REFEREE 1 (**responses in bold**)

This manuscript used the differences in ^{13}C and ^{14}C discrimination of the geogenic CO_2 emitted at mofette soils and atmospheric CO_2 in reference soils to distinguish C fixed by plants from C fixed by autotrophic microorganisms. The results show that CO_2 fixation by autotrophic microorganisms contributes significantly to soil organic matter formation and alters the isotope signatures in mofette soils. The experiment seems well designed and the study is overall well presented.

Thank you for handling our manuscript and for your constructive and helpful comments. Replies to specific comments are given below.

However, a number of aspects were raised which were commented directly in the manuscript.

1. Page2 Line7-11. It is inconclusive to infer that the negative $\delta^{13}C$ shift was caused

by the activity of chemolithoautotrophic microorganisms with the quantitative data even under dark incubation. The soils used for isotope analysis (labelling experiment) were different from those for quantitative PCR analysis, which makes me doubt more about the consistency of the analysis. Furthurmore, I think it is inappropriate to classify aceto- genic microorganisms, methanogenic microorganisms and chemo-lithoautotrophic mi- croorgansims into different catalogs.

We agree that the term chemolithoautotrophic microorganisms might be misleading and does not represent the organisms that we wanted to target with qPCR analyses. Our target was to get information about the potential of CO_2 fixation through the Calvin Benson Basham Cycle (CBB) in the mofette soils. This is important for our mass balance approach, because the metabolic cycle determines the $\delta^{13}C$ signature of the microbial end-member in the isotope mass balance. These data should be complementary to information about acetogenic and methanogenic pathways from previous studies (Beulig et al. 2014, see manuscript for reference; see also discussion, 4.3). The term chemoautotrophic microorganisms should refer to organisms using the CBB cycle for CO_2 fixation. We agree that other microbes also use the CBB Cycle. Similarly, not all

chemolithoautotrophs use the CBB Cycle, although it is the most common metabolic cycle. Therefore you are correct and the term chemolithoatutrophic microorganisms is not correct in this context. It was changed to "autotrophic microorganisms using the CBB cycle". The data for the qPCR was taken from same soil as for the labelling experiments (mofette soil 1), although they were sampled at different time points. This was clarified in the text.

2. Page2 Line 13. Change "organisms" to "microorganisms".

Done

3. Page2 Line 14. Consider adding "carbon" before "observed reservoir effects" here.

Done

4. Page3 Line 2-4. Many published papers concerned this issue. Specify the differ- ences of your study from these published researches.

We added a sentence to clarify that we try to target the question of whether CO_2 fixation influences carbon isotope signatures, and that we try to use a quantitative approach by means of natural abundance carbon isotope values, that distinguishes it from other studies dealing with CO_2 fixation in soils.

5. Page3 Line 30. "indicates"

Done

6. Page4 Line 23-25. Do the cbbL genes encode Form I RubisCO or Form II RubisCO, or both Form I and Form II RubisCO? Make it clear.

7. Page 4 Line 25. How many subclasses do Form I RubisCO have? Why chose cbbL IA and cbbL IC in this study?

Answers to 6 and 7: We tried to improve this section according to the referee's suggestion. cbbL encodes for Form I RubisCO and we choose Form I genes encoding for subclasses 1A and 1C, because they can give us information as to weather CO_2 is assimilated by obligate or facultative microorganisms. Further, cbbM, encoding for

Form II RubisCO, can give information if microbes with special adaption to anaerobic environments are important CO_2 fixing microorganisms.

8. Page5 Line 16-17. How far is Mofette 1 from Mofette2? If close, why the geochemical properties are so different? How to fix the sampling sizes of these two mofettes?

Mofette 2 is approximately 500 m distant from mofette 1. The geochemical properties are very different, because the two mofettes differ in size. This is mentioned in the text (page 5, line 25). Mofette 1 is considerably smaller than mofette 2. Thus, plant litter inputs from the top is greater in mofette 1 compared to mofette 2, because plants growing at the rim of the structure can more easily fall into the bare centre of the mofette structure.

9. Page5 Line 29-30. Page 6 Line 12. Please clarify the sampling time of soil and plant samples respectively.

10. Page6 Line14. Why take the vegetation samples at 2 meter intervals? Did you collect Eriophorum vaginatum, Deschampsia cespitosa and Filipendula ulmaria from each intervals?

Answers to 9 and 10. Dates for all three sampling campaigns were clarified in the text, as was the sampling strategy. We took vegetation samples (Eriophorum vaginatum) in the direct vicinity (rim) of both mofette structures. In addition, we took samples from a transect crossing mofette 2, because mofette 2 represents an undisturbed hummock structure without the disturbances associated with secondary exhalation structures found in the overall smaller mofette 1. In mofette 2, the vegetation changed with increasing distance from the central CO_2 exhalation hummock (which was bare of vegetation). This was clarified in the text.

