

Pulse labelling of deep soil layers in forest with $^{13}\text{CH}_4$: testing a new method for tracing methane in the upper horizons, understorey vegetation and tree stems using laser-based spectrometry.

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This is a pre-copyedited, author-produced version of an article accepted for publication in Biogeochemistry following peer review. The article is available online at:
<https://doi.org/10.1007/s10533-021-00775-x>

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Funding (information that explains whether and by whom the research was supported)

This study was part of the EMEFOR project, which received financial support from the ADEME – REACTIF programme (convention n° 12-60-C0057), the French agency for ecological transition. This work was supported partly by the French PIA project “Lorraine Université d’Excellence”, reference ANR-15-IDEX-04-LUE. The installation of the experimental site was mainly supported by the French National Forest Office (ONF). UMR 1434 is supported by the ARBRE Laboratory of Excellence (ANR-11-LABX-0002-01). The isotope analyser belongs to the French National Research Infrastructure “AnaEE France” (ANR-11-INBS-0001).

Conflicts of interest/Competing interests (include appropriate disclosures)

‘Not applicable’

Availability of data and material (data transparency)

Data are available at the following link: <http://doi.org/10.5281/zenodo.3929102>

Acknowledgments

We are grateful to S. Martin, E. Dalle, P. Courtois for their technical assistance and to the colleagues who kindly helped us in the field (B. Amiaud, S. Chauchard, D. Desalme, N. Marron and P. Priault). We also thank B. Longdoz, I. Houillon and E. Delogu for the adaptation of the flux gradient model. We also thank the colleagues of the “Biogéochimie des Ecosystèmes Forestiers” laboratory who are in charge of the experimental forest where we did the experiment and to K. Klumpp who allowed us to use the isotope analyser.

Keywords

Laser-based spectrometry / Soil methane oxidation /Greenhouse gas /Stable isotopes /Methane diffusion

Abstract

Methane emissions from plants in wetlands are mainly due to internal transport, from the anoxic soil layers where methane is produced, to the atmosphere. This pathway has not yet been clearly demonstrated for upland forest vegetation, where methane can be produced in deep soil layers. We developed a new method to trace methane transfer from the deep soil. We conducted a $^{13}\text{CH}_4$ pulse labelling at 40-cm soil depth and then monitored $^{13}\text{CH}_4$ in the upper horizons, at the soil surface (with or without understorey vegetation) and emitted by tree stems until the total disappearance of the labelled gas. Most of the injected $^{13}\text{CH}_4$ was oxidized in the soil despite high soil water content. The understorey vegetation did not contribute to $^{13}\text{CH}_4$ emission by the soil. We prove that tree stems can emit methane produced in an upland forest soil, even when the said soil is a net methane sink. We conclude that pulse labelling with $^{13}\text{CH}_4$ and tracing by laser-based spectrometry is a promising tool approach to study the transport of methane from production to emission.

Introduction

Upland forest soils are one of the main biospheric sinks for atmospheric methane worldwide (Feng et al. 2020). Net soil methane uptake results from the balance of two opposite fluxes: methane is produced by methanogens driven by soil oxygen status (Le Mer and Roger 2001), and methane is consumed by methanotrophs. Soil methane uptake presents seasonal variations driven mainly by soil water content (SWC), which affects gas transport.

At the ecosystem level, vegetation can also affect the methane sink (Carmichael et al. 2014). Vegetation can enhance either methane emission or methane consumption. This depends mainly on soil water status and whether methane is being produced in the soil. In wetlands, the aerenchyma of some understorey plants acts like a pipe, allowing the methane produced in the anoxic soil horizons to diffuse directly into the atmosphere (Morrissey and Livingston 1992; Schimel 1995; Greenup et al. 2000; Bhullar et al. 2013). In upland forests, understorey vegetation either has no clear impact on methane emissions at high SWC or, in well-aerated conditions with low SWC, it enhances soil methane uptake (Halmeenmäki et al. 2017; Subke et al. 2018; Plain et al. 2019).

