

1 **Solid fraction of separated digestate as soil improver – implications for soil fertility and**  
2 **carbon sequestration**

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24 **ABSTRACT**

25 **Purpose**

26 This study investigated the C and N mineralisation potential of solid fractions (SFs) from co-  
27 digested pig manure after P-stripping (P-POOR SF) in comparison with P-rich SFs, as a  
28 means to estimate their organic matter stability in soil. Compost (COMP) and biochar (BCHR)  
29 (made from P-POOR SF) were also included in the study as reference biosolids.

30 **Methods**

31 The SFs were incubated in a sandy-loam soil under moist conditions to determine production  
32 of CO<sub>2</sub> and mineral N. At specified intervals, CO<sub>2</sub> evolution in the mixtures was measured via  
33 the alkali trap method and titration over a period of 81 days while mineral N was measured  
34 using a flow analyser after KCl extraction over a period of 112 days.

35 **Results**

36 The various SFs showed similar patterns of C mineralisation (15%-26% of added total C in 81  
37 days) that were clearly higher than for COMP and BCHR (6% and 7%, respectively).  
38 Temporary N immobilisation was observed in biosolids with a high C/N ratio. The effective  
39 organic matter (EOM) of the SFs was calculated based on the C mineralisation data and  
40 varied between 130 and 369 kg tonne<sup>-1</sup>.

41 **Conclusions**

42 The SF with a reduced P content had a high EOM/P ratio which is beneficial in areas where P  
43 status of the soil is already high. Moreover, the N mineralisation patterns confirm that a high  
44 C/N ratio may also reduce risks for N leaching due to temporary N immobilisation.

45

46 **Keywords** : soil organic matter; solid fraction; digestate; C/N ratio; mineralization

47 **1. Introduction**

48 Over the last decade, European Union (EU) policy oriented towards developing a circular and  
49 resource-efficient economy has encouraged a rapid increase of anaerobic digestion (AD)  
50 installations. The number of AD installations in the EU rose from 6,227 to 17,662 between  
51 2009 and 2016 (EBA, 2018), with a main focus on AD of residues from food and feed  
52 industries, sewage sludge, animal manure and landfill waste. While biogas is the primary  
53 product from AD, millions of tonnes of digestate are generated annually as a by-product.  
54 Digestate requires appropriate management or disposal to prevent over-fertilisation in  
55 regions with an excess of manure and digestate as well as storage problems. Digestate is  
56 typically separated mechanically into liquid and solid fractions as a simple procedure to  
57 overcome transport constraints and facilitate application to soil (Holm-Nielsen et al. 2009).  
58 The liquid fraction (LF) typically contains significant amounts of nitrogen (N), mainly in the  
59 form of plant available ammonium-N, and can be used as a N-fertiliser (Sigurnjak et al.  
60 2017). The solid fraction (SF), on the other hand, has a high dry matter content, is rich in  
61 phosphorus (P) and organic carbon (C), and has the potential to be used as a P fertiliser and  
62 an organic soil improver.

63  
64 The recently approved Fertiliser Regulation (European Commission, 2019) makes a  
65 distinction between “organic soil improvers” and “solid organic fertilisers” primarily based  
66 on the nutrient content and the intended use of the biosolid. While solid organic fertilisers  
67 are added as sources of readily available plant nutrients, organic soil improvers are added  
68 primarily as sources of organic matter (OM) in order to ameliorate soil physicochemical and  
69 biological properties. When OM is added to soil, microorganisms use it as a source of energy  
70 thereby emitting CO<sub>2</sub> via respiration (Juma, 1999). Hence, only the fraction of OM that is less

71 degradable remains and eventually contributes to soil organic matter. This stable fraction of  
72 the original OM that remains after 1 year is often referred to as the “effective organic  
73 matter” (EOM) (Veeken et al. 2017). In order to be classified as an organic soil improver,  
74 Veeken et al. (2017) proposed that the EOM to mineral-N ratio must be higher than 150 and  
75 the EOM to phosphate ( $P_2O_5$ ) ratio must be higher than 35.

76  
77 SFs from AD processing have a high C and P content, and so the C/P ratio may be  
78 problematic for their application as soil improvers in regions with restrictions on P input (e.g.  
79 Belgium and the Netherlands). However, these products are marketed in other countries as  
80 P-fertilisers, thus, representing a loss of organic C from such regions. A recent study has  
81 shown that up to 80% P can be recovered from SFs of digestate at reasonable costs using a  
82 P-stripper system known as Re-P-eat (Schoumans et al. 2017). This process involves addition  
83 of sulphuric acid to release the phosphate from the SF followed by adding a base to  
84 precipitate the solubilised P. Two products are then obtained – a mainly crystalline struvite  
85 ( $NH_4MgPO_4 \cdot 6H_2O$ ) and a P-POOR SF (Regelink et al. 2019; Schoumans et al. 2017). While the  
86 recovered struvite can be sold as a P fertiliser, the P-POOR SF may open opportunities for  
87 keeping C in regions with P-rich soils.

88  
89 Sustaining an adequate soil organic matter content plays an important role in maintaining  
90 and improving soil microbial health and soil structure, which is crucial for attaining  
91 sustainable crop production (Chatterjee et al. 2017). Moreover, European policy has  
92 recognized soil organic carbon (SOC) as an instrument to reduce  $CO_2$  emission through soil C  
93 sequestration (Lugato et al. 2014). Finally, organic soil improvers have also proven their  
94 potential utilization to reduce metal mobility in soils exhibiting elevated concentrations, and

95 by doing so alleviate associated environmental risks (Van Poucke et al. 2019; Egene et al.  
96 2018). Therefore, there is a need for increased utilization of C-rich biosolids in agriculture to  
97 improve soil organic matter and increase soil C reserves.

98

99 There are a few studies that have looked at the C and N mineralisation potential of  
100 digestates in soils (Peters & Jensen, 2011; de la Fuente et al. 2013; Möller, 2015). These  
101 studies highlighted the relationships between digestate composition and C and N  
102 mineralisation dynamics. However, the effect of P-stripping of SFs of digestates on its OM  
103 decomposition and consequent C and N mineralisation has not been investigated. This  
104 makes it necessary to assess the dynamics of active C and N pools in the SFs of digestate to  
105 determine how much organic C and N will mineralise in current and future growing seasons.  
106 The aim of this study was to evaluate the dynamics of C- and N-mineralisation in soil  
107 amended with solid digestate following different post-digestion treatments, compared to  
108 biochar and compost (as reference biosolids), in order to quantify the mineralisable C and N.  
109 To achieve this, the C and N decomposition kinetics of the different biosolids were studied  
110 through incubation experiments and were related to some of their inherent agrochemical  
111 characteristics (e.g. C/N ratio, mineral N, pH). Any such relationships should provide useful  
112 insight into the soil improving properties of SF of digestate and may be useful in estimating  
113 appropriate application rates. Furthermore, the potential of long-term application of the SFs  
114 as sources of soil organic matter in P-rich soils was evaluated.