The goal of the transect sampling was to have plant samples that experience a full mixture of the two presumed end members (geogenic and atmospheric CO_2), so we could determine if $\delta^{13}C$ and $\Delta^{14}C$ values follow a linear trend with increasing CO_2 concentrations. This was necessary to make predictions for mofette SOM, because mofette SOM is derived from plants that are likely exposed to fluctuating CO_2

concentrations. In order to make predictions for mofette SOM from vegetation $\delta^{13}C$ and $\Delta^{14}C$ values, it is therefore necessary to test, weather plants follow a linear trend in $\delta^{13}C$ and $\Delta^{14}C$ values within a CO_2 gradient, as well as the if the relationship between $\delta^{13}C$ and $\Delta^{14}C$ of plants is linear with increasing CO_2 concentrations, or if fractionation of plants is influenced by elevated CO_2 concentrations. The good correlation of $\delta^{13}C$ and $\Delta^{14}C$, even with increasing CO_2 concentrations indicated that our model used for prediction of ^{13}C values from ^{14}C is valid for plant-derived SOM (assuming, for the moment, no radioactive decay influence on ^{14}C).

11. Page6 Line7-8. Mofette and reference soil cores used for geochemical properties analysis were divided into depth intervals from 0-10cm, 10-25cm and 25-40cm according to the sampling strategy. However, results (Table 1) showed these soil cores were sectioned into 0-5cm, 5-10cm, 10-20cm, 20-30cm and 30-40cm.

Table 1 shows the geochemical data from the sampling campaign conducted in September 2014, when we took samples for the second labelling experiment. The data given in table 1 represent the unlabelled (time zero) geochemical data from soils sampled for the second labelling experiment. We choose to use these data, to present $\delta^{13}C$ isotope values with a higher spatial resolution, which gives a more detailed insight into small scale variations in the geochemical and stable isotope changes in both, mofette and reference soil with depth. However, we do not have radiocarbon data from this sampling campaign. Therefore, we included $\Delta^{14}C$ values from the first sampling campaign into table 1, which has a broader depth resolution. This is now clarified in the table caption.

12. Page6 Line10-11. Confirm that the stable isotope and radiocarbon analysis were performed in triplicate for each mixed sample.

All stable isotope analyses were conducted in triplicate. For sampling campaign 1, we used a mixed homogenized sample composited from three cores. This homogenized sample was subsampled three times for stable isotope analyses. For radiocarbon, we

measured only one subsample. Therefore, uncertainties given for radiocarbon analyses from sampling campaign 1 represent the analytical uncertainty (see revised figure caption 3A)

13. Page6 Line 23-24. Why remove the top of the Oh horizon?

We removed the Oh layer because we wanted avoid CO_2 uptake by phototrophic organisms like algae.

14. Page9 Line 4. The temperature may affect microbial activity. The incubation temperature was10◦C in the first labelling experiment and it was set to 12◦C in the second labelling experiment. How do you set the temperature?

The temperature given for experiment 1 is a mistake in the text. Both experiments were conducted at 12◦C. This has been corrected in the text.

15. Page10 Line 19-20. Was the DNA extraction performed in triple?

All DNA analyses were performed in triplicate.

16. Page11 Line1-7. What about the amplification reaction and program?

The exact protocol for amplification reactions and the program used have been inserted in the text (page 11, line 22-30).

17. Page12 Line 12-13. It was inconsistent with the sampling method part, where you showed that the replicates of respective depth intervals were mixed (Page6 Line 8-9).

This is correct. We did not perform a t-test on radiocarbon data, because the error in Δ14C values represent only the analytical precision. This was corrected in the text.

18. Page12 Line 20-24. The mofette soils and reference soils are close to each other (5 or 18 meters), however, the TOC contents in these soils are so different. And the variations of TOC contents along soil depths are also quite different. Why?

The differences in C content, pH and C/N ratio are caused by permanently anoxic conditions in mofette soil compared to the reference soil and by the addition of plant material as well as microbial carbon that has been partly derived from autotrophic organisms. The reasons for the observed differences are also discussed in detail in section 4.1.

19. Page13 Line4-5. What about the radiocarbon and stable isotope concentrations of CO_2 sampled at different depth (5cm, 15cm, 25cm, 40 cm)?

The radiocarbon and $\delta^{13}C$ values of sampled CO_2 are given in line 27.

20. Page13 Line 6. I don't think "Measured" in this sentence is necessary.

Done

21. Page15 Line 12. What do CFE and bulk mean in table2?

CFE means values obtained from the first experiment, where uptake rates were determined by CFE extractions and bulk refers to experiment 2, where $^{13}CO_2$ incorporation directly into bulk organic carbon was determined, without extracting microbial biomass. It was clarified in the text.

22. Page16 Line2. The ratios of cbbL IC to 16s RNA in this study are almost two orders magnitude higher than those in other published papers (table3), it is hard to believe that the ratios range from 7% to 37% in these investigated soils. Even the cbbL IA copy numbers at 5-10cm in Mofette1 is 1.40E+08±1.69E+08. These results did throw my doubt on their quantitative PCR.

The high number of 16s RNA and cbbL 1C and 1A genes correspond with the low C/N ratios and high CO2 uptake rates found in Mofette 1. Together with the geochemical

data, and the activity measurements, the high number of 16S rRNA genes as well as genes encoding for RubisCO reflect a large number of microorganisms in these soils, which finally leads to an increased contribution of microorganisms to SOM

23. Page16 Line 23. I don't understand what "processed OM" are here.

Processed OM refers to partly degraded OM. This was clarified in the text.

24. Page16 Line28-31. I didn't see the connection between RbusiCO encoding genes and microbially assimilated carbon. This would depend more on the activity of CO2 assimilation microorgansims.

