



A biorefinery approach to exploit digestate as key feedstock in the energy–nutrient nexus

D4.3 Composition and agro-environmental performance of produced fertilising products and soil enhancers

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Author(s):	Çağrı Akyol and Thais Guedes Silveira
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1. Objective

An agro-environmental assessment of the phosphorus (P)-rich and nitrogen (N)-rich products, as well as the nutrient-poor but organic carbon (OC)-rich by-product of the P-recovery route from digestate was performed. Briefly, the N-recovery from digestate was performed via N-stripping/scrubbing, resulting in N-rich products, ammonium sulphate (AS) and ammonium citrate (AC). The N dynamics of these products, together with the raw digestate and its liquid fraction (LFD), as well as the N-stripped digestate and its liquid fraction (LFND), were assessed via a soil incubation test. The P-recovery from digestate was performed from the solid fraction of digestate (SFD) and solid fraction of N-stripped digestate (SFND) via acid leaching followed by precipitation, forming struvite-like precipitates. Additionally, the P-recovery was also performed from the LFD via struvite crystallization, forming a final struvite product. Fe-modified biochar adsorption was also performed in the LFD, resulting in a P-rich biochar. All these P-rich products were assessed as potential P-fertilizers via pot trials. Finally, after the P-acid leaching from the SFD and SFND, P-poor and N- and P-poor fractions were left rich in OC, so their soil enhancer potential was evaluated via a C incubation experiment.

2. Set-up/Parameters

2.1 N incubation

The biobased fertilizing products from the N-recovery route were obtained from stripping and scrubbing tests: two digestates (N-stripped and N-rich), liquid fractions of these digestates (separated via centrifugation), and two scrubbing waters (AS and AC). Although scrubbing was also tested using CO₂, the resulting scrubbing water, ammonium bicarbonate, was not rich in enough N to be used as a fertilizer (the recovery of N in water-rich CO₂ as scrubbing water was below the go/no-go criterion of 80% of the benchmark, ammonium sulfate, with a total of only 0.7 g N/kg of ammonium bicarbonate). Soil samples without the addition of fertilizers were also included as a control. A total of 192 tubes (8 treatments × 4 replicates × 6 sampling days) were used in the experiment. The soil incubation experiment was run for 120 days to determine the N dynamics in the soil in comparison to those in the synthetic fertilizer calcium ammonium nitrate (CAN, 16% N) by measuring the plant-available mineral N in the soil.

The soil samples were collected from a silty loam soil field (composed of sand-silt-clay corresponding to 5.5%-75.3%-19.2%) in Liège, Belgium. The digestate used in this work is derived from a bench-scale anaerobic digestion (AD) of cow manure together with the N-stripped digestate collected after the stripping-scrubbing of the same cow manure-based digestate. The characterization of both digestates is reported in “D3.3 Mineral P-fertilizer, extracted and re-precipitated from the solid fraction of digestate”.

A preincubation step was performed one week before the incubation. Pre-air-dried soil with 35% water-filled pore space (WFPS) was maintained in the dark at 18 °C for one week. The amount of fertilizer added to the soil was fixed at 170 kg N.ha⁻¹. The steps performed in the soil incubation were based on methodology reported elsewhere (Saju et al., 2022). The moisture content in the tubes was kept constant at 50% WFPS, with weekly checks and adjustments in the tubes where water loss occurred. Parafilm with pinholes was applied on the top of each tube to prevent moisture loss by evaporation, and the tubes were kept in a dark room at 18 °C. The steps performed to start the soil incubation are presented in the

Figure 1.