115

116

117 **2. Materials and methods**

118 **2.1. Biosolid collection and characterization**

119 Six biosolids (Table 1) were used: four SFs that went through different post-digestion  
120 treatment steps, biochar and compost. All SFs were collected in 2018 from full scale AD  
121 installations of either Groot Zevert Vergisting (GZV) (Beltrum, Netherlands) or AM Power  
122 (Pittem, Belgium). Briefly, the AD plant of AM Power has a total utilizable volume of 20,000  
123 m<sup>3</sup> producing 7,360 and 7,435 kWh day<sup>-1</sup> of thermal and electrical energy, respectively, by  
124 the anaerobic digestion of organic biological waste. GZV is an AD plant with a volumetric  
125 capacity of 15,000 m<sup>3</sup> producing 10 Mm<sup>3</sup> of biogas by mesophilic digestion of animal manure  
126 (74%) and various organic waste products from the agro-food industry plus glycerin (26%) as  
127 co-substrates.

128  
129 In this study, the SFs are differentiated primarily based on the type of post-digestion  
130 treatment(s) of the digestates. The digestate from AM Power underwent solid-liquid  
131 separation with a decanter centrifuge after which the SF was passed through a fluidized bed  
132 dryer at 60 °C to obtain a dried SF denoted as DRY-SF. Three SFs were obtained from the  
133 digestate of GZV: (i) SF separated by a decanter centrifuge (DEC-SF) (ii) SF separated by a  
134 screw press (SCP-SF) and (iii) SF separated by a decanter centrifuge followed by P-stripping  
135 (P-POOR SF). The P-stripping process, called Re-P-eat, was developed by Wageningen UR and  
136 has been demonstrated at pilot scale (Schoumans et al. 2017). The process involves  
137 acidifying (H<sub>2</sub>SO<sub>4</sub>) the SF of digestate to pH 5 to solubilize mineral P followed by the solid-  
138 liquid separation of the acid slurry to obtain a P-rich acid solution and a P-poor SF. Ca(OH)<sub>2</sub>  
139 or Mg(OH)<sub>2</sub> is then added to the acid solution to precipitate the P as calcium phosphate or  
140 struvite (Schoumans et al. 2017).

141  
142 Biochar (BCHR), produced via slow pyrolysis of the P-POOR SF at 400 °C, and was included in  
143 the study. In addition, commercial compost (COMP) produced by Attero (Venlo, The  
144 Netherlands) from source-separated household and garden waste was used as reference  
145 material.

146  
147 All biosolids were collected in polyethylene sampling bottles and stored at 4 °C. The dry  
148 matter (DM) content was determined by drying to constant weight (48 h) at 80 °C and was  
149 calculated as percentage of wet weight. OM was measured based on loss-on-ignition (LOI) of  
150 dried solids by incineration at 550 °C in a muffle furnace for 4 h. The pH and electrical  
151 conductivity (EC) were measured potentiometrically using an Orion-520A pH-meter and a  
152 WTW-LF537 (GE) conductivity electrode, respectively, in a 1:5 (w w<sup>-1</sup>) wet solids to deionised  
153 water ratio. Total C, N and Sulphur (S) were determined via Elemental analysis (Variomax  
154 CNS analyzer, Elementar, Germany). Ammonium (NH<sub>4</sub><sup>+</sup>-N-) and nitrate (NO<sub>3</sub><sup>-</sup>-N) were  
155 analysed in a 1:10 (w w<sup>-1</sup>) suspension of wet solid and 1 M KCl shaken end-over-end for 30  
156 min. The extracts were filtered (Whatman No. 45) and analysed for their NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N  
157 contents with a continuous flow auto-analyser (Chemlab System 4, Skalar, the Netherlands).  
158 Total P, potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) were determined  
159 using an inductively coupled plasma emission spectrometry (ICP-OES) (Varian Vista MPX,  
160 USA) after wet digestion of the incinerated solids with 5 mL of 6M HNO<sub>3</sub> and 5 ml of 3M  
161 HNO<sub>3</sub> at 150 °C (Van Ranst et al. 1999). Water soluble carbon (WSC) and hot water  
162 extractable carbon (HWEC) were determined on fresh samples according to a modified  
163 method of Ghani et al. (2003). For WSC, 3 g of each biosolid and 30 ml of distilled water was  
164 weighed into 50 ml polypropylene centrifuge tubes, which were shaken end-over-end for 30

165 minutes. The tubes were centrifuged for 20 min at 3500 rpm and the supernatants were  
166 filtered (Whatman No. 45), and transferred into vials for C analysis. To determine the HWEC,  
167 30 ml of distilled water was added to the residues from the previous step, shaken briefly to  
168 re-suspend the solids, capped well and placed in a hot water bath at 80 °C for 16 h. The  
169 extracts were centrifuged for 20 min at 3500 rpm, filtered (Whatman No. 45), and  
170 transferred into vials for C analysis. Total C (inorganic and organic C), in both the first and  
171 second extracts, was determined using a TOC-analyser (TOC-5000, Shimadzu, Tokyo Japan).

## 172 **2.2 Soil characteristics**

173 Soil for the incubation experiments was taken from the topsoil layer (0-30 cm) of an  
174 unfertilised agricultural field in Evergem (Belgium). The soil texture was sandy-loam (87%  
175 sand, 8.5% silt and 4.5% clay), with a pH-H<sub>2</sub>O of 7.1, initial C content of 118 mg kg<sup>-1</sup>, and an  
176 initial exchangeable N content of 1.7 mg NH<sub>4</sub><sup>+</sup>-N kg<sup>-1</sup> and 21 mg NO<sub>3</sub><sup>-</sup>-N kg<sup>-1</sup>. Prior to  
177 incubations, the soil was air-dried and sieved through a 2-mm screen.

## 178 **2.3 C incubation experiment**

179 Organic C mineralisation was evaluated by measurement of soil CO<sub>2</sub> respiration (De Neve &  
180 Hofman, 2000). First, the soil was wetted with distilled water to attain a moisture content,  
181 corresponding to a 50% water filled pore space (WFPS), taking into account the moisture  
182 content of the air-dried soil and tested organic materials. The tested organic materials were  
183 mixed with 117 g of soil at an application rate of 9000 kg OC ha<sup>-1</sup> (or 28 g C kg soil<sup>-1</sup>). These  
184 mixtures were filled into PVC tubes (7.2 cm height, 6.8 cm diameter) and gradually  
185 compacted during filling to attain an apparent bulk density of 1.4 g cm<sup>-3</sup>. The conversion



186 from kg ha<sup>-1</sup> to g kg<sup>-1</sup> soil was based on the surface area of the tubes. The percentage of  
187 WFPS in the soil was calculated from the equation (Linn and Doran 1984):

188 
$$\text{WFPS \%} = (\text{GWC} \times \text{BD}) / (1 - (\text{BD}/\text{PD})) \times 100 \quad \text{Equation (1)}$$

189 where GWC is the gravimetric water content (g water g soil<sup>-1</sup>), BD is the dry bulk density (Mg  
190 m<sup>-3</sup>), and PD is the particle density (Mg m<sup>-3</sup>). The PVC tubes containing the soil-biosolid  
191 mixtures were placed in 1 L Mason jars and just before closing them airtight, glass vials  
192 containing 15 ml 1 M NaOH were placed to capture CO<sub>2</sub> evolved. Controls were performed  
193 without soil samples and with unamended soil. The incubation was done under controlled  
194 conditions at 10 °C for 81 days. At predetermined sampling times (1, 2, 3, 5, 9, 14, 21, 28, 36,  
195 45, 59 and 81 days), the glass vials were removed for determination of amounts of evolved  
196 CO<sub>2</sub> by titration of the NaOH with 1 M HCl after precipitation of carbonates with excess BaCl<sub>2</sub>  
197 (Anderson, 1982). During each sampling time, moisture was adjusted to 50% WFPS and the  
198 jars were left opened for at least 2 hours to allow replenishment of oxygen.

