Open access • Journal Article • DOI:10.1016/J.SOILBIO.2014.04.030

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Published on: 01 Sep 2014 - Soil Biology & Biochemistry (Pergamon)

Topics: Soil carbon, Soil organic matter and Mineralization (soil science)

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Soil Biology & Biochemistry 76 (2014) 149–160

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

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ARTICLE INFO

Article history:
Received 18 December 2013
Received in revised form
25 April 2014
Accepted 29 April 2014
Available online 17 May 2014

Keywords:
Priming effect
Triggering
Soil organic matter destabilisation
Stable isotope spectroscopy
PLFA
Biolog test

ABSTRACT

Input of organic matter to soil may stimulate microbial activity and alter soil carbon storage by modifying the mineralization of native soil organic carbon (SOC). Assessing the age of SOC affected by the altered mineralization is a major challenge as the destabilisation of old SOC would be much more damageable for the overall carbon budget than the mobilization of recent SOC.

Here, we investigated the microbial populations sequentially activated after the addition of a labile substrate. We questioned whether they have distinct metabolic potential and we characterised the age of the native SOC they primed. We used soils from Congolese *Eucalyptus* plantations that were previously under savannah and which old and recent SOC exhibited different $\delta^{13}C$. Soils were amended with glucose, in an amount sufficient to induce microbe growth, and incubated for one week. The $\delta^{13}C$ of respired CO_2 was continuously recorded using a tuneable diode laser spectrometer (TDLS). The combination of two glucose treatments with different $\delta^{13}C$ signatures allowed partitioning the various sources of CO_2 over time (recent SOC, old SOC and glucose). This was combined with phospholipids fatty acids (PLFA) analyses and potential metabolic activities measurements after 40 h and seven days of incubation.

A peak of glucose mineralization occurred after 17 h of incubation. Before this peak (Stage 1), some specific communities with a strong feeding preference for recent SOC were activated. After the glucose peak (Stage 2), over-mineralization of native SOC occurred for some days. The recent C3 SOC was first preferentially used (Stage 3), while the old C4 SOC was destabilised in a later stage (Stage 4). Metabolic functions and composition of microbial communities also differed between Stages 3 and 4. Microbial populations collected at Stage 4 were slower compared to Stage 3, but more efficient in decomposing nutrient-containing substrates. Gram negative bacteria (16:1w7c and 18:1w7c) were stimulated at Stage 3 only, while Gram negative bacteria (cy17:0) were stimulated at both Stages 3 and 4.

Our results demonstrated that the input of labile substrate alters the microbial community composition, potential metabolic activities, and the SOC pools utilisation. They pointed out the necessity to assess the age of destabilised SOC when investigating the impact of priming on carbon storage in soil.

1. Introduction

Agricultural practices favouring the return of plant residues to soil are recommended by the International Panel for Climate Change (IPCC, 2004) as a means to mitigate the raise of atmospheric carbon (C) responsible for climate change. Indeed it is generally

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http://dx.doi.org/10.1016/j.soilbio.2014.04.030
0038-0717/

admitted that the additional input of fresh organic matter increases soil C stocks (Lal, 2005), and improves soil fertility through the release of nutrients from the additional input. In turn, this also increases primary production and C sequestration in plant biomass (Versini et al., 2013). However, the entrance of easily mobilizable organic substrates, (i.e. glucose, cellulose) into the soil can temporary activate diverse microbial populations and affect the cycling of soil organic carbon (SOC) (Kuzyakov, 2010). Such effect could be especially harmful to the environment and opposite to the expected goal when SOC is over-mineralized in an amount exceeding the stabilisation of the added substrate, but also if stable SOC is destabilised.

The short-term change in SOC dynamics induced by adding a substrate is called "priming effect" (PE) (Löhnis, 1926). Two types of PE occur within a temporal sequence. The first type of PE is an early phenomenon called "apparent priming effect" occurring during the first hours after the substrate addition (Blagodatkaya and Kuzyakov, 2008). Dormant and potentially active microorganisms are triggered: they accelerate their metabolism by mineralizing their storage compounds (de Nobili et al., 2001). The activity of the triggered microbial populations stops quickly when the energy provided by the added substrate is insufficient to synthesize enzymes and to grow. If the degradation of the added substrate allows enzymes production, the second type of PE called "real priming effect" is induced. The real priming effect is negative when the activated microorganisms mobilize the added substrate only. In this case, the mineralization of SOC temporary decreases until the complete consumption of the preferred substrate. In contrast, the real priming effect is positive when the activated microorganisms mineralise both the SOC and the added substrate.

A theory developed by Fontaine and coauthors in 2003 received a favourable echo in the soil science community. They hypothesized that the increase of SOC mineralization after labile substrate addition involves the succession of different microbial communities. The labile substrate favours the activity and development of fast growing microbes, called r-strategists, compared to slow growing microbes called K-strategists. After labile substrate consumption, the r-strategists may prolong their activity by using labile SOC for a few days. In the meanwhile, the K-strategists that benefit from r-strategist necromass or hydrolysis sub-products are slowly activated. They display enzymes necessary to mineralise complex compounds and are responsible for enhanced mineralization of recalcitrant SOC.

The hypothesis of Fontaine et al. (2003) has not been fully validated yet. Some changes in the structure of microbial populations have been shown after the addition of substrate (*e.g.* Nottingham et al., 2009; Bird et al., 2011; Dungait et al., 2011). However most studies only permitted to identify two sources for the mineralised CO_2: the added substrate and the native SOC. No distinction was made between the relative contributions of old recalcitrant and recent labile sources in the overmineralization of native SOC in spite of their importance for the overall C balance. Only few studies were designed to identify more than two CO_2 sources (Kuzyakov and Bol, 2004; Blagodatskaya et al., 2011, 2014), but they did not focused on the temporal evolution of these SOC sources.

In our study we tested the hypothesis that glucose addition destabilizes old SOC by sequentially activating different microbial populations, the first ones feeding on recent labile SOC and the second ones on old recalcitrant SOC. Here, we applied a glucose solution on soils sampled from two adjacent C3 *Eucalyptus* plantations afforested on a former C4 savannah. The isotopic composition of CO_2 released from the soil was recorded with a high temporal resolution (every 30 min) using isotope infrared spectroscopy to determine the relative contribution of CO_2 sources. The combination of two glucose treatments with different $\delta^{13}C$ signatures allowed partitioning the various sources of CO_2 over time (recent C3 SOC, old C4 SOC and glucose). To relate changes of CO_2 sources with functional changes of microbial populations, we also measured phospholipid fatty acid (PLFA) known as biomarker of microbial communities (Amelung et al., 2008), and we determined metabolic profiles of microorganisms using Biolog EcoPlates.

2. Materials and methods

2.1. Soil samples

The studied sites are two *Eucalyptus* plantations established on a native savannah at the experimental station of Kondi (CRDPI – CIRAD) in Congo (4°35′ S, 11°75′E). Mean air temperature is 25 °C with very low seasonal variations. Annual precipitations average 1200 mm with a dry season from mid-May to end of September. The soil is a Ferralic Arenosol (FAO) containing more than 80% of sand. Two adjacent plots of a native savannah were planted with *Eucalyptus*. Plot 1 was planted with the clone PF1 1-41 (density of 530 trees per hectare) in January 1992, harvested and afforested a second time in 2001 with the most commonly used hybrid in Congo (*E. urophylla x E. grandis, clone UG 18-52;* density of 800 trees per hectare). Plot 2 was afforested in 2001 with the *Eucalyptus* clone PF1 1-41 (density of 530 trees per hectare). The starter fertilization at the afforestation of the native savannah in 1992 or 2001 was: 10.3 kg ha^{-1} of N, 10.3 kg ha^{-1} of P and 16.7 kg ha^{-1} of K. For the second rotation of Plot 1 in 2001, 43.2 kg N ha^{-1} were also added. Despite low SOC and nitrogen contents (Table 1), the mean primary production over a 7-year rotation is about 20 m^3 ha^{-1} yr^{-1} for the clone PF1 1-41 and 30 m^3 ha^{-1} yr^{-1} for the *UG* 18-52 clone.

The first five centimetres of the mineral soil were collected in December 2008, after almost 17 years and 7 years of afforestation with *Eucalyptus* growing in Plot 1 and Plot 2, respectively. The collected soils were named accordingly 17 yr Euca soil (Plot 1) and 7 yr Euca soil (Plot 2). 7 yr Euca soil was less enriched in recent C3 SOC ($\delta^{13}C = -19.2‰$) compared to 17 yr Euca soil ($\delta^{13}C = -23.7‰$) as a direct consequence of the time elapsed since the conversion of savannah into *Eucalyptus* plantation (Table 1).

2.2. Soil incubation

The 7 yr and the 17 yr Euca soils were air-dried after sampling, sieved (<4 mm) and pre-incubated at 20 °C and 11.5% moisture content for six weeks. The isotopic composition of the CO_2 was promptly measured during the preincubation period (see below for method description). It was −24.7‰ and of −26.7‰ for the 7 yr and 17 yr Euca soil, respectively. This illustrated that the recent C3 SOC contributed more to the respiration than the old C4 SOC (65%–70% in the 7 yr Euca soil, and 80–85% in the 17 yr Euca soil - values depending on the choice of C3 and C4 end-members for the linear mixing model). Subsequently, any increment in the low flux of C4 CO_2 will induced a larger shift in CO_2-$\delta^{13}C$ than the same increment

Table 1
Characteristics of soil samples collected in the 0–5 cm horizon. *Data from Epron et al., 2009.

