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1 **Can stable isotopes quantify soil carbon build-up from organic fertilizers?**

2 Floriane Jamoteau^{abc*}, Jérôme Balesdent^{ct}, Isabelle Basile-Doelsch^c, Emmanuel
3 Tillard^{de}, Antoine Versini^{ab}

4 ^a*CIRAD, Internal Research Unit (UPR) Recycling and Risk, Station de La Bretagne, 97743 Saint-*
5 *Denis, Réunion, France;* ^b*Internal Research Unit (UPR) Recycling and Risk, Université de*
6 *Montpellier, CIRAD, Montpellier, France;* ^c*CEREGE, Aix-Marseille Université, CNRS, IRD, Collège*
7 *de France, INRA, F-13545 Aix-en-Provence, France;* ^d*CIRAD, Joint Research Unit (UMR)*
8 *Mediterranean and Tropical Livestock Systems, 97410 Saint-Pierre, Réunion, France;* ^e*Joint Research*
9 *Unit (UMR) Mediterranean and Tropical Livestock Systems, Université de Montpellier, CIRAD,*
10 *Montpellier, France*

11 * Corresponding author: jamoteau@cerege.fr

12 [†] *deceased, 19 July 2020*

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14 **Can stable isotopes quantify soil carbon build-up from organic fertilizers?**

15 Application of organic fertilizers (OF) can supply carbon (C) to the soil in crop fields.
16 OF-derived C (OF-C) is often estimated using the differential method that can be biased
17 due to indirect effects of OF on soil C. This study tested three methods to quantify OF-
18 C: (i) the widespread differential method, (ii) the synchronic isotopic method comparing
19 plots with and without OF and (iii) the asynchronous isotopic method mimicking a trial
20 without a control plot. These methods were implemented on an Arenosol and an
21 Andosol supplied during 13 years with slurry or compost. The results highlighted the
22 relevance of using the isotopic synchronic method, which focuses on the direct effect of
23 OFs on the soil organic matter (without bias of vegetation change) and considers control
24 soil's evolution. Considering limit of resolution in the Arenosol, the method was
25 suitable after 4 years of fertilisation for an initial $\delta^{13}\text{C}$ difference of 7.5 ‰ between OF
26 and soil, and after 9 years for a difference of 3.5 ‰. A sensitivity analysis showed that
27 particular attention must be paid to OF- $\delta^{13}\text{C}$ variability to guarantee the method
28 validity. The method proved to be suitable to study the factors controlling the OF-C fate
29 in tropical soils.

30 Keywords: carbon, carbon stable isotopes, ^{13}C , organic fertilizers, Arenosol, Andosol

31 **1. Introduction**

32 Ensuring high organic carbon contents in agricultural soils appears to be an effective
33 way of meeting the triple challenge of food security, adaptation to climate change and
34 mitigation of anthropogenic emissions (4p1000 initiative, [1]). Organic fertilization—
35 currently encouraged by the development of the circular economy—is a key means of
36 increasing organic C stocks in agricultural soils. In agroecosystems benefitting from
37 organic fertilizer (OF) amendments, there are three main sources of soil organic C (Figure
38 1): (1) native soil organic matter (SOM) (derived from C inputs preceding organic
39 fertilization; initial-C), (2) carbon derived from crops (via aboveground and belowground
40 C inputs provided by crops; crop-C), and (3) carbon from OFs (OF-C).

41 Carbon isotope methods using ^{14}C isotopes [2], [3] or stable ^{13}C isotopes [4]–[6] help track
42 the fate of C in various ecosystems. There are natural variations in the $^{13}\text{C}/^{12}\text{C}$ ratio according

43 to the photosynthesis pattern in two types of plants: C₃-plants with a $\delta^{13}\text{C}$ value in the -29 to -
44 25 ‰ range, and C₄-plants with a $\delta^{13}\text{C}$ value in the -16 to -12 ‰ range [7], [8] . These
45 natural $\delta^{13}\text{C}$ variations can also be attributed to various factors as stomata regulation by
46 microclimate or nitrogen availability [9], [10] or carbon gain from mycorrhizal fungi [11]–
47 [13]. $\delta^{13}\text{C}$ has been used to quantify carbon changes throughout soil profiles, leading to a C
48 age distribution to 1 m depth [14], but also on various soil components and soil fractions
49 [15]–[17]. Quantification of OF-C contribution is generally calculated by a stock difference or
50 comparison: the C stock in the control soil at a given depth (often 0-30 cm) is subtracted from
51 the C stock in the fertilized soil at an equivalent depth [18]–[21]. But the C-stock difference
52 does not take into account the effect of OFs on native SOM mineralization rates (i.e. so-called
53 priming effect) neither crop-C inputs if vegetation's changes occurred [22], [23]. In the same
54 way that isotopic mass budget are used to trace C₃/C₄ vegetation changes, $\delta^{13}\text{C}$ can be used
55 to monitor long-term carbon inputs and OF impacts on soil carbon [8], [24]–[27]. However,
56 isotopic mixing budgets can be applied only if the isotopic resolution limit is reached (i.e
57 there is a statistical significant difference between isotopic signatures of the end-members)
58 and if the sensitivity of the different parameters of the isotopic equation is analysed [8].

59 Several studies that have quantified OF-C using the isotopic method are summarized in
60 Table 1 as a function of the experimental duration and sampling frequency. Short-term
61 experiments (up to 120 days) have been carried out using field experimental designs [28]–[31]
62 as well as in mesocosms (through $\delta^{13}\text{C}$ of CO₂) [32], [33]. Two stages of decomposition
63 processes were observed over time [32], [30]: a first stage corresponding to the
64 biodegradation of a readily available and more liquid phase accumulated over time in the
65 upper soil; a second phase, 48 h after OF amendment, corresponded to the biodegradation of
66 the slurry solid phase [30].

67 However, only few isotopic studies have been focused on long-term OF retention [34]–

68 [37]. All of them, except Nicoloso et al., 2018 [37], focused on a temperate climatic zone.
69 Long-term single quantification experiments have been conducted over 1 year [38], 25 years
70 [37] and 42 years [39]. However, single quantification did not reveal temporal changes in soil
71 C dynamics over time. Long-term chronosequence experiments have also been carried out
72 [34]–[36]. Kristiansen *et al.* (2005) [35] and Thomsen and Christensen (2010) [36] used
73 aboveground fresh maize biomass and sheep faeces to amend four Danish fields (including an
74 Alfisol Hapludalf and an Inceptisol Orchrept) over periods of 9 and 14 years, respectively
75 (with total input of 32,000 to 51,100 kg C.ha⁻¹). Gerzabek *et al.*, 1997 [34] applied animal
76 manure on a Swedish Eutric Cambisol over 42 years (with total input of 84 000 kg C.ha⁻¹)
77 (Table 1). These studies showed: (1) that OF- $\delta^{13}\text{C}$ may vary in long-term experiments [34],
78 and (2) that C derived from OFs and vegetation had jointly contributed 13-55 % of the soil C
79 at the end of the experiments [34]–[36].

80 To the best of our knowledge, no long-term chronosequence studies involving the $^{13}\text{C}/^{12}\text{C}$
81 isotopic proxy have been performed in tropical or subtropical environments to track the fate
82 of OF-C in soils. Natural variations in ^{13}C are highly prevalent in tropical areas under C₃
83 forests and C₄ crops. As mineralization and degradation processes are faster in tropical
84 conditions, the isotopic method should be relevant for studying long-term dynamics of OF-C
85 incorporation into tropical soils. A 13-year grassland trial was conducted on an Arenosol and
86 on an Andosol with repeated fertilization using slurry and compost in a tropical area
87 (Réunion, France). We tested the applicability of the ^{13}C isotopic method for quantifying soil
88 C inputs through long-term OF amendments in tropical soils. Tree quantification methods
89 were tested in this study: (1) the widespread differential method; (2) the isotopic budget in a
90 synchronous calculation system with a comparison of amended and unamended control plots
91 (the synchronic method) and (3) the isotopic budget in an asynchronous system with
92 amended plots and plots at the start of the experiment (asynchronic method, experimental

93 design without control plots).

94 **2. Material and methods**

95 **2.1 Study sites**

96 The experiment was conducted on the volcanic island of Réunion (Indian Ocean) on two
97 different soils [40]: (1) an Arenosol, i.e. a typically sandy soil located in the southwestern part
98 of the island around Saint-Joseph (21°22'57'' S; 55°36'28'' E), 10 m a.s.l with about 1000-
99 1500 mm rainfall and mean annual temperature of 23.3°C. The soil was under *Chloris*,
100 *Paspalum* and *Panicum* cover (C₄-plants); (2) an Andosol, i.e. a soil containing allophanes
101 which are nanometric aluminosilicates resulting from the alteration of volcanic glasses,
102 located in the southern-central part of the island (21°12'60'' S; 55°34'45'' E) around La
103 Plaine des Cafres, 1,600 m a.s.l with about 1500 to 2000 mm rainfall and a mean annual
104 temperature of 14.2°C. The soil was under *Festuca*, *Dactylis* and *Bromus* cover (C₃-plants).

105 **2.2 Experimental design**

106 For both sites, the experimental design included three different fertilization treatments
107 applied from 2005 to 2017 and repeated in three block. Plots measured 3.6 m² and were
108 separated by 0.3 m wide edge strips (Figure S1). The composition of the three treatments
109 were: bovine manure compost and bovine slurry as two OF treatments and mineral
110 fertilization as control treatment. For convenience, bovine manure compost and bovine slurry
111 are respectively called compost and slurry hereafter. OFs were applied on plots when pastures
112 were harvested (6-8 times per year). OF inputs were estimated at around 9,060 kg C.ha.⁻¹yr⁻¹
113 for bovine manure compost and 19,760 kg C.ha.⁻¹yr⁻¹ for bovine slurry (considering a mean of
114 seven applications). The same amount of the same OF was applied at both sites (Table 2).
115 Mineral fertilization consisted of 120 kg of mineral N. No carbon was added in this mineral
116 fertilization modality so these plots were considered as controls in the isotopic calculations. C

117 in control plot soils thus included C from the initial SOM and C input from vegetation
118 throughout the experiment (Figure 1).

119 ***2.3 Soils and organic fertilizers sampling***

120 Amended soils and control soils were sampled yearly from years 3 to 13 (experiment
121 started at year 0, i.e. 2004) for the Arenosol and from years 1 to 13 for the Andosol. The top
122 15 cm of soil was sampled with an auger in November or October each year before OF
123 application. OFs were sampled yearly from years 2 to 9. Soils and OF were dried at 60°C and
124 sieved to 2 mm before storage at 20°C. Subsamples (5 g) were dried and ground in a mixer
125 mill (MM400, Retsch) to ensure the overall representativeness of the analyzed samples.

126 As there was an insufficient quantity of remaining soil, control plot samples were not
127 analysed from the Arenosol block 1 in year 3, from the Andosol block 3 in year 10 and
128 control plots from the three Andosol blocks in year 2.

