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# High nitrogen availability but limited potential carbon storage in anaerobic digestates from cover crops

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## Abstract

Cover crops are increasingly used for biogas production, a renewable energy source, without competing for food production. The behavior of the resulting digestates after soil application is poorly understood, which prevents their efficient recycling in agriculture and the environmental assessment of their application. The objective of this study was to quantify the nitrogen availability and potential carbon storage of cover crop-issued digestates after soil application. A total of 10 raw digestates, 2 liquid phases, and 3 solid phases after phase separation were sampled. Main cover crops used in the sampled biogas plants were winter barley, rye and maize. Classical physicochemical analyses and laboratory incubations to study their C and N mineralization were conducted. Despite a moderate C mineralization of raw and liquid digestates after 91 days, their initial limited carbon content induced, in the end, a low contribution to soil organic carbon (13 and 11 kg remaining C Mg<sup>-1</sup> FM, respectively), similar to a pig slurry and much lower than a bovine manure. With a higher initial carbon content and

26 lower C mineralization, the contribution of solid digestates to carbon storage could be higher if  
27 applied at a sufficient rate. Organic N mineralization of raw and liquid digestates was moderate,  
28 but their N availability was high (3 and 4 kg available N Mg<sup>-1</sup> FM, respectively) thanks to their  
29 mineral nitrogen contents, similar again to a pig slurry. In contrast, that of solid digestate was  
30 almost null with a very low mineral N content and no organic N mineralization. Finally, all the  
31 digestates also brought significant amounts of P and K.

32 **Keywords:** digestate, cover crop, mineralization, carbon, nitrogen.

### 33 **Acknowledgment**

34 We thank the LDAR laboratory technicians in charge of the digestate analyses and the farmers  
35 for agreeing with digestate sampling.

## 36 **1 Introduction**

37 To increase renewable energy production the production of cover crops for anaerobic digestion  
38 is promoted in various countries (Marsac et al., 2019; Riau et al., 2021; Szerencsits et al.,  
39 2016). For example, the French agency for ecological transition published a prospective study  
40 about the complete substitution of fossil gas by renewable gas, which included the  
41 generalization of the use of cover crops for biogas production (with 50 10<sup>6</sup> Mg of dry matter  
42 harvested per year) (Ademe, 2018). In comparison to dedicated crops, the use of cover crops  
43 has the advantage to not compete with food production because harvested cover crops for  
44 biogas replace bare soil or non-harvested cover crops. Anaerobic digestion also results in the  
45 production of digestates that are used as organic fertilizers. Contrary to digestates from  
46 livestock effluents or dedicated crops, which are rather well known (e.g., Nkoa (2014), Möller  
47 & Müller (2012)), information on the digestates of cover crops is still rather scarce. To our  
48 knowledge, except for the chemical composition of two digestates reported by Marsac et al.  
49 (2019), there are no published results on the digestates of cover crops from real anaerobic  
50 plants. This lack of information prevents an adequate assessment of their agronomic and  
51 economic values in relation to the potential savings on mineral fertilizer. Moreover, the potential

52 contributions of carbon digestate to soil organic matter (Cayueta et al., 2010) and of nitrogen  
53 digestate to the N supply to crops (Brockmann et al., 2018) are key factors to consider in the  
54 environmental assessment of organic waste recycling. These contributions are closely related  
55 to their C and N contents, but also to their C and N mineralization dynamics, which can be  
56 highly variable for different organic wastes (Lazicki et al., 2020; Levvasseur et al., 2021).  
57 Especially for digestates (excluding digestates of cover crops), the literature has reported both  
58 N immobilization (use of mineral N by microbial biomass during decomposition of organic  
59 matter) or net mineralization as well as low to high C mineralization (Cavalli et al., 2017; de la  
60 Fuente et al., 2013; Levvasseur et al., 2021). Specific results for digestates of cover crops  
61 are thus needed.

