Autotrophic fixation of geogenic CO_2 by microorganisms contributes to soil organic matter formation and alters isotope signatures in a wetland mofette

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Abstract

To quantify the contribution of autotrophic microorganisms to organic matter formation (OM) in soils, we investigated natural CO_2 vents (mofettes) situated in a wetland in NW Bohemia (Czech Republic). Mofette soils had higher SOM concentrations than reference soils due to restricted decomposition under high CO_2 levels. We used radiocarbon ($\Delta^{14}C$) and stable carbon isotope ratios ($\delta^{13}C$) to characterize SOM and its sources in two moffetes and compared it with respective reference soils, which were not influenced by geogenic CO_2.

The geogenic CO_2 emitted at these sites is free of radiocarbon and enriched in ^{13}C compared to atmospheric CO_2. Together, these isotopic signals allow us to distinguish C fixed by plants from C fixed by autotrophic microorganisms using their differences in ^{13}C discrimination. We can then estimate that up to 27 % of soil organic matter in the 0-10 cm layer of these soils was derived from microbially assimilated CO_2.

Isotope values of bulk SOM were shifted towards more positive $\delta^{13}C$ and more negative $\Delta^{14}C$ values in mofettes compared to reference soils, suggesting that geogenic CO_2 emitted from the soil atmosphere is incorporated into SOM. To distinguish whether geogenic CO_2 was fixed by plants or by CO_2 assimilating microorganisms, we first used the

proportional differences in radiocarbon and $\delta^{13}C$ values to indicate the magnitude of discrimination of the stable isotopes in living plants. Deviation from this relationship was taken to indicate the presence of microbial CO_2 fixation, as microbial discrimination should differ from that of plants. $^{13}CO_2$-labelling experiments confirmed high activity of CO_2 assimilating microbes in the top 10 cm, where $\delta^{13}C$ values of SOM were shifted up to 2 ‰ towards more negative values. Uptake rates of microbial CO_2 fixation ranged up to 1.59 ± 0.16 μg gdw^{-1} d^{-1}. We inferred that the negative $\delta^{13}C$ shift was caused by the activity autotrophic microorganisms using the Calvin Benson Basham Cycle, as indicated from quantification of cbbL/cbbM marker genes encoding for RubisCO by quantitative polymerase chain reaction (qPCR) and by acetogenic and methanogenic microorganisms, shown present in the moffettes by previous studies. Combined $\Delta^{14}C$ and $\delta^{13}C$ isotope mass balances indicated that microbially derived carbon accounted for 8 to 27 % of bulk SOM in this soil layer.

The findings imply that autotrophic microorganisms can recycle significant amounts of carbon in wetland soils and might contribute to observed radiocarbon reservoir effects influencing $\Delta^{14}C$ signatures in peat deposits.

1 Introduction

Microbial assimilation of CO_2 is a ubiquitous process in soils, and can be accomplished by a wide variety of microorganisms using different metabolic pathways (Berg, 2011; Wood et al., 1941). RubisCO, the most important carboxylating enzyme for obligate and facultative chemo- or photoautotrophic microorganisms that fix CO_2 using the Calvin Benson Bassham Cylce (CBB) has been shown to be highly abundant in agricultural, forest and volcanic soils (Nanba et al., 2004; Tolli and King, 2005; Selesi et al., 2007). Direct uptake of CO_2 into microbial biomass (MB) and soil organic matter (SOM) by photoautotrophic and chemoautotrophic organisms has been measured in paddy rice and agricultural upland soils (Liu and Conrad, 2011; Wu et al., 2015; Wu et al., 2014), as well as under manipulating experimental conditions, like H_2 amendment (Stein et al., 2005) or addition of reduced sulphur compounds (Hart et al., 2013). Autotrophic acetogenic organisms, using the Wood-Ljungdahl Pathway for CO_2 fixation, are important groups in wetland and forest soils (Küsel and Drake, 1995; Ye et al., 2014). In addition, many heterotrophic soil microorganisms fix CO_2 in order to maintain their metabolic cycle by anaplerotic reactions, either to form new

sugars for cell wall synthesis or to excrete organic acids for nutrient mobilization (Feisthauer et al., 2008; Miltner et al., 2005; Santruckova et al., 2005). Global estimates of microbial CO_2 fixation in soils range between 0.9 and 5.4 PgC per year (Yuan et al., 2012). However, it still remains unclear how much of assimilated CO_2 is stored and contributes to the formation of soil organic matter (SOM). In this study we aim at evaluating the impact of autotrophic microorganisms on carbon isotope signatures of SOM. We further aim at quantifying the contribution of autotrophs to SOM by means of natural abundance ^{14}C and ^{13}C isotope signatures in a unique environment.

Microbial utilization of CO_2 and its incorporation into SOM is also potentially an important mechanism influencing the isotope signatures of SOM (Ehleringer et al., 2000; Kramer and Gleixner, 2006). Stable carbon ($\delta^{13}C$) and radiocarbon (^{14}C) isotope signatures are important tools for determining turnover of soil organic matter and dating ancient sediments (Balesdent et al., 1987; Hughen et al., 2004; Trumbore, 2000).

Stable isotope variations in soil reflect mass-dependent fractionation processes (Werth and Kuzyakov, 2010). In many well-drained soils, there is a well-documented increase in $\delta^{13}C$ with depth that has been variously attributed to selective preservation/decomposition of different components of organic matter, recent declines in atmospheric $\delta^{13}C$ due to the Suess effect, or microbial fractionation (summarized in Ehleringer et al. 2000). Enzymatic fractionation during assimilation of CO_2 can also lead to changes in $\delta^{13}C$ values of synthesized organic matter (Hayes, 2001; Robinson and Cavanaugh, 1995; Whiticar, 1999). Carboxylation processes by heterotrophic microorganisms have been hypothesized to be responsible for the increase in $\delta^{13}C$ values with depth in aerated upland soils (Ehleringer et al., 2000).

Radiocarbon signatures reflect the time elapsed since the C being measured was fixed from the atmosphere, and are corrected (using measured $\delta^{13}C$ values) to remove mass dependent fractionation effects. The radiocarbon signature of CO_2 in soil pore space can be depleted or enriched in ^{14}C compared to organic matter found at the same depth, depending on the age of C being mineralized (Trumbore, 2006). Because soil pore space CO_2 can have quite different isotopic signatures compared to SOM at the same depth, microbial assimilation of CO_2 may influence SOM ^{14}C signatures and therefore bias estimates of carbon turnover and radiocarbon age by generating reservoir effects (Pancost et al., 2000).

In turn, comparing both, radiocarbon and stable isotope values of SOM, MB and their sources might allow quantifying the potential contribution of autotrophic microorganisms to SOM, because a mismatch of both isotopes in quantifying SOM sources indicates either fractionation of ^{13}C by carboxylation processes of different enzymes or depletion or enrichment of ^{14}C by the use of soil CO_2 (Kramer and Gleixner, 2006).

In order to test the hypothesis that microbial CO_2 fixation contributes to SOM formation and alters isotope signatures in soil depth profiles, we investigated wetland mofettes in NW Bohemia. Mofettes are cold exhalations of geogenic CO_2 from wetland soils with high CO_2 concentrations. The exhaling volcanic-derived CO_2 has a distinct isotopic signature, is enriched in $\delta^{13}C$ by about 5 ‰ and free of radiocarbon compared to atmospheric CO_2. This unique feature allows us to use geogenic CO_2 as a natural isotopic tracer, because CO_2 assimilating microorganisms take up an isotopically different CO_2 source compared to plants growing in the area, which use a mixture of geogenic and atmospheric CO_2. We used three approaches to evaluate the importance of CO_2 fixation for SOM generation in mofettes and its impact on carbon isotope values:

1) We measured natural abundance ^{13}C and radiocarbon signatures of SOM, CO_2 and plant material in mofette and reference soils, in order to identify areas where C derived from microbial CO_2 fixation altered isotope signatures of bulk SOM from expected plant signals and quantified C derived from microbial CO_2 fixation by isotope mass balances.

2) We conducted isotope-labelling experiments with $^{13}CO_2$ in order to quantify the rate of CO_2 fixation by microorganisms in soil profiles of two CO_2 vents and compared these to reference soils away from the vents.

3) We complemented existing data about microbial community and activity in wetland mofettes (Beulig et al., 2014), by assessing the importance of microorganisms using the Calvin Benson Basham Cycle for CO_2 fixation. This was especially important to infer whether differences in kinetic isotope effects compared to plants were feasible given the pathways of microbial C fixation. Therefore, we quantified cbbL and cbbM marker genes encoding for Form I and II RubisCO, respectively. Form I RubisCO consists of eight small and eight large subunits. It can be subdivided into two groups, the “red” and “green” like groups, which can be further subdivided into Form 1A, 1B and 1C and 1D, respectively (Yuan et al., 2012; Tolli and King, 2005). Form II

RubisCO consists only of large subunits. Because of its low CO_2 affinity and high O_2 sensitivity, it represents an early form, evolved under anaerobic conditions and high CO_2 concentrations (Alfreider et al., 2003). Form II RubisCO might be favourable under conditions prevailing in mofettes. cbbL 1A was identified mainly in obligate autotrophic bacteria and cbbL 1C in facultative autotrophic bacteria (Tolli and King, 2005). cbbM encodes for autotrophic organisms living under anaerobic conditions (Selesi et al., 2005).

