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

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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 to trace the fate of photosynthetic carbon in plant-soil systems in natural light and at outdoors temperature. This method was applied to the study of soil organic carbon turnover in temperate grasslands. We tested the hypothesis that a low disturbance by grazing and cutting of the grassland increases the mean residence time of carbon in coarse (>0.2 mm) soil organic fractions.

Grassland monoliths ($0.5\times0.5\times0.4$ m) were sampled from high and low disturbance treatments in a long-term (14 yrs) grazing experiment and were placed during two years in the mesocosms. During daytime, the canopy enclosure in each mesocosm was supplied in an open flow with air at mean CO_2 concentration of 425 μmol mol^{-1} and $\delta^{13}C$ of -21.5 ± 0.27‰. Fully labelled mature grass leaves reached a $\delta^{13}C$ of -40.8 (±0.93) and -42.2‰ (±0.60) in the low and high disturbance treatments, respectively, indicating a mean ^{13}C labelling intensity of 12.7‰ compared to unlabelled control grass leaves. After two years, the delta ^{13}C value of total soil organic matter above 0.2 mm was reduced in average by 7.8‰ in the labelled monoliths compared to controls. The isotope mass balance technique was used to calculate for the top (0–10 cm) soil the fraction of ^{13}C labelled carbon in the soil organic matter above 0.2 mm (i.e. roots, rhizomes and particulate organic matter). A first order exponential decay model fitted to the unlabelled C in this fraction shows an increase in mean residence time from 22 to 31 months at low compared to high disturbance. A slower decay of roots, rhizomes and particulate organic matter above 0.2 mm is therefore likely to contribute to the observed increased in soil carbon sequestration in grassland monoliths exposed to low disturbance.

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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). Transformation of plant litter is a key process of the carbon and nutrient cycles, which drives C mineralization and C accumulation in the soil organic matter (Tateno and Chapin, 1997). Detrital carbon accumulation accounts for most of an ecosystem's capacity to store organic carbon belowground within a few years and is largely conditioned by the plant turnover rate (Cebrian and Duarte, 1995). Root litter transformation is an important determinant of the carbon cycle in grassland ecosystems, which is affected both by the root litter quality and by the rhizosphere activity (Personeni and Loiseau, 2004, 2005).

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)

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(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 approaches were developed by using CO_2 enrichment experiments (FACE) to obtain a ^{13}C depleted signature in plant-soil systems (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). Nevertheless, ^{13}C 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 was first used with seedlings grown hydroponically or on sand in phytotrons (Deléens et al., 1983; Schnyder et al., 1992, 2003).

This method has been applied to the mesocosm experiment previously described by Klumpp et al. (2007), showing reduced soil carbon sequestration at high compared to low disturbance. We have tested the hypothesis that a low disturbance by grazing and cutting of the grassland increases the mean residence time of carbon in coarse (>0.2 mm) soil organic fractions.

The aim of the present study was: i) to change during several months the ^{13}C isotope signature of photosynthates from plant-soil systems grown in natural light and at outdoors temperature using a steady state $^{13}CO_2$ labelling facility; ii) to apply this method to study the role of disturbance for soil carbon turnover in grassland ecosystems.

2 Materials and methods

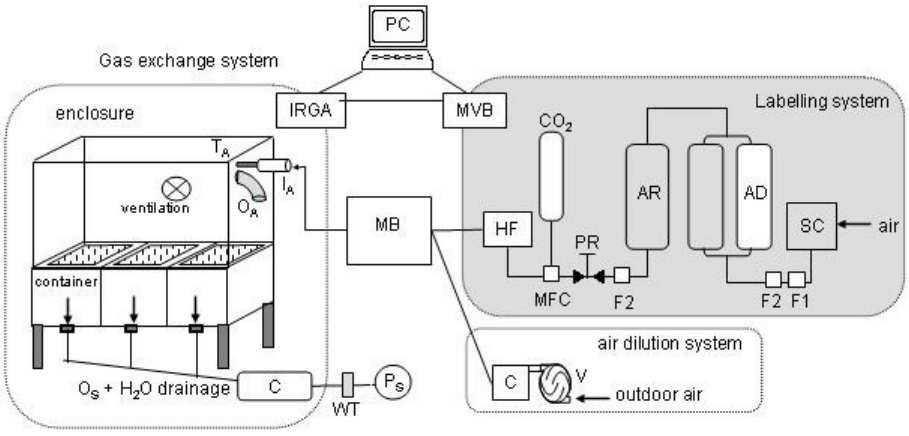
2.1 Set-up of controlled system for long-term $^{13}CO_2/^{12}CO_2$ labelling