62 The objective of this study was to quantify the potential N availability and potential C storage  
63 related to the application of digestates made exclusively from cover crops and agro-industrial  
64 wastes, and with no animal manures as co-substrates. based on physico-chemical analyses  
65 of 15 digestates and laboratory incubations to study their C and N mineralization. The main  
66 hypothesis tested was whether cover crop-issued digestates had a high N availability and a  
67 limited C storage potential as other types of digestates.

## 68 **2 Materials and methods**

69 Digestates were sampled from 13 anaerobic digestion plants (wet mesophilic process) mainly  
70 located in Ile-de-France (Paris area, northern France). There were 10 raw digestates, 2 liquid,  
71 and 3 solid phases of digestate after phase separation (screw press). The 13 digesters differed  
72 in terms of feedstock (Online Resource, Table S1), with a proportion of cover crop (in % weight  
73 of fresh matter) ranging from 16% to 100% (mean proportion equal to 55%). Maize (*Zea mays*  
74 *L.*) was the main summer cover crop. It was typically sown immediately after the harvest of a  
75 winter crop (e.g., grain winter barley) in the end of June, ensiled in mid-October, and followed  
76 either by a winter cereal or a spring crop. Winter barley (*Hordeum vulgare L.*) and rye (*Secale*  
77 *cereal L.*) were the main winter cover crops. They were typically sown in the end of September  
78 or in the beginning of October, ensiled in the beginning of May, and followed by a spring crop

79 (grain maize usually). The other digested wastes were mainly sugar beet pulp, cereal wastes,  
80 and food wastes. The hydraulic retention time (digester and postdigester) was usually greater  
81 than 100 days.

82 The raw digestates were sampled in the postdigester, while liquid and solid digestates were  
83 sampled just after phase separation. Samples were immediately frozen at -20°C to avoid any  
84 digestate evolution before analysis. The references for the analytical methods are in Table S2  
85 (Online Resource). Dry matter, organic C, Kjeldahl N, N-NH<sub>4</sub>, P, and K contents were analyzed  
86 (3 replicates, except for digesters 12 and 13) and compared to well-known organic fertilizers:  
87 pig slurry and bovine solid manure (mean characteristics retrieved from Houot et al. (2014)).  
88 In addition, soil and digestate mixtures were incubated for 91 days under controlled conditions  
89 (28°C, pF 2.5 corresponding to a soil water content of 0.17 g g<sup>-1</sup>) to study the mineralization of  
90 organic C and organic N under standardized conditions (adapted from FD U44-163 and FD  
91 U42-163). Ninety-one days of incubation was estimated to represent one year in the field in  
92 the temperature conditions of central France (Levassasseur et al., 2021). The soil used for  
93 incubation was a decarbonated luvisol with a low carbon content (Online Resource, Table S3).  
94 Raw and liquid digestates were incubated fresh, whereas solid digestates were dried and  
95 ground before incubation. The application rate varied from 700 to 2000 mg C kg<sup>-1</sup> dry soil. For  
96 solid digestates poor in mineral N, mineral N (KNO<sub>3</sub>) was added in excess to reach at least a  
97 soil mineral N content of 35 mg N kg<sup>-1</sup>. This avoided any mineral N deficiency, which could  
98 have limited the digestate decomposition rate, and highlighted the potential N immobilization  
99 (Recous et al., 1995). The CO<sub>2</sub> evolved, and the soil mineral N was measured to determine  
100 the mineralized C and N. The net C and N mineralized from each digestate were computed by  
101 subtracting the mineralized C and N of an unamended soil (control). The proportions of the net  
102 mineralized C and N from the digestates were obtained by dividing the net C and N mineralized  
103 from each digestate by the total amount of added organic C and organic N by the digestate,  
104 respectively. Additional information about the incubation experiment is given in Table S4  
105 (Online Resource).