Using this information, we aimed to quantify the amount of C derived from microbial assimilation of CO_2 into soil organic matter within soil profiles, and assess its potential to alter isotope signatures of SOM.

2 Materials and methods

2.1 Site description

The study site (50°08´48´´ N, 12°27´03´´E) is located in the northwestern part of the Czech Republic (Bohemia). The area is part of a continental rift system, where deep tectonic faults provide pathways for ascending gases and fluids from the upper earth`s mantle (Kämpf et al., 2013). Mofettes are surficial, low temperature exhalations of mantle derived CO_2. Macroscopically, they form a complex of landscape features. At centre is a spot of typically 0.5 to 1 meter bare soil. From this central spot, almost pure CO_2 emanates to the atmosphere. The mofette centre is surrounded by a raised hummock that extends 1 to 20 m away from the spot. The investigated mofettes are situated on the floodplain of the river Plesna and are part of a wetland. Geogenic CO_2 emanates with an average discharge of up to 0.62 tons CO_2 d^{-1} per spot (Kämpf et al., 2013). The surrounding hummock is built up by different vascular plant communities. *Eriophorum vaginatum* and *Deschampsia cespitosa* are dominating plant species in the immediate proximity of the central vent and hummock structure, respectively. *Filipendula ulmaria* represents typical floodplain vegetation.

We investigated two mofettes that differed in size. Mofette 1 had a spot-diameter of 0.6 m, whereas the diameter of Mofette 2 was 1.5 m. We also sampled soils away from the influence of the mofette-exhaled CO_2 (deemed reference soils). These soils are vegetated and experience periodic anoxic conditions due to waterlogging, as evidenced by gleyed soil features and porewater geochemistry (Mehlhorn et al., 2014). In Mofettes 1 and 2, the local water table is elevated by ascending CO_2 and O_2 is mainly displaced by the CO_2 stream,

leading to anoxic (but not necessarily water-logged) conditions (Bräuer et al., 2011). According to the World Reference Base for soil resources (WRB, 2007), mofette soils are characterized as Histosols with pronounced reductomorphic features (reduced Y horizons) due to the influence of up-streaming CO_2. Reference soils are classified as 'gleyic' Fluvisols (Beulig et al., 2014).

2.2 Sampling of soils, plants and gases for bulk geochemical and isotope measurements

For bulk $\delta^{13}C$ and radiocarbon analyses soil cores were taken from the central, un-vegetated part of the mofette structure and reference soils. Reference soils lacking CO_2 emissions were identified with a portable landfill gas analyser (Visalla GM70 portable CO_2 sensor) in close proximity to each vent structure. Reference soils 1 and 2 were defined 5 and 18 meters distant from the central vent structures, respectively. Samples for bulk stable isotope and radiocarbon analyses were taken in November 2013. In order to account for soil heterogeneity, three soil cores (I.D. 5 cm) were taken from a plot of 50 x 50 cm from mofette and reference soils. Because mofette and reference soils were characterised by very different soil features, soil cores were not divided according to horizons, but depth intervals. Based on visual inspection, soil cores were divided into depth intervals from 0-10 cm, 10-25 cm and 25-40 cm. Replicates of the respective depth intervals were mixed and sieved to 2 mm. Roots and plant debris were removed by handpicking. The sieved soil was subsequently dried at 40° and prepared for stable isotope, radiocarbon and C/N analysis.

In April 2014, vegetation samples were taken from the same plot as soil cores, in order to characterize the isotopic composition of the plant material contributing to mofette SOM. Vegetation samples in the direct proximity of both mofettes were represented by *Eriophorum vaginatum*. Vegetation samples were also taken by clipping plants at 2cm height at 2 meter intervals along a transect that crossed moffett 2, allowing us to test how the isotope signatures ($\delta^{13}C$ and $\Delta^{14}C$) of plants changed with different mixtures of ambient and geogenic CO_2. Mofette 2 is an exposed hummock, dominated by an un-vegetated central region of CO_2 exhalation. One to two meters distant from the central exhalation, the dominant plant species was *Deschampsia cespitosa,* and at greater distances the dominant plant was *Filipendula ulmaria*. The collected samples were dried at 40° C, ground and prepared for stable isotope, radiocarbon and C/N analysis.

CO_2 was sampled from the centre of each mofette by filling 250 ml evacuated stainless steel cylinders through a perforated lance from four different soil depths (5, 15, 25, 40 cm), in order to determine its radiocarbon and stable isotope signature.

2.3 Soil sampling for $^{13}CO_2$ labelling experiments

Mofette soils were sampled for two labelling experiments in November 2013 and September 2014, respectively. For the first experiment, 10 x 10 cm soil monoliths, extending to 10 cm depth were sampled from each soil in November 2013. After removing the top of the Oh horizon (about 1 cm thickness), the remaining material was divided into three subsamples. Each replicate was homogenized within a sterilized plastic bag, put under an anoxic N_2 atmosphere and cooled at 4° until further processing in the lab within the same day.

For a second experiment, three soil cores (I.D. = 5 cm) were taken from 0 to 40 cm of each mofette and reference soil and subsampled from 0-5, 5-10 10-20, 20-30 and 30-40cm. 5g subsamples from each core were transferred immediately after core recovery to a sterilized 12 ml Labco® Exetainer, flushed with N_2 to preserve anoxia, sealed and brought to the laboratory at 4°C for further processing. To obtain background (i.e. with no influence of added label) values for isotopic composition, one set of subsamples was dried and prepared for TOC, C/N, pH and $\delta^{13}C$ analyses as described above.

2.4 Sampling for DNA extraction

Samples for DNA extraction were taken in May 2014 from Mofette 1 and Reference 1. Samples were taken from 0-5, 5-10, 10-20, 20-30 and 30-40 cm. Three replicates of 30 g were sampled from each depth, and homogenized under anoxic conditions. Subsequently, subsamples of 5 g were transferred to 50 ml tubes, cooled with dry ice and transported under an Ar atmosphere to the laboratory for molecular analyses.

2.5 Analyses of geochemical parameters and natural abundance isotope signatures of vegetation and soil samples

Soil pH was determined in a 0.01 M $CaCl_2$ solution with a soil:solution ratio of 1:2.5 using a WTW pH meter. The precision of pH measurements was better than 0.1 (n=3). Total C and N concentration of soil and plant samples were determined on a “Vario EL” (Elementar

Analysesysteme GmbH, Germany). Gravimetric water content was determined after drying soils for 48h at 105° and C and N content are reported per g dry soil weight.

Stable C isotope signatures of bulk soil and plant samples were determined on an isotope ratio mass spectrometer (DELTA+XL, Finnigan MAT, Bremen, Germany) coupled to an elemental analyser (NA 1110, CE Instruments, Milan, Italy) via a modified ConFloII™ interface (EA-IRMS). Stable carbon isotope ratios are reported in the delta notation that expresses $^{13}C/^{12}C$ ratios as $\delta^{13}C$-values in per mil (‰) relative to the international reference material Vienna Pee Dee Belemnite (V-PDB, Coplen et al., 2006):

$$\delta^{13}C = \left[\frac{\frac{^{13}C}{^{12}C}_{sample}}{\frac{^{13}C}{^{12}C}_{reference}} - 1 \right] \times 1000 \quad (1)$$

Analytical precision of all samples was better than 0.1 ‰.

For discussing microbially mediated isotope effects the isotope discrimination value Δ is used, which expresses the isotopic difference between two compounds in ‰:

$$\Delta_{x-y} = \delta_x - \delta_y \quad (2)$$

Where δ_x and δ_y refer to $\delta^{13}C$ values of the product and reactant, respectively.

The radiocarbon content of soil and plant samples was determined by accelerator mass spectrometry at the Jena ^{14}C facilities (Steinhof et al., 2004). Subsamples of soil containing 1 mg of carbon were combusted quantitatively and the developed CO_2 was catalytically reduced to graphite at 625°C by H_2 reduction. To simplify comparison with stable isotope ratios, radiocarbon activities are reported in $\Delta^{14}C$, which is the ‰ deviation of the $^{12}C/^{14}C$ ratio from the international oxalic acid universal standard. The $\Delta^{14}C$ value of the sample is corrected for mass dependent isotope fractionation to a common value of -25 ‰ (Mook and van der Plicht, 1999). The standard is corrected for radioactive decay between 1950 and the year (y) of the measurement (2014).

$$\Delta^{14}C = \left[\frac{\frac{^{14}C}{^{12}C}_{sample,-25}}{0.95 \frac{^{14}C}{^{12}C}_{Ox1,-19} \times \exp^{\left(y - \frac{1950}{8267}\right)}} \right] \times 1000 \quad (3)$$

Errors reported for radiocarbon measurements represent the analytical error of homogenized mixed samples in ‰. Analytical precision of all radiocarbon measurements was better than 3 ‰.