129 ***2.4 C analyses and C stock calculations***

130 The carbon content was measured in air dried samples using elemental analyzer isotopic
131 ratio mass spectrometry (Flash EA 1112 Series and ThermoFinnigan DELTApplus IRMS). 10-
132 20 mg and 3-6 mg samples were analysed for the Arenosol and the Andosol, respectively.
133 IAEA-CH-6 was used as a control of the measurement accuracy and IAEA-CH-7 as an
134 international lab standard [41]. Analysed soil humidity ranged about 1.7 % on the Arenosol
135 and 8.9 % on the Andosol (dried at 105°C). Thus, a carbon content correction link to residual
136 water were perform following the C analysis

137 Soil carbon stocks were assessed by equation (1).

$$138 \quad S = \rho * e * C \quad (1)$$

139 where S is the stock in kg C.m^{-2} , ρ is the bulk density in g.cm^{-3} , e is the soil thickness (0.15 m)

140 and C is the carbon content in g C.kg^{-1} soil. Bulk density measurements were performed on
 141 the Arenosol (5-10 cm) in year 0 and year 14 and on the Andosol (0-10 cm) in year 3 and year
 142 16. We used the Alexander law to estimate the bulk density over time (equation 2, Figure S2,
 143 [42]). Alexander coefficients underwent least squares adjustment on carbon linear regression
 144 data, by fitting both measured values in each soils.

$$145 \quad \rho = A_{coef} - A_{slope} * \sqrt{C} \quad (2)$$

146 with A_{coef} Alexander coefficient, A_{slope} the Alexander slope and C the total carbon content in
 147 gC.kg^{-1} soil. The bulk density data are given in Figure S2.

148 **2.5 $\delta^{13}\text{C}$ analysis and $\Delta\delta^{13}\text{C}$ calculations**

149 $\delta^{13}\text{C}$ Isotope measurements were done using the same elemental analyzer isotopic ratio
 150 mass spectrometry procedure as for the carbon content analysis. IAEA-CH-6 (-10.5 ‰) was
 151 used for calibration and IAEA-CH-7 as measurement of precision (control yielded as mean of
 152 -32.15 ± 0.15 ‰) [41]. $\delta^{13}\text{C}$ was calculated as follows:

$$153 \quad \delta^{13}\text{C}(\text{‰}) = \left(\frac{\left(\frac{^{13}\text{C}}{^{12}\text{C}} \right)_{sample}}{\left(\frac{^{13}\text{C}}{^{12}\text{C}} \right)_{reference}} - 1 \right) * 1000 \quad (3)$$

154 VPDB was used as reference standard for $\delta^{13}\text{C}$ units.

155 $\Delta\delta^{13}\text{C}$ is the difference between initial $\delta^{13}\text{C}$ of control plots at year 0 ($\delta^{13}\text{C}_{CTLsoilT0}$, CTL stands
 156 for control) and vegetation $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{veg}$) or difference between $\delta^{13}\text{C}_{CTLsoilT0}$ and OF- $\delta^{13}\text{C}$
 157 ($\delta^{13}\text{C}_{OF}$) such as:

$$158 \quad \Delta\delta^{13}\text{C}(\text{‰}) = \left| \delta^{13}\text{C}_{CTLsoilT0} - \delta^{13}\text{C}_{veg} \text{ or } \delta^{13}\text{C}_{OF} \right| \quad (4)$$

159 **2.6 Estimation of OF-C proportions in amended soils**

160 Three methods for quantifying OF-C were tested in this study: the differential method (the

161 most common), the synchronic isotopic method and the asynchronous isotopic method.

162 2.6.1 Differential method

163 The differential method estimates the proportion of OF-derived carbon (f_{COF} , equation 5) by
164 dividing the OF-C ($C_{OFsoil} - C_{CTLsoil}$) by the total carbon of the amended plots (C_{OFsoil}).

$$165 f_{COF} = \frac{C_{OFsoil} - C_{CTLsoil}}{C_{OFsoil}} \quad (5)$$

166 The carbon stock ($\text{kgC} \cdot \text{ha}^{-1}$ in 0-15 cm) derived from OF (S_{COF}) was calculated with equation
167 (6) where S is the total carbon stock ($\text{kg C} \cdot \text{ha}^{-1}$):

$$168 S_{COF} = S * f_{COF} \quad (6)$$

169 2.6.2 Synchronic isotopic method

170 In the synchronic isotopic method, the proportion of OF-derived C was calculated using the
171 mixing model (7) (adapted from Balesdent *et al.*, 1987 [4]):

$$172 f_{COF} = \frac{\delta^{13}C_{OFsoil} - \delta^{13}C_{CTLsoil}}{\delta^{13}C_{OF} - \delta^{13}C_{CTLsoil}} \quad (7)$$

173 where f_{COF} is the proportion of OF-C, $\delta^{13}C_{OFsoil}$ is the isotopic signature of the amended soil,
174 $\delta^{13}C_{CTLsoil}$ is the isotopic signature of soil under mineral fertilization and $\delta^{13}C_{OF}$ is the isotopic
175 signature of the applied OF. By this synchronic method, f_{COF} was estimated on a yearly basis,
176 with the same sampling year of $\delta^{13}C_{CTLsoil}$ and $\delta^{13}C_{OFsoil}$. The carbon stock ($\text{kg C} \cdot \text{ha}^{-1}$ in 0-15
177 cm) derived from OF (S_{COF}) was calculated with equation 6.

178

179 In this study, samples from year 3 in block 1 were missing for the Arenosol, as well as from
180 years 2 and 10 in block 3 for the Andosol. The calculation of f_{COF} was therefore not possible
181 for those years.

182 **2.6.3 Asynchronic isotopic method**

183 In the asynchronic isotopic method, the proportion of OF-derived C was calculated by the
184 mixing model (8) (adapted from Balesdent *et al.*, 1987) :

185
$$f_{COF} = \frac{\delta^{13}C_{OFsoil} - \delta^{13}C_{CTLsoilT0}}{\delta^{13}C_{OF} - \delta^{13}C_{CTLsoilT0}} \quad (8)$$

186 where f_{COF} is the proportion of OF-C, $\delta^{13}C_{OFsoil}$ is the isotopic signature of amended soil,
187 $\delta^{13}C_{CTLsoilT0}$ is the isotopic signature of soil under mineral fertilization at year 0 and $\delta^{13}C_{OF}$ is
188 the isotopic signature of the applied OF.

189 Samples from year 0 were missing, so we estimated the $\delta^{13}C_{CTLsoilT0}$ of each plot using linear
190 regressions (Figure 2). We performed a linear regression whereby all control plots for a given
191 soil type were considered and kept the same slope but change the initial $\delta^{13}C$ of year 0 block
192 wise (Figure 2). Each control plot thus had its own $\delta^{13}C$ estimation over time with linear
193 regression being applied to reduce experimental variation (see Discussion part 4.1.2). The
194 carbon stock ($kgC \cdot ha^{-1}$ in 0-15 cm) derived from OF (S_{COF}) was calculated with equation 6.

195 **2.7 Statistical analysis and overcoming block effect**

196 All statistical analyses were performed with *lm* and *t.test* functions of the R software package
197 (R Core Team, 2016). To ensure the limit of resolution of the three methods (used in Figure
198 4), t-test were performed : between total C content of controls and amended plots for the
199 differential method, between $\delta^{13}C_{CTLsoil}$ and $\delta^{13}C_{OFsoil}$ for the isotopic method and between
200 $\delta^{13}C_{CTLsoilT0}$ and $\delta^{13}C_{OFsoil}$ for the asynchronic isotopic method. T-test between $\delta^{13}C_{CTLsoil}$ and
201 $\delta^{13}C_{OF}$ were performed to discuss resolution and sensitivity analysis for isotopic methods (see
202 part 4.2).

203 **2.8 Sensitivity analysis of the isotope mixing equation**

204 We ranked the impact of each variable of the mixing equation 7. We first determined the

205 percentage deviation from the mean (standard deviation divided by the mean on T13) for the
206 three parameters: $\delta^{13}\text{C}_{\text{OFsoil}}$, $\delta^{13}\text{C}_{\text{CTRLsoil}}$ and $\delta^{13}\text{C}_{\text{OF}}$. Then we carried out a random selection
207 of 1000 values for the three variables according to the deviation percentage calculated in T13.
208 Using equation 7, 1000 f_{COF} values were thus obtained for the Arenosol compost, Arenosol
209 slurry and Andosol compost treatments.

210 The weight of each variable in the mixing equation was obtained thanks to the *anova* function
211 in the R software package (R Core Team, 2016), using the 3x1000 random variables from
212 $\delta^{13}\text{C}_{\text{OFsoil}}$, $\delta^{13}\text{C}_{\text{CTRLsoil}}$ and $\delta^{13}\text{C}_{\text{OF}}$ and the log (f_{COF}).

213

214 **3. Results**

215 **3.1 Control soil C content and $\delta^{13}\text{C}$**

216 The organic carbon content in Arenosol control plots (under mineralization) increased from
217 $10.5 \pm 0.0 \text{ g.kg}^{-1}$ in year 3 to $17.9 \pm 1.8 \text{ g.kg}^{-1}$ in year 13. The control plots $\delta^{13}\text{C}$ (Figure 2a)
218 increased over time. According to linear regressions, $\delta^{13}\text{C}$ increased from -18.2 ‰ to -16.8‰
219 in block 1, from -17.0‰ to -15.6‰ in block 2 and from -17.0‰ to -15.5‰ in block 3. The
220 Arenosol controls showed the same overall variation pattern over time but with a slight shift
221 in the initial value for estimated year 0. Including all block, the $\delta^{13}\text{C}$ from year 0 (-18.2 to -17
222 ‰) indicated that the organic matter content was from mixed C₃ and C₄ origins, with a
223 predominance of C₄. Vegetation $\delta^{13}\text{C}$ in this Arenosol trial was about -13.9 ‰ (Table 2).

224 The organic carbon content in Andosol control plots increased from $112 \pm 7 \text{ g.kg}^{-1}$ in year 1 to
225 $126 \pm 7 \text{ g.kg}^{-1}$ in year 13. The control plot $\delta^{13}\text{C}$ remained stable over time (Figure 2b).
226 According to linear regressions, $\delta^{13}\text{C}$ values were about -23.8 ‰ to -24.5 ‰ in the three
227 blocks. Block 3 had a slight shift in the initial value for estimated year 0. Including all block,
228 the $\delta^{13}\text{C}$ from year 0 (-23.8 to -24.1 ‰) indicated that the organic matter content was from

229 mixed C₃ and C₄ origins, with a predominance of C₃. Vegetation $\delta^{13}\text{C}$ in this Andosol trial
230 was about -27.8 ‰ (Table 2).