106 The contributions to the soil organic matter of the digestate and to the N supply were computed  
107 as follows (the nitric N content of the digestate is neglected):

$$108 \quad C_{remaining} = C_{org} (1 - C_{mine91}) \quad (1)$$

$$109 \quad N_{available} = N_{NH4} + N_{org} N_{mine91} \quad (2)$$

110 where  $C_{remaining}$  is the quantity of remaining C in the digestate (kg C Mg<sup>-1</sup> fresh matter (FM)),  
111  $C_{org}$  is the organic carbon content of the digestate (kg C Mg<sup>-1</sup> FM),  $C_{mine91}$  is the proportion of  
112 mineralized organic carbon after 91 days of incubation,  $N_{available}$  is the quantity of available N  
113 in the digestate (kg N Mg<sup>-1</sup> FM),  $N_{NH4}$  is the ammoniacal N content of the digestate (kg N Mg<sup>-1</sup>  
114 FM),  $N_{org}$  is the organic nitrogen content of the digestate (kg N Mg<sup>-1</sup> FM) and  $N_{mine91}$  is the  
115 proportion of mineralized organic nitrogen after 91 days of incubation.

116 The indicator of residual organic carbon ( $I_{ROC}$ ) proposed by Lashermes et al. (2009) was also  
117 determined from the biochemical fractions of the digestates (Van Soest and Wine, 1967) and  
118 the proportion of carbon in the digestate that was mineralized during a very short incubation (3  
119 days).  $I_{ROC}$  has been defined as a predictor of C remaining from exogenous organic matter  
120 (EOM) after long-term incubation of EOM with soil under controlled conditions, and was shown  
121 to represent the C remaining in soils after years under field conditions (Levavasseur et al.,  
122 2020). Its original calibration was made on a database without digestates. The objective was  
123 thus to determine whether  $I_{ROC}$  was also a good predictor of residual digestate C at the end of  
124 the incubation.

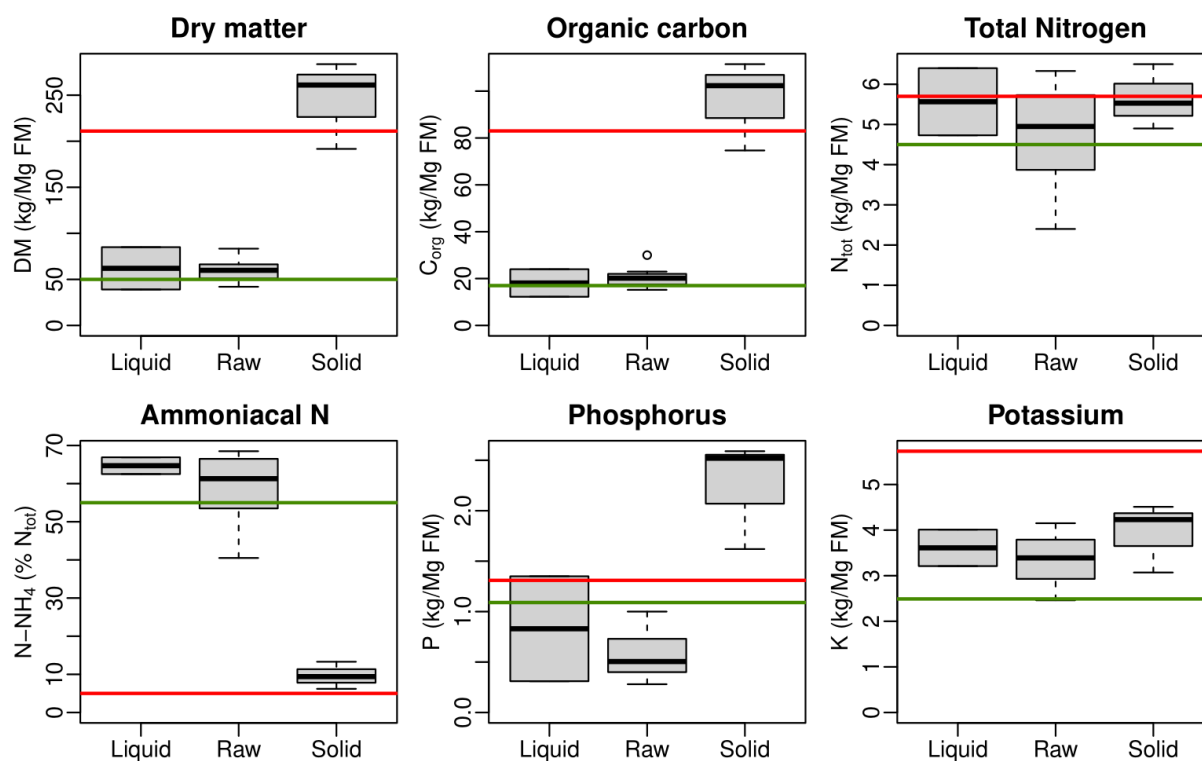
125 To analyze the correlations between digestate characteristics and digester feedstocks, a  
126 correlation matrix was analyzed (R Development Core Team, 2013). Only raw digestates were  
127 used for this analysis because of the limited number of liquid and solid digestates in our  
128 dataset.