We know from the study from Beulig et al. (2014), that acetogenic and methanogenic microorganisms are active in the mofette soil, especially in the top 10 cm. This was also confirmed by a metatranscriptomic approach (Beulig et al., in press). In this study we did complementary analyses to evaluate the importance of mciroorganisms using the Calcin Benson Cycle. This is important, in order to derive the isotopic signature of microbial carbon that is derived from CO2 fixation. This is discussed in detail in section 4.3 in the discussion. Obviously, RubisCO is also a pathway that contributes to CO2 assimilation and has to be considered by defining a microbial isotope end-member in the isotope mass balance.

25. Page17 Line7-20. The discussion is too weak. Try not to repeat what was already presented in the methods and results.

The discussion in this section was streamlined.

26. Page18 Line 19. For the correlation analysis in figure 4, the lateral axis rep- resents the cbbL gene abundance, while the circle marker means cbbL, cbbM gene abundances. Which marker gene do you use for this analysis?

Yes, the axis title is wrong. The data points represent total number of cbbL and cbbM genes.

27. P19. Line 2-4 Which kinds of chemoautotrophic bacteria were involved in the Calvin Benson Cycle according to the metatranscriptomic analysis? The good correlation of cbbL/cbbM marker genes and CO2 fixation rates indicated that the fixed carbon derived from autotrophic bacteria, not chemoautotrophic bacteria.

28. P19. Line 5-6 Is any data support your state that type I RubisCO is the dominant type in the mofette?

Type I RubisCO is the dominant type, because it is most abundant in the mofette soil.

REFEREE 2

The manuscript describes a study investigating the autotrophic CO2 fixation by soil microorganisms and their contribution to soil organic matter (SOM) in mofette soils compared to reference soils. The particular composition of the geogenic CO_2 at these sites allows estimating the contribution of plant-derived, SOM-derived and CO_2-derived C in soil microbial biomass and SOM. The approach taken by the authors includes tracing the isotopic composition (both ^{13}C and ^{14}C) into soil microbial biomass and SOM, but also molecular analyses of genes involved in autotrophic CO_2 fixation. This allows the authors to study the process and relate it to potentially responsible microorganisms.

The results show that a significant percentage of soil C in the investigated mofette soils is derived from geogenic C, and that cbbL 1C was more abundant than the other genes investigated. The manuscript thus describes an important process which has been mostly neglected in the past. It is well written and organized, and thus it should be published. However, some relatively minor revisions to the manuscript will further improve it.

Thank you for these encouraging and constructive comments. Our answers to specific comments are in bold face, below.

Here are some more detailed comments:

Labelling experiments: Were unlabelled controls included or are analyses of the starting materials used to correct for background values? Also, how were the different CO_2 concentrations in the air during the labelling experiment accounted for when assessing the data?

We used $\delta^{13}C$ values of autoclaved controls as background values. All autoclaved controls showed zero enrichment after labelling, compared to the samples that were not autoclaved. The non-autoclaved samples had enrichments of up to +80 ‰ in unfumigated extracts, although enrichment was always higher after fumigation (see page 18, line 18-21). This is most probably caused by formation of

secondary metabolites of the microorganisms that were synthesised from labelled CO_2 and excreted, presumably acetate. However, the $\delta^{13}C$ value of autoclaved samples does not accurately represent the natural abundance $\delta^{13}C$ value of the microbial biomass, because autoclaving disturbed the sample and also could have made some of the plant material extractable. Natural abundance $\delta^{13}C$ values of microbial biomass are presumably more negative than the values obtained from autoclaved samples and also more negative than bulk SOM.

p. 14571, top paragraph: When looking at Fig. 1, I wonder whether the effect of radioactive decay need not be taken into account already here. For the reference soils, delta^{13}C is almost constant, whereas Delta^{14}C varies, indicating different ages. How would this affect the relationship between the two isotopic signatures?

It is true that the decreasing ^{14}C with depth in the reference soils indicate aging of SOM with depth. However, interestingly, there is no change in $\delta^{13}C$ values with depth. This means, that $\delta^{13}C$ values are not changed with increasing decomposition stages in these soils, as observed in other soils, where there is usually an increase in $\delta^{13}C$ values with depth. Theoretically, it is possible to apply the model from eq. 9 to the reference soil. This we have done in the attached plot (see figure 187 **below).**

