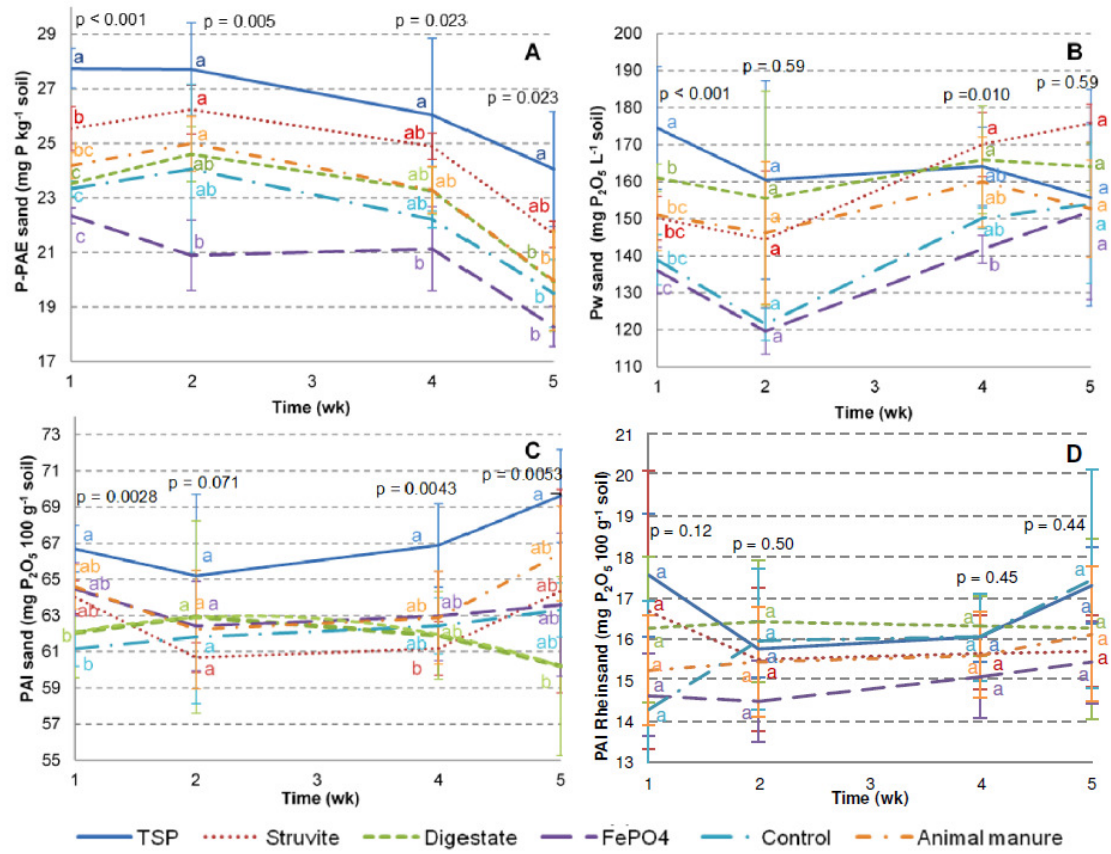


Fig. 2

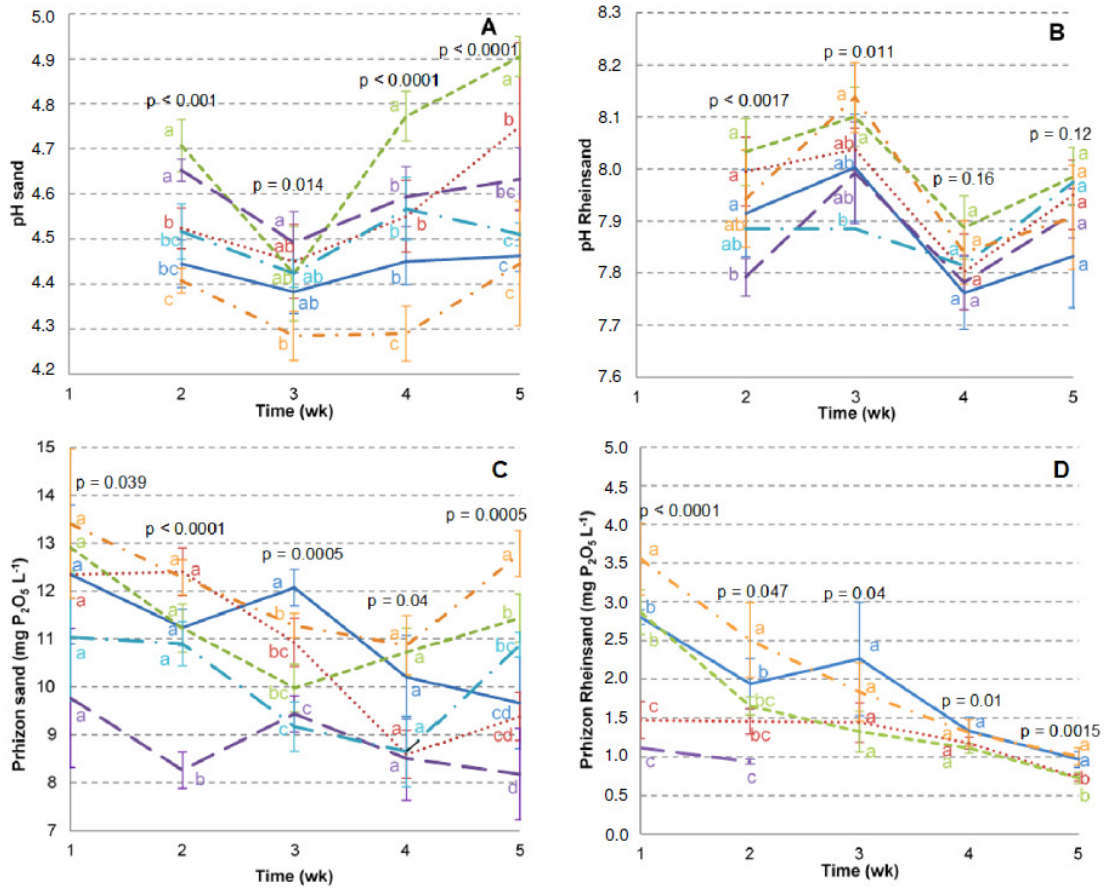


