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Disentangling the effects of applying pig slurry or its digestate to winter wheat or a catch crop on dissolved C fluxes

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Abstract

The anaerobic digestion industry, which is still developing, generates biogas from organic waste products. A co-product of this process, digestate, is increasingly produced and can be recycled on agricultural land as an alternative to mineral fertilizers. Biogas digestate is a recent product whose chemical composition differs from that of its source material, and additional data still need to be acquired on its effects on dissolved carbon fluxes. The objectives of this study were to assess (i) the effects of applying biogas digestate on dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) fluxes with different winter crops, (ii) the dynamics of DOC and DIC concentrations during the drainage season, and (iii) the annual dynamics of DOC and DIC fluxes along the soil profile. The study examined effects of applying biogas digestate, pig slurry,

27 or a mineral fertilizer to winter wheat and two catch crops (mustard and a multispecies crop)
28 on DOC and DIC fluxes in the soil. Lysimeters at 40 cm (topsoil) and 90 cm (subsoil) depths were
29 monitored from 2014-2023, from November to March (i.e., 9 winter drainage seasons). During
30 the drainage season, the DOC concentration was highest with digestate, and its timing
31 depended on development of the cover crop: from the beginning of the drainage season for
32 mustard and the multispecies crop and around February for wheat. Applying digestate
33 increased the topsoil DOC fluxes (mean of $35.7 \pm 13.7 \text{ kg}\cdot\text{ha}^{-1}$ with digestate vs. $21.0 \pm 6.7 \text{ kg}\cdot\text{ha}^{-1}$
34 with the other treatments), particularly under mustard. Topsoil DIC fluxes were highest with
35 pig slurry due to higher mineralization than that with digestate (mean of $59.1 \pm 22.8 \text{ kg}\cdot\text{ha}^{-1}$
36 with pig slurry vs. $46.2 \pm 16.3 \text{ kg}\cdot\text{ha}^{-1}$ with the other treatments). In the subsoil, DOC fluxes were
37 low ($6.2 \pm 4.1 \text{ kg}\cdot\text{ha}^{-1}$) and DIC fluxes were high ($80.0 \pm 45.7 \text{ kg}\cdot\text{ha}^{-1}$), with no difference among
38 treatments.

39
40

41 1. Introduction

42

43 The anaerobic digestion industry has developed throughout the 21st century in Europe (Scarlat
44 et al., 2018), which counted more than 18,900 biogas plants in 2019 (Iglesias et al., 2021). Since
45 the energy produced is sustainable, it could be an interesting contribution to one of the
46 European Union's targets for 2030, which is to increase the percentage of renewable energy to
47 45% of the total energy consumption. Anaerobic digestion can generate biogas from organic
48 waste products from livestock. Thus, digestate, a co-product of anaerobic digestion, is
49 increasingly produced.

50

51 Despite the digestion, digestate retains high nutrients concentrations (Czekała, 2022) and can
52 be recycled on agricultural land as an alternative to synthetic mineral fertilizers. Digestate also
53 contains organic carbon (C), which could improve soil quality and increase the amount of C
54 stored in the subsoil by providing dissolved organic C (DOC), which is then stabilized by
55 interacting with the mineral phase (Rumpel and Kögel-Knabner, 2011). Storing organic C in the
56 soil is one of the solutions showcased to reduce the impact of climate change and to preserve
57 soils and food security, as in the French initiative “4 per 1000” launched in 2015 during the
58 COP21 (Chenu et al., 2019).

59

60 The composition of digestate differs from that of the organic waste product from which it is
61 derived. Most of the labile fraction of the initial product, such as carbohydrate-like molecules,
62 is consumed by microorganisms during anaerobic digestion. Thus, the digestate concentrates
63 recalcitrant molecules such as lignin and non-hydrolyzable lipids that have higher biological
64 stability (Tambone et al., 2009). Because of this distinct composition, digestate may have
65 different effects on the soil, and more specifically on fluxes of elements dissolved in soil
66 water. An excess of these fluxes could lead to negative consequences and threaten aquatic
67 ecosystems, since DOC can transport pollutants (Dunnivant et al., 1992) or form carcinogenic
68 trihalomethanes during water purification (Li et al., 1996). Moreover, dissolved inorganic C
69 (DIC) contains dissolved CO₂, some of which is emitted into the atmosphere when groundwater
70 joins the surface water (Kindler et al., 2011). Thus, it is essential to assess effects of applying
71 digestate on dissolved C fluxes in the soil.

72 To do so, it is also important to consider plants, which also influence these fluxes. Winter crops
73 have many benefits, such as increasing the soil organic matter content, conserving mineral
74 nitrogen (N), and preventing soil erosion and weed development (Hartwig and Ammon, 2002).

75

76 Crops can provide organic matter to the soil via the degradation of residues (roots, leaves,
77 stems) and root exudation (Kalbitz et al., 2000). Root exudates are low molecular weight
78 compounds (Nguyen, 2009) and thus, can contribute to the DOC pool. About 30-60% of the
79 carbon fixed by photosynthesis by annual plants is translocated to the roots and up to 70% of
80 this carbon can be released into the soil (Neumann and Römheld, 2000). Crops can also provide
81 inorganic C to the soil by root respiration (Viktor and Cramer, 2003).

82

83 Despite a growing interest in studying digestate and the effects of applying it on the soil, this
84 product is relatively new, and additional data still need to be acquired, particularly in
85 association with different crop species. The objectives of this study were to assess (i) the effects
86 of biogas digestate from pig slurry on DOC and DIC fluxes in different cropping contexts, (ii) the
87 dynamics of DOC and DIC concentrations during the drainage season, and (iii) the annual
88 dynamics of DOC and DIC fluxes along the soil profile. We compared the soil water collected by
89 lysimeters at 40 and 90 cm depths for three treatments: biogas digestate from pig slurry, raw
90 slurry, and mineral nitrogen (the control). We hypothesized that there could be more DOC
91 when applying digestate due to its recalcitrant chemical composition.

92

93 **2. Materials and methods**

94

95 *2.1 Study site and experimental design*

96

97 The study site, EFELE, forms part of the SOERE PRO network (<https://www6.inra.fr/valor-pro>)

98 (see Morvan et al. (2020) for a complete description). EFELE is located near Rennes, in western

99 France (48.1019444° N, -1.7955555° W), and its experiment, started in 2012, compares effects

100 of applying pig slurry or digestate derived from pig slurry on soil and water quality to those of

101 mineral N fertilization. The experiment follows a complete randomized block design with 4
 102 replicates, with each block consisting of 9 plots of 113 m² each.

103 The crop rotation (Table 1) consists of a 2-year succession of maize (*Zea mays*) and winter
 104 wheat (*Triticum aestivum*) with a N catch crop of white mustard (*Sinapis alba*) or a multispecies
 105 catch crop composed of white mustard, rapeseed (*Brassica napus*), clover (*Trifolium*
 106 *alexandrinum*), and phacelia (*Phacelia tanacetifolia*) sown at the beginning of September (2
 107 months after the wheat harvest). The mineral fertilizer, pig slurry and digestate treatments
 108 were applied every year in April to the growing wheat or before the sowing of maize (Table 2).

109 Organic waste products application rates were determined by their N fertilizer value, in
 110 compliance with the European Union Nitrates Directive (91/676/EEC). The annual application
 111 rates and the inputs of mineral and organic N varied among the 9 years of the experiment
 112 analyzed in the present study (2014-2023) (Table 2). The rate of mineral N fertilizer applied to
 113 the crop was calculated using the mineral N balance-sheet method recommended in France
 114 (Machet et al., 2017).