Soil	Time since afforestation years	C-content g kg^{-1}	$\delta^{13}C$ ‰	N-content g kg^{-1}
7 yr Euca Soil	7	5.9 ± 0.4	−19.2 ± 0.5	0.38 ± 0.01
17 yr Euca Soil	17, two rotations	9.2 ± 0.5	−23.7 ± 0.1	0.52 ± 0.02
Native savannah	–	5.7 ± 0.7	−14.8 ± 0.3*	0.36 ± 0.07

in the high flux of C3 CO_2. The effect will be more pronounced in the 17 yr Euca soil where C4-SOC respiration is very low. For this reason, we expect C4 priming to be more easily detected in 17 yr Euca soil and C3 priming in 7 yr Euca soils.

The pre-incubated soils were poured in flat containers and sprayed with a glucose solution (200 μg C g $soil^{-1}$) or with distilled water (control treatment) (Fig. 1). The raise in moisture content was only 1% to avoid any pulse of respiration due to soil rewetting (Meisner et al., 2013). The amount of added glucose C slightly higher than the soil microbial C (from 60 to 130 μg C g $soil^{-1}$ (Versini et al., 2014)) was expected to stimulate microbial growth and to induce a real priming effect (Blagodatskaya et al., 2007; Blagodatskaya and Kuzyakov, 2008). Each soil was submitted to two different glucose treatments: a "^{13}C-enriched-glucose" addition (glucose $\delta^{13}C = -9.8‰$ – Sigma, France) and a "normal-glucose" addition (the $\delta^{13}C$ of glucose equals the $\delta^{13}C$ of CO_2 measured during the preincubation period). The "normal glucose" substrates were obtained by mixing the glucose product at −9.8‰ with another glucose product at −26.7‰ (Groningen University, The Netherlands). As demonstrated by Kuzyakov and Bol (2004), the decomposition of glucose was completely independent on its isotopic composition. The combination of the "^{13}C-enriched-glucose" and "normal-glucose" treatments allowed partitioning the various sources of CO_2. In addition, the continuous recording of the CO_2-$\delta^{13}C$ shifts in the "normal-glucose" modality (see method description below) provided some insights in the sources of native SOC affected by the priming effect and was useful to define sampling dates for microbial analyses while the experiment was running. Indeed, the $\delta^{13}C$ signature of the CO_2 released by the mineralization of the normal-glucose substrate was expected to only slightly mask the changes in signature of the CO_2-$\delta^{13}C$ released from native SOC.

The amended soil was finally mixed, sieved (<4 mm) and distributed in 2l-jars (1 kg dry soil per jar). Incubations were performed in triplicate at 25 °C for one week. After 40 h and 7 days of incubation 30 g of dry soil were collected from each jar using a corer (1 cm ∅) to perform enzymatic tests and PLFA analyses.

2.3. PLFA analyses

The soil microbial community was characterized by phospholipids fatty acids (PLFA) (Amelung et al., 2008). Lipids were extracted from 4 g of freeze-dried soil by using a modified Bligh and Dyer extraction (Boschker et al., 1999). The PLFA fraction was then isolated on silica columns and derivatized to volatile fatty-acid methyl esters. Carbon content and isotopic composition of PLFA were measured by gas chromatography–isotope ratio mass spectrometry (Middelburg et al., 2000). A detailed description of the PLFA extraction procedure and analysis can be found in Boschker (2004).

Gram negative ($Gram^-$) bacteria were tracked using some specific mono-unsaturated fatty acids (16:1ω7c, i17:1ω7 and 18:1ω7c) and cyclopropyl fatty acids (cy17:0 and cy19:0) (the latter also characterizes anaerobic Gram positive bacteria, that were unexpected in our sandy soil). Gram positive ($Gram^+$) bacteria were characterized by terminally branched saturated fatty acids (i15:0, a15:0, i16:0, i17:0 and a17:0). Within $Gram^+$ bacteria, Actinobacteria, belonging to the phylum of Actinobacteria, were specifically identified by methyl-branching on the tenth atom of the C chain (10Me16:0 and 10Me18:0). Fungi were characterized by 18:2ω6c (Zelles, 1999; Amelung et al., 2008).

We tried to partition the glucose versus C3 and C4 SOC sources in PLFA, but the accuracy of the isotope measurement of PLFA in our samples (about 2‰) was too low to get significant conclusions.

2.4. Community level physiological profiles

We analysed the catabolic diversity of microbial populations using the Biolog™ method reported by Garland (1996). This technique consists of direct incubation of soil extract on Biolog microplates and provides the pattern of potential C-substrate utilisation. It is based on tetrazolium dye reduction as an indicator of sole-C-source utilization and tests 31 of the most used C sources. Biolog™ ECO plates have been used in ecological studies to estimate metabolic potential of microbial communities (Stefanowicz, 2006).

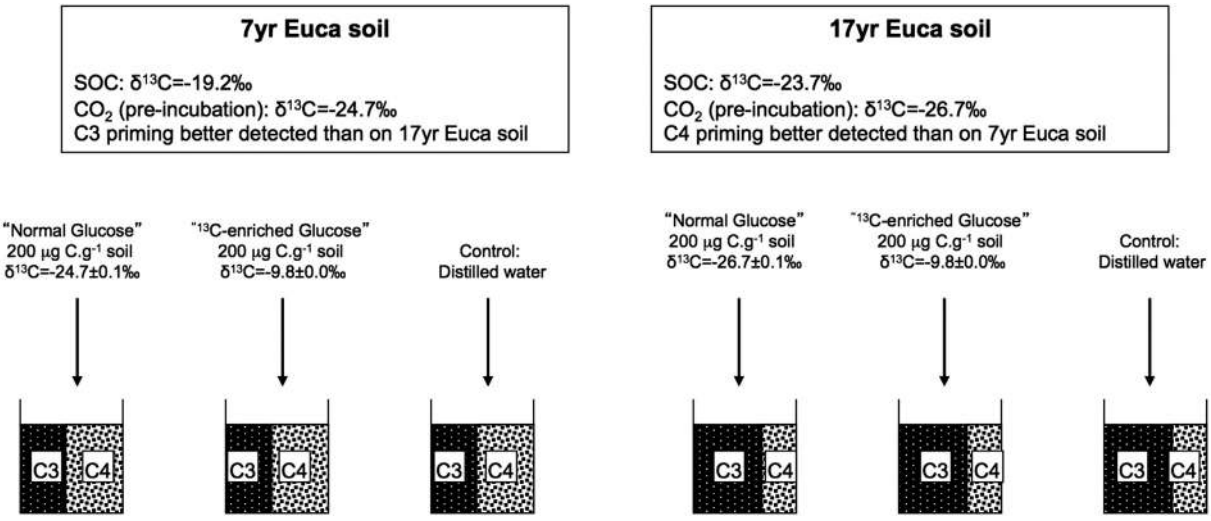

Fig. 1. Schematic representation of the approach chosen to determine the age of the primed SOC. In any soil amended with glucose, the isotope composition of respired CO_2 was obtained by summing the $\delta^{13}C$ of CO_2 released from SOC and the $\delta^{13}C$ of CO_2 released from glucose, each term being weighted by its contribution to the respiration flux (see Eq. (4)). This equation has two unknowns, the $\delta^{13}C$ of CO_2 released from SOC and the relative contribution of glucose-C (or SOC) to the respiration. The equation was written for "normal glucose treatment" and for "^{13}C enriched glucose treatment". The system of two equations with two unknowns could be resolved. We performed the experiment on two soils differing by the date of afforestation. We indeed expected contrasted sensibility for detection of C3 and C4 SOC primings due to different intensities in the C3 and C4-CO_2 fluxes recorded during a pre-incubation.

One gram of freshly ground soil was suspended in 10 ml of sodium pyrophosphate (0.1%) solution and shaken for 20 min. Sample suspensions settled for 10 min, were then decanted and diluted to 1/10 with a NaCl solution (0.85%). Each well of Biolog plates was inoculated with 150 µl of sample supernatant. Three analytical replicates were performed for each substrate. Plates were incubated at 25 °C and the optical density was read for each plate at 16-h intervals over a period of 160 h. The average well colour developments (AWCD) of the different replicates were calculated according to Garland (1996) where AWCD equals the sum of the difference between the optical density of control (no substrate) and substrate wells divided by 31 (number of substrate wells in ECO plates). AWCD 0.5 (55-h maximum slope) was also used in statistical analyses.

2.5. Soil CO_2 efflux and $\delta^{13}C$

Soil respiration and CO_2 isotopic composition were determined using a Tuneable Diode Laser Spectroscopy (TDLS) (TGA100A; Campbell Scientific Inc.) coupled with a multiplexer measuring alternatively the three replicates of each treatment: control, "normal glucose" and "^{13}C-enriched glucose". In comparison with IRMS, laser spectroscopy has the advantage to allow automatic recording of the C isotopes in CO_2 at a very high frequency (Bai et al., 2011). In our device each replicate was measured during one minute every 30 min. Briefly, the diode laser produced linear wavelength scans centred on selected absorption lines of $^{12}CO_2$ and $^{13}CO_2$. The laser radiation was absorbed proportionally to the concentration of these gases in the sample cell. Three gas standard tanks were used for calibration (Air Product, 0.5% certified for CO2 concentrations), they were measured during one minute every five minutes (Marron et al., 2009). The ^{12}CO2 and ^{13}CO2 concentrations of the standards were respectively 297.21/3.19, 397.22/4.28 and 1189.17/12.83 µmol mol^{-1} (measurements done by PTEF-INRA Lorraine).