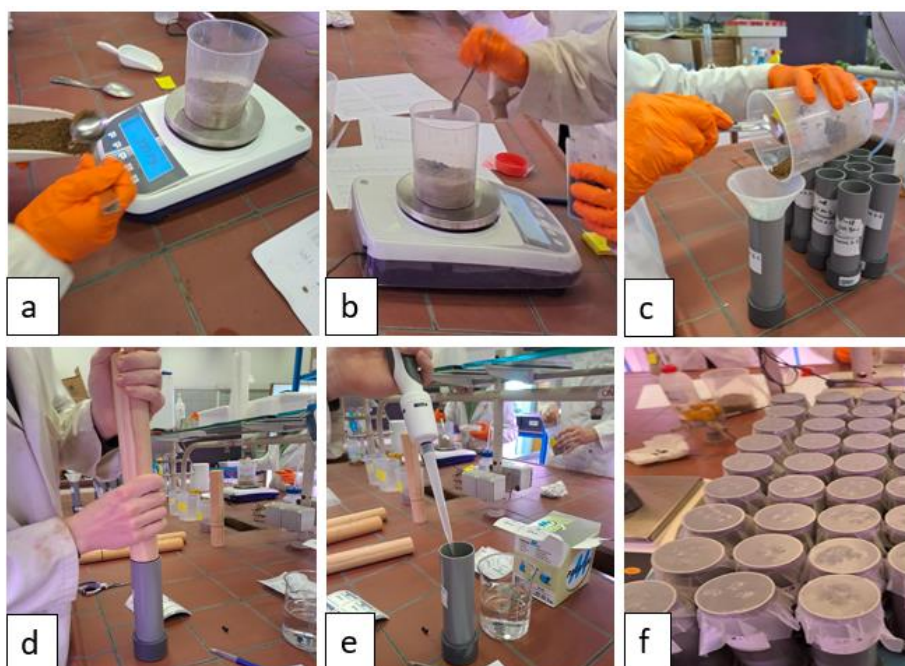


Figure 1 – Steps performed in the soil incubation experiment for further evaluation of the N dynamics of the products in the soil. The images correspond to a) The weighing of soil; b) The addition of the BBF at a proper weight; c) The mixing of the BBF and soil followed by its addition in the PVC tube; d) The compression of the mixture in the PVC tube; e) The addition of water to maintain the WFPS at 50%; f) The addition of the parafilm with pinholes, followed by their transference to a dark room.

Approximately every 20 days, quadruplicates of each treatment were sampled for further analyses of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ for possible “net N release” calculations. The nitrogen net release ($\text{N}_{\text{rel,net}}$) was calculated for each biobased fertilizer tested, corresponding to the difference between the mineral N present in the soil after the addition of the biobased fertilizer, referred to as the “treatment”, and the mineral N present in the soil without fertilizer, referred to as the “control” (De Neve & Hofman, 1996).

2.2 Pot trials

The P-rich biobased fertilizers (BBFs) were tested as potential P-fertilizers in pot trials with ryegrass as a crop and river sand as a low-available P soil. Triple super phosphate (TSP) was used as a reference as a commercial mineral fertilizer and pots without application of P-fertilizer were used as a control. Additionally, AC was applied in separate treatments, with TSP, as well as with one struvite-like product, and compared to a pot with only soil and AC, to investigate any possible enhancement in P-uptake by the plant in the presence of AC. The AC was added in the mentioned treatments at a rate of 170 kg N.ha^{-1} , as a N-fertilizer for the mentioned pots. A pre-incubation step was performed with 40% of the water-holding capacity (WHC) of the river sand maintained in the dark for 1 week. During the experiment, the moisture content was kept at 70% WHC. The pots with the ryegrass and BBFs are illustrated in Figure 2.

The P application rate of the fertilizers was $60 \text{ kg P}_2\text{O}_5\cdot\text{ha}^{-1}$. The products were applied in quadruplicates for a pot trial of 13 weeks, with harvesting every 4 weeks, except for the 1st harvest after 5 weeks. A total of 52 pots were studied (13 treatments x 4 replicates). The biomass and P plant uptake at each harvest, from each BBF treatment was compared with the TSP and the control, and the phosphorus fertilizer replacement value (PFRV) was calculated by the end of each harvest, the latter corresponding to the P uptake in the BBF treatment subtracted by the uptake in the control, divided by the value obtained in the reference TSP.