231 **3.2 Organic fertilizer $\delta^{13}\text{C}$**

232 OF and trials vegetation characteristics are summarized in Table 2. The OF signatures were
233 about -20.3 ± 2.8 ‰ for compost and -24.4 ± 1.3 ‰ for slurry over the eight study years (years 2
234 to 9; Table 2; Figure S3). In the Arenosol trial, the $\delta^{13}\text{C}$ difference between the Arenosol (at
235 T0) and applied organic fertilizers ($\Delta\delta^{13}\text{C}$) were from 2.9 ± 0.4 ‰ with compost and 6.9 ± 0.4
236 ‰ with slurry. As OFs- $\delta^{13}\text{C}$ was from -24.4 to -20.3 ‰ and vegetation- $\delta^{13}\text{C}$ of -13.9 ‰,
237 quantification of OF-C avoided C from vegetation in equation 7. Differences between
238 $\delta^{13}\text{C}_{\text{soilT0}}$ and OFs- $\delta^{13}\text{C}$ for the Arenosol trial were high enough for isotopic OF-C
239 quantification.

240 In the Andosol trial, the $\delta^{13}\text{C}$ difference between the Andosol (at T0) and applied organic
241 fertilizers ($\Delta\delta^{13}\text{C}$) was about 3.8 ± 0.1 ‰ with compost and 0.2 ± 0.1 ‰ with slurry. As OFs-
242 $\delta^{13}\text{C}$ was from -24.4 to -20.3 ‰ and vegetation- $\delta^{13}\text{C}$ of -27.8 ‰, quantification of OF-C
243 avoided C from vegetation either in equation 7. Differences between $\delta^{13}\text{C}_{\text{soilT0}}$ and compost-
244 $\delta^{13}\text{C}$ in the Arenosol trial were high enough for isotopic OF-C quantification. However, as
245 $\delta^{13}\text{C}$ from $\delta^{13}\text{C}_{\text{soilT0}}$ with slurry was close, isotopic quantification of OF-C only failed.

246 **3.3 $\delta^{13}\text{C}$ and carbon content of OF-amended soils**

247 The temporal change of $\delta^{13}\text{C}$ of the Arenosol (Figure 3a) trial showed that amended soils
248 diverged from controls over time. In year 3, mean $\Delta\delta^{13}\text{C}$ between controls to compost
249 amended plots was about 1.0 ± 0.2 ‰ and in slurry amended plots about 1.4 ± 0.05 ‰. In year
250 13, mean $\Delta\delta^{13}\text{C}$ reached 2.4 ± 0.3 ‰ in compost amended plots and 3.3 ± 0.1 ‰ in slurry
251 amended plots. In the Andosol trial, with regard to $\delta^{13}\text{C}$, only compost amended plots
252 significantly differed from control plots over time (Figure 3b). In year 1, mean $\Delta\delta^{13}\text{C}$ between

253 controls to compost amended plots was about 0.9 ± 0.1 ‰ (about 0.3 ± 0.1 ‰ in slurry amended
254 plots). In year 13, mean $\Delta\delta^{13}\text{C}$ reached 1.8 ± 0.1 ‰ in compost amended plots (0.2 ± 0.0 ‰ in
255 slurry amended plots).

256 Meanwhile the total carbon content of the Arenosol increased from 12.1 ± 1.3 to 38.6 ± 6.1
257 g.kg^{-1} between years 3 and 13 in the compost amended plots (Figure 4a) and from 13.2 ± 1.4 to
258 29.5 ± 0.1 g.kg^{-1} in the slurry amended plots (Figure 4b). The total carbon content of the
259 Andosol increased from 111.3 ± 3.9 to 152.7 ± 5.9 g.kg^{-1} between years 1 and 13 in the compost
260 amended plots (Figure 4c) and from 107.1 ± 4.7 to $136.2.1 \pm 6.9$ g.kg^{-1} in the slurry amended
261 plots (Figure 4d).

262 ***3.4 Quantification of soil OF-C via the differential, isotopic synchronic and isotopic*** 263 ***asynchronic methods***

264 In Figure 4, to fully understand the significance of the three methods, we represented every
265 quantification method and displayed its significance by t-test student using stars (see method
266 part 2.7).

267 In the Arenosol with compost inputs, compost-C quantifications increased over time
268 (Figure 4a), i.e. in year 13, 53 % with the differential method, 56 % with the isotopic
269 synchronic method and only 31 % with the asynchronic isotopic method. The p-value were
270 significant only in the final 2 years (years 12 and 13) with the differential method and from
271 year 9 to 13 with the isotopic synchronic method. In the Arenosol with slurry inputs, slurry-C
272 contribution also increased over time (Figure 4b), i.e. in year 13, 39 % with the differential
273 method, 40 % with the isotopic synchronic method and only 28% with the asynchronic
274 isotopic method. The p-values were more significant in the final years for the three methods
275 and the most significant using the synchronic isotopic method. In the last year, in the
276 Arenosol trial, the synchronic isotopic methods estimated more OF-C than the other methods

277 and was more p-values significant. The OF-C estimations were roughly the same for the
278 differential method and lower for the asynchronous method.

279 In the Andosol with compost inputs, compost-C contribution also increased over time
280 (Figure 4c), i.e. in year 13, 17 % with the differential method, 43 % with the isotopic
281 synchronic method and 36% with the asynchronous isotopic method. The differential method
282 revealed lower OF-C proportions over the years. All methods were p-value significant from
283 year 7 to year 13, the last year all methods had the same significance. In the Andosol with
284 slurry inputs, as it was not possible to use the isotopic methods, OF-C was only estimated by
285 the differential method (Figure 4d). Slurry-C did not significantly increase over time. In year
286 13, the estimation reached 8 % and the p-values were not significant.

287 Using the synchronic method and only significant quantifications years (Figure 4; p-values
288 <0.05), the OF-C stock estimations were plotted against total OF inputs (Figure 5). OF-C
289 content in the Arenosol was higher in compost amended plots than in slurry amended plots
290 (23% and 8%, respectively). The final carbon stock in 0-15 cm (meaning retention percentage
291 from linear regressions of Figure 5) in the compost amended Arenosol was about 27,094
292 $\text{kgC}\cdot\text{ha}^{-1}$ and 20,552 $\text{kgC}\cdot\text{ha}^{-1}$ with slurry inputs. Compost-C content in the Andosol was
293 higher than in the Arenosol (36 % and 23 %, respectively). Andosol and Arenosol only
294 differed with respect to the soil type and climatic conditions; the same compost was spread
295 over this 13-year experiment. The final compost-C stock in the Arenosol reached 27,094
296 $\text{kgC}\cdot\text{ha}^{-1}$ and 42,408 $\text{kgC}\cdot\text{ha}^{-1}$ in the Andosol.

297 **4. Discussion**

298 *4.1 Relevance of the 3 methods for quantifying OF contribution to SOM*

299 **4.1.1. Isotopic methods focuses on the direct effect of OFs on the SOM**

300 The synchronic isotopic method estimated more OF-C than the differential method in the

301 Arenosol and Andosol trials (Figure 4). Lynch *et al* [38] also found that the synchronic
302 isotopic method estimated higher proportion of OF-C than the differential method among
303 three fertilization treatments (i.e. corn silage compost, dairy manure compost and sewage
304 sludge compost). This difference in OF-C proportion could be explained by two non-
305 exclusive factors:

306 (i) The repeated OF applications that may have modified the vegetation and its C
307 inputs. It has been reported that OF can alter the plant community composition
308 [43], [44] leading to different C inputs relative to control plots [45], [46]. In the
309 Andosol trial, modification of the vegetation composition was revealed by the
310 replacement of *Dactylis* (C₃ plant-group) in the mineral-fertilized control plots, and
311 of *Bromus* (C₃ plant-group) in the plot with OF amendment (Tillard, pers. comm.).
312 Lower belowground biomass production in the OF plots would likely result in
313 underestimation of the direct OF contribution to SOM with the differential method
314 (as measured in the Andosol, Figure 4c). In the Arenosol trial, the vegetation
315 composition did not change during the experiment (Tillard, pers. comm.).

316 (ii) Mineralization of the initial soil organic C (Figure 1) could be higher in the OF-
317 amended plots, e.g. through a priming effect [22], [23]. The priming effect involves
318 significant changes in soil organic matter renewal [22], possibly caused by OF
319 input. The latter could accelerate mineralization of the initial C and thus gradually
320 replace part of this fraction. In such cases, the direct contribution of the OF would
321 be underestimated by the differential method. Organic fertilization is a common
322 practice leading to considerable input of relatively labile C into agricultural soils.
323 However, studies dealing with OF-induced SOM priming-effect remain very scarce
324 most likely due to the methodological challenge of labeling OF with ¹³C.

325 To conclude, the differential method based on total carbon did not allow quantification of

326 OF-C regardless of vegetation changes or mineralization of initial soil organic C. The
327 isotopic method, on the other hand, measures the proportion of each element by the
328 isotopic signature. It can directly measure the fate of C contributed by OFs without
329 artefacts related to indirect effects. Where the differential approach measures the overall
330 effect of the organic fertilization practice, the isotopic approach measures the fate of C
331 from the OF in the soil and ignores the indirect effects of the practice (e.g. changes of
332 vegetation or priming).

333 **4.1.2. Synchronic isotopic method take into consideration SOM evolution in the control**

334 The synchronic isotopic method estimated significantly more OF-C than the asynchronous
335 isotopic method in the Arenosol trial (Figure 4a-b). Differences were smaller in the Andosol
336 trial (Figure 4c). The distinction between the asynchronous and synchronic method was the
337 control value used in the mixing model (equations 7 and 8). By the asynchronous method, it
338 was considered that the $\delta^{13}\text{C}$ signature of the control soil over time was equal to the $\delta^{13}\text{C}$ of
339 the amended plot before OF application. By the synchronic method, the $\delta^{13}\text{C}$ of the amended
340 and control soils were measured on the same sampling date, on two adjacent plots. Variations
341 in the control $\delta^{13}\text{C}$ could thus be subtracted when implementing this method. A change in the
342 $\delta^{13}\text{C}$ pattern in the control soils over time was observed (Figure 2) and attributed to C input
343 from vegetation (SOM from vegetation in Figure 1) during the experiment. The impact of the
344 vegetation on control soil $\delta^{13}\text{C}$ variation pattern was larger in the Arenosol than in the
345 Andosol (Figure 3). The asynchronous method cannot integrate vegetation changes during the
346 time of the experiment, which probably explain the high differences of C quantification
347 observed between asynchronous and synchronic methods for the Arenosol. Due to the temporal
348 changes in the controls, the synchronic method appeared therefore as the most accurate
349 isotopic method when control plots are available. The asynchronous method is nevertheless
350 more cost-effective because there are fewer samples to analyse than with the synchronic

351 method. The experiment is also simpler to implement because it does not require a control
352 plot but just requires the soil signature prior to OF spreading. However, the asynchronous
353 method can be used with confidence only if the soil is at isotopic equilibrium (control plot
354 $\delta^{13}\text{C}$ constant over the course of the experiment).

355 In our case, the synchronic isotopic method was better for determining the OF field
356 potential to supply SOM. The differential method could be used to estimate the overall effect
357 of organic fertilization, integrating both direct and indirect effects of OF applications. The
358 differential method remains the simplest to carry out, whereas the isotopic method can only be
359 applied with an appropriate $\delta^{13}\text{C}$ system (OF- $\delta^{13}\text{C}$ differentiated from other $\delta^{13}\text{C}$ sources). Yet
360 sometimes in case of minor OF-C inputs, the total carbon content is not enough to
361 differentiate amended plots from controls [29].

