**Biogeosciences
Discussions**

Long-term steady state ^{13}C labelling to investigate carbon turnover in plant soil systems

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Abstract

We have set up a facility allowing steady state $^{13}CO_2$ labeling of short stature vegetation (12 m^2) for several years. ^{13}C labelling is obtained by scrubbing the CO_2 from outdoors air with a self-regenerating molecular sieve and by replacing it with ^{13}C depleted (−34.7±0.03‰) fossil-fuel derived CO_2 The facility, which comprises 16 replicate mesocosms, allows tracing the fate of photosynthetic carbon in plant-soil systems in natural light and at outdoors temperature.

This method was applied during 2 yrs to temperate grassland monoliths (0.5×0.5×0.4 m) sampled in a long term grazing experiment. During daytime, the canopy enclosure in each mesocosm was supplied in an open flow (0.67–0.88 volume per minute) with modified air (43% scrubbed air and 57% cooled and humidified ambient air) at mean CO_2 concentration of 425 μmol mol^{-1} and $\delta^{13}C$ of −21.5±0.27‰. Above and belowground CO_2 fluxes were continuously monitored. The difference in $\delta^{13}C$ between the CO_2 at the outlet and at the inlet of each canopy enclosure was not significant (−0.35±0.39‰). Due to mixing with outdoors air, the CO_2 concentration at enclosure inlet followed a seasonal cycle, often found in urban areas, where $\delta^{13}C$ of CO_2 is lower in winter than in summer. Mature C_3 grass leaves were sampled monthly in each mesocosm, as well as leave from pot-grown control C_4 (*Paspalum dilatatum*). The mean $\delta^{13}C$ of fully labelled C_3 and C_4 leaves reached −41.4±0.67 and −28.7±0.39‰ respectively. On average, the labelling reduced by 12.7‰ the $\delta^{13}C$ of C_3 grass leaves. The isotope mass balance technique was used to calculate the fraction of "new" C in the soil organic matter (SOM) above 0.2 mm. A first order exponential decay model fitted to "old" C data showed that reducing aboveground disturbance by cutting increased from 22 to 31 months the mean residence time of belowground organic C (>0.2 mm) in the top soil.

1 Introduction

About two-thirds of terrestrial C is found belowground and the amount of organic carbon that is stored in the soil (1.5×10^{18} g C) is globally about twice that of the total C in atmosphere (Schlesinger, 1997). Below-ground C generally has slower turnover rates than above-ground carbon, as most of the organic carbon in soils (humic substances) is produced by the transformation of plant litter into more persistent organic compounds (Jones and Donelly, 2004).

Carbon sequestration can be determined directly by measuring changes in C pools (Conant et al., 2001) and, or by simulation (e.g. Smith et al., 2005). However, to gain further understanding on C turnover in different soil fractions, the use of radiocarbon tracers and of stable isotopes has proven to be essential (e.g. Trumbore, 2000; Verburg et al., 2004). Isotope techniques, often in combination with other methods (e.g. gas exchange) stand out among the few tools available to track C fluxes in terrestrial ecosystems. Carbon isotopes have been used as tracers including radioactive short lived ^{11}C (half time 20.5 min) and long lived ^{14}C (5760 yr) (Stevenson, 1986), as well as stable ^{13}C (Balesdent et al. 1988). The natural atmospheric ^{14}C activity can be used to date the accumulation of C in fractions with slow turnover time (>50 year). Artificial atmospheric ^{14}C activity, such as the "bomb ^{14}C" studies, can date shorter lived (>10 years) C pools from undisturbed soils (e.g. Trumbore, 2000).

Under the Kyoto Protocol (available at http://www.unfccc.de), biospheric sinks and sources of C can be taken into account in attempts to meet "Quantified Emission Limitation or Reduction Commitments" (QELRCs) for the first commitment period (2008–2012). To better understand how land use and management factors affect carbon turnover in the short-term (<5 yrs), the fate of photosynthates in plant-soil systems should be traced at this time scale.

The development of the ^{13}C isotope mass balance technique has allowed to calculate the amount of "new" carbon in soils after shifting cultivation from C_3 to C_4 plant species (or conversely from C_4 to C_3) (Balesdent et al., 1988; Conen et al., 2006; Derner et al.,

2006). However, this method cannot be applied to temperate and high latitude/altitude ecosystems which lack C_4 species.

Recently, novel techniques were developed by making use of the ^{13}C depleted signatures obtained in CO_2 enrichment experiments (e.g. Loiseau and Soussana, 1999; Personeni et al., 2004; Trueman and Gonzales-Meler, 2005; Dijkstra et al., 2004). However, this method cannot be used to investigate C cycling under ambient CO_2.

^{13}C pulse-labeling experiments in the field allow detailed studies of the temporal relationships between carbon fixation and its delivery to a defined sink (e.g. exudates, soil biota) (Ostle et al., 2000). However, pulse labeling studies cannot be used to understand multi-year processes as the pulse is diluted over time.

