Response of phosphorus dynamics to sewage sludge application in an agroecosystem in northern France
David Houben, Etienne Michel, Cécile Nobile, Hans Lambers, Ellen Kandeler, Michel-Pierre Faucon

To cite this version:

HAL Id: hal-02176387
https://hal.archives-ouvertes.fr/hal-02176387
Submitted on 8 Jul 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Response of phosphorus dynamics to sewage sludge application in an agroecosystem in northern France

David Houben¹*, Etienne Michel¹, Cécile Nobile¹, Hans Lambers², Ellen Kandeler³, Michel-Pierre Faucon¹*

¹AGHYLE, UniLaSalle, 19 rue Pierre Waguet, 60026 Beauvais, France.
²School of Biological Sciences and Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley (Perth), WA 6009, Australia.
³Institute of Soil Science and Land Evaluation, Soil Biology, University of Hohenheim, Emil-Wolff Str. 27, 70599 Stuttgart, Germany.

*Corresponding authors
Email: david.houben@unilasalle.fr; michel-pierre.faucon@unilasalle.fr

Abstract

Sewage sludge is a phosphorus (P) source alternative to P fertiliser derived from rock phosphate, but its impact on soil processes driving P cycling in agroecosystems requires further study. In order to optimise the use of sludge for sustainable P fertilisation, we need to elucidate the drivers of P dynamics. The present study aims at determining how different sludges (heated sludge, HS and composted sludge, CS) affect soil P pools and dynamics. A field experiment was established and soil was amended either with sludge or with inorganic P (triple superphosphate, TSP). Soil samples were collected five times during a vegetation period, and analysed for Hedley P fractions, microbial P and phosphatase activity. Phosphorus dynamics in soil was strongly influenced by P concentrations in sludge. About one year after application, sludge with the highest P concentration (HS) was as effective as TSP to improve soil P availability. The P source of TSP was immediately available for plant uptake, but the high
phosphatase activity of the HS treatment evidenced that soil microorganisms released phosphatases which can hydrolyse HS-derived organic P compounds. In addition, the high content of microbial P in the HS treatment suggests that soil microorganisms assimilate P into their own biomass. By contrast, sludge with the lowest P concentration (CS) enriched primarily the weakly-soluble soil P fractions, resulting in lower P availability compared with that in the TSP treatment. Our findings suggest that both high P concentration and slow, but continuous microbial breakdown of organic P substrates derived from HS allow using this resource as an important source for plant mineral nutrition. This study stresses the need to both characterise P concentrations and P forms in sludge, prior to their application in the field.

Keywords
Hedley fractionation; Microbial phosphorus; Phosphatase; Phosphorus availability; Waste management
1. Introduction

Phosphorus (P) is a limiting nutrient for the productivity of many agroecosystems and indispensable to feed an ever-increasing human population (Filippelli, 2008; Withers et al., 2015). Currently, most of the P used in chemical fertilisers is derived from phosphate rocks that are finite and located in only a few places on Earth (Edixhoven et al., 2013; Reijnders, 2014). Developing sustainable fertilisation practices based on the use of renewable resources such as P-rich waste is thus essential to ensure long-term food security (Dawson and Hilton, 2011; Houben et al., 2017). Among P-rich wastes, sewage sludge appears an excellent candidate because it represents the largest component of recycled P, and due to the world’s population growth, this reserve is expected to increase (Lwin et al., 2017). Its use in agriculture will likely increase, requiring continued vigilance in assessing the impacts of the presence of potential pollutants such as metals, organic contaminants (e.g., pharmaceuticals and personal care products), emerging contaminants, viruses and other pathogens (Clarke and Smith, 2011). Since 1986, Council Directive No. 86/278/EEC has governed the use of sewage sludge in the European Union by prescribing testing of sludge and soil for a number of potential pollutants. This Directive has been implemented into the national legislation of member states, most often with stricter limits than that prescribed in the Directive (Kirchmann et al., 2017).

Because sewage sludge has a much lower P concentration than mineral P fertilisers, large amounts of sludge need to be transported to and applied on farms which may result in higher costs compared with mineral P fertilisers (Mackay et al., 2017a). Moreover, in contrast to conventional P fertilisers in which P is predominantly in a soluble form readily available to plants, sewage sludge contains a range of P forms with varying availability to plants (Kahiluoto et al., 2015). Phosphorus is present as a mixture of inorganic P forms including calcium (Ca) phosphates and amorphous aluminium (Al)- or iron (Fe)-bound P, while organic P generally represents a small fraction (Xie et al., 2011a). We already have solid evidence from different
laboratory studies that the concentration of P in the soil solution, which is the form of P for plant uptake, after adding sewage sludge is controlled by the forms of P in sludge which depend on the origin and the treatment process of sludge (Frossard et al., 1996; Maguire et al., 2001). However, very little is known on changes in other soil P forms, and processes that are often driven by functional traits of plants and soil microorganisms (e.g., mycorrhizal colonisation, release of extracellular enzymes involved in P cycling) (Mackay et al., 2017b; Requejo and Eichler-Löbermann, 2014).