362 ***4.2 Resolution and sensitivity of the OF isotopic method***

363 The synchronic isotopic method was found to be the most suitable in our study, but certain
364 precautions should be taken regarding its use. As stated by the pioneers of the ^{13}C natural
365 abundance method, it is necessary to ensure that the limit of resolution is exceeded and to
366 assess the risk of bias due to error propagation [4], [8].

367 The limit of resolution is reached when the effects of OF application on SOM is sufficiently
368 strong that a significant difference in the considered parameter can be established between
369 two samples. This requirement applies equally to the isotopic method (parameter is $\delta^{13}\text{C}$) and
370 to the differential method (parameter is TOC). For the isotopic method, it is therefore
371 necessary to ensure that the $\delta^{13}\text{C}_{\text{OF}}$ is significantly different from the $\delta^{13}\text{C}_{\text{CTRLsoil}}$. Even more
372 importantly, the $\delta^{13}\text{C}_{\text{OFsoil}}$ and $\delta^{13}\text{C}_{\text{CTRLsoil}}$ should be significantly different to compute from
373 the mixing model the proportion of OF-derived soil-C at a given date. The first condition (t-
374 test between $\delta^{13}\text{C}_{\text{OF}}$ and $\delta^{13}\text{C}_{\text{CTRLsoil}}$) was acquired for all sampled years. The second condition

375 (t-test between $\delta^{13}\text{C}_{\text{OFsoil}}$ and $\delta^{13}\text{C}_{\text{CTRLsoil}}$) was not acquired for some years at the beginning of
376 the experiment, which is why the final OF-C retention by isotopes was not calculated on these
377 years (Figure 5). According to the isotopic mixing equations (equations 7 and 8), the
378 quantification accuracy is higher when the OF signature differs from that of the soil. The
379 standard deviation of OF-C quantification between the three plots decreased as the $\Delta\delta^{13}\text{C}$
380 increases. The OF must have a high soil $\Delta\delta^{13}\text{C}$ and a stable temporal signature in time to
381 ensure accurate quantification from the beginning of the experiment.

382 Whatever the number of repetitions performed, attention should be paid to the risk of bias
383 due to the propagation of errors in the mixing equation [8]. The sensitivity analysis (see part
384 2.8) of the mixing equation according to natural variations in the parameters revealed that the
385 variable with the least impact was $\delta^{13}\text{C}_{\text{CTRLsoil}}$, then $\delta^{13}\text{C}_{\text{OFsoil}}$, whereas $\delta^{13}\text{C}_{\text{OF}}$ was the
386 variable with the greatest impact (Table S2).

387 Variability in the $\delta^{13}\text{C}_{\text{CTRLsoil}}$, although low, could be attributed to the block variability
388 (Figure 2; block 1 for the Arenosol and block 3 for the Andosol). In the Arenosol trial, the
389 maximum $\delta^{13}\text{C}$ variation between blocks was up to 1.2 ‰ and up to 0.4 ‰ in the Andosol
390 trial. This block variability revealed short-scale variability. The Arenosol trial was conducted
391 in a high school test plot that had been cultivated for a long time. The cropping history may
392 vary over short distances. A solution would be to process blocks independently. Reduce the
393 distance between controls and OF amended plots could lower the $\delta^{13}\text{C}$ spatial variability.
394 Working on a per-block basis reduced our spatial variability but it would be essential to check
395 a similar signature of the unamended plots prior to the first inputs.

396 The $\delta^{13}\text{C}_{\text{OFsoil}}$ variability could also have two explanations. (i) The first concerns the
397 sampling variability inherent to long-term trials (equipment and operator changes). This
398 sampling variability may be reduced by linear regressions that average these values. (ii) And
399 due to high OF incorporations, soil horizon must change over time (swelling) especially for

400 andosol which have high OM-stabilization capacities [47], [48]. A 0-15 cm horizon sampled
401 at the start of an experiment might not correspond to the same horizon 13-years later.

402 $\delta^{13}\text{C}_{\text{OF}}$ was the variable with the greatest impact on the equation. OFs applied to the soils
403 over the 13 years of the experiment were subjected to temporal variations, with standard
404 deviations of about 2.8 ‰ for the compost and 1.3 ‰ for the slurry (Figure S3). Such isotopic
405 variation was previously observed over 6 years by Gerzabek *et al.* (1997) [34] in fields
406 amended with green manure compost (standard deviation about 1.12 ‰), animal manure (0.77
407 ‰) and peat (0.36 ‰). The composition of OF parent materials can be a major source of $\delta^{13}\text{C}$
408 variation. A slight change in the livestock diet can have a marked impact on the $\delta^{13}\text{C}$ of
409 faeces. The $\delta^{13}\text{C}$ of the plant fraction of the OF can also vary since compounds like lignin can
410 be ^{13}C depleted relative to other plant tissues [49], [50]. Consequently, variations in parent
411 material proportions over time (litter/faeces ratio) was probably a major source of variability
412 for OF- $\delta^{13}\text{C}$ throughout the study. Another source of variability could be related to OF
413 transformations that may occur during storage and the composting process, leading to isotopic
414 fractionation. C biotransformation might increase the $\delta^{13}\text{C}$ ratio of OF during the composting
415 process. Here with yearly quantification for 9 years out of 13, OF-variations factor was the
416 largest of the 3 factors in the mixing model equation 7. Regular intra- and inter-annual $\delta^{13}\text{C}$
417 analysis is therefore strongly recommended to ensure the validity of the results obtained with
418 the mixing equation.

419 ***4.3 An isotopic method to study the factor controlling soil OF-C sequestration***

420 This study case demonstrated that the synchronic isotopic method can be used to investigate
421 factors controlling OF-C retention.

422 Less compost-C was stabilized over time in the Arenosol as compared to the Andosol (23 %
423 and 36 % retention, respectively), underlying the effect of the soil and the climate since the

424 same amount of OF were applied at both sites. The impact of the soil type was suggested by
425 Kristiansen *et al.* (2005), who reported a retention percentage ranging from 11 % to 15 % on
426 four arable soils [35]. In contrast, Thomsen and Christiansen (2010) did not observe any soil
427 type influence [36]. In our study, the Réunion Andosol contains poorly crystalline material
428 [47] while storing large amounts of organic matter that has a low renewal rate [48]. The soil
429 type-dependent retention could be explained by the high retention capacity of the Andosol
430 compared to the Arenosol, but it could also be explained by the cooler climatic conditions that
431 prevailed in the Andosol plot, with higher rainfall than in the Arenosol plot. Cooler climatic
432 conditions can induce slower organic matter decomposition and renewal in soils and thus
433 slower organic matter mineralization. The combined effect of the soil mineralogy and climate
434 affected the long-term stabilization (13 years) of the applied OFs, with higher stabilization in
435 the Andosol trial.

436 Furthermore, the Arenosol trial showed a retention of 8% for the slurry but of 23% for the
437 compost, demonstrating a second impact related to the quality of the OF. A difference in
438 retention related to the OF nature was also observed by Lynch *et al.* (2006) [38] and Thomsen
439 and Christensen (2010) [36]. Lynch *et al.* (2006) [38] observed higher retention for corn
440 silage compost (89 %), and lower rates for sewage sudge compost (63 %) and dairy manure
441 compost (42 %). Thomsen and Christensen (2010) observed higher retention for sheep faeces
442 (30%) than for aboveground fresh maize biomass (19 %)[36]. In our case, compost-derived
443 carbon has a composting step where a large part of the labile carbon may have been removed.
444 Slurry did not have this composting stage, thus explaining why a large part of applied slurry-
445 derived C might be directly mineralized. Moreover, as the slurry applications are massive,
446 there may be a toxic impact on microorganisms that could slow down OF decomposition. In
447 our case, the extent of C retention differed by threefold when comparing the two OFs. The
448 nature of the applied organic materials was therefore a major factor in the long-term (13

449 years) stabilization of carbon.

450 By focusing directly on the fate of OF-C in the soil, the synchronic isotopic method thus may
451 provide an opportunity to study the mechanisms of sequestration in the soil of C applied with
452 organic fertilizers.

453 **5. Conclusion**

454 We compared three methods for quantifying carbon from organic fertilizers (OF-C) in an
455 Arenosol and an Andosol trial under tropical climatic conditions, i.e. the differential method,
456 the synchronic isotopic method and the asynchronous isotopic method. The differential method
457 was the simplest to use since it did not require a specific $\delta^{13}\text{C}$ system for OF-C calculation.
458 However, the indirect effects (changes in vegetation and mineralization link to OFs input)
459 cannot be subtracted from it. This effect had a major influence on the final compost-C
460 quantifications in the Andosol trial, with $17\pm 8\%$ found using the differential method
461 compared to $43\pm 3\%$ with the synchronic isotopic method. The asynchronous method would
462 be very useful when there are no control plots, but the control system without OF input must
463 be isotopically stable through time, unlike the Arenosol control system here. Here, $\delta^{13}\text{C}$
464 variations of controls plots were detected from the crop-C over time. The isotopic synchronic
465 method was therefore the most appropriate for subtracting crop-C from the OF-C. Yet there
466 are other methodological variabilities to consider, such as, spatial $\delta^{13}\text{C}$ variations, sampling
467 variability in long-term studies and mostly OF- $\delta^{13}\text{C}$ variations over time. The application of
468 the synchronous isotopic method revealed potentialities to quantify the OF-C retention factors
469 (e.g. soil type influence, OF nature as well as climate type). In our case study the compost-C
470 retention rate in the Andosol was up to 36% whereas compost-C in the Arenosol reached only
471 23%, revealing the marked influence of climate and soil type. The slurry-C retention rate in
472 the Arenosol was only about 8% compared to compost-C (up to 23%), revealing a OF nature

473 influence, thus highlighting the importance of the OF nature on long-term C stabilization.

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484 prematurely at the age of 62. Jérôme was a senior scientist at INRAE and one of the most
485 renowned French soil scientists of his generation. Jérôme was a recognized pioneer in the use
486 of stable C isotopes for studying SOM dynamics in C3/C4 transitions. Here we would like to
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488 the soil science community.

489 Data are available upon request to the corresponding author.

490 **Disclosure statement**

491 No potential conflict of interest was reported by the author(s).

492 **References**

- 493 1. Soussana JF *et al.* Matching policy and science: Rationale for the ‘4 per 1000 - soils for food
494 security and climate’ initiative. *Soil and Tillage Research* 188, 3–15 (2019).
- 495 2. Trumbore SE. Comparison of carbon dynamics in tropical and temperate soils using
496 radiocarbon measurements. *Global Biogeochemical Cycles* 7, 275–290 (1993).
- 497 3. Mathieu JA, Hatté C, Balesdent J *et al.* Deep soil carbon dynamics are driven more by soil
498 type than by climate: a worldwide meta- analysis of radiocarbon profiles. *Global Change Biology* 21,
499 4278–4292 (2015).
- 500 4. Balesdent J, Mariotti A and Guillet B. Natural ^{13}C abundance as a tracer for studies of soil
501 organic matter dynamics. *Soil Biology and Biochemistry* 19, 25–30 (1987).
- 502 5. Glaser B. Compound-specific stable-isotope ($\delta^{13}\text{C}$) analysis in soil science. *Journal of Plant*
503 *Nutrition and Soil Science* 168, 633–648 (2005).
- 504 6. Boutton TW, Archer SR, Midwood AJ *et al.* $\delta^{13}\text{C}$ values of soil organic carbon and their use
505 in documenting vegetation change in a subtropical savanna ecosystem. *Geoderma* 82, 5–41 (1998).