To investigate the fate of carbon in plant-soil systems, we have further developed a steady-state $^{13}CO_2$ labeling technique, which has first been used in plant physiology (Deléens et al., 1983; Schnyder et al., 1992, 2003). Hence, for the first time, we have been able to change in natural light and at outdoors temperature the ^{13}C isotope signature of photosynthates during two and half years, thereby gradually replacing with "new" ^{13}C labeled carbon the "old" plant and soil carbon. This method has been applied to the experiment previously described by Klumpp et al. (2007), who reported the role of past and current disturbance level for belowground carbon storage in grassland mesoscoms.

The aim of the present study was: i) to setup a controlled system for continuous long term $^{13}CO_2$ labeling which can trace the fate of carbon in soil organic matter fractions; ii) to assess the role of disturbance for below-ground soil carbon turnover in grassland ecosystems.

2 Materials and methods

2.1 $^{13}CO_2/^{12}CO_2$ labelling system

The facility is shown schematically in Fig. 1 and comprises four components: labeling, air dilution, 16 mesocosms and a gas exchange system. Ambient air is compressed, CO_2 and H_2O of ambient air is scrubbed by passing through a molecular sieve and then replaced by fossil-fuel derived CO_2 being depleted in ^{13}C ($\delta^{13}C - 34.7 \pm 0.03‰$). Thereafter, the ^{13}C depleted air is humidified and mixed with ambient temperature regulated air and distributed to canopy enclosures. The main parts of the labeling system are: i) a screw compressor (Air Worthington-Creyssenac, 50AX6, Lyon, France), ii) a self-regenerating adsorption dryer (Schnyder et al., 1992) (KEN 300, Zander, Essen, Germany) which generates CO_2 free dry air (residual CO_2 below 1μmol CO_2 mol^{-1}, pressure dew point $-70°C$) at a rate of up to 5000 standard liters per minute (SLPM) regulated by a pressure regulator, iii) an air tank, iv) gas cylinders containing fossil fuel derived CO_2 and v) a humidifier ($1 m^3$; $1 m^2$ cross corrugated cellulosic pads, CMF, Varades, France). The flow rates of the labeling system are controlled by pressure regulators and the CO_2 injection rate by a mass flow meter (Tylan, 0–3 SLPM). To regenerate the molecular sieve, approximately 30% of the CO_2 free dry air is diverted to the adsorber chamber. For each of the two adsorber chambers, drying and regeneration were alternated during a 6 min cycle. At night time (one hour after sunset until one hour before sunrise), the supply of CO_2 free dry air was stopped which allowed for a full regeneration of the labeling system.

The labeling system was coupled to an air dilution system. This allowed to vary the degree of ^{13}C labeling by mixing ^{13}C depleted air with ambient air in variable proportions and to apply the labeling system to continental climate with hot summers (e.g. 38°C during summer 2003). For air dilution, ambient air was sampled at a height of 3 m by a centrifuge fan at flow rates up to 400 L min^{-1}. Then ambient air passed through a cooler (378 kWh) being automatically regulated by the temperature differ-

ence between enclosure outlets and ambient air. Finally, ambient air and ^{13}C-depleted air were mixed in a $1\,m^3$ metal box (Fig. 1). At the outlet of the mixing box, thermally insulated PVC pipes delivered the air to the 16 canopy enclosures.

2.1.1 Gas exchange measurement system

Each open-flow canopy enclosure (Fig. 1) had an average flow rate between 500 and $650\,L\,min^{-1}$ during day, which gave with the internal volume of $742\,L$ per enclosure an air exchange rate of 40–50 times per hour and a mean air residence time of 1 min 30 s (Klumpp et al., 2007). During night (one hour after sunset until one hour before sunrise), the flow rate was automatically reduced to $150–250\,L\,min^{-1}$ to regenerate the molecular sieves of the labeling system. The air flow in each enclosure was monitored continuously by an Averaging Pitot tube connected to a differential pressure gauge (Annubar Rosemount, Dietrich Standard Inc., North, USA).

From start of growing season, in April 2003, the CO_2 concentration in enclosures was held from sunrise to sunset at $425\pm39\,\mu$mol mol^{-1} (mean $\pm$ s.d. of 30 min measurements, data not shown) by injection of ^{13}C depleted CO_2 (-34.7 ± 0.03‰). During night time, enclosures were provided with ambient air. Following Casella and Soussana (1997) and Schapendonk et al. (1997), a fraction of the air flowing through the canopy enclosure of a mesocosms was pumped (KNF, Neuberger, Germany) at a constant flow rate ($3.5\pm0.2\,L\,min^{-1}$) through the soil column of each monolith. The flow rate though the soil column was adjusted according to preliminary trials (Klumpp et al., 2007). The CO_2 concentration between outlet and inlet of each enclosure was measured in differential mode every 20 min for a period of 1 min with an IRGA (LI6262, LICOR Nebraska, USA). Both the ambient air and the inlet air CO_2 concentrations were measured in absolute mode with a second IRGA (Maihak, UNOR100, Germany). The two IRGAs were calibrated every two weeks with a $480\,\mu$L CO_2 L^{-1} standard (Messer-Griesheim, Germany). Soil respiration (Klumpp et al., 2007), soil temperature and air humidity of each enclosure as well as external PAR, temperature and humidity were

monitored every 30 and 40 min, respectively.