Figure 2 – Pots of ryegrass with BBFs in river sand as soil, maintained for 13 weeks, for the evaluation of the BBFs as potential alternative P-rich fertilizers to the commercial mineral fertilizer TSP.

2.3 C incubation

The nutrient-poor solid products obtained after the different acid leaching of the solid fraction of the two digestates were grouped and mixed as follows: P-poor fractions from the acid leaching of the solid fraction of the N-rich digestate (PPSD); and P-poor fractions from the acid leaching of the solid fraction of N-stripped digestate (PPND). The C incubation measures the soil CO_2 respiration and by that value, we can assess the C mineralization (De Neve & Hofman, 2000; Egene et al., 2021). These products were added in quadruplicate to PVC tubes (7.2 cm height and 6.8 cm diameter), with the same soil as the one used in the N incubation at a rate of $9000 \text{ kg Total Organic C (TOC)}\cdot\text{ha}^{-1}$, compacted to $1.4 \text{ g}\cdot\text{cm}^{-3}$. Tubes with only soil and jars with only NaOH vials were added as controls. These tubes were added in glass jars and on top of the tubes a vial with 15 mL of NaOH (1 mol/L) was added to capture the CO_2 released. On certain days the NaOH vial was removed, BaCl_2 was added, and the solution was titrated with HCl (0.5 mol/L) to calculate the mineralized C in each treatment. The experiment was conducted in the dark, at 19°C , for 92 days. The moisture content was kept at 50% WFPS. The C-mineralization was calculated in percentage, by subtracting the cumulative CO_2 -C value of the control from the evolved from the treatment, divided by the TOC added in the treatment. The setup is presented in Figure 3.



Figure 3 - Carbon mineralization setup for 92 days: PVC tubes filled with soil and organic carbon-rich products, with the addition of a NaOH vial to trap the CO₂ emitted by the microorganisms, for the assessment of these products as potential soil enhancers. These jars were maintained in the dark throughout the experiment.

3 Results

3.1 Soil incubation

The products tested in the soil incubation were characterized for dry matter (DM), organic matter (OM), total nitrogen (TN), Total ammonium nitrogen (TAN), and nitrate, as well as total carbon, pH-KCl and Electrical conductivity (EC). The characterization of the products is presented in the Table 1.

Table 1 - Characteristics of the products applied in the soil incubation experiment.

Products	Parameters							
	pH-KCl	EC (mS/cm)	DM (%)	OM (%)	Total C (%)	Total N (g/kg)	NH ₄ -N (g/kg)	NO ₃ -N (g/kg)
Digestate	8.43 ± 0.12	20.36 ± 0.20	5.76 ± 0.26	79.27 ± 0.07	2.08 ± 0.38	4.15 ± 0.10	2.32 ± 0.01	< 0.001
N-stripped digestate	8.42 ± 0.02	13.60 ± 0.25	8.58 ± 0.66	67.56 ± 0.22	2.25 ± 0.27	2.27 ± 0.06	0.18 ± 0.01	< 0.001
LF of digestate	8.47 ± 0.03	24.32 ± 0.26	2.21 ± 0.11	91.42 ± 2.15	0.95 ± 0.07	3.42 ± 0.08	2.36 ± 0.08	< 0.001
LF of N- stripped digestate	8.27 ± 0.03	16.48 ± 0.16	2.77 ± 0.25	84.69 ± 2.34	1.30 ± 0.01	1.27 ± 0.02	0.06 ± 0.01	< 0.001
AS	0.88 ± 0.02	232.33 ± 2.47	8.02 ± 0.04	n.a.	n.a.	4.52 ± 0.07	4.22 ± 0.12	0.22 ± 0.38
AC	4.04 ± 0.01	21.24 ± 0.28	3.66 ± 0.05	n.a.	1.67 ± 0.01	4.41 ± 0.06	4.08 ± 0.16	1.06 ± 0.36

n.a. = not applicable; AS: Ammonium sulphate; AC: Ammonium citrate; LF: Liquid fraction.

