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

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

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

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

1. Introduction

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

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

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

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

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

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

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

design without control plots).

2. Material and methods

2.1 Study sites

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

2.2 Experimental design

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

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

2.3 Soils and organic fertilizers sampling

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

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

2.4 C analyses and C stock calculations

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

Soil carbon stocks were assessed by equation (1).

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

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)

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

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

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

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

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

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

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

$\Delta\delta^{13}\text{C}$ is the difference between initial $\delta^{13}\text{C}$ of control plots at year 0 ($\delta^{13}\text{C}_{CTL\text{soil}T0}$, CTL stands for control) and vegetation $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{veg}$) or difference between $\delta^{13}\text{C}_{CTL\text{soil}T0}$ and OF- $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{OF}$) such as:

$$\Delta\delta^{13}\text{C}(\text{‰}) = \left| \delta^{13}\text{C}_{CTL\text{soil}T0} - \delta^{13}\text{C}_{veg} \text{ or } \delta^{13}\text{C}_{OF} \right| \quad (4)$$

2.6 Estimation of OF-C proportions in amended soils

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

most common), the synchronic isotopic method and the asynchronic isotopic method.

2.6.1 Differential method

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

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

The carbon stock (kgC.ha⁻¹ in 0-15 cm) derived from OF (S_{COF}) was calculated with equation (6) where S is the total carbon stock (kg C.ha⁻¹):

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

2.6.2 Synchronic isotopic method

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

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

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

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

2.6.3 Asynchronic isotopic method

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

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

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

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

2.7 Statistical analysis and overcoming block effect

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

2.8 Sensitivity analysis of the isotope mixing equation

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

percentage deviation from the mean (standard deviation divided by the mean on T13) for the 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 of 1000 values for the three variables according to the deviation percentage calculated in T13. Using equation 7, 1000 f_{COF} values were thus obtained for the Arenosol compost, Arenosol slurry and Andosol compost treatments.

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

3. Results

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

The organic carbon content in Arenosol control plots (under mineralization) increased from $10.5 \pm 0.0 \text{ g.kg}^{-1}$ 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) increased over time. According to linear regressions, $\delta^{13}\text{C}$ increased from -18.2 ‰ to -16.8‰ in block 1, from -17.0‰ to -15.6‰ in block 2 and from -17.0‰ to -15.5‰ in block 3. The Arenosol controls showed the same overall variation pattern over time but with a slight shift in the initial value for estimated year 0. Including all block, the $\delta^{13}\text{C}$ from year 0 (-18.2 to -17 ‰) indicated that the organic matter content was from mixed C_3 and C_4 origins, with a predominance of C_4 . Vegetation $\delta^{13}\text{C}$ in this Arenosol trial was about -13.9 ‰ (Table 2).

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

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

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

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

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

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

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

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

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

3.4 Quantification of soil OF-C via the differential, isotopic synchronic and isotopic asynchronous methods

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

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

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

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

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

4. Discussion

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

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

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

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

- (i) The repeated OF applications that may have modified the vegetation and its C inputs. It has been reported that OF can alter the plant community composition [43], [44] leading to different C inputs relative to control plots [45], [46]. In the Andosol trial, modification of the vegetation composition was revealed by the replacement of *Dactylis* (C₃ plant-group) in the mineral-fertilized control plots, and of *Bromus* (C₃ plant-group) in the plot with OF amendment (Tillard, pers. comm.). Lower belowground biomass production in the OF plots would likely result in underestimation of the direct OF contribution to SOM with the differential method (as measured in the Andosol, Figure 4c). In the Arenosol trial, the vegetation composition did not change during the experiment (Tillard, pers. comm.).
- (ii) Mineralization of the initial soil organic C (Figure 1) could be higher in the OF-amended plots, e.g. through a priming effect [22], [23]. The priming effect involves significant changes in soil organic matter renewal [22], possibly caused by OF input. The latter could accelerate mineralization of the initial C and thus gradually replace part of this fraction. In such cases, the direct contribution of the OF would be underestimated by the differential method. Organic fertilization is a common practice leading to considerable input of relatively labile C into agricultural soils. However, studies dealing with OF-induced SOM priming-effect remain very scarce most likely due to the methodological challenge of labeling OF with ¹³C.

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

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

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

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

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

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

4.2 Resolution and sensitivity of the OF isotopic method

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

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

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

Whatever the number of repetitions performed, attention should be paid to the risk of bias due to the propagation of errors in the mixing equation [8]. The sensitivity analysis (see part 2.8) of the mixing equation according to natural variations in the parameters revealed that the 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 variable with the greatest impact (Table S2).

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

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

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

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

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

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

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

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

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

years) stabilization of carbon.

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

5. Conclusion

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

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

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This paper is dedicated to Jerome Balesdent, Research Director at INRAE who died prematurely at the age of 62. Jérôme was a senior scientist at INRAE and one of the most renowned French soil scientists of his generation. Jérôme was a recognized pioneer in the use of stable C isotopes for studying SOM dynamics in C3/C4 transitions. Here we would like to pay tribute to the great soil scientist and generous man he was. He will be greatly missed by the soil science community.

Data are available upon request to the corresponding author.

Disclosure statement

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

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612

Table list

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

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

Figure list

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

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

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

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

Figure 5. Carbon stock derived from organic fertilizers as a function of cumulated C inputs from organic fertilizer amendments (0-15 cm soil layer) using the synchronic isotopic method. Linear model of the Andosol compost amended plots with retention of 36 % ($R^2=0.93$), 23 % ($R^2=0.94$) in the Arenosol and 8 % ($R^2=0.98$) in the Arenosol slurry amended plot. Each point represents the average of the three plots and the error bars are the standard deviation (only two blocks in year 3 for the Arenosol and year 10 for the Andosol). The insert compares data from previous studies with the regression lines of the different modalities. Note that data from literature had a 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]				
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 sillage	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 extention 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	continous	-25.9 to -27.3 (estimated from litterature)	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				
				[cm]				[‰ VPDB]	[‰ VPDB]	[‰ VPDB]	[%]	[kgC.ha ⁻¹]	[%]	[kgC.ha ⁻¹]
Chrono-sequence	Gerzabek <i>et al.</i> , 1997 [34]	Uppsala, Sweden	Eutric Cambisol	0 - 20	Green manure	42 years	Continuous	-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	Continuous	-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	Continuous	-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	continuous	-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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655

656 **Figure 1.**

657

658 **Figure 2.**

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Supplementary information

Table S1. Percentage of OF-C according to the methods, i.e. differential, synchronic isotopic and asynchronous isotopic. Mean of three OF-C estimations $\pm$ standard deviation (only two blocks in year 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

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

	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

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

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

	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.**