2.1.2 Mesocosms

A mesocosm consists of an aboveground canopy enclosure of $0.74\,m^3$ (L 1.8×W 0.55×H 0.75 m) and a belowground compartment of $0.30\,m^3$ (1.5×0.5×0.4 m). The canopy enclosure consists of a metal frame covered with a transparent polyethylene film (60 μ). One side in polycarbonate was equipped with two plastic tubes of 20 cm length and 8cm inner diameter, used as air inlet and outlet (Fig. 1). A temperature sensor and a gas sample tube connected to an IRGA through a multi valve block allowed to monitor air temperature and CO_2 concentration at the outlet.

The belowground compartment consisted of three metal containers, each with a volume of $0.14\,m^3$ (0.6×0.6×0.4 m) and a hole at the bottom for drainage water (Fig. 1). Each of these containers contained a grassland monolith (0.5×0.5×0.4 m) framed in a 5 side stainless steel box with drain holes at the bottom. Spaces between monolith and box were airtight sealed. The drainage holes of the three boxes were connected to a 20 L canister (Fig. 1). A pump connected to the canister pumped air continuously through the soil column (see gas exchange measurements).

2.2 Grassland monoliths and management

The grassland (soil, plant community structure) type and procedures to select and extract grassland monoliths were described by Klumpp et al. (2007). Briefly, in June 2002, 56 monoliths (0.5×0.5×0.4 m) were extracted from two semi-natural grassland plots, being subjected to two contrasted sheep grazing treatments during the last 14 years (Louault et al., 2005): one grazing event per year (extensive plot), and 5 grazing events per year and one cut (intensive plot). 24 monoliths of each treatment were placed in the 16 mesocosms. The enclosures were placed in natural light and at outdoors temperature and air humidity was adjusted to field conditions. 4 monoliths of each field treatment were kept outdoors as unlabelled controls. Monoliths were wa-

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tered to provide a soil water potential of approximately 30 kPa.

On five occasions per year the monoliths of the intensive field treatment were cut simultaneously at 5 cm stubble height (high disturbance treatment, H) and artificial urine was applied evenly after each cut in order to simulate N returns at grazing. The remaining monoliths (24 monoliths in enclosures and 4 outdoor controls) sampled in the extensive field treatment were neither cut nor fertilised (low disturbance treatment, L). The harvested phytomass was separated into live and dead plant parts, oven dried and analysed for δ^{13}C.

2.3 Monitoring of ^{13}C labeling

2.3.1 Supplied CO_2

To determine δ^{13}C of CO_2 in canopy enclosures, air was sampled every 14 days at enclosure outlets during daytime. Air samples were collected in 10 ml air-tight vials (BD Vacutainer, UK) through a y-branch-connection (equipped with 2 needles) which was inserted in the continuous air flow going to the IRGA. Vials were flushed for 2 min and CO_2 concentration was measured at the same time. Vials were analyzed for δ^{13}C (see ^{13}C isotope analyses).

2.3.2 Plant material

To monitor the ^{13}C-signature of the aboveground vegetation, last mature green grass leaves were harvested monthly in each enclosure and in outdoor controls. Harvested leaves were oven dried and analyzed for their δ^{13}C signature.

2.3.3 C_4 control

In April 2004, a pot (∅ 30 cm) with a C_4 grass (*Paspalum dilatatum*) was placed in each enclosure to monitor labeling quality (Loiseau and Soussana, 1999). During the

growing season, young grass leaves were harvested every 3 weeks and analyzed for their δ^{13}C content.

2.4 Soil organic matter fractions

Soil harvest and analyses were described by Klumpp et al. (2007). Briefly, soils were sampled once before start of ^{13}C labeling and then 5 times during the experiment (in June and September 2003, April and September 2004 and April 2005). At each soil harvest a vertical soil slice (40×6×10 cm) was split into horizontal layers (0–10, 10–20 and 20–30 cm depth) in each mesocosm. The soil layers were air dried and the organic matter (OM) fractions were separated with water by passing through a series of three brass sieves with successive mesh sizes (1.0, 0.2 and 0.05 mm) (wet sieving). The remaining material in each sieve was separated into the organic and mineral fraction by density flotation in water (Loiseau and Soussana, 1999). Organic fractions were oven dried and analyzed for δ^{13}C.

The δ^{13}C of the total soil organic matter (SOM) above 0.2mm, containing roots, rhizomes, coarse (>1 mm) and fine (1 mm$>×>0.2$ mm) particulate organic matter (POM) and aggregated organic matter (AOM, 0.2 mm$>×>0.05$ mm) was calculated by weighing the δ^{13}C signature of each organic fraction by its dry weight.

2.5 ^{13}C- samples

2.5.1 ^{13}C-isotope analyses

Soil and vegetation samples were oven dried for 48 h at 60°C, grounded to homogenous powder and analyzed for C-content and δ^{13}C by elemental analyzer (EA1110, Carlo Erba, Milano, Italy) coupled (Conflo III) with a mass spectrometer (Delta plus; FinniganMAT, Bremen, Germany). The δ^{13}C in CO_2 from outdoors and labelling atmosphere were measured on a gas chromatography isotope ratio mass spectrometer (Gas system, Fisons, Loughborough, UK).