- 506 7. O’Leary MH. Carbon Isotopes in Photosynthesis. *BioScience* 38, 328–336 (1988).
- 507 8. Balesdent J and Mariotti A. Measurement of Soil Organic Matter Turnover Using ^{13}C Natural
508 Abundance. in *Mass spectrometry of soils* 83–111 (1996).
- 509 9. Farquhar GD, Ehleringer JR and Hubick KT. Carbon Isotope Discrimination and
510 Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40, 503–537 (1989).
- 511 10. Gebauer G, Schulumacher MI, Krstić B *et al.* Biomass production and nitrate metabolism of
512 *Atriplex hortensis* L. (C3 plant) and *Amaranthus retroflexus* L. (C4 plant) in cultures at different levels
513 of nitrogen supply. *Oecologia* 72, 303–314 (1987).
- 514 11. Gebauer G, Preiss K and Gebauer AC. Partial mycoheterotrophy is more widespread among
515 orchids than previously assumed. *New Phytologist* 211, 11–15 (2016).
- 516 12. Giesemann P *et al.* Dark septate endophytes and arbuscular mycorrhizal fungi (Paris-
517 morphotype) affect the stable isotope composition of ‘classically’ non-mycorrhizal plants. *Functional*
518 *Ecology* 34, 2453–2466 (2020).
- 519 13. Giesemann P, Rasmussen HN, Liebel HT *et al.* Discreet heterotrophs: green plants that
520 receive fungal carbon through Paris-type arbuscular mycorrhiza. *New Phytologist* 226, 960–966
521 (2020).
- 522 14. Balesdent J *et al.* Atmosphere–soil carbon transfer as a function of soil depth. *Nature* 559,
523 599–602 (2018).
- 524 15. Jastrow JD, Miller RM and Boutton TW. Carbon Dynamics of Aggregate-Associated Organic
525 Matter Estimated by Carbon-13 Natural Abundance. *Soil Science Society of America Journal* 60, 801–
526 807 (1996).
- 527 16. Liao JD, Boutton TW and Jastrow JD. Organic matter turnover in soil physical fractions
528 following woody plant invasion of grassland: Evidence from natural ^{13}C and ^{15}N . *Soil Biology and*
529 *Biochemistry* 38, 3197–3210 (2006).
- 530 17. Bol R, Poirier N, Balesdent J *et al.* Molecular turnover time of soil organic matter in particle-
531 size fractions of an arable soil. *Rapid Communications in Mass Spectrometry* 23, 2551–2558 (2009).
- 532 18. Lou Y, Wang J and Liang W. Impacts of 22-year organic and inorganic N managements on
533 soil organic C fractions in a maize field, northeast China. *CATENA* 87, 386–390 (2011).
- 534 19. Paetsch L, Mueller CW, Rumpel C *et al.* Urban waste composts enhance OC and N stocks
535 after long-term amendment but do not alter organic matter composition. *Agriculture, Ecosystems &*
536 *Environment* 223, 211–222 (2016).
- 537 20. Liu H *et al.* 16-Year fertilization changes the dynamics of soil oxidizable organic carbon
538 fractions and the stability of soil organic carbon in soybean-corn agroecosystem. *Agriculture,*
539 *Ecosystems & Environment* 265, 320–330 (2018).
- 540 21. Liang F *et al.* Two-decade long fertilization induced changes in subsurface soil organic carbon
541 stock vary with indigenous site characteristics. *Geoderma* 337, 853–862 (2019).
- 542 22. Kuzyakov Y, Friedel JK and Stahr K. Review of mechanisms and quantification of priming
543 effects. *Soil Biology and Biochemistry* 32, 1485–1498 (2000).
- 544 23. Fontaine S, Mariotti A and Abbadie L. The priming effect of organic matter: a question of
545 microbial competition? *Soil Biology and Biochemistry* 35, 837–843 (2003).

- 546 24. Angers DA and Giroux M. Recently Deposited Organic Matter in Soil Water-Stable
547 Aggregates. *Soil Science Society of America Journal* 60, 1547–1551 (1996).
- 548 25. Balesdent J. Les isotopes du carbone et la dynamique des matières organiques des sols.
549 *Cahiers Agricultures* 7, 201-206 (1) (1998).
- 550 26. Dignac MF *et al.* Carbon-13 natural abundance as a tool to study the dynamics of lignin
551 monomers in soil: an appraisal at the Closeaux experimental field (France). *Geoderma* 128, 3–17
552 (2005).
- 553 27. Rasse DP *et al.* Lignin turnover in an agricultural field: from plant residues to soil-protected
554 fractions. *European Journal of Soil Science* 57, 530–538 (2006).
- 555 28. Amelung W, Bol R and Friedrich C. Natural ^{13}C abundance: a tool to trace the incorporation
556 of dung-derived carbon into soil particle-size fractions. *Rapid Communications in Mass Spectrometry*
557 13, 1291–1294 (1999).
- 558 29. Bol R, Amelung W, Friedrich C *et al.* Tracing dung-derived carbon in temperate grassland
559 using ^{13}C natural abundance measurements. *Soil Biology and Biochemistry* 32, 1337–1343 (2000).
- 560 30. Glaser B *et al.* Short-term sequestration of slurry-derived carbon and nitrogen in temperate
561 grassland soil as assessed by ^{13}C and ^{15}N natural abundance measurements. *Journal of Plant*
562 *Nutrition and Soil Science* 164, 467–474 (2001).
- 563 31. Carpenter-Boggs LA, Carlson BR, Higgins SS *et al.* Decomposition of Dairy Manure
564 Assessed in the Field by Monitoring Natural Abundance of ^{13}C . *Soil Science Society of America*
565 *Journal* 78, 1949–1952 (2014).
- 566 32. Rochette P, Angers DA and Côté D. Soil Carbon and Nitrogen Dynamics Following
567 Application of Pig Slurry for the 19th Consecutive Year I. Carbon Dioxide Fluxes and Microbial
568 Biomass Carbon. *Soil Science Society of America Journal* 64, 1389–1395 (2000).
- 569 33. Angers DA, Rochette P, Chantigny MH *et al.* Use of ^{13}C abundance to study short-term pig
570 slurry decomposition in the field. *Soil Biology and Biochemistry* 39, 1234–1237 (2007).
- 571 34. Gerzabek MH, Pichlmayer F, Kirchmann H *et al.* The response of soil organic matter to
572 manure amendments in a long-term experiment at Ultuna, Sweden. *European Journal of Soil Science*
573 48, 273–282 (1997).
- 574 35. Kristiansen SM, Hansen EM, Jensen LS *et al.* Natural ^{13}C abundance and carbon storage in
575 Danish soils under continuous silage maize. *European Journal of Agronomy* 22, 107–117 (2005).
- 576 36. Thomsen IK and Christensen BT. Carbon sequestration in soils with annual inputs of maize
577 biomass and maize-derived animal manure: Evidence from ^{13}C abundance. *Soil Biology and*
578 *Biochemistry* 42, 1643–1646 (2010).
- 579 37. Nicoloso RS, Rice CW, Amado TJC *et al.* Carbon saturation and translocation in a no-till soil
580 under organic amendments. *Agriculture, Ecosystems & Environment* 264, 73–84 (2018).
- 581 38. Lynch DH, Voroney RP and Warman PR. Use of ^{13}C and ^{15}N natural abundance techniques
582 to characterize carbon and nitrogen dynamics in composting and in compost-amended soils. *Soil*
583 *Biology and Biochemistry* 38, 103–114 (2006).
- 584 39. Gerzabek MH, Haberhauer G and Kirchmann H. Soil Organic Matter Pools and Carbon-13
585 Natural Abundances in Particle-Size Fractions of a Long-Term Agricultural Field Experiment
586 Receiving Organic Amendments. *Soil Science Society of America Journal* 65, 352–358 (2001).

- 587 40. USS Working Group WRB. World Reference Base for Soil Resources 2014, update
588 2015. International soil classification system for naming soils and creating legends for soil maps.
589 World Soil Resources Reports (2015).
- 590 41. Coplen TB *et al.* After two decades a second anchor for the VPDB $\delta^{13}\text{C}$ scale. Rapid
591 Communications in Mass Spectrometry 20, 3165–3166 (2006).
- 592 42. Alexander EB. Bulk Densities of California Soils in Relation to Other Soil Properties 1. Soil
593 Science Society of America Journal 44, 689–692 (1980).
- 594 43. Liu W, Zhu YG, Christie P *et al.* Botanical composition, production and nutrient status of an
595 originally *Lolium perenne*-dominant cut grass sward receiving long-term manure applications. Plant
596 Soil 326, 355 (2009).
- 597 44. Blumenthal DM, LeCain DR and Augustine DJ. Composted manure application promotes
598 long-term invasion of semi-arid rangeland by *Bromus tectorum*. Ecosphere 8, e01960 (2017).
- 599 45. De Deyn GB, Cornelissen JHC and Bardgett RD. Plant functional traits and soil carbon
600 sequestration in contrasting biomes. Ecol. Lett. 11, 516–531 (2008).
- 601 46. Steinbeiss S *et al.* Plant diversity positively affects short-term soil carbon storage in
602 experimental grasslands. Global Change Biology 14, 2937–2949 (2018).
- 603 47. Levard C *et al.* Structure and distribution of allophanes, imogolite and proto-imogolite in
604 volcanic soils. Geoderma 183–184, 100–108 (2012).
- 605 48. Basile-Doelsch I, Balesdent J and Rose J. Are Interactions between Organic Compounds and
606 Nanoscale Weathering Minerals the Key Drivers of Carbon Storage in Soils? Environ. Sci. Technol.
607 49, 3997–3998 (2015).
- 608 49. Benner R, Fogel ML, Sprague EK *et al.* Depletion of ^{13}C in lignin and its implications for
609 stable carbon isotope studies. Nature 329, 708–710 (1987).
- 610 50. Cayet C and Lichtfouse E. $\delta^{13}\text{C}$ of plant-derived n-alkanes in soil particle-size fractions.
611 Organic Geochemistry 32, 253–258 (2001).
- 612

613 **Table list**

614 **Table 1. Summary of similar OF-C estimation experiments using ^{13}C isotopes from the**
615 **literature.** *C proportion estimation using C3 of -27‰ and C4 of -13‰ and not the soil at the
616 beginning of the experiment or without OF. **Estimation by linear regression (see Figure 1).