2.6 Labelling experiments

The first labelling experiment traced the flow of fixed CO_2 directly into microbial biomass (MB), evaluated rates of CO_2 uptake associated with biological activity and compared the proportion of labelled MB in mofettes with reference soils. From each field replicate sample, 20 g aliquots were taken and put into sterilized 120 mL boro-silicate bottles with butyl rubber stoppers inside a glove box containing an N_2 atmosphere. From these subsamples, three replicates were prepared for incubation with $^{13}CO_2$. In order to obtain control samples without biological activity, an additional aliquot of each sample was prepared and autoclaved for 2 hours at 160° and 60 bar.

Soil samples were incubated under anoxic conditions with $^{13}CO_2$ at N_2:CO_2 ratios equivalent to those experienced by the soils in the field: mofette soils were incubated with a 100 vol. % $^{13}CO_2$ atmosphere using sterile techniques and reference soils were incubated with a 10 vol. % $^{13}CO_2$ and 90 vol. % N_2 atmosphere. In order to account for soil respiration and to maintain a constant label, the headspace of every sample was removed and renewed every 3 days. The samples were incubated for 14 days in the dark at 12°C. Living and autoclaved control samples were treated identically.

After 14 days, the jars were flushed with N_2 and the soil samples were homogenized and split. One part was air dried for bulk ^{13}C analysis and the other part was prepared for extraction of the microbial biomass C by chloroform fumigation extraction (CFE) (Vance et al., 1987). CFE extracts microbial biomass C by lysing the cells with chloroform and releasing the products of cell lysis into a salt solution as dissolved organic carbon (DOC). In order to enhance extraction efficiency and to minimize the losses for extracted C by microbial degradation, the protocol from Vance et al. (1987) was slightly modified (Malik et al., 2013). The concentration of dissolved microbial biomass C (MB-DOC) and its stable carbon isotope ratio were determined by a high performance liquid chromatography system coupled to an IRMS (HPLC/IRMS) system (Scheibe et al., 2012). This method allows direct determination of concentration and carbon isotopic value of DOC in the liquid phase by coupling a LC-IsoLink system (Thermo Electron, Bremen, Germany) to a Delta+ XP IRMS (Thermo Fisher

Scientific, Germany). A detailed description of the apparatus and measurement procedure is given in Scheibe et al. (2012).

The amount of microbial biomass was determined by subtracting the amount of MB-DOC of un-fumigated samples from MB-DOC of fumigated samples and dividing with a proportionality factor K_c that accounts for the extraction efficiency:

$$C_{mic} = \frac{DOC_{fum} - DOC_{unfum}}{K_C} \quad (4)$$

A value of 0.45 was used for *Kc* according to Amha et al. (2012). The isotope ratio of microbial biomass C can be derived by applying an isotope mass balance:

$$\delta^{13}C_{MB} = \frac{\delta^{13}C_{fum} \times C_{fum} - \delta^{13}C_{unfum} \times C_{unfum}}{C_{fum} - C_{unfum}} \quad (5)$$

The net CO_2 fixation rate was calculated by determining the increase in ^{13}C from the label compared to the unlabelled control, and is normalized for C content (either total soil or microbial-C). The excess ^{13}C can be derived from the $^{13}C/^{12}C$ ratio of the sample before and after the labelling:

$$ExcessC[mg] = \frac{^{13}C_{labeled}}{^{12}C_{labeled}} \times C_{sample}[mg] - \frac{^{13}C_{unlabeled}}{^{12}C_{unlabeled}} \times C_{sample}[mg] \quad (6)$$

The $^{13}C/^{12}C$ ratio can be obtained from the measured $\delta^{13}C$ as follows:

$$\frac{^{13}C}{^{12}C_{sample}} = \left(\frac{\delta^{13}C_{measured}}{1000} + 1 \right) \times 0.011237 \quad (7)$$

where 0.01123 is the $^{13}C/^{12}C$ ratio of the international V-PDB standard (Craig, 1957).

A second labelling experiment was performed in order to obtain uptake rates as a function of depth for mofette and reference soils. After sampling 5 g of soil into 12 ml Labco® Exetainers as described above, mofette samples were flushed with 100 vol. % $^{13}CO_2$, and reference soils with 10 vol. % $^{13}CO_2$ and 90 vol. % N_2. Soils were incubated for 7 days in the dark at 12°C. The headspace of all samples was exchanged after 3 days of incubation. After 7 days, vials were opened and flushed with N_2 for 2 min and evacuated to remove any

sorbed or dissolved $^{13}CO_2$. Soil samples were subsequently air dried at 60°C and prepared for bulk ^{13}C analysis as described above. The measured enrichment in ^{13}C was used to measure uptake rates according Eq. (6).

2.7 DNA extraction and quantitative PCR

Total nucleic acid extractions of 0.7 g homogenised soil from mofette 1 and reference 1 were performed in triplicates according to the protocol of Lueders et al. (2004). Co-extracted organic soil compounds were removed by sequential purification with gel columns (S-400 HR; Zymo Research, Irvine USA) and silica columns (Powersoil Total RNA Kit in combination with the DNA Elution Accessory kit; MO BIO Laboratories, Carlsbad CA). Nucleic acid extraction efficiency was checked by agarose gel electrophoresis.

Copy numbers of 16S rRNA, cbbL 1A, cbbL 1C and cbbM genes in extracted DNA were determined using quantitative PCR (qPCR). qPCR was performed on a Mx3000P instrument (Agilent, Santa Clara, CA, USA) using Maxima SYBR Green Mastermix (Thermo Scientific) and the primer combinations Uni-338 F-RC and Uni-907 R (16S rRNA, (Weisburg et al., 1991), F-cbbM and R-cbbM (cbbM, (Alfreider et al., 2003)), F-cbbL and R-cbbL (cbbL IA, (Alfreider et al., 2003)) as well as F-cbbL IC and R-cbbL IC (cbbL 1C, (Alfreider et al., 2003)) as described by Herrmann et al. (2012). Cycling conditions for 16S rRNA genes as well as cbbL and cbbM genes consisted of denaturation for 10 min at 95°C, followed by 50 cycles with 4 temperature steps (1. 95°C at 30 s; 2. 55 and 57°C at 30 s for cbbL and cbbM/16S rRNA genes, respectively; 3. 72°C at 45 s; 4. data acquisition at 78°C and 15 s). Standard curves were constructed using plasmid CB54 for 16S rRNA and standard curves for cbbL and cbbM marker genes were constructed from ten times dilution series of mixtures of plasmids containing cbbL and cbbM inserts as described in Herrmann et al. (2015). PCR inhibitors were tested by ten times dilution series of representative samples. For the investigated samples 5 µl of DNA was taken as template for gene copy quantification of 16S rRNA, cbbL and cbbM.

2.8 Mass balance calculations

The unique isotopic composition of geogenic CO_2 and combined measurements of radiocarbon and stable isotopes allows identification of plant and microbial end-members for quantifying the importance of these two sources of SOM. Geogenic CO_2 ($\Delta^{14}C$ = -1000‰,

$\delta^{13}C$ = -2 ‰) is quite different from atmospheric CO_2 ($\Delta^{14}C$ ~+20‰, $\delta^{13}C$ = -7 ‰) in both isotopes. Therefore, $\Delta^{14}C$ values can be used to determine the overall fraction of geogenic CO_2 that is assimilated by plants or microorganisms in the mofette by using the end-members $\Delta^{14}C_{geogenic\ CO2}$ and $\Delta^{14}C_{air}$. A conventional mixing model for determining the fraction of geogenic CO_2 in SOM can be calculated according to:

$$SOM_{geogenic}[\%] = \frac{\Delta^{14}C_{SOM} - \Delta^{14}C_{air}}{\Delta^{14}C_{geogenicCO2} - \Delta^{14}C_{air}} \times 100 \quad (8)$$

This mass balance assumes that changes in $\Delta^{14}C_{SOM}$ caused by radioactive decay of ^{14}C are small compared to contributions from geogenic CO_2.

The same mass balance can be applied for calculating the fraction of geogenic CO_2 with stable isotope values. The end-members for this calculation are $\delta^{13}C$ values of plants, which grew solely on geogenic CO_2 or solely on ambient air CO_2. Plant $\delta^{13}C$ values are expected to be around 20 ‰ depleted in ^{13}C compared to the respective CO_2 source due to enzymatic fractionation, which has to be considered in determining the $\delta^{13}C$ end-member value.

We used the correlations between $\delta^{13}C$ and $\Delta^{14}C$ of plant material to prove that enzymatic discrimination of plants is constant in the vicinity of the mofette, despite potentially fluctuating CO_2 concentrations. If $\Delta^{14}C$ and $\delta^{13}C$ values of plants show a linear correlation, $\Delta^{14}C$ values of SOM can be used to derive $\delta^{13}C$ values that should be expected, if the organic matter is solely derived from plants according the mixing model:

$$\delta^{13}C_{model} = \delta^{13}C_{plant\_geo} \times \left(\Delta^{14}C_{SOM\_mofette} \times m + t\right) + \delta^{13}C_{plant\_air} \times \left(1 - \left(\Delta^{14}C_{SOM\_mofette} \times m + t\right)\right) \quad (9)$$

where $\delta^{13}C_{plant\_geo}$ and $\delta^{13}C_{plant\_air}$ are the measured plant input end-members exhibiting the most depleted (i.e. highest exposure to geogenic CO_2) and most enriched (exposure to atmospheric CO_2) $\Delta^{14}C$ values, respectively. $\Delta^{14}C_{SOM\_mofette}$ are measured radiocarbon values at a certain depth within the mofette soil. m and t are the slope and intercept of the regression between measured $\delta^{13}C$ and $\Delta^{14}C$ plant values. The model calculates the $\delta^{13}C_{SOM}$ that corresponds to measured $\Delta^{14}C_{SOM}$ values, if all SOM would be derived from plant material. Deviation from the model indicates input of C sources other than plants with distinct isotopic compositions.