2.5.2 ^{13}C methods and terminology

Isotope ratios are reported as $\delta^{13}C$ values relative to V-PDB standard (‰) and expressed as:

$$\delta^{13}C = [(R_{sample} - R_{standard})/R_{standard}] * 10^3 \tag{1}$$

where R is $^{13}C/^{12}C$ ratio of the standard and sample. The discrimination (Δ) between product ($\delta^{13}C_{sample}$) and source was:

$$\Delta = [(\delta^{13}C_{source} - \delta^{13}C_{sample})/(1000 + \delta^{13}C_{sample})] \times 10^3 \tag{2}$$

Gas samples and solid samples (soil and plant material) were measured against working laboratory standard gases, previously calibrated against IAEA secondary standards. The fraction of "new" C derived from ^{13}C-labelling (fC_{new}) in soil organic fractions was calculated by a mass balance equation:

$$fC_{new} = (\delta^{13}C_{sample} - \delta^{13}C_{control})/(\delta^{13}C_{input} - \delta^{13}C_{control}) \tag{3}$$

Where $\delta^{13}C_{sample}$ is the $\delta^{13}C$ of the sample, $\delta^{13}C_{control}$ is the $\delta^{13}C$ value before start of labelling and $\delta^{13}C_{input}$ is the $\delta^{13}C$ value of a fully labelled plant derived material entering the soil (see Results).

3 Results and discussion

3.1 System accuracy and $\delta^{13}C$ signature

3.1.1 ^{13}C labelling

After scrubbing CO_2 and H_2O from ambient air, the residual CO_2 concentration in CO_2-free air was below 1 ppm, except for some hours of high outdoor air temperature (>35°C) during summer 2003 heat wave where residual CO_2 concentration could reach 10 ppm (data not shown).

Inlet and outlet δ^{13}C values were compared for enclosures with vegetation during the experiment (i.e. monthly air sampling at noon) and for enclosures without vegetation. In the latter case, an enclosure was placed on an even solid surface under the same conditions (air flow, natural light and temperature). The comparison resulted in a mean absolute δ^{13}C-difference between enclosure inlet and outlet of -0.35 ± 0.39‰ (P>0.5, repeated measure ANOVA) and -0.19 ± 0.47‰ (P>0.5) for enclosures with and without vegetation, respectively (data not shown). These non significant δ^{13}C-differences between enclosure inlet and outlet indicated that: i) seasonal variations in δ^{13}C fractionation processes caused by photosynthesis (e.g. Pataki et al., 2004) were not measurable due to high air flows and ii) enclosures were not causing leaks that could change δ^{13}C values.

The CO_2 concentration delivered by the labelling system reached, on average, $425\pm5\,\mu$mol mol^{-1} (daily mean, $\pm$s.e.). However, due to mixing with outdoors air, the CO_2 concentration at enclosure inlet followed a seasonal cycle often found in urban areas, where due to fossil fuel combustion and lack of plant photosynthesis CO_2 concentration is higher in winter than in summer (e.g. Pataki et al., 2004) (Fig. 2a).

^{13}C fractionation processes during photosynthesis modify instantly the δ^{13}C of CO_2 (O'Leary, 1981), thereby the CO_2 in the atmosphere surrounding the plants becomes ^{13}C enriched relative to the CO_2 at the inlet. In a well-mixed gas exchange enclosure, the ^{13}C signature of CO_2 sensed by plants during photosynthesis corresponds to the δ^{13}C measured at the enclosure outlet (Evans et al., 1986; Schnyder et al., 2003). In our experiment, the δ^{13}C measured at enclosure outlet indicated ^{13}C depletion in winter and enrichment in summer time (Fig. 2b). This was explained by CO_2 mixing with outdoors air, which contributed on average to 57% of the CO_2 flux supplied to the enclosures. The CO_2 from outdoors air (mean δ^{13}C-11.4) had a depleted ^{13}C signature from fossil fuel combustion and plant/soil respiration in winter time and an enriched ^{13}C signature from plant photosynthesis in summer and spring time (Pataki et al., 2004). The plant supplied CO_2 had on average a δ^{13}C$_{offered}$ of -21.5 ± 0.27‰ during the two growth periods.

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Additional to the monthly air sampling, we checked the labelling by calculating (in 30 min time steps) the ^{13}C signature of CO_2 provided to enclosures inlet ($\delta^{13}C_{estimated}$) with a mass balance equation:

$$\delta^{13}C_{estimated} = \frac{qCO_{2air} \cdot \delta^{13}C_{air} + qCO_{2inject} \cdot \delta^{13}C_{inject} + qCO_{2decarb} \cdot \delta^{13}C_{decarb}}{qCO_{2air} + qCO_{2inject} + qCO_{2decarb}}$$

5 where $\delta^{13}C_{air}$, $\delta^{13}C_{inject}$ and $\delta^{13}C_{decarb}$ are the measured $\delta^{13}C$ values of CO_2 from outdoors air, from CO_2 cylinders and from decarbonated air, respectively. qCO_{2air}, $qCO_{2inject}$ and $qCO_{2decarb}$ are the flow rates of CO_2 supplied by outdoors air, by CO_2 injection from cylinders and by decarbonated air, respectively. $qCO_{2inject}$ was adjusted to 1.55 standard L min^{-1} by a mass flow-meter. qCO_{2air} reached 2.01±0.14 standard
10 L min^{-1} and $qCO_{2decarb}$ was equal to zero (except during some days above 35°C in summer 2003).