617 **Table 2. Quality and amount of carbon inputs from the two organic fertilizers applied and**
618 **vegetation C data.** The two organic fertilizers are averages of 8 samples from years 2 to 9. Vegetation
619 is a single measurement.

620

621 **Figure list**

622 **Figure 1. Schematic representation of carbon origins in experiments with organic inputs.** Initial
623 SOM reflects the C pool before the start of the experiment. SOM from OF are displayed in yellow,
624 SOM from vegetation in green and initial SOM in brown.

625 **Figure 2. Variations in soil $\delta^{13}\text{C}$ in control plots with mineral fertilization of: a) the Arenosol and**
626 **b) the Andosol.** $\delta^{13}\text{C}$ is reported for topsoils (0-15 cm) in three experimental blocks (block 1: black
627 triangles; block 2: open circles; block 3: black diamonds). Lines correspond to the linear regression of
628 $\delta^{13}\text{C}$ in each block, with a common slope for a given soil type.

629 **Figure 3. Variations in soil $\delta^{13}\text{C}$ in plots amended with organic fertilizers implied on: a) the**
630 **Arenosol and b) the Andosol.** Topsoils (0-15 cm) receiving compost (squares) and slurry (diamonds)
631 are compared to the control mean (triangles). Each point is the average of three blocks and error bars
632 are \pm one standard deviation of 3 plots (one per block; only two blocks in year 3 in the Arenosol and
633 year 10 in the Andosol). $\delta^{13}\text{C}$ of OFs is displayed as a continuous line over time (green and yellow),
634 the error bar is the standard deviation of values (n=8). (*), (**), And (***), indicates p-value
635 significant differences between controls and amended, $P < 0.05$, <0.01 or <0.001 , respectively

636 **Figure 4. C content and origin of carbon in the Arenosol and Andosol topsoils (0-15 cm)**
637 **according to the quantification method.** The origin was inferred from the differential method
638 (orange), synchronic isotopic method (red) and asynchronous isotopic method (blue). The dotted error
639 bars are the standard deviation of three total C quantifications (only two blocks in year 3 for the
640 Arenosol and year 10 for the Andosol). The solid line error bars are standard deviations of three OF-C
641 quantifications. Negative OF-C values using the differential method were set at 0. (*), (**), And
642 (***), indicates the resolution limit of the methods by student t-test evaluation ($P < 0.05$, <0.01 or
643 <0.001 , respectively; see method par 2.7)

644 **Figure 5. Carbon stock derived from organic fertilizers as a function of cumulated C inputs**
645 **from organic fertilizer amendments (0-15 cm soil layer) using the synchronic isotopic method.**
646 Linear model of the Andosol compost amended plots with retention of 36 % ($R^2=0.93$), 23 %
647 ($R^2=0.94$) in the Arenosol and 8 % ($R^2=0.98$) in the Arenosol slurry amended plot. Each point
648 represents the average of the three plots and the error bars are the standard deviation (only two blocks
649 in year 3 for the Arenosol and year 10 for the Andosol). The insert compares data from previous
650 studies with the regression lines of the different modalities. Note that data from literature had a
651 sampling horizon thickness ranging from 4 to 20 cm.

652 **Table 1.**

Sampling frequency	Publication	Location	Soil type	Sampled depth	Type of OF applied	Time of experiment	Number of OF applications through time	$\delta^{13}\text{C}$ of sources			OF-C in the soil at the end of the experiment	Total OF-C spread	Retention of OF-C	Total OF-C in soil
								OF	Vegetation	Soil at the beginning of the experiment or without OF				
				[cm]				[% VPDB]	[% VPDB]	[% VPDB]	[%]	[kgC ₁ .ha ⁻¹]	[%]	[kgC ₁ .ha ⁻¹]
Single	Amelung <i>et al.</i> , 1999 [28]	North Wyke, Devon, southwest of England	Gleyic cambisol	1 - 5	C3 dung	70 days	Single	-25.6	-27.4	-	Results on fractions only			
					C4 dung			-15.4	-27.4	-				
	Bol <i>et al.</i> , 2000 [29]	North Wyke, Devon, southwest of England	Gleyic cambisol	1 - 5	C3 dung	150 days	Single	-25.7	-27.4	-27.9	-	14,900	-	-
					C4 dung			-15.4	-27.4	-27.9	11		12.6	1,877
	Gerzabek <i>et al.</i> , 2001 [39]	Uppsala, Sweden	Eutric Cambisol	0 - 20	Green manure	42 years	Continuous	-27.3	C3	-26.03	Results on fractions only			
					Animal manure			-27.5	C3	-26.03				
					Peat			-25.6	C3	-26.03				
					Sewage sludge			-25.5	C3	-26.03				
	Glaser <i>et al.</i> , 2001[30]	North Wyke, Devon, southwest of England	Dystric gleysol	0 - 2	C3 dung	14 days	Single	-30.7	C3	-30	-	-	-	-
					C4 dung			-21.3	C3	-30	<1 (0.11)	1,100	36	396
	Lynch <i>et al.</i> , 2006 [38]	Marshwind Farm, Masstown, Truro, Nova Scotia, Canada	Orthic humoferric podzol	0 - 5	Composting corn silage	1 year after applications	Spread over 1 year	-14.0	C4	-28.03	33	9,200	89	8,188
					Dairy manure compost			-26.6	C4	-28.03	19	10,000	42	4,200
Sewage sludge compost					-25.9			C4	-28.03	32	10,900	63	6,867	
Liquid dairy manure					-27.3			C4	-28.03	-	2,900	-	-	
Carpenter Boggs <i>et al.</i> , 2014 [31]	Washington university, Puyallup Research and extension center, USA	Mesic Fluventic Haploxerolls	0 - 15	Dairy manure	120 days	Single	-20.9	-30.0	-26.9	<0.01	2,722	-	-	
Nicoloso <i>et al.</i> , 2018 [37]	North farm of Kansas University, Manattan, USA	Well-drained Kennebec silt loam	0-5; 5-15; 15-30	Chilled tillage, additions of fresh cattle manure (from year 0 to 12) and composted organic waste (from year 13 to 25)	25 years	continuous	-25.9 to -27.3 (estimated from literature)	C4 (converted in year 0 from C3 crop)	Year 2 after OF spreading :-18.9 to -19.1 (0-5 cm); -15.8 to -16.5 (15-30 cm)	-	98,800	27*	26,300*	
				No tillage, additions of fresh cattle manure (from year 0 to 12) and composted organic waste (from year 13 to 25)						-		12*	11,900*	

Sampling frequency	Publication	Location	Soil type	Sampled depth	Type of OF applied	Time of experiment	Number of OF applications through time	$\delta^{13}\text{C}$ of sources			OF-C in the soil at the end of the experiment	Total OF-C spread	Retention of OF-C	Total OF-C in soil		
								OF	Vegetation	Soil at the beginning of the experiment or without OF						
								[‰ VPDB]	[‰ VPDB]	[‰ VPDB]						
Chrono-sequence	Gerzabek <i>et al.</i> , 1997 [34]	Uppsala, Sweden	Eutric Cambisol	0 - 20	Green manure	42 years	Continuou s	-27.3	C3	-26.03	-	-	-	-		
					Animal manure			-27.5	C3	-26.03	55	84,000	0	30,131		
					Peat			-25.6	C3	-26.03	-	-	-	-		
	Kristiansen <i>et al.</i> , 2005 [35]	Soil from Lundgaard in Askov, Denmark	Sand	0 - 20	Above ground fresh maize biomass	14 years	Continuou s	-10	C4	-27.2	25	51,100	11	5,621		
								Askov, Denmark	Sandy loam	-10	C4		-27.2	18	14	7,154
								Soil from Roskilde in Askov, Denmark	Sandy loam	-10	C4		-25.4	27	14	7,154
								Soil from Rønhave in Askov, Denmark	Sandy loam	-10	C4		-26.2	31	15	7,665
	Thomsen and Christensen, 2010 [36]	Soil from Lundgaard in Askov, Denmark	Sand	0 - 20	Above ground fresh maize biomass	9 years	Continuou s	-12.0	C4	-27.5	15	32,000	19	6,080		
					Sheep faeces			-13.7	C4	-27.5	22		30	9,600		
		Askov, Denmark	Sandy loam		Above ground fresh maize biomass			-12.0	C4	-27.5	13		19	6,080		
					Sheep faeces			-13.7	C4	-27.5	22		30	9,600		
	This study	Saint-Joseph, La Réunion island, France (near Madagascar)	Arenosol	0 - 15	Bovine manure compost	13 years	continuo us	-20.3	-14.0	-18.2 to -17**	57	117,800	23	27,094		
					Bovine slurry			-24.4	-14.0	-18.2 to -17**	41	256,900	8	20,552		
		Le plaine des cafres, La Réunion island, France (near Madagascar)	Andosol		Bovine manure compost			-20.3	-27.8	-24.3 to -23.8**	41	117,800	36	42,408		
Bovine slurry					-24.4			-27.8	-24.3 to -23.8**	-	256,900	-	-			

653 **Table 2.**

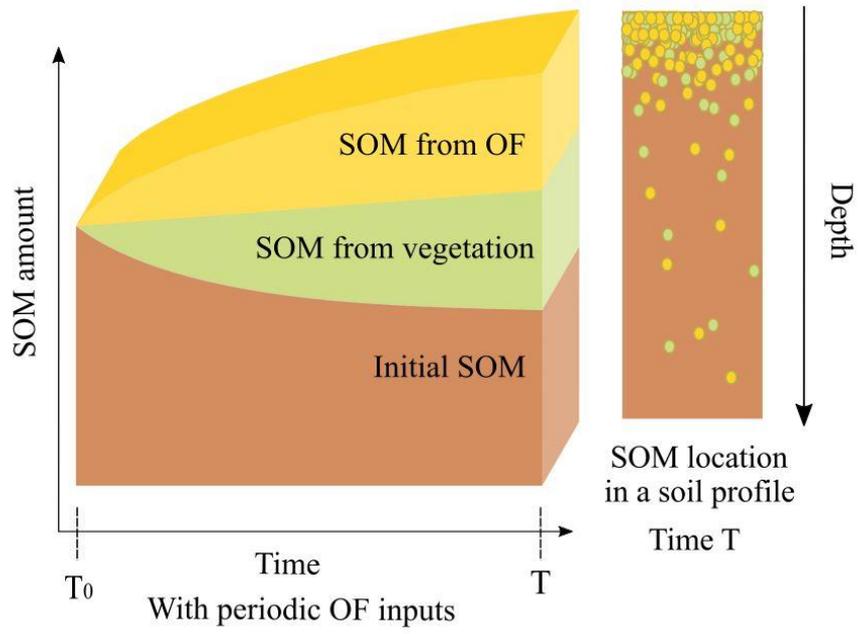
	C air-dried matter	N Kjeldahl	Dry mass (105°C)	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$ temporal standard deviation	Annual input
	[g.kg ⁻¹]	[g N.kg ⁻¹ of dry mass 105°C]	[g.100 g ⁻¹]	[‰ VPDB]	[‰ VPDB]	[kg C.ha. ⁻¹ .yr ⁻¹]
Bovine manure compost	213	22	46.7	(n= 8) -20.3	2.8	9064
Bovine slurry	374	43	9.3	(n= 8) -24.3	1.3	19760
C ₄ vegetation in the Arenosol trial	417			(n= 1) -13.9		
C ₃ vegetation in the Andosol trial	414			(n= 1) -27.8		