Total soil CO2 efflux (CO_2_tot) and its $\delta^{13}C$ ($\delta^{13}C_{CO_2\_tot}$) were calculated using the two following equations:

$$C_{CO_2\_tot} = \frac{([CO_2]_{out} - [CO_2]_{in}) \times P \times F}{8.314 \times T \times W} \quad (1)$$

where $[CO_2]_{in}$ and $[CO_2]_{out}$ are the CO_2 concentrations in the incubator influx and outflux, P is the atmospheric pressure, F is the flow inside the bottle, W is the weight of soil incubated in each bottle, T is the temperature (°K), 8.314 J mol^{-1} is the ideal gas constant.and

$$\delta^{13}C_{CO_2\_tot} = \frac{\frac{[^{13}CO_2]_{out} - [^{13}CO_2]_{in}}{[^{12}CO_2]_{out} - [^{12}CO_2]_{in}}}{R_{PDB}} - 1 \quad (2)$$

where R is isotopic ratio of VPDB (0.011179602).

In order to gain in $\delta^{13}C$ sensitivity in the measurement of low respiration fluxes, a tank of compressed air was used to stabilize the $^{12}CO_2$ and $^{13}CO_2$ concentrations of the incoming gas instead of using the ambient atmosphere, and the influx was set to 270 ml min^{-1}. Moisture content was readjusted every day to compensate the flush of air circulating in the incubators. Data were excluded when they deviated by more than 5% from a running average computed from three consecutive measurements on the same incubator (what typically happened during and after periods of incubator opening or due to instrument malfunctions).

For glucose treatments, data are given as mean and standard deviation of the one-minute measurement made on the three independent replicates.

For control treatment, as the respiration signal was low, we computed for each replicate the average values of CO_2 efflux and $\delta^{13}C$ and their 95% confident interval from data recorded over the whole incubation period. These averaged values were further used to determine if the SOC mineralization in amended treatments was significantly different from the SOC mineralization in the control treatments.

2.6. Analysis of data recorded by the tuneable diode laser

The isotopic composition of the total produced CO_2, $\delta^{13}C$–CO_{2_tot}, was partitioned into SOC and glucose substrate sources according to the following equation

$$\delta^{13}C_{CO_2\_tot} = \delta^{13}C_{CO_2\_glc} \times f_{glc} + \delta^{13}C_{CO_2\_SOC} \times \left(1 - f_{glc}\right) \quad (3)$$

with f_{glc} the fraction of CO_2 efflux corresponding to glucose mineralization, $\delta^{13}C_{CO_2\_glc}$ the isotopic composition of the CO_2 produced from glucose and $\delta^{13}C_{CO_2\_SOC}$ the isotopic composition of the CO_2 produced from SOC.

Assuming no or negligible fractionation during glucose mineralization (Ekblad et al., 2002), Eq. (3) becomes:

$$\delta^{13}C_{CO_2\_tot} = \delta^{13}C_{glc} \times f_{glc} + \delta^{13}C_{CO_2\_SOC} \times \left(1 - f_{glc}\right) \quad (4)$$

Eq. (4) written for the two modalities of glucose addition ("normal glucose", glc_{normal}, and "^{13}C-enriched glucose", $glc_{enriched}$) provided a system of two equations with two unknowns, f_{glc} and $\delta^{13}C_{CO_2\_SOC}$:

$$\begin{cases} \delta^{13}C_{CO_2\text{-}tot_{(glc_{normal})}} = \delta^{13}C_{glc_{normal}} \times f_{glc} + \delta^{13}C_{CO_2-SOC} \times \left(1 - f_{glc}\right) \\ \delta^{13}C_{CO_2\text{-}tot_{(glc_{enriched})}} = \delta^{13}C_{glc_{enriched}} \times f_{glc} + \delta^{13}C_{CO_2-SOC} \times \left(1 - f_{glc}\right) \end{cases}$$

Solving the system, we get for any time:

$$f_{glc} = \frac{\delta^{13}C_{CO_2\_tot_{(glc_{normal})}} - \delta^{13}C_{CO_2\_tot_{(glc_{enriched})}}}{\delta^{13}C_{glc_{normal}} - \delta^{13}C_{glc_{enriched}}} \quad (5)$$

and whatever the modality of glucose addition:

$$\delta^{13}C_{CO_2\_SOC} = \frac{\delta^{13}C_{CO_2\_tot} - \delta^{13}C_{glc} \times f_{glc}}{\left(1 - f_{glc}\right)} \quad (6)$$

The respiration from native SOC, $C_{CO_2\_SOC}$, was computed as follows:

$$C_{CO_2\_SOC} = C_{CO_2\_tot} \times \left(1 - f_{glc}\right) \quad (7)$$

To assess the uncertainty in source partitioning, first order Taylor series approximations of the variances of f_{glc}, $\delta^{13}C_{CO_2\_SOC}$ and $C_{CO_2\_SOC}$ were calculated using partial derivatives (Phillips and Gregg, 2001). Detailed calculations are given in Appendix:

$$\sigma^2_{f_{glc}} = \frac{\sigma^2_{\delta^{13}C_{CO_2\_tot\ (glc_{normal})}} + \sigma^2_{\delta^{13}C_{CO_2\_tot\ (glc_{enrichedl})}}}{\left(\delta^{13}C_{glc_{normal}} - \delta^{13}C_{glc_{enriched}}\right)^2} \quad (8)$$

and

$$\sigma^2_{\delta^{13}C_{CO_2\_SOC}} = \frac{\sigma^2_{\delta^{13}C_{glc_{normal}}} \cdot \left(\delta^{13}C_{CO_2\_tot_{(glc_{enriched})}} - \delta^{13}C_{CO_2\_SOC}\right)^2 + \sigma^2_{\delta^{13}C_{glc_{enriched}}} \cdot \left(\delta^{13}C_{CO_2\_tot_{(glc_{normal})}} - \delta^{13}C_{CO_2\_SOC}\right)^2}{\left(\delta^{13}C_{glc_{normal}} - \delta^{13}C_{glc_{enriched}} - \delta^{13}C_{CO_2\_tot_{(glc_{normal})}} + \delta^{13}C_{CO_2\_tot_{(glc_{enriched})}}\right)^2} + \frac{\sigma^2_{\delta^{13}C_{CO_2\_tot_{(glc_{normal})}}} \cdot \left(\delta^{13}C_{glc_{enriched}} - \delta^{13}C_{CO_2\_SOC}\right)^2 + \sigma^2_{\delta^{13}C_{CO_2\_tot_{(glc_{enriched})}}} \cdot \left(\delta^{13}C_{glc_{normal}} - \delta^{13}C_{CO_2\_SOC}\right)^2}{\left(\delta^{13}C_{glc_{normal}} - \delta^{13}C_{glc_{enriched}} - \delta^{13}C_{CO_2\_tot_{(glc_{normal})}} + \delta^{13}C_{CO_2\_tot_{(glc_{enriched})}}\right)^2} \quad (9)$$

$$\sigma^2_{C_{CO_2\_SOC}} = \left(C_{CO_2\_tot}\right)^2 \times \sigma^2_{f_{glc}} + \sigma^2_{C_{CO_2\_tot}} \times \left(1 - f_{glc}\right)^2 \quad (10)$$

As the values and uncertainties for flux and $\delta^{13}C$ of SOC respiration in amended treatments were not measured but derived from the resolution of equations, classical statistical tests could not be used to compare amended treatments with control treatments. We then considered that control and amended treatments were significantly different when their 95% confidence intervals did not overlap.

3. Results

3.1. Changes in CO_2 efflux and isotopic composition

Soil respiration for control treatment was about 0.12 and 0.15 mg C kg soil^{-1} h^{-1} for 7 yr and 17 yr Euca soil, respectively (Table 2). Glucose addition induced a strong emission of CO_2 emission 17 h after the incubation started (peak of 10 mg C kg soil^{-1} h^{-1}, Fig. 2). Respiration sharply dropped to 1 mg C kg soil^{-1} h^{-1} after 20 h of incubation and continued to slightly decrease until the end of the incubation.

In the control treatments the mean CO_2-$\delta^{13}C$ was −23.6‰ and −27.0‰ in the 7 yr and the 17 yr Euca soil, respectively (Table 2). Large variations around the mean values were due to the low sensitivity of the TDLS for such low respiration. The CO_2-$\delta^{13}C$ was strongly affected by glucose addition. Signal from soils amended with "^{13}C enriched glucose" at −9.8‰ tended towards this value at the peak of CO_2 emission (Fig. 2 – top). The $\delta^{13}C$ decreased after the peak, but remained above the $\delta^{13}C$ of CO_2 from the control soils over the 7 days of monitoring.

The $\delta^{13}C$ of CO_2 from soils amended with "normal-glucose" substrate showed a different pattern (Fig. 2 – bottom). Four stages were identified. Stage 1 corresponded to the first six hours of incubation during which the $\delta^{13}C$ of CO_2 decreased towards about −30‰. In Stage 2, between 6 h of incubation and the peak of CO_2 efflux, the isotopic composition rapidly increased to reach about the $\delta^{13}C$ value of the added glucose (−24.7‰ and −26.7‰ for 7 and 17 yr Euca soil, respectively), supporting the assumption of none or negligible fractionation during glucose mineralization made to convert Eq. (3) into Eq. (4). In Stage 3, after the peak and until about 50–60 h of incubation, the CO_2-$\delta^{13}C$ displayed lower values than the added glucose substrates. Finally, in Stage 4, after 50–60 h of incubation and until the end of the experiment, the CO_2-$\delta^{13}C$ slightly increased. It reached values similar to the $\delta^{13}C$ of added glucose in 7 yr Euca soil, and values a bit higher than the CO_2-$\delta^{13}C$ emitted from control treatment in 17 yr Euca soil.