2.9 Statistical analyses

Reported results (e.g. $\delta^{13}C$ values, microbial biomass), represent the mean of three independent replicates. Uncertainties reported for radiocarbon data represent analytical precision of a homogenised sample comprised of three independent soil cores. Differences of $\delta^{13}C$ in mofette and reference soils as well as between soil depth intervals were analysed using Student`s t-test. Significant differences are reported at $p < 0.05$.

3 Results

3.1 pH, bulk TOC and C/N

Soil pH ranges from 3.0 to 3.5 in mofette soils and is higher in reference soils (averaging 4.4), without significant trends with depth (Table 1). Total organic carbon (TOC) contents are high (~12 - 20% C) in the surface 5 cm of both mofette and reference soils. In the reference soil, TOC decreases with depth to concentrations of 3 % C below 20 cm. In contrast, TOC concentrations in both mofettes decrease below 5 cm (~6 to 16 %) and increase subsequently to more than 30 % below 20 cm.

Organic matter quality as indicated by C/N ratio also highlights differences between mofette and reference soils. High C/N ratios ranging from 25 to 30 are found below 20 cm depth in both mofettes, whereas C/N ratios decrease rapidly as low as 16.5 to 9 (for mofette 1 and 2, respectively) in the upper 10 cm (Table 1). In both reference soils, C/N ratios remain constant throughout the profile at 10 to 14 (Table 1).

3.2 Radiocarbon and stable isotope ratios of bulk SOM, plants and CO_2

Consistent with our expectation, we found that geogenic CO_2 is free of radiocarbon (-1000 ‰) and has an average $\delta^{13}C$ value of -2.36 ± 0.6 ‰.

Radiocarbon concentrations of SOM in both mofettes are generally more depleted by several hundred ‰ compared to reference soils (table 1). In reference soils, $\Delta^{14}C$ values decrease uniformly with depth from -60 ‰ and -34 ‰ in the top 10 cm to values of -280 ‰ and -163 ‰ at 40 cm depth in reference soil 1 and 2, respectively, reflecting radioactive decay (table 1).

$\delta^{13}C_{SOM}$ in mofettes has average values of -26.99 ± 0.33 ‰ and -26.38 ± 0.54 ‰ in mofette 1 and 2, respectively. In both mofettes $\delta^{13}C_{SOM}$ decreases slightly (but not significantly) below 20 cm depth ($p = 0.39$ and 0.49 in mofette 1 and 2, respectively) (table 1). Both reference soils have $\delta^{13}C_{SOM}$ of -28.08 ± 0.4 ‰ with no distinct depth trend in reference 1 ($p = 0.96$) and a slight but not significant decrease in reference 2 ($p = 0.35$) below 20 cm. At every depth, reference soils are 1 to 2 ‰ depleted in ^{13}C compared to mofette $\delta^{13}C_{SOM}$ throughout the soil profile ($p < 0.05$) (table 1).

Carbon isotope signatures in vegetation samples surrounding the mofette range from -29.95 ± 0.16 ‰ to -23.81 ± 0.30 ‰ in $\delta^{13}C$ and from -10.3 ‰ to -807.7 ‰ in $\Delta^{14}C$. Variations in the two isotopes are highly correlated, and plants with most positive $\delta^{13}C$ and most negative $\Delta^{14}C$ were found closest to the mofette and vice versa (figure 1). The linear fit to the strong ($R^2 = 0.86$) relationship between ^{13}C and ^{14}C found in vegetation material (figure 1) is used to determine parameters for the mixing model (Eq. 9). The intercept of the line with the y-axis yields a value of -22.79 ‰ and represents the $\delta^{13}C$ end-member value of plant material, which is fully labelled with geogenic CO_2 ($\delta^{13}C_{plant\_geo}$, or t in Eq. (9)). For the other endmember, $\delta^{13}C_{plant\_air}$, we used the $\delta^{13}C$ value of plants from the reference site that exhibited the most positive $\Delta^{14}C$ value, which yields $\delta^{13}C_{plant\_air}$ of -29.15 ‰. The corresponding $\Delta^{14}C$ value, i.e. the value closest to atmospheric radiocarbon concentrations, was -10.3 ‰ (= $\Delta^{14}C_{plant\_air}$). This is less than $\Delta^{14}C$ measured in CO_2 in clean background air in the year of sampling (~+20‰) and indicates either that the reference site experiences some influence of geogenic CO_2 or the influence of local fossil fuel release in the region.

The slope of the relationship fit to plant samples (m in Eq. (9)) is what would be expected for a linear mixture of plant material of the two end-member atmospheres (pure geogenic and pure air). Plant derived SOM would be expected to fall with this mixing line. The majority (71 %) of reference soil values are within the 95 % confidence interval of this expected slope (figure 1). In reference soils, ^{14}C declines with soil depth, while ^{13}C remains nearly constant. Mofette SOM generally has lower ^{13}C values than would be expected if they had the same linear relationship as plant material, and ^{14}C signatures are all much lower than those of the reference soil (figure 1). Only 5 % of mofette SOM values fall within the 95 % confidence interval of the regression line.

3.3 Mass balance calculations

Radiocarbon signatures of SOM indicate that, on average, 55 to 65 % of carbon accumulated in the mofette is derived from geogenic CO_2 (assuming end-members of -10 ‰ for $\Delta^{14}C$ air and -1000‰ for $\Delta^{14}C$ geogenic CO_2). The calculated proportion increases with depth. By doing the same mass-balance calculation with $\delta^{13}C$ values, (with - 22.47 ‰ as geogenic CO_2 end-member and - 29.15 ‰ as reference end-member), one obtains lower proportions of 34 - 44 % geogenic C compared to the radiocarbon mass balance. This mismatch in quantifying the proportion of geogenic C suggests that $\delta^{13}C_{SOM}$ values differ from what we would expect if they were completely derived from plant inputs.

Equation (9) can be used to predict $\delta^{13}C$ SOM values corresponding to measured radiocarbon values, assuming that all carbon would be derived from unaltered plant material. Calculated $\delta^{13}C_{SOM}$ values are 1-2 ‰ more positive at all depths ($p < 0.05$) compared to observations (figure 2 B), i.e. measured $\delta^{13}C_{SOM}$ values are depleted in ^{13}C compared to a signal that would be expected, if SOM would have preserved its original plant $\delta^{13}C$ signature.

3.4 Quantification of microbial CO_2 fixation activity

The analysis of bulk SOM and plant material revealed that mofette and reference soils are distinct in their radiocarbon as well as stable isotope values, indicating incorporation of geogenic CO_2 into mofette SOM either by plants or by microorganisms. Both isotopes show a bias in quantifying the amount of SOM derived from geogenic CO_2 by the same isotope mass balance, which suggests the presence of another source of carbon than plants, presumably microorganisms, that depletes $\delta^{13}C$ values. CO_2 fixing microorganisms might be a potential source with a distinct $\delta^{13}C$ value. In order to assess the activity of CO_2 fixing microorganisms as well as their spatial distribution along the soil profile, we conducted two isotope-labelling experiments.

In the first experiment we traced $^{13}CO_2$ directly into microbial biomass (MB) within the first 10 cm of the soil profile. After incubating the soils with $^{13}CO_2$, MB within all soils showed high enrichment in ^{13}C, except in autoclaved control soils. Microbial biomass extracts of autoclaved controls had $\delta^{13}C$ values ranging between -24.10 ± 0.38 to -27.55 ± 0.14 ‰, in both, fumigated and unfumigated samples, which is close $\delta^{13}C$ values obtained from bulk soil measurements (table 2). This confirms that mainly biological processes mediated CO_2 incorporation. In un-sterilized samples, unfumigated extracts showed enrichment in ^{13}C in all

mofette and reference soils. The $\delta^{13}C$ of unfumigated samples ranged from -14.29 ± 0.8 ‰ to +80.47 ± 9.46 ‰ and are therefore enriched in ^{13}C compared to controls ($p < 0.05$). However, in all cases ^{13}C enrichment was higher after fumigation ($p < 0.05$). $\delta^{13}C$ values of fumigated samples ranged between 143.76 ± 3.93 ‰ and 227.04 ± 2.63 ‰.

The calculated rate of CO_2 uptake expressed per gram microbial biomass in the top 10 cm of soil (table 2) was higher in mofettes compared to reference soils ($p < 0.05$) ranging between 287 ± 85 and 271 ± 58 ug^{-1} gMB^{-1} d^{-1} in mofettes compared to 139 ± 32 and 99 ± 36 ug^{-1} gMB^{-1} d^{-1} in reference soils (table 2).