Fig. 3

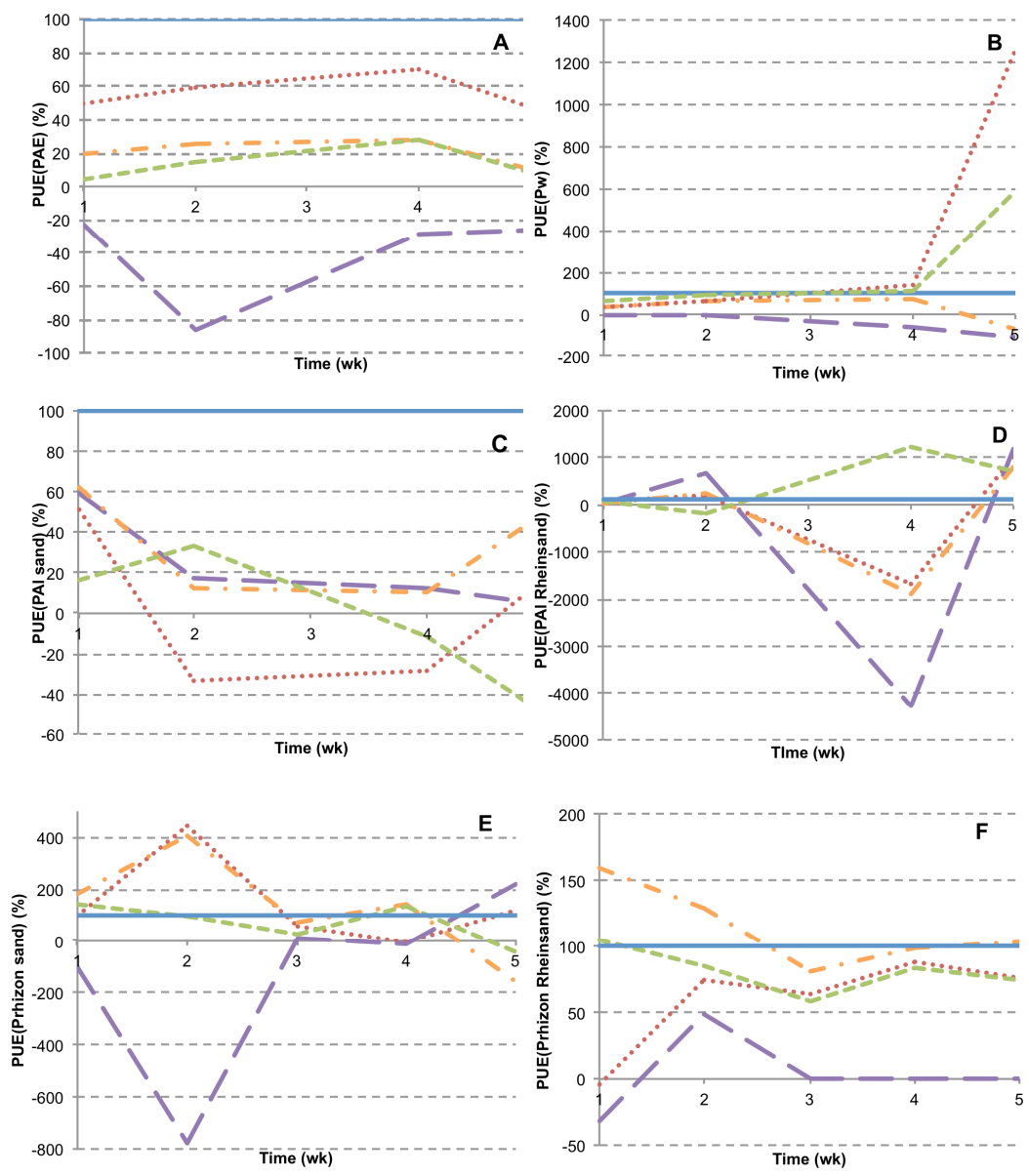


Fig. 4