The facility is shown schematically in Fig. 1 and comprises four components: labeling system, air dilution, 16 mesocosms and a gas exchange system (previously described by Klumpp et al., 2007). More precisely, 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 by $-21‰$ compared to outdoor air ($\delta^{13}C$ $-34.7\pm0.03‰$). Thereafter, the ^{13}C depleted air is humidified, mixed with outdoor air, temperature regulated and distributed to canopy enclosures. The main parts of the labeling system are: a screw compressor, a self-regenerating adsorption dryer (Schnyder et al., 1992) generating CO_2 free dry air at a rate of up to 5000 standard liters per minute (SLPM) and a residual CO_2 below 1 μmol CO_2 mol^{-1}, an air tank, gas cylinders containing fossil fuel derived CO_2 and a humidifier (1 m^3; 1 m^2 cross corrugated cellulose pads). 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). During daytime, to regenerate the molecular sieve of the adsorbtion dryer, approximately 30% of the CO_2 free dry air is diverted to the adsorber chamber, where drying and regeneration alternated in 6 min cycles. 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 adsorption dryer.

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 outdoor 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 difference 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

The gas exchange system was previously described and discussed by Klumpp et al. (2007). Briefly, 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. During night (one hour after sunset until one hour before sunrise), the flow rate was automatically reduced to



		Abbreviation	Reference
Labelling 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)	Zander, Essen, Germany
	F1	Oil and water condensate drain	Zander, Essen, Germany
	F2	Oil-, water- and particle filter	
	PR	Pressure regulator	Tylan General, Eching Germany
	MFC,	Mass flow controller 0-5 l min^{-1}	Messer, Lyon, France
	CO_2	Cylinder containing CO_2 of fossil origin	
	MVB	Multi valve block	
Air dilution system	HF	Humidificator	CMF, Varades, France
	MB	Mixing vessel (1 m^3)	
	C	Temperature regulated cooler	AuverFroid, Clermont-Ferrand, France
	V	Air flow controllable centrifuge fan	
Enclosure	I_A	Inlet aboveground	
	O_A	Outlet aboveground	
	T_A	Temperature sensor aboveground	
	O_s	Outlet belowground	
	Ps	Pump belowground	
	WT	Water trap	
	C_D	Canister for drainage water	
	IRGA	Infrared gas analyzers	LI6262, LICOR, Nebraska, USA; Maihak, Unor 100, Germany
	PC	Computer	

Fig. 1. Long term steady state ^{13}C labelling facility to investigate carbon turnover in plant-soil system.

150–250 L min^{-1} to regenerate the molecular sieves of adsorption dryer. 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±39 μmol mol^{-1} (mean ± s.d. of 30 min measurements, data not shown) by injection of ^{13}C depleted CO_2 (−34.7±0.03‰). The mean CO_2 concentration inside the enclosures was close to outdoors CO_2 concentration (mean difference of 13.2±9.5 μmol mol^{-1}). During night time, the enclosures were provided with outdoors 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±0.2 L min^{-1}) through the soil column of each monolith. A small pressure head was maintained by the open-flow system. This pressure head and the depression caused by the pump eliminated back-diffusion of CO_2 from the soil (e.g. Casella and Soussana, 1997). The flow rate though the soil column was adjusted according to preliminary trials, showing, that a flow rate of 3.5 L min^{-1} suppresses diffusion of soil CO_2 in the above ground compartment without changing significantly soil respiration rates. 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 μL 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 (L 1.5×W 0.5×H 0.4 m). The canopy enclosure consists of a thin metal frame covered with a transparent polyethylene film (60 μ, transmitting 90% of the incident PAR). One side in polycarbonate was equipped with two plastic tubes of 20 cm length and 8 cm inner diameter, used as air inlet and outlet (Fig. 1). Air mixing inside enclosures was provided by a fan. 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 (L 0.6×W 0.6×H 0.4 m) and a hole at the bottom for drainage water (Fig. 1). Each of these containers contained a grassland monolith (L 0.5×W 0.5×H 0.4 m) framed in a 5 side stainless steel box with drain holes at the bottom. Gaps between monolith and box were filled with a thermally insulating material (vermiculite) and the surface was 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 Experiment with 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 (L 0.5×W 0.5×H 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). Plot 1 was cut once and sheep grazed 4 times yr^{-1} and, hence, represented a high disturbance regime (H). Plot 2 was sheep grazed only once a year (low disturbance regime, L). 24 monoliths of each treatment were placed in the 16 mesocosms (3 monoliths per enclosure). 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. Depending on season, monoliths were watered once to three times a week to target a soil volumetric water content of 33% corresponding to a soil water potential of ca. −30 kPa. All enclosures received the same irrigation volumes.