The N-mineralization ($N_{\text{net,min}}$) on the 120th day, expressed in percentage of the total N applied initially, was calculated as the difference between the $N_{\text{rel, net}}$ on the last day, and day 0. The highest value was

achieved from LF of N-stripped digestate (16%). For the samples with AS, AC, and CAN, all products composed of 100% inorganic forms of N, N immobilization (corresponding to lower $N_{rel, net}$ on the chosen day than on day 0) occurred on the 120th day. Since N-mineralization indicates the conversion of organic N to its mineral forms (Zhang et al., 2017), this can explain the mineralization only identified in the samples with organic N content.

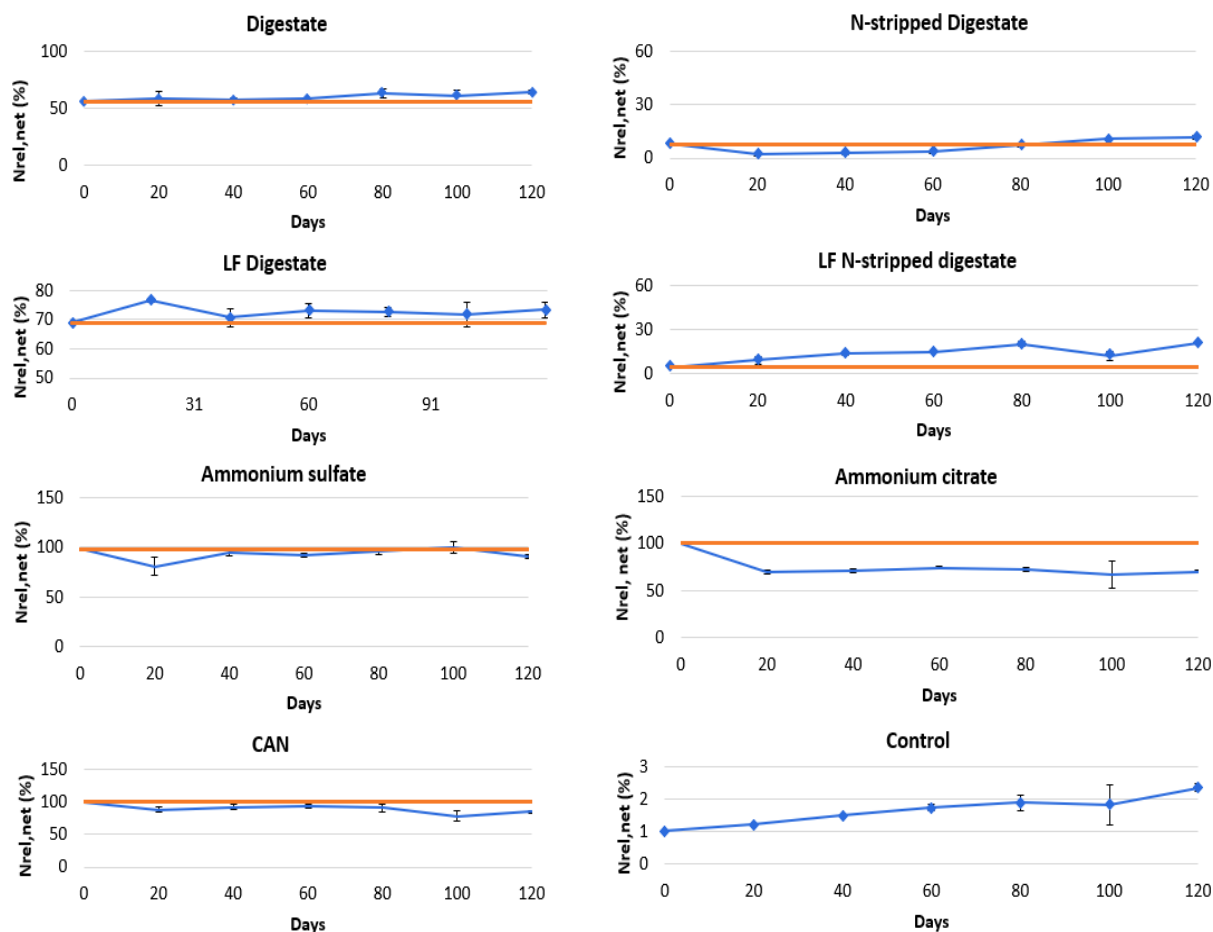


Figure 4. Net N-release ($N_{rel, net}$) % of the N input of potential fertilizers during the 120 days of soil incubation experiment. The orange line in the graphs represents the percentage of mineral N in the fertilizer applied on day 0. The values above the line correspond to N-mineralization and below the line N-immobilization.

3.2 Pot trials

The nutrient and metal content of the P-rich BBFs was assessed, as well as their DM, OM, and total organic carbon (TOC), which are given in Table 2. The P concentration in the grass in each treatment, after the four cuts is illustrated in Figure 5. Additionally, the PFRV obtained after each cut is shown in Figure 6.

Table 2 - Characteristics of the dried P-rich biobased fertilizers applied in the pot trials (Struvite-like precipitates dried at 50°C). PD: Struvite-like precipitate from P-leached solid fraction of digestate (SFD); PN: Struvite-like precipitate from P-leached solid fraction of N-stripped digestate (SFND); SU: Sulfuric acid applied in the P-leaching; AS: Ammonium sulfate used in the P-leaching; WA: Waste sulfuric acid applied in the P-leaching; AC: Ammonium citrate; BIO_UM: Fe-modified biochar with adsorbed P from Liquid fraction of digestate (LFD); STRU_UM: Struvite from LFD.