Tree stems can have an influence on the methane budget, even in upland forests: they can emit methane and thus decrease the net methane uptake of the ecosystem (Wang et al. 2016), though in some cases, methane uptake by tree stems has been observed (Machacova et al. 2016). The origin of the methane emitted by tree stems is still unclear. In wetland forests, the methane emitted by tree stems may have been produced in the soil and transported upward, a process which should be favoured by the presence of aerenchyma (Pangala et al. 2017). In upland forests, methane emission has been related to higher methane concentrations in the soil than in the atmosphere (Maier et al. 2018; Plain et al. 2019). While deep-rooted tree species can access deep soil horizons where methane may be produced, the transport of methane through the stems of these trees has not yet been clearly demonstrated (Pitz and Magonigal 2017; Covey and Magonigal 2019). In addition, methane emissions from tree stems is not always related to soil water status. Methane can be produced locally by saprotrophic fungi on the surface of the stems (Lenhart et al. 2012) or by archaea in the heartwood (Yip et al. 2019; Flanagan et al. 2021).

Labelling experiments with either stable or radioactive methane isotopes have been conducted to separate methane production and methane consumption in the soil and to estimate the contribution of the two processes to net methane uptake (von Fischer and Hedin 2002; Nielsen et al. 2017; Pedersen et al. 2018). However, so far, this approach has not been used to trace the transfer of methane from the soil to the atmosphere through tree stems and understorey vegetation, though this would be a promising approach to determine if plants have a significant influence on the methane budget of an upland forest.

We therefore developed a method to pulse-label the deep-soil layer with $^{13}\text{CH}_4$ and to trace the labelled CH_4 through the upper horizons of the soil to its emission by understorey vegetation, bare soil and tree stems. Our objectives were (i) to test whether this approach is suitable, and to estimate (ii) what fraction of the labelled methane was oxidised in the soil when the soil water content was high; (iii) to what extent understorey vegetation contributed to soil emissions, and (iv) whether tree stems emitted CH_4 originating in the soil.

Materials and Methods

Labelling experiment

The labelling experiment was conducted in the state-owned forest of “Hauts Bois” (north-eastern France) on an acidic riptic Luvisol with limited waterlogging due to a temporary perched water table (Plain et al. 2019; Bonnaud et al. 2019). Labelling was done at the end of March before budburst and therefore before tree transpiration resumed. To label the deep soil layer (-40 cm, just above the base of the temporary water table) with $^{13}\text{CH}_4$ and $^{13}\text{CO}_2$ and trace the labelled gases in the upper soil layers (0, 5, 25 cm), we used a membrane tube system installed 3.5 years ago and described in Plain et al. (2019) to create a gas concentration equilibrium between the air within the tubes and the surrounding (a schematic diagram of the experimental setup is provided in supplementary Figure 1).

We used a mass-flow controller to inject the labelled gases into a closed loop system at 40-cm in depth. A pump circulated the air in the loop at a flow of less than 0.8 l min^{-1} . One-hundred-and-twenty-five ml of pure $^{13}\text{CH}_4$ (99.2% Eurisotop, Cambridge Isotope Laboratory Inc., Andover, USA) were introduced first, followed by 2500 ml of $^{13}\text{CO}_2$ labelled at 50% (Eurisotop). The $^{13}\text{CO}_2$ was only a reference tracer since it is not consumed in the soil. Labelling was performed on March 22 at 10:00 UTC (time 0 in the graphs) and labelled gases were traced during 42 h.

The labelled $^{13}\text{CH}_4$ and $^{13}\text{CO}_2$ were traced at different depths in the soil (-25, -5 and 0 cm). The tubes at each of the three depths were connected to an isotopic trace gas analyser (see below). Before each measurement, the air inside the closed loop was homogenised for 7.5 minutes at a flow of less than 0.8 l min^{-1} . Soil O_2 concentrations were measured on the air circulating in the closed loop with an oxygen sensor (SO210 Apogee instruments, Logan, USA).

$^{13}\text{CH}_4$ and $^{13}\text{CO}_2$ effluxes to the atmosphere were measured on i) bare soil (two opaque PVC chambers, $20 \times 20 \times 20 \text{ cm}$), ii) understorey vegetation (two transparent acrylic plastic chambers, $20 \times 20 \times 25 \text{ cm}$, one on soil covered with *Juncus effusus* and one on soil covered with *Glyceria striata*, two aerenchymatous plants), and iii) tree stems (two chambers made of two transparent polymethyl methacrylate half-cylinders placed 0.2 m above the soil, one on a *Quercus petraea* stem and one on a *Salix caprea* stem). The trees were 6 m in height.

CH_4 and CO_2 concentrations and isotopic measurements were taken with an isotopic trace gas analyser (G2201-i Picarro Inc., Santa Clara, USA) connected to the soil device, the chambers and two calibration gas tanks by way of a manifold that could switch between lines, thus allowing to measure for 420 s each chamber every 128 minutes and each soil depth every 74 minutes.