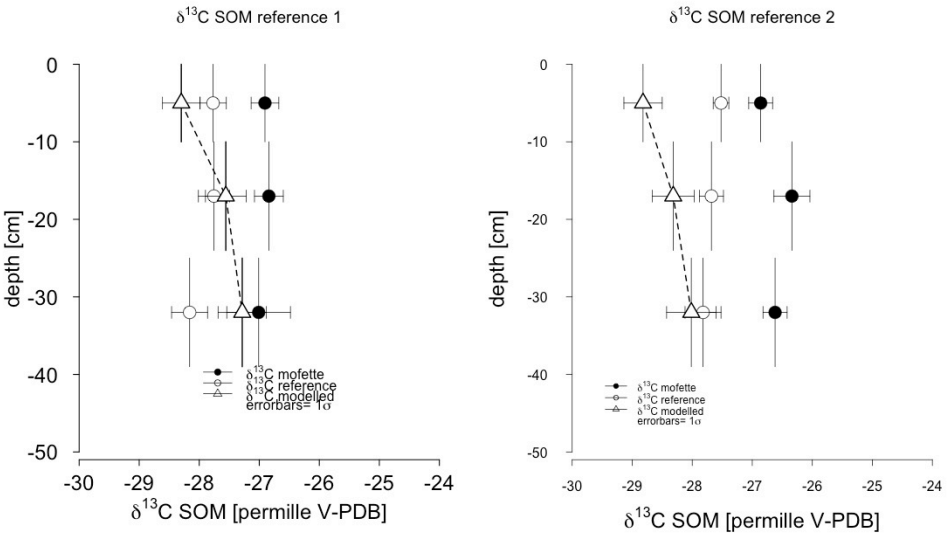

Figure 184: Adapted model for reference soils.

The results indicate that measured $\delta^{13}C$ values for both reference soils are more positive in the first 5 cm, whereas they should increase with depth according to the model. However, in both soils $\delta^{13}C$ values show the opposite trend and get more depleted with depth. One has to consider that the process assumed responsible for the shift in the mofette model is different than for the prediction for the reference soil in the figure 187. In the reference soil the model assumes that Δ14C is mainly determined from the amount of incorporated geogenic CO_2, whereas radioactive decay is of minor importance compared to geogenic CO_2 and can be corrected with ^{14}C decay derived from the reference soil. The model prediction shown in figure 187 shows implies that there is no linear relationship between aging of organic matter (as implied by $\Delta^{14}C$ values) and $\delta^{13}C$ values. An increase in $\delta^{13}C$ values with increasing decomposition of the organic matter is therefore not supported by the relationship of $\Delta^{14}C$ and $\delta^{13}C$ values in the reference soil. CO_2 fixation might be an explanation for this, because it adds depleted carbon via microbial biomass to the soil and might "shift" $\delta^{13}C$ back towards more negative values.

p. 14575, line 5-bottom of page: I certainly agree with the authors that radioactive has to be taken into account when estimating the contribution of the different pools to SOM or microbial biomass. However, I have some doubts about how good their approach to do so was. The authors claim that after correction for radioactive decay, all but one calculated value for delta13C in the horizons deeper than 10 cm match the measured values. In total, however, this means that 3 out of 6 calculated values match the measured ones, the other 3 don't. So it is difficult to judge whether this correction really did a good job. The authors mention different organic matter dynamics in the mofette and the reference soils as a potential source of error. If there are any indications for this, this should be discussed more in detail.

Indeed, there is some evidence that carbon dynamics are slower in the deepest layer sampled in mofette 2. All soils in the floodplain are subjected to fluctuating water levels. However, in the mofettes these fluctuations are attenuated, because within the central part of the exhalation water table is elevated by the upstreaming CO_2. Mofette 2 is considerably larger than mofette 1 and the CO_2 discharge is somewhat higher (see answer 8 to referee #1). Mofette soil 2 is therefore likely water-saturated throughout the

whole year, in contrast the all other soils, although we have no direct evidence, because we did not measured the water level throughout the whole year. However, we have indirect evidence, because during the sampling campaign in September, it was not possible the gain soil cores from the deepest point with our auger (this is the reason, why this depth is missing from table 1 for that date). Permanently waterlogged conditions might lead to much lower C turnover and the model-correction of radioactive decay with $\Delta^{14}C$ from the reference soil might not be valid. This means that the modelled $\delta^{13}C$ values are biased toward too positive values.

p. 14578, line 1-11: An additional potential reason for the increase with depth of CO2 fixation normalized to C in the reference soil, which could be included in the discussion, might be that the deeper parts of the profile are adapted to higher CO2 concentrations in the soil air.

This is a very good point, we now include it in the text.

minor editorial comments:

p. 14556, line 2: I think it is a bit too ambitious to claim quantifying the actual con- tribution of autotrophic microorganisms to SOM formation. I suggest to speak about "potential contribution".

Done. We changed the phrase according to the referees suggestion.

p. 14559, line 25 (and other places): Check for consistent use of either "form I Ru- bisCO" or "type I RubisCO"

OK, we checked and used the uniform terms Form I and II RubisCO

p. 14559, line 28: reword "cbbL 1A comprise obligate autotrophic bacteria"; this sounds odd to me.

Ok, we changed the sentence according to the referees suggestion

p. 14562, line 16: "unlabelled": up to now, no label was mentioned. Maybe: "To obtain background values for the isotopic compositions..."

This was rephrased according to the referee‘s suggestion

p. 14564, equation 3: Check if this complex equation is printed correctly.

We checked the equation and compared to other published versions. It is correct

p. 14564, line 20: Replace "Mofette" by "Soil"

Done

p. 14567, line 13 (and other places): "anoxic restrictions": Should that be "anoxic conditions"?

We changed the paragraph according to referee #1.

p. 14570, line 1 and 3: Not all of the values given here are consistent with Table 1.

The values were corrected, thank you for pointing this out.

p. 14572, line 19: I could not find these numbers in the corresponding table.

This is true, for clarity we included solely values that were obtained after fumigation. Values given in table 2 should illustrate the enrichment of the microbial biomass compared to the background values.

p. 14576, line 1: replace "alternation" by "alteration"

Done

p. 14577, top paragraph: I suggest to focus on the decay corrected data here.