Element	Fertilizer applied in the pot trials							
	PD-SU	PD-AS	PD-WA	PN-SU	PN-AS	PN-WA	BIO-UM	STRU-UM
TOC (%)	39.76 ± 0.06	37.20 ± 0.07	24.42 ± 0.67	25.21 ± 6.05	19.41 ± 4.11	31.76 ± 0.18	30.97 ± 1.53	15.86 ± 0.03
P (g/kg)	60.84 ± 1.21	70.46 ± 2.26	67.99 ± 0.80	40.58 ± 0.60	80.17 ± 1.81	62.12 ± 3.51	10.70 ± 1.41	28.00 ± 3.93
N (g/kg)	41.59 ± 0.87	51.00 ± 5.53	36.07 ± 3.79	40.86 ± 0.29	41.57 ± 2.02	33.17 ± 0.29	10.41 ± 1.14	30.15 ± 0.32
K (g/kg)	10.65 ± 0.35	7.42 ± 0.19	10.22 ± 0.53	13.09 ± 0.44	9.10 ± 0.16	12.43 ± 0.57	< 0.05	33.13 ± 1.70
Mg (g/kg)	38.66 ± 1.44	49.91 ± 1.84	36.95 ± 0.36	20.08 ± 0.74	59.44 ± 1.20	22.24 ± 1.20	1.02 ± 0.79	28.58 ± 0.29
Ca (g/kg)	48.93 ± 1.19	35.06 ± 0.84	65.19 ± 3.67	63.54 ± 1.32	33.59 ± 1.72	102.13 ± 8.14	14.01 ± 2.44	64.76 ± 0.70
Na (g/kg)	8.48 ± 0.24	61.75 ± 1.22	5.88 ± 0.35	6.15 ± 0.07	6.14 ± 0.77	6.87 ± 0.52	0.13 ± 0.18	29.74 ± 0.48
S (g/kg)	28.18 ± 1.19	26.59 ± 0.60	29.01 ± 2.57	31.69 ± 1.37	22.48 ± 0.39	32.15 ± 3.41	0.65*	4.59 ± 0.13
Fe (g/kg)	4.19 ± 0.07	3.81 ± 0.21	4.81 ± 0.54	7.49 ± 0.21	47.44 ± 1.57	6.59 ± 0.73	272.35 ± 12.45	4.37 ± 0.17
Mn (g/kg)	1.20 ± 0.02	1.52 ± 0.04	1.85 ± 0.06	1.18 ± 0.03	19.57 ± 0.69	1.55 ± 0.09	3.18 ± 0.21	0.70 ± 0.01
Zn (g/kg)	0.44 ± 0.01	0.57 ± 0.02	0.47 ± 0.02	0.52 ± 0.01	8.87 ± 0.47	0.46 ± 0.03	42.94 ± 1.91	0.93*
Cu (g/kg)	0.84 ± 0.02	0.71 ± 0.01	0.47 ± 0.02	0.71 ± 0.01	4.32 ± 0.15	0.60 ± 0.04	0.12 ± 0.01	0.20*
Al (g/kg)	0.77 ± 0.03	0.59 ± 0.04	0.59 ± 0.11	1.53 ± 0.04	5.45 ± 0.21	1.12 ± 0.15	< 0.05	4.21 ± 0.24
Cr (g/kg)	0.01*	0.01 ± 0.01	0.01*	0.01*	0.07*	0.01*	0.07*	0.08*
Co (g/kg)	< 0.002	< 0.002	< 0.002	< 0.002	0.01*	< 0.002	0.03 ± 0.01	< 0.002
Ni (g/kg)	0.01*	< 0.001	< 0.001	< 0.001	0.03 ± 0.03	< 0.001	0.15 ± 0.07	0.06 ± 0.01
Cd (g/kg)	< 0.002	< 0.002	< 0.002	< 0.002	0.01 ± 0.00	< 0.002	< 0.002	< 0.001
Pb (g/kg)	< 0.005	< 0.005	< 0.005	< 0.005	0.03 ± 0.00	< 0.005	< 0.005	< 0.005