3.2. Sources of emitted CO_2

The isotopic signature of CO_2 was used to partition the relative contributions of glucose (f_{glc}) and SOC to respiration. Calculations could only be performed after six hours of incubation, when the $\delta^{13}C$ of emitted CO_2 stand within the range of the $\delta^{13}C$ of the two glucose substrates used as end-members in Eq. (5). The kinetics of glucose and SOC relative contributions to soil respiration were similar for the soils from the two plots. Glucose substrate strongly dominated the respiration peak. Then, the proportion of CO_2 originating from glucose slightly decreased to 50% of total efflux at the end of the seven-day incubation.

The supply of glucose substantially affected the mineralization of native SOC (Fig. 3). A significant increase in the mineralization of native SOC was observed during few hours after the peak of respiration in the 7 yr Euca soil, and during almost the whole Stage 3 in the 17 yr Euca soil. The large 95% confidence interval for the CO_2 production in 7 yr Euca control soil (Table 2) indeed strongly reduces the significance of differences observed between control and amended treatments.

Fig. 4 displays the $\delta^{13}C$ of CO_2 emitted from native SOC calculated according to Eq. (6). A shift in isotopic composition towards values lower than the control treatment signature indicates a raise in the relative contribution of recent C3 material derived from *Eucalyptus* to the mineralised native SOC. Conversely, values higher than control soils demonstrate a raise in the relative contribution of old C4 material derived from savannah. As explained in the section "soil incubation" of the Material and Methods, the detection of C3 SOC priming was easier in 7 yr Euca soil and conversely, the detection of

Table 2
Mean and 95% confidence interval for CO_2 efflux and carbon isotopic composition recorded over the 7 day incubation period in each control incubator. *n*: number of measurements per replicate.

Soil	Replicate	*n*	CO_2 efflux		$\delta^{13}C$	
			Mean mg C h^{-1} kg soil^{-1}	95% Confidence interval mg h^{-1} kg soil^{-1}	Mean ‰	95% Confidence interval ‰
7 yr Euca Soil	1	252	0.146	[0.142 : 0.150]	−21.4	[−21.6:−21.1]
	2	241	0.110	[0.107 : 0.112]	−23.5	[−23.8:−23.3]
	3	254	0.108	[0.105 : 0.112]	−25.8	[−26.0:−25.5]
17 yr Euca Soil	1	149	0.150	[0.146 : 0.153]	−26.0	[−26.3:−25.7]
	2	114	0.151	[0.148 : 0.155]	−27.5	[−27.7:−27.2]
	3	45	0.169	[0.165 : 0.173]	−27.4	[−27.7:−27.1]

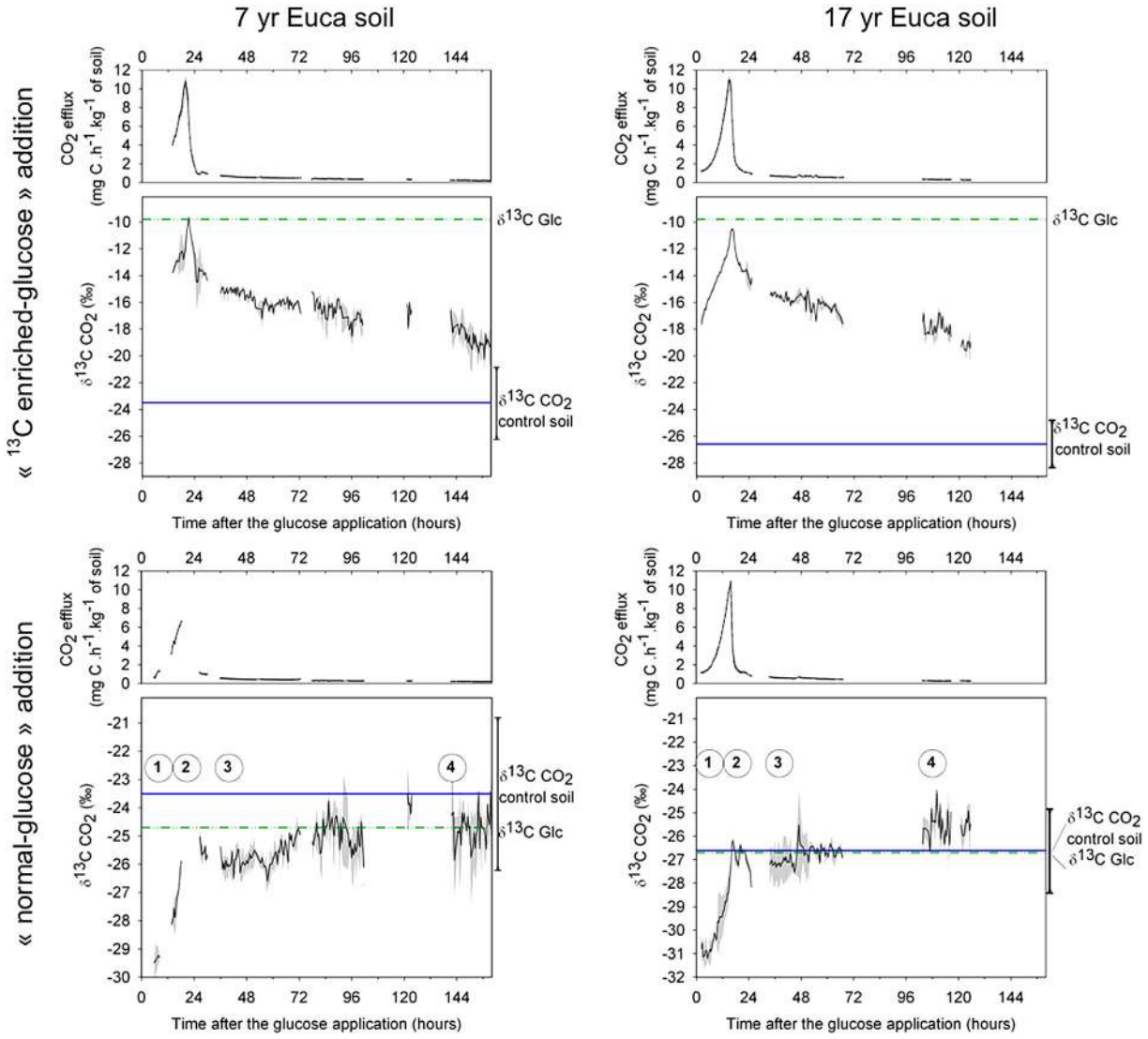

Fig. 2. CO_2 efflux rate and isotopic composition recorded every 30 min, after addition of "^{13}C-enriched-glucose" (top panel), or "normal-glucose" (bottom panel). Data recorded on soils from the plot afforested in Eucalyptus for 7 years are on the left, data recorded on soils sampled from the plot afforested in Eucalyptus for 17 years are on the right. For amended soils, the grey area shows the standard deviation around the mean black curve ($n = 3$). The blue line indicates the mean isotopic composition measured over the whole incubation period in control soils, and also gives the CO_2 isotope composition of amended soil before glucose addition. The bars on the left of the graphics indicate the standard deviation of the mean CO_2-δ^{13}C of control soils. The green dash-double dot line provides the isotopic composition of the added glucose substrate. Four stages, referred as Stages 1, 2, 3 and 4, were identified from the direct visualisation of the shift in CO_2-δ^{13}C in the "normal-glucose" modality - as the δ^{13}C of CO_2 emitted from native SOC was not masked by the δ^{13}C of CO_2 emitted from the glucose. Stage 1 corresponds to the triggering phase, Stage 2 to the peak of respiration, Stage 3 to a phase of decrease in δ^{13}C of CO_2 emitted from native SOC compared to controls, Stage 4 to a phase of increase in δ^{13}C.

C4 SOC priming was easier in 17 yr Euca soil. An increase in the relative contribution of recent C3 SOC was showed in both 7 yr and 17 yr Euca soil amended with glucose after the peak of respiration. It was significant for 60 h in 7 yr Euca soil but only for few hours in 17 yr Euca soil. A significant raise in the relative contribution of old C4 SOC was then observed during Stage 4 in 17 yr Euca soil.

3.3. PLFA profiles

PLFA data were first utilised to estimate the microbial biomass (Blagodatskaya and Kuzyakov, 2013). Using the conversion factor provided by Bailey et al. (2002) who observed that 1 nmol PLFA correspond to a flush of 2.4 μg C released by fumigation, and the 0.45 factor for the efficiency of the biomass extraction by fumigation (Jenkinson et al., 2004), we estimated that microbial biomass accounted for about 90 μg C.g soil^{-1} in the control treatments. In the 7 yr Euca soil, the addition of glucose increased the PLFA by 73% at Stage 3, and 29% at stage 4 (Table 3). In the 17 yr Euca soil, the addition of glucose increased the microbial biomass by ca 20% at both Stages 3 and 4. Analysis of individual PLFA biomarkers revealed that Gram$^-$ bacteria were strongly stimulated by glucose addition (Table 3, Fig. 5). Gram$^+$ bacteria were also stimulated during Stage 3, but only in 7 yr Euca soil. No changes were observed in PLFA specific of Actinobacteria and Fungi.