In addition to supplying P to the plant-available soil P pool through dissolution of inorganic P and mineralisation of organic P, sludge application can also alter P dynamics in soil by modifying abiotic and biotic soil properties (Faucon et al., 2015). Like other organic amendments, the presence of sludge in soil may enhance the activity of phosphatases (Bastida et al., 2008). Phosphatases are a broad group of enzymes that catalyse the hydrolysis of organic P, leading to the release of available ortho-phosphate. Recent findings have shown that organic P, rather than available P, is the most important P fraction in regulating phosphatase activity in soil (Margalef et al., 2017). Transformation of organic P through enzymatic reactions and its subsequent potential immobilisation by microbial biomass play a fundamental role in P dynamics, and is likely affected by the source of P (Saha et al., 2008). However, although studies about the effects of sewage sludge addition on soil biological properties have been numerous, they generally focused on one type of sludge (Criot et al., 2007) and were carried out under controlled conditions, which do not fully mimic field scenarios. As a result, the extent of biological processes involved in situ in P cycling (i.e. organic P hydrolysis, P immobilisation) under different sludge sources has not been clearly identified, and it appears difficult to generalise previous results to different types of sewage sludge applied under field conditions.

Given the interest in using sewage sludge for P fertilisation, it is crucial to find out how its properties mediate both chemical and microbial processes affecting P cycling in
agroecosystems. In particular, unravelling the role of sludge application in changes in soil P forms over time by characterising both chemical and microbial soil properties involved in P dynamics may shed light on the pathway of P release in sludge-amended soils. Ultimately, this knowledge helps predict the prospective P availability and establish the dose, frequency, and timing of sludge application. Therefore, this study aimed at gaining a better insight into the effects of sludge application on P lability and availability under field conditions, taking into account the impacts on soil microbial activities. We monitored shifts in soil P fractions over time (Hedley fractionation method; Tiessen and Moir, 2008) and determined phosphatase activity and microbial P in a field experiment using soil amended with two types of sewage sludge with different P concentrations. In order to estimate the potential of sludge to supply plant-available P relative to that by conventional P fertilisers, we also performed treatments with triple superphosphate (TSP). We hypothesised that i) changes in soil P availability would be driven by P composition of the sludge, and ii) sludge would increase microbial alkaline phosphatase activity, possibly resulting in higher P availability over time.

2. Materials and methods

2.1. Sewage sludge properties

Two types of sewage sludge treated in different processes were provided by the Parisian public sanitation service (Seine Aval wastewater treatment plant, SIAAP, Paris). The first sludge (hereafter called heated sewage sludge, HS) was derived from a treatment process comprising P precipitation using ferric chloride, an anaerobic digestion, followed by a dewatering by thickening and thermal conditioning (heat exchange and heating at 195° C and 20 bars). The second sludge (hereafter called composted sewage sludge, CS) was treated by anaerobic digestion followed by composting. Four biochemical fractions were determined using
a modified Van Soest method (AFNOR, 2009): soluble (SOL), hemicellulose-like (HCE), cellulose-like (CEL) and lignin-like (LIG). The indicator of residual organic carbon (C), $I_{ROC}$, which represents the proportion of stable organic C was then calculated as (Lashermes et al., 2009):

$$I_{ROC} = 44.5 + 0.5 \times SOL - 0.2 \times CEL + 0.7 \times LIG - 2.3 \times MinC3$$

where $I_{ROC}$, SOL, CEL and LIG are expressed in % total organic C in sludge and MinC3 is the proportion (%) of mineralised organic C during the first three days of incubation (AFNOR, 2009). Organic C concentration, C/N ratio (AFNOR, 2013, 2012) and water-soluble P (WSP) concentrations (García-Albacete et al., 2012) were also determined. Phosphorus forms in sewage sludge which were determined through the SMT (Standards, Measurements and Testing programme) protocol. The SMT method is a harmonised protocol proposed by the European Commission for sequential extraction of P initially in sediments which was then extended to other materials, including sewage sludge (García-Albacete et al., 2012; Medeiros et al., 2005).

Briefly, inorganic P (IP) was extracted with 1 M HCl for 16 h and residues of this extraction were calcined for 3 h at 450 °C and then again extracted with 1 M HCl for organic P (OP). Nonapatite inorganic P (NAIP) associated with oxides and hydroxides of Fe, Al or Mn was extracted with 1 M NaOH for 16 h, and then some part of this extract was treated with 3.5 M HCl. The residues of this extraction were extracted for apatite P (AP) associated with Ca (Ca–P) with 1 M HCl for 16 h. Total P (TP) is the sum of OP and IP. As shown in Table 1, HS contains three times more total P than CS, while AP, NAIP and OP concentrations are 2.6, 11.4 and 2.8 times greater in HS than in CS.
2.2. Study site and experimental design

A field experiment was established in September 2015 in Beauvais, North of France (49°28’N; 2°4’W). The experimental site is included in a long-term (> 20 years) cropland field with an oilseed rape – winter wheat – winter barley rotation and an organic and mineral fertilisation based on soil tests, crop requirements, and timed to crop uptake. Cattle manure (25 Mg ha⁻¹) and lime amendments were applied, respectively, every three years, and six years before this experiment. Reduced tillage (5 cm of soil deep rotary harrow) was practiced since 2010. The oceanic climate is characterised by an average precipitation of 669 mm year⁻¹. Average minimum and maximum temperatures vary from 1 to 6.7°C in winter, 5 to 14.5°C in spring, 12 to 23°C in summer and from 7.2 to 15.3°C in autumn. The studied soil was classified as a Haplic Luvisol (IUSS Working Group WRB, 2015). The soil texture was a silt loam with 12% sand, 66% silt and 22% clay. The soil pH_H2O was 7.53, the organic carbon concentration was 24 g kg⁻¹ and the cation exchange capacity was 15 cmol_c kg⁻¹. Given its soil properties and long-term fertilisation and cropping history, the study site is considered representative of the agricultural areas receiving the SIAAP sludge.

Five treatments were tested: two sludge amendments (HS and CS) were applied at a rate of 4.78 Mg dry matter ha⁻¹ (i.e. 7.5 Mg fresh matter ha⁻¹), which is based on common practices of farmers who use SIAAP sludge. Two mineral controls (triple superphosphate) adding the same quantity of P as in each sludge were also included (MHS for HS and MCS for CS) as well as a control without P fertilisation (C). A randomised complete block with four replicates per treatment was used. Each plot had an area of 40 m² (10 x 4 m) as used in Gallet et al. (2003). The experimental field had an area of 1000 m². The homogeneity of soil properties within the study area was previously assessed by a topsoil (0-5 cm) sampling (20 samples collected) and chemical characterisation. Before spreading sludge, the soil was tilled with a stubble cultivator in order to make the spreading homogeneous (5 cm depth). All the treatments were carried out
on the 15th of September 2015. Then, the soil was again tilled with a harrow to incorporate the sludge following the French legislation. Winter barley (Viva, two-rowed cultivar) was sown on the 9th October 2015. Nitrogen application of 130 kg ha\(^{-1}\) was based on a nitrogen balance calculation.

2.3. Plant sampling and analysis

Plants were harvested on 26th June 2016 and the crop was weighed on board of a harvester. After harvest, plants were dried at 70°C for 72 h, weighed and then ground to 250 µm prior to analysis. Phosphorus concentrations in straw and grain were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo Scientific XSERIES2) after digesting the dried biomass in \textit{aqua regia}. Briefly, 0.2 g of plant powder was mixed with 8 mL of concentrated HNO\(_3\) and 2 mL of concentrated HCl directly in a microwave Teflon vessel (Lange et al., 2014) with a control without plant. The vessels (Easyprep 432175A, CEM µWaves) were placed in the microwave system (Mars 5, CEM Corporation, Charlotte, USA). The digestion programme consisted of a ramp time of 5 min to reach 180 °C and digestion was performed for 15 min. The power was set at 1600 W (Lange et al., 2014). The digest was then diluted to approximately 30 g (accurately weighed) and then stored until being analysed with ICP-MS.

2.4. Soil sampling and analysis

Two composite soil samples were collected at the surface (0 – 10 cm) within each plot in September (before and one week after fertilisation), January (during winter), May (during spring) and July (a few days after harvesting) to characterise P fractions, Olsen-P and soil chemical properties. Subsamples were used either fresh for microbial P and alkaline
phosphatase activity (May and July) analyses or oven-dried (35°C, 48 h), crushed and sieved through a 2-mm plastic sieve. Soil pH and electrical conductivity were measured in suspensions by shaking 2 g of soil with water (1:5 ratio). The Hedley et al. (1982) sequential fractionation method as modified by Tiessen and Moir (2008) was used to fractionate soil P. This method uses a sequence of increasingly strong extractants that remove labile inorganic P (Pi) and organic P (Po) forms first, then stable P forms. Briefly, 0.5 g of air-dried soil (ground to pass through a 2 mm sieve) was successively extracted with resin strips in deionised water (plant-available Pi), 0.5 M NaHCO$_3$ (pH 8.5) (Pi and Po adsorbed onto the soil surface), 0.1 M NaOH (Pi and Po held more strongly by sorption to surfaces of Al and Fe oxides), 1 M HCl (P associated to Ca, derived from primary mineral-apatite). The last fraction (residual-P; stable Po forms and relatively insoluble Pi forms) was determined using a microwave digestion (Mars 5, CEM Corporation, USA) with aqua regia and hydrofluoric acid, EN 13 656 (Gaudino et al., 2007). Labile P was considered the sum of resin-P and NaHCO$_3$-extracted P (Cross and Schlesinger, 1995; Tiessen and Moir, 2008). The Pi concentration in the extracts was determined colourimetrically using the molybdate blue method according to Murphy and Riley (1962) at 712 nm, as recommended by Tiessen and Moir (2008) to reduce possible interference from traces of organic matter. Total P (P$_{tot}$) in the extracts was determined by ICP-MS and the concentration of Po was estimated by subtracting Pi from P$_{tot}$. In addition to Hedley’s fractionation, Olsen-P concentration was determined (Olsen, 1954).

2.5. Microbial phosphorus

Microbial P was determined by hexanol fumigation (Bergkemper et al., 2016) and extraction with anion-exchange membranes (VWR, 551642S). Anion-exchange membrane strips were prepared by initially shaking in 0.5 M NaHCO$_3$. For each sample, three portions of fresh soil (2 g on a dry-weight basis) were weighed into 50 mL bottles with 30 mL deionised
water and two anion-exchange membrane strips. One bottle received 1 mL of hexanol and the
samples were shaken for 16 h. The membranes were then removed and rinsed in deionised water
and the phosphate ($P_{fumigated}$) recovered by shaking for 1 h in 20 mL (HCl 0.5 M). Another bottle
only contained water and was treated the same way as previously ($P_{non-fumigated}$). To determine
the amount of P retained by soil particles and complexation after fumigation incubation, a
defined P concentration ($KH_2PO_4$), which was equal to the measured P concentrations in
fumigated subsamples, was added to additional non-fumigated, but otherwise identically treated
subsamples. Phosphorus concentrations were analysed colourimetrically (Murphy and Riley,
1962) using a spectrophotometer (712 nm). The ratio of recovered P to added P was used to
calculate the $P_{mic}$ concentration as follows (Nassal et al., 2018):

$$P_{mic} [\mu g \ g^{-1}] = \frac{(P_{fumigated} [\mu g \ g^{-1}] - P_{non-fumigated} [\mu g \ g^{-1}])}{(P_{recovered} [\mu g \ g^{-1}] / P_{added} [\mu g \ g^{-1}])}$$

2.6. Alkaline phosphatase activity

According to the pH of the soil (7.53), alkaline phosphomonoesterase (alkaline
phosphatase) activity was assayed as an enzyme involved in P cycling. Alkaline phosphatase
activity was assayed by the method of Tabatabai and Bremner (1969), which involves the
determination of p-nitrophenol released by incubation at 37°C for 1 h of 1 g soil with 0.2 ml
toluene, 4 ml universal buffer and 1 ml substrate. Sodium p-nitrophenyl phosphate was used as
substrate for assay of phosphatase activities (Eivazi and Tabatabai, 1977). The quantity of p-
nitrophenol produced by alkaline phosphatase was measured using a spectrophotometer (410
nm).
2.7. Statistical analyses

All recorded data were analysed using descriptive statistics (mean ± standard error) and normality was determined using the Shapiro-Wilk test. The data were subjected to one-way ANOVA and Tukey’s post-hoc test to compare treatments, which had a normal distribution. Data without normal distribution were subjected to the Kruskall-Wallis test and Mann-Whitney post-hoc test. Two-way ANOVAs were performed considering time as a fixed factor and fertilisation type as variable. All statistical analyses were performed using R software version 3.5.0. (R Core Team, 2017) and the package Rcmdr (Fox, 2005).

3. Results

3.1. Sewage sludge properties

Table 1 indicates that CS and HS had a similar I_{ROC} and C/N ratio, while HS was slightly more alkaline than CS, and had a higher C concentration. For each sludge, NAIP was the predominant P form. The proportion of AP was slightly higher in HS, while the proportions of WSP and OP were similar between both sludges. More importantly, the total P concentration in HS was three times as high as that in CS, suggesting that the main difference between both sludges is not P fraction, but total P concentration.

3.2. Changes in labile P concentration over time

The effect of sludge addition on the labile P concentration (i.e. the sum of resin-P and NaHCO₃-P concentration) was strongly affected by the type of sludge applied (Fig. 1). After 124 days, the labile P concentration in the presence of HS was greater than that of the no-P control (C), but lower than the mineral control (MHS) ($\chi^2= 9.85$, df=2, p value<0.01). However,
after 240 days, the labile P concentration in HS was as high as that in MHS ($\chi^2=8.77$, df=2, p value<0.05). Unlike HS, labile P concentrations in the presence of CS were similar to that of C (p > 0.05) and significantly lower (p < 0.05) than its mineral control (MCS) for the duration of the experiment (Fig. 1). Despite differences in labile P concentrations among fertilisation treatments, P concentrations and quantity in shoot (straw) and grain were not significantly different among treatments (Supplementary Fig. 1, 2, 3).

3.3. Phosphorus forms and microbial-P in soil

Among the studied forms, only HCl-P was unaffected by fertilisation treatment, while only Resin-P was unaffected by time (Table 2). NaHCO$_3$-P, NaOH-P and residual P fractions were significantly affected by fertilisation treatment and also by time (Table 2). After 307 days (Fig. 2), HS significantly increased the NaHCO$_3$-Pi fraction and decreased the residual-P fraction compared with MHS and C. The NaOH-Po fraction was also significantly greater in the presence of HS compared with that in the other treatments. Moreover, only HS significantly increased the concentration of microbial-P in the soil (Fig. 3). In contrast with HS, CS had no effect on P forms in soil compared with both C and its mineral control (MCS).

3.4. Soil pH

Soil pH was influenced by fertilisation treatment and time (Table 2). The soil amended with MHS was significantly (p < 0.05) more acidic over the entire experimental period, while the pH did not differ significantly among the other treatments (Supplementary Fig. 4).
3.5. Alkaline phosphatase activity

Alkaline phosphatase activity was significantly increased by the application of HS while no significant difference was observed for the other treatments (Fig. 3).

4. Discussion

Most of previous studies investigating the P fertiliser potential of waste products such as sewage sludge have only focused on change in available P concentration after their application, disregarding how other soil P forms are affected. Here, our findings suggest, however, that P availability may change with time due to chemically and biologically controlled shifts in soil P fractions, which are themselves mediated by P concentrations in sludge.

4.1. Effect of sludge on labile P concentration over time

Monitoring labile P concentration over time is necessary to ensure that P supplies will meet crop demand. Labile P can be continuously supplied to the soil solution, for instance, by organic P mineralisation through microbial activity and by mineral P desorption through chemical reactions (Chen et al., 2004; Sanyal and Datta, 1991). Chemical extractions have been extensively used to evaluate P lability in soils (Sharpley, 2009), even though they may be inappropriate in detecting changes produced by natural environmental soil modification such as cycles of alternating oxidation and reduction conditions (Scalenghe et al., 2014). Here, as recommended by Cross and Schlesinger (1995), we consider labile P to be the sum of resin-extractable P + NaHCO$_3$-extractable P. According to these authors, P extracted by ion exchange resins and NaHCO$_3$ solutions represents the most likely contributors to plant-available P over
the course of a growing season, because it cycles readily through the microbial community and
is made available to plant roots through a variety of chemical and biological processes. Our
results reveal that HS significantly increased the labile P concentration over time (by 100%
after 124 days). After 240 days, the HS treatment showed the same concentration of labile P as
the mineral control (MHS). According to Huang et al. (2012), who observed an increase in P
availability in sludge-amended soil after 100 days of incubation, the strong increase of labile P
concentration in the presence of HS likely results from the continuous mineralisation of its large
organic P pool (9.49 g kg\(^{-1}\)). This increase in P availability with time suggests that sludge with
a high organic P concentration must be regarded as a slow-release P fertiliser. This slow-release
P fertiliser behaviour has also been reported by Lemming et al. (2017). The authors showed that
when applying a sludge similar to HS to a soil with pH 7.8, the efficiency in terms of available
P relative to TSP increased from 20% to 46% after 84 days. Since many crops have high P
demands in the early stage of their life cycle (Fageria, 2016), for example wheat plants take up
50-60% of their P in the first six weeks of growth (Römer and Schilling, 1986), there is a risk
that the slow release of P from HS negatively impacts early plant growth. This might, however,
be compensated by adding fertiliser mixtures of soluble P (e.g., TSP) and HS. In contrast with
HS, CS did not influence the available P concentration. Since both sludges did not differ in their
P fractions, it is likely that the much lower total P concentration in CS was the predominant
driver of the lack of increase in P availability after addition to the soil (Welch et al., 2002). Due
to its low total P concentration, the potential of CS to be used as a P fertiliser is thus limited, at
least during the first year after its application, in such cropping systems. One possibility to
mobilise the sparingly available CS-derived P might be to develop cropping systems with
species having highly efficient P-acquisition strategy such as Lupinus albus L. (e.g., by
intercropping it with cereals) (Hallama et al., 2019; Lambers et al., 2013). Further researches
are however needed to determine to what extent this would counteract the limited P fertiliser
potential of CS. Taken together, our results indicate that the efficacy of sludge to increase P availability in soils would depend on the concentration and the forms of P added and the cropping system used.

It is important to note that the lack of any improvement of P concentration and quantity in plants in spite of the increase in available P concentration in soil after the addition of HS likely results from the already high P concentrations in these soils as a result of the long-term addition of P fertiliser (Gallet et al., 2003). According to Glæsner et al. (2019), a significant increase in P uptake might be expected in the long run, as P applied with sludge remained highly plant available in the soil after long-term application.

4.2. Phosphorus forms and concentrations in sludge influence P forms in soil

Interactions between P forms in sludge and P forms in soil are not fully understood so far, most studies focusing only on total and water-extractable P concentrations. As shown by other researchers using pot experiments (Frossard et al., 1996; Meyer et al., 2018; Nanzer et al., 2014), our results indicate that coupling the determination of P concentration and forms in sludge is, however, essential. Indeed, depending on total P concentration, P forms in sludge drive P distribution in various soil pools, which, in turn, impact P availability. By contrast to HS, CS had no effect on P distribution in soil pools compared with its mineral control (MCS). Since CS and HS had a relatively similar P composition (OP is ca 20% while insoluble AP+NAIP is ca 80% in both sludges), the lack of any effect of CS is thus most likely related to its much lower total P concentration.

The application of HS increased the NaHCO$_3$-P pool, which is considered readily available to plants and is strongly related to P uptake by most crops (Saleque et al., 2004). However, some crops with highly efficient P-acquisition strategies (e.g., white lupine, canola)
can access additional P fractions (Gardner et al., 1983; Hoffland et al., 1989). These results confirm the high potential of HS as a P fertiliser.

The application of HS also strongly increased the NaOH-P (Pi +Po) concentration. The NaOH-Pi pool is considered P sorbed to Al and Fe (hydr)oxides while NaOH-Po has been found to be predominantly associated with organic matter, namely fulvic and humic acids (Cassagne et al., 2000; Schroeder and Kovar, 2006; Tiessen and Moir, 2008). Although Al and Fe oxides are important P-sorbing components in soil (Hinsinger, 2001; Houben et al., 2011), it is unlikely that they act as a significant sink for P added by HS, since, even in the mineral controls, the fraction of P in the NaOH-Pi was not significantly increased compared with the control. The higher proportion of NaOH-Pi in HS-amended soil might be due to the high NAIP content in HS which results from the pre-treatment of this sludge (i.e. iron chloride precipitation) (Kahiluoto et al., 2015). On the other hand, the significantly higher NaOH-Po fraction following the application of HS probably results in part from the high concentration of organic P in this sludge (Malik et al., 2013; Smith et al., 2006). As a slowly exchangeable P pool (Frossard et al., 2000), the NaOH-P pool can be mobilised when P in soil solution is depleted from the soil by plant uptake (Guo et al., 2000; Saleque et al., 2004). Beck and Sanchez (1994) reported that NaOH-P is the dominant pool related to availability of P to plants in an 18-year continuously-cultivated and fertilised cropping system because it maintains the levels of plant-available P through P mineralization. According to Crews and Brookes (2014), the NaOH-P pool holds P for years to decades before crop demand shifts the equilibrium in the soil solution and causes P to be released. In addition to increasing the readily-available P pool in soil, our findings suggest that the addition of HS might also supply available P in the long run. Further investigations will however, be necessary to determine the contribution of the NaOH-P pool to long-term P release and thus better predict the dynamics of P in sewage sludge-amended soils.
Microbial biomass plays a key role in P availability in agroecosystems. It acts as a buffer by immobilising P from the soil solution and potentially preventing it from bonding with soil particles (Crews and Brookes, 2014). Microbial P is released when cells are disrupted, e.g., in response to sudden changes in soil temperature, water content, and carbon availability (Turner et al., 2003) or due to predation (Bonkowski, 2004), which results in an increase in available P (Oehl et al., 2001). Our results indicate that sludge application may have a strong impact on the microbial-P pool. By contrast to CS, which showed a similar microbial-P pool to its mineral control (MCS), HS application significantly increased the microbial-P pool compared with its mineral control (MHS). Andriamananjara et al., (2016) also observed a significant increase in microbial-P concentration after sewage sludge application compared with TSP. Consistently with Crews and Brookes (2014), this can be related to the increase of the NaOH-Po fraction brought about by this treatment, resulting in a stronger assimilation of P into soil microorganisms. Two different mechanisms might lead to the enrichment of P in soil microorganisms: 1. Soil microorganisms might grow faster due to the accelerated decomposition of organic P compounds, or 2. Soil microorganisms might change the stoichiometry of their cells towards a lower C/P ratio. Independently on the mechanism of P accumulation of soil microorganisms, the mean residence time of P in soil microorganisms is only 18 to 39 days and depends on soil P availability (Spohn and Widdig, 2017). Therefore, soil microorganisms represent a short-term storage of easily-available P fractions in soil, and store P somewhat longer in the case of restricted P access from soils. The P fraction stored in soil microorganisms is not only released by death of soil microorganisms, but also by the feeding behaviour of soil animals that require carbon resource to a higher extent than nutrients like P and N. This phenomenon, which was first described by Clarholm (1985) for protozoa, is now extended to a wider group of soil animals (protists, nematodes) and to other nutrients like...
P (Trap et al., 2016). Consequently, the importance of food webs in agroecosystems for biotic release of P derived from organic resources (like sludge) needs further study (Richardson and Simpson, 2011). Additional work including the characterisation of microbial biomass and structure of microbial community using phospholipid fatty acid analysis, sequencing and quantification of genes involved in P dynamic in soil (i.e. phoB and phoR) (Anderson et al., 2011) might be of help to better understand this microbial P increase in the presence of sludge.

4.4. Effect of sludge on alkaline phosphatase activity

The relationship between phosphatase activities and P in soil amended with sludge is still poorly understood, despite extensive investigations (Xie et al., 2011b). Phosphatase activities are expected to increase after application of organic matter, resulting in higher P availability in soil (Garg and Bahl, 2008). However, the response of phosphatase activities to applied organic waste also depends upon its initial constituents, amount and size, and must be investigated prior its application (Criquet et al., 2007; Saha et al., 2008; Tejada et al., 2008; Xie et al., 2011b) (Criquet et al., 2007; Saha et al., 2008; Tejada et al., 2008). Increases of alkaline phosphatase activity after the application of organic amendments have been primarily attributed to both the supply of easily decomposable organic compounds and change in soil pH (Dick et al., 2000; Garg and Bahl, 2008). In our study, application of HS did not affect soil pH. For this treatment, it can therefore be inferred that the supply of large amounts of substrates, including substrates for phosphatases (Bachmann et al., 2014), was the main process responsible for the stimulation of enzyme production by soil microorganisms (including mycorrhizal fungi). The stimulation of phosphatase activity is largely due to increased microbial numbers in the soil which, with time, cause a build-up of enzymes (Feder, 1973). Therefore, the higher alkaline phosphatase activity in the presence of HS is consistent with the higher NaOH-Po fraction and the subsequent increase in microbial-P concentration measured under this treatment. Unlike HS,
CS application did not increase alkaline phosphatase activity, most likely because it did not increase the soil organic P pool due to its low total P concentration, as discussed above (Margalef et al., 2017; Requejo and Eichler-Löbermann, 2014). In line with the recent efforts directed toward increasing P availability by mobilising recalcitrant soil P (Menezes-Blackburn et al., 2017), gaining insight into the factors responsible for the higher phosphatase activity in the presence of HS might help develop strategy to transform organic P to inorganic P available for plants. More generally, our data are consistent with the literature showing a positive effect of sewage sludge on the soil enzyme activities, with a positive correlation between rate of sludge application and enzyme activity (Fernandes et al., 2005; Siebielec et al., 2018).

4.5. Implications

Moving toward more sustainable sources for managing the P nutrition in agroecosystems, it is increasingly suggested to replace mineral fertilizers by P-rich materials originating from waste materials (Dawson and Hilton, 2011). In addition to studying the release of available P from these soil amendments, it is however essential to gain further insights into their indirect effects on the soil P pools, especially by paying attention to modifications of soil biota (Faucon et al., 2015). Here, our results showed that changes in soil biological properties after the addition of sewage sludge with high total P concentration (and 20% as organic P) contributed to increase the labile P concentration to a level as high as that of conventional fertiliser. It is necessary, however, to conduct further studies to identify the relative contribution of soil-derived P and sludge-derived P to this P lability increase, especially through the use of isotopic labelling techniques.

5. Concluding Remarks
This study showed that the impact of sewage sludge with similar P fractions on P dynamics in agroecosystem is predominantly driven by total P concentration in sludge. Our findings suggest that, despite a relatively low water-soluble P concentration, the addition of sewage sludge with high total P concentration and 20% of total P as organic P has a great potential to improve sustainable P fertilisation, since it increased alkaline phosphatase activity and microbial-P, temporarily protecting P from fixation by soil particles. The positive effect of sludge with high total P concentration (and 20% as organic P) on the microbial-P pool and phosphatase activity is a pivotal result to understand the effects of organic waste on agroecosystem functioning, and, ultimately, to improve sustainable P fertilisation. Overall, our results stress the need that sludge provider measure both P concentration and P forms in sludge prior to their application in the field. The perspective is to elucidate the sludge’s long-term effects on P pools and availability over the whole crop rotation in several soil types to define new sustainable practices of P fertilisation by taking into account both microbial and plant functional traits involved in P mobilisation/acquisition.

Acknowledgments

We thank the SIAAP wastewater treatment for funding and Thomas Pucheu from SEDE Environnement for technical discussion. We also thank Vincent Hervé, Adeline Dubos, Sabine Rudolph, Petru Jitaru and Philippe Jacolot for technical assistance. Olivier Pourret, Sabine Houot, Frédéric Gérard and Isabelle Trinsoutrot-Gattin are acknowledged for their valuable comments to this research.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.
References


Fageria, N.K., 2016. The Use of Nutrients in Crop Plants. CRC Press.


582 Lange, B., Faucon, M.-P., Meerts, P., Shutcha, M., Mahy, G., Pourret, O., 2014. Prediction of the edaphic factors influence upon the copper and cobalt accumulation in two


**Figures**

**Figure 1. Release of labile phosphorus (resin-P + NaHCO$_3$-extractable P) over time.** C: No-P control; CS: Composted Sludge; MCS: Mineral control of Composted Sludge; HS: Heated Sludge; MHS: Mineral control of Heated Sludge. Different letters indicate significant differences between treatments (P<0.05). Capital letter indicate differences between composted-sludge/Mineral control of CS/ No-P control, lower case letter between heated sludge/ Mineral control of HS/ No-P control. Values are the mean of four replicates ± standard error. Significant differences have been tested using Kruskal Wallis test for the comparison HS-MHS-C and One Way ANOVA for the comparison CS-MCS-C.

**Figure 2. Effect of sewage sludge application on phosphorus (P) fractionation (Hedley scheme) after 307 days.** C: No-P control; CS: Composted Sludge; MCS: Mineral control of Composted Sludge; HS: Heated Sludge; MHS: Mineral control of Heated Sludge. The means with the same letters are not significantly different. Values are the mean of four replicates± standard error. Significant differences has been revealed by a Kruskall-Wallis test with a Mann-Whitney post hoc test (Resin-P and NaOH-P) or One Way ANOVA with Tukey post hoc test. Phosphorus fractions are ordered by their availability (highest for Resin-P and lowest for Residual-P). *: P < 0.05 ; **: P < 0.01 ; ***: P < 0.001.

**Figure 3. Alkaline phosphatase activity (left y-axis) and microbial-phosphorus (P) (right y-axis) after 307 days.** C: No-P control; CS: Composted Sludge; MCS: Mineral control of Composted Sludge; HS: Heated Sludge; MHS: Mineral control of Heated Sludge. Significant differences has been revealed using a Kruskall Wallis test with a Mann Withney post hoc test (for alkaline phosphatase) and One Way ANOVA followed by Tukey post hoc test. Values are the mean of four replicates ± standard error. The means with the same letters are not significantly different at the 5 % level.
Phosphorus fraction (%)

- Resin-P
- NaHCO3-Pi
- NaHCO3-Po
- NaOH-Pi
- NaOH-Po
- HCl-P
- Residual-P

C  |  CS  |  MCS  |  HS  |  MHS
---|------|-------|------|------
50 | 46   | 47    | 29   | 38   |
24 | 27   | 25    | 28   | 23   |
5  | 7    | 5     | 9    | 5    |
7  | 7    | 6     | 11   | 7    |
9  | 7    | 6     | 9    | 13   |
2  | 2    | 4     | 3    | 8    |
Tables

Table 1. Chemical properties of sludge. \( I_{ROC} \): proportion of stable organic matter; Corg: organic carbon; WSP: Water-soluble phosphorus; AP: Apatite phosphorus; NAIP: Non-apatite inorganic phosphorus; OP: Organic phosphorus. Carbon and P concentrations are expressed in dry matter of sludge (g kg\(^{-1}\)MS). Values in parentheses represent the percentage of P in each fraction (%).

<table>
<thead>
<tr>
<th>Sludge</th>
<th>( I_{ROC} )</th>
<th>pH</th>
<th>Corg (g kg(^{-1}))</th>
<th>C/N ratio</th>
<th>WSP (g kg(^{-1}))</th>
<th>AP (g kg(^{-1}))</th>
<th>NAIP (g kg(^{-1}))</th>
<th>OP (g kg(^{-1}))</th>
<th>Total P (g kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>79.9%</td>
<td>7.4</td>
<td>185</td>
<td>9.5</td>
<td>0.03</td>
<td>12.46</td>
<td>0.83</td>
<td>3.32</td>
<td>16.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.2)</td>
<td>(74.9)</td>
<td>(5.0)</td>
<td>(19.9)</td>
<td></td>
</tr>
<tr>
<td>HS</td>
<td>80.8%</td>
<td>8.4</td>
<td>251</td>
<td>12.3</td>
<td>0.08</td>
<td>32.18</td>
<td>9.49</td>
<td>9.17</td>
<td>50.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.2)</td>
<td>(63.2)</td>
<td>(18.6)</td>
<td>(18.0)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Summary of Two Way ANOVA analysis.

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>Time</th>
<th>Treatment x Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Df</td>
<td>MS</td>
<td>F</td>
</tr>
<tr>
<td>pH</td>
<td>4</td>
<td>0.7383</td>
<td>14.346</td>
</tr>
<tr>
<td>Olsen-P</td>
<td>4</td>
<td>128613</td>
<td>104.216</td>
</tr>
<tr>
<td>Resin-P</td>
<td>4</td>
<td>296.34</td>
<td>56.393</td>
</tr>
<tr>
<td>NaHCO(_3)-P</td>
<td>4</td>
<td>91.25</td>
<td>27.183</td>
</tr>
<tr>
<td>NaOH-P</td>
<td>4</td>
<td>89.77</td>
<td>29.82</td>
</tr>
<tr>
<td>HCl-P</td>
<td>4</td>
<td>56.92</td>
<td>1.898</td>
</tr>
<tr>
<td>Residual-P</td>
<td>4</td>
<td>658.5</td>
<td>18.343</td>
</tr>
</tbody>
</table>

NS: non significant; **: P < 0.01; ***: P < 0.001
Supplementary Materials

Response of phosphorus dynamics to sewage sludge application in an agroecosystem in northern France

David Houben¹*, Etienne Michel¹, Cécile Nobile¹, Hans Lambers², Ellen Kandeler³, Michel-Pierre Faucon¹*

¹AGHYLE, UniLaSalle, 19 rue Pierre Waguet, 60026 Beauvais, France.

²School of Biological Sciences and Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley (Perth),WA 6009, Australia.

³Institute of Soil Science and Land Evaluation, Soil Biology, University of Hohenheim, Emil-Wolff Str. 27, 70599 Stuttgart, Germany.

*Corresponding authors

Email: david.houben@unilasalle.fr; michel-pierre.faucon@unilasalle.fr
Supplementary Figure 1. Phosphorus concentration in winter barley at crop maturity. C: No-P control; CS: Composted Sludge; MCS: Mineral control of Composted Sludge; HS: Heated Sludge; MHS: Mineral control of Heated Sludge. Values are average (n = 4) ± standard error. Columns with the same letter do not differ significantly at the 5% level.

Supplementary Figure 2. Dry biomass at crop maturity. C: No-P control; CS: Composted Sludge; MCS: Mineral control of Composted Sludge; HS: Heated Sludge; MHS: Mineral control of Heated Sludge. Values are average (n = 4) ± standard error. Columns with the same letter do not differ significantly at the 5% level.
Supplementary Figure 3. Phosphorus exported by shoot at full tillering and flowering stages. C: No-P control; CS: Composted Sludge; MCS: Mineral control of Composted Sludge; HS: Heated Sludge; MHS: Mineral control of Heated Sludge. Values are average (n = 4) ± standard error. Columns with the same letter do not differ significantly at the 5% level.

Supplementary Figure 4. Soil pH. C: No-P control; CS: Composted Sludge; MCS: Mineral control of Composted Sludge; HS: Heated Sludge; MHS: Mineral control of Heated Sludge.