115

116 *Table 1. Two-year crop rotation of winter wheat (W), a catch crop (Cc, mustard or a multispecies crop*
 117 *composed of mustard, rapeseed, clover, and phacelia), and maize (M). The drainage season is*
 118 *represented in dark gray. Mineral nitrogen fertilizer, pig slurry, or digestate from pig slurry is applied in*
 119 *April of each year (circled).*

	J	F	M	(A)	M	J	J	A	S	O	N	D	J	F	M	(A)	M	J	J	A	S	O	N	D
120	W	W	W	W	W	W	W		Cc	Cc	Cc	Cc	Cc	Cc	Cc	M	M	M	M	M	M	M	W	W

121

122 The 2020-2021 season was unusual, because a catch crop of mustard instead of winter wheat
 123 was sown after the maize harvest. Unlike the other seasons with a mustard catch crop, the
 124 latter was sown later, at the end of September, and its cover developed less.

125 *Table 2. Application rates and amounts of organic carbon (C) and mineral nitrogen (N) applied to the*
 126 *given crop for the three treatments (mineral N fertilizer, pig slurry, or digestate from pig slurry) from*
 127 *2014-2022. Mineral N equals the sum of the ammoniacal N of the organic waste product and the N from*
 128 *the mineral fertilizer.*

Treatment	Content	2014	2015	2016	2017	2018	2019	2020	2021	2022
		Maize	Wheat	Maize	Wheat	Maize	Wheat	Maize	Maize	Wheat
Mineral fertilizer	N (kg N.ha ⁻¹)	102	120	96	80	126	145	120	124	149
	Application rate (t.ha ⁻¹)	25	32.8	23.4	22.5	21.2	40	41	41	48.1
Pig slurry	Organic C (kg C.ha ⁻¹)	519	1023	724	796	684	617	537	1880	120
	Mineral N (kg N.ha ⁻¹)	114	160	98	89	102	92	101	226	148
	Application rate (t.ha ⁻¹)	28	27.3	40	24.4	24.7	40	41	41	48.8
Digestate	Organic C (kg C.ha ⁻¹)	556	694	361	641	414	570	884	967	682
	Mineral N (kg N.ha ⁻¹)	132	135	92	111	103	110	121	176	214

129

130 *2.2 Soil description*

131 The soil is classified as a Luvisol-redoxisol derived from aeolian silt deposited on schist material
 132 (see Morvan et al. (2020) for details). The main characteristics of the 0-30 cm layer at the
 133 beginning of the experiment were 14.2% clay, 71.0% silt, 14.8% sand, 1.1% organic C, a C:N
 134 ratio of 9.6, and pH of 6.1 (slightly acidic).

135 *2.3 Climate data*

136 Climate data were obtained from an automatic weather station located at the study site
 137 (Campbell Scientific CR10 0 0 logger and weather station). The climate is mild oceanic
 138 temperate, with a mild winter and warm summer. From 2014-2022, the mean annual
 139 temperature was 12.8°C, annual rainfall averaged 681 mm, and Penman potential
 140 evapotranspiration was 731 mm, respectively.

141

142 *Table 3. Drainage sampling period, winter rainfall, and drainage for each drainage season studied (mean*
 143 *± 1 standard deviation). Winter rainfall equals the sum of rainfall received during the sampling period*
 144 *(from 1 November to 31 March of the following year).*

Drainage season	Winter crop	Winter rainfall (mm)‡	Sampling period†	No. of sampling dates	Drainage (mm)	
					40 cm depth	90 cm depth
2014-2015	Winter wheat	295 (- 24)	8 Dec. 2014-24 Mar. 2015	7	226 ± 30	298 ± 45
2015-2016	Mustard	384 (+ 65)	5 Jan. 2016-14 Mar. 2016	6	213 ± 21	236 ± 46
2016-2017	Winter wheat	253 (- 66)	1 Feb. 2017-16 Mar. 2017	4	97 ± 8	27 ± 15
2017-2018	Mustard	367 (+ 48)	21 Dec. 2017-28 Mar. 2018	7	262 ± 33	281 ± 43
2018-2019	Winter wheat	279 (- 40)	27 Dec. 2018-20 Mar. 2019	3	149 ± 25	120 ± 13
2019-2020	Mustard	512 (+ 193)	14 Nov. 2019-23 Dec. 2019	5	170 ± 30	191 ± 35
2020-2021	Mustard	301 (- 18)	2 Nov. 2020-1 Mar. 2021	6	241 ± 44	221 ± 37
2021-2022	Winter wheat	220 (- 99)	13 Dec. 2021-30 Mar. 2022	5	135 ± 21	124 ± 31
2022-2023	Multispecies	377 (+ 58)	5 Dec. 2022-22 Mar. 2023	5	204 ± 51	158 ± 36

† First date of collection – Last date of collection

‡ Values in parentheses are the difference compared to the 30-year mean (319 mm).

145 *2.4 Drained water sampling*

146 In pits opened in spring 2011 on six plots, two pairs of fiberglass wick lysimeters were installed
 147 in each plot, one in the topsoil (40 cm depth) and one in the subsoil (90 cm depth). Fiberglass
 148 wick lysimeters have been shown to be most effective at collecting soil leachate (Sansoulet et
 149 al., 2008) and can sample the large volumes of water necessary for subsequent analyses. Each
 150 lysimeter (area of 0.125 m²) is made from a 50 × 25 × 2 cm stainless steel plate, with the
 151 fiberglass wick unfolded onto the plate before applying the plate up against the soil layers
 152 above. The entire wick goes through a central hole in the plate and down a Tygon® tube to a
 153 receiving glass flask (10 L). Six flasks were equipped with ultrasonic sensors (Telemecanique
 154 Sensor XX918A3C2M12) to monitor the filling dynamics of the flasks and to collect the water
 155 rapidly after the end of the drainage, before its composition could change. Drainage is relatively

156 moderate under the climate conditions at the EFELE site, and the number of sampling dates
157 per season ranged from 3-10 depending on the amount of winter rainfall.

158 *2.5 Sample collection and analysis*

159 Drainage water was sampled as soon as possible after the end of the drainage event. Water
160 samples were filtered at 0.45 μm and stored at 4°C until analysis. DOC and DIC concentrations
161 were determined using a TOC-V_{CSH} analyzer (Shimadzu Corp., Kyoto, Japan) using thermal
162 oxidation.

163 *2.6 Water balance and calculation of fluxes*

164 DOC and DIC fluxes were calculated by multiplying the drainage estimated from the water
165 balance by the DOC and DIC concentrations, respectively. The water balance was predicted
166 using the STICS (multidisciplinary simulator for standard crops) model (Brisson et al., 1998,
167 http://www6.paca.inra.fr/stics_eng), with the model being initialized for the first date of water
168 collection by the lysimeters and predicting drainage from the soil layers as a function of soil
169 field capacity.

170 The 2019-2020 drainage season was unusual: due to high rainfall (512 mm) (Table 3) the
171 groundwater table rose into the soil profile at the end of December, which disrupted drainage,
172 and the water sampling had to be stopped early. Consequently, this season was excluded when
173 calculating the fluxes (Table 4), but its undisrupted sampling dates (14 Nov.-23 Dec. 2019) were
174 kept when analyzing concentration dynamics (Figures 2-4).

175 *2.7 Statistical analysis*

176 All statistical analysis was performed using R software (R Core Team, 2017). The influence of
177 crop, fertilization type, drainage, and their interactions on annual fluxes of DOC and DIC were

178 assessed using analysis of variance (ANOVA). The assumptions of ANOVA were checked by using
 179 the Shapiro-Wilk and Bartlett's tests. When needed, the data were transformed to satisfy these
 180 assumptions. Differences were considered significant at $p < 0.05$, and means were compared
 181 using Tukey's range test. When the latter's parametric conditions could not be met, Kruskal-
 182 Wallis and Dunn's tests were performed instead.