We think it is better to show both, the ^{14}C decay corrected and uncorrected preditions of ^{13}C. The predictions for using ^{14}C data uncorrected for radio-decay serve to illustrate the overall depletion of ^{13}C-SOM values compared to vegetation values. The decay corrected data in turn show, that after calibrating the model, ^{13}C- depletion is still occurring where we measure the greatest abundance and activity of autotrophic microorganisms.

Table 3: At least in the printed version, this table is difficult to read because the columns are too small and therefore the averages and the standard deviations were printed in two lines. Maybe a different layout would help.

Done

General: The numbering of the Figures does not match their first occurrence in the text.

Apologies, this is now fixed.

List of relevant changes in the text:

(page numbers refer to pages in this document)

page 17, line 8

page 18, line 5-8

page 19, line 30 ff.

page 21, line 25 ff.

page 26, line 27 ff.

page 31, line 17 f.

page 32, line 21 ff.

page 33, line 5 ff.

page 34, line 10 ff.

page 35, line 31 ff.

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 ug gdw^{-1} d^{-1}. We inferred that the negative $\delta^{13}C$ shift was caused by the activity ~~of chemo-lithoautotrophic~~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 $\Delta^{14}C$~~radiocarbon~~ 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 organisms 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~~chemolithoautotrophic microorganisms~~. This was~~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~~the dominating forms in soils by qPCR~~ (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.~~We investigated genes of two subclasses of Form I (cbbL 1A and cbbL 1C), as well as cbbM, encoding for From II RubisCO.~~ cbbL 1A was identified mainly in ~~comprise~~ obligate autotrophic bacteria and cbbL 1C in facultative autotrophic bacteria (Tolli and King, 2005). cbbM encodes for autotrophic organisms living under anaerobic conditions~~restriction~~ (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

~~Soil and plant samples were acquired between November 2013 and September 2014.~~ For bulk $\delta^{13}C$ and radiocarbon analyses soil cores were taken from the central, unvegetated 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, v~~V~~egetation 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. ~~in April 2014.~~ Vegetation samples in the direct proximity of both mofettes were represented by *Eriophorum vaginatum*.~~At each~~ ~~Additionally to vegetation sampling in the direct vicinity of the mofette, v~~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 ~~along a CO_2 gradient, in order to test weather the~~how the isotope ~~signal~~signatures ($\delta^{13}C$ and $\Delta^{14}C$) of plants ~~follows a linear relationship with increasing~~changed with different mixtures of ambient

and geogenic CO_2. ~~concentrations and can therefore be used for a linear mixing model~~ sources.~~.~~ ~~Therefore, point, the dominant plant species was collected by clipping the plant at 2 cm height.~~ ~~v~~Vegetation was sampled at 2 meter intervals along a 20 m transect that transected ~~mofette 2.~~ Mofette 2 ~~represents~~is an exposed hummock, ~~without disturbance of smaller~~dominated by a un-vegetated central region of CO_2 exhalation ~~(WITH NO VEGETATION???)~~. ~~1~~One to ~~to~~ 2 meters ~~away~~distant from the central exhalation, the dominant plant species ~~changed~~was ~~to~~ *Deschampsia cespitosa*, ~~-~~and ~~subsequently~~at greater distances the dominant plant was ~~to~~ *Filipendula ulmaria*~~, most distant from the central mofette structure. At every sampling point, the dominant plant species was collected by clipping the plant at 2 cm height~~. 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 compositions~~s~~, one ~~Another unlabelled~~ set of subsamples was ~~prepared~~ 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):

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

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 ‰:

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

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).

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

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~~Mofette~~ 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 120°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:

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

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) ~~by~~ 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, obtained from Herrmann et al. (2015). PCR inhibitors were tested by ten times dilution series of representative samples. For the investigated samples 5 ul of DNA was taken as template for gene copy quantification of 16S rRNA, cbbL and cbbM. ~~The quantified functional marker genes are indicative for nitrifiers and sulphur-oxidizers (cbbL-1A), photosynthetic organisms (cbbl-1C) as well as chemo- and phototrophic organisms living solely under anoxic restrictions (cbbM) (Selesi et al., 2005).~~

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:

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

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 $\delta^{13}C$ ~~and $\Delta^{14}C$ values~~ 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 ‰.

~~Measured R~~radiocarbon concentrations of SOM ~~for SOM range between -550 ‰ and -800 ‰ ~~in both mofettes are generally more depleted by several hundred ‰ ~~than ~~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 ~~an ~~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 (~~f~~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 ($\delta^{13}C_{plant\ geo}$~~($\delta^{13}C_{plant\ geo}$~~, 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 ~~$\delta^{13}C/\Delta^{14}C$ relationship of plants~~ (figure 1). In reference soils, ~~but in general have relatively constant ^{13}C values, while~~ ^{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 23 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 ~~to bulk~~ $\delta^{13}C$ 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 32). 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~~2~~).