Soil temperature (T_s) and soil water content (SWC) were measured continuously at depths of 10 and 25 cm with two water content reflectometers (CS650; Campbell Scientific). The SWC of the first 7 cm was measured with a soil moisture sensor (ThetaProbe ML2, Delta-T Devices, Cambridge, UK). SWC at each depth was converted into an air-filled porosity value (AFP) with bulk soil density (Epron et al. 2016).

Data analyses

All calculations and analyses were done on the R software (R Core Team 2017).

Fluxes (F_{CH_4} or F_{CO_2} respectively) were calculated from the slopes of the linear variations in $[\text{CO}_2]$ and $[\text{CH}_4]$ over time (Plain et al. 2019) after discarding the first 330 seconds needed to fully flush the cell of the analyser.

The atom fraction of ^{13}C in CO_2 and CH_4 was calculated based on mean $\delta^{13}\text{C}$ in CO_2 and CH_4 and the Vienna Pee Dee Belemnite standard isotope ratio for carbon (0.0111802). Excess $x^E(^{13}\text{C}-\text{CO}_2)$ and $x^E(^{13}\text{C}-\text{CH}_4)$ were calculated as the difference in the atom fractions measured at the same soil depth after labelling $x(^{13}\text{C})_{\text{lab}}$ and the mean of the two days of measurement before labelling $x(^{13}\text{C})_{\text{unlab}}$ multiplied by the concentrations of either CO_2 or CH_4 measured at the same depth:

$$x^E(^{13}\text{C}) = (x(^{13}\text{C})_{\text{lab}} - x(^{13}\text{C})_{\text{unlab}}) \times C \quad (1)$$

Excess ^{13}C in soil fluxes, $x^E(F^{13}\text{C}-\text{CO}_2)$ and $x^E(F^{13}\text{C}-\text{CH}_4)$, were calculated in the same way using fluxes instead of concentrations.

$x(F^{13}\text{C})_{\text{unlab}}$ was calculated based on the $\delta^{13}\text{C}$ measured before labelling.

Because stem emission of $^{13}\text{CH}_4$ was low, the range of variation in $^{13}\text{CH}_4$ in the chambers was too small to calculate a significant slope with a linear regression function. We therefore used the average atom fractions in the stem chamber and the $^{12}\text{CH}_4$ -fluxes ($F^{12}\text{C}-\text{CH}_4$) to calculate the excess.

The amount of labelled methane consumed within the soil was estimated according to a flux gradient model (Goffin et al. 2014):

$$C(z) = \frac{\partial \text{AFP} \times [\text{CH}_4]}{\partial t} + \frac{\partial}{\partial z} (Ds \times \frac{\partial [\text{CH}_4]}{\partial z}) \quad (2)$$

where $C(z)$ is consumption ($\text{mol CH}_4 \text{ m}^{-3} \text{ s}^{-1}$), AFP the air-filled porosity, $[^{13}\text{CH}_4]$ the concentration in $^{13}\text{CH}_4$ ($\text{mol CH}_4 \text{ m}^{-3}$), t time (s), z depth (m), D_s the effective soil diffusion coefficient of $^{13}\text{CH}_4$ ($\text{m}^2 \text{ s}^{-1}$), and $D_s \frac{\partial [^{13}\text{C}]}{\partial z}$ the diffusive flux as determined by Fick's law. D_s is a function of soil temperature and soil water content. The dependency on soil water content has been established from laboratory experiments and its dependence on T from the ideal gas law (Goffin et al. 2014). The total consumption in the first 25 cm of the soil was calculated by adding the consumption in all the layers, then adding the result of each time step of the model multiplied by the time interval between each step.

Results and discussion

Oxidation of labelled methane in the soil profile

The air-filled porosity (AFP), and the methane and oxygen concentrations in the soil profile during the labelling experiment are presented in supplementary Figure 2. The upper 25 cm of the soil were well-oxygenated despite the low AFP at the time of the year we conducted the experiment.