183 Four statistical models were applied:

- 184 • Model 1: to the concentration dataset, by drainage date and depth:

$$185 \quad Y = \mu + Tr_i + \varepsilon_i \quad (1)$$

186

- 187 • Model 2: to the seasonal cumulative fluxes of the entire dataset, by depth:

$$188 \quad Z = \mu + Tr_i + Cr_j + Dr_k + (TrCr)_{ij} + (CrDr)_{jk} + (TrDr)_{ik} + \varepsilon_{ijk} \quad (2)$$

189

- 190 • Model 3: to the seasonal cumulative fluxes dataset, by crop and depth:

$$191 \quad Z = \mu + Tr_i + Dr_j + (DrTr)_{ij} + \varepsilon_{ij} \quad (3)$$

192

- 193 • Model 4: to the seasonal cumulative fluxes dataset, by drainage season, crop and depth:

$$194 \quad Z = \mu + Tr_i + \varepsilon_i \quad (4)$$

195

196 Where Y is a response variable (DOC or DIC concentration on a given drainage date), Z is a
 197 response variable (total amount of leached DOC or DIC fluxes), μ is population mean, Dr is the
 198 amount of drained water, Cr is the type of crop, Tr is the organic waste product treatment, and
 199 ε is the residual error.

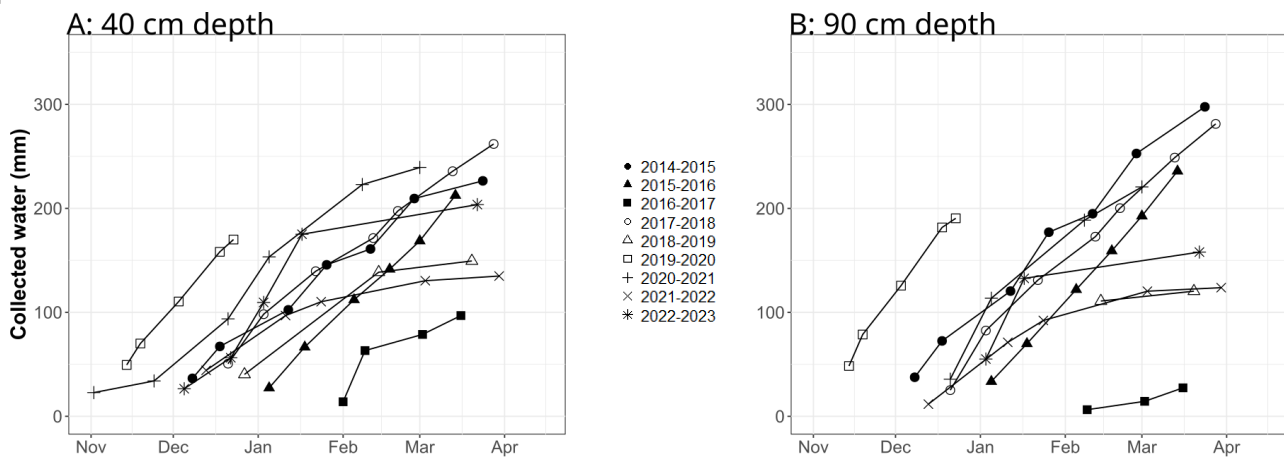
200 3. Results

201

202 3.1 Drained water

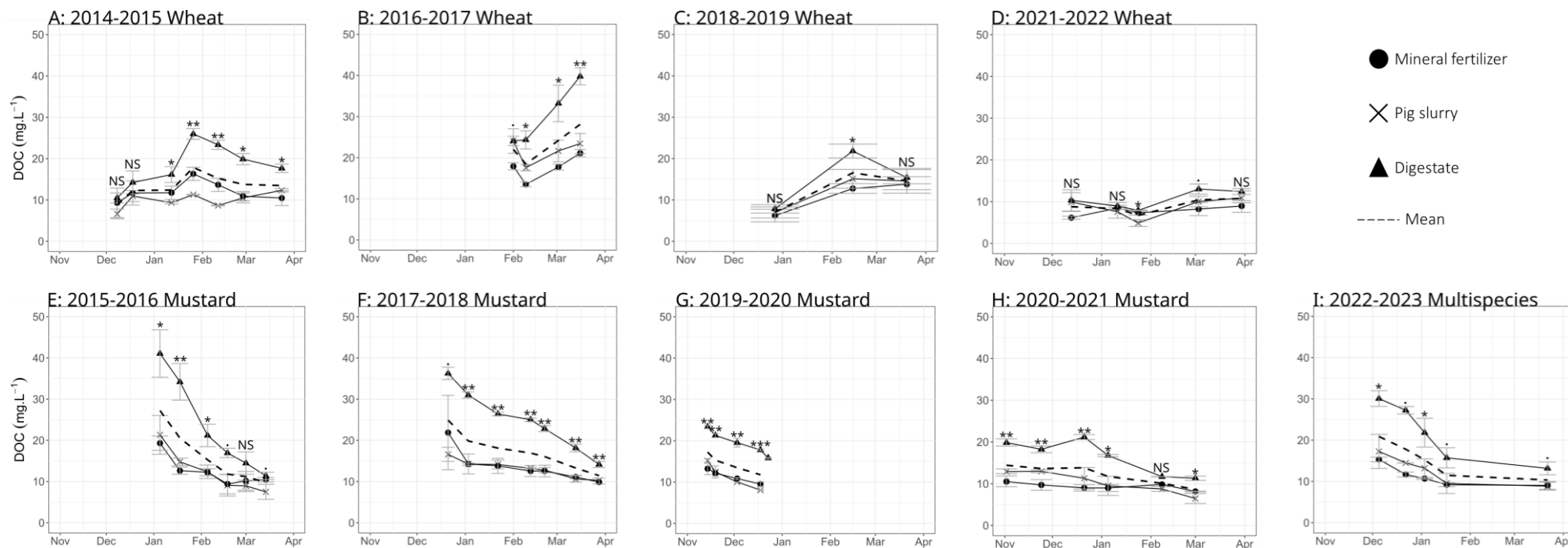
203 The cumulative volume of drained water ranged from 97 ± 8 (2016-2017) to 262 ± 33 mm
204 (2017-2018) at 40 cm depth and 27 ± 15 (2016-2017) to 298 ± 45 mm (2014-2015) at 90 cm
205 depth. At both depths, drainage varied greatly among seasons (Figure 1), in accordance with
206 the high variability in rainfall (220-512 mm) (Table 3). Most drainage seasons started between
207 November and January, except for 2016-2017, which started in February, lasted only one
208 month, had much less rainfall (253 mm, compared to the mean of 332 mm), and resulted in
209 very low drainage volumes, particularly at 90 cm depth (Table 3). The seasons with the lowest
210 rainfall and drainage volumes, such as 2016-2017, 2018-2019, and 2021-2022, coincided with
211 the growing of wheat (Table 3). Overall, similar amounts of water were collected at 40 ($189 \pm$
212 54 mm) and 90 cm (184 ± 86 mm) during each drainage season; thus, water flowed through
213 the soil profile and was not retained between the two depths.

214



215

216 Figure 1. Mean cumulative amount of leached water collected by the lysimeters at (A) 40 cm and (B) 90
217 cm depths during each drainage season. See Table 3 for the standard errors.