On five occasions per year, a high current disturbance was applied (24 monoliths in enclosures and 4 outdoor controls) by cutting simultaneously at 5 cm stubble height and applying artificial urine (5 g N m^{-2}) consisting of 80% urea, 10% hippuric acid, 5% allantoin N and 5% creatine N (Doak, 1952) in order to simulate N returns at grazing (see Klumpp et al., 2007). The harvested phytomass was separated into live and dead plant parts, oven dried and analysed for δ^{13}C. For the low current disturbance monoliths were neither cut nor fertilised. At the start of the experiment, half of the enclosures of the low and high field disturbance were switched to the opposite disturbance treatment (Klumpp et al., 2007).

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 analyzed monthly for δ^{13}C (see ^{13}C isotope analyses).

2.3.2 Plant material

To monitor the ^{13}C-signature of the aboveground vegetation, 20 last mature green grass laminae (tip to ligula) were harvested monthly in each enclosure and in outdoor controls by clipping. For each enclosure (and outdoor control) harvested leaves were oven dried and analyzed for their δ^{13}C signature.

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 free organic matter fractions (OM) 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; Six et al., 2001). Organic fractions were oven dried and analyzed for δ^{13}C.

The δ^{13}C of the total soil organic matter (SOM) above 0.2 mm, 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-determination and calculation

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 δ^{13}C values relative to V-PDB standard (‰) and expressed as:

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

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:

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

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:

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

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

2.5.3 Follow up of ^{13}C labeling

Additional to the monthly air sampling, we estimated (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:

$$\delta^{13}C_{estimated}= \frac{qCO_{2air}\cdot\delta^{13}C_{air}+qCO_{2inject}\cdot\delta^{13}C_{inject}+qCO_{2decarb}\cdot\delta^{13}C_{decarb}}{qCO_{2air}+qCO_{2inject}+qCO_{2decarb}} \quad (4)$$

where $\delta^{13}C_{air}$, $\delta^{13}C_{inject}$ and $\delta^{13}C_{decarb}$ are the measured δ^{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 L min^{-1} and $qCO_{2decarb}$ was equal to zero (except for a few days above 35°C in summer 2003).

2.6 Data analysis

Differences in ^{13}C values for leaves and for gas samples were analyzed by repeated measure ANOVA. The amounts of "new" and of "old" C were fitted using non linear regression models with log transformed data. Since in these regression models there were no significant differences among the two current disturbance treatments ($p>0.95$), the data from the two current disturbance treatments were pooled. Hence, we compare the two field disturbance treatments (H and L), each with 8 replicate enclosures.

3 Results and discussion

3.1 System accuracy and δ^{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, two enclosures were placed on an even gas-tight 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 indicate that δ^{13}C fractionation processes caused by photosynthesis were not measurable due to high air flows.

The CO_2 concentration delivered by the labelling system reached, on average, 425±5 μmol mol^{-1} (daily mean, ±s.e.). However, due to mixing with outdoors air, the CO_2 concentration at enclosure inlet followed a seasonal cycle, characterized by a higher 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