Labelled methane and CO_2 were recovered in all three soil layers (Fig. 1). The labelled methane was transported rapidly through the soil since the peak in the upper soil layers had already appeared only two to four hours after labelling. At 25 cm in depth, the $[^{13}\text{CH}_4]$ reached 1500 ppb, but maxima were lower in the upper layers where they only reached 40 ppb and 2 ppb at, respectively, -5 and 0 cm in depth. After ten hours, excess $^{13}\text{CH}_4$ was already very low, especially in the upper soil (23, 1.8 and 0.1 ppb at respectively -25, -5 and 0 cm in depth). After 24 hours, no more labelled methane could be detected in the upper soil layers and only 0.2 ppb remained at 25 cm in depth. A rapid diffusion of labelled methane through 45 cm of a stagnogleyic soil planted with ryegrass had already been reported by Shaw et al. (Shaw et al. 2014), but with an AFP twice as high.

The labelled methane in the soil profile disappeared much faster than the labelled CO_2 (Fig. 1), which suggests that processes other than diffusion are at play for methane. Indeed, through a flux gradient model, we estimated that 70 mg of labelled methane was oxidized in the top 25 cm of soil during the first ten hours, representing 73% of the total $^{13}\text{CH}_4$ injected into the soil. The high rate of methane oxidation we observed contrasts with two previous labelling experiments involving ^{13}C -labelled methane: oxidation represented only 1% of the added labelled methane in vegetated peat excavated in mesocosms (Nielsen et al. 2017), and only 7% in a mixture of rice paddy soil and river clay in which rice seedlings were growing under greenhouse conditions (Groot et al. 2003). In the latter two studies, oxidation only occurred in the vicinity of the roots due to the oxygenation of the rhizosphere by the plants. The high rate of methane oxidation in our soil, even during a period of low AFP and high SWC, agrees with previous results at our site where, despite the presence of a temporary perched water table, the soil is a net methane sink almost year round (Epron et al. 2016).

Emission of labelled methane by the soil

The net methane flux at the soil-atmosphere interface was negative, between -4.4 and -9.4 on bare soil and between -3.4 and -6.5 $\mu\text{g m}^{-2} \text{ h}^{-1}$ on soil covered with understorey vegetation (Fig. 2). The soil was therefore a weak methane sink at low AFP, and this was consistent with previous results for the site (Epron et al. 2016).

A small amount of $^{13}\text{CH}_4$ (less than 10 $\mu\text{g m}^{-2} \text{ h}^{-1}$) was emitted from both bare soil and soil covered with understorey vegetation, with a large variability among the four chambers. Maximum emissions were observed between two to four hours after labelling (Fig. 3). After ten hours, no more labelled methane was released from the soil. In contrast, excess $^{13}\text{CO}_2$ was still measurable in the different soil chambers after 42 hours (0.11 $\text{mg m}^{-2} \text{ h}^{-1}$; Fig. 3). In a previous study on mesocosms filled with agricultural soil (Shaw et al. 2014), the emission of labelled methane also only occurred for eight to ten hours after labelling, with a peak in emissions about three hours after the introduction of the labelled methane.

For soil both with and without vegetation, the rate of decrease in labelled methane emissions over time was similar, and maximum labelled methane emissions exhibited a similarly large range in both cases. We cannot firmly conclude whether or not there was an effect of the presence of understorey species with aerenchyma on soil methane emissions at our site since we chose to favour measuring frequency over number of repetitions. But beyond the question of the number of replicates, the lack of a marked difference in labelled methane emissions between soil with or without vegetation is in agreement with previous results for the site, where there was no influence of understorey vegetation on the net flux of methane during periods of methane production in the soil (Plain et al. 2019). While the

role of aerenchymatous plants in methane transport from the soil to the atmosphere has been well established for wetlands (Schimel 1995; Nielsen et al. 2017), the lack of effect in our study may reflect the depth at which we injected the methane in relation with the shallow rooting system of the two understorey species.

Emission of labelled methane by tree stems

Tree stems did indeed emit methane during the measuring period (between 3.5 and 5.8 $\mu\text{g m}^{-2} \text{h}^{-1}$, Fig 2) and were therefore a methane source, even though the soil remained a net methane sink, thus confirming our previous observations (Plain et al. 2019).

The tree stems also emitted labelled methane. The peak was reached less than five hours after labelling and no more labelled methane was detectable after 16 hours (Fig 3). We can conclude that in this upland forest, *Quercus petraea* and *Salix caprea*, both species that do not have stem aerenchyma, are able to transport methane produced at depth. This is in agreement with our previous observations that stem methane emissions were correlated to soil status and that the methane emitted could therefore, at least partially, have a soil origin (Plain et al. 2019).