#### 199 **2.4 N incubation experiment**

200 The evolution of N mineralisation was determined by measuring temporal changes in the  
201 mineral N (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N). The air-dried and sieved soil was brought to 35% of WFPS  
202 and was pre-incubated for 1 week at 21.5 °C. This step was necessary to stabilize the  
203 microbial community in the soil. After pre-incubation, each of the biosolids was applied at a  
204 rate of 9000 kg OC ha<sup>-1</sup> (as in the C incubation) and thoroughly mixed with approximately  
205 272 g of pre-incubated soil. Table 2 shows the corresponding total N, organic N and mineral  
206 N dosages. The soil-biosolid mixtures were placed in PVC tubes (18.2 cm height, 4.61 cm  
207 diameter), again, at a bulk density of 1.4 g cm<sup>-3</sup> and the WFPS in the tubes was brought up to  
208 50% by adding distilled water. In total, 256 PVC tubes (8 treatments x 4 replicates x 8

209 sampling times) were covered with pin-holed gas permeable parafilm, placed on trays in  
210 completely randomized design (CRD) and incubated for 112 days at 21.5 °C. Destructive  
211 sampling was done bi-weekly in which 32 intact tubes (4 treatments x 4 replicates) were  
212 removed. After mixing well, 10g of soil per tube was analyzed for its mineral N contents as  
213 described in 2.1. The sum of soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N build-up in excess to the unamended soil  
214 was considered as the amount of N mineralised per sampling time.

## 215 2.5 Data and statistical analysis

216 The cumulative amount of mineralised C, evolved as CO<sub>2</sub> (cumulative % C mineralised) from  
217 each of the tested biosolids was calculated as the difference between the cumulative CO<sub>2</sub>-C  
218 evolved from the unamended (control) soil and the biosolid-treated soil. Cumulative  
219 amounts of mineralised C were expressed as a percentage of the amount of TOC applied  
220 (Equation 2) and is hereafter referred to as %C mineralised (%C<sub>min</sub>).

221

$$222 \quad \%C_{min} = \frac{(C_{min,treatment} - C_{min,control})}{TOC_{applied}} \times 100 \quad \text{Equation (2)}$$

223 A second order kinetic model was fitted to the %C<sub>min</sub> data for each of the tested biosolid  
224 using the Grapher Statistical software (Version 10). The second order kinetic model  
225 (Equation 3) suggests that the mineralisation or decomposition is proportional to the  
226 product of the substrate concentrations and of the microorganisms derived from the  
227 substrate (Sleutel et al. 2005) and is expressed by:

228

$$229 \quad C(t) = C_A - \frac{C_A}{1 + k_2 a (1-a) C_A t} \quad \text{Equation (3)}$$

230

231 Where  $C(t)$  is the cumulative amount of C mineralised at time  $t$ ,  $C_A$  is the amount of  
232 mineralisable C,  $a$  is the fraction of decomposed substrate that becomes part of microbial  
233 biomass, and  $k_2$  is the second order C-mineralisation rate constant. As a simplification,  $k_2$  and  
234  $a$  were determined together as a single variable  $k_2a(1-a)$ . The model parameters were then  
235 used to extrapolate the stable organic fraction left in the soil after a period of 365 days (*i.e.*  
236 the humification coefficient).

237  
238 Net N mineralisation (Equation 4) in the biosolid-amended treatments and unamended  
239 control was calculated by subtracting the mineral N ( $N_{min}$ ) content of the treatment at day 0  
240 from the  $N_{min}$  in the sample at all subsequent measurements and expressed as percentage of  
241 organic N ( $N_{org}$ ) and total N ( $N_{tot}$ ), as follows:

242  
243 
$$\text{Net mineralisation: } (t; \% \text{ organic } N) = \frac{N_{min}(t=x) - N_{min}(t=0)}{N_{org}} \quad \text{Equation (4)}$$

244 
$$(t; \% \text{ total } N) = \frac{N_{min}(t=x) - N_{min}(t=0)}{N_{tot}}$$

245  
246 Treatment effects were compared using one-way ANOVA and the Tukey's honestly  
247 significant difference (HSD) test. Pearson's correlation analysis was performed to test  
248 relationships between variables. All tests were carried out at a probability ( $P$ ) level of 0.05  
249 using SPSS 22.0 software for Windows.

## 250 **3. Results**

### 251 **3.1. Biosolid characteristics**

252 The agrochemical characteristics of the tested biosolids are summarized in Table 3. With the  
253 exception of P-POOR SF, the pH of the tested biosolids were alkaline (7.3-8.5) which is  
254 consistent with previously reported pH values of digestate and digestate products  
255 (Albuquerque et al. 2012; Pognani et al. 2009). The acidic pH of the P-POOR SF (5.7) was  
256 attributed to the P-stripping process that involved the addition of acid ( $H_2SO_4$ ). Pyrolysis of  
257 the P-POOR SF to produce BCHR greatly altered its agrochemical properties, with a  
258 noticeably, higher pH, total carbon (TC) and total nitrogen (TN) contents (Table 3).  
259  
260 TC, TN and inorganic N content in the solid fractions were variable. DRY-SF had the highest  
261 TC ( $275\text{ g kg}^{-1}$ ) and TN ( $30\text{ g kg}^{-1}$ ) while DEC-SF had the lowest TC ( $102\text{ g kg}^{-1}$ ) and SCP-SF the  
262 lowest TN ( $5.0\text{ g kg}^{-1}$ ). This variability is reflected in the  $C/N_{tot}$  ratios which ranged between  
263 9.2 and 36 (Table 3). The tested biosolids were generally characterized by very low amounts  
264 of  $NO_3^-$ -N. The  $NH_4^+$ -N/TN ratio, which provides an indication of the amount of readily plant-  
265 available N, was low in all the tested biosolids. It was lowest in DRY-SF and BCHR due to  
266 drying and pyrolysis, respectively, which are processes that result in a significant loss of  $NH_4$ .  
267  
268 Moderate amounts of TN was present in the SFs, mostly in the form of organic N, as could be  
269 expected since the solid-liquid separation of digestate concentrates the major part of the  
270 mineral N in the LF. In general, their OM and N contents did not show much variability (74–  
271 89% OM,  $5.0$ – $5.7\text{ g kg}^{-1}$  TN). However, high variability was observed in the total P content  
272 with DEC-SF having 1.7 and 5 times more P than SCP-SF and P-POOR SF, respectively. DRY-SF

273 was characterized by a relatively low OM content (59%) and higher concentration of  
274 nutrients, including TN ( $30 \text{ g kg}^{-1}$ ) and TP ( $14 \text{ g kg}^{-1}$ ), compared to the other SFs.