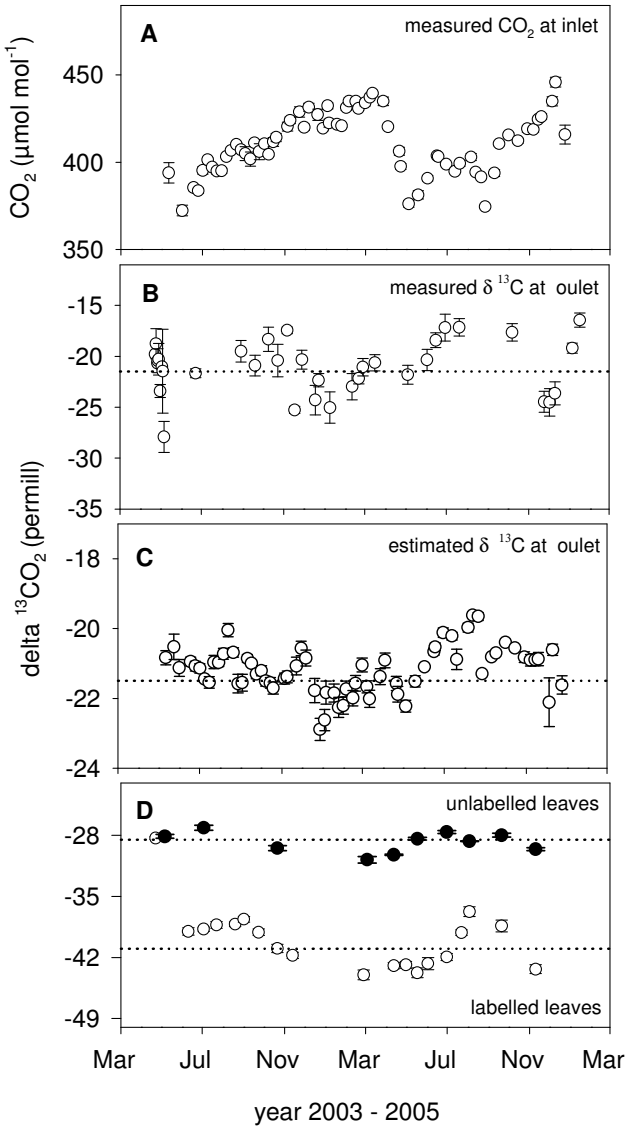


Fig. 2. (A) Measured CO_2 concentration at the canopy enclosure inlet, **(B)** δ^{13}C at canopy enclosure outlet, **(C)** estimated δ^{13}C of CO_2 supplied to canopy enclosure inlet and **(D)** δ^{13}C of labelled C_3 grass leaves during experimental period (April 2003 to March 2005) and of unlabelled C_3 control grasses grown outdoors. Data are means (±SE) of 16, 14 and 4 replicates for labelled C_3 and unlabelled C_3 controls, respectively. Dotted lines represent the mean δ^{13}C values of supplied CO_2 (−21.5‰) (A, B) and of C_3 leaves of labelled (−41.4‰) and unlabelled control (−28.7‰) plants (D).

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 $\delta^{13}C_{offered}$ of −21.5±0.27‰ during the two growth periods.

The calculated labelling intensity, $\delta^{13}C_{estimated}$ (Fig. 2c), and the measured δ^{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 δ^{13}C difference of −0.47±0.5‰). Therefore, even during periods without direct δ^{13}C analyses, the labelling intensity can be estimated by calculating $\delta^{13}C_{estimated}$. The seasonal pattern of $\delta^{13}C_{estimated}$, which was close to the monthly measured δ^{13}C at enclosure outlet (Fig. 2b) and was most susceptible to changes in δ^{13}C of outdoor air ($\delta^{13}C_{air}$). In our experiment the δ^{13}C of outdoors air was in average −11.4‰ due to urban activity and could not be controlled by the experimental set up.

In our experiment, a small pressure head was maintained in the aboveground compartment by the open-flow system and air was sampled from the soil, which eliminated back-diffusion of CO_2 from the soil surface. This may have disturbed the O_2 and CO_2 concentration profile in the soil. Nevertheless, the CO_2 concentration at the outlet of the below-ground compartment varied between 1000 and 2000 ppm and was thus far above the concentration inside the shoot enclosure. We have used a low air flow rate (3.5 L min^{-1}) and a well structured sandy loam (50% sand) soil with a large macroporosity. Therefore, only 4–6% of the air filled pore space was sampled each minute, which reduced the disturbance of the soil gas concentration profiles.

3.1.2 Plant material

The C_3 grass leaves harvested each month had mean δ^{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 more depleted in ^{13}C (P<0.001; repeated measure ANOVA) than unlabelled controls grown outdoors. The lower δ^{13}C values of C_3 leaves compared to air surrounding the leaves indicated the selectivity for ^{12}C (i.e. discrimination Δ, O'Leary, 1981) of the carbon fixing enzyme Rubisco. The mean discrimination (Δ^{13}C) over the experimental period reached 17.0±0.6 (April 2003 to April 2005). This was irrespective of the disturbance level by cutting in the mesocosms (P>0.1, repeated measure ANOVA, data not shown). Labelled and control leaves showed a seasonal pattern (Fig. 2d), being isotopically enriched in summer (−40.4±0.3‰ in the second year) and depleted in winter (−44.0±0.6‰), 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 δ^{13}C were similar inside and outside (controls) of the labelled mesocosms (Fig. 2d). Hence, during the experiment, the mean change in δ^{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.