3.4. Changes in enzymatic activity

Effects of glucose addition on catabolic diversity of soil microbial populations were evaluated by substrate utilization patterns, using Biolog tests. Glucose addition significantly reduced the latency stage and the time to reach AWCD 0.5 whatever the stage and the soil sample (Table 4a). Moreover, the latency stage and the delay to reach AWCD 0.5 were significantly lower at Stage 3 compared to Stage 4 whatever the soil sample (Table 4a). For glucose treatments,

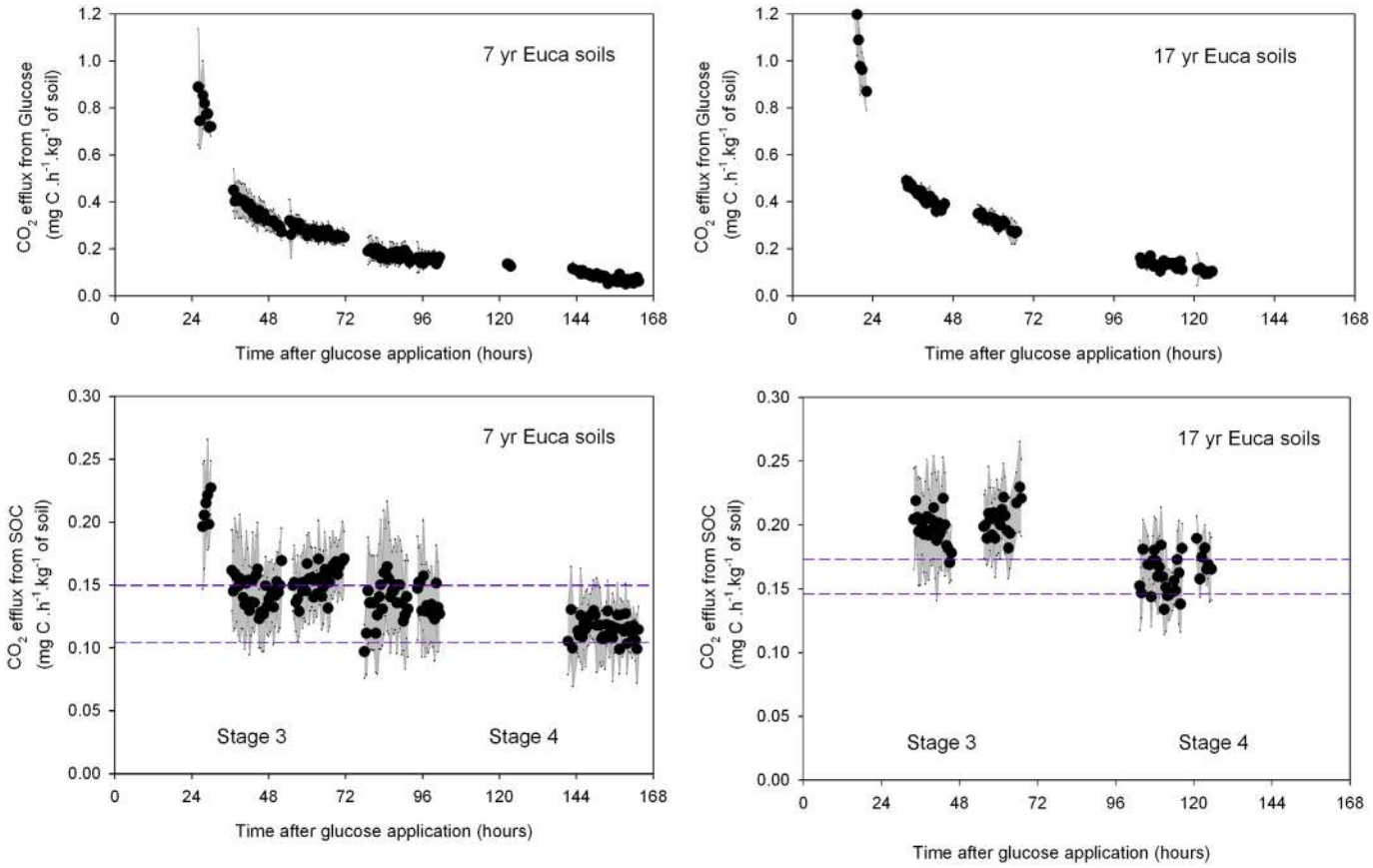

Fig. 3. CO_2 produced from the incubated glucose (top panel) and of the native SOC (bottom panel) calculated according to Eq. (5) from data recorded every 30 min. Results for 7 yr Euca Soil are on the left, results for 17 yr Euca Soil are on the right. Fluxes are presented with the associated uncertainty as a grey area (see Section 2.6). The CO_2 efflux from control soil is also given on graphics showing CO_2 production from native SOC, as a 95% confidence interval standing within the dashed lines.

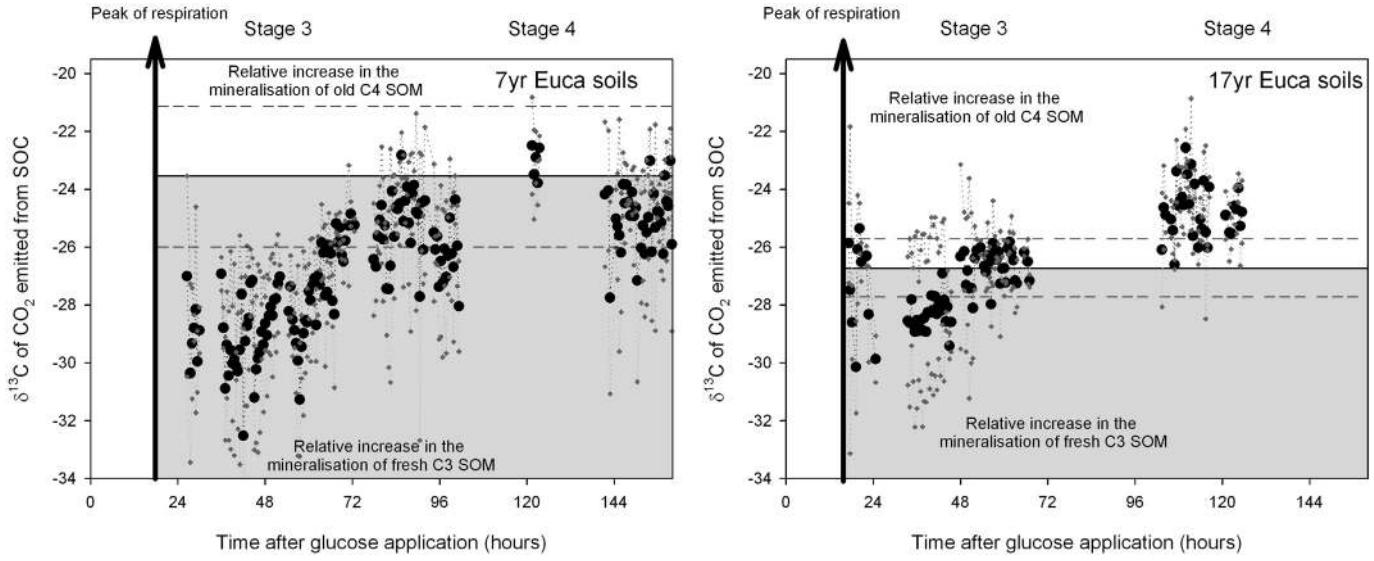

Fig. 4. Change in $\delta^{13}C$ of CO_2 emitted from native SOC after glucose addition. Dark dots stand for $\delta^{13}C$ values computed from Eq. (6). Grey crosses showed the uncertainty associated with calculation as given by Eq. (9). Results for 7 yr Euca Soil are on the left panel, for the 17 yr Euca Soil on the right. The dark line separating the grey and white area indicates the mean $\delta^{13}C$ of CO_2 from control soils, and also give the $\delta^{13}C$ of CO_2 from amended soil before glucose addition. The dash-lines indicate the 95% confidence interval of $\delta^{13}C$ of CO_2 in control soils. Any shift into the grey zone shows a relative increase in the contribution of C3 recent C to the bulk SOC mineralization. Conversely, any shift into the white zone shows a relative increase in the contribution of C4 old C to the bulk SOC mineralization. Control and amended treatments are significantly different in $\delta^{13}C$ when the 95% confident interval of the control and the uncertainty computed for glucose treatment do not overlap.

Table 3
Difference in individual PLFA concentrations between glucose and control treatments at stage 3 and stage 4 (after 40 h and 7 days of incubation, respectively). Measurements were done on a composite sample deriving from sampling in each triplicate treatment. Data from glucose treatments (Glc) are provided as the average and standard deviation (Sd) of "normal glucose" and "enriched glucose" modalities. n.a.: non available.