275

276 The WSC and HWEC, which have been reported to constitute the highly labile pool of C in  
277 organic materials (Ghani et al. 2003; Weigel et al. 2011), represented  $< 2\%$  and  $\leq 3\%$ ,  
278 respectively, of the total C in all the tested biosolids. This suggests that majority of the C in  
279 were present as the stable carbon fraction.

280

281

282 **3.2. C mineralisation**

283 The  $C_{min}$  of the tested biosolids was calculated according to Equation 2. The results were  
284 expressed in terms of the percentage of C added and mineralisation curves were plotted  
285 (Fig. 1). At the end of the incubation period, the highest  $C_{min}$  was observed in SCP-SF (23%) at  
286 5% significance level. The  $C_{min}$  of DEC-SF, DRY-SF and P-POOR SF did not significantly  
287 ( $P<0.05$ ) differ from each other but were significantly higher than in COMP (6%) and biochar  
288 (4%). In general, the curves indicate that the mineralisation of the SFs followed similar  
289 patterns that clearly differed from the compost and biochar treatments.

290  
291 The % $C_{min}$  curves for the different biosolids were fitted to a second-order kinetic model  
292 (Equation 3) with high coefficients of determination (Table 4). The amount of mineralisable C  
293 ( $C_A$ ) followed the same pattern of  $C_{min}$ : SCP-SF>DEC-SF>P-POOR SF>DRY-SF>BCHR>COMP.  
294 Humification coefficients (HC), which is defined as the percentage of OC that remains after  
295 one year, was calculated for each biosolid using the second order kinetic model. Among the  
296 SFs, SCP-SF had the lowest HC (51%) which indicates that it contained the least stable forms  
297 of OC, while other SFs contained the more stable forms of OC with HCs between 70 and 75  
298 (Table 4). The variation in HC may be explained by differences in  $C_A$  in the tested biosolid  
299 which showed a positive linear relationship between HC and  $C_A$  ( $r=0.99$ ,  $P<0.05$ ). The HC of  
300 compost in this study (93%) was in agreement with values (95 and 93%) reported by De Neve  
301 et al. (2003) and Postma & Ros (2016), respectively. Table 6 shows that HC was positively  
302 correlated with  $N_{org}/N_{tot}$  ratio ( $r=0.82$ ,  $P<0.05$ ) and negatively correlated with  $N_{min}/N_{tot}$  ratio  
303 ( $r=-0.82$ ,  $P<0.05$ ).

304

305

306 **3.3. Estimating carbon retention potential**

307 The humification coefficients (HC) can be expressed in kg tonne<sup>-1</sup> of DM to derive the  
308 effective organic matter (EOM), which is defined as the OM that is left in the soil after 365  
309 days (Veeken et al. 2017). Among the solid digestates tested, the highest EOM calculated  
310 was in DRY-SF at 369 kg tonne<sup>-1</sup> (Table 5). This means that after an initial application of 1  
311 tonne of biosolid, 369 kg of OM will remain in the soil after one year.

312

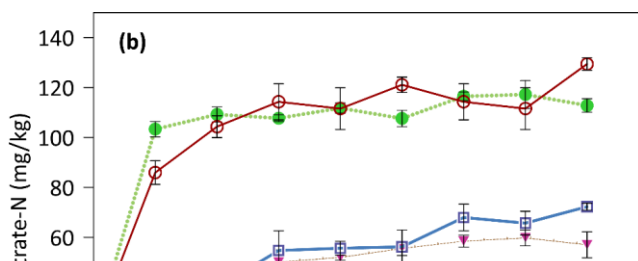
313 The OM/P ratio which gives an indication of the quantity of biosolid that can be applied  
314 within P limits was also determined (Table 5). DRY-SF had an OM/P ratio of 36 kg kg<sup>-1</sup>, of  
315 which 27 kg kg<sup>-1</sup> was EOM. For the P-POOR SF instead, the EOM/P ratio was 204 kg kg<sup>-1</sup>  
316 meaning that 7.5 times more EOM per kg of added P can be applied to soil as compared to  
317 DRY-SF.

318 **3.4. N-mineralisation**

319 At the start of the incubation, the mineral N in all treatments was present mainly as NH<sub>4</sub><sup>+</sup>-N  
320 (Fig. 2a). However, by the 14<sup>th</sup> day, majority of the NH<sub>4</sub><sup>+</sup>-N was already nitrified (Fig. 2b).  
321 NO<sub>3</sub><sup>-</sup>-N increased steadily throughout the duration of the incubation and was highest in DRY-  
322 SF and DEC-SF with 129 and 113 mg kg<sup>-1</sup>, respectively after day 112. The pattern of total  
323 mineral N evolution and that of NO<sub>3</sub><sup>-</sup>-N were similar since increase in NH<sub>4</sub><sup>+</sup>-N concentration  
324 were negligible.

325

326 N mineralisation was calculated as a percentage of the N<sub>org</sub> present in the tested biosolid  
327 (Fig. 3). N immobilisation occurred in BCHR, P-POOR SF and SCP-SF while DEC-SF, DRY-SF and  
328 COMP yielded modest near-linear N mineralisation patterns during the incubation, with the



329 highest  $N_{org}$  mineralisation of 28% (in DEC-SF). N is more likely to be net mineralised from  
330 substrates with low C/N ratio while substrates with C/N ratio > 20 tend to cause net N  
331 immobilisation in soil (Mendham et al. 2004; Muhammad et al. 2011; Nicolardot et al. 2001;  
332 Wagner & Wolf, 1999). This was reconfirmed in this study, where % $N_{org}$  mineralisation and  
333  $C/N_{tot}$  ratio were strongly negatively correlated ( $r=-0.88$ ,  $P<0.05$ ) (Table 6). The high  
334 immobilisation of N from SCP-SF was likely due to its high  $C/N_{org}$  ratio (76) in combination  
335 with its high  $NH_4^+$ -N that was readily available for fast initial microbial immobilisation  
336 (Calderón et al. 2005). P-POOR SF incorporation also induced temporary net N  
337 immobilisation in the first 56 days of incubation, and this may also be attributed to the high  
338  $C/N_{org}$  ratio (32) of this biosolid. The mineralisation patterns of SCP-SF and P-POOR SF,  
339 however, do suggest a re-mineralisation of the immobilised N.

340



## 4. Discussion

### 4.1. Agrochemical characteristics of tested biosolids

The high alkalinity of the solid fractions (with the exception of P-POOR SF) may influence soil pH and nutrient bioavailability when added to soil. For example, nitrification in soil is highest at pH between 7.5–8.0 while ammonification is optimal between pH 6.0–8.0 (Wood 1988).