TC = Total Carbon; TOC = Total Organic carbon;

*The standard value was below 0.01

The P concentration in the harvested grass in the treatments in which struvite-like products were used as P-fertilizers was similar between them in the 4 cuts, and similarly to the TSP during the 13 weeks. A reduction in the P concentration happened from the 1st to the 4th cut, a phenomenon also observed by Bogdan et al. (2021), although the P concentration in the last two cuts had only a small difference. Additionally, no significant impact on P-uptake was observed when AC was added to the treatments with TSP and the struvite-like product PD_SU when compared to the control with only AC.

The PFRV of all the products in which AC was added had low PFRV when compared to the struvite-like products. This is related to the low biomass harvested in those treatments. The treatments with biochar resulted in low P concentration and low PFRV as well, below 50% after the 4th cut. This might be related to the insufficient P-release or the need for a longer time for the P to be released from the biochar.

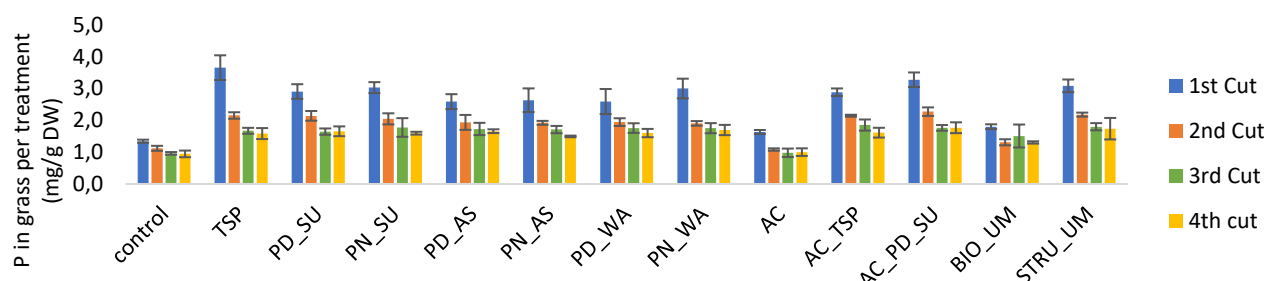


Figure 5 - Phosphorus concentration in the plants per cut for each treatment.