The second labelling experiment measured CO_2 fixation activity along the whole soil profile with samples taken from depth intervals between 1 to 40 cm. Tracer uptake was measured only in bulk SOM. In both soils, uptake rates decrease with depth (figure 3). In the top 5 cm, uptake rates were higher in mofette soils compared to reference soils. Below 20 cm, rates decrease to values of 0.14 ± 0.03 ug gdw^{-1} d^{-1} in both mofettes and 0.09 ± 0.02 ug gdw^{-1} d^{-1} in reference soils. Normalizing the uptake rates to soil carbon content (ug gC^{-1} d^{-1}) instead of soil mass, removes the depth-dependence of uptake rates in reference soils ($p < 0.05$), but not in mofette soils (figure 3).

3.5 Quantification of 16s rRNA and marker genes for RubisCO

Results of 16S rRNA and RubisCO encoding marker genes are listed in table 3. The abundance of 16S rRNA genes per gram soil is a measure of the total abundance of microorganisms in the soil (Fierer et al., 2005). Gene copy numbers per gram soil of 16S rRNA genes were more abundant in the top 5 cm of the mofette soil. They decrease with depth, in both, mofette and reference soil ($p < 0.05$), but the decrease is more rapid in the mofette. The same holds true for marker genes encoding for RubisCO. CbbL IC is the most abundant marker gene in both soils, whereas it is more abundant in the reference soil compared to the mofette. CbbL 1C is one order of magnitude more abundant than cbbL 1A and cbbM in both, reference and mofette soils. cbbL:16S rRNA ratios range between 0.07 ± 0.03 and 0.19 ± 0.04 in the mofette soil and stays fairly constant with depth ($p = 0.61$). In the reference soil the ratio decreases slightly with depth from 0.37 ± 0.16 to 0.17 ± 0.04, but values are consistently greater than in the mofette soil.

4 Discussion

4.1 Carbon sources in mofette soils

Low C/N ratios, as found in the top 10 cm of both mofettes, reflect microbially degraded OM (Rumpel and Kogel-Knabner, 2011) and C/N ratios as low as 9 (top 10 cm of mofette 2) suggest a high contribution of microbial biomass to bulk SOM (Wallander, 2003). A significant contribution of microbial biomass carbon at these depths is also supported by very high 16S rRNA copy numbers, extracted from mofette 1, which are one order of magnitude higher than known from other soils (Fierer et al., 2005). Also numbers of RubisCO encoding genes are two orders of magnitude more abundant than in agricultural soils (Selesi et al., 2007) and twice as high as in organic rich paddy rice fields (Wu et al., 2015), suggesting microbial carbon derived from CO_2 assimilation as an important carbon source. Further evidence is given by the isotope data, as mofette SOM at 0 to 10 cm differs from a pure plant signal. The deviation of $\delta^{13}C_{SOM}$ towards more negative values compared to plant signatures suggests that microbialy derived carbon in shallower depths is fractionated against ^{13}C, which provides further evidence that autotrophic microorganisms contribute significantly to mofette SOM.

Below 20 cm, inceasing C contents in both mofettes are accompined with a steep increase in C/N, which is attributed to lower proportions of microbial carbon and accumulation of undecomposed plant organic matter, as suggested from studies at other mofette sites (Rennert et al., 2011).

4.2 Quantification of SOM isotope shifts by combined $\Delta^{14}C$ and $\delta^{13}C$ mass-balances

TOC, C/N ratios and the abundance of 16S rRNA genes in mofette soils all suggest that microbial carbon might constitute a significant part of bulk SOM. The isotope mass balance model can be used to assess the contribution of plant vs. microbial derived carbon. The approach assumes that microbially derived carbon is distinct either in its ^{14}C or its ^{13}C isotope ratio compared to plant carbon. The isotope mass balance model derived from equation 9 shows that microbial carbon that is added to SOM has to be depleted in $\delta^{13}C$ compared to plant inputs, leading to an overall negative $\delta^{13}C$ shift in bulk SOM of 1-2 ‰ compared to a pure plant signal at all depths (figure 2 B).

However, the model assumes that the radiocarbon content of mofette SOM solely depends on the amount of fixed geogenic CO_2 and does not consider radioactive decay. ^{14}C depletion by radioactive decay, especially with soil depth, can lead to an overestimation of fixed geogenic CO_2 and consequently to an overestimation of the shift in $\delta^{13}C$ values. In order to account for ^{14}C depletion by radioactive decay, $\Delta^{14}C$ values of reference soil SOM can be subtracted from $\Delta^{14}C_{SOM\_mofette}$ in Eq (9).

After correcting the model for radioactive decay, the calculated $\delta^{13}C_{SOM}$ depletion still matches the data for the first 10 cm of both mofettes, where measured $\delta^{13}C$ values are more negative than calculated ones (figure 2 C). Below 10 cm, the calculated $\delta^{13}C_{SOM}$ coincides with measured values in both mofettes, suggesting that SOM $\delta^{13}C$ preserved the signal of the plant source and only radioactive decay lead to the initial $\delta^{13}C$ shift in the model (figure 2 C). This supports findings from previous studies, where carbon accumulation accompanied with high C/N ratios was attributed to accumulation of poorly decomposed plant material (Rennert et al., 2011). The only exception from this pattern is at 30 - 40 cm in mofette 2, where measured $\delta^{13}C$ values are still more negative than calculated ones, even after correction for radioactive decay (figure 2 C). This might be caused by extremely low carbon dynamics, e.g. due to permanently waterlogged conditions, which would lead to an overestimation of the $\delta^{13}C$ isotope shift in the model. Although water levels fluctuate in the floodplain, permanently waterlogged conditions are likely to occur deeper in moffete 2, where high CO_2 discharge rates might lead to an elevation of the water table. Waterlogged conditions lead to low carbon turnover, and correction of radioactive decay with reference soil values might not be sufficient, because reference soils at these depths are only temporally waterlogged. This might explain the mismatch of measured and calculated $\delta^{13}C$ values at the deepest sampling point in mofette 2 and would indicate a potential bias of modelled C-isotope signatures towards too positive $\delta^{13}C$ values.

Another source of error in the model is accumulation of recalcitrant compounds within the SOM pool, like lignin or lipids, which might also lead to a shift in $\delta^{13}C$ values compared to the original bulk plant material (Benner et al., 1987; Werth and Kuzyakov, 2010). The accumulation of phenolic compounds is usually accompanied with an increase in C/N ratios (Hornibrook et al., 2000; Werth and Kuzyakov, 2010), which is not the case in the top 10 cm of the mofette soil. Therefore, lignin accumuation is not likely to have caused the depletion in

the top 10 cm of both mofettes. Nevertheless, increased lignin accumulation might also be the reason for the observed depletion in $\delta^{13}C$ below 20 cm depth in mofette 2.

Therefore, the model shows that $\delta^{13}C$ values in the top 10 cm of both mofettes are significantly lower than expected for SOM derived from plants alone, indicating significant addition of $\delta^{13}C$ depleted carbon. Below 10 cm depth, the calculated and measured $\delta^{13}C$ values agree after correcting for possible sources of error, like radioactive decay and alteration of $\delta^{13}C$ due to decomposition processes.

Microbial carbon that is added to mofette SOM by several CO_2 fixation pathways is likely to be depleted in $\delta^{13}C$ because of enzymatic fractionation processes (Fuchs, 2011). The deviation in $\delta^{13}C$ in the top 10 cm of both mofettes also is in accord with high CO_2 fixation rates and the abundance of functional marker genes for CO_2 fixation at this depth (figure 4). This implies that microbial carbon derived from CO_2 assimilating organisms is a major driver of the observed $\delta^{13}C_{SOM}$ depletion.

4.3 Quantification of microbial carbon C derived from CO_2 fixation

In order to quantify the proportion of CO_2-derived microbial carbon from the observed isotope shift, it is important to know the metabolic pathway that was used for CO_2 fixation and its corresponding isotope fractionation factor. Beulig et al. (in press, 2016) investigated by metatranscriptomic and metagenomic approaches microbial key processes in mofette soil 1. Consistent with our quantification of cbbL/cbbM marker genes, Beulig et al., (in press, 2016) detected high frequencies of transcripts encoding key enzymes for the Calvin Benson Cycle as well as the Reductive Acetyl CoA Cycle. The Acetyl CoA Cycle is used by acetogens, methanogens and sulphate reducers for catabolism and anabolism (Drake et al. 2006). According to Beulig et al. (in press, 2016), transcripts of key enzymes for the Acetyl CoA pathway in the mofette soil are also related to these groups. Most transcripts encoding for the Calvin Benson Cycle were related to chemoautotrophic bacteria and algae, living under anaerobic restrictions. The activity of autotrophic bacteria using the Calvin Benson Cylce is also supported by our data, as shown by the good correlation of cbbL/cbbM marker genes and uptake rates (figure 4).