Although DEC-SF, SCP-SF and P-POOR SF were obtained from digestates derived from the same digested feedstock of GZV (Table 1), they showed a large variation in their total P content. This suggests that the type of post-digestion treatment (i.e. solid-liquid separation technique, P-stripping) may have a stronger influence on P composition of the SFs than on other nutrient parameters. P-POOR SF not only had the lowest P content but also the lowest K concentration. This was because leaching of P also removes K due to their similar mobility kinetics (Nishanth & Biswas, 2008).

The loss of moisture from drying resulted in increased concentration of nutrients in DRY-SF. Although the mineral N content was low ( $2.0 \text{ g kg}^{-1}$ ), the high organic N level may lead to a steady supply of N to crops provided there would be continuous mineralisation. These characteristics suggest that DRY-SF is more suited for use as a solid organic fertiliser rather than as organic soil improver. Furthermore, its high DM content (84%) may ease the storage and transportation of the DRY-SF.

There was no significant correlation between either WSC or HWEC and %Cmin ( $r=0.11$  and  $r=0.25$ , respectively;  $P<0.05$ ). For instance, the WSC and HWEC were highest in compost even though %Cmin in compost was very low. This may be due to the inherent heterogeneity of the feedstocks as well as differences in processing technologies applied. These results suggest that

WSC and HWEC may not be good indicators of labile OM, at least, for the biosolids investigated in this study.

#### 4.2. C and N mineralisation

The incorporation of the different biosolids significantly modified the C and N dynamics of the soil. Some studies (Flavel & Murphy, 2006; Wang et al. 2004) have shown that dissimilarities in decomposition rates (i.e. %C<sub>min</sub>) may be due to differences in the nature of organic carbon compounds present in the substrates. That is, mineralisation patterns in soils are mainly influenced by the initial composition of the organic material (Heal et al. 1997; Aerts, 1997; Teklay et al. 2007). For example, the C/N<sub>tot</sub> ratio may influence substrate decomposition rate such that substrates with a high C/N<sub>tot</sub> ratio above 25 are poorly decomposable (Nicolardot et al. 2001). In this study, however, SCP-SF had the highest C/N<sub>tot</sub> ratio (36) and yet showed fast degradability. This was likely caused by both higher C and N availability, which was reflected by the lower WSC/WHEC to N<sub>min</sub> ratio (Table 3). Such an occurrence will favour the preferential utilisation of labile biosolid-derived C by soil microorganisms and cause high C mineralisation. The corollary effect of high C mineralisation in SCP-SF treatment was the rapid N immobilisation observed in the first 28 days of the N incubation experiment (Fig. 3).

To an extent, P-POOR SF showed similar behaviour as SCP-SF but with moderate C mineralisation and less rapid N immobilisation. N mineralisation in P-POOR-SF was inhibited at the beginning but caught up to the unamended control at around Day 70, indicating N was likely immobilised by soil microorganisms and then re-released due to microbial turnover. In addition, the low pH of P-POOR-SF may have slowed down the turnover of microbial biomass and extended the microbial immobilisation as (a) low pH slows down bacterial cell division

(Bååth et al. 1998) and (b) low pH favours the prevalence of soil fungi with longer life-spans (Gyllenberg and Eklund 1974). It is important to note that the mineralisation patterns of SCP-SF and P-POOR SF do suggest a re-mineralisation of the immobilised N. Therefore, both biosolids may not be suitable for supplying N to crops grown during the season of application but can serve as an N source in the subsequent growing season.

The amendment of DEC-SF induced similar amount of C mineralised (Fig. 1) but greater N mineralisation when compared to DRY-SF (Fig. 1 and Fig. 2c). This observation may be explained by the "microbial N mining" theory (Craine et al. 2007; Moorhead & Sinsabaugh, 2006) which postulates that N deficiency can enhance microbial decomposition of labile substrates in order to obtain N from recalcitrant OM. That is, the relatively higher C/N<sub>tot</sub> ratio in DEC-SF might have driven the soil microorganisms to decompose more N-rich OM for their N requirement when compared to DRY-SF where N was not limited. Considering their continued net mineralisation of organic N (Fig. 3), DEC-SF and DRY-SF appears to hold the highest N fertiliser value. Given the rather slow pace of N release, their intended use should probably be to fertilise the soil rather than the crop, as practiced in organic farming, in order to improve soil quality and fertility (Webb et al. 2010).

Despite its low C/N ratio, the C mineralisation of compost progressed slowly (6% after 81 days) indicating it to be more stable in soil than the digestate SFs here tested. That is because the undecomposed C in compost exists in complex forms that is inherently resistant to decomposition (Sana and Soliva, 1987; Robertson & Groffman, 2006). Similarly, the C in BCHR is recalcitrant, but it is not inert and can be slowly mineralised (Singh & Cowie, 2014; Zimmerman, 2010). BCHR had a high C/N<sub>org</sub> ratio and yet showed very little N immobilisation. This was

attributed to its high humification coefficient (91%) meaning that is no need for mineral N to sustain microbial growth.

#### 4.3. Implications for using solid digestates as an organic soil improver

The relative proportions of TOC mineralised from the tested biosolids reflect their different biostabilities in soil. Estimates of humification coefficients (HC) and effective organic matter (EOM) are good indicators of long term availability of OM in soil after initial application of biosolids. The high EOM of DRY-SF (369 kg tonne<sup>-1</sup>) represents a significant potential source of soil organic matter. However, its use as an organic soil improver may be limited because restrictions on P input in some EU regions (e.g. Belgium, the Netherlands and Denmark) constrain the application of P-rich materials (Amery & Schoumans, 2014). The P-POOR SF, with its high OM/P proves to be far more advantageous in such regions. In fact, with the exception of BCHR, P-POOR SF can be applied to soil at least 2.3 times more than any of the other biosolids tested (Table 5). Furthermore, its low N-content permits the application of large amounts without violating the European Nitrates Directive (91/676/EEG; European Commission, 1991). The slow N mineralisation of P-POOR SF also makes it ideal for use as an exclusively organic soil improver in regions with restrictions on P and N additions to soils.

P-stripping, as an additional treatment step, may thus be a promising technique for valorisation of SFs of digestate. Pyrolysis of the SFs (of P-POOR SF in this study) increased OM stability but still appears unwarranted to promote pyrolysis as a sustainable post-treatment, especially considering the high production costs involved (Campbell et al. 2018). Composting of SFs has been proposed by some studies as a viable alternative to increase its biological stability (de la Fuente et al. 2013; Torres-Climent et al. 2015). However, a study carried out by Tambone et al.

(2015) showed that composting did not remarkably increase the stability of SFs and Möller (2015) argued that digestate composting may reduce the fertiliser's nutrient value and may cause greenhouse gas emissions. Given the already high stability of the tested SFs, except for SCP-SF, it again seems likely that composting would not further valorise the SF as a soil improver and the extra economic costs involved may be unfavourable. In any case, the here observed differences in the composition and mineralisation rates of SFs from digestate processing highlight the need for detailed analyses before using them as soil amendments.