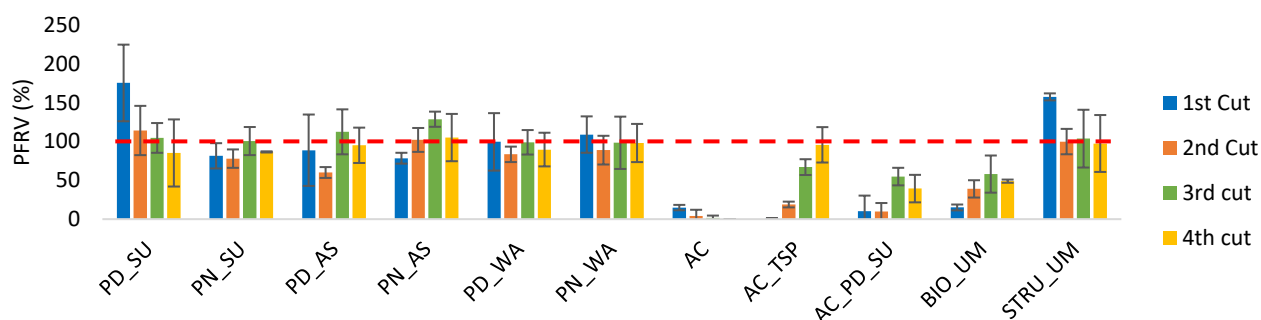


Figure 6 - PFRV calculated after each cut for each treatment. The dashed red line corresponds to the reference TSP. TSP: Mineral fertilizer; PD: Struvite-like precipitate from P-leached solid fraction of digestate (SFD); PN: Struvite-like precipitate from P-leached solid fraction of N-stripped digestate (SFND); SU: Sulfuric acid applied in the P-leaching; AS: Ammonium sulfate applied in the P-leaching; WA: Waste sulfuric acid applied in the P-leaching; AC: Ammonium citrate; BIO_UM: Fe-modified biochar with adsorbed P from Liquid fraction of digestate (LFD); STRU_UM: Struvite from LFD.

All the treatments in which a struvite-like precipitate was added as P-rich fertilizer had mostly similar PFRV throughout the experiment, in which for most of them the highest value was obtained after the 1st cut, with up to 175% for PD_SU, following a decrease in the 2nd cut, with a slight increase or decrease in the 3rd cut, depending on the struvite product, but with a similar PFRV after the 3rd and 4th cut, although the latter had a slightly lower value for most of them. Overall, at least 85% PFRV was obtained for all the struvite-like precipitates after the final cut, with up to 105% PFRV for PN_AS. Even higher values could be obtained in the longer term, since four months was reported by Bogdan et al. (2021) as the minimum to obtain a near-complete dissolution rate from struvite products, as it is expected that struvite has a lower release pattern when compared to TSP. Therefore, all the tested struvite-like precipitates are potential P-rich BBFs alternatives to the conventional mineral TSP.

3.3 C-mineralization

The nutrients, TOC, as well as the DM and OM of the nutrient-poor products were analyzed, as presented in Table 3. The carbon mineralization (% added C) of the P-poor products in the soil is presented in Figure 7. After 92 days only 15% of C-mineralization was obtained with PPSD, and similarly,

16% for the PPND. In the literature, the reported value for P-poor SFD was equally low, with approximately 16% (Egene et al., 2021). These low values of C-mineralization indicate that a small amount of the C initially present in the nutrient-poor products was mineralized and converted to CO₂ by the microorganisms in the soil via respiration. Therefore, this can indicate that in the long term, the biggest part of the C in these products would still be present in the soil and act as soil enhancers.

Table 3 - Characteristics of the nutrient-poor, OC-rich solid products tested in the C-incubation experiment. The nutrient content is expressed on a fresh matter basis.