The $\delta^{13}C_{estimated}$ (Fig. 2c) and the measured $\delta^{13}C$ at the enclosure outlet did not differ significantly during the time course of the experiment (P>0.1; repeated measure ANOVA, data not shown, absolute $\delta^{13}C$ difference of −0.47±0.5‰). Therefore, even
15 during periods without direct $\delta^{13}C$ analyses, the labelling intensity was monitored by calculating $\delta^{13}C_{estimated}$. The seasonal pattern of $\delta^{13}C_{estimated}$, which was close to the monthly measured $\delta^{13}C$ at enclosure outlet (Fig. 2b) also, indicated that $\delta^{13}C_{estimated}$ was most susceptible to changes in $\delta^{13}C$ of outdoor air ($\delta^{13}C_{air}$). In our experiment the $\delta^{13}C$ of outdoors air was in average −11.4‰ due to urban activity and could not be
20 controlled by the experimental set up.

3.1.2 Plant material

The C_3 grass leaves harvested each month had mean $\delta^{13}C$ values of −41.4±0.67 and −28.7±0.39‰ for ^{13}C-labelled and control monoliths, respectively (Fig. 2d, means of April to November 2004). The C_3 grass leaves grown in labelled mesocosms were

therefore significantly (P<0.001; repeated measure ANOVA) more depleted in ^{13}C than unlabelled controls grown outdoors. $\delta^{13}C$ values of C_3 leaves were significantly lower than that of the air surrounding the leaves, indicating the selectivity for ^{12}C (i.e. discrimination Δ, O'Leary, 1981) of the carbon fixing enzyme Rubisco. The C_4 grass leaves were less depleted (-23.2 ± 0.2‰) than the C_3 grasses (-41.4 ± 0.7‰) because C_3 plants discriminate more against $^{13}CO_2$ (Δ 20–28‰) than C_4 plants (Δ2–15‰) (O'Leary, 1981). The mean discrimination ($\Delta^{13}C$) over the experimental period reached 17.0 ± 0.6 and 1.6 ± 0.3‰ for the C_3 (April 2003 to April 2005) and C_4 (April 2004 to 2005) grasses, respectively. This was irrespective of the disturbance level by cutting in the mesocosms (P>0.1, repeated measure ANOVA, data not shown).

Both, labelled (C_3 and C_4) and unlabelled control leaves showed a small seasonal pattern (Fig. 2d), being isotopically enriched in summer (-40.4 ± 0.3‰ in the second year) and depleted in winter (-49.9 ± 0.1‰) (P<0.001, data not shown), indicating changes in plant metabolism induced by abiotic factors such as water stress, high temperatures and high radiation intensities which decrease stomatal conductance (Farquhar et al., 1989; Brugnoli and Farquhar, 2000). Interestingly, seasonal changes in $\delta^{13}C$ were similar inside and outside (controls) of the labelled mesocosms (Fig. 2d). Hence, during the experiment, the mean change in $\delta^{13}C$ signature of C_3 grass leaves caused by labelling was equal to -13.6 ± 0.7‰. This shows that the labelling facility generated a fairly constant change in delta ^{13}C signature of leaves, despite seasonal variations, occurring in both, outdoors and inside the mesocosms.

The mean $\delta^{13}C$ of fully labelled plant shoots reached -41.4‰.Several studies reported a $\delta^{13}C$ difference of 1–2‰ between roots and shoots in grass plants (Klumpp et al., 2005; Hobbie and Werner, 2004; Schweizer et al., 1999), showing that roots are isotopically enriched compared to shoots. Accordingly, we set the $\delta^{13}C_{input}$ of "new" C entering the belowground compartment at a $\delta^{13}C$ of -40.4‰, which corresponds to a ^{13}C enrichment of 1‰ of roots compared to shoots.

3.2 Effects of grassland disturbance level on soil carbon turnover

Klumpp et al. (2007) have shown that belowground carbon storage was higher for monoliths previously acclimated for 14 yrs to low disturbance by grazing and cutting. Notably, values for belowground carbon storage calculated from the balance of gas exchanges were consistent with the increment in soil organic carbon content directly measured during the experiment. Moreover, the belowground carbon storage was positively correlated to tissue density and diameter of roots and rhizomes (R=0.50; P<0.05; data not shown). These results confirm that root diameter and root density are plant functional traits that control the decomposition rate and the mean residence time of carbon in the soil litter continuum (Personeni and Loiseau, 2005)