It is unlikely that the labelled methane was dissolved and transported in the xylem sap because labelling was done before tree transpiration resumed. Before budburst, the main methane pathway into the tree stems is probably through passive gaseous diffusion or effusion (Meronigal et al. 2020). We demonstrated that this can occur in tree species that do not have stem aerenchyma. However, the labelled methane emitted by the stems (1.6 $\text{ng m}^{-2} \text{h}^{-1}$) was only a thousandth of the total methane emitted by these same stems during the measuring period. This result indicates that either high methane concentrations exist in even deeper layers of soil, accessible to tree roots, and that this methane could have diluted the labelled methane, or that, even in young trees, a large part of the methane emitted by the tree stems was of authigenic origin (Wang et al. 2016; Barba et al. 2019; Covey and Meronigal 2019).

To our knowledge, this is the first time that pulse-labelling of the deep soil layer with $^{13}\text{CH}_4$ was undertaken *in situ* in a forest to underline the dynamics of the methane in the upper horizons and at the soil surface, and the origin of the methane emitted by tree stems. This method is appropriate for estimating both the amount of methane transported through and consumed in the soil, and the amounts released by the soil, the understorey vegetation and trees. Progress in isotope analyser technology should make it possible to gain precision and speed for better coverage of both temporal and spatial variability.

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Figure 1. Average temporal variations of excess ^{13}C in (a) CO_2 and (b) CH_4 concentrations measured at different soil depths for the 42 hours following labelling on March 22 at 10 am. The black points represent measurements at -5 cm, dark grey at -10 cm and light grey at -25 cm depths. Error bars represent the standard error of the mean of the concentrations recorded during the last 90 seconds ($n=21$).