Organisms	7 yr Euca soil								17 yr Euca soil							
	Stage 3				Stage 4				Stage 3				Stage 4			
	Control	Glc	Sd	Glc	Control	Glc	Sd	Glc	Control	Glc	Sd	Glc	Control	Glc	Sd	Glc
	(nmol C g^{-1} soil)			% of control	(nmol C g^{-1} soil)			% of control	(nmol C g^{-1} soil)			% of control	(nmol C g^{-1} soil)			% of control
Bacteria (G+)	76.4	135.1	10.6	177%	68.9	87.0	1.5	126%	75.4	86.1	15.6	114%	70.3	82.4	0.2	117%
Actinobacteria	13.1	13.4	0.8	102%	12.5	14.2	1.3	113%	12.0	12.3	2.5	103%	11.6	12.9	0.0	111%
Bacteria (G-)	86.9	146.3	6.4	168%	78.7	110.2	7.0	140%	102.8	130.6	16.4	127%	81.4	107.3	0.0	132%
Fungi	2.2	3.0	n.a.	139%	0.0	4.0	n.a.	n.a.	8.9	8.3	0.1	93%	6.0	7.0	n.a.	117%
Non specific	84.0	155.5	9.0	185%	74.1	97.8	0.6	132%	89.5	101.4	16.9	113%	81.1	95.3	0.1	118%
Total	**262.6**	**453.3**	**5.3**	**173%**	**243.3**	**313.2**	**10.7**	**129%**	**288.7**	**338.6**	**50.9**	**117%**	**250.3**	**304.9**	**22.0**	**122%**

microbial populations were significantly faster at Stage 4 compared to Stage 3 in degrading simple carbohydrates as well as amino acids, amines and glycerol-P, but slower in degrading polymers and carboxilic acids (Table 4b).

4. Discussion

4.1. Advantages and limitations of the three sources partitioning method

The distinction between old and recent C pools affected by priming effect is a major challenge as the mobilisation of long-term preserved SOC would be much more damageable for the overall C budget than the mobilisation of fresh SOC. Recently, Blagotatskaya et al. (2011, 2014) successfully applied a ^{14}C labelled substrate on C3–C4 soil to disentangle old and recent SOC contributing to the over production of CO_2. However the use of a radioactive substrate may require adapted laboratory facilities, depending on national regulations. We chose another approach that combined two treatments of glucose addition, with different $\delta^{13}C$ signature, with the resolution of a system of equations. Compared to experiments using radioactive substrate, this approach to trace respiration sources by using stable isotopes does not require adapted laboratory facilities but faced the problems of ^{13}C fractionation and of soil CO_2 admixing with atmospheric CO_2, which limits data accuracy (Midwood and Millard, 2011; Pausch and Kuzyakov, 2012). The

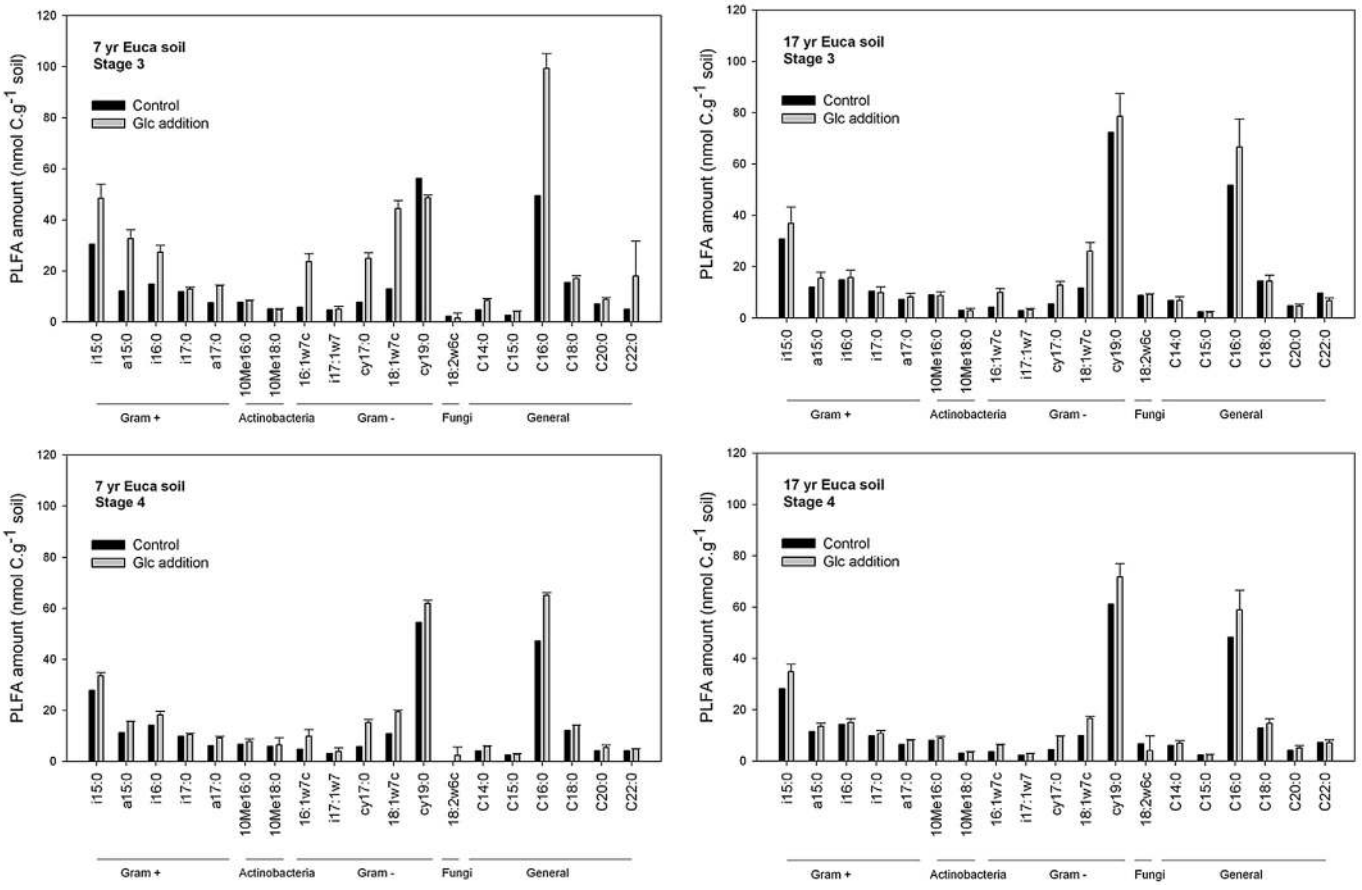

Fig. 5. PLFA in glucose and control treatments at stage 3 and stage 4 (after 40 h and 7 days of incubation, respectively). Results for 7 yr Euca Soil soil are on the left panel, for the 17 yr Euca Soil on the right. Measurements were done on a composite sample deriving from sampling in each triplicate treatment. Data from glucose treatments (Glc) are provided as the average and standard deviation of "normal glucose" and "enriched glucose" modalities.

Table 4a
Comparison of soil microbial metabolic responses (AWCD 0.5 and latency stage) of 7 yr and 17 yr Euca soil. Mean and standard deviation of AWCD and latency stages were calculated from triplicate 7 yr and 17 yr Euca Soil plates by using three soil suspension. For each soil sample and each stage, differences in AWCD 0.5 and latency stage between the control and glucose treatments were performed with a one-way ANOVA (Scheffe's F test). Bold values indicate a p-value <0.05.

Soil sample	7 yr Euca soil				17 yr Euca soil			
Stage	Stage 3		Stage 4		Stage 3		Stage 4	
Modalities	Control	Glucose	Control	Glucose	Control	Glucose	Control	Glucose
AWCD 0.5 (days)	4.6 ± 0.4	**3.0 ± 0.1**	6.0 ± 0.2	**3.4 ± 0.1**	4.7 ± 0.2	**3.0 ± 0.2**	7.4 ± 0.1	**3.3 ± 0.2**
Latency stage (days)	2.0 ± 0.1	**1.2 ± 0.1**	4.1 ± 0.2	**2.1 ± 0.2**	2.2 ± 0.1	**1.2 ± 0.2**	3.8 ± 0.4	**2.1 ± 0.2**

continuous record by the TDLS of the $^{12}CO_2$ and $^{13}CO_2$ concentrations in the incoming air flushing the incubators reduced the uncertainty related to atmospheric CO_2 contribution to the isotope signal. It indeed provides the atmosphere background, what a traditional IRMS cannot do. However, inevitably, the accuracy of C-sources partitioning decreased over the course of our experiment, as respiration decreased. Nevertheless, we were able to determine the contribution of glucose and glucose derived metabolites to CO_2 emission versus SOC contribution with less than 5% of uncertainty during the first days of the experiment, and with around 10–20% of uncertainty at the end of the incubation. The good accuracy in the isotope signature further allowed reporting significant changes in the relative contributions of recent C3 or old C4 to the mineralization of SOC. As the recent C3 SOC contributed less to the basal respiration in 7 yr Euca soil than in 17 yr Euca soil, the sensitivity for detecting an increase in C3 respiration was better in 7 yr Euca soils, yet. Conversely, the sensitivity for detecting an increase in C4 respiration was better in 17 yr Euca soils.

Table 4b
Substrates preferentially consumed (Biolog microplates) by soil microbial populations whatever the soil between the modalities 40 h (stage 3) and 7 days (stage 4).