218

219 Figure 2. Dissolved organic carbon (DOC) concentrations (mg.L⁻¹) (mean of two lysimeter replicates) in topsoil (40 cm) water during the 9-year study under winter
 220 wheat (A to D), a mustard catch crop (E to H), or a multispecies catch crop (I) for the three treatments: mineral nitrogen fertilizer, pig slurry, or digestate from
 221 pig slurry. Sampling during the 2019-2020 drainage season was interrupted after 23 Dec. 2019. Error bars are standard deviations. The treatment effect was
 222 tested for each date using ANOVA. Significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; . $p < 0.1$; NS $p > 0.1$.

223 3.2 Effect of the crop on DOC and DIC concentrations

224 Trends in topsoil DOC concentrations differed by crop. For the seasons under wheat, except for
225 2016-2017 and 2021-2022, DOC concentrations increased from the beginning of the drainage
226 season, peaked in February-March, and then decreased (Figure 2.A-D). Mean DOC
227 concentrations of the four seasons started the season at $11.7 \pm 6.6 \text{ mg.L}^{-1}$ and ended the season
228 at $16.7 \pm 8.4 \text{ mg.L}^{-1}$. For the 2016-2017 season, whose drainage started later, mean DOC
229 concentrations were higher throughout the season (seasonal mean: $23.2 \pm 7.2 \text{ mg.L}^{-1}$, 1.6 times
230 higher than that of the four seasons of $14.2 \pm 7.0 \text{ mg.L}^{-1}$). During the 2021-2022 season, the
231 DOC concentrations remained relatively stable.

232 For the five seasons under the catch crop of mustard (Figure 2.E-H) or multispecies catch crop
233 (Figure 2.I), trends in mean topsoil DOC concentrations differed markedly from the those
234 observed under wheat and were repeatable. They started the season at $20.9 \pm 8.7 \text{ mg.L}^{-1}$ and
235 continuously decreased to end the season at $9.7 \pm 2.4 \text{ mg.L}^{-1}$ (seasonal mean: $14.5 \pm 6.8 \text{ mg.L}^{-1}$).
236 Compared to the seasons under wheat, initial DOC concentrations under the catch crops were
237 1.8 times higher and 0.6 times lower at the end of the drainage season, with a similar seasonal
238 mean. Regarding the unusual 2020-2021 season, during which mustard was sown instead of
239 wheat after the maize harvest, DOC concentrations at the beginning of the season were lower
240 ($14.4 \pm 4.8 \text{ mg.L}^{-1}$) than the mean DOC concentration of the five seasons under a catch crop,
241 but the concentrations at the end of the season ($8.6 \pm 2.5 \text{ mg.L}^{-1}$) were similar.

242 Subsoil DOC concentrations remained low and stable during all seasons and did not differ
243 among the crops. Mean seasonal DOC concentrations under wheat ($6.2 \pm 9.1 \text{ mg.L}^{-1}$) were
244 similar to those under the mustard and multispecies catch crops ($4.3 \pm 6.1 \text{ mg.L}^{-1}$) (Figure S1.A-
245 I).

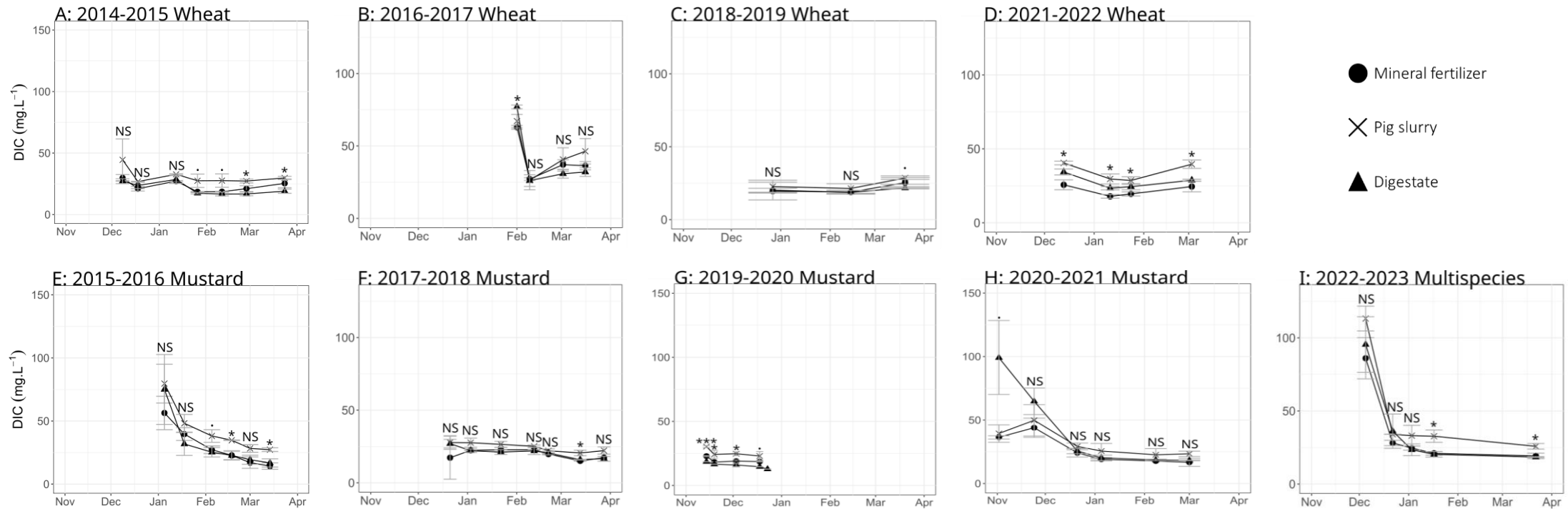
246 Regardless of the crop, mean seasonal DIC concentrations were $29.0 \pm 16.8 \text{ mg.L}^{-1}$ in the topsoil
247 (Figure 3) and $50.5 \pm 24.1 \text{ mg.L}^{-1}$ in the subsoil (Figure S2). Trends in topsoil DIC concentrations
248 differed from those of DOC concentrations. They did not vary during several seasons, but did
249 decrease during three seasons of a mustard or multispecies catch crop (2015-2016, 2020-2021,
250 and 2022-2023) and one season of wheat (2016-2017). Under wheat, mean DIC concentrations
251 were $29.1 \pm 12.1 \text{ mg.L}^{-1}$ in the topsoil (Figure 3.A-D) and $51.0 \pm 24.5 \text{ mg.L}^{-1}$ in the subsoil (Figure
252 S2.A-D). Under mustard and multispecies catch crops, DIC concentrations were 29.0 ± 19.0
253 mg.L^{-1} in the topsoil (Figure 3.E-I) and $50.3 \pm 24.0 \text{ mg.L}^{-1}$ in the subsoil (Figure S2.E-I).

254

255 *3.3 Treatment effects on DOC and DIC concentrations*

256 To assess effects of the treatment on DOC and DIC concentrations, ANOVA model (1) was
257 applied and detected significant differences among crops in the dynamics of DOC
258 concentrations. In the topsoil under wheat, DOC concentrations differed little among
259 treatments at the beginning of the drainage season but did differ later, being higher with
260 digestate (mean of $17.6 \pm 7.3 \text{ mg.L}^{-1}$ for the four seasons on the first date when differences
261 became significant) than with pig slurry or mineral fertilizer ($11.5 \pm 4.2 \text{ mg.L}^{-1}$) (Figure 2.A-D).
262 During the 2014-2015, 2016-2017, and 2018-2019 seasons, DOC concentrations increased
263 under digestate in February-March. Under the mustard and multispecies catch crops, mean
264 DOC concentrations at the beginning of the drainage season were significantly higher with
265 digestate ($30.1 \pm 8.8 \text{ mg.L}^{-1}$) than with pig slurry or mineral fertilizer ($16.4 \pm 3.7 \text{ mg.L}^{-1}$) (Figure
266 2.E-I). DOC concentrations with digestate decreased consistently to become similar to those
267 with pig slurry or mineral fertilizer at the end of the drainage season ($9.7 \pm 2.4 \text{ mg.L}^{-1}$). Mean
268 DOC concentrations in the subsoil did not differ among treatments (Figure S1).