## 129 **3 Results and discussion**

### 130 **3.1 Chemical composition of digestates**

131 The chemical composition of raw digestates, liquid digestates, and pig slurry were similar (**Fig.**  
132 **1**, Table S5), with low dry matter (mean values of 61, 62 and 50 kg Mg<sup>-1</sup> FM, respectively) and  
133 organic carbon content (21, 18 and 17 kg Mg<sup>-1</sup> FM, respectively), moderate nitrogen content  
134 (4.7, 5.6 and 5.5 kg Mg<sup>-1</sup> FM, respectively), and a similar to slightly higher proportion of nitrogen  
135 under mineral form for digestates (59, 65 and 55% total N, respectively). Solid digestates had  
136 similar characteristics to bovine manure. The variability of raw digestates was moderate, with  
137 coefficients of variation lower than 25% except for phosphorus. The contents of organic carbon  
138 and total phosphorus were positively correlated with dry matter content (Online Resource, Fig.  
139 S1).

140 The characteristics of our digestates were in the ranges reported in the review of Möller &  
141 Müller (2012) for various types of digestates. Our digestates were very similar to the digestate  
142 of silage maize reported by Wolf et al. (2014) for C and N contents. Despite different  
143 feedstocks, the present digestates were also very similar to the 20 digestates of various  
144 substrates studied by Risberg et al. (2017). For example, the raw digestates had a mean C  
145 content, total N content, and proportion of N under mineral form equal to 21 kg Mg<sup>-1</sup> FM,  
146 4.7 kg Mg<sup>-1</sup> FM, and 59%, respectively, in comparison to 17 kg Mg<sup>-1</sup> FM, 4.8 kg Mg<sup>-1</sup> FM, and  
147 71%, respectively, in Risberg et al. (2017) (recalculated).



148

149 **Fig. 1** Chemical composition of the studied digestates compared to a pig slurry (green line)  
 150 and a solid bovine manure (red line). The digestate samples included 10 raw digestates, 2  
 151 liquid digestates and 3 solid digestates