Substrate		Stage 3 vs Stage 4	p-value
Miscellaneous	Pyruvic Acid Methyl Ester	=	0.124
	Glucose-1- Phosphate	=	0.064
	D,L-α-Glycerol Phosphate	<	**0.039**
Polymeres	Tween 40	>	**0.024**
	Tween 80	>	**0.036**
	α- Cyclodextrin	=	0.739
	Glycogen	>	**0.037**
Carbohydrates	D-Cellobiose	<	**0.023**
	α-D-Lactose	<	**0.043**
	β-Methyl-D- Glucoside	=	0.056
	D-Xylose	<	**0.039**
	i-Erythritol	=	0.874
	D-Mannitol	<	**0.000**
	N-Acetyl-D- Glucosamine	=	0.140
Carboxilic acids	D- Glucosaminic Acid	>	**0.001**
	D-Galactonic Acid γ-Lactone	>	**0.002**
	D- Galacturonic Acid	=	0.284
	2-Hydroxy Benzoic Acid	>	**0.003**
	4-Hydroxy Benzoic Acid	>	**0.003**
	γ-Hydroxybutyric Acid	=	0.419
	Itaconic Acid	=	0.294
	α-Ketobutyric Acid	=	0.429
	D-Malic Acid	=	0.356
Amino acids	L-Arginine	=	0.147
	L-Asparagine	<	**0.041**
	L- Phenylalanine	<	**0.026**
	L-Serine	<	**0.043**
	L-Threonine	<	**0.028**
	Glycyl-L- Glutamic Acid	=	0.425
Amines Amides	Phenylethyl- amine	<	**0.032**
	Putrescine	<	**0.029**

Difference in the substrates consumption between stage 3 and 4 was performed at AWCD 0.5 with a one-way ANOVA (Scheffe's F test, $n = 3$). Bold values indicate a p-value < 0.05. Legend: = , not significant differences; > significantly more consumed at stage 3; < significantly more consumed at stage 4.

4.2. Evidence of triggering on microbial populations feeding on recent C3 SOC

In our experiment, the use of a laser spectrometry offered the unique opportunity to capture changes in the respiration sources at a hour timescale (Griffis et al., 2004; Plain et al., 2009; Epron et al., 2011), while studies on priming sources traditionally collect CO_2 trapped in NaOH vessels at a daily or weekly timescale. During Stage 1, the mineralization of glucose had started, as demonstrated by the shift towards glucose $\delta^{13}C$ in the "enriched glucose" treatment (Fig. 2), where the glucose $\delta^{13}C$ clearly differed from the $\delta^{13}C$ of CO_2 produced in control soil. But the glucose mineralization was quite low yet. Such a lag phase in glucose mineralization was already reported in other studies and interpreted as a phase of microbe reactivation and growth (De Nobili et al., 2001; Schneckenberger et al., 2008; Zyakun et al., 2013), what was confirmed by the raise in PLFA amounts (Table 3). A strong decrease of $\delta^{13}C$ of CO_2 during Stage 1 was observed in the "normal-glucose" treatments (Fig. 2). In these "normal-glucose" treatments the added glucose had a C isotope composition similar to the $\delta^{13}C$ of CO_2 produced in control soil. The $\delta^{13}C$ decrease resulted either (i) from a change in sources of mineralised SOC immediately after glucose addition or (ii) from isotope fractionation during glucose mineralisation. We posit that the main reason would be the preferential utilisation of the recent C3 organic matter. Not all microbes responsible for the basal respiration would be rapidly triggered by the glucose addition, but only some specific microbial populations feeding on C3 material. Zyakun et al. (2013) indeed demonstrated by measuring respiratory coefficient that the reactivation of microbes occurring rapidly after glucose addition involves the mineralization of stored lipids, known to be ^{13}C depleted. This interpretation of a sole stimulation of some microbe feeding on recent C3 SOC would be consistent with the further observation that the microbial communities active at stage 3 after the peak of glucose consumption preferentially utilise C3 SOC. If any isotope fractionation occured at Stage 1 it would be due to kinetics effect. Measurements of CO_2 $\delta^{13}C$ performed at the peak of glucose consumption (Stage 2) indeed indicated that thermodynamic fractionation during glucose mineralisation was minor.

4.3. Characteristics of microbial populations sequentially active

The triggered populations utilised the added glucose-C at Stage 2 and the recent C3 SOC at Stage 3 as glucose-C became rapidly included in microbial metabolites and less accessible (Derrien et al., 2007; Hill et al., 2008). At Stage 4, changes in the isotopic signature of mineralised SOC in the 17 yr Euca soil amended with glucose indicated that the active populations preferentially mineralised the old C4 SOC. In the conditions of the Biolog test, these populations collected at Stage 4 consumed the tested substrates slower than populations from Stage 3, and interestingly they exhibited a higher potential for the decomposition of nutrient-containing substrates (Table 4a and 4b). The soils of our experiment are known to be poor in available nitrogen (Mareschal et al., 2013). We postulated that

the populations efficient in N mining outcompeted the populations active at Stage 3 when available nitrogen became limiting. They found their N-resource in the old C4 SOC, old soil organic matter being N-rich compared with recent one due to nitrogen immobilisation by microbes during decomposition (von Lützow et al., 2007). The relation between old SOC mining and N availability is not univocal, yet. Blagodatskaya et al. (2011) invoked the mining of old SOC to meet needs in N as we did, but Waldrop and Firestone (2004a) showed that addition of mineral N can enhanced old SOC mineralization through an increase oxidative activity. These opposite responses indicate that the enhanced mineralization of old SOC depends on the factor limiting the activity of the microbial populations, *e.g.* energy, nitrogen … (Hamer and Marschner, 2005; Fontaine et al., 2011; Nottingham et al., 2012; Sullivan and Hart, 2013).

Gram$^-$ bacteria were particularly important in the response to substrate addition, as also found by other authors (Waldrop and Firestone, 2004b; Notthingham et al., 2009; Bastida et al., 2013; Dungait et al., 2013) and could be designed as r-strategists. A complementary experiment that we performed adding 200 μg C. soil^{-1} of 99% ^{13}C labelled glucose in 17 yr Euca soil revealed that the growth of microbe was supported by glucose substrate at Stage 3 while native SOC was also included in the newly formed biomass at Stage 4 (See Supplementary). PLFA markers for Gram$^+$ bacteria experiment did not exhibit the same dynamics in the two soils of our experiment. Observations made by other groups indicate they usually do not rapidly take advantage of labile substrate addition. Waldrop and Firestone (2004b) and Bird et al. (2011) observed a response of Gram$^+$ populations several months after substrate addition whereas Bastida et al. (2013) revealed a response only delayed by few days compared to Gram$^-$ populations. In line with these priming experiments, Kramer and Gleixner (2008) demonstrated that at the steady state Gram$^-$ bacteria preferentially use recent SOC and Gram$^+$ bacteria old SOC.

4.4. Implications of pulse-addition of labile substrates on the preservation of carbon in soil

After seven days of incubation 50% of the glucose-C was still retained in soil (ca. 100 μg g^{-1} of soil), largely exceeding the amount of native SOC that had been over-mineralised (ca. 10 μg g^{-1} of soil). At the end of the experiment it can be reasonably assumed that the glucose-C was included in microbial metabolites and that in average it will be preserved over years to decades (Derrien et al., 2006). Balancing the C input into the soil by the output is not sufficient to assess the soil storage potential yet. Changes in the duration of SOC storage must also be inspected. The shifts in the isotope composition of respired native SOC showed that the glucose addition induced first a destabilisation of the recent pool of native SOC for a couple of days and then a destabilisation of the old pool of native SOC (Fig. 4). The latter may seriously impact the C storage in our soils. We could neither precisely determine the age of the old organics that were destabilised (apart than they were older than 7 or 17 yr old, depending on the soil), nor determine if the destabilisation occurred for a few days or persisted over time. Even if the effect is short, it is of primary importance to consider it as pulse addition of labile substrate frequently occurs in most plant-soil systems. Pulse-inputs are typical for root exudation and breaking-down of dead microbial cells releasing soluble compounds in the rhizosphere (Kuzyakov, 2010). Soluble extracellular enzymes also scavenge substrates away from the rhizosphere and generate flush of easily available compounds. Therefore, the potential of a soil to preserve C is strongly dependent on the age of the SOC utilised by microbes after sporadic but regular supply of labile substrate. Some previous studies (Blagodatskaya et al., 2008, 2011) reported that the rate of C input determines the intensity of the priming: (i) low input is insufficient to induce microbe growth and solely causes a microbe biomass turnover, (ii) input in an amount similar to microbe biomass induces a positive priming – as in our experiment, (iii) while input several time higher than the microbe biomass induces a zero or negative priming due to the preferential use of the added substrate rather than SOC. Blagodatskaya et al. (2011) reported that higher biomass turnover caused by a low input only affects the recent SOC, in accordance with our observations during Stage 1. We may also expect that despite a balance favouring C sequestration, a high substrate addition may alter the duration of C storage. Recent SOC would be less mobilised than in normal conditions due to the great accessibility of added substrate, but a high amount of nutriments would be required, leading to a destabilisation of old SOC in nutrient-poor soil.

5. Conclusions

To assess the impact of pulse-addition of labile substrate on the C storage in soil, the amount of preserved substrate should be compared to the over-mineralized SOC, but shifts in the age of mineralised SOC should also be investigated. Our study demonstrated that the pulse input of glucose induced major changes in microbial community structure and SOC utilisation patterns, inducing first a preferential use of recent SOC but then a destabilisation of old SOC. This study making use of TDLS to partition the native SOC sources of respired CO_2 at a very high frequency calls for other experiments using the same kind of device, preferentially in soils with a high basal respiration so that to gain in signal sensitivity, and if possible on longer period of time.

Nutrient limitation could be responsible for the shift from recent to old SOC sources and would then exert a strong control on the duration of carbon preservation in soil. It appears crucial to verify this idea on soils with different levels of fertility.