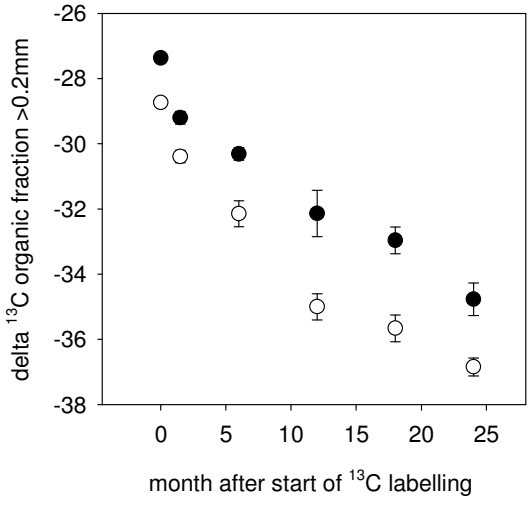


Fig. 3. Changes of the δ^{13}C of total soil organic matter above 0.2 mm (SOM>0.2 mm, including roots, rhizomes and particulate organic matter) in the top soil (0–10 cm) during the labelling experiment. Monoliths acclimated to low (solid symbols) and to high (open symbols) field disturbance. Data are means ±SE of 8 replicate mesocosms.

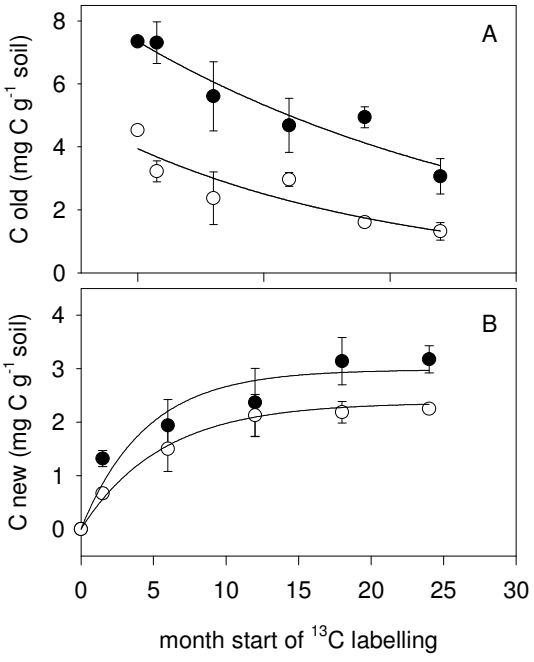


Fig. 4. Changes in the contents (mg C g^{-1} dry soil) of "old" **(A)** and "new" C **(B)** in the SOM above 0.2 mm of the top soil (0–10 cm) layer after start of ^{13}C labelling, for monoliths acclimated for 14 yrs to low (full symbols) and high (open symbols) disturbance in the field. The fate of "old" C and the input of "new" C 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).

The mean δ^{13}C of fully labelled plant shoots reached −41.4‰. Several studies reported a δ^{13}C difference of 1–2‰ between roots and shoots in grass plants grown under controlled and field conditions (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 δ^{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 than to high 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.

Changes in δ^{13}C values of SOM>0.2 mm (i.e. roots, rhizomes and particulate organic matter) 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, illustrating the effects of grassland management by grazing and cutting on plant community structure and carbon cycling (Hooper and Vitousek, 1998). Plant community structure was suggested to affect ^{13}C signature of root litter in grasslands (Dijkstra et al., 2004). Personeni and Loiseau (2004) reported more depleted δ^{13}C values for particulate organic matter fractions (POM) of *Lolium perenne* compared to *Dactylis glomerata*. Possible reasons for these differences in isotopic signatures in grasslands exposed to contrasted disturbance levels are: i) contrasted 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 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 at low compared to high disturbance (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 had a higher fraction of particulate OM (0.2>×>0.05 mm) (P<0.01, data not shown) with a more depleted δ^{13}C value than monoliths adapted to low disturbance (data not shown).

During the 24 months of labelling, the gradual δ^{13}C depletion of SOM (>0.2 mm) 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

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: $C_{old}=a.e^{-k.t}$; $C_{new}=a'.(1-k'.t)$, 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±0.018	<0.001	
		$MT_{1/2-new}$ (month)	3.48		
	Low	a' (mg C g^{-1} soil)	3.047±0.298	<0.001	0.92**
		k' (month^{-1})	0.196±0.072	<0.05	
		$MT_{1/2-new}$ (month)	3.54		

the δ^{13}C also declined towards more ^{13}C depleted values (P<0.001, data not shown). SOM above 0.2 mm (P<0.001) had less negative δ^{13}C values in monoliths adapted to low compared to high disturbance, suggesting a higher fraction of "new" (^{13}C depleted) carbon in the high compared to low disturbance treatment (Fig. 3).