269 Mean DIC concentrations in the topsoil with pig slurry, digestate, and mineral fertilizer were
270 33.5 ± 16.3 , 27.9 ± 19.5 , and $25.7 \pm 13.2 \text{ mg.L}^{-1}$, respectively (Figure 3). Mean DIC
271 concentrations in the subsoil did not differ among treatments (Figure S2).



272

273 Figure 3. Dissolved inorganic carbon (DIC) concentrations (mg.L⁻¹) (mean of two lysimeter replicates) in the topsoil (40 cm)
 274 study under winter wheat (A to D), a mustard catch crop (E to H), or a multispecies catch crop (I) for the three treatments: mineral nitrogen fertilizer,
 275 pig slurry, or digestate from pig slurry. Sampling of the 2019-2020 drainage season was interrupted after 23 Dec. 2019. Error bars are standard
 276 deviations. The treatment effect was tested for each date using ANOVA. Significance: *** p < 0.001; ** p < 0.01; * p < 0.05; . p < 0.1; NS p > 0.1.

277 *3.4 Inter-seasonal variability in DOC and DIC fluxes*

278 Annual DOC fluxes ranged from 10.5-50.5 kg.ha⁻¹ in the topsoil and 1.0-14.1 kg.ha⁻¹ in the
279 subsoil, while annual DIC fluxes ranged from 26.0-86.9 kg.ha⁻¹ in the topsoil and 19.9-174.4
280 kg.ha⁻¹ in the subsoil (Table 4). Mean DOC fluxes were particularly low during the 2021-2022
281 season in both the topsoil (11.7 ± 1.6 kg.ha⁻¹) and subsoil (1.4 ± 0.7 kg.ha⁻¹). The dissolved C
282 fluxes varied greatly depending on the drainage season and the crop.

283 When applying ANOVA model (2), the Crop , Drainage , and Treatment had significant ($p <$
284 0.001) effects on topsoil DOC fluxes. The Treatment:Crop and Crop:Drainage interactions were
285 also significant ($p <$ 0.001), implying that the Treatment effect must be interpreted as a function
286 of the Crop effect, and the Crop effect as a function of the Drainage effect. The Crop:Drainage
287 interaction was in accordance with the fact that the seasons with lower rainfall and drainage
288 volumes (2016-2017, 2018-2019, and 2021-2022 seasons) coincided with the growing of wheat
289 (Table 3). Mean DOC fluxes in the topsoil were significantly ($p <$ 0.001) higher for the three
290 seasons under mustard (33.4 ± 11.2 kg.ha⁻¹) than for the season under the multispecies crop
291 (27.1 ± 9.3 kg.ha⁻¹) or the four seasons under wheat (20.0 ± 9.5 kg.ha⁻¹). The mean DOC fluxes
292 under the crops resulted not only from the Crop effect, but from that of the Crop:Drainage
293 interaction. Thus, ANOVA model (3) was applied to test the Drainage effect separately for each
294 crop. It revealed that the Drainage effect was significant for wheat ($p <$ 0.001) and nearly so for
295 mustard ($p =$ 0.051), with no Treatment:Crop interaction for wheat but a significant
296 Treatment:Crop interaction ($p <$ 0.05) for mustard. This effect of the Treatment:Crop
297 interaction led to the application of Model (4) to each drainage season (section 3.5).

298 Applying ANOVA model (2) to subsoil DOC fluxes indicated that only Crop had a significant
299 effect ($p <$ 0.001). Mean DOC fluxes were 9.2 ± 4.4 kg.ha⁻¹ under mustard and 4.4 ± 2.6 kg.ha⁻¹
300 under wheat and the multispecies crop.

301 For topsoil DIC fluxes, applying ANOVA model (2) revealed that Crop, Drainage, and Treatment
302 had significant ($p < 0.001$) effects and no interactions. Mean DIC fluxes were significantly higher
303 under mustard and the multispecies crop ($62.4 \pm 16.5 \text{ kg}\cdot\text{ha}^{-1}$) than under wheat (38.5 ± 14.3
304 $\text{kg}\cdot\text{ha}^{-1}$). In the subsoil, only Crop and Drainage had a significant effect ($p < 0.001$), with $120.1 \pm$
305 $38.2 \text{ kg}\cdot\text{ha}^{-1}$ under mustard, $68.1 \pm 11.9 \text{ kg}\cdot\text{ha}^{-1}$ under the multispecies crop, and 52.9 ± 33.2
306 $\text{kg}\cdot\text{ha}^{-1}$ under wheat.

307

308 *3.5 Treatment effect on DOC and DIC fluxes*

309 When applying ANOVA model (2) without separation by crop, the Treatment effect was
310 significant for both DOC ($p < 0.001$) and DIC fluxes ($p < 0.001$) in the topsoil but not in the
311 subsoil. To test the Treatment effect while avoiding the Drainage effect, ANOVA model (4) was
312 applied to each drainage season.

313 In the topsoil, except for the 2021-2022 season, which showed no difference among
314 treatments, DOC fluxes were always higher with digestate (mean of $35.7 \pm 13.7 \text{ kg}\cdot\text{ha}^{-1}$ for the
315 eight seasons) than with pig slurry or mineral fertilizer, regardless of the crop (Table 4). DOC
316 fluxes with pig slurry and mineral fertilizer for the eight seasons were similar (mean of $21.0 \pm$
317 $6.7 \text{ kg}\cdot\text{ha}^{-1}$) and nearly 2.0 times lower than those with digestate. DIC fluxes in the topsoil were
318 significantly higher with pig slurry ($59.1 \pm 22.8 \text{ kg}\cdot\text{ha}^{-1}$) than with mineral fertilizer or digestate
319 ($46.2 \pm 16.3 \text{ kg}\cdot\text{ha}^{-1}$). The Treatment effect, tested individually for each season to avoid the
320 Drainage effect, was significant only for the 2014-2015 and 2021-2022 seasons, which were
321 under wheat.

322 Table 4. Dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) fluxes ($\text{kg}\cdot\text{ha}^{-1}$) (mean of two lysimeter replicates) in the topsoil and subsoil during
 323 the 8-season study under winter wheat, a mustard catch crop, or a multispecies catch crop for the three treatments: mineral nitrogen fertilizer, pig slurry, or
 324 digestate from pig slurry. The 2019-2020 season was excluded from the flux dataset. Letters indicate significant differences among DOC fluxes (lowercase) or DIC
 325 fluxes (uppercase) based on ANOVA for each drainage season, depth, and crop separately.