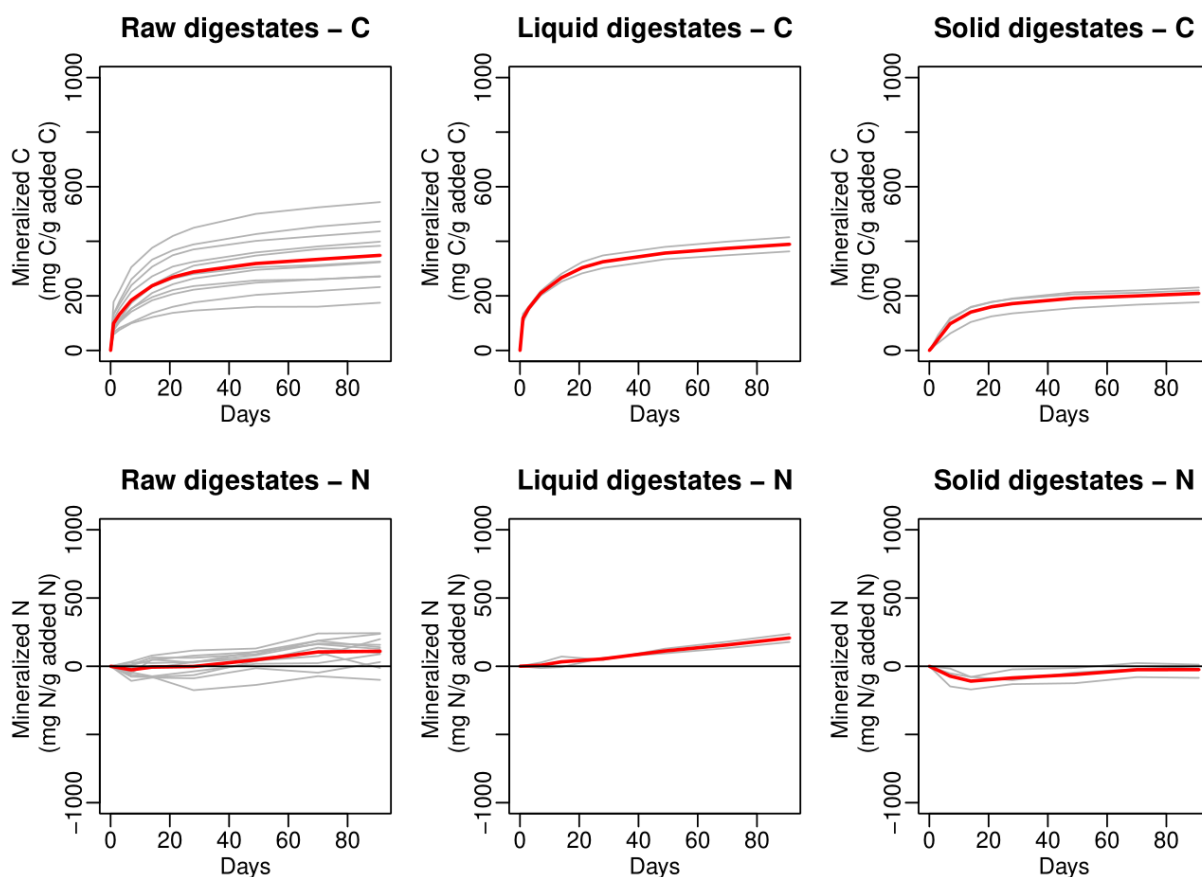
### 152 3.2 Organic carbon and nitrogen mineralization

153 During incubation, we observed a first phase of rapid C mineralization for two to three weeks,  
 154 followed by slower linear mineralization (**Fig. 2**), regardless of the digestate type. The mean  
 155 proportion of C mineralized for raw digestates after 91 days was 360 mg C g<sup>-1</sup> added C, and  
 156 the variability was moderate (standard deviation equal to 109 mg C g<sup>-1</sup> added C). The C  
 157 mineralization of liquid digestates was similar (389 mg C g<sup>-1</sup> added C on average), while that  
 158 of solid digestate was lower (209 mg C g<sup>-1</sup> added C on average). Focusing on raw digestates,  
 159 the C mineralization after 91 days was strongly and negatively correlated with  $I_{ROC}$  ( $R=0.92$ ,  $p$   
 160 value < 0.001, Fig. S1, Table S5 for  $I_{ROC}$  values), which confirmed the usefulness of this  
 161 indicator to estimate the potential remaining digestate C in soil, although  $I_{ROC}$  was calibrated  
 162 with a dataset of EOM without digestates (Lashermes et al., 2009). C mineralization was also  
 163 positively but weakly correlated with the total nitrogen ( $R=0.76$ ,  $p$  value < 0.05, Fig. S1, Online  
 164 Resource).