## 5. Conclusions

The C and N mineralisation potential of P-POOR SF was compared with conventional SFs of digestate to study the stability of the present OM. The results showed that the humification coefficient does not correlate with the C/N ratio, HWEC or WSC. It therefore seems that the nature of the organic matter was the main factor controlling C mineralisation in the treatments. Temporary N immobilisation was observed in P-POOR SF and SF after screw press (SCP-SF) and this was attributed to their high C/N<sub>org</sub> ratios. In biochar (BCHR), no N immobilisation was observed despite its high C/N<sub>org</sub> ratio which is due to the high humification coefficient (91%) of the biosolid meaning that is no need for mineral N to sustain microbial growth. The other tested biosolids, SF after decanter (DEC-SF), SF after decanter and drying (DRY-SF) and compost, showed a near linear positive N mineralisation. There was no correlation between the HC and %N mineralisation. When considering the amount of effective organic matter (EOM) that can be applied to soils within the phosphate limits, i.e. the EOM/P ratio, P-POOR SF had the highest potential to be used as an organic soil improver among the SFs. As such, reducing the P concentration in SFs facilitates their application in many European soils. These results highlight the agricultural value of SFs of digestate for soil organic matter, and their potential limitations in P-rich soils.

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**Table 1.** Description of the studied organic materials

Product	Name	Feedstock	Post-digestion treatment at AD plant	
			Solid-liquid separation	Additional treatment
DEC-SF	Solid fraction	74% pig manure and 26% co-substrates	Decanter centrifuge	-
SCP-SF	Solid fraction	74% pig manure and 26% co-substrates	Screw press	-
P-POOR SF	Solid fraction	74% pig manure and 26% co-substrates	Decanter centrifuge	P stripping (Re-P-eat)
DRY-SF	Solid fraction	Organic biological waste	Decanter centrifuge	Drying at ~60 °C
COMP	Commercial compost	Source-separated household and garden waste		
BCHR	Biochar	P-POOR SF	-	Pyrolysis at 400 °C

DEC-SF: solid fraction after decanter, SCP-SF: solid fraction after screw press, P-POOR SF: solid fraction decanter and P stripping,

DRY-SF: solid fraction after decanter and drying, COMP: compost, BCHR: biochar produced from P-POOR SF.

**Table 2.** Dosages in kg ha<sup>-1</sup> for total organic carbon (TOC), total N (N<sub>tot</sub>), organic N (N<sub>org</sub>) and mineral N (N<sub>min</sub>) of the tested products as implied in the C incubation and N mineralisation experiment

<b>Product</b>	<b>TOC</b>	<b>N<sub>tot</sub></b>	<b>N<sub>org</sub></b>	<b>N<sub>min</sub></b>
DEC-SF	9000	500	306	194
SCP-SF	9000	252	118	134
P-POOR SF	9000	309	283	26
DRY-SF	9000	983	763	220
COMP	9000	788	776	12
BCHR	9000	316	316	0.42

DEC-SF: solid fraction after decanter, SCP-SF: solid fraction after screw press, P-POOR SF: solid fraction decanter and P stripping, DRY-SF: solid fraction after decanter and drying, COMP: compost, BCHR: biochar produced from P-POOR SF.

**Table 3.** Agrochemical characteristics of the tested organic materials (n = 3 ± standard deviations)

Parameter	DEC-SF	SCP-SF	P-POOR SF	DRY-SF	COMP	BCHR
pH	8.4 ± 0.1	8.5 ± 0.0	5.7 ± 0.0	7.8 ± 0.1	8.5 ± 0.0	7.3 ± 0.0
EC (mS cm <sup>-1</sup> )	2.4 ± 0.2	1.1 ± 0.0	1.4 ± 0.0	6.1 ± 0.0	0.7 ± 0.0	2.8 ± 0.0
DM (%)	25 ± 0.6	37 ± 0.4	34 ± 0.1	84 ± 0.7	54 ± 0.4	98 ± 0.2
OM (% of DM)	74 ± 2.9	89 ± 0.2	89 ± 0.1	59 ± 1.5	30 ± 0.8	75 ± 0.0
TOC (g kg <sup>-1</sup> )	102 ± 4.0	177 ± 2.2	161 ± 0.9	275 ± 4.3	70 ± 2.5	643 ± 0.2
WSC (% of TOC)	1.7 ± 0.0	0.9 ± 0.1	0.3 ± 0.0	0.8 ± 0.1	1.7 ± 0.1	0.04 ± 0.0
HWEC (% of TOC)	3.0 ± 0.3	1.0 ± 0.1	0.6 ± 0.0	1.2 ± 0.0	4.3 ± 0.2	0.05 ± 0.0
N <sub>tot</sub> (g kg <sup>-1</sup> )	5.7 ± 0.1	5.0 ± 0.1	5.5 ± 0.2	30 ± 0.1	6.1 ± 0.2	23 ± 0.1
N <sub>min</sub> (g kg <sup>-1</sup> )						
NH <sub>4</sub> <sup>+</sup> -N	2.2 ± 0.1	2.7 ± 0.0	0.46 ± 0.0	2.0 ± 0.0	0.22 ± 0.0	0.03 ± 0.0
NO <sub>3</sub> <sup>-</sup> -N	0.002 ± 0.0	0.003 ± 0.0	0.001 ± 0.0	0.1 ± 0.0	0.01 ± 0.0	0.001 ± 0.0
C/N <sub>tot</sub>	18	36	29	9.2	11	28
C/N <sub>org</sub>	29	76	32	9.8	12	28
N <sub>min</sub> /N <sub>tot</sub> (%)	39	54	8.0	7.0	2.0	0.0
Elements (g kg <sup>-1</sup> )						
P	5.6 ± 0.2	3.3 ± 0.1	1.1 ± 0.0	14 ± 1.1	1.7 ± 0.2	3.1 ± 0.2
Ca	5.8 ± 0.8	4.1 ± 0.4	3.4 ± 0.6	18 ± 1.1	12 ± 0.8	12 ± 1.0
Mg	3.5 ± 0.5	1.7 ± 0.3	0.7 ± 0.2	3.5 ± 0.2	1.7 ± 0.1	9.1 ± 1.2
K	3.2 ± 0.3	2.3 ± 0.2	0.8 ± 0.0	9.2 ± 0.7	2.4 ± 0.1	11 ± 0.2
Al	0.93 ± 0.1	0.34 ± 0.1	0.35 ± 0.0	2.8 ± 0.2	3.2 ± 0.4	1.6 ± 0.0

EC: electrical conductivity, DM: dry matter, OM: organic matter, TOC: total organic carbon, WSC: water soluble carbon, HWEC:

hot water extractable carbon, N<sub>tot</sub>: total nitrogen, N<sub>min</sub>: mineral nitrogen, N<sub>org</sub>: organic nitrogen, DEC-SF: solid fraction after



decanter, SCP-SF: solid fraction after screw press, P-POOR SF: solid fraction decanter and P stripping, DRY-SF: solid fraction after decanter and drying, COMP: compost, BCHR: biochar produced from P-POOR SF.

**Table 4.** Kinetic C-mineralisation parameter values and fit, and humification coefficients (HC) for the tested products at 10 °C. Parameter data obtained with a second order kinetic model (Equation 3)

Parameters	DEC-SF	SCP-SF	P-POOR SF	DRY-SF	COMP	BCHR
$C_A$	36.27	67.44	34.29	29.05	7.065	9.793
$K_2a(1-a)$	$3.57 \times 10^{-4}$	$9.62 \times 10^{-5}$	$3.16 \times 10^{-4}$	$5.33 \times 10^{-4}$	$6.30 \times 10^{-3}$	$2.38 \times 10^{-3}$
$R^2$	0.997	0.989	0.999	0.998	0.955	0.982
HC (%)	70	51	73	75	93	91

DEC-SF: solid fraction after decanter, SCP-SF: solid fraction after screw press, P-POOR SF: solid fraction decanter and P stripping, DRY-SF: solid fraction after decanter and drying, COMP: compost, BCHR: biochar produced from P-POOR SF.

**Table 5.** Composition of organic fertilising products in kg per tonne product

Parameter	DEC-SF	SCP-SF	P-POOR SF	DRY-SF	COMP	BCHR
DM (kg tonne <sup>-1</sup> )	251	365	335	840	535	982
OM (kg tonne <sup>-1</sup> )	185	324	298	492	160	737
HC (%)	70	51	73	75	93	91
EOM (kg tonne <sup>-1</sup> )	130	165	217	369	148	671
P (kg tonne <sup>-1</sup> )	5.6	3.3	1.1	13.7	1.7	3.0
OM/P (kg kg <sup>-1</sup> )	33	97	279	36	93	244
EOM/P (kg kg <sup>-1</sup> )	23	49	204	27	86	222
N mineralisation						
as % of N <sub>tot</sub>	22	-7	-6	25	15	5
as % of N <sub>org</sub>	81	-13	-7	27	15	7

**Commentato [CEE1]:** Kg/t was used since we are showing application rates as used in the field. The format is in agreement with that used in Inge's report "Phosphorus recovery from co-digested pig slurry"  
<https://edepot.wur.nl/476731>

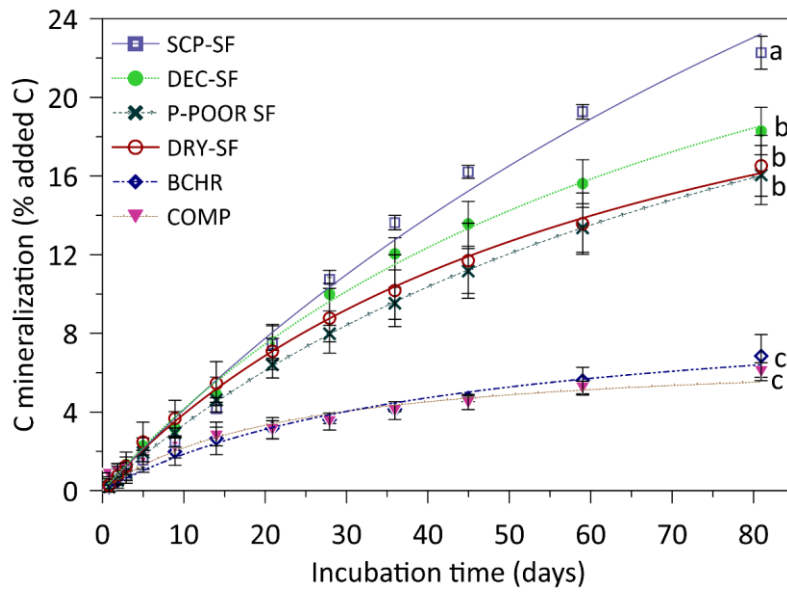
DM: dry matter, OM: organic matter, HC: humification coefficient, EOM: effective organic matter, N<sub>tot</sub>: total nitrogen, N<sub>org</sub>: organic nitrogen, DEC-SF: solid fraction after decanter, SCP-SF: solid fraction after screw press, P-POOR SF: solid fraction decanter and P stripping, DRY-SF: solid fraction after decanter and drying, COMP: compost, BCHR: biochar produced from P-POOR SF

1 **Table 6.** Pearson correlation coefficients among parameters related to product  
 2 characteristics and C and N mineralisation dynamics

	TOC	N <sub>tot</sub>	N <sub>min</sub>	C/N <sub>tot</sub>	C/N <sub>org</sub>	N <sub>min</sub> /N <sub>tot</sub>	N <sub>org</sub> /N <sub>tot</sub>	WSC	HWEC	C <sub>A</sub>	HC	%C <sub>min</sub>	%N <sub>min</sub>
TOC	1												
N <sub>tot</sub>	-0.372	1											
N <sub>min</sub>	-0.353	-0.062	1										
C/N <sub>tot</sub>	0.273	-0.372	0.005	1									
C/N <sub>org</sub>	0.310	0.441	-0.515	0.310	1								
N <sub>min</sub> /N <sub>tot</sub>	-0.390	-0.482	0.855 <sup>*</sup>	0.366	-0.404	1							
N <sub>org</sub> /N <sub>tot</sub>	0.390	0.482	-1.000 <sup>**</sup>	-0.366	0.404	-1.000 <sup>**</sup>	1						
WSC	-0.547	-0.095	0.821 <sup>*</sup>	-0.434	-0.623	0.617	-0.617	1					
HWEC	-0.496	0.234	0.285	-0.849 <sup>*</sup>	-0.606	-0.071	0.071	0.500	1				
C <sub>A</sub>	-0.340	-0.358	0.762	0.548	-0.475	0.825 <sup>*</sup>	-0.825 <sup>*</sup>	0.376	-0.146	1			
HC	0.355	0.327	-0.794	-0.497	0.502	-0.824 <sup>*</sup>	0.824 <sup>*</sup>	-0.436	0.112	-0.996 <sup>**</sup>	1		
%C <sub>min</sub>	-0.397	-0.234	0.825 <sup>*</sup>	0.334	-0.572	0.759	-0.759	0.110	-0.252	0.941 <sup>**</sup>	-0.966 <sup>**</sup>	1	
%N <sub>min</sub>	-0.065	0.400	0.331	-0.880 <sup>**</sup>	-0.104	0.015	-0.015	0.700	0.572	-0.340	0.276	-0.106	1

