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► To cite this version:

Florent Levavasseur, Caroline Le Roux, Patrice Kouakou, Vincent Jean-Baptiste, Sabine Houot. High Nitrogen Availability but Limited Potential Carbon Storage in Anaerobic Digestates from Cover Crops. Journal of Soil Science and Plant Nutrition, 2022, 22, 10.1007/s42729-022-00853-5 . hal-03653740

HAL Id: hal-03653740

<https://hal.inrae.fr/hal-03653740>

Submitted on 24 Aug 2023

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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⁻¹ 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⁻¹ 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 50 10⁶ 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-NH₄, 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⁻¹) 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⁻¹ dry soil. For
96 solid digestates poor in mineral N, mineral N (KNO₃) was added in excess to reach at least a
97 soil mineral N content of 35 mg N kg⁻¹. 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 CO₂ 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⁻¹ fresh matter (FM)),
111 C_{org} is the organic carbon content of the digestate (kg C Mg⁻¹ 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⁻¹ FM), N_{NH4} is the ammoniacal N content of the digestate (kg N Mg⁻¹
114 FM), N_{org} is the organic nitrogen content of the digestate (kg N Mg⁻¹ 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.

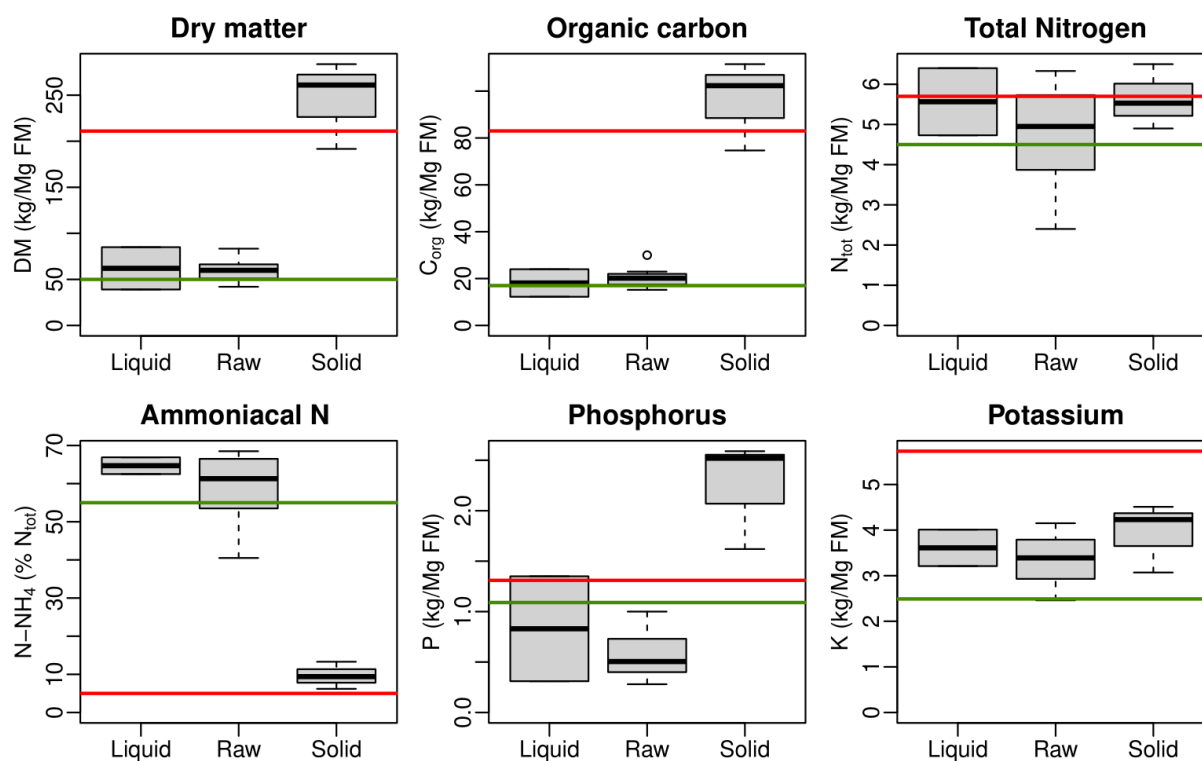
125 To analyze the correlations between digestate characteristics and digester feedstocks, a
126 correlation matrix was analyzed (R Development Core Team, 2013). Only raw digestates were
127 used for this analysis because of the limited number of liquid and solid digestates in our
128 dataset.

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⁻¹ FM, respectively) and
133 organic carbon content (21, 18 and 17 kg Mg⁻¹ FM, respectively), moderate nitrogen content
134 (4.7, 5.6 and 5.5 kg Mg⁻¹ 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⁻¹ FM,
146 4.7 kg Mg⁻¹ FM, and 59%, respectively, in comparison to 17 kg Mg⁻¹ FM, 4.8 kg Mg⁻¹ FM, and
147 71%, respectively, in Risberg et al. (2017) (recalculated).