165 On average, the mineralization of organic N was low and linear for raw and liquid digestates  
166 **(Fig. 2)**. In contrast, solid digestates exhibited a first phase of immobilization for approximately  
167 two weeks, followed by slow remineralization. The mineralization of organic N after 91 days  
168 was moderate for raw digestates ( $101 \text{ mg N g}^{-1}$  added N) with a relatively high variability  
169 (standard deviation equal to  $107 \text{ mg N g}^{-1}$  added N); the mineralization after 91 days ranged  
170 from a net immobilization of  $-100 \text{ mg N g}^{-1}$  added N to a net mineralization of  $242 \text{ mg N g}^{-1}$   
171  $\text{added N}$ . The net mineralization of organic N from liquid digestates was higher ( $207 \text{ mg N g}^{-1}$   
172  $\text{added N}$ ), but that of solid digestate was lower and negative ( $-25 \text{ mg N g}^{-1}$  added N, still a  
173 net immobilization after 91 days despite remineralization). The N mineralization after 91 days  
174 for raw digestates was strongly and negatively correlated with total nitrogen content ( $R=-0.84$ ,  
175  $p \text{ value} < 0.01$ , Fig. S1, Online Resource). Contrary to previous studies with various organic  
176 wastes (Lazicki et al., 2020; Levavasseur et al., 2021), N mineralization was not correlated  
177 with the  $C:N_{org}$  ratio.

178 Raw and liquid digestates from cover crops exhibited a higher C mineralization and a lower N  
179 mineralization than the digestate of maize and rye silage (and 14% of chicken manure)  
180 reported by Reuland et al. (2022), with slightly different incubation conditions. Raw and liquid  
181 digestates from cover crops exhibited higher C and N mineralization than other types of  
182 digestates studied by Levavasseur et al. (2021), who found mean C and N mineralization of  
183  $274 \text{ mg C g}^{-1}$  added C and  $-18 \text{ mg N g}^{-1}$  added N, respectively. These latter values were  
184 similar for the solid digestates studied here. As already shown for other types of digestates  
185 (Cavalli et al., 2017; de la Fuente et al., 2013), phase separation leads to a solid phase of  
186 digestates with net N immobilization and lower C mineralization than raw or liquid digestates.



187

188 **Fig. 2** Observed mineralized organic carbon and nitrogen for the raw (n=10), liquid (n=2), and  
189 solid (n=3) digestates during laboratory incubations. Each gray line represents a digestate  
190 incubation, while the red lines represent the mean mineralization for all digestates

### 191 3.3 Effect of digester feedstock on digestate characteristics

192 The proportion of cover crops in the digester feedstock was significantly and negatively  
193 correlated with total nitrogen content ( $R=-0.81$ ,  $p$  value  $< 0.01$ , Fig. S1, Online Resource). This  
194 effect was weak and mainly induced by the digestate from cover crops only. These results are  
195 in line with Risberg et al. (2017) who found a limited effect of digester feedstock on digestate  
196 characteristics, but in contradiction to Guilayn et al. (2019), who found the opposite. Our study  
197 contains, however, fewer samples than that of Guilayn et al. (2019) and less diverse feedstock.

198 The proportion of cover crops in the digester feedstock was significantly and negatively  
199 correlated with C mineralization ( $R=-0.73$ ,  $p$  value  $< 0.05$ , Fig. S1, Online Resource), but not  
200 with N mineralization. Among the other feedstocks, only the proportion of food waste in the  
201 digester was significantly and positively correlated with the proportion of C mineralized

202 (R=0.72, p value < 0.05, Fig. S1, Online Resource). However, these latter substrates  
203 concerned only two digesters.