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

~~The investigated mofettes are characterized by low pH values, permanently anoxic conditions and TOC accumulation throughout the soil profile, in contrast to nearby reference soils, where C contents accumulate preferentially in the organic rich A horizon and pH values are higher. pH values in mofette soils are lower than organic acid buffers. Based on odour, H_2S oxidation might be responsible for observed low pH values. C/N ratios in both mofette soils indicate a change in SOM quality with depth.~~ Low C/N ratios, as found in the top 10 cm of both mofettes, reflect ~~biologically~~ microbially degraded~~highly processed~~ 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~~3~~ 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 23 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 23 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 23 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 ~~at lower depths~~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 ~~lowest depth of~~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~~Alewell et al., 2011~~; 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 ~~depleted compared~~lower than expected for SOM derived from ~~to a pure~~ plants ~~signal~~ alone, indicating significant ~~due to~~ addition of $\delta^{13}C$ depleted carbon. Below ~~, whereas below~~ 10 cm depth, the calculated and measured $\delta^{13}C$ values ~~coincide~~ 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 ~~coincides well~~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 ~~respective~~ corresponding isotope fractionation factor. Beulig et al. (~~in prep~~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 ~~prep~~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 ~~prep~~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 ~~chemo~~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 ~~chemo~~autotrophs or algae using Form~~type~~ I RubisCO, the dominant form~~type~~ 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~~s~~ 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 ~~in~~ 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~~2~~). ~~While w~~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~~is~~ are not directly influenced by geogenic CO_2. However~~,~~ the rate measurements~~s~~ suggest increasing importance of CO_2 assimilating microorganisms for carbon stocks with depth. ~~Also~~ 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 a higher substrate availability for RubisCO or other carboxylases with depth.

In contrast to the mofette soil, which is characterized as an organic rich histosol, ~~the~~ 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 ~~proofs~~indicates ~~and increasing ageing of~~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 ~~were~~ are influenced by incorporation of geogenic CO_2 fixed not only by plants, but also by ~~microbial CO_2~~

~~fixation~~microbes, as indicated by deviation of $\delta^{13}C$ values from those expected ~~from~~ if plant C inputs ~~alone~~were the sole source of SOM-C. The unique isotopic composition of geogenic CO_2 and the different enzymatic fractionation of plants and microorganisms allow~~ed~~ us to quantify microbially derived C ~~by~~ 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 represents background (i.e. without addition of label) data obtained from sampling in September 2014. Radiocarbon data was obtained in November 2013. Uncertainties ~~of~~in geochemical and $\delta^{13}C$ data represent ±1σ standard deviation (n=3). Uncertainties ~~of~~ 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 ~~CFE~~ [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~~s~~ 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

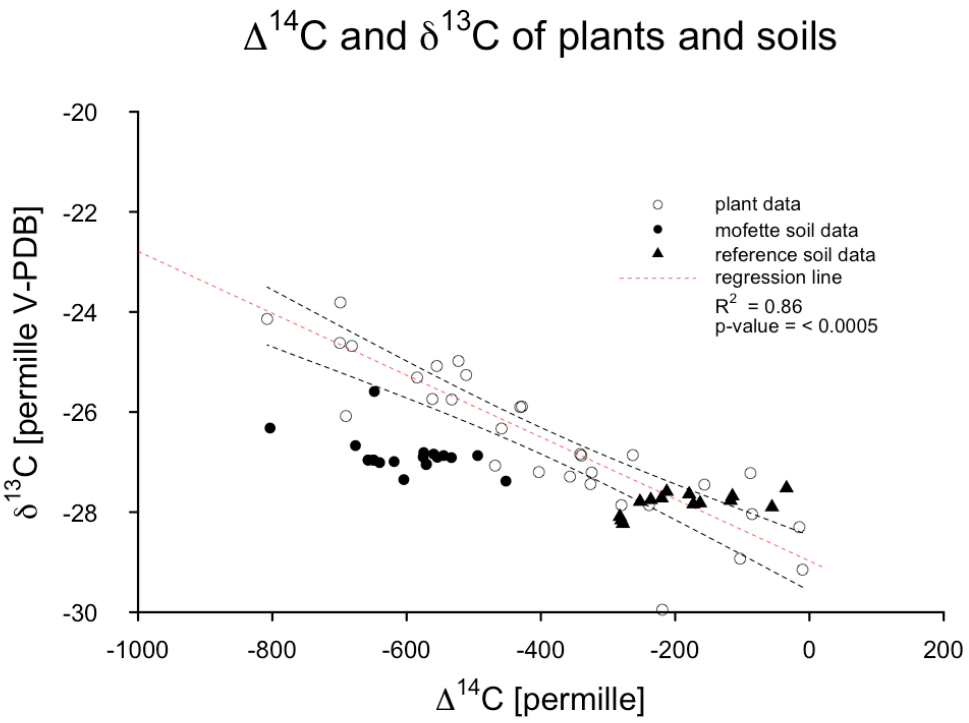
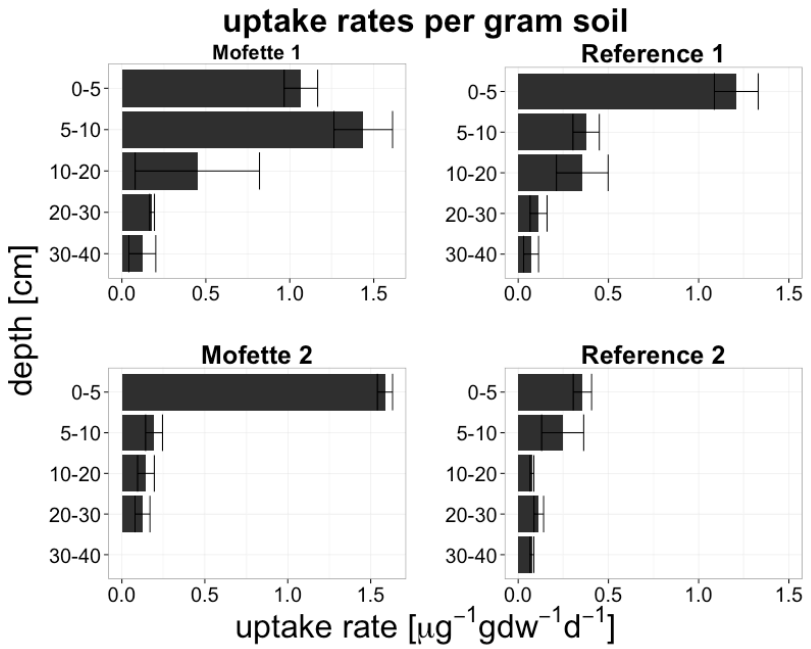