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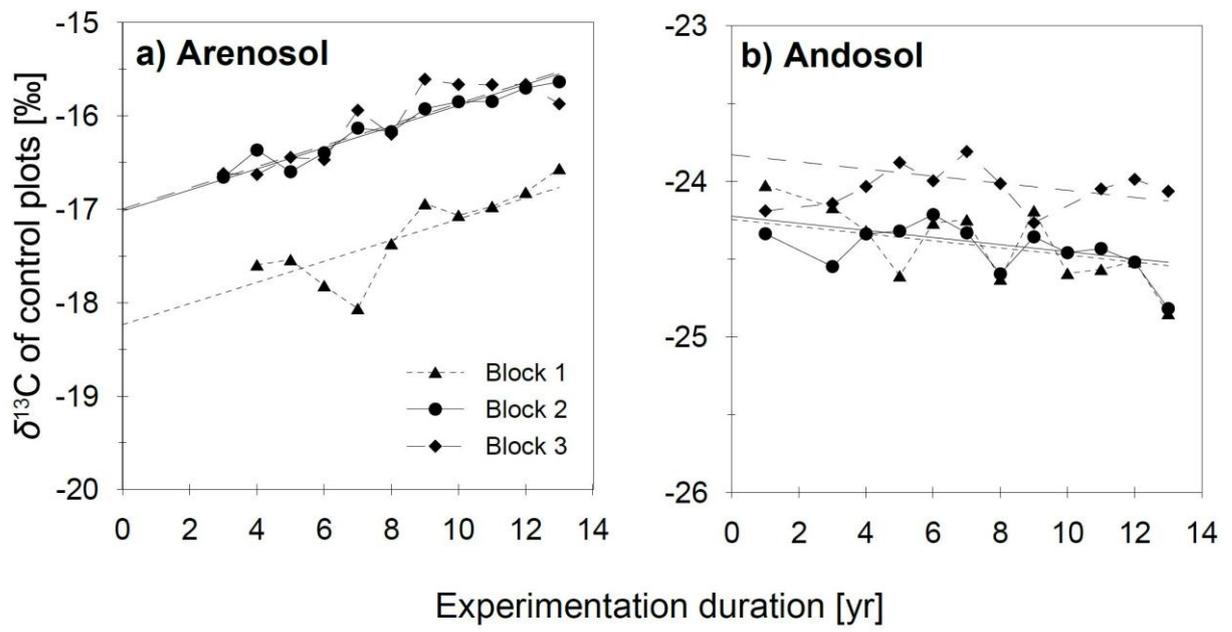
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656 **Figure 1.**

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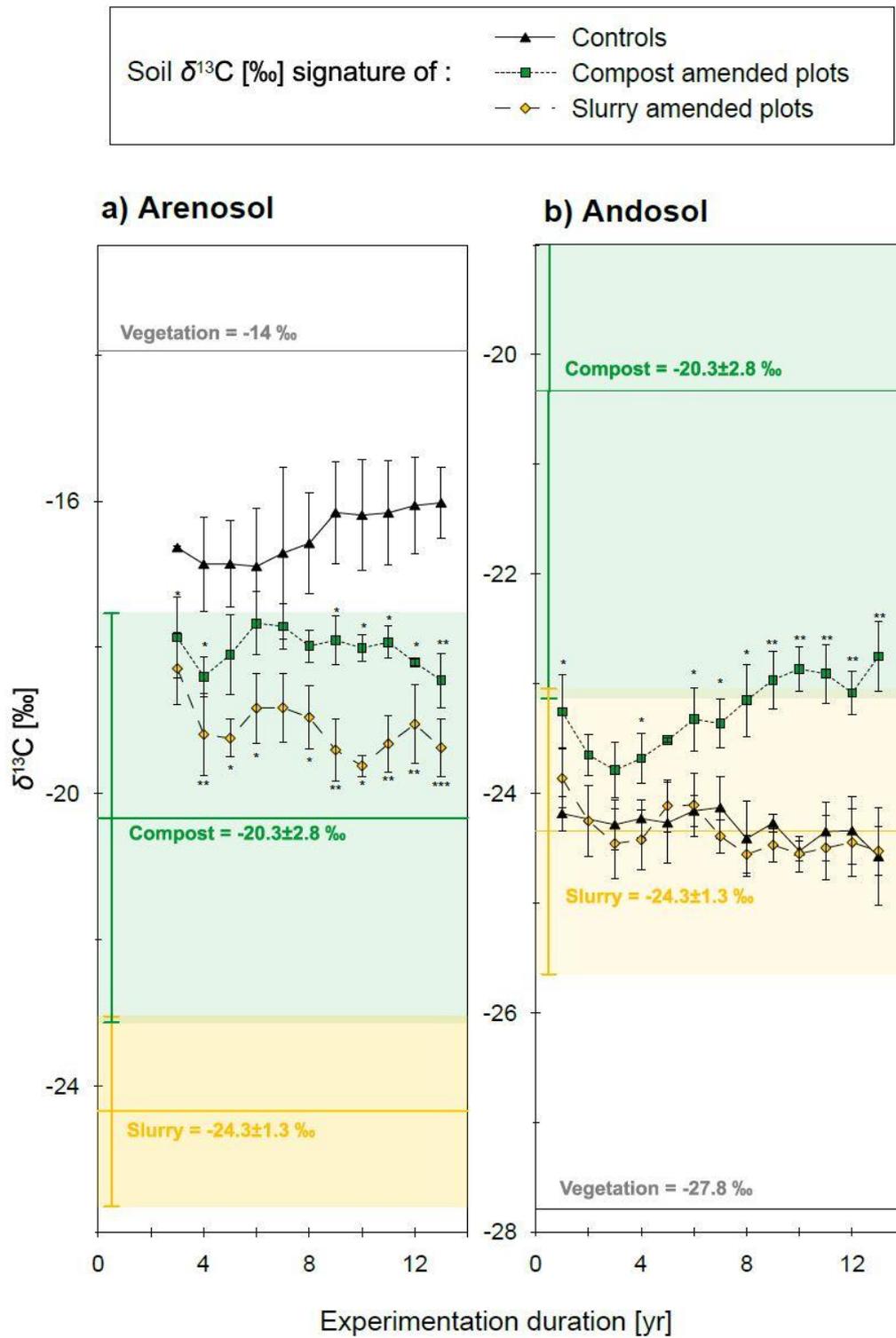


