Biogeosciences Discuss., 12, C6793–C6799, 2015
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Biogeosciences
Discussions
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Interactive comment on "Autotrophic fixation of geogenic CO_2 by microorganisms contributes to soil organic matter formation and alters isotope signatures in a wetland mofette" *by* M. E. Nowak et al.

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Received and published: 20 October 2015

Dear referee,

Thank you for handling our manuscript. I would like to send you answers to your comments. The comments were constructive and helpful and I hope that we could improve our manuscript where it was necessary as well as clarify and dispel reservations, where it was possible.

Comment 1: We agree that the term chemolithoautotrophic microorganisms might be

a misleading term and do not represent the organisms that we wanted to target with qPCR analyses. Our target was to get information about the potential of CO2 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 δ13C value of the microbial end-member in the isotope mass balance. This 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 reefer to organisms using the CBB cycle for CO2 fixation and I agree that also other microbes than chemolithoautotrophic organisms use CBB Cycle. In turn not all chemolithoautotrophes use the CBB Cycle, although it is the most common metabolic cycle. Therefore the term is not correct in this context and I changed it to "autotrophic microorganism using the CBB cycle". The data for the qPCR was taken from the same soil than for the labelling experiments (mofette soil 1), although they were sampled at different time points.

Comment 2: Ok

Comment 3: Ok

Comment 4: We added a sentence, which clarifies that we try to target the question if CO2 fixation influences carbon isotope signatures and that we try to use a quantitative approach by means of natural abundance carbon isotope values, which was not done in other studies dealing with CO2 fixation in soils.

Comment5: Ok

Comