



Research article

Acidification and alkalization pretreatments of biowastes and their effect on P solubility and dynamics when placed in soil

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ABSTRACT

Background: Sustainability concerns as well as recent increases in fertilizer prices exacerbates the need to optimise the use of biowastes as fertilizers. For this reason, we investigated how different pretreatments affect the P dynamics when biofertilizers are placed in the soil.

Methods: Sewage sludge (SS), sewage sludge ash (SS-ash), meat and bone meal (MBM), and the solid fraction of biogas digestate (BGF) were pretreated with H₂SO₄, NaOH, and Ca(OH)₂ and incubated for 2 and 12 days, respectively, in a one-dimensional reaction system for detailed studies of the interactions in the biomaterial-soil interface and the soil adjacent to the placement zone.

Results: Our results showed that acidification and treatment with NaOH increased the P solubility of the biomaterials. The P loss from the biomaterial layer to the soil was correlated with water-extractable P in the biomaterials (0.659) and water-extractable P in the soil (0.809). Acidification significantly increased the total amount of P depleted from the biomaterial to the soil whereas NaOH pre-treatment did not. However, for NaOH-treated SS and SS-ash, the apparent recoveries were significantly higher compared to the acidification due to a decrease in soil P sorption capacity as the soil pH increased due to residual alkalinity in the biomaterials.

Conclusions: Acidification showed promising results by increasing the P solubility of all the biomaterials, and the alkalization of SS and SS-ash with NaOH by increasing the apparent recovery in the soil. However, further studies are needed to assess the effects of these treatments on plant growth and P uptake.

1. Introduction

Phosphorus is a limited resource that deserves special attention, as there are still uncertainties regarding the longevity, depletion, and geographic locations of rock phosphate reserves. The European Union listed rock phosphate as a critical raw material (European Commission, 2017a), and recently this situation has worsened due to high increases in mineral fertilizer prices as a result of the Russian invasion of Ukraine (Ibendahl, 2022).

Therefore, biomaterials that can potentially replace mineral P fertilizer merit research to reduce the exploitative dependence on rock phosphate reserves (Geissler et al., 2018; Koppelaar and Weikard, 2013). Sewage sludge, sewage sludge ash, meat and bone meal, and the solid fraction of biogas digestate are among biomaterials that have been studied and can potentially be used as P fertilizers. However, the P use efficiency of these biomaterials varies widely depending on their origin and is usually lower than that of mineral P fertilizer. Möller et al. (2018) reviewed the literature, including the gray literature, on alternative P

fertilizers and identified several hundred studies on these sources. Average P efficiencies compared to water-soluble P fertilizer were as low as about 10% for meat and bonemeal ash, 30% for untreated ashes from sewage sludge, 40% for meat and bone meal, 60% for sewage sludge with chemical P removal through flocculation with Fe/Al, and as high as 90% for sewage sludge with only biological P removal.

The localized application is an alternative that has been studied to increase the P use efficiency of biomaterials (Lemming et al., 2016; Wang et al., 2016). In a previous study, it has been shown that placement of biomaterial in a “hot-spot” in the soil can lead to a decrease of root growth in the soil outside the “hot spot” zone, thereby inflicting opportunity costs on the plant that prevent an overall beneficial effect on growth (Lemming et al., 2016). Thus, in a larger project, we explore the overarching hypothesis that placement of nutrient-rich bioresources close to the seed may be beneficial for plant acquisition of nutrients only if the placement ‘hot spot’ can deliver sufficient nutrients to over-compensate for the decrease in soil-derived nutrients that results from root proliferation in or near the “hot spot”. In this context, it is relevant

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to investigate whether the solubility of P in bioresource materials can be increased to alleviate the effect of increased root growth in the 'hot-spot' zone.

To achieve this, chemical pretreatments of biomaterials by acidification or alkalinization can be relevant. Such pretreatments have been shown to affect P solubility and/or uptake by various crops. For instance, the use of sulfuric acid is a common practice used in Denmark since the 2000s to reduce the pH and minimize ammonia emissions from animal slurry (Fangueiro et al., 2015). Acidification of the raw or anaerobically digested slurry solid fraction also increased the P solubility of these biomaterials and the P uptake by maize (Regueiro et al., 2020). Furthermore, the application of NaOH to sewage sludge was studied to increase the efficiency of anaerobic digestion (Grübel and Suschka, 2015) and has been found to increase the content of water-soluble P in the sludge (Xu et al., 2015). Alkalinization of sewage sludge is also used for sanitization treatments, and lime is the cheapest chemical that can be used to reduce disease vectors (Anderson et al., 2015). Results obtained by Alvarenga et al. (2017) showed that liming of sewage sludge increased the P uptake by barley, while Bauer et al. (2019) reported that the co-application of poultry litter ash with lime increased plant available P. However, the effects of the placement of pretreated biomaterials in the soil have not yet been studied.

Biomaterials are expected to release increasing amounts of phosphate when placed in soil after pretreatment by acidification or alkalinization. The release is likely predominantly due to phosphate diffusion (Lambers, 2022; Silberbush and Barber, 1983), while other mechanisms, e.g. biological turnover and transport through fungal highways (Ruess and Ferris, 2004), and even particle migration (Chetti et al., 2016), may sometimes also be relevant. Pretreatments not only affect the speciation of P in the biomaterials in terms of solubility, but any residual alkalinity or acidity may also affect the soil and thus the prevailing conditions for phosphate reactions in soil by adsorption, precipitation, and occlusion, as discussed by Barrow (1983), Frossard et al. (1995), and Lombi et al. (2006). Thus, it is to be expected that a substantial part of the phosphate released from biomaterials can be immobilized by the soil to a greater or lesser extent, but that this will also depend on the changes in soil pH caused by the residual acidity or alkalinity of the (un)treated biomaterials.

Application of biomaterials and mineral fertilizers leads to a more or less heterogeneous distribution of resources and the formation of nutrient-rich patches in the soil (Hodge, 2004) which have seldom been studied under controlled laboratory conditions (Magid et al., 2006). Thus, whether by design or happenstance, the contact between soil and a biomaterial is generally limited to discrete bands or patches at a well-defined depth where the tillage implements have affected the soil (Magid et al., 2006). In addition to that, the formation of nutrient rich areas in the soil due to localized or band application has received little attention, with recent studies elucidating the P dynamics in the

concentrated mineral fertilizer bands. The processes at the soil–biomaterial interface are therefore crucial for understanding the changes in soil nutrient availability following a localized application and in soils where the fertilizer has been mixed by tillage. For that purpose, we used a one-dimensional reaction system that allows detailed studies of the biomaterial–soil interface and the time-dependent diffusion of phosphate (and other ions) into the soil (Fig. 1), inspired by Kuchenbuch and Jungk (1982).

Based on this background, we hypothesize that.

1. Diffusion is the most predominant driver of P movement from the biomaterial into the soil, while particle migration and microbial uptake and transport may be notable in some cases;
2. P depletion from biomaterial to the adjacent soil is related to its water extractable P content;
3. Pretreatments change the release of phosphorus and other elements from the biomaterials, affecting the soil chemical composition, the pH, and thus the P dynamics in the soil.

To test these hypotheses, we selected four biomaterials with contrasting P speciation and solubility (Brod et al., 2015; Nanzer et al., 2019; Ylivainio et al., 2021): Fe-precipitated dewatered sewage sludge (SS), sewage sludge ash (SS-ash) derived from the aforementioned SS, meat and bone meal (MBM) and the solid fraction of biogas digestate (BGF All four biomaterials were pretreated with H₂SO₄, NaOH, and Ca (OH)₂.

2. Materials and methods

2.1. Biomaterials

The chemical compositions of the biomaterials assessed in this study are presented in Table 1.

The dewatered sewage sludge (SS) and sewage sludge ash (SS-ash) were collected at the BIOFOS wastewater treatment plant (www.biofos.dk) in Avedøre, Greater Copenhagen, which combines biological and chemical (ferric chloride sulphate) P removal. At this plant, the sewage sludge undergoes the following processes: mesophilic anaerobic digestion, dewatering, drying, and mono-incineration producing the ash (SS-ash). More information about SS and SS-ash processing can be found in López-Rayó et al. (2016) and Lemming et al. (2020).

The solid fraction of biogas digestate (BGF) was sampled from Maabjerg Energy Center (Holstebro, Denmark: www.maabjergenergycenter.dk). The reactor substrate is composed of cattle manure (approx. 70%), pig slurry (20%), chicken manure (8–9%), and food waste (1–2%). More information about the BGF is provided by Liu et al. (2019).

The meat and bone meal (MBM) represented category 1 material

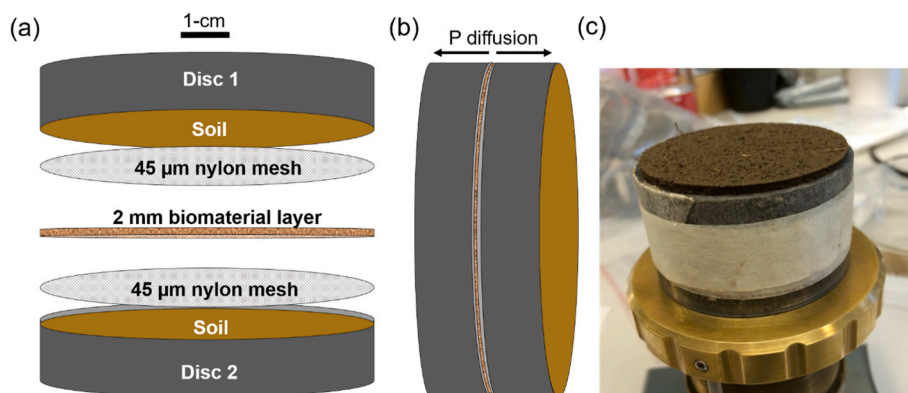


Fig. 1. Demonstration of the soil disc-columns and biomaterial layer set-up (a), the layout of how the soil columns were incubated (b), and the soil column after incubation, being moved upwards in the specially designed slicing piston (c).

Table 1

Dry matter content and chemical characteristics of the four biomaterials assessed in this study: sewage sludge = SS; sewage sludge ash = SS-ash; biogas fiber = BGF; meat and bone meal = MBM. The biomaterials composition, including the total P, were measured with ICP-OES after microwave digestion with HNO₃, H₂O₂, and HF.

		SS	SS-ash	BGF	MBM
pH	(H ₂ O, 1:5)	8.76	8.79	9.07	5.82
Dry matter	%	72	30	75	97
B	mg kg ⁻¹ (dw)	43	112	36	5.8
Cd ^a		0.9	3	n.d.	n.d.
Cr ^a		36	124	10	4
Cu ^a		323	1262	82	16
Mn		271	918	489	27
Zn ^a		1079	2834	414	159
Al	g kg ⁻¹ (dw)	10	37	1.4	0.3
Ca		57.1	134.4	36.7	67
Fe		52	132	7.1	1.7
K		3.5	14	11.3	7.1
Mg		5.1	13.9	17.4	1.6
Na		1.5	4.6	4	6.3
S		19.9	25.1	9.8	7.7
P		31.7	73.7	34.0	28.4
C		229	1.8	325	428
N		38.9	0.6	20.4	96.3
C:N	Ratio	5.9	3	15.9	4.4
(Fe + Al)/P	Molar ratio	1.27	1.57	0.16	0.04
Ca/P		1.39	1.41	0.83	1.82
Cd	mg kg ⁻¹ P	21.7	30.3	–	–

^a according to the Danish executive order on the use of waste for agricultural purposes (Miljøstyrelsen, 2018) the heavy metal content limits are Cr: 100 mg of kg⁻¹; Cu 1000 mg kg⁻¹; Zn: 4000 mg kg⁻¹; Cd: 0.8 mg kg⁻¹ or 100 mg of Cd kg⁻¹ P.

collected from the Daka SecAnim plant (Hedensted, Denmark: www.secanim.dk). This material is of animal origin, mostly from livestock farming and slaughterhouse waste. More information about the use of meat and bone meal in agriculture can be found in Möller (2015).

2.2. Experimental set-up

2.2.1. Acidification and alkalization treatments

Based on findings from preliminary tests, the following procedure was chosen to treat the materials:

All biomaterials were treated in 50 mL centrifuge tubes with H₂SO₄, NaOH, and Ca(OH)₂. The H₂SO₄ and NaOH solutions were applied directly to the biomaterials and homogenized. For each 2 g of biomaterial (fresh weight), 1 mL of solution was applied (ratio 2:1 wv⁻¹). For the Ca(OH)₂ treatments, the appropriate amount of dried powder was added to the biomaterial. After that, 1 mL of deionized water was added per g of biomaterial (total weight) to help the mixing process, and it was homogenized by using disposable spoons. The treated biomaterials were left at room temperature for 15 min, and subsequently randomly placed in the oven at 65 °C for 48 h, as preliminary tests had indicated that water-extractable P (WEP) and pH of the treated and dried biomaterials did not change over time when the biomaterials were thermally dried after the treatment.

2.2.2. Titration curves

The procedure described in section 2.2.1 was used to assess the effects of different H₂SO₄, NaOH, and Ca(OH)₂ concentrations on the pH and WEP of the biomaterials. Each biomaterial was treated in three replicates by placing 5 g-samples in 50-mL centrifugation tubes.

SS, MBM, and BGF were treated with seven different concentrations of H₂SO₄: 0.25 M, 0.5 M, 0.75 M, 1 M, 1.5 M, 2 M, and 2.5 M. For the SS-ash, eight different concentrations of H₂SO₄ were applied: 0.5 M, 1 M, 1.5 M, 2 M, 2.5 M, 5 M, 7.5 M, and 10 M. All four biomaterials were treated with the same concentrations of NaOH (1 M, 1.5 M, 2 M, and 2.5

M) and the same amounts of Ca(OH)₂ (10%, 20%, 33%, and 40% dry weight of Ca(OH)₂ per fresh weight of biomaterial).

2.3. Incubations

The soil used in this experiment was sampled at the University of Copenhagen's experimental farm in Taastrup (Denmark, 55°40'N, 12°16'E), from the unfertilized treatment (negative control) of the CRUCIAL long-term fertilization trial initiated in 2003 and cultivated mainly with spring cereals. The soil was a low-P sandy loam (clay 12.6%, silt 14.3%, and sand 69.8%) classified as Luvisol (FAO classification), as previously described by Gómez-Muñoz et al. (2018), and had a pH of 6.8 (1:5 H₂O), a water holding capacity (WHC) of 31 g of water per 100 g of dry soil and a WEP content of 1.78 mg kg⁻¹ dry soil. More detailed information about the CRUCIAL long-term trial and the soil used in this study can be found in Lemming et al. (2019). After being collected, the soil was air-dried and sieved to 4 mm.

A one-dimensional reaction system was used to determine the effects of the chemical treatments on the P dynamics in the soil (Fig. 1). For that, the soil was watered to reach 70% of the water holding capacity (21% moisture) and packed to a bulk density of 1.3 g cm⁻³ in plastic discs (height: 18 mm; diameter: 60 mm; 66.13 g of dry soil). Each experimental unit was set up as soil disc columns with two soil discs attached. One disc (Disc 1) was filled with soil (18 mm) and the other disc was filled with soil to a height of 16 mm. (Disc 2). A 2-mm biomaterial layer was placed above disc 2. To avoid direct contact between the biomaterial and the soil, a 45 µm nylon mesh was placed on each side of the biomaterial layer (Fig. 1, a).

Based on the results from the titration curves performed as described in section 2.2.2., the following treatments were selected and applied to all four biomaterials: untreated, 2.5 M H₂SO₄, 2.5 M NaOH, and 33% Ca(OH)₂. A negative control without biomaterial application was also incubated. In the SS, SS-ash, and MBM treatments, 4 g of dried material was applied in the 2 mm layer. For the BGF treatments, 2 g of dried material was applied in the 2 mm layer due to its low density.

Six replicates of each treatment were randomly distributed in a closed box and incubated horizontally at 15 °C and 70% of relative humidity (Fig. 1, b). After two days, three random replicates of each treatment were sampled. The other three replicates were sampled after 12 days of incubation.

After sampling, the discs were separated and placed in a specially designed slicing piston that can move the soil column upward by 1 mm (Fig. 1, c). Each 1 mm-thick layer was carefully cut off with a long, sharp razor knife. For each disc, the soil column was sliced at millimeter intervals from 0 to 7 mm away from the biomaterial layer. The samples with equal distance from the biomaterial layer in the two discs of the same experimental unit were pooled (Fig. 1, b). Immediately after sampling and homogenizing, two subsamples were collected from the soil layers of 0–1 mm, 1–2 mm, 4–5 mm, and 6–7 mm for the analysis of pH and WEP. The biomaterial layer was weighed and three subsamples were collected for analysis of water content, pH, total P, and WEP. A subsample was collected from the biomaterials incubated for 12 days for the sequential P extractions (described in section 2.3.1.).

2.3.1. Sequential extractions

The four untreated and treated (H₂SO₄, NaOH, and Ca(OH)₂) biomaterials were sequentially extracted before being incubated and after 12 days of incubation (Section 2.3), using a modified and simplified Hedley P fractionation scheme (Alvarenga et al., 2017; Hedley et al., 1982). The modified fractionation consisted of the sequential extraction of four fractions of an equivalent of 0.5 g dry matter of the biomaterial with three replicates. The first extraction was performed by adding 30 mL of deionized water (1:60 ratio), extracting for 16 h in an end-over-end shaker, and centrifuging (15 min, 5000 rpm). The supernatant was removed and filtered. The remaining solids were used for the following extraction with 35 mL of 0.5 M NaHCO₃. The same

procedures: 35 mL of extractant, 16 h shaking, centrifugation (15 min, 5000 rpm), and filtration of supernatant were used for the subsequent extractions with 0.1 M NaOH and 1 M HCl. The fifth fraction, residual P, was determined as the difference between the total P and the sum of the P content of the H₂O, NaHCO₃, NaOH, and HCl fractions.

2.3.2. Soil P sorption capacity

H₂SO₄ and NaOH (0.1 M, 0.25 M, 0.5 M, 1 M, 1.5 M, 2 M, and 2.5 M) were applied to the air dried soil in sufficient amounts to reach 50% of the water holding capacity. After 2 h, samples were collected and pH and water-extractable P of the treated soil were measured. To measure the P sorption capacity of the soil, samples at pH 1.7, 3.9, 4.5, 6.3, 8.3, 9.5, and 11.9 were collected and extracted with water (1:10 w/w). Spiking solutions with 0 mg, 0.1 mg, 0.5 mg, 1 mg, 2 mg, and 5 mg of P were applied in each sample. Samples were shaken for 16 h, centrifuged, filtered and the ortho-P content was measured (NSW, 1995).

2.4. Chemical analyses

The pH of soil and biomaterials was determined after suspension in MilliQ water (ratio 1:5) by shaking for 1 h and allowing to stand for an additional hour. The WEP fraction of the soil and biomaterials was determined by weighing an equivalent of 0.5 g of dry matter of substrate into a 50-mL centrifugation tube, adding 30 mL of deionized water (1:60 ratio), and shaking for 1 h in an end-over-end shaker. The extracts were subsequently filtered through Whatman no. 5 filter paper.

The ortho-P content in all extracts was analyzed using the molybdenum blue method on the flow injection analyzer (FIAStar 5000, Foss Analytical, Denmark). The elemental composition of the biomaterials before and after 12 days of incubation was analyzed by ICP-OES after microwave-assisted digestion with HNO₃, H₂O₂, and HF.

2.5. Calculations

The 3-parameter exponential decay equation (Eq. 1) can be used to model WEP diffusion in the soil (Hao et al., 2002):

$$y = a.e^{-bx} + y_0$$

where: y is the WEP concentration at a certain distance from the biomaterial layer (mg kg soil⁻¹); x is the distance (mm); y_0 is the background, which is the minimum value in the curve (mg kg soil⁻¹); a is the WEP content in the surface at a certain distance (mg kg soil⁻¹); and b indicates the rate of change of WEP at different distances. For the model, we subtracted the negative control values (considered as background) from the results obtained in the experiment.

The depletion of elements from the biomaterials was calculated as the amount of the element applied at time 0 that was released from the biomaterial layer to the soil columns during the 12 days of incubation, according to Eq. (2):

$$\text{Depletion (\%)} = 100x \frac{(dw_{t12} \times \text{total content } t12) - (dw_{t0} \times \text{content } t0)}{(dw_{t0} \times \text{content } t0)} \quad (2)$$

where $dw_{12\text{ d}}$ is the total dry weight of the biomaterial after 12 days of incubation in g, the content at 12 d is the total content of the element in the biomaterial after 12 days of incubation in mg g⁻¹, $dw_{0\text{ d}}$ is the total dry weight of the biomaterial in the 2-mm layer at time 0 (4 g for SS, SS-ash, and MBM, and 2 g for BGF), and the content at 0 d is the total content of the element in the biomaterial at time 0 in mg g⁻¹.

The P apparent recovery (AR) in the first 7-mm from the biomaterial layer was calculated to allow the comparison between treatments, according to Eq. (3):

$$\text{AR (\%)} = 100x \frac{(\text{WEP treatment} - \text{WEP negative control})}{\text{Total P applied with the P source}} \quad (3)$$

where AR is the apparent recovery, WEP treatment is the amount of P as WEP in the first 7 mm of soil from the biomaterial layer for a given treatment in mg, WEP negative control is the amount of P as WEP in the first 7 mm of the soil disc in the negative control in mg, i.e. when no P source was applied between the soil discs, and total P applied with the P source is the amount of P applied at time 0 in mg.

The P sorption capacity of the soil was calculated as:

$$\text{PSC (mg per 100 g)} = \frac{(\text{P spiking} - \text{P increase measured})}{\text{Soil dry weight}} \times 100$$

where PSC is the P sorption capacity in mg of P per 100 g of soil, P spiking is the amount of P added with the spiking solution (a range from 0.1 to 5 mg of P), P increase measured is the difference between the P measured and the P of the sample in water (in mg), soil dry weight is the dry weight of the sample.

2.6. Statistical analysis

All the experiments were set up in a randomized complete block design. The statistical analyses were performed using the software IBM SPSS Statistics 27.0 and the graphs were generated using SigmaPlot version 14.0. Differences between treatments within each biomaterial were tested by using a one-way ANOVA. The homogeneity of variance of the data was verified by using Levene's test and the Kolmogorov-Smirnov test was used to verify that the data followed a normal distribution. Student's T-tests (<0.05) and Tukey's HSD test (<0.05) were used for the comparison of means. Pearson correlation coefficient and polynomial regression were performed to assess the correlation between different parameters. The specific analyses applied for each parameter are indicated in the Figure and Table captions.

3. Results and discussion

3.1. Effects of treatments on pH and P fractions of the biomaterials

The sequential P extraction method proposed by Hedley et al. (1982) has been widely used to determine the P fractions and to predict the P availability in soils and biobased fertilizers. Recently, P fractionation has been debated after Barrow et al. (2020) claimed that "Fractionation procedures designed to measure chemically specified phosphate fractions in soil are fallacious and should be abandoned". Gu and Margenot (2020) argue that this is overstated, but that it is necessary to disabuse the notion that fractions necessarily represent specific P compounds. In this study, we chose to use a simplified extraction procedure, in which we interpret P fractions as operationally defined rather than as specific P compounds. In this way, it was possible to demonstrate the effects of the treatments and the incubation on the P fractions in the biomaterials. These changes are discussed below and can be seen in Table 2 (0 d, before incubation) and 4 (12 d, after incubation). In this simplified extraction procedure, the residual fraction also accounts for the total organic P.

3.1.1. Sewage sludge (SS) and sewage sludge ash (SS-ash)

The sequential extractions results showed that most of the P was present in the HCl-P fraction for both SS (58%) and SS-ash (85%) (Table 2). Both biomaterials were chemically treated with FeClSO₄ and previous studies have shown that most of the P in sludge treated with Fe-salts was extractable by NaOH (Alvarenga et al., 2017, Øgaard and Brod, 2016; Ylivainio et al., 2021). However, this was not the case in our study.

Interestingly, Lemming et al. (2020) also found larger HCl-P fractions than NaOH-P fractions in several Danish sewage sludges and sewage sludge ashes. Their explanation was the fact that the Danish sewage sludge has a very high Ca content, presumably due to the hardness of Danish potable water. Thus, the SS assessed in this study had

Table 2

Distribution of the total P into different inorganic P fractions of four biomaterials (sewage sludge = SS; sewage sludge ash = SS-ash; biogas fiber = BGF; meat and bone meal = MBM) untreated and treated (2.5 M H₂SO₄, 2.5 M NaOH, and 3:10 (w/w) Ca(OH)₂) at time 0 (0 d) in mg of P per 100 mg of P applied in each treatment.

		WEP	Bicarbonate	NaOH	HCl	Residual P ^a
mg of P 100 mg ⁻¹ of applied P						
SS	Untreated	4.0 ± 0.3 b	6.1 ± 0.1 b	15.2 ± 3.0 a	57.9 ± 4.0 a	16.8 ± 1.5 b
	H ₂ SO ₄	54.8 ± 4.4 a	4.8 ± 0.2 c	18.1 ± 0.4 a	7.9 ± 0.6 b	14.4 ± 3.9 b
	NaOH	45.6 ± 1.5 a	3.1 ± 0.2 d	2.8 ± 0.2 ± c	48.5 ± 1.3 a	0.01 ± 0.0 c
	Ca(OH) ₂	0.01 ± 0.0 c	12.6 ± 0.4 a	9.7 ± 0.2 b	51.0 ± 0.7 a	26.7 ± 1.3 a
SS-Ash	Untreated	0.01 ± 0.0 c	0.9 ± 0.01 b	6.6 ± 0.3 b	84.6 ± 3.5 a	7.9 ± 3.3
	H ₂ SO ₄	29.3 ± 2.2 a	3.4 ± 0.3 a	11.7 ± 0.8 a	46.9 ± 3.0 b	8.6 ± 3.3
	NaOH	12.2 ± 0.7 b	1.2 ± 0.1 b	0.5 ± 0.1 c	79.8 ± 4.2 a	13.4 ± 4.6
	Ca(OH) ₂	2.5 ± 0.1 c	3.7 ± 0.4 a	0.8 ± 0.1 c	69.9 ± 1.7 a	15.9 ± 1.8
BGF	Untreated	14.0 ± 0.2 c	15.9 ± 0.8 b	5.8 ± 0.7 b	35.9 ± 0.9 b	29.0 ± 1.3 a
	H ₂ SO ₄	70.2 ± 5.4 a	13.3 ± 0.2 b	4.6 ± 0.3 b	11.5 ± 0.9 c	0.37 ± 5.6 b
	NaOH	27.3 ± 0.4 b	27.6 ± 0.3 a	8.8 ± 0.1 a	36.6 ± 1.3 b	0.01 ± 0.0 b
	Ca(OH) ₂	0.01 ± 0.0 d	29.6 ± 1.1 a	5.3 ± 0.2 b	65.1 ± 0.8 a	0.01 ± 0.0 b
MBM	Untreated	9.1 ± 0.3 c	4.4 ± 0.2 b	1.6 ± 0.1	81.5 ± 5.4 ab	3.4 ± 5.4 b
	H ₂ SO ₄	77.7 ± 1.3 a	6.2 ± 0.4 a	0.9 ± 0.1	15.3 ± 1.0 c	0.0 ± 0.1 c
	NaOH	15.2 ± 0.6 b	3.3 ± 0.2 b	1.7 ± 0.1	70.8 ± 4.9 b	8.9 ± 4.6 a
	Ca(OH) ₂	0.1 ± 0.0 d	4.1 ± 0.2 b	1.1 ± 0.1	91.3 ± 9.4 a	3.5 ± 9.5 b

Different lowercase letters indicate a significant difference in the P fraction within the biomaterial at 0 d (Tukey HSD, <0.05). Values after ± indicate the standard error (n = 3).

^a The residual P was calculated as the difference between the total P and the sum of water-extractable P (WEP), bicarbonate extractable P, NaOH extractable P, and HCl extractable P.

a calcium content greater than sewage sludge from a number of countries examined in other studies (i.e. Finland and Sweden (Ylivainio et al., 2021); Brazil (Rodrigues et al., 2021); and Norway (Øgaard and Brod, 2016)). The same was found for the SS-ash calcium content and Ca/P molar ratio (i.e. Germany (Herzel et al., 2016) and Switzerland (Nanzer

et al., 2014a)). Indeed, Nanzer et al. (2014b) demonstrated that sewage sludge with a high Ca/P ratio favored the formation of apatite P in ashes. This can be a plausible explanation for the effects of the acidification and alkalization treatments found in this study.

The acidification of SS and SS-ash decreased the pH to below 2.0, and

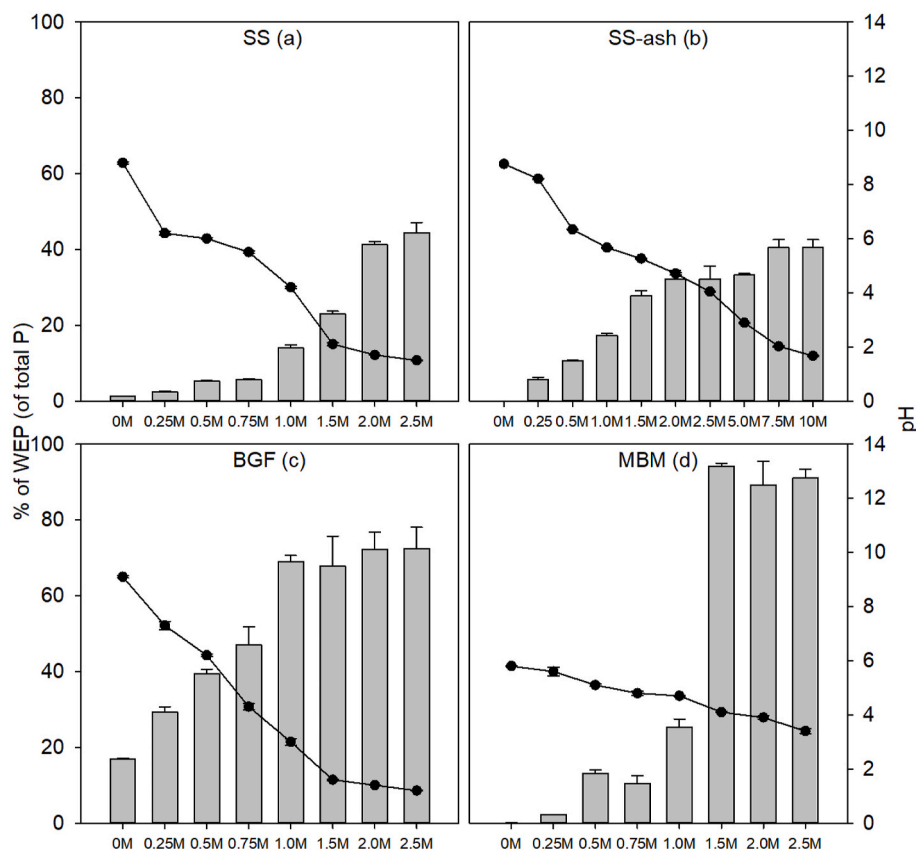


Fig. 2. Effects of the application of seven different concentrations of H₂SO₄ on the pH (solid line) and water-extractable P (% WEP of the total P, bars) of four different biomaterials: sewage sludge (SS, a), sewage sludge ash (SS-ash, b), biogas fiber (BGF, c), and meat and bone meal (MBM, d). The standard error is indicated above the bars and at each point in the line.

the WEP increased to about 40% of the total P when higher concentrations of sulfuric acid were applied (Fig. 2). Xu et al. (2015) assessed the P release and speciation of P in sewage sludge and also found that at pH 2, about 36% of the total P was released, mainly from inorganic P and apatite P. As can be seen in Table 2, the acidification of SS and SS-ash significantly decreased the HCl-P pool, which may indicate that most of the P in the WEP fraction originated from this pool. According to Petzet et al. (2012), the pH between 2.5 and 3.5 is the optimum range for Ca-P bond dissolution, however, it may also favor the formation of Al-P in sewage sludge, which is in agreement with our findings that the acidification of the SS-ash (pH ~ 4, Table S1) significantly increased the NaOH-P pool.

On the other hand, at pH below 2, the Fe-, Al-, and Ca-bound P in the sewage sludge may be in almost complete dissolution in long-term equilibrium (Xu et al., 2015); thus, the WEP showed the highest values at pH below 2 for both biomaterials in our study (2 M for SS and 7.5 M for SS-ash), indicating that the strongest acidification may have dissolved not only the Ca-P but also some of the Al- and Fe-bound P.

The alkalization with NaOH increased the pH of SS and SS-ash to about 11.5 (2.5 M) and the WEP to about 33% and 13% of total P, respectively (Fig. 3). According to Xu et al. (2015), the alkalization of sewage sludge to pH 11 mainly releases P from non-apatite inorganic fractions and organic P. Our results show that the NaOH treatment significantly reduced the NaOH-P pool, and the residual P came close to zero. The residual P in our study included both the organic P and the non-extractable inorganic P forms. Accordingly, there was only a significant decrease in the NaOH-P pool in the SS-ash (Table 2), following the logic that the NaOH treatment would transfer the P from the NaOH-P pool to the WEP pool.

Alkalization with Ca(OH)₂ showed contrasting effects on the WEP

of the SS and the SS-ash. For SS, it almost eliminated the WEP fraction, whereas for SS-ash, it increased to around 2.5% (Fig. S1). The sequential extraction results show that the Ca(OH)₂ treatment significantly increased the bicarbonate-P and reduced the NaOH-P fractions in both SS and SS-ash (Table 2). Our observations are consistent with several other studies that found an increasing P availability and uptake when biomaterials were pre-treated or co-applied with lime (Alvarenga et al., 2017; Bauer et al., 2019; Øgaard and Brod, 2016; Ylivainio et al., 2021). The increase of the bicarbonate-P pool of SS and SS-ash can be explained by two facts: i) according to Alvarenga et al. (2017, citing Lindsay, 1979), the Fe and Al oxides are less positively charged as the pH increases, and the Fe- and Al-P tend to become more easily extractable; ii) the formation during pretreatment of mono- and dicalcium phosphates (Ca₂-P) and octacalcium phosphates (Ca₈-P), which are partially available to the plant (Shariatmadari et al., 2007) and can be extracted by 0.5 M sodium bicarbonate (Shen et al., 2004).

3.1.2. Biogas digestate solid fraction (BGF)

Of all biomaterials, BGF had the highest initial WEP (~17%). This value was further increased as the concentration of H₂SO₄ increased to 1 M, reaching around 70% of the total P and showing no major changes as the concentration increased to 2.5 M. However, the pH, decreased from 3.9 when treated with 1 M to below 2 when treated with 1.5 M H₂SO₄ (Fig. 2). In the anaerobic digestion reactor, phosphates can partially crystallize as calcium – magnesium phosphates and struvite, precipitate and remain in the solid fraction (Möller and Müller, 2012). Dissolution of Ca-bound P may be favored at a pH below 4 (Valsami-Jones et al., 1998), which is consistent with our observation that even with stronger acidification and a further decrease in pH, the WEP was not increased above 70% of the total P (Fig. 2).

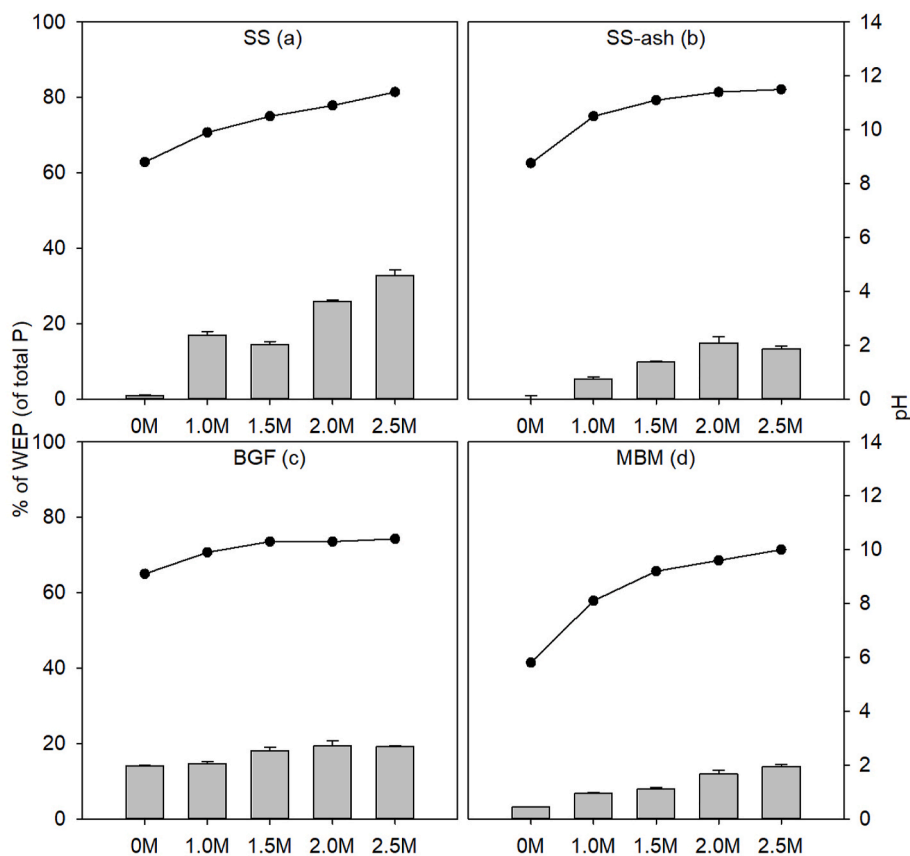


Fig. 3. Effects of the application of four different concentrations of NaOH on the pH (solid line) and water-extractable P (% WEP of the total P, bars) of four different biomaterials: sewage sludge (SS, a), sewage sludge ash (SS-ash, b), biogas fiber (BGF, c), and meat and bone meal (MBM, d). The standard error is indicated above the bars and at each point in the line.

The alkalization of BGF with NaOH slightly increased the WEP to about 20% of the total P (Fig. 2), and the sequential extraction results show that the NaOH treatment almost doubled the WEP and bicarbonate-P fractions. In contrast, alkalization with Ca(OH)₂ almost eliminated the WEP (Fig. 3) and significantly increased the bicarbonate-P and the HCl-P pool. A high Ca/P molar ratio favors the formation of tetracalcium phosphate and CaO, which in the presence of humidity can lead to the formation of apatite (Nanzer et al., 2014b). Ca₂-P and Ca₈-P can be extracted by 0.5 M sodium bicarbonate (Shen et al., 2004), which explains the increase in the bicarbonate-P pool, whereas the apatite P formed can be extracted by HCl (Wang et al., 2013), explaining the increase in the HCl-P pool.

3.1.3. Meat and bone meal (MBM)

The sequential extraction results showed that the total P in MBM was 80% HCl-P (Table 2). This is in agreement with the literature, as most of the meat and bone meal P originates from the bones in the form of hydroxyapatite (Jeng et al., 2007), which can be dissolved by acid (Wang et al., 2013). Brod et al. (2015) studied a meat and bone meal in which 3% of the total P was extracted by water and 75% by 1 M HCl.

While acidified MBM showed the smallest pH decrease, from 5.5 (untreated) to 3.7 (acidified with 2.5 M), it released the largest amount of P to solubilized form (Fig. 2). This is in accordance with the fact that the apatite-P can be solubilized when the pH is below 4 (Valsami-Jones et al., 1998). The alkalization of MBM with NaOH significantly increased the WEP fraction, whereas the Ca(OH)₂ almost eliminated the WEP fraction and increased the HCl-P fraction to around 90% (Fig. 3 and Table 2), probably due to the formation of additional apatite P (Nanzer et al., 2014b).

3.2. Incubations in the 1-dimensional reaction system

3.2.1. Effects of the localized application of treated biomaterials on P dynamics

In the incubation experiments, we investigated the effects of treatments, time, and distance to the biomaterial layer on the soil WEP content. The acidification (Fig. 4) and alkalization with NaOH (Fig. 5) substantially increased the WEP contents in the soil when compared to the untreated biomaterials, whereas the alkalization with Ca(OH)₂ significantly reduced it (Fig. S2).

The acidified SS did not show a significant difference among the distances 1-mm, 2-mm, and 5-mm after 2 d, indicating a very rapid release and diffusion of phosphate that would occur if the concentration gradient between biosolid and the soil was very steep. In agreement with this, there were no significant differences between any of the distances after 12 d. In contrast, after 2 d, the acidified SS-ash was not significantly different from the untreated. However, at 12 d, the WEP values were significantly higher around 100 mg of P kg⁻¹ soil (Fig. 4a and b), indicating a slower P release from the biomaterial layer.

For the BGF, the acidification treatment led to a very substantial increase in soil WEP at all distances already at 2 d when compared to the untreated material, with no significant difference between distances. At 12 d, the WEP was significantly increased for all the distances when compared to 2 d. This agrees with the large increase in WEP of BGF due to acidification discussed in the previous section. For the NaOH-treated BGF, there was a significant difference at 2 d and 12 d compared to the untreated BGF, however, within each distance, there was no significant difference between 2 d and 12 d (Fig. 5, c). For the MBM, the acidification treatment led to greater WEP levels in the soil, with significant increases in the 5-mm and 7-mm distances at 12 d (Fig. 4, d). However, the alkalization only showed a significant effect on soil WEP in the first

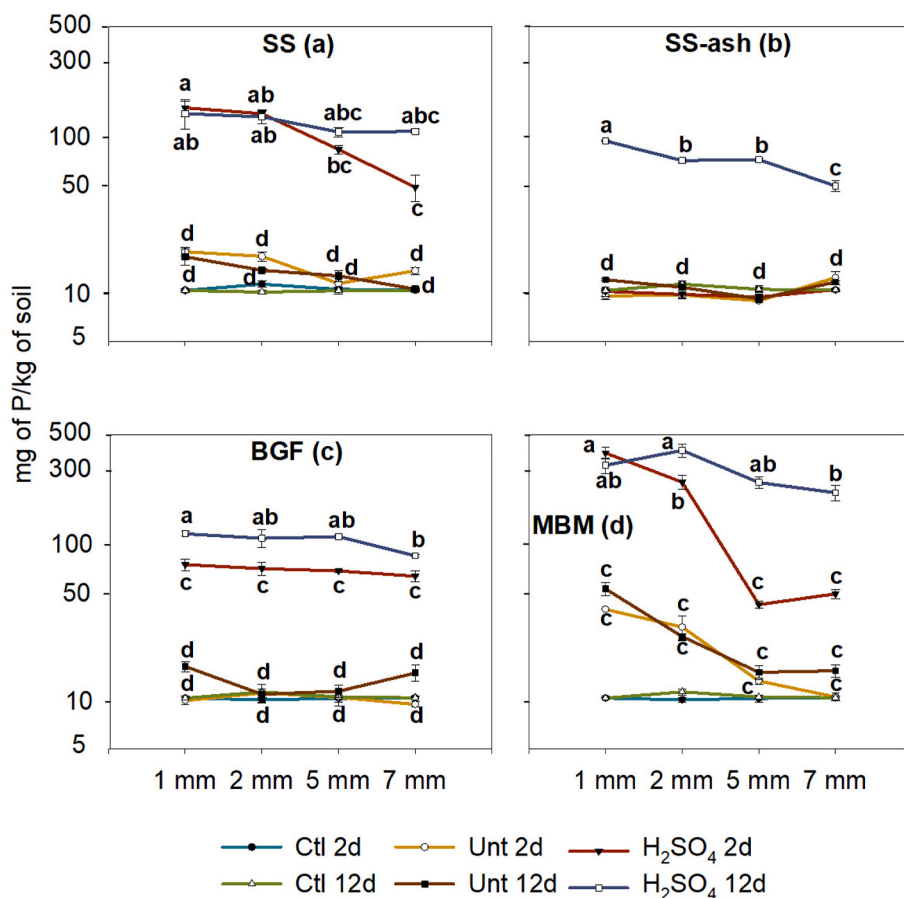


Fig. 4. Water extractable P (WEP, mg of P per kg of dry soil) in four 1-mm soil slices (1 mm = 0–1 mm; 2 mm = 1–2 mm; 5 mm = 4–5 mm; 7 mm = 6–7 mm) apart from the biomaterial layer incubated for 2 days (2 d) and 12 days (12 d) in a 2 mm layer between two low-P soil columns. The four biomaterials (sewage sludge = SS; sewage sludge ash = SS-ash; biogas fiber = BGF; meat and bone meal = MBM) were applied untreated (Unt.) and treated with 2.5 M H₂SO₄. For the control (Ctl), the soil was incubated under the same conditions without fertilizer application. The y-axes are on a log scale. Different letters indicate a significant difference (Tukey HSD, p < 0.05).

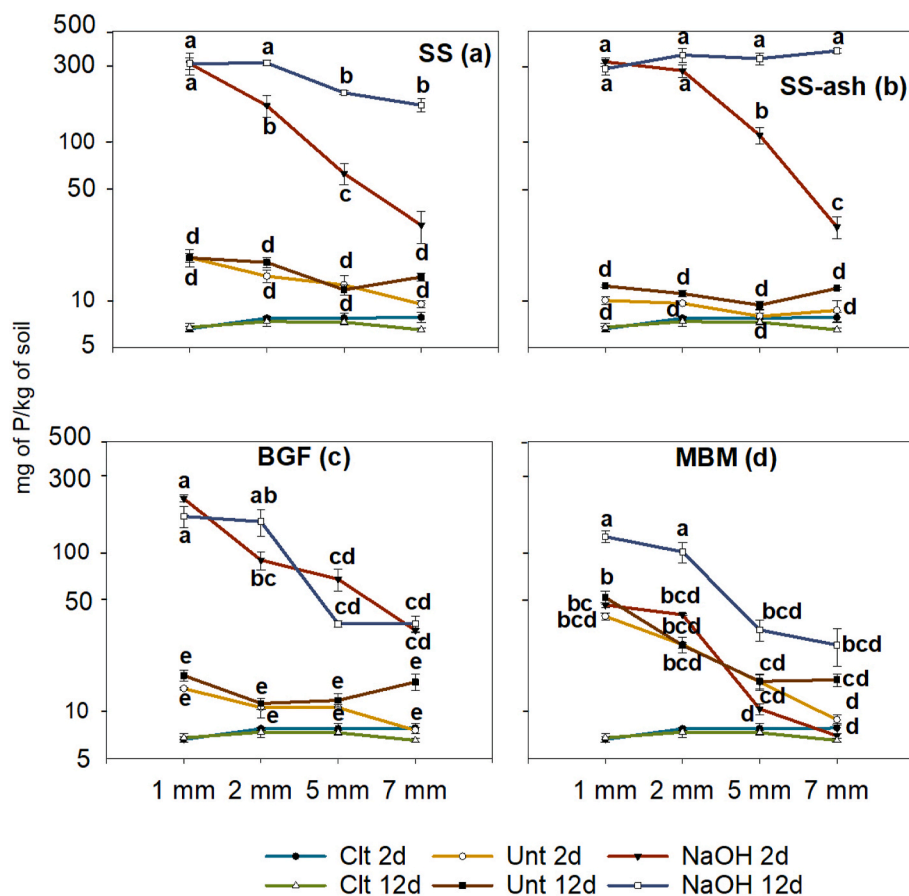


Fig. 5. Water extractable P (WEP, mg of P per kg of dry soil) in four soil slices (1 mm = 0–1 mm; 2 mm = 1–2 mm; 5 mm = 4–5 mm; 7 mm = 6–7 mm) apart from the biomaterial layer incubated for 2 days (2 d) and 12 days (12 d) in a 2 mm layer between two low-P soil columns. The four biomaterials (sewage sludge = SS; sewage sludge ash = SS-ash; biogas fiber = BGF; meat and bone meal = MBM) were applied untreated (Unt.) and treated with 2.5 M NaOH. For the control (Ctl), the soil was incubated under the same conditions without fertilizer application. The y-axes are on a log scale. Different letters indicate a significant difference (Tukey HSD, $p < 0.05$).

2 mm (Fig. 5, d).

For all four biomaterials, the treatment and distances had a significant effect on soil pH. The acidified and NaOH-treated SS and BGF showed a greater effect on soil pH, reducing it to below 4 and increasing it to above 10, respectively, even at 7-mm from the biomaterial layer at 12 d. For both acidified MBM and SS-ash, the soil pH remained above 6 after 12 d at all the distances (Table S1).

The 3-parameters exponential decay equation ($y = a \cdot e^{-bx} + y_0$) can be used to model the WEP diffusion in the soil (Hao et al., 2002). As can be seen in Table S2, for all the untreated biomaterials at 2 d and 12 d, the values obtained from the incubation in the 1-D reaction system fitted the model well and gave realistic values for all the 3 parameters. However, this was not the case for some of the treated biomaterials. For all the Ca(OH)₂-treated materials (except SS-ash), the model gave errors or negative values. This is probably because this treatment significantly reduced the WEP contents in the soil (Fig. S2), giving negative y_0 values and/or low b values (< 0.001). Hao et al. (2002) have shown that liming reduces the diffusion from a soluble source of P (KH₂PO₄) in the soil. In our study, the biomaterials had relatively low WEP (Table 2) and the Ca(OH)₂ treatment virtually eliminated the P diffusion.

Similarly, the model showed an error for the NaOH-treated SS and SS-ash at 12 d and for the acidified SS-ash, BGF, and MBM for both incubation periods, or at least one of the parameters was negative or close to zero. As can be seen in Figs. 5 and 6, the WEP contents in these cases were relatively high even at 7-mm from the biomaterial layer. These treatments clearly increased the potential P diffusion to distances well beyond 7-mm, and thus overwhelmed our experimental setup. Based on preliminary studies, we expected that 7-mm would be the largest diffusion distance for treated materials. In contrast, Hao et al. (2002) found that the application of KH₂PO₄ increased the WEP content up to 17 mm away. Indeed, the results of Rech et al. (2018) indicated that P

from TSP would diffuse up to 20 mm from the application area after 14 days. Therefore, the setup we used with the 1-dimensional reaction system should be extended to longer diffusion lengths for future studies on treated materials. It was adequate to assess diffusion-related WEP increases in soil for the untreated biomaterials and some of the treated materials, though. Thus, we maintain that the first part of our first hypothesis is plausible: “diffusion is the most predominant driver of P movement from the biomaterial into the soil”.

3.2.2. Changes in P fractions in biomaterials over the course of incubation

The sequential extractions of the biomaterials after the incubation showed that for all the acidified and NaOH-alkalinized biomaterials (except BGF), the WEP content was significantly reduced after 12 d. A reduction was observed in all the fractions of the acidified SS, BGF, and MBM. The acidification treatment showed the highest P depletion for all the biomaterials, around 50% for the SS and SS-ash and 77% for the BGF (Table 3). These results are similar to the reduction in the WEP fraction when comparing the initial WEP content in the biomaterials with that after incubation (Tables 2 and 3). Indeed, the P depletion was significantly correlated with the biomaterials’ initial WEP (Pearson’s correlation: 0.659, $p < 0.01$; Table 3). This indicates that most of the P released to the soil was derived from the WEP fraction and, therefore, confirms our second hypothesis: “P depletion from the biomaterial to the adjacent soil is related to its water extractable P content”.

However, for the acidified SS-ash, the WEP fraction only decreased from 29.3 to 11.9% (–17.4%), values much lower than the 52.6% of P released to the soil. The HCl-P fraction of the acidified SS-ash was significantly reduced after 12 d, which may indicate that the P from this pool may have been transferred to the WEP pool during the incubation and was released to the soil at slower rates. Nevertheless, after 12 d, there was also a significant increase in the NaOH-P fraction of the

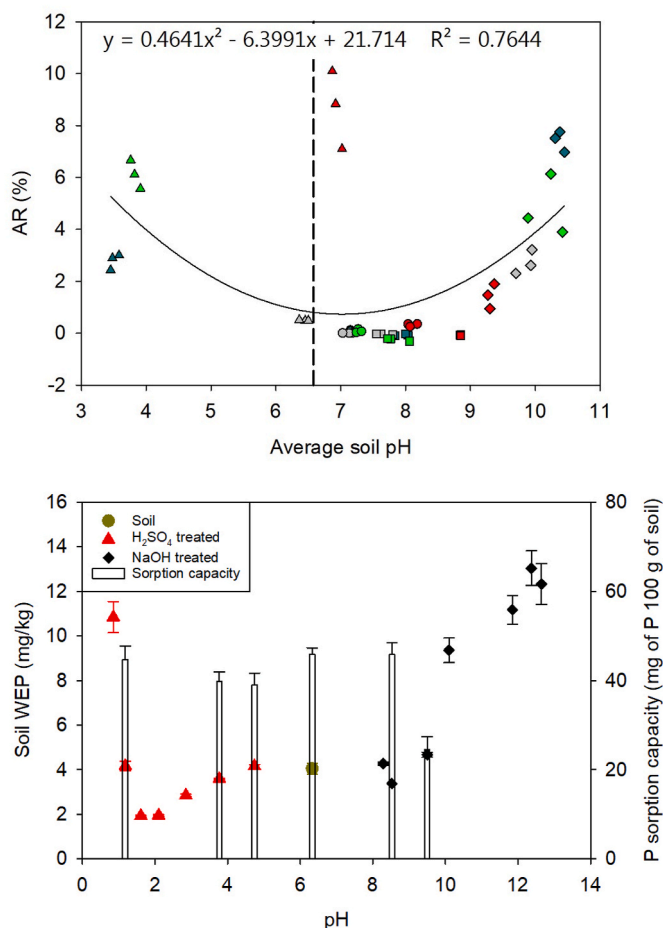


Fig. 6. Correlation between apparent recovery in the soil (AR, %) and soil pH. Acidified MBM was removed from the polynomial regression as outliers. Vertical dotted line indicates soil initial pH (6.6) ●: untreated; ▲: acidified; ◆: NaOH; ■: Ca(OH)₂. Blue: SS; gray: SS-ash; green: BGF; red: MBM (Top). Soil WEP and P sorption capacity at different pH when treated with H₂SO₄ and NaOH (Bottom).

acidified SS-ash, which may indicate that some of the P released from the HCl-P due to the acidification may have become bound to other elements. As can be seen in Table S2, the pH of the acidified ash significantly increased from 4.02 to 4.47 after 12 days of incubation. This change in pH over time might favor the fixation of the WEP by Al (Penn and Camberato, 2019).

Although large amounts of P were released to the soil and the WEP pool was significantly reduced for most biomaterials after 12 d, considerable amounts of P still remained in the biomaterial layer as WEP. The acidification significantly increased the sum of WEP in the biomaterial and in the soil (AR) for the SS-ash (12.4% of the total P applied) and the MBM (39.9%), whereas the NaOH treatment significantly increased the sum for the SS (24.3%) and the BGF (25.6%) (Table 3). Lemming et al. (2016) showed a concentration of maize root proliferation in the zone where the biomaterial is placed. Thus, it is expected that the plant benefits not only from the P released as WEP in the surrounding soil but also from the P remaining as WEP in the placement zone.

3.2.3. Effects of treatments on P depletion and apparent recovery (AR)

There was a substantial change in the elemental contents of all untreated biomaterials after 12 d incubation, indicating a strong interaction with the surrounding soil (Table S3). Of the total P initially applied, between 7% was released to the soil from MBM and about 23% from SS and BGF. However, only a very small fraction of the total P applied with

the untreated biomaterials was recovered as WEP in the soil (AR), from 0% (SS-ash) to 0.31% (MBM) (Table 3).

As can be seen in Fig. S3, for the SS, BGF, and MBM, microbial growth was observed at the soil interface. This clearly indicates that the localized application of these biomaterials is stimulating microbial growth in the surrounding soil and that these microorganisms are immobilizing the P from the biomaterials (Richardson and Simpson, 2011; Spohn and Kuzyakov, 2013). Thus, while diffusion is the main driver of transport, microbial growth and incorporation of P causes a reduction in the WEP contents in soils adjacent to these materials, and most likely also transport through the soil via fungal highways (Ruess and Ferris, 2004).

Although no microbial activity was observed in the untreated SS ash at either the soil interface or in the biomaterial layer, 13.9% of the total P applied was released to the soil. As indicated by arrows in Fig. S3, a reddish color was observed at the soil interface, which may indicate that nano- and microparticles (<45 μm) (Krejcirikova et al., 2019) migrated from the SS-ash through the nylon mesh into the soil. Micro- and nanoparticle migration in the soil has previously been observed by (Wang et al., 2022; Xu et al., 2021). Together, the evidence of microbial transformation and transport (and SS, BGF, and MBM) and particle migration (SS ash) underpin the second part of our first hypothesis: particle migration and microbial uptake and transport may be notable P transporting mechanisms.

In this study, we applied strong chemical treatments to the biomaterials and assessed the soil surrounding the placement zone. Therefore, changes in soil pH were caused by the large amount of elements released from the biomaterial into the surrounding soil (Table S3), mainly due to residual acidity/alkalinity effects from the treatments. This is confirmed by the fact that for some treatments, the soil pH was above 9 in the first 7-mm for the biomaterial layer for all the NaOH-treated biomaterials and below 4 for the acidified SS and BGF. These were also the pH values at which we found the highest AR. Pearson's correlations indicated that, in this study, soil pH had no linear relationship with AR (−0.003) (Table 3). However, a polynomial regression showed a strong correlation between soil pH and AR (Fig. 6). It is notable that the lowest AR values, with the exception of acidified MBM, were found at neutral pH, a range commonly claimed to provide the highest phosphate availability (Penn and Camberato, 2019; Price, 2006). According to Meyer et al. (2021) findings, in non-calcareous soils, the Al-P precipitations regulates the P availability.

Pearson's correlation in Table 3 also shows that the AR was significantly correlated to the biomaterial P depletion over 12 days (0.461, $p < 0.05$) and the initial WEP of treated (and untreated) biomaterials (0.809, $p < 0.01$). Interestingly, although the P depletion of the NaOH-treated SS and SS-ash showed no significant difference compared to the untreated materials and was significantly lower than the acidified treatment, the AR for both was higher at 2 d and 12 d (Table 3). As can be seen in Fig. 4, the soil WEP contents reached a maximum of around 100 and 200 mg P kg^{−1} for the acidified SS-ash and SS, whereas in the NaOH-treated SS-ash and SS, these values were increased to a greater extent, around 300 mg P kg^{−1}. This could be explained by the differences in the soil pH (Table S1, Fig. 6). The degree of P saturation in loamy soils was found to be negatively correlated with pH and positively correlated with WEP (Renneson et al., 2015), which may explain the fact that although the acidified treatment released more P to the soil for SS and SS-ash, the AR was greater in the NaOH treatments. As can be seen in Fig. 6b, when the pH was decreased to 1.8, the soil WEP was reduced by half and the P sorption capacity of the soil did not change. When the pH increased due to the NaOH treatment, the soil WEP increased and the P sorption capacity was reduced from 46 to 24 mg of P per 100 g of soil, at pH 12. Thus, we can confirm that the effects of the acidified treatments on soil pH do not result in a release of P from the soil; however, these results may indicate that the alkalization of biomaterials with NaOH increases the soil WEP not only through the P released from the biomaterial, but also due to the alkalinity effects on the soil and, as a

Table 3

Mass balance of the total P applied into different P inorganic fractions of four biomaterials (sewage sludge = SS; sewage sludge ash = SS-ash; biogas fiber = BGF; meat and bone meal = MBM) untreated and treated (2.5 M H₂SO₄, 2.5 M NaOH, and 3:10 (w/w) Ca(OH)₂) after 12 days of incubation (12 d) in each treatment and the P depletion, apparent recovery (AR, at 2 d and 12 d), in mg of P per 100 mg of P applied. Pearson's correlation (AR and P release, AR and soil average pH, AR and biomaterial initial WEP, and P release and biomaterial initial WEP).

		WEP		Bicarbonate		NaOH		HCl		P depletion 12d	AR 2d	AR 12d	WEP in biomaterial + soil 12d
mg of P 100 mg ⁻¹ of applied P													
SS	Untreated	2.1 ± 0.2	-	5.6 ± 0.1	=	22.3 ± 0.4	=	41.3 ± 0.4	-	22.4 ± 8.0 b	0.07 ± 0.01	0.09 ± 0.01	2.2 ± 0.2 c
	H ₂ SO ₄	8.6 ± 0.4	-	2.3 ± 0.1	-	14.7 ± 0.7	-	3.8 ± 0.3	-	50.3 ± 3.9 a	2.4 ± 0.2	2.8 ± 0.2	11.4 ± 0.5 b
	NaOH	16.9 ± 2.2	-	2.2 ± 0.03	-	6.9 ± 0.5	-	39.8 ± 1.2	-	28.3 ± 2.5 b	3.7 ± 0.2	7.4 ± 0.2	24.3 ± 3.1 a
	Ca(OH) ₂	0.18 ± 0.1	=	5.0 ± 2.1	=	6.3 ± 1.7	=	25.9 ± 3.7	-	-10.3 ± 21 c	-0.05 ± 0.01	-0.07 ± 0.02	0.18 ± 0.1 c
SS-Ash	Untreated	0.3 ± 0.04	=	0.76 ± 0.1	=	0.04 ± 0.01	-	83.5 ± 1.2	=	13.9 ± 2.6 b	-0.04 ± 0.01	0.00 ± 0.0	0.3 ± 0.01 c
	H ₂ SO ₄	11.9 ± 1.3	-	3.7 ± 0.6	=	18.3 ± 0.6	+	32.8 ± 2.4	-	52.6 ± 4.6 a	-0.04 ± 0.01	0.5 ± 0.01	12.4 ± 1.4 a
	NaOH	2.4 ± 0.2	-	0.8 ± 0.04	-	1.34 ± 0.05	+	66.2 ± 2.0	=	10.9 ± 3.0 b	1.5 ± 0.08	2.7 ± 0.2	5.1 ± 0.2 b
	Ca(OH) ₂	0.01 ± 0.00	-	2.7 ± 0.04	=	0.5 ± 0.06	=	77.3 ± 0.6	=	-0.5 ± 13 c	-0.02 ± 0.01	-0.03 ± 0.01	0.01 ± 0.00 c
BGF	Untreated	18.7 ± 1.2	+	19.9 ± 1.5	=	0.07 ± 0.01	-	24.7 ± 1.6	-	22.6 ± 1.5 b	0.01 ± 0.03	0.09 ± 0.03	18.8 ± 1.5 ab
	H ₂ SO ₄	9.9 ± 0.5	-	1.4 ± 0.3	-	0.13 ± 0.01	-	2.4 ± 0.5	-	77.0 ± 1.1 a	3.7 ± 0.2	6.12 ± 0.3	16.1 ± 0.8 b
	NaOH	20.8 ± 3.2	=	17.2 ± 0.9	-	9.0 ± 0.3	=	39.6 ± 2.1	=	23.2 ± 8.0 b	4.5 ± 0.2	4.8 ± 0.6	25.6 ± 2.9 a
	Ca(OH) ₂	4.6 ± 0.6	+	28.7 ± 1.5	=	8.7 ± 0.3	+	83.3 ± 2.9	+	3.0 ± 4.0 c	-0.12 ± 0.02	-0.25 ± 0.03	4.6 ± 0.6 c
MBM	Untreated	3.4 ± 0.2	-	3.6 ± 0.3	=	1.53 ± 0.04	=	72.4 ± 6.4	=	7.1 ± 2.6 b	0.3 ± 0.04	0.31 ± 0.03	3.7 ± 0.3 c
	H ₂ SO ₄	31.2 ± 0.1	-	5.9 ± 0.6	-	1.1 ± 0.2	-	31.4 ± 8.0	-	35.4 ± 9.2 a	4.5 ± 0.3	8.7 ± 0.7	39.9 ± 0.2 a
	NaOH	12.4 ± 0.4	-	2.8 ± 0.06	=	1.9 ± 0.1	=	62.5 ± 5.2	=	10.0 ± 2.1 b	0.37 ± 0.01	1.44 ± 0.2	13.8 ± 0.5 b
	Ca(OH) ₂	0.5 ± 0.02	+	2.5 ± 0.2	-	0.4 0.01	-	81.2 ± 5.0	=	10.4 ± 12 b	-0.04 ± 0.01	-0.08 ± 0.01	0.5 ± 0.03 d
Pearson's correlation	AR x P depletion		AR x soil pH		AR x initial WEP		P depletion x initial WEP						
	0.461*		-0.003		0.809**		0.659**						

The signs in front of the values mean indicate = no significant difference, + a significant increase, and - a significant decrease on the respective fraction after 12 days of incubation when compared to the same fraction at 0 d (Student's T-test, <0.05). Different uppercase letters indicate a significant difference in the P depletion and AR within the biomaterial at 12 d (Tukey HSD, <0.05). Values after ± indicate the standard error (n = 3). For Pearson's correlation: *p < 0.05; **p < 0.01.

consequence of that, the P released from the soil.

Another factor that could explain the differences in the AR of NaOH-treated and acidified SS is the combination of the release of other elements and soil pH. The SS released large amounts of Al and Fe to the soil from the biomaterial layer (Table S3), which had a pH of 1.74 at 12 d (Table S4). As mentioned in the previous section, the Fe-, Al-, and Ca-bound P in sewage sludge may be in almost complete dissolution at pH 1.74 in long-term equilibrium (Xu et al., 2015). However, these elements were released into the soil with different pH conditions. The average soil pH in the first 7-mm was around 3.5, which is ideal for the formation of Fe-P (Penn and Camberato, 2019) and might have been favored by the large amounts of Fe released from the SS to the soil. This indicates that our third hypothesis is plausible: "pretreatments change the release of phosphorus and other elements from the biomaterials, affecting the soil chemical composition, the pH, and thus the P dynamics in the soil".

3.3. Outlook of the applicability of pre-treatments and use as fertilizers

3.3.1. Application of pre-treatments

Acidification of pig slurry is a practice adopted in Denmark since the 2000s (Fangueiro et al., 2015) and has been included by the European Commission (2017b) in the "best available techniques (BAT) reference

document for intensive rearing of poultry and pigs". Acidification was also studied as a potential sanitization method (Anderson et al., 2015), as a pH below 2.5 was found to be efficient in controlling microbial growth (Zhu et al., 2006). In our study, we observed that after 12 d, in the untreated sewage sludge layer there was visible microbial growth (Fig. S3) and the pH was 6.98. By contrast, microbial growth was not visibly apparent in the acidified (pH 2.15), NaOH-treated (pH 10.4), and Ca(OH)₂-treated (pH 12.4) SS at 12 d (Fig. S4, Table S4). Thus, the acidification and NaOH treatments may also be effective in sanitizing the sewage sludge and inhibiting microbial growth when applied to the soil.

The direct use of sewage sludge as fertilizer still generates much debate (Magid et al., 2020). In the European Union in 2012, about half of the sewage sludge produced is reused (direct field application and composting) and a quarter is incinerated (Kelessidis and Stasinakis, 2012). However, the final use (Donatello and Cheeseman, 2013) and legislations (Miljøstyrelsen, 2018) varies among the countries and some countries are moving to mono-incineration of sewage sludge. In Switzerland, the use of sewage sludge in agriculture has been banned since 2003 (Swiss Federal Council, 2003) due to the potential risks from organic contaminants (Smith, 2009). Thus, different sanitization processes are often used before the application of sewage sludge in the field

(Kahiluoto et al., 2015). Sanitization using lime is widely used because of its effectiveness and low costs compared to other treatments (Anderson et al., 2015). Studies using lime showed that at a pH range of 9–10, *Salmonella* spp. and fecal coliforms are killed (Vinnerås et al., 2003), and at a pH above 11, helminth eggs can be controlled (Bina et al., 2004).

The use of meat and bone meal for animal feeding has been banned in the European Union since the 2000s (European Commission, 2000) but it can be used as organic fertilizer due to its high content of N (~8%), P (~4%), and Ca (~10%) (Kivelä et al., 2015). However, a part of the MBM from livestock slaughterhouses cannot be used for agricultural purposes due to the risk of being infected by transmissible spongiform encephalopathy (Möller, 2015), commonly known as mad cow disease. For this reason, up to 50% of the meat and bone meal (mainly from category 1) is currently incinerated (Möller, 2015). In the early 2000s, the treatment with NaOH of potentially contaminated carcasses was proposed and used in the US (Thacker and Kastner, 2004). In our study, we found that the alkalization of MBM with NaOH significantly increased the WEP fraction in the material (Table 2) and increased the AR from soil (Table 3) when compared to the untreated material.

3.3.2. Use of treated biomaterials as fertilizers

Our results showed that the pre-treatments assessed in this study may provide further benefits to the biomaterials in terms of fertilizer use efficiency. The application of Ca(OH)₂ significantly increased the bicarbonate-P fraction of SS and SS-ash, which is in agreement with previous studies (Alvarenga et al., 2017; Huang et al., 2008, Øgaard and Brod, 2016). Results of Alvarenga et al. (2017) showed that the increase in the bicarbonate fraction due to the liming treatment correlated with an increase in P uptake by barley. The increase in P uptake after a liming treatment was also observed by Bauer et al. (2019) and Øgaard and Brod (2016) in ryegrass. Kahiluoto et al. (2015), however, found a decrease in plant P availability after a liming stabilization treatment of sewage sludge.

NaOH-treated SS and SS-ash showed higher soil WEP contents compared to those in the Ca(OH)₂ treatments. Thus, this could be a more efficient treatment to increase the P use efficiency of sewage sludge when placed in the soil. NaOH prices are much higher than the lime prices (Xu and Cheng, 2011), but our results show that only a small amount of NaOH (1.5 M) needs to be applied to the sewage sludge to reach a pH above 11. In addition, the difference in price could be compensated by the increase in the P fertilizer efficiency.

Recently, meat and bone meal from pork slaughterhouses (category 2) have been commercialized as pellets in Denmark (Daka SA, n.d.) and in Sweden, where it has been recommended to be placed in the soil, reaching around 85% of mineral N fertilizer equivalent (Delin et al., 2018). However, Christiansen et al. (2020) found a low P fertilizer equivalent (17–37%) when meat and bone meal was applied localized in a layer in the soil. The results of Damaceno et al. (2019) indicated that the acidification of bone meal increased the relative fertilizer efficiency from 16% to 73%. Acidification of the digestate solid fraction has been recently studied by (Regueiro et al., 2020). They found that the acidified digestate solid fraction significantly increased the P uptake by maize compared to the untreated solid fraction when applied localized.

We expect therefore that the localized application of these pre-treated biomaterials would create a larger P-rich zone in the soil with

a greater amount of P available to the plant. However, we also found that large amounts of other elements, i.e. aluminum and ammonium, are released into the soil. This may create a toxicity zone for the plant roots, avoiding access to the placement zone (Pan et al., 2016; Wang et al., 2016). Therefore, further studies with plants are needed to assess the effects of acidification of all the biomaterials and NaOH treatments of SS and SS-ash on plant growth and P uptake.

4. Conclusions

Using a one-dimensional reaction system, we compared the composition of the biomaterials before and after incubation and found that the acidified materials released high amounts of P to the soil. Surprisingly, we also found relatively high amounts of P being released from the untreated biomaterials, however, very small amounts of this were recovered as WEP in the soil. We hypothesize that the localized application of untreated biomaterials stimulated the growth of microbes, which transported nutrients from the biomaterial layer through the soil profile. However, further studies are needed to elucidate the interactions between the treatments, biomaterials and soil-microorganisms.

The results obtained in this study show that commonly used chemical treatments can change P fractions of biomaterials, with acidification showing better results in terms of increasing the P solubility of all the biomaterials. However, when applied localized into the soil the NaOH-treated SS and SS-ash showed most promising results. Therefore, our results paves the way to the application of pretreatments to increase the phosphorus use efficiency from biowastes. Further studies using plants will help support these findings.

Credit author statement

Pietro Sica: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Clara Kopp: Methodology, Investigation, Writing – review & editing, Dorette Muller-Stover: Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing, Supervision, Funding acquisition, Jakob Magid: Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pietro Sica reports financial support was provided by European Commission Marie Skłodowska-Curie Actions.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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