Figure 1. Correlation between $\delta^{13}C$ and $\Delta^{14}C$ of plants growing around the mofette structure. Dependent on the exposure to geogenic CO_2, plants incorporate different amounts of geogenic CO_2, which complicates isotope mass balance calculations for mofette SOM. However, both isotopes are highly correlated in sampled plant ~~vegetation~~ material, which allows ~~to predict~~prediction of $\delta^{13}C$ ~~comparing~~ SOM isotope values ~~with the aid of~~with~~from~~ plant $\Delta^{14}C$ ~~values~~. 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~~signal, although~~and ~~they~~ do not ~~show a~~ increase with depth, as is ~~usually~~often observed in soil depth profiles. Parameters of the regression ~~can be used for calculating~~ 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).



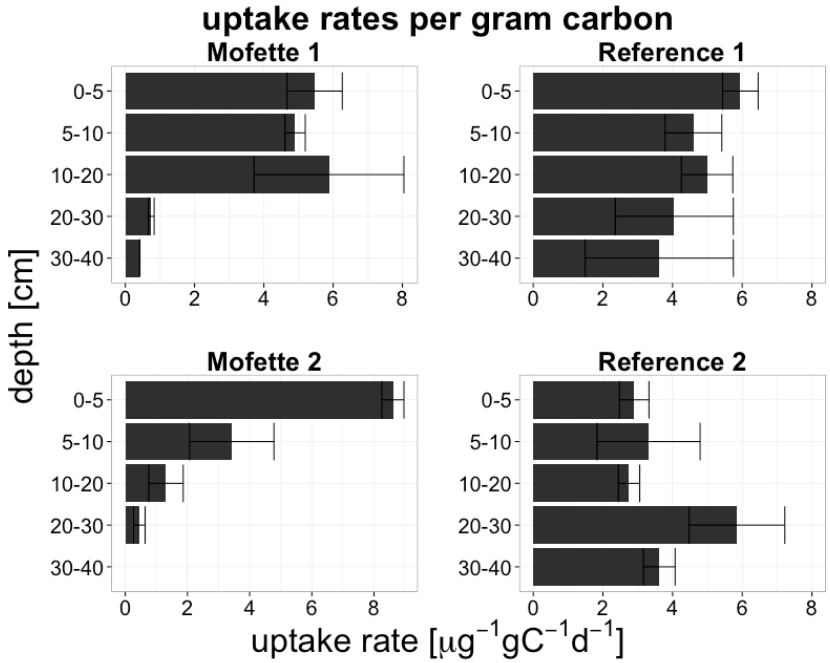


Figure ~~3~~2: CO_2 u~~U~~ptake rates along depth profiles of mofette and reference soils as determined by bulk measurements from experiment 2. In both mofettes, uptake rates are highest in the top 10 cm and show a trend towards decreasing values at lower depths, especially below 20 cm. Uptake rates in reference soils ~~are~~ also decreas~~e~~~~ing~~ with depth, but ~~stay fairly the same~~are nearly constant if normalized to organic carbon content. In contrast, ~~,~~ ~~which is not true for mofette soils (~~uptake rates per organic carbon decline with depth in the

mofette soils). This suggests an increasing importance of autotrophic organisms with soil depth in the reference soil.