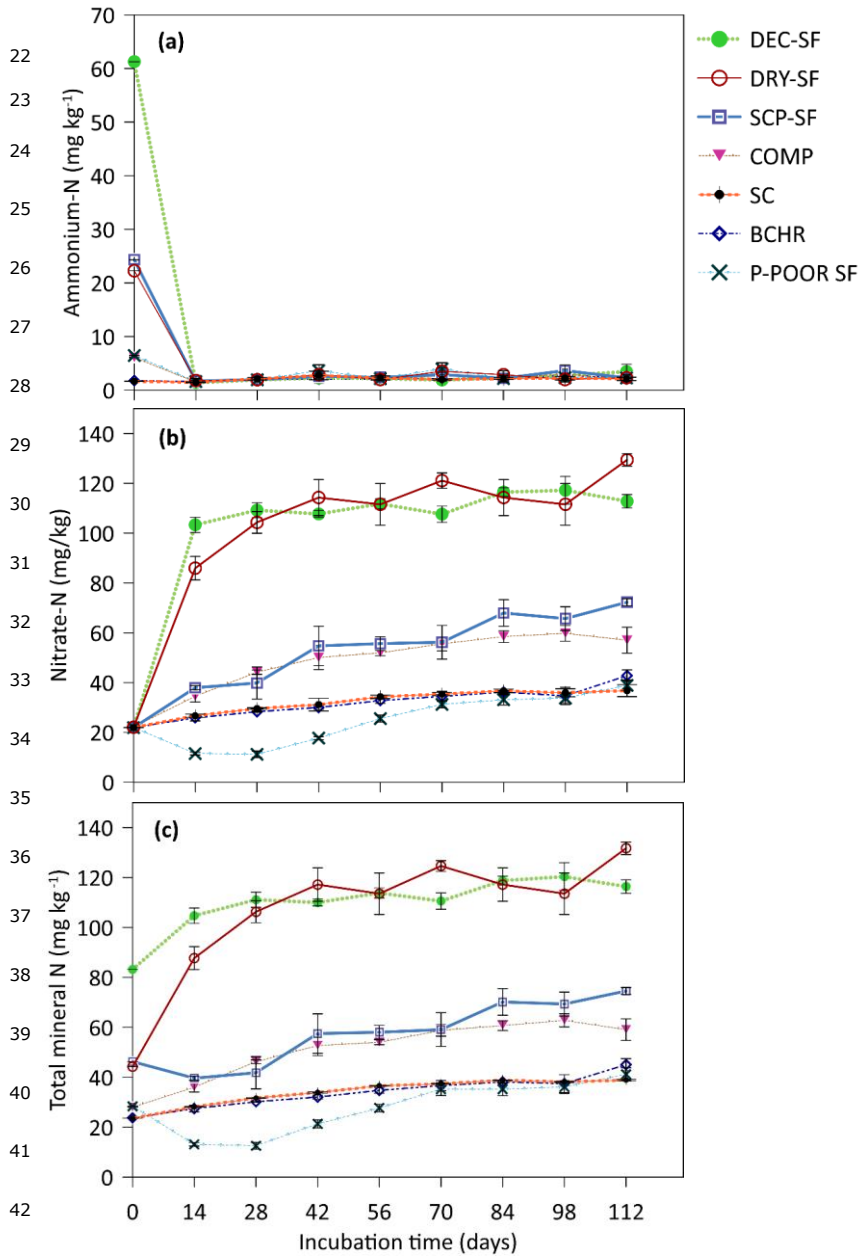
3 TOC (g kg<sup>-1</sup>): total organic carbon, N<sub>tot</sub> (g kg<sup>-1</sup>): total nitrogen, N<sub>min</sub> (g kg<sup>-1</sup>): mineral nitrogen, N<sub>org</sub> (g kg<sup>-1</sup>): organic  
 4 nitrogen, WSC (% of TOC): water soluble carbon, HWEC (% of TOC): hot water extractable carbon, N<sub>org</sub>: organic nitrogen,  
 5 C<sub>A</sub>: amount of mineralisable C, HC: humification coefficient (%), %C<sub>min</sub>: C mineralized as % of added C after 81 days of  
 6 incubation, %N<sub>min</sub>: N mineralised as % of added organic N after 112 days of incubation

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 12 **Fig. 1.** Cumulative amount of C mineralised after addition of 9000 kg OC ha<sup>-1</sup> to soil during  
 13 the 81-day incubation period at 10 °C (mean value ± standard deviation, n=3). Lines  
 14 represent the curve-fitting result; symbols are experimental data. Different lower case  
 15 letters indicate significant differences between C mineralisation means at day 81. DEC-SF,  
 16 solid fraction after decanter; SCP-SF, solid fraction after screw press; P-POOR SF, solid  
 17 fraction decanter and P stripping; DRY-SF, solid fraction after decanter and drying; COMP,  
 18 compost; BCHR, biochar produced from P-POOR SF

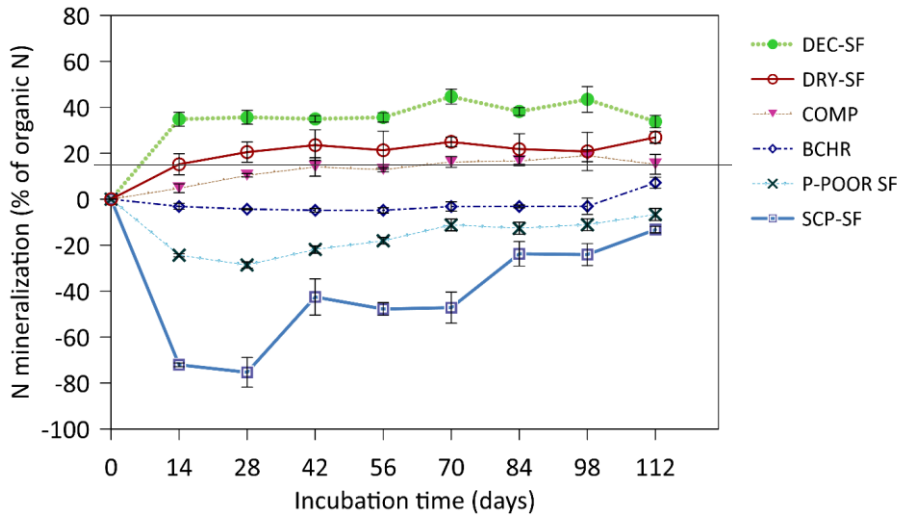
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44 **Fig. 2.** Evolution of (a) ammonium-N (b) nitrate-N and (c) total mineral N expressed in mg kg<sup>-1</sup>  
 45 in the unamended soil and soil amended with the tested products during 112-day incubations

46 at 21.5 °C (mean value  $\pm$  standard deviation, n=4; where absent, error bars fall within  
47 symbols). SC, soil control; DEC-SF, solid fraction after decanter; SCP-SF, solid fraction after  
48 screw press; P-POOR SF, solid fraction decanter and P stripping; DRY-SF, solid fraction after  
49 decanter and drying; COMP, compost; BCHR, biochar produced from P-POOR SF  
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53 **Fig. 3.** N mineralisation (% of organic N supplied (equation 4) from the tested products  
54 during 112 days of incubation) (mean value  $\pm$  standard deviation, n=4; where absent, error  
55 bars fall within symbols). DEC-SF, solid fraction after decanter; SCP-SF, solid fraction after  
56 screw press; P-POOR SF, solid fraction decanter and P stripping; DRY-SF, solid fraction after  
57 decanter and drying; COMP, compost; BCHR, biochar produced from P-POOR SF

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