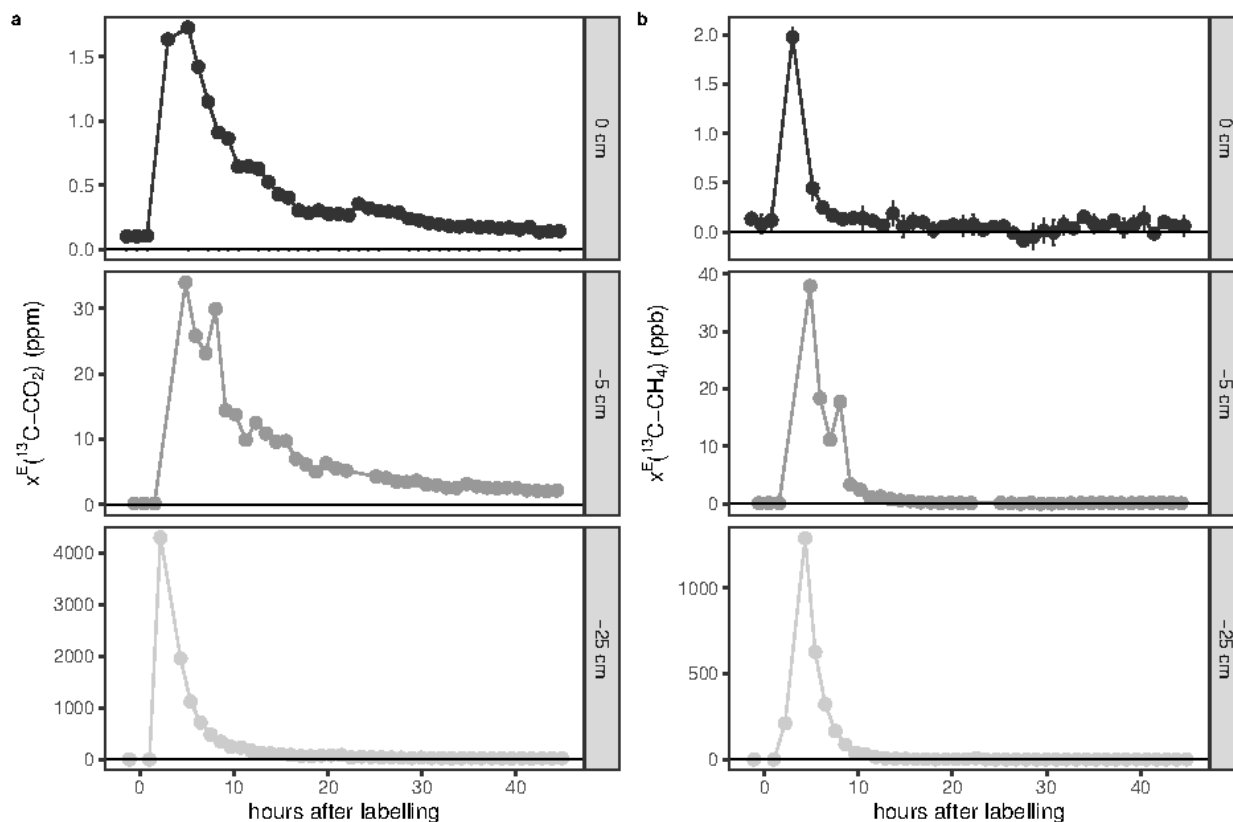


Figure 2. Average net $^{12}\text{CH}_4$ fluxes measured in two chambers (Ch) on bare soil, two on soil covered with understorey vegetation and two on tree stems for the 42 hours following labelling on March 22 at 10 am. Error bars represent the standard error of the mean of 25 measurements.