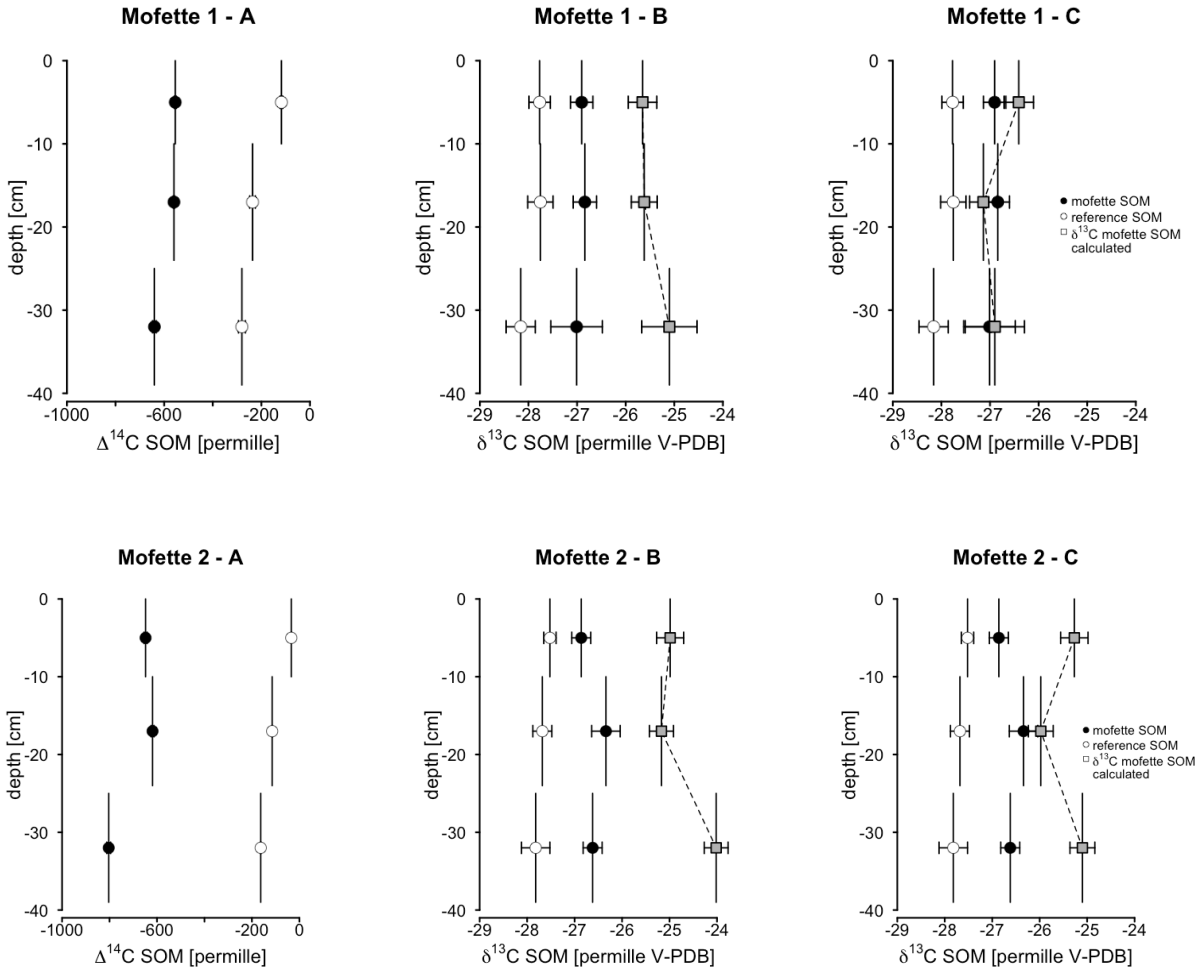


Figure 23: Depth profile of ^{14}C and ^{13}C signatures of SOM in mofette and reference soils.

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 ~~run~~analyzed.

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

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

way as the reference soil~~. Radiocarbon values of mofette SOM depends not only on incorporated geogenic CO_2 but also on depletion of ^{14}C by radioactive decay.~~ These estimated $\delta^{13}C$ values ~~, which were corrected for radioactive decay, correspond well~~agree with measured values below 20 cm depth~~s~~ but remain ~~still~~ depleted compared to ~~a~~ what is expected from a pure plant ~~signal~~ 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 ~~calculated~~ estimated and measured values below 20 cm depth in (B) can be explained by radioactive decay.

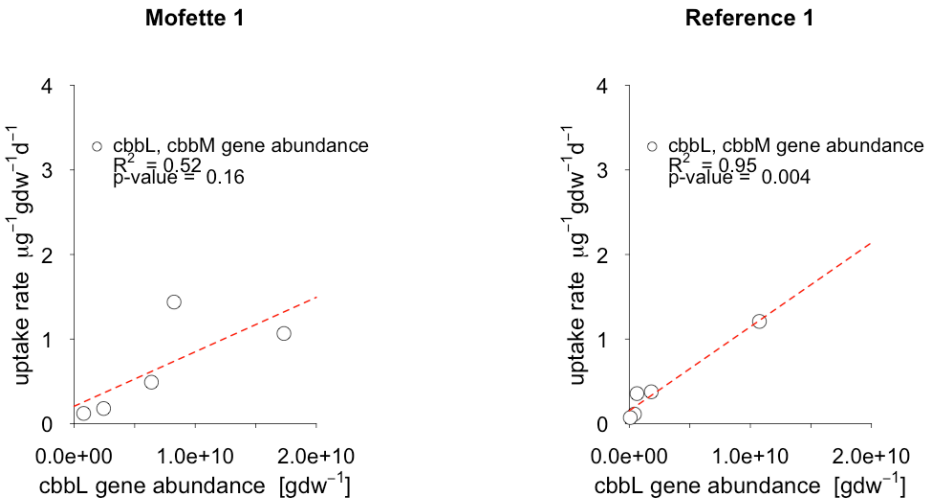


Figure 4: Correlation of marker genes encoding for RubisCO and measured uptake rates in mofette soil 1 and reference soil 1 in the soil depth profile from 0 to 40 cm depth. The good correlation in the reference soil indicates high contribution of chemolithoautotrophic microorganisms to measured uptake 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.