Changes in δ^{13}C values of SOM>0.2 mm (i.e. roots, rhizomes and particulate organic matter) and of aggregated soil organic matter (AOM, 0.2>×>0.05 mm) in the top soil layer (0–10 cm) during the labelling experiment are shown in Fig. 3. Interestingly, initial δ^{13}C values of SOM >0.2 mm were significantly different (P<0.001) between monoliths adapted to high (−28.7±0.10‰) and to low (−27.4±0.14‰) disturbance. Possible reasons for these differences in isotopic signatures are: i) different decomposition rates of distinct biochemical components of plant litter (Melillo et al., 1989; Agren et al., 1996) and ii) the contribution and δ^{13}C values of different particle sizes (e.g. 1 mm, 0.2 mm) to soil OM (Balesdent et al., 1988; Personeni and Loiseau, 2004). Findings from the same field site, showing a higher cellulose content in the plant material from the light (low disturbance) compared to heavy (high disturbance) grazing treatments (Picon-Cochard et al., 2004) confirm the role of differences in biochemical components such as cellulose, which is enriched in ^{13}C (Schweizer et al., 1999; Gleixner et al., 1993). Moreover, monoliths adapted to high disturbance in the field during the last 14 yrs had a higher fraction of particulate OM (1.0>×>0.2 mm)(P<0.01, data not shown) with a more depleted δ^{13}C value than monoliths adapted to high disturbance (data not shown). Personeni and Loiseau (2004) reported more depleted δ^{13}C values for particulate organic matter fractions of *Lolium perenne* compared to

Dactylis glomerata, suggesting that plant community structure also has a large impact on ^{13}C signature of root litter in grasslands (Dijkstra et al., 2004).

During the 24 months of labelling, the gradual δ^{13}C depletion of SOM (>0.2 mm) and of AOM in the upper most 10 cm (Fig. 3) indicated that the soil litter continuum was steadily filled with "new" ^{13}C-depleted carbon (P<0.001). Similar trends were found for the 10–20 cm and 20–30 cm soil depths, where the δ^{13}C also declined towards more ^{13}C depleted values (P<0.001, data not shown). Both, SOM above 0.2 mm (P<0.001) and AOM (P<0.05) had less negative δ^{13}C values in monoliths adapted to low compared to high disturbance, indicating a higher fraction of "new" (^{13}C depleted) carbon in the high compared to low disturbance treatment (Fig. 3).

The fate of "old" C (F_{old}) and the input of "new" C (F_{new}) into SOM (>0.2 mm) were fitted to first order exponential decay (Loiseau and Soussana, 1999; Personeni and Loiseau, 2005) and rise to maximum models, respectively (Fig. 4):

$$C_{old} = ae^{-k.t} \tag{4}$$

$$C_{new} = a'(1 - e^{-k'.t}) \tag{5}$$

where t is time in months; a is the initial amount of old C at $t=0$ (start of the labelling experiment); a' is the potential accumulation of "new" C (mg C g^{-1} soil) in the compartment. k is the first order decay constant of "old" organic C that leaves the OM compartment above 0.2 mm. k' is the rate constant of accumulation of "new" C in the soil in SOM above 0.2 mm. The mean residence time of "old" C (MRT$_{old}$, months) was calculated as:

$$MRT_{old} = 1/k \tag{6}$$

The time for half-potential accumulation of "new" C in the SOM above 0.2 mm (MT$_{1/2-new}$) was calculated as:

$$MT_{1/2-new} = -\ln(1/2)/k' \tag{7}$$

k values of 0.032 and 0.045 were found, giving mean residence time of C of 31.2 and 22.0 months (Fig. 4; Table 1) for low and high disturbance levels, respectively. These values show a fast decay of OM through the breakdown of particulate OM in fractions below 0.2 mm and the respiration and mineralization of part of the root and
5 litter carbon. Since k values were significantly different (P<0.05, data not shown), the decay was slower at low compared to high disturbance level. Low disturbance treatment was dominated by plant species with coarse and dense roots and rhizomes, which are likely to last longer and decompose slower than the fine roots observed at high disturbance level (data not shown).

10 First order exponential models indicated that the potential accumulation (a', 3.0 and 2.3 mg C g^{-1} soil, at L and H) of "new C" was substantially lower than the initial amount of "old" C at the start of the experiment (a, 7.4 and 4.0 mg C g^{-1} soil at L and H) (Table 1). Likewise, k' values indicated that half of the potential accumulation of "new" C was reached after 3.5 month in both disturbance levels.

15 During the experiment, "new" C entered first the roots and rhizomes, before being released in the soil through rhizodeposition (i.e. root exudates, root and rhizome litter). Furthermore, at the low compared to high disturbance level, shoot litter may also contribute to the supply of "new" C to the soil. The "new" C remained in the litter continuum above 0.2 mm until its breakdown into smaller particle sizes and its respiration and
20 mineralization. Another factor contributing to the disappearance of both "old" and "new" carbon is the activity of the soil fauna (macro-decomposers, e.g. earthworms) (Hoope et al., 2000; Seeber et al., 2006), which was able to transport part of the litter to deeper soil layers and which contributed to the breakdown of coarse litter into finer fractions.

25 Given the short residence time of "old" C (below 36 months), it is likely that a large part of the "new" C which was deposited into the soil compartment vanished into smaller particle sizes (<0.2 mm) before the end of the 2 years of the experiment. This explains why the potential accumulation of "new" C (a') was found to be much lower than the initial amount of "old" C (a; Table 1). For the same reason, half the maxi-

mum accumulation of "new" C was reached within a few months only, as some "new" C started to escape the compartment above 0.2 mm after a few months only.