326

Depth	Treatment	C flux	Wheat				Mustard			Multispecies	Mean
			2014-2015	2016-2017	2018-2019	2021-2022	2015-2016	2017-2018	2020-2021	2022-2023	All seasons
40 cm	Mineral fertilizer	DOC	29.5 ± 4.5 ^{b†}	12.6 ± 0.3 ^c	14.9 ± 0.2 ^b	10.5 ± 0.2 ^a	26.2 ± 3.8 ^b	30.4 ± 4.4 ^b	21.7 ± 7.2 ^b	21.5 ± 0.5 ^b	20.9 ± 7.8
		DIC	53.9 ± 0.8 ^B	28.2 ± 0.3 ^A	26.4 ± 1.9 ^A	30.3 ± 0.7 ^B	62.4 ± 7.6 ^A	37.6 ± 8.5 ^A	55.7 ± 4.1 ^A	65.1 ± 4.3 ^A	45.0 ± 15.9
		Total	83.4	40.8	41.3	40.8	88.6	68	77.4	86.6	65.9 ± 21.6
	Pig slurry	DOC	23.7 ± 0.2 ^b	16.8 ± 0.3 ^b	16.8 ± 1.4 ^b	11.6 ± 1.7 ^a	25.3 ± 3.2 ^b	25.0 ± 0.8 ^b	28.9 ± 2.9 ^{ab}	20.8 ± 3.1 ^b	21.1 ± 5.7
		DIC	72.4 ± 2.0 ^A	32.8 ± 2.5 ^A	28.6 ± 3.6 ^A	47.8 ± 1.2 ^A	86.9 ± 6.4 ^A	45.7 ± 3.1 ^A	81.3 ± 11.0 ^A	76.9 ± 15.2 ^A	59.1 ± 22.8
		Total	96.1	49.6	45.4	59.4	112.2	70.7	110.2	97.7	80.2 ± 27.1
	Digestate	DOC	44.5 ^a	22.1 ± 1.6 ^a	23.6 ± 2.0 ^a	13.1 ± 1.7 ^a	49.6 ± 3.2 ^a	50.5 ± 2.3 ^a	43.1 ± 0.6 ^a	39.0 ± 1.3 ^a	35.7 ± 13.7
		DIC	50.9 ± 2.3 ^B	28.3 ± 2.6 ^A	26.0 ± 1.7 ^A	36.8 ± 1.5 ^C	64.6 ± 11.7 ^A	40.1 ± 1.8 ^A	73.9 ± 3.8 ^A	58.9 ± 0.6 ^A	47.4 ± 17.2
		Total	95.4	50.4	49.6	49.9	114.2	90.6	117	97.9	83.1 ± 28.9
90 cm	Mineral fertilizer	DOC	6.1 ± 0.1 ^a	5.6 ± 0.9 ^a	1.3 ± 1.4 ^b	1.0 ± 0.4 ^a	14.1 ± 5.6 ^a	10.7 ± 5.5 ^a	5.1 ± 2.5 ^a	3.4 ± 0.1 ^a	5.9 ± 4.9
		DIC	105.2 ± 17.4 ^A	27.7 ± 3.6 ^A	30.9 ± 9.5 ^A	40.7 ± 5.6 ^C	131.1 ± 38.2 ^A	87.5 ± 9.8 ^A	99.8 ± 15.2 ^A	55.3 ± 9.3 ^A	72.3 ± 39.5
		Total	111.3	33.3	32.2	41.7	145.2	98.2	104.9	58.7	78.2 ± 42.3
	Pig slurry	DOC	5.6 ± 1.7 ^a	5.9 ± 1.6 ^a	3.3 ± 0.6 ^{ab}	2.2 ± 0.3 ^a	5.7 ± 0.5 ^a	8.9 ± 1.9 ^a	5.7 ± 2.4 ^a	3.1 ± 0.2 ^a	5.1 ± 2.3
		DIC	122.2 ± 2.6 ^A	30.6 ± 4.7 ^A	31.6 ± 2.5 ^A	52.1 ± 4.9 ^A	174.4 ± 17.5 ^A	87.9 ± 0.4 ^A	131.2 ± 3.3 ^A	77.6 ± 5.2 ^A	88.4 ± 49.8
		Total	127.8	36.5	34.9	54.3	180.1	96.8	136.9	80.7	93.5 ± 52.0
	Digestate	DOC	5.0 ± 0.7 ^a	8.7 ± 5.0 ^a	6.4 ± 0.8 ^a	1.0 ± 0.3 ^a	10.3 ± 4.0 ^a	10.5 ± 3.8 ^a	11.5 ± 8.4 ^a	6.4 ± 2.2 ^a	7.5 ± 4.5
		DIC	88.5 ± 2.3 ^A	19.9 ± 8.4 ^A	42.2 ± 4.2 ^A	42.9 ± 9.7 ^B	169.8 ± 25.2 ^A	74.6 ± 4.4 ^A	124.7 ± 38.7 ^A	71.5 ± 7.9 ^A	79.3 ± 48.8
		Total	93.5	28.6	48.6	43.9	180.1	85.1	136.2	77.9	86.7 ± 50.7

† Standard deviation

327

328 *3.6 Total dissolved C balance between topsoil and subsoil*

329 The annual mean of total dissolved C (DOC+DIC) for mineral fertilizer and pig slurry was $65.9 \pm$
330 21.6 and 80.2 ± 27.1 $\text{kg}\cdot\text{ha}^{-1}$, respectively, at 40 cm and 78.2 ± 42.3 and 93.5 ± 52.0 $\text{kg}\cdot\text{ha}^{-1}$,
331 respectively, at 90 cm (Table 4). According to linear regressions between total dissolved C at 40
332 and 90 cm, there was 1.8 times more dissolved C at 90 cm than at 40 cm with mineral fertilizer
333 ($R^2 = 0.668$) and 1.6 times more with pig slurry ($R^2 = 0.822$) (Figure S3). For both treatments,
334 there was much more DIC than DOC (22.1 - 168.7 $\text{kg}\cdot\text{ha}^{-1}$ more) at 90 cm (Figure S4.B).

336 With digestate, the dissolved C at 40 cm (83.1 ± 28.9 $\text{kg}\cdot\text{ha}^{-1}$) and 90 cm (86.7 ± 50.7 $\text{kg}\cdot\text{ha}^{-1}$)
337 were similar and more balanced than those of the other treatments, with only 1.2 times more
338 total C fluxes at 90 cm depth ($R^2 = 0.794$) (Figure S3).

339

340 **4. Discussion**

341

342 *4.1 Independent and interacting effects of treatment and crop on DOC and DIC*
343 *concentrations*

344

345 Mean DOC concentrations in the topsoil (14.4 ± 6.8 $\text{mg}\cdot\text{L}^{-1}$, over nine seasons) were quite high
346 despite a low organic C content (1.1%). Those concentrations were similar to those observed
347 in three European cropland experiments (12 $\text{mg}\cdot\text{L}^{-1}$) by Kindler et al. (2011). Mean DOC
348 concentrations under mustard and mineral fertilizer (11.5 ± 3.5 $\text{mg}\cdot\text{L}^{-1}$, over four seasons) were
349 higher than those observed by Walmsley et al. (2011) on Irish cropland (Eutric Cambisol) under
350 mustard in the topsoil with a similar fertilization rate (4.4 ± 0.2 $\text{mg}\cdot\text{L}^{-1}$). In the subsoil, DOC
351 concentrations (5.0 ± 7.4 $\text{mg}\cdot\text{L}^{-1}$, over nine seasons) were lower than those observed by Kindler
352 et al. (2011) (12 $\text{mg}\cdot\text{L}^{-1}$). DIC concentrations over nine seasons in the present study were similar
353 to those observed by Kindler et al. (2011) in both the topsoil (29.0 ± 16.8 vs. 35 $\text{mg}\cdot\text{L}^{-1}$,
354 respectively) and the subsoil (50.5 ± 24.1 vs. 57 $\text{mg}\cdot\text{L}^{-1}$, respectively). As Herbrich et al. (2017)

355 observed in Germany, DOC concentrations decreased and DIC concentrations increased as
356 depth increased.