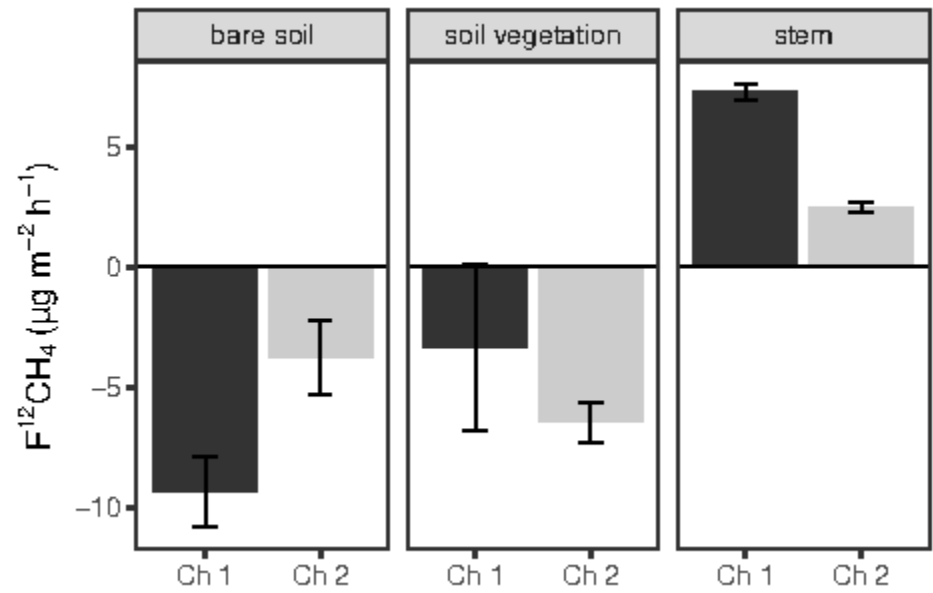
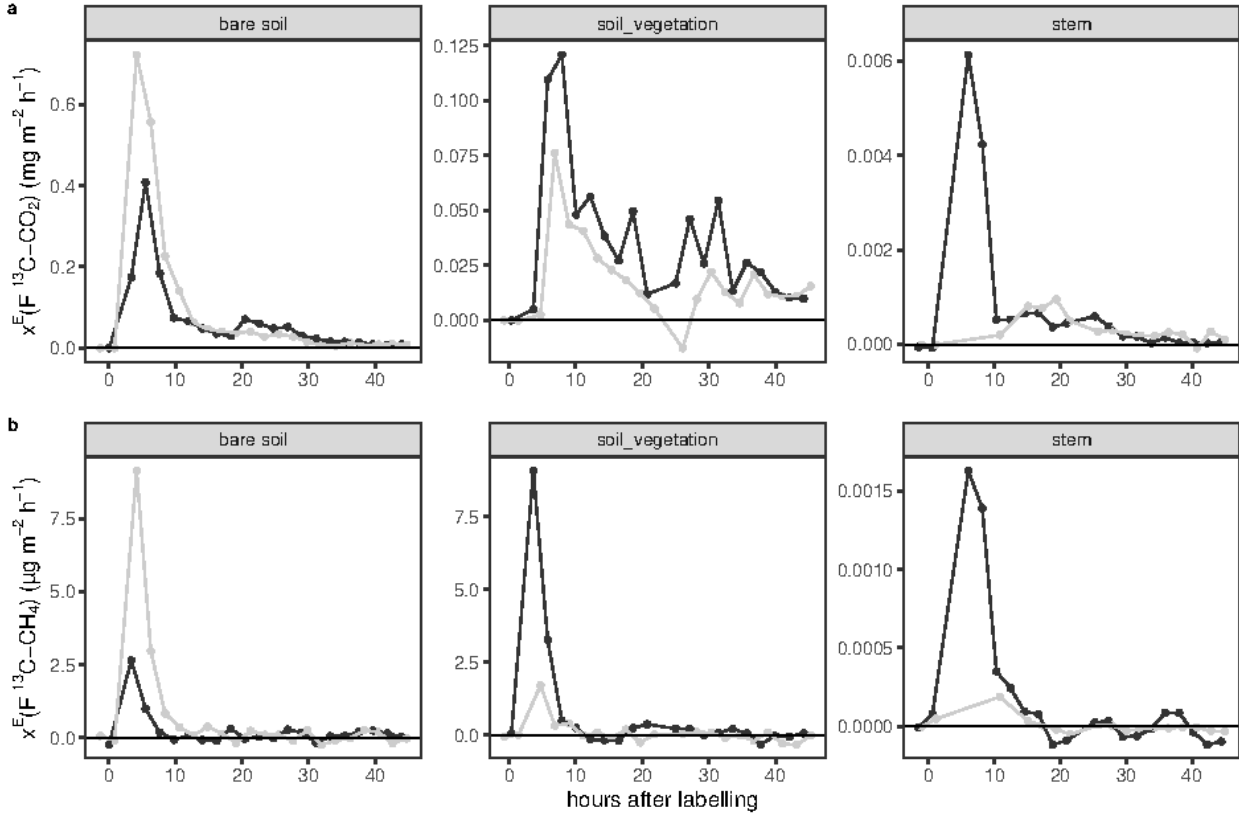
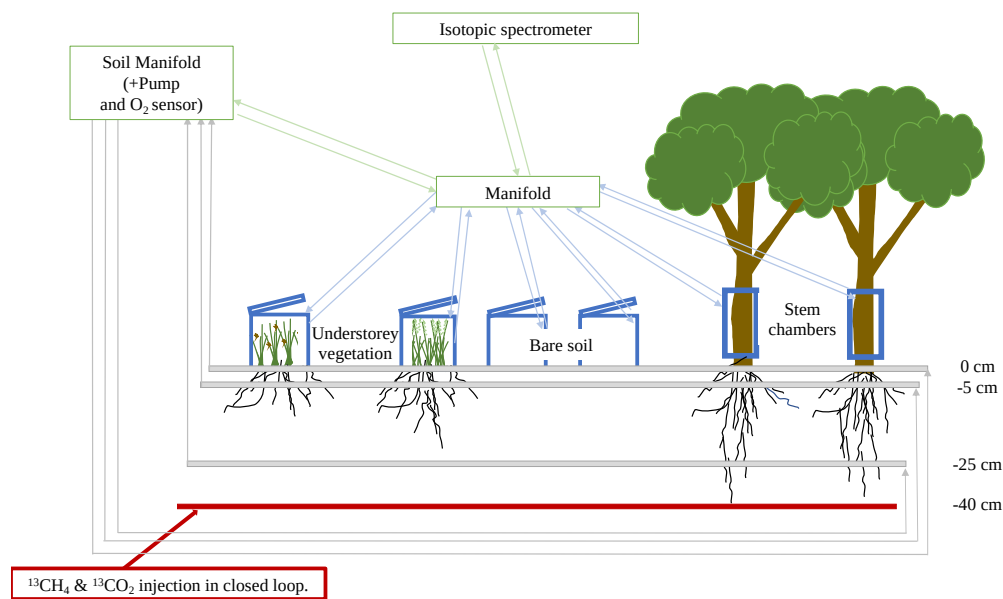