### 204 **3.4 Nutrients supply and potential C storage at usual rates**

205 Considering the usual rates of application for raw, liquid, and solid digestates, which were 40,  
206 40, and 10 Mg ha<sup>-1</sup>, respectively (according to a survey of the farmers associated with the  
207 digesters), the quantity of nutrients applied to the field was computed, with the mean digestate  
208 characteristics presented in section 3.1 (Table 1). At these rates, raw and liquid digestates  
209 brought a large amount of available N (mainly N-NH<sub>4</sub>) and phosphorus, similar to the amount  
210 of mineral N and P applied yearly in northern France (Moinard et al., 2021), while K was in  
211 excess. In contrast, the total C and remaining C applied were rather low in comparison to the  
212 application of solid bovine manure at a usual rate of 30 Mg ha<sup>-1</sup>. They may, however, contribute  
213 to SOC storage in the case of repeated applications, as suggested by Tambone et al. (2019).  
214 The application of solid digestate provided a limited amount of nutrients, mainly P and K, and  
215 a limited amount of total C and remaining C. In addition to the difference in the quantity applied,  
216 the comparison of raw, liquid, and solid digestates must be made cautiously; these digestates  
217 come from different digesters with different feedstocks and processes that may impact the  
218 digestate characteristics (Bareha et al., 2021).

219 Finally, the nitrogen of digestate available to plants will depend on pedoclimatic conditions and  
220 their insertion in cropping systems. For example, the available N mainly relies on ammoniacal  
221 N; thus the limitation of ammonia volatilization will be a key issue, as already highlighted by  
222 many authors on digestates (Riva et al., 2016). Concerning phosphorus, its availability  
223 depends on the feedstock of the biogas plant, on the considered phase (liquid/solid) and on  
224 soil pH, but can be high (Tuszynska et al., 2021).

225 Table 1 Mean input with the studied digestates (10 raw digestates, 2 liquid phases, and 3 solid  
226 phases) applied at usual rates in comparison with typical organic fertilizers

Parameter	Raw digestate	Liquid digestate	Solid digestate	Pig slurry	Bovine manure
Fresh matter (Mg ha <sup>-1</sup> )	40	40	10	40	30
Dry matter (Mg ha <sup>-1</sup> )	2.4	2.5	2.5	2.0	6.3
Organic C (kg ha <sup>-1</sup> )	821	725	962	680	2490

Remaining C (after one year in the field) <sup>a</sup> (kg ha <sup>-1</sup> )	519	437	762	373	1743
Total N (kg ha <sup>-1</sup> )	189	223	56	180	171
N-NH <sub>4</sub> (kg ha <sup>-1</sup> )	107	141	3	99	9
Available N (after one year in the field) (kg ha <sup>-1</sup> )	114	157	2	115	14
P (kg ha <sup>-1</sup> )	23	33	22	44	39
K (kg ha <sup>-1</sup> )	134	144	39	100	172

227 <sup>a</sup> The remaining C was computed as remaining C after 91 days of incubation

## 228 **4 Conclusion**

229 Raw and liquid digestates from cover crops appeared as potential efficient N fertilizers, similar  
230 to pig slurry, mainly due to their high content of mineral N. Applied at usual rates (40 Mg ha<sup>-1</sup>),  
231 they could bring a significant portion of the N, P, and K required by an intensive cereal crop  
232 rotation in central France. On the other hand, their contribution to soil organic carbon is limited  
233 but could be significant with repeated applications. In contrast, the direct N availability of solid  
234 digestate was very limited, while its contribution to soil organic carbon could be higher if applied  
235 at a large enough rate. It could also possibly be a valuable substitution for mineral P fertilizer.  
236 This better knowledge of such rather new digestates from cover crops could be used to better  
237 manage their use by farmers and allow for an environmental assessment of their recycling in  
238 agriculture with the help of soil-crop models calibrated with these data.

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242 l'Alimentation) and GRDF. GRDF is the France's main natural gas distribution system operator  
243 and is committed to promoting the use of renewable gas as a crucial part of the energy  
244 transition.

### 245 **Employment**

246 Vincent Jean-Baptiste is employed by GRDF.

247 **Financial and non financial interests**

248 The authors have no relevant financial or non-financial interests to disclose.

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