148

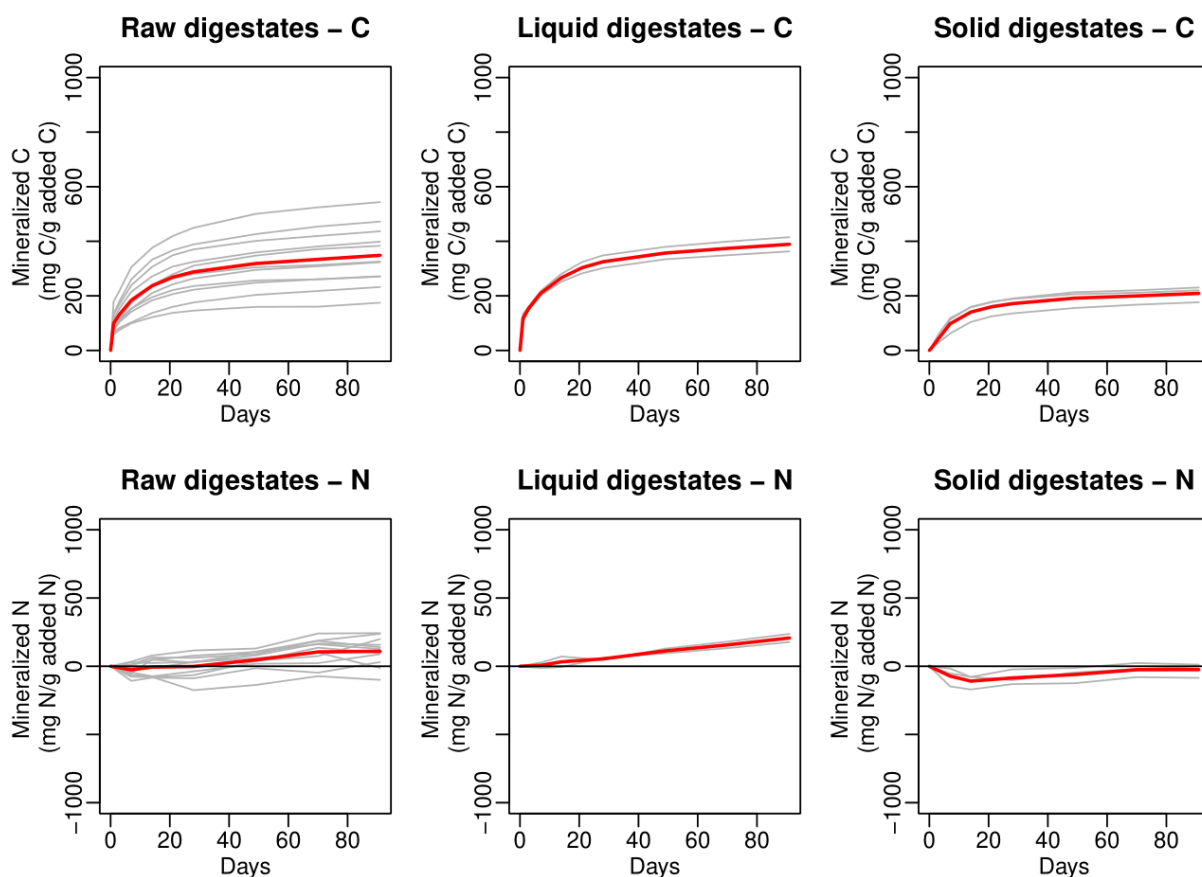
149 **Fig. 1** Chemical composition of the studied digestates compared to a pig slurry (green line)
 150 and a solid bovine manure (red line). The digestate samples included 10 raw digestates, 2
 151 liquid digestates and 3 solid digestates

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⁻¹ added C, and
 156 the variability was moderate (standard deviation equal to 109 mg C g⁻¹ added C). The C
 157 mineralization of liquid digestates was similar (389 mg C g⁻¹ added C on average), while that
 158 of solid digestate was lower (209 mg C g⁻¹ 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 mg N g⁻¹ added N) with a relatively high variability
169 (standard deviation equal to 107 mg N g⁻¹ added N); the mineralization after 91 days ranged
170 from a net immobilization of -100 mg N g⁻¹ added N to a net mineralization of 242 mg N g⁻¹
171 added N. The net mineralization of organic N from liquid digestates was higher (207 mg N g⁻¹
172 added N), but that of solid digestate was lower and negative (-25 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 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 mg C g⁻¹ added C and -18 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⁻¹, 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-NH₄) 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⁻¹. 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 ⁻¹)	40	40	10	40	30
Dry matter (Mg ha ⁻¹)	2.4	2.5	2.5	2.0	6.3
Organic C (kg ha ⁻¹)	821	725	962	680	2490

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

227 ^a 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⁻¹),
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.

239 **Statements and Declarations**

240 **Funding**

241 This work was funded by the French Ministry of Agriculture (Ministère de l'Agriculture et de
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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