Parameters	Products assessed as soil enhancers	
	PPSD	PPND
Total organic carbon (% DM)	39.76 ± 0.06	37.20 ± 0.07
DM (%)	9.18 ± 0.15	10.88 ± 0.19
OM (%)	77.31 ± 24.18	85.74 ± 30.65
N (g/kg)	3.42 ± 0.01	2.98** ± 0.17
P (g/kg)	0.53 ± 0.03	0.90 ± 0.01
K (g/kg)	0.68 ± 0.03	1.23 ± 0.01
Mg (g/kg)	0.50 ± 0.02	0.92 ± 0.01
Ca (g/kg)	1.50 ± 0.07	2.45 ± 0.02
Na (g/kg)	0.16*	0.27*
S (g/kg)	2.93 ± 0.17	4.31 ± 0.06

*These standard deviations were below 0.01; PPD: P-poor product from acid leaching of the solid fraction of digestate; PPND: P-poor product from acid leaching of the solid fraction of N-stripped digestate.

**The N content of the PPND was initially lower than the value on the table, however, one of the batches of N-stripped digestate had higher N content, and due to a limited amount of N-stripped digestate that batch had to be included with the other batches, all of them being mixed, therefore resulting in a higher N value for the PPND.

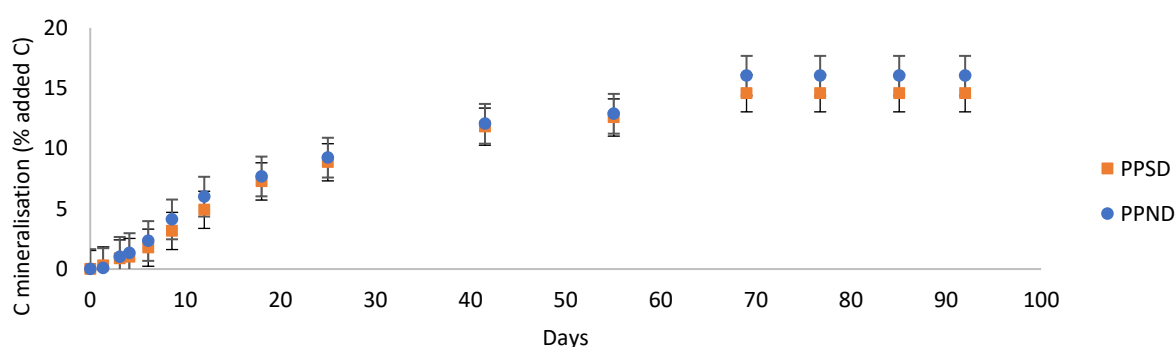


Figure 7 – Cumulative amount of carbon mineralized after the addition of the nutrient-poor products in the soil. PPSD: P-poor after acid leaching of the solid fraction of digestate; PPND: P-poor after acid leaching of the solid fraction of N-stripped digestate.

Conclusions/Remarks

- After 120 days of soil incubation the highest $N_{rel, net}$ was observed in the soil mixed with the scrubbing water AS (91%), with a value similar to the one reached by CAN (85%), indicating its great potential as a substitute to the conventional mineral N fertilizer;
- The samples of soil mixed with LF of digestate and AC resulted in $N_{rel, net}$ equal to 73%, and 70%, respectively, and the soil mixed with digestate had a $N_{rel, net}$ equal to 64%, all values corresponding to a good fertilizing potential.
- All the struvite-like products are good alternatives to the TSP as P-rich fertilizers, since they performed similarly to the commercial mineral fertilizer, with PFRV after the final harvest from 85-105%.
- The nutrient-poor products obtained from the P-leaching of the SFD and SFND had low C-mineralization, around 15%, after 92 days of C-incubation, which can be an indication of their high potential to improve the soil quality in the long term, acting as soil enhancers.

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Contact: thais.guedessilveira@ugent.be; cagri.akyol@ugent.be; erik.meers@ugent.be

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Project partners: Biogas-E, KU Leuven, Ghent University, Marmara University, VCM, OSTIM