4 Conclusion

These results show the potential of ^{13}C steady state labelling for investigating the fate of carbon in the plant-soil continuum. By combining isotope analyses of soil OM compartments and of soil CO_2 efflux, a detailed understanding of the fate of carbon can be gained for model ecosystems that can be subjected to contrasted management and environmental treatments. The labelling facility presented here represents a compromise between the costs of the equipment (which scales with the decarbonated air flow) and the intensity and accuracy of the ^{13}C labelling (which declines with increasing outdoors air supply). A large array of applications at different time scales can be envisaged, ranging from short-term labelling of plant and soil carbon to long-term labelling of more stable soil OM fractions. The method does not involve the need for continuous measurements of the ^{13}C signature of the labelling atmosphere, as we have shown that this signature can be calculated with sufficient accuracy.

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Table 1. First order exponential models of "old" C decay and "new" C accumulation in pasture monoliths adapted to low (L) and high (H) disturbance and continuously labelled with ^{13}C. The fate of "old" C (C_{old}) and the accumulation of "new" C (C_{new}) into SOM (>0.2 mm) in 0–10 cm soil layer were fitted to first order exponential decay and rise to maximum models, respectively: $C_{old}=a.e^{-k.t}$; $C_{new}=a'.(1 - e^{-k'.t})$, where t is time in months; a is the initial amount of old C at $t=0$ (start of the labelling experiment); a' is the potential accumulation of "new" C (mg C g^{-1} soil) in the compartment. k is the first order decay constant of "old" organic C. k' is the rate constant of accumulation of "new" C. Mean residence time of "old" C (MRT_{old}, months) was calculated as: $MRT_{old}=1/k$; the time for half-potential accumulation of "new" C ($MT_{1/2-new}$) was calculated as: $MT_{1/2-new}=-\ln(1/2)/k'$. Results are means of 8 replicates per treatment.

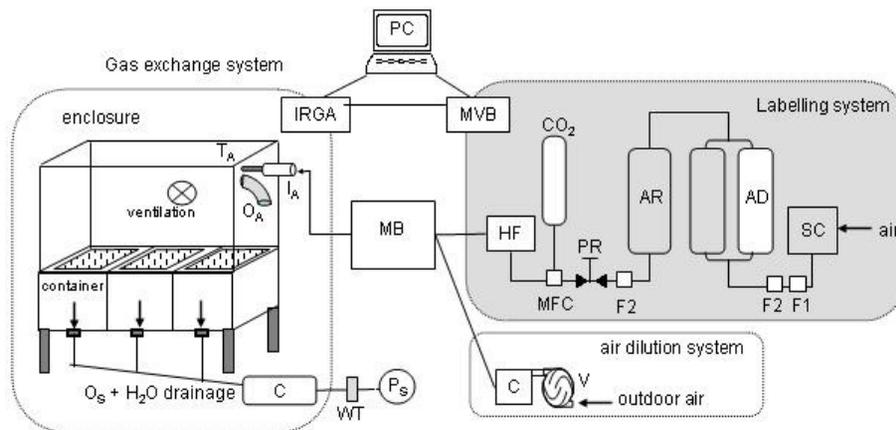| C pool	Disturbance level	Coefficient (mean ± s.e.)			P	Model R^2
"Old" C	High	a (mg C g^{-1} soil)	3.94	±0.43	<0.001	0.79**
		k (month^{-1})	0.045	±0.014	<0.05	
		MRT_{old} (month)	22.0			
	Low	a (mg C g^{-1} soil)	7.35	±0.38	<0.001	0.91**
		k (month^{-1})	0.032	±0.005	<0.01	
		MRT_{old} (month)	31.2			
"New" C	High	a' (mg C g^{-1} soil)	2.27	±0.05	<0.001	0.99***
		k' (month^{-1})	0.199	±0.017	<0.001	
		$MT_{1/2-new}$ (month)	3.48			
	Low	a' (mg C g^{-1} soil)	3.045	±0.298	<0.001	0.92**
		k' (month^{-1})	0.196	±0.071	<0.05	
		$MT_{1/2-new}$ (month)	3.54			