The fate of "old" C (unlabelled) and the input of "new" C (^{13}C labelled) (mg C g^{-1} soil) 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):

$$C_{old} = ae^{-k.t} \quad (5)$$

$$C_{new} = a'(1 - e^{-k'.t}) \quad (6)$$

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:

$$MRT_{old} = 1/k \quad (7)$$

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

$$MT_{1/2-new} = -ln(1/2)/k' \quad (8)$$

k values of 0.032 and 0.045 were found, giving mean residence time of "old" C in SOM>0.2 mm of 31.2 and 22.0 months (Fig. 4; Table 1) for monoliths acclimated to low and high disturbance, respectively. The different k values indicated a slower decay at low compared to high disturbance level.

With *Lolium perenne*, mean residence times of 55 and 11 months (for litter fractions >0.2 mm) were reported for a monoculture exposed for 10 yrs to elevated CO_2 (van Kessel et al., 2006) and in a two year decomposition experiment using labelled root litter (Personeni and Loiseau, 2004), respectively. In the latter experiment, which compared grass species in monocultures, 95% of the variability in litter decomposition rates was explained by a root morphology trait, the mean specific root length.

In our experiment, the plant species richness reached 41 species and the abundance of *Lolium perenne* in the plant community was always below 10% (Klumpp et al., 2007). The low disturbance treatment was dominated by plant species with coarse and dense roots and rhizomes (Klumpp and Soussana, 2007[1]), which are likely to last longer and decompose slower than the fine roots observed at high disturbance level. However, differences in microbial communities and in mycorrhizae between disturbance treatments could also play a significant role in the decay rate of carbon in the detrital pathway (Rangel-Castro et al., 2005).

[1]Klumpp, K. and Soussana, J. F.: Plant functional traits and species richness control carbon sequestration in grassland mesocosms, Ecol. Lett., in revision, 2007.

First order exponential models indicated that the potential accumulation of "new" C was higher at low compared to high disturbance (a', 3.0 and 2.3 mg C g^{-1} soil, at L and H; $P<0.01$) and was for both treatments 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). Thus, soil organic C pools were not fully filled at the end of the experiment, suggesting that a large part of the "new" C which was deposited into the soil compartment was decomposed before the end of the two years of the experiment. This would explain why the potential accumulation of "new" C (a') was found to be lower than the initial amount of "old" C (a; Table 1). For the same reason, half the maximum accumulation of "new" C was reached after 3.5 month on average, as some "new" C started to vanish from the compartment above 0.2 mm after a few months only.

During ^{13}C labelling , "new" C entered first shoots then roots and rhizomes, before being released in the soil through rhizodeposition (i.e. root exudates) and decomposed litter. However, at the low disturbance level shoot litter decomposition also contributed to the supply of "new" C to the soil, which could explain the higher potential accumulation of "new" C (a') in the L compared to H treatment. Another factor contributing to the disappearance of both "old" and "new" carbon might be the activity of the soil fauna (macrodecomposers, e.g. earthworms) (Ostle et al., 2007; Hooper et al., 2000; Seeber et al., 2006), which could transport part of the litter to deeper soil layers and break down coarse litter into finer fractions. Indeed, there was a high presence of earthworms in the high disturbance treatment (data not shown).

A detailed understanding of the C cycling belowground can be gained by investigating with this method carbon turnover in different organic matter size fractions and at different depths. Moreover, the ^{13}C signature of the respired carbon can be studied giving access to the rate of decomposition of the "old" versus "new" carbon pools.

4 Conclusion

These results show the potential of ^{13}C steady state labelling for investigating the fate of carbon in the plant-soil continuum. A large array of applications at different time scales can be envisaged, ranging from short-term labelling of plant and soil carbon pools to long-term labelling of more stable soil organic matter fractions. 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 herbaceous ecosystems. We have shown here that this method allows calculating the mean residence time of carbon in soil organic matter fractions. Moreover, we have shown that changes in grassland disturbance regime alter the rate of detrital C mineralization and accumulation in the soil organic matter. Since detrital carbon accumulation accounts for most of an ecosystem's capacity to store organic carbon belowground within a few years, this quantification is important for understanding the role of grassland management on belowground C sequestration.

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