Carbon that is fixed by autotrophs or algae using Form I RubisCO, the dominant form in the mofette, is depleted by -27 to -30 ‰ compared to the source CO_2 ($\Delta \approx$ -27 to -30 ‰) (Hayes, 2001; Pancost and Damste, 2003). A similar value can be expected for acetate formed

from geogenic CO_2 during acetogenesis. In systems where acetate is not limiting, depletion is less pronounced ($\Delta \approx$ -32 ‰) than in acetate-limited systems ($\Delta \approx$ -58.6 ‰) (Conrad, 2005; Gelwicks et al., 1989). A value of -32 ‰ is in accordance with acetate $\delta^{13}C$ values measured by Beulig et al. (2014) in a mofette study from the same area. Therefore, given a $\delta^{13}C$ value of geogenic CO_2 of around -2 ‰, the C end-member derived from microbial CO_2 fixation adds carbon with an average $\delta^{13}C$ value of -30 to -34 ‰ to bacterial biomass and SOM in mofettes. Taking the differences between measured and calculated $\delta^{13}C$ (with and without correction for radioactive decay, respectively) for mass balance calculation according to equation 8, microbially fixed geogenic CO_2 carbon in the top 10 cm of the mofette soil can make up between 8 ± 2 % and 15 ± 4 % in mofette 1 and between 23 ± 4 % and 27 ± 5 % in mofette 2.

4.4 Importance of microbial CO_2 fixation for isotope ratios in peat soils

Our data provide evidence that assimilation of CO_2 by several groups of autotrophic microorganisms contributes to SOM formation derived from CO_2. Recycling of CO_2 in peat deposits has been proposed to cause 'reservoir' effects in radiocarbon, biasing dating of peat (Kilian et al., 1995). As an explanation, Pancost et al. (2000) proposed recycling of $\Delta^{14}C$ depleted methane that diffuses from the catotelm layer up the peat profile, where it is oxidized by methanotrophic organisms and subsequently assimilated by mycorrhizal fungi living in association with *Ericaceae* rootlets. However, the authors could not find evidence from biomarker analyses of methanotrophic or fungal organisms and attributed recycling of ^{14}C depleted CO_2 to plants. Our findings suggest that other groups besides fungi are involved in CO_2 recycling, namely CO_2 utilizing autotrophic microorganisms. Pancost et al. (2000) estimated that 20 % of C in the investigated peat is derived from this recycling process. This proportion is very similar to our estimates for autotrophic fixation of CO_2 in the 0-10 cm of mofette soil. Hence we would propose that direct fixation of CO_2 could be a major process influencing peat radiocarbon signatures.

4.5 Importance of CO_2 fixation for soil carbon in reference soils

When normalized for the mass of carbon (as opposed to mass of soil), rates of CO_2 fixation in the reference soil at depth remain similar to values at the surface (figure 3). We cannot use the isotope-mixing model to estimate the amount of C derived from CO_2 fixation in the reference soil, because the soil atmosphere as well as plants at the reference soil are not directly influenced by geogenic CO_2. However the rate measurements suggest increasing

importance of CO_2 assimilating microorganisms for carbon stocks with depth. In addition, the high relative abundance of RubisCO marker genes relative to 16S rRNA genes suggest that autotrophic organisms constitute a substantial part of the microbial community throughout the soil profile. Their activity is also indicated by the strong correlation between RubisCO marker genes and uptake rates ($R^2 = 0.94$, $p < 0.05$) (figure 4). Higher CO_2 concentrations, which are usually observed with depth, might also lead to an increase of CO_2 assimilation, because of higher substrate availability for RubisCO or other carboxylases with depth.

In contrast to the mofette soil, which is characterized as an organic rich histosol, reference soils are classified as gleysols, with high organic carbon contents only in the A horizon. They are characterized by frequently changing redox conditions due to groundwater fluctuations, which might provide sufficient electron donors and acceptors for chemolithoautotrophic microorganisms (Akob and Küsel, 2011).

Beulig et al. (2014) characterized the microbial community of a reference soil at the same study site. The authors found that Proteobacteria constituted a substantial part of the microbial community. Many Proteobacteria are facultative autotrophs using the CBB cycle and have a facultative anaerobe metabolism (Badger and Bek, 2008). They would be therefore able to assimilate CO_2 also under the experimental conditions.

A contribution of phototrophic and chemoautotrophic microorganisms to SOM has been demonstrated already by other studies (Hart et al., 2013; Yuan et al., 2012), but solely for top soils. Wu et al. (2014) and Wu et al. (2015) investigated soil depth profiles up to 15 cm depth , but found no significant incorporation below 5 cm depth in upland and paddy soils under not manipulating experimental conditions, like illumination .

Our data suggest that autotrophic microorganisms are active even in the reference-subsoil. Microorganisms using the CBB cycle would add ^{13}C-depleted carbon to SOM. Indeed, $\delta^{13}C$ profiles of both reference soils do not show shifts towards more positive values with depth, as is usually observed from other Gleysols, although radiocarbon data indicates that SOM becomes older with depth (Alewell et al., 2011; Bol et al., 1999). Further, both reference soils have C/N ratios close to 10 throughout the soil profile, which normally indicates a higher contribution of microbial C to SOM (Rumpel and Kogel-Knabner, 2011). This strongly suggests a contribution of autotrophic microorganisms to carbon stocks in the subsoil, though ultimately its influence on the C isotopic signature of SOM at depth must be further evaluated.

Conclusions

$\delta^{13}C$ and $\Delta^{14}C$ values of SOM in wetland mofettes are influenced by incorporation of geogenic CO_2 fixed not only by plants, but also by microbes, as indicated by deviation of $\delta^{13}C$ values from those expected if plant C inputs were the sole source of SOM-C. The unique isotopic composition of geogenic CO_2 and the different enzymatic fractionation of plants and microorganisms allows us to quantify microbially derived C using combined ^{14}C and ^{13}C mass balances, because microbial carbon is more depleted than plant C. Other parameters, like C/N ratio, 16S rRNA and cbbL gene abundance also indicate addition of C fixed from geogenic CO_2 by microbes. According to the isotope mass balances, microbial carbon derived from CO_2 fixation accounts for 8 - 27 % of bulk SOM in mofette soils. The significant contribution of autotrophic microorganisms to SOM also implies that they might be able to cause reservoir effects in radiocarbon by recycling of old CO_2, as has been already suggested for peat soils.

Further, high CO_2 fixation rates, especially in mineral horizons of the reference soil, as well as the high of RubisCO marker genes indicate a significant contribution of autotrophic microorganisms to subsoil carbon.

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Table 1. Geochemical soil properties of mofette and reference soils. $\delta^{13}C$ and geochemical data represent background (i.e. without addition of label) data obtained from sampling in September 2014. Radiocarbon data was obtained in November 2013. Uncertainties in geochemical and $\delta^{13}C$ data represent $\pm 1\sigma$ standard deviation (n=3). Uncertainties in radiocarbon values represent analytical precision of a homogenized mixed sample.

	pH	TOC [w-%]	C/N	Water content [%]	$\delta^{13}C$	$\Delta^{14}C$
Mofette 1						
0-5	3.68	19.64 ± 1.20	15.95	53	-26.90 ± 0.15	- 554.3 ± 2.0
5-10	3.59	26.54 ± 0.08	16.52	52	-27.55 ± 0.21	
10-20	3.68	11.53 ± 0.18	15.12	57	-26.71 ± 0.18	- 559.7 ± 2.1
20-30	3.43	16.33 ± 0.59	21.65	51	-26.79 ± 0.12	
30-40	3.40	34.00 ± 1.25	31.40	56	-27.01 ± 0.23	- 640.2 ± 1.9
Reference 1						
0-5	4.13	25.85 ± 1.72	14.37	69	-27.98 ± 0.32	- 117.5 ± 2.8
5-10	4.07	12.40 ± 0.60	14.18	49	-28.10 ± 0.24	
10-20	4.00	3.16 ± 0.26	14.52	42	-27.80 ± 0.13	- 236.3 ± 2.7
20-30	3.91	3.14 ± 0.13	12.93	31	-27.79 ± 0.16	
30-40	3.69	2.81 ± 0.50	15.88	30	-28.23 ± 0.09	- 280.2 ± 2.5
Mofette 2						
0-5	3.80	8.66 ± 0.69	8.95	52	-26.01 ± 0.14	- 648.1 ± 1.2
5-10	3.76	5.87 ± 1.11	8.97	53	-26.26 ± 0.24	
10-20	3.79	11.41 ± 0.95	9.72	50	-26.76 ± 0.19	- 618.7 + 1.3
20-30	3.52	28.72 ± 1.42	19.74	56	-27.10 ± 0.59	
30-40	-	-	-	-	-	-
Reference 2						
0-5	4.50	12.48 ± 0.31	12.16	45	-27.91 ± 0.12	-34.1 ± 2.2
5-10	4.51	7.59 ± 0.21	11.52	42	-28.85 ± 0.21	
10-20	4.48	2.94 ± 0.15	10.30	46	-28.11 ± 0.05	-114.7 ± 1.9
20-30	4.46	1.91 ± 0.10	11.85	40	-27.82 ± 0.30	
30-40	4.43	1.80 ± 0.04	10.19	35	-28.23 ± 0.06	-162.9 ± 1.9

Table 2: Microbial biomass C and comparison of uptake rates determined during experiment 1 with CFE and bulk measurements. Uncertainties represent ±1σ standard deviation (n=3).