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Fig. 1. Long term steady state ^{13}C labelling facility to investigate carbon turnover in plant-soil system. SC, Screw compressor (Air Worthington-Creyssenac, 50AX6, Lyon, France); AD, adsorption dryer (KEN300, Zander Essen, Germany, molecular sieve with zeolith, activated aluminum oxide); AR, air reservoir (1 m^3); F1, Oil and water condensate drain; F2, oil-, water- and particle filter (Zander, Essen, Germany); PR, pressure regulator; HF, humidificator; C, temperature regulated cooler; I$_A$, inlet aboveground; MFC, mass flow controller 0–5 l min^{-1} (Tylan General, Eching Germany); MB, mixing vessel (1 m^3); MVB, multi valve block; CO$_2$, cylinder containing CO$_2$ of fossil origin (Messer, Lyon, France); canopy enclosure, cuvette with plastic film (180×55×75) cm; O$_A$, outlet aboveground; O$_s$ outlet soil; IRGA, infrared gas analyzers (LI6262, LICOR, Nebraska, USA; Maihak, Unor 100, Germany); PC, central control and data acquisition system; Ps pump for sampling of soil compartment; T$_A$, temperature sensor aboveground; V, air flow controllable ventilation; WT, water trap.
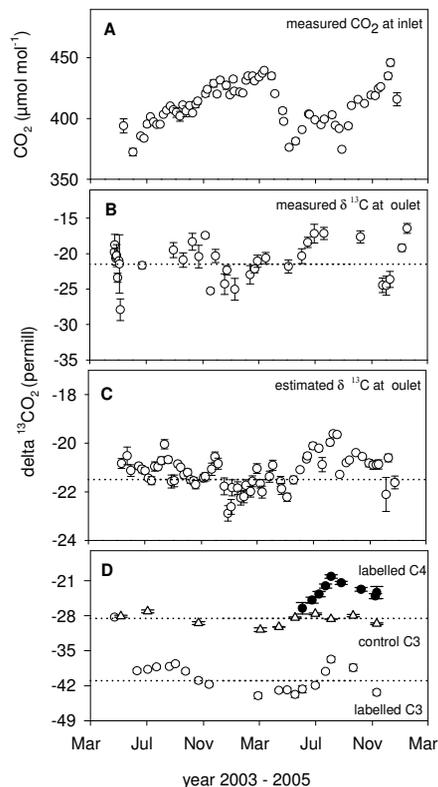
Fig. 2. (A) Measured CO_2 concentration at the canopy enclosure inlet, **(B)** $\delta^{13}C$ at canopy enclosure outlet, **(C)** estimated $\delta^{13}C$ of CO_2 supplied to canopy enclosure inlet and **(D)** $\delta^{13}C$ of labelled C_3 and C_4 (*Paspalum dilatatum*) grass leaves during experimental period (April 2003 to March 2005) and of unlabelled C_3 control grasses grown outdoors. Data are means (±SE) of 16, 14 and 4 replicates for labelled C_3, labelled C_4 and unlabelled C_3 controls, respectively. Dotted lines represent the mean $\delta^{13}C$ values of supplied CO_2 (−21.5‰) (B, C) and of C_3 leaves of labelled (−41.4‰) and unlabelled control (−28.7‰) plants (D).

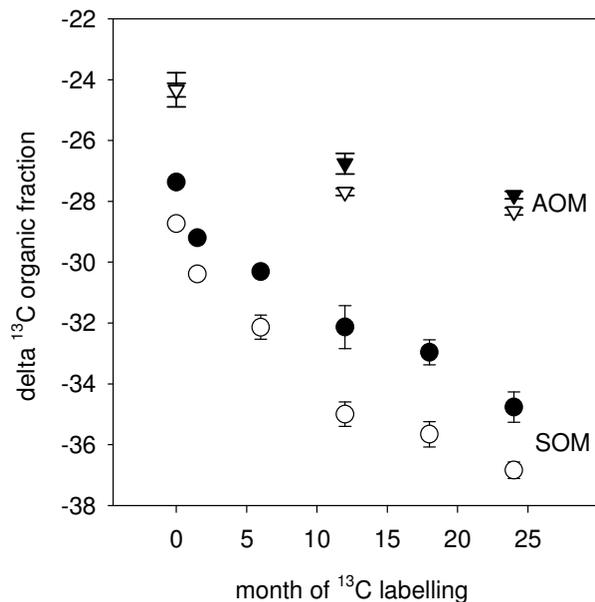**Fig. 3.** Changes of the δ^{13}C of total soil organic matter above 0.2 mm (SOM>0.2 mm, including roots, rhizomes and particulate organic matter) (circles) and of aggregated organic matter (AOM, 0.2>×>0.05 mm)(triangles) in the top soil (0–10 cm) during the labelling experiment. Monoliths exposed to low (solid symbols) and to high (open symbols) field disturbance. Data are means ±SE of 8 replicate mesocosms.
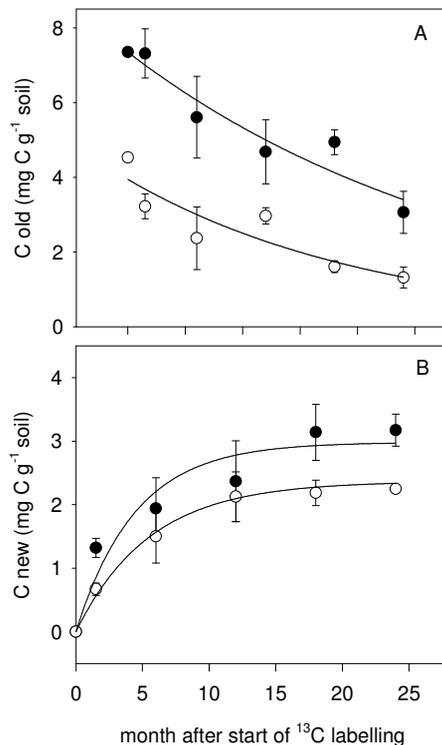
Fig. 4. Changes in the contents (mg C g^{-1} dry soil) of "old" **(A)** and "new" C **(B)** in the SOM above 0.2 mm of the top soil (0–10 cm) layer after start of ^{13}C labelling for monoliths adapted for 14 yrs to low (full symbols) and high (open symbols) disturbance in the field. The fate of "old" C (F$_{old}$) and the input of "new" C (F$_{new}$) were fitted to first order exponential decay and rise to maximum models, respectively: $C_{old}=a.e^{-k.t}$; $C_{new}=a'.(1-e^{-k'.t})$ (abbreviations and coefficients of regression lines see Table 1).

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