357

358 In the topsoil, the temporal dynamics of DOC concentrations depended on the type of crop,
359 with the degree of response depending on the treatment, and the concentrations were
360 particularly high for digestate. Two factors can explain the difference observed between
361 cropping contexts.

362

363 First, regardless of the treatment, DOC concentrations at the beginning of the season under
364 wheat ($11.7 \pm 6.6 \text{ mg.L}^{-1}$) were 2 times lower than those under a catch crop ($20.9 \pm 8.7 \text{ mg.L}^{-1}$),
365 which suggests that the latter had a larger leachable DOC pool. The crop before the mustard
366 and multispecies catch crop was wheat, and the crop before wheat was maize (Table 1). Abiven
367 et al. (2011) observed that maize roots contained more lignin than wheat roots (212.0 ± 16.1
368 vs. $134.5 \pm 13.8 \text{ mg C lignin.g C plant}^{-1}$, respectively) but that their lignin was less biodegradable
369 (C-type lignin:V-type lignin ratio of 3.90 vs. 1.47, respectively). After the harvest of wheat in
370 July, its roots decomposed in the soil and provided soluble molecules of low molecular weight
371 that contributed to the DOC concentrations measured at the beginning of winter drainage, as
372 well as DIC from microbial respiration (Dang et al., 2022; He et al., 2015).

373

374 Second, the mustard and multispecies catch crops were sown in September and had two
375 additional months to develop and produce root exudates than the wheat, which was sown in
376 November and had developed little when the drainage season started. Under wheat, DOC
377 concentrations increased around February, which could correspond to the end of the tillering
378 stage. Since tiller number and root development are positively correlated (O'Brien, 1979), the

379 higher root biomass may have increased root exudation, thus increasing DOC concentrations
380 (Ge et al., 2015; Lu et al., 2004). The results of the 2020-2021 season tend to support this
381 hypothesis. In this season, a mustard catch crop was sown instead of wheat after the harvest
382 of maize, and it was also sown later than those during the other seasons with a catch crop and
383 thus less developed when the drainage season started. DOC from the decomposition of maize
384 roots and from fresh mustard exudates contributed less to the DOC pool, which resulted in
385 lower DOC concentrations at the beginning of the 2020-2021 drainage season than those of
386 the catch-crop-seasons that followed wheat and were sown earlier. These differences between
387 cropping contexts could explain why topsoil DOC concentrations peaked at the beginning of
388 the drainage season for mustard and the multispecies crop but in the middle of the drainage
389 season for wheat.

390

391 Other factors can explain why digestate seemed to increase the differences between crops
392 more than the other treatments did. The higher DOC concentrations with digestate could have
393 been related to the digestate's chemical composition, since some digestates of pig slurry
394 contain molecules with auxin-like properties that come from the anaerobic digestion of
395 aromatic amino acids (Scaglia et al., 2017a, 2015b). The auxin effect of digestate is a lead that
396 could be explored in further studies on the digestate used in this study. Auxin can stimulate
397 root development (Saini et al., 2013), which can provide more DOC through exudation and
398 absorb more nutrients. Nitrate absorption tends to basify the rhizosphere (Bloom, 1996;
399 Morot-Gaudry et al., 2006), which can increase the solubility of organic matter (Andersson et
400 al., 2000).

401

402 Regardless of the crop, pig slurry could have provided more DOC than digestate did when
403 organic waste products were applied in spring (April-May). Based on the application rates
404 (Table 2) and concentrations of DOC in pig slurry (6.0 mg.g^{-1} ; Morvan, personal communication)
405 and digestate (3.8 mg.ml^{-1} ; Monard et al., 2020), we estimated that pig slurry and digestate
406 could have provided ca. 200 and 135 kg.ha^{-1} of DOC, respectively. Both treatments also could
407 have provided a small amount of DIC; for example, pig slurry could have provided ca. 28 kg.ha^{-1}
408 of total inorganic carbon based on a concentration of $0.84 \text{ mg inorganic C.g}^{-1}$ (Morvan,
409 personal communication). During the 8 months between treatment application and the
410 beginning of drainage, some of the DOC provided by the treatment likely mineralized. During
411 anaerobic digestion, microorganisms consume some of the more labile fraction, which
412 increases digestate concentrations of more biologically stable molecules such as lignins,
413 hemicellulose, and non-hydrolyzable lipids (Marcato et al., 2009; Tambone et al., 2009). These
414 molecules can be a source of DOC to the topsoil without undergoing much mineralization. Since
415 pig slurry may have provided more DOC, and this DOC was more bioavailable, it may have
416 resulted in higher DIC production than digestate. In summary, digestate yielded a larger
417 leachable DOC pool that could have been due to its less-degradable chemical composition and
418 its potential influence on root development and exudation via auxin-like molecules. Further
419 analyses on the dissolved organic matter composition are needed to determine which sources
420 supplied the DOC pool.

421
422 *4.2 Annual dynamics of DOC and DIC fluxes along the soil profile depending on the*
423 *treatment*

424
425 In the topsoil, mean winter DOC losses from leaching ($10.5\text{-}50.5 \text{ kg.ha}^{-1}$, over eight seasons)
426 were consistent with those observed by Kindler et al. (2011) in three European cropland
427 experiments ($31 \pm 12 \text{ kg.ha}^{-1}$). Under a catch crop, the DOC fluxes ($31.8 \pm 10.9 \text{ kg.ha}^{-1}$, over four

428 seasons) were higher than those observed by Walmsley et al. (2011) ($22 \pm 2 \text{ kg}\cdot\text{ha}^{-1}$). In the
429 subsoil, the DOC fluxes observed by Kindler et al. (2011) were higher ($30 \pm 2 \text{ kg}\cdot\text{ha}^{-1}$) than those
430 observed in the present study ($6.2 \pm 4.1 \text{ kg}\cdot\text{ha}^{-1}$, over eight seasons). In the topsoil, DIC leaching
431 losses ($50.5 \pm 19.5 \text{ kg}\cdot\text{ha}^{-1}$, over eight seasons) were more than 2 times lower than those
432 observed by Kindler et al. (2011) ($112 \pm 30 \text{ kg}\cdot\text{ha}^{-1}$) and 6 times lower than those observed by
433 Walmsley et al. (2011) ($299 \pm 39 \text{ kg}\cdot\text{ha}^{-1}$). In the subsoil, DIC fluxes ($80.0 \pm 45.7 \text{ kg}\cdot\text{ha}^{-1}$, over
434 eight seasons) were lower than those observed by Kindler et al. (2011) ($133 \pm 7 \text{ kg}\cdot\text{ha}^{-1}$).

435

436 We summarized the sources that contributed to the DOC and DIC fluxes at 40 and 90 cm depths
437 and their annual dynamics to explain the differences observed among the three treatments
438 (Figure 4). At 40 cm, DOC fluxes were higher under digestate (Figure 4.C), while DIC fluxes were
439 higher under pig slurry (Figure 4.B). As mentioned, application of pig slurry or digestate in spring
440 could explain these differences, with more DOC potentially provided by pig slurry which
441 mineralized into DIC. Chantigny (2003) highlighted an increase in soil-soluble organic C after
442 treatment application, followed by rapid decomposition, which could explain why the topsoil
443 DOC fluxes of pig slurry and mineral fertilizer did not differ (Figure 4.A). In comparison, since
444 digestate provided more recalcitrant DOC, the latter could persist longer in the soil. The crops
445 also input C into the system. As mentioned, the mustard and multispecies catch crops likely had
446 more DOC coming from fresh root exudation and decomposition from the roots of the previous
447 crop than wheat did. Some of this DOC may have been mineralized as well, and root respiration
448 could also have provided DIC (Viktor and Cramer, 2003).