	$\delta^{13}C$ extract (after fumigation) [‰]	$\delta^{13}C$ control [‰]	Uptake rate/g soil [ug gdw^{-1} d^{-1}]	Uptake rate/g MB [ug gMB^{-1} d^{-1}]	% labelled MB
Mofette 1					
CFE 0 - 10 cm	233.24 ± 11.19	-25.94 ± 0.36	0.17 ± 0.03	287 ± 85	0.88 + 0.33
Bulk 0 -10 cm	-21.19 ± 0.62	-26.28 ± 0.10	0.77 ± 0.23	-	-
Reference 1					
CFE 0 - 10 cm	182 ± 5.44	-23.65 ± 0.54	0.59 ± 0.05	139 ± 32	0.40 ± 0.13
Bulk 0 -10 cm	-12.82 ± 0.95	-27.55 ± 0.14	2.65 ± 0.36	-	-
Mofette 2					
CFE 0 - 10 cm	124.51 ± 10.66	-24.10 ± 0.38	0.06 ± 0.02	271 ± 58	0.8 ± 0.16
Bulk 0 -10 cm	-21.37 ± 0.99	-26.49 ± 0.08	0.66 ± 0.15	-	-
Reference 2					
CFE 0 - 10 cm	158.05 ± 4.01	-26.46 ± 0.21	0.25 ± 0.09	99 ± 36	0.20 ± 0.10
Bulk 0 -10 cm	-17.44 ± 0.81	-27.21 ± 0.22	0.71 ± 0.16	-	-

Table 3: Quantification of 16S RNA, cbbL and cbbM marker genes. Uncertainties represent ±1σ standard deviation (n=3).

	Depth [cm]	16S rRNA	cbbM	cbbL 1A	cbbL 1C	cbbL 1C/ 16sRNA
Mofette 1	0 - 5	7.50E+10 ± 1.42E+07	5.70E+08 ± 3.21E+08	9.45E+08 ± 4.86E+08	9.23E+09 ± 4.55E+09	0.12 ± 0.06
	5 - 10	1.65E+10 ± 5.35E+06	2.21E+08 ± 1.28E+08	1.40E+08 ± 1.69E+08	1.46E+09 ± 1.20E+09	0.11 ± 0.04
	10 - 20	3.35E+09 ± 0.51E+06	1.49E+07 ± 8.45E+06	1.83E+07 ± 1.22E+07	6.02E+08 ± 1.25E+08	0.17 ± 0.03
	20 - 30	5.94E+09 ± 9.02E+05	1.62E+07 ± 1.23E+07	1.12E+07 ± 4.07E+06	3.98E+08 ± 1.53E+08	0.07 ± 0.03
	30 - 40	7.62E+08 ± 9.39E+04	8.53E+05 ± 3.02E+05	1.71E+06 ± 5.23E+05	7.91E+07 ± 2.18E+07	0.10 ± 0.03
Reference 1	0 - 5	4.63E+10 ± 3.01E+07	3.43E+08 ± 3.18E+08	1.14E+09 ± 4.74E+08	1.58E+10 ± 7.20E+09	0.37 ± 0.23
	5 - 10	2.98E+10 ± 2.02E+07	2.01E+08 ± 5.98E+07	2.69E+08 ± 1.52E+08	7.78E+09 ± 8.12E+08	0.28 ± 0.08
	10 - 20	2.81E+10 ± 4.83E+07	1.31E+08 ± 4.73E+07	3.06E+08 ± 1.59E+08	5.95E+09 ± 1.50E+09	0.21 ± 0.06
	20 - 30	1.24E+10 ± 4.37E+07	9.75E+07 ± 3.99E+07	9.11E+07 ± 3.90E+07	2.25E+09 ± 6.84E+08	0.18 ± 0.03
	30 - 40	4.65E+09 ± 9.61E+07	1.57E+08 ± 9.26E+07	3.47E+07 ± 2.20E+07	5.95E+08 ± 1.78E+08	0.10 ± 0.06

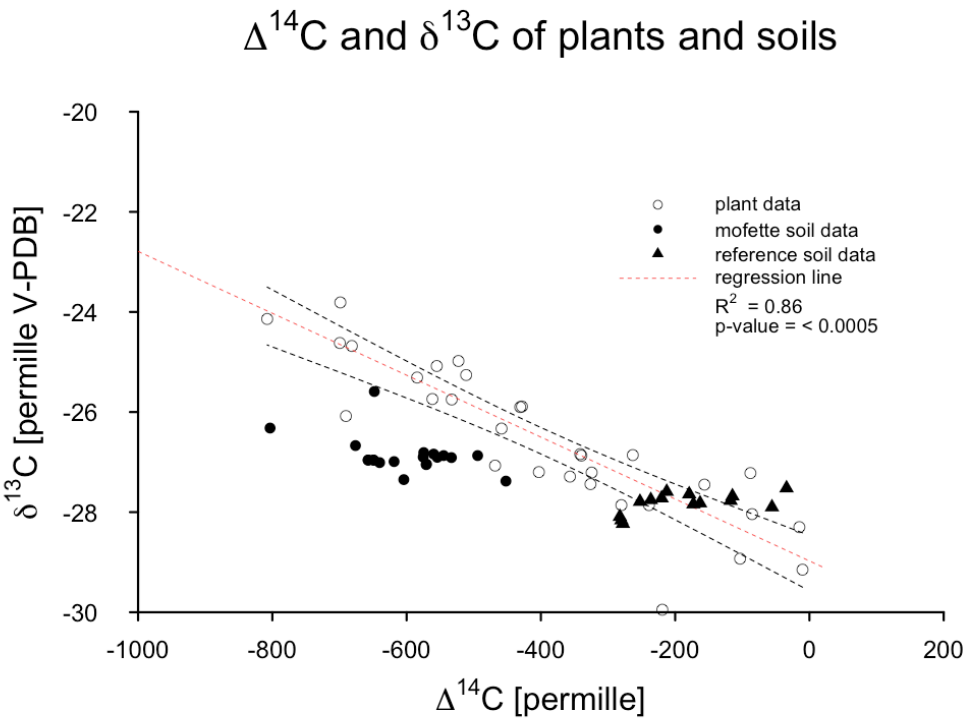
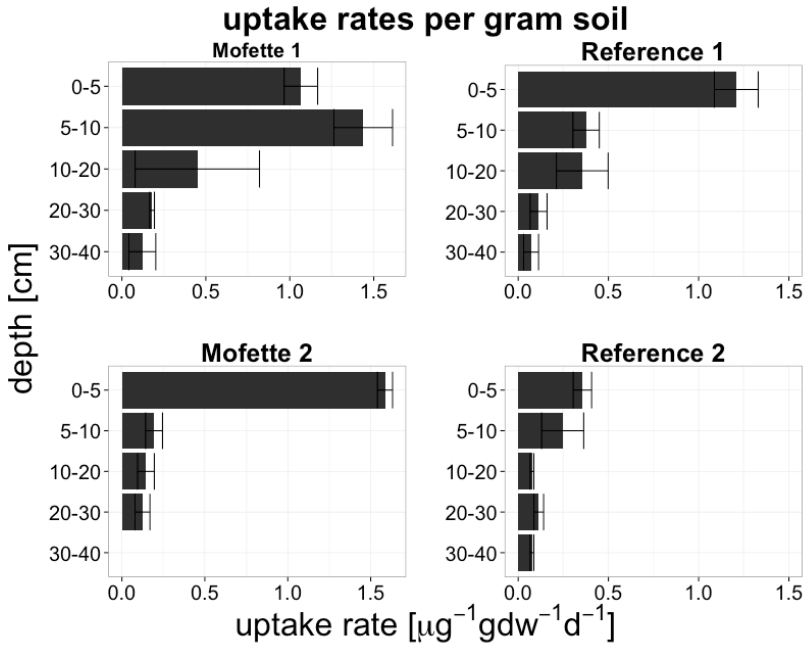


Figure 1. Correlation between $\delta^{13}C$ and $\Delta^{14}C$ of plants growing around the mofette structure. Dependent on the exposure to geogenic CO_2, plants incorporate different amounts of geogenic CO_2, which complicates isotope mass balance calculations for mofette SOM. However, both isotopes are highly correlated in sampled plant material, which allows prediction of $\delta^{13}C$ SOM isotope values from plant $\Delta^{14}C$. Most data points measured from mofette SOM fall outside 95% confidence levels of the regression, which suggests a deviation of mofette SOM $\delta^{13}C$ values from a pure vegetation signal. Reference SOM $\delta^{13}C$ values fall mainly within the observed plant $\delta^{13}C$ values, and do not increase with depth, as is often observed in soil depth profiles. Parameters of the regression are used to predict the $\delta^{13}C_{SOM}$ values expected in mofette soils that correspond to measured radiocarbon values, assuming that all carbon would be plant derived (Eq. 9).