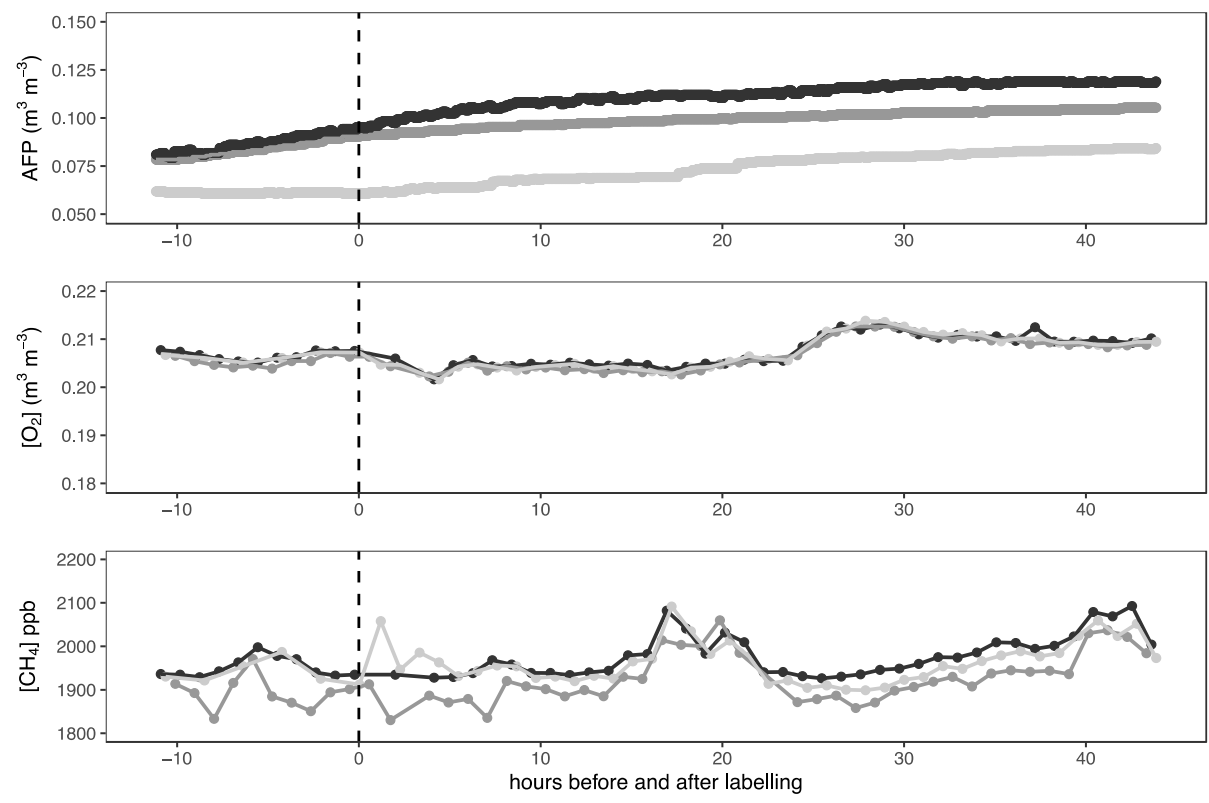
Figure 3. Temporal variations of excess ^{13}C in (a) CO_2 and (b) CH_4 fluxes recovered in each chamber (bare soil, soil with vegetation and tree stems) for the 42 hours following labelling on March 22 at 10 am.





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Supplementary figure 1. Schematic diagram of the experimental setup (vertical cross-section):
The thick red line is a porous membrane tubes at 40-cm in depth which was used to inject the labelled gases (close loop with a pump allowing an air circulation lower than 0.8 Lmin⁻¹), and the thick grey lines are the porous tubes which were used to trace the labelled gases at the different depth in the soil (-25, -5 and 0 cm). The blue boxes are the different chambers (soil with or without understorey vegetation and stem) which were used to trace the efflux of the labelled gases. The green boxes are the isotope spectrometer, the soil manifold that switches between the three soil depths, and the manifold that switches between the soil profile, the chambers and the calibration gases. An oxygen sensor (SO210 Apogee instruments, Logan, USA) was used to measure the O₂ concentrations in the porous tubes at the different depths. The blue, grey and green arrows are the tubing connections between the manifolds and the analyser. The large red arrows correspond to the diffusive transport of the labelled gases from depth to the atmosphere.



Supplementary figure 2. Temporal variations of (a) air-filled porosity (AFP), (b) O_2 concentrations and (c) CH_4 concentrations at different depths in the soil before and after labelling on March 22 at 10 UTC (vertical dashed black line). For AFP, the black points represent measurements at -5 cm, dark grey at -10 cm at and light grey at -25 cm depth. For O_2 and CH_4 , black points represent measurements at 0 cm, dark grey at -5 cm at and light grey at -25 cm depth. The soil temperature at 10 cm depth ranged from 6.7 to 8.2°C (data not shown).