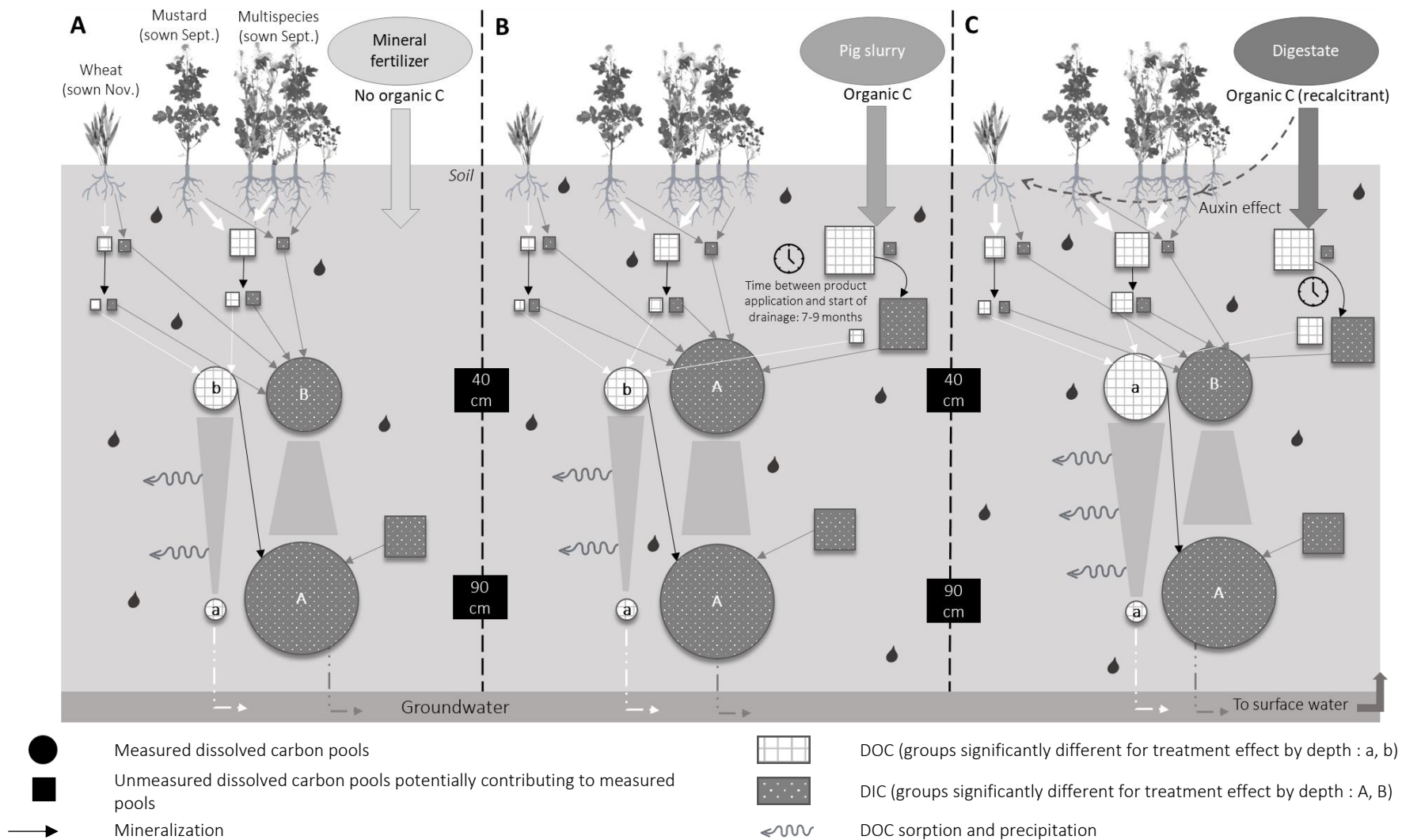
449

450 At 90 cm depth, DIC fluxes were higher, DOC fluxes were lower, and treatments no longer
451 differed. The decrease in DOC fluxes from 40 to 90 cm is consistent with the transfer of DOC

452 in the soil profile explained by Kaiser and Kalbitz (2012), in which DOC moving down soil
453 layers undergoes sorption and precipitation reactions that temporarily immobilize it and also
454 undergoes microbial biodegradation (Leinemann et al., 2018; Scott and Rothstein, 2014). The
455 imbalance in total dissolved C with pig slurry and mineral fertilizer, with an excess of DIC in
456 the subsoil, suggested an input of DIC between 40 and 90 cm. It may have come from the
457 mineralization of older organic matter that remained associated with the reactive mineral
458 phase (i.e., iron and aluminum oxides) and underwent desorption or dissolution (Kaiser and
459 Kalbitz, 2012). Moreover, wheat and mustard roots can exceed 40 cm (Aziz et al., 2017;
460 Panigrahi and Panda, 2003) and still produce DIC through respiration. The near balance in
461 total dissolved C between 40 and 90 cm with digestate suggests that the DIC pool at 90 cm
462 may simply have come from the DIC pool at 40 cm and decomposition of the biodegradable
463 portion of the DOC pool from 40 cm, with no other input. However, studies by Schmidt and
464 Martínez (2019) and Coward et al. (2018) observed preferential adsorption of aromatic or
465 hydrophobic molecules on the reactive mineral phase. Thus, the aromatic molecules in
466 digestate (Marcato et al., 2009) can be retained more easily in the soil than those in pig slurry
467 and mineral fertilizer, which contain lower concentrations of aromatic molecules. Additional
468 DIC from the mineralization of older organic matter (Kaiser and Kalbitz, 2012) and root
469 respiration, which can increase if the roots are more developed due to the auxin effect
470 (Scaglia et al., 2017a, 2015b), could have compensated for the C lost through sorption and
471 precipitation and thus approached a balance in total dissolved C. These processes may explain
472 why the difference between treatments in the topsoil decreased as the soil water passed
473 through the soil profile. The DOC that became associated with the mineral phase could
474 remain stored in the subsoil for a long period (Rumpel and Kögel-Knabner, 2011), particularly

475 with digestate, due to its potential high concentration of preferentially adsorbed molecules.

476 This hypothesis could be supported by analyzing the composition of the digestate used.



477

478 Figure 4. Conceptual diagram of the potential pools that contribute to the dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) pools measured
479 at 40 and 90 cm depths and explain the differences between three treatments: (A) mineral nitrogen fertilizer, (B) pig slurry, and (C) digestate from pig slurry.

480 **Conclusion**

481 This study highlighted that application of digestate from pig slurry, depending on the cropping
482 context, increased the topsoil DOC concentration, particularly under a catch crop. The
483 differences in DOC concentrations among treatments, with higher concentrations with
484 digestate application, occurred at distinct moments during the drainage season depending on
485 the crop: from the beginning of the season for mustard and multispecies catch crops and
486 around February for wheat. Topsoil DIC fluxes were higher with pig slurry. In the subsoil, the
487 treatments did not differ, having low DOC fluxes and high DIC fluxes.

488 The present study provided new references for both DOC and DIC fluxes for several organic
489 waste products and crops, with good repeatability over 9 drainage seasons. In particular, it
490 added new insights into higher DOC fluxes after application of digestate than after that of its
491 original pig slurry or a mineral fertilizer, which did not differ. This study also has the advantage
492 of exploring dissolved fluxes in both the topsoil and the subsoil.

493

494 **Declaration of Competing Interest**

495 The authors declare that they have no known competing financial interests or personal
496 relationships that could have appeared to influence the work reported in this article.

497

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499

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513

514

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