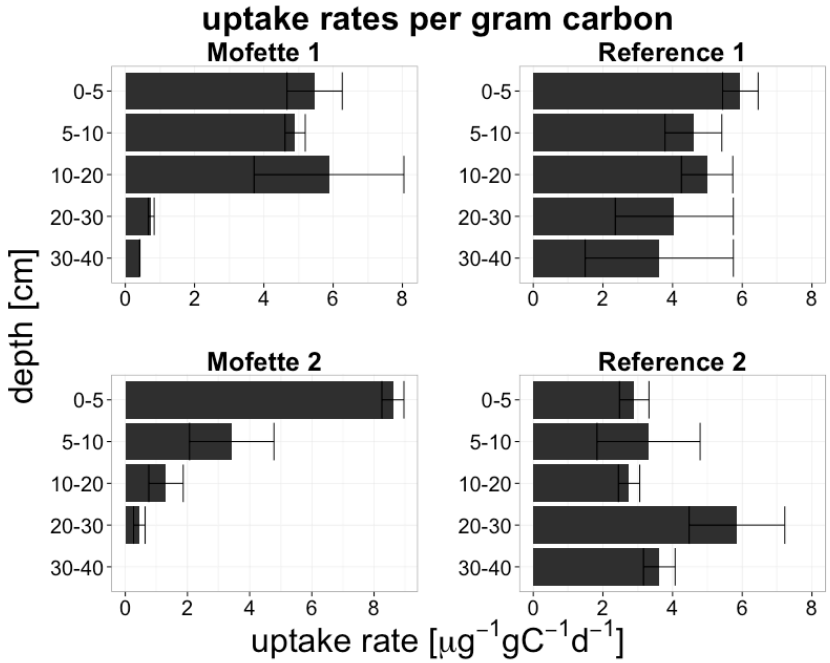


Figure 3: CO_2 uptake rates along depth profiles of mofette and reference soils as determined by bulk measurements from experiment 2. In both mofettes, uptake rates are highest in the top 10 cm and show a trend towards decreasing values at lower depths, especially below 20 cm. Uptake rates in reference soils also decrease with depth, but are nearly constant if normalized to organic carbon content. In contrast, uptake rates per organic carbon decline with depth in the mofette soils. This suggests increasing importance of autotrophic organisms with soil depth in the reference soil.

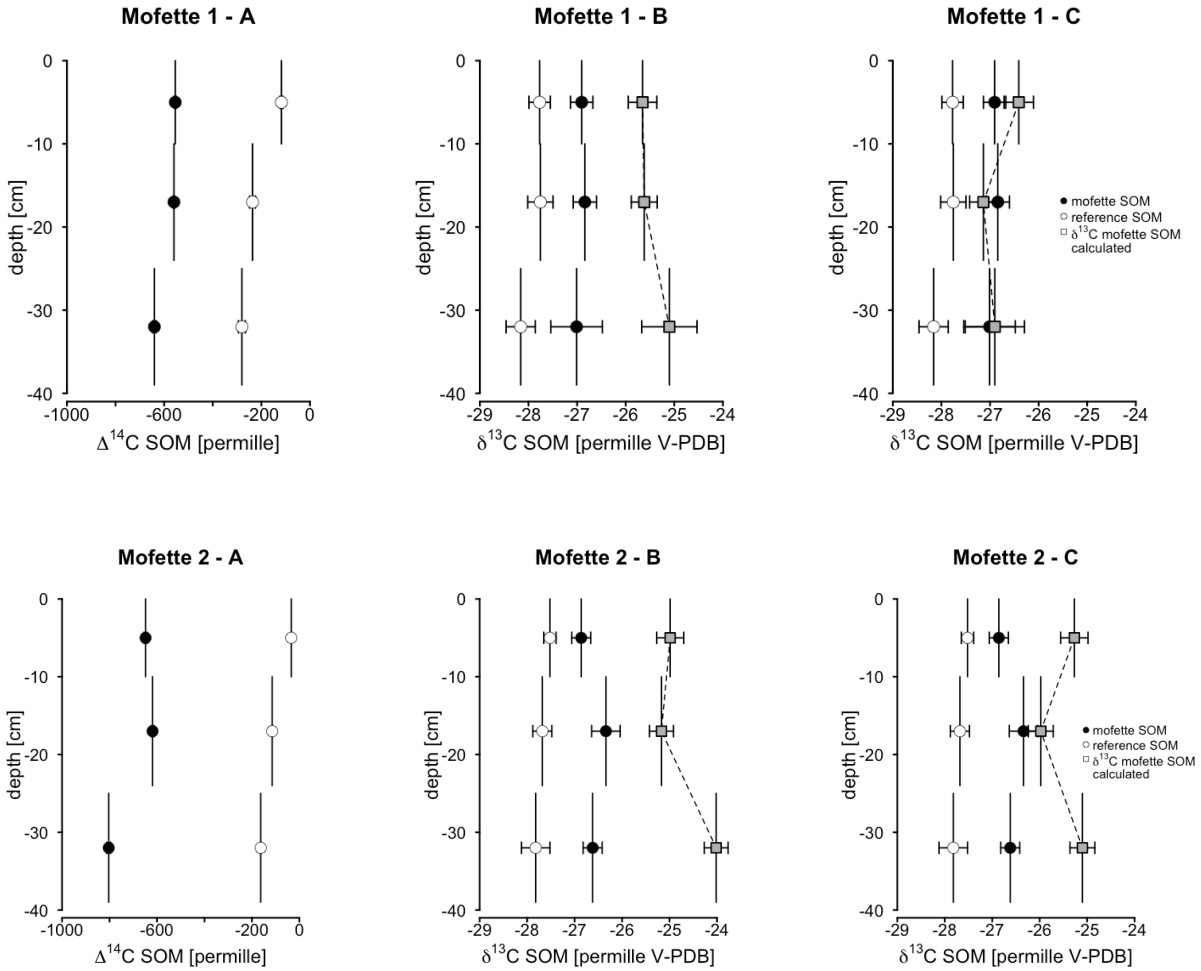


Figure 2: Depth profile of ^{14}C and ^{13}C signatures of SOM in mofette and reference soils.

A) Radiocarbon values in mofette soils are more depleted than reference soils, reflecting incorporation of geogenic CO_2 either by plants or by microorganisms. Error bars reflect analytical precision because only one homogenized sample was analyzed.

B) $\delta^{13}C$ values in both mofettes are also shifted towards geogenic CO_2, but to a smaller extent than radiocarbon values. Gray squares in $\delta^{13}C$ depth profiles show values of $\delta^{13}C$ in mofette SOM estimated using Eq (9). Measured $\delta^{13}C$ values are more depleted than estimated values at all depths.

C) Estimated $\delta^{13}C$ values, assuming eq (9) but with 14C values that have been corrected for radioactive decay assuming that SOM ages with depth in the same way as the reference soil

These estimated $\delta^{13}C$ values agree with measured values below 20 cm depth but remain depleted compared to what is expected from a pure plant SOM source in the top 10 cm. This suggests that the observed depletion in the top 10 cm of both mofette soils is caused by addition of ^{13}C depleted microbial carbon, derived from fixed CO_2. In contrast, the mismatch between estimated and measured values below 20 cm depth in (B) can be explained by radioactive decay.

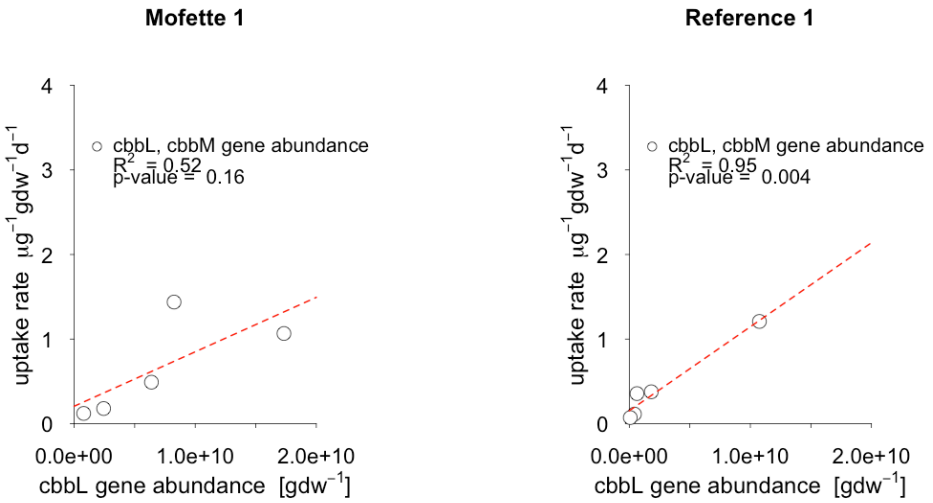


Figure 4: Correlation of marker genes encoding for RubisCO and measured uptake rates in mofette soil 1 and reference soil 1 in the soil depth profile from 0 to 40 cm depth. The good correlation in the reference soil indicates high contribution of chemolithoautotrophic microorganisms to measured uptake rates. In the mofette soil R^2 is considerable lower, most probably, because also other CO_2 fixation cycles that the CBB cycle, like the Acetyl-CoA cycle, are important pathways in these soils.