658 **Figure 2.**

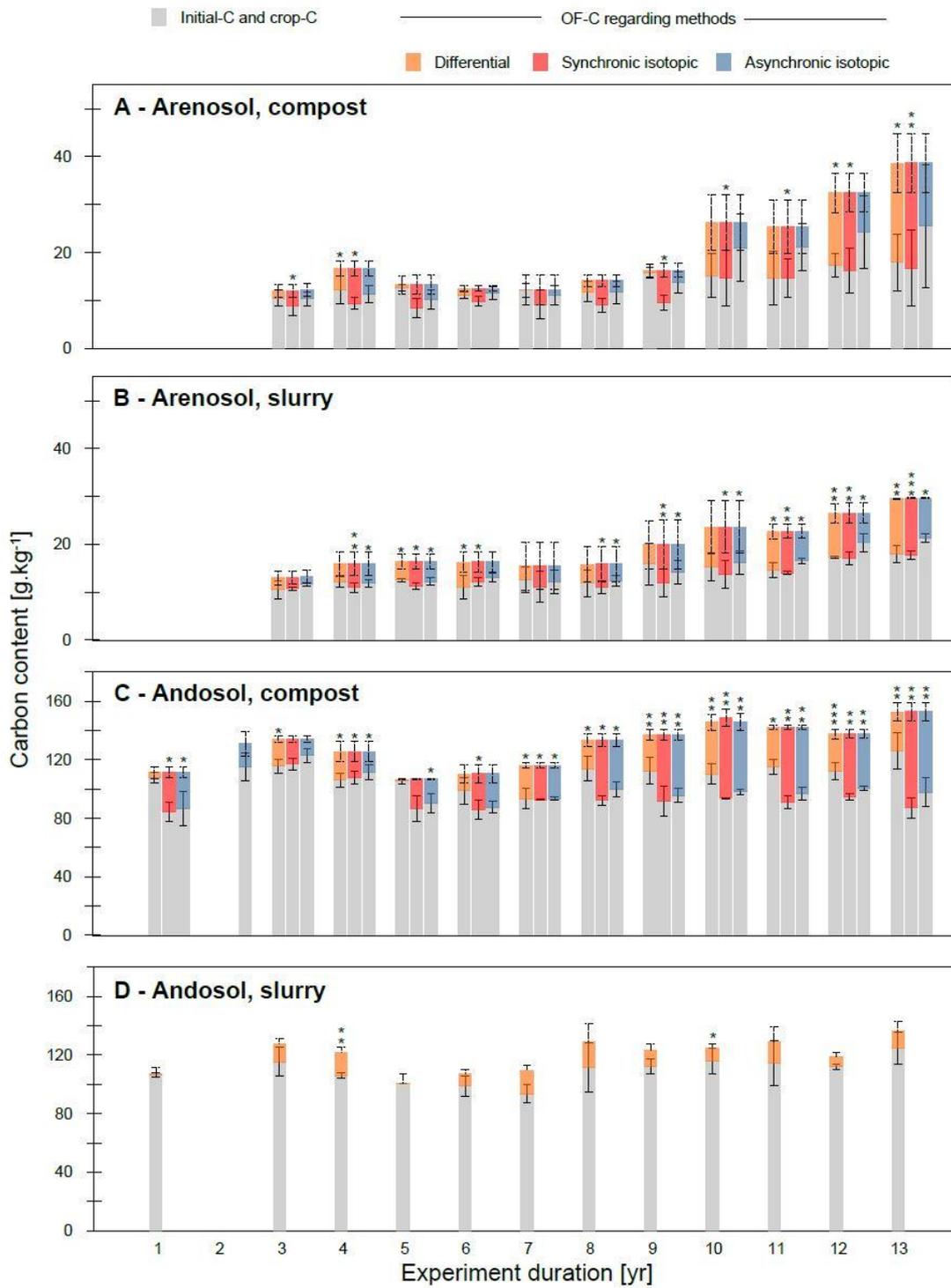


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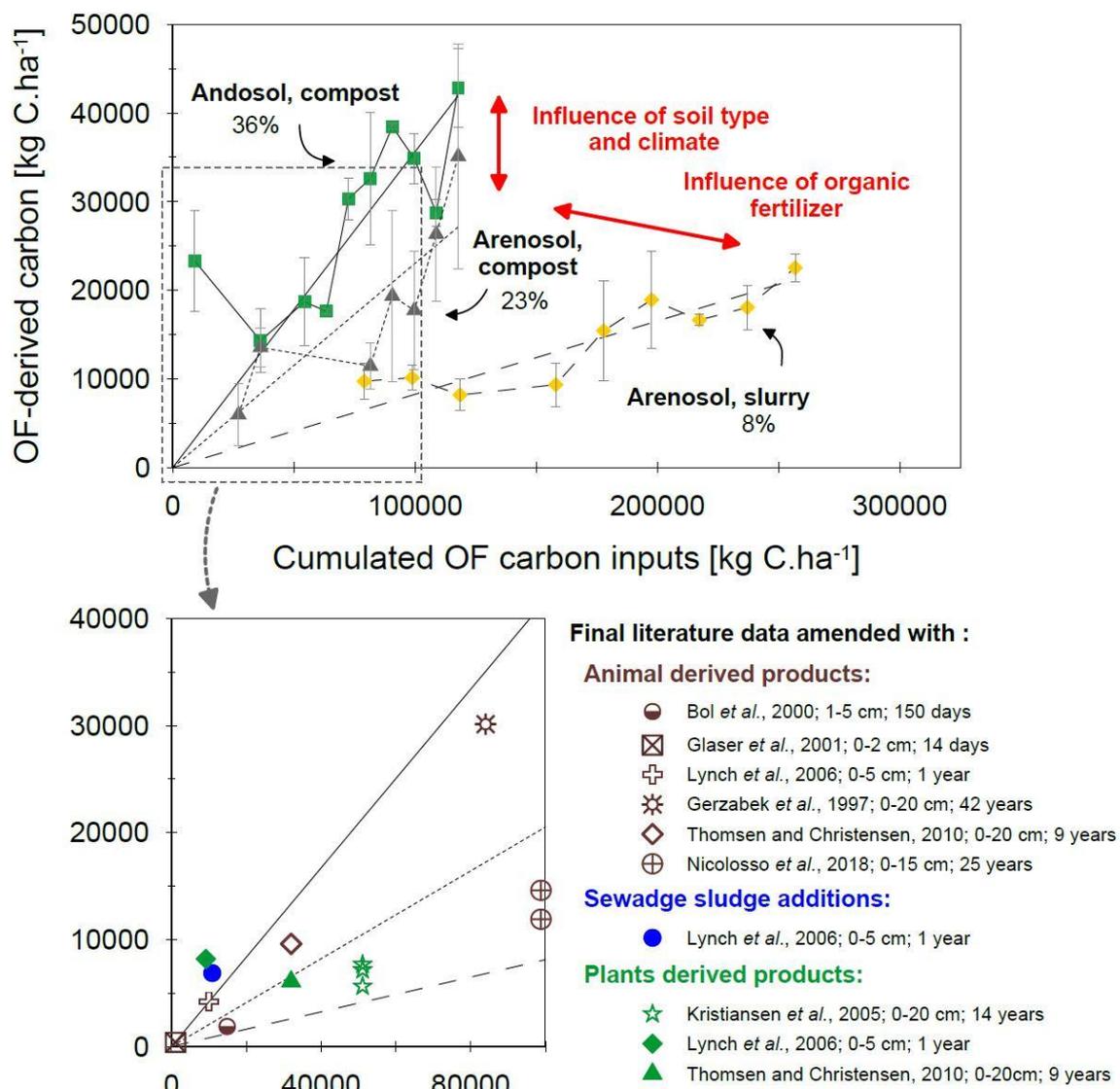
660 **Figure 3.**



661 **Figure 4.**



662 **Figure 5.**



663 **Supplementary information**

664 **Table S1. Percentage of OF-C according to the methods, i.e. differential, synchronic isotopic and**
 665 **asynchronic isotopic.** Mean of three OF-C estimations \pm standard deviation (only two blocks in year
 666 3 for the Arenosol and year 10 for the Andosol).

	Arenosol, compost			Arenosol, slurry			Andosol, compost		
	Difference	Synchronic	Asynchronic	Difference	Synchronic	Diachronic	Difference	Synchronic	Asynchronic
T1	-	-	-	-	-	-	3 \pm 3	24 \pm 6	22 \pm 10
T2	-	-	-	-	-	-	-		
T3	11 \pm 13	26 \pm 12	15 \pm 11	18 \pm 12			14 \pm 4		
T4	26 \pm 15	44 \pm 3	32 \pm 8	22 \pm 5	31 \pm 2	26 \pm 1	15 \pm 3	14 \pm 3	11 \pm 4
T5	5 \pm 4			23 \pm 3	32 \pm 2	26 \pm 4	1 \pm 2		
T6	12 \pm 6			31 \pm 12	26 \pm 3	20 \pm 4	10 \pm 8	22 \pm 5	21 \pm 3
T7	0 \pm 1			16 \pm 14			20 \pm 6	20 \pm 0	20 \pm 1
T8	15 \pm 13			23 \pm 14	31 \pm 3	22 \pm 4	14 \pm 6	31 \pm 3	25 \pm 5
T9	2 \pm 4	41 \pm 6	15 \pm 13	18 \pm 17	40 \pm 5	28 \pm 5	18 \pm 6	33 \pm 7	30 \pm 3
T10	41 \pm 10	42 \pm 14	16 \pm 26	35 \pm 4	42 \pm 5	31 \pm 7	24 \pm 4	37 \pm 1	33 \pm 2
T11	40 \pm 12	42 \pm 7	15 \pm 17	35 \pm 5	39 \pm 2	27 \pm 3	19 \pm 4	36 \pm 3	32 \pm 3
T12	47 \pm 3	49 \pm 9	24 \pm 22	35 \pm 2	36 \pm 5	23 \pm 7	19 \pm 4	31 \pm 1	27 \pm 1
T13	53 \pm 7	56 \pm 12	31 \pm 30	39 \pm 6	40 \pm 3	28 \pm 3	17 \pm 8	43 \pm 3	36 \pm 5

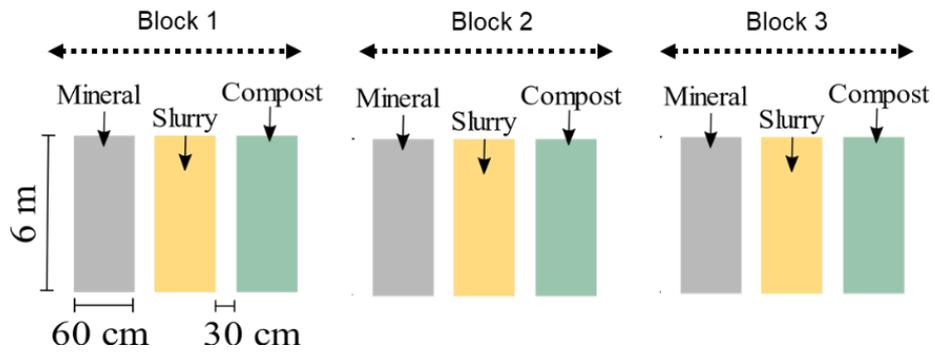
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668 **Table S2. Weight of each parameter in the sensitivity analysis of the isotopic mixing equation.**
 669 Average $\delta^{13}\text{C}$ is the average $\delta^{13}\text{C}$ from three plots at T13. The variation is the standard deviation
 670 divided by the average. Importance variable method detailed in part 2.8.

671

	Equation variable	Average $\delta^{13}\text{C}$	Variation [%]	Weight of the variable [%]
Arenosol compost	$\delta^{13}\text{C}_{\text{OF}}$	-20.33	14	95
	$\delta^{13}\text{C}_{\text{OFsoil}}$	-18.45	2	5
	$\delta^{13}\text{C}_{\text{CTRLsoil}}$	-16.02	3	1
Arenosol slurry	$\delta^{13}\text{C}_{\text{OF}}$	-24.35	6	58
	$\delta^{13}\text{C}_{\text{OFsoil}}$	-19.37	2	26
	$\delta^{13}\text{C}_{\text{CTRLsoil}}$	-16.02	3	16
Andosol compost	$\delta^{13}\text{C}_{\text{OF}}$	-20.33	14	92
	$\delta^{13}\text{C}_{\text{OFsoil}}$	-22.75	1	6
	$\delta^{13}\text{C}_{\text{CTRLsoil}}$	-24.58	2	2

672 **Figure S1. Schematic representation of the experimental design of Arenosol and Andosol trials.**
673 Consisting of nine plots divided into three blocks with three different treatments, leading to three plots
674 per modality. The plots were randomly distributed within each block.



675 **Figure S2. Bulk density estimations (results and parameters) using Alexander's law on total**
 676 **carbon content.** Note that a linear regressions on the total carbon from Figure 4 data was done to fit
 677 Alexander's law by the least squared method with measurements performed on the Arenosol (5-10
 678 cm) in year 0 an year 14 and on the Andosol (0-10 cm) in year 3 and 16. Alexander coefficients
 679 underwent least squares adjustment on carbon linear regression data, by fitting both measured values
 680 in each soils.

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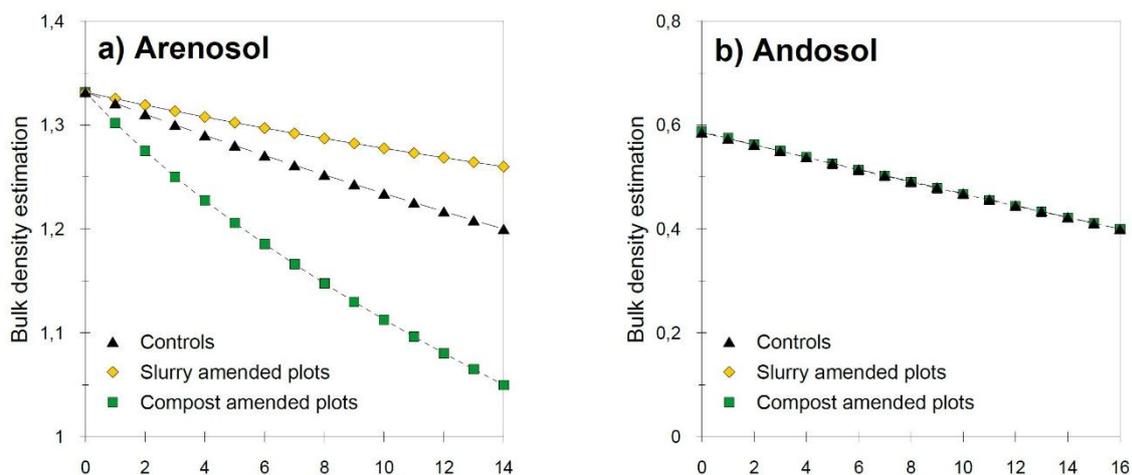
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	Time after experimentation start [yr]			Time after experimentation start [yr]	
	Controls	Compost amended soils	Slurry amended soils	Controls	Compost amended soils
Alexander's coefficient (A_{coef})	1.74	1.58	1.46	3.61	1.71
Slope (A_{slope})	0.13	0.09	0.04	0.30	0.11

689 **Figure S3, OF- $\delta^{13}\text{C}$ over time from years 2 to 9 for the bovine manure compost (green square)**
690 **and bovine slurry (yellow dot) treatments.**