Ackowledgements

This work was funded by the Fabelor research program (12000176A). The UMR 1137 and the UR 1138 are supported by the French National Research Agency through the Laboratory of Excellence ARBRE (ANR-12- LABXARBRE-01). We thank the technical staff of the CR2PI for taking care over years of the C3C4 chronosequence in Congo, Jacques Ranger for helping with soil sampling, Nicolas Richet and André Clerc for technical support, Frans Stellaard for offering its C3-glucose, Mélanie Maître and Charlotte Burgault for data treatment, Jean-Paul Laclau, Rémi d'Annunzio and Louis Mareschal for discussions and the two referees for their comments. Pierre-Emmanuel Courty gratefully acknowledge the Swiss National Science Foundation (ambizione grant PZ00P3_136651). Special thoughts to Anna and Paulette.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.04.030.

Appendix

The solution (Eq. (8)) to the general Taylor series equation for the variance of f_{glc} (Eq. (5)) can be determined as follows

$$f_{glc} = \frac{\delta^{13}C_{CO_2\_tot_{(glc_{normal})}} - \delta^{13}C_{CO_2\_tot_{(glc_{enriched})}}}{\delta^{13}C_{glc_{normal}} - \delta^{13}C_{glc_{enriched}}} \quad (A1)$$

Let's denote

$\delta^{13}C_{CO_2\_tot_{(glc_{normal})}}, \delta^{13}C_{CO_2\_N}$

$\delta^{13}C_{CO_2\_tot_{(glc_{enriched})}}, \delta^{13}C_{CO_2\_E}$

$\delta^{13}C_{glc_{normal}}, \delta^{13}C_{glc\_N}$

$\delta^{13}C_{glc_{enriched}}, \delta^{13}C_{glc\_E}$

The partial derivatives for f_{glc} are:

$$\frac{\partial f_{glc}}{\partial \delta^{13}C_{CO_2\_N}} = \frac{1}{\delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E}}$$
$$\frac{\partial f_{glc}}{\partial \delta^{13}C_{CO_2\_E}} = \frac{-1}{\delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E}}$$
$$\frac{\partial f_{glc}}{\partial \delta^{13}C_{glc\_N}} = \frac{-\left(\delta^{13}C_{CO_2\_N} - \delta^{13}C_{CO_2\_E}\right)}{\left(\delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E}\right)^2} \quad (A2)$$
$$\frac{\partial f_{glc}}{\partial \delta^{13}C_{glc\_E}} = \frac{\left(\delta^{13}C_{CO_2\_N} - \delta^{13}C_{CO_2\_E}\right)}{\left(\delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E}\right)^2}$$

These derivatives are substituted into

$$\sigma^2 f_{glc} = \left(\frac{\partial f_{glc}}{\partial \delta^{13}C_{CO_2\_N}}\right)^2 \cdot \sigma^2_{\delta^{13}C_{CO_2\_N}} + \left(\frac{\partial f_{glc}}{\partial \delta^{13}C_{CO_2\_E}}\right)^2 \cdot \sigma^2_{\delta^{13}C_{CO_2\_E}} + \left(\frac{\partial f_{glc}}{\partial \delta^{13}C_{glc\_N}}\right)^2 \cdot \sigma^2_{\delta^{13}C_{glc\_N}} + \left(\frac{\partial f_{glc}}{\partial \delta^{13}C_{glc\_E}}\right)^2 \cdot \sigma^2_{\delta^{13}C_{glc\_E}} \quad (A3)$$

to give

$$\sigma^2 f_{glc} = \frac{\sigma^2_{\delta^{13}C_{CO_2\_N}} + \sigma^2_{\delta^{13}C_{CO_2\_E}}}{\left(\delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E}\right)^2} + \frac{\left(\sigma^2_{\delta^{13}C_{glc\_N}} + \sigma^2_{\delta^{13}C_{glc\_E}}\right) \cdot \left(\delta^{13}C_{CO_2\_N} - \delta^{13}C_{CO_2\_E}\right)^2}{\left(\delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E}\right)^4} \quad (A4)$$

This equation can be approximated by

$$\sigma^2 f_{glc} = \frac{\sigma^2_{\delta^{13}C_{CO_2\_N}} + \sigma^2_{\delta^{13}C_{CO_2\_E}}}{\left(\delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E}\right)^2} \quad (A5)$$

Similarly, the solution (Eq. (9)) to the general Taylor series equation for the variance of $\partial^{13}C_{CO_2\_SOC}$ (Eq. (6)) can be determine as follows:

For the normal glucose modality, we have

$$\delta^{13}C_{CO_2\_SOC} = \frac{\delta^{13}C_{CO_2\_N} - \delta^{13}C_{glc\_N} \times f_{glc}}{\left(1 - f_{glc}\right)} \quad (A6)$$

with $f_{glc} = \frac{\delta^{13}C_{CO_2\_N} - \delta^{13}C_{CO_2\_E}}{\delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E}}$

Eq. (A6) can also be written as

$$\delta^{13}C_{CO_2\_SOC} = \frac{\delta^{13}C_{glc\_N} \times \delta^{13}C_{CO_2\_E} - \delta^{13}C_{glc\_E} \times \delta^{13}C_{CO_2\_N}}{\delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E} - \delta^{13}C_{CO_2\_N} + \delta^{13}C_{CO_2\_E}} \quad (A7)$$

Let's denote

$$\alpha = \delta^{13}C_{glc\_N} \times \delta^{13}C_{CO_2\_E} - \delta^{13}C_{glc\_E} \times \delta^{13}C_{CO_2\_N}$$
$$\beta = \delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E} - \delta^{13}C_{CO_2\_N} + \delta^{13}C_{CO_2\_E}$$

The partial derivatives for $\partial^{13}C_{CO_2\_SOC}$ are:

$$\frac{\partial \delta^{13}C_{CO_2\_SOC}}{\partial \delta^{13}C_{glc\_N}} = \frac{\beta.\delta^{13}C_{CO_2\_E} - \alpha}{\beta^2} = \frac{\delta^{13}C_{CO_2\_E} - \delta^{13}C_{CO_2\_SOC}}{\beta}$$
$$\frac{\partial \delta^{13}C_{CO_2\_SOC}}{\partial \delta^{13}C_{glc\_E}} = \frac{-\beta.\delta^{13}C_{CO_2\_N} + \alpha}{\beta^2} = -\frac{\delta^{13}C_{CO_2\_N} - \delta^{13}C_{CO_2\_SOC}}{\beta}$$
$$\frac{\partial \delta^{13}C_{CO_2\_SOC}}{\partial \delta^{13}C_{CO_2\_N}} = \frac{-\beta.\delta^{13}C_{glc\_E} + \alpha}{\beta^2} = -\frac{\delta^{13}C_{glc\_E} - \delta^{13}C_{CO_2\_SOC}}{\beta}$$
$$\frac{\partial \delta^{13}C_{CO_2\_SOC}}{\partial \delta^{13}C_{CO_2\_E}} = \frac{\beta.\delta^{13}C_{glc\_N} - \alpha}{\beta^2} = \frac{\delta^{13}C_{glc\_N} - \delta^{13}C_{CO_2\_SOC}}{\beta} \quad (A8)$$

These derivatives are substituted into

$$\sigma^2\delta^{13}C_{CO_2\_SOC} = \left(\frac{\partial \delta^{13}C_{CO_2\_SOC}}{\partial \delta^{13}C_{glc\_N}}\right)^2 \cdot \sigma^2_{\delta^{13}C_{glc\_N}} + \left(\frac{\partial \delta^{13}C_{CO_2\_SOC}}{\partial \delta^{13}C_{glc\_E}}\right)^2 \cdot \sigma^2_{\delta^{13}C_{glc\_E}} + \left(\frac{\partial \delta^{13}C_{CO_2\_SOC}}{\partial \delta^{13}C_{CO_2\_N}}\right)^2 \cdot \sigma^2_{\delta^{13}C_{CO_2\_N}} + \left(\frac{\partial \delta^{13}C_{CO_2\_SOC}}{\partial \delta^{13}C_{CO_2\_E}}\right)^2 \cdot \sigma^2_{\delta^{13}C_{CO_2\_E}} \quad (A9)$$

to give

$$\sigma^2\delta^{13}C_{CO_2\_SOC} = \frac{\sigma^2_{\delta^{13}C_{glc\_N}} \cdot \left(\delta^{13}C_{CO_2\_E} - \delta^{13}C_{CO_2\_SOC}\right)^2 + \sigma^2_{\delta^{13}C_{glc\_E}} \cdot \left(\delta^{13}C_{CO_2\_N} - \delta^{13}C_{CO_2\_SOC}\right)^2}{\left(\delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E} - \delta^{13}C_{CO_2\_N} + \delta^{13}C_{CO_2\_E}\right)^2} + \frac{\sigma^2_{\delta^{13}C_{CO_2\_N}} \cdot \left(\delta^{13}C_{glc\_E} - \delta^{13}C_{CO_2\_SOC}\right)^2 + \sigma^2_{\delta^{13}C_{CO_2\_E}} \cdot \left(\delta^{13}C_{glc\_N} - \delta^{13}C_{CO_2\_SOC}\right)^2}{\left(\delta^{13}C_{glc\_N} - \delta^{13}C_{glc\_E} - \delta^{13}C_{CO_2\_N} + \delta^{13}C_{CO_2\_E}\right)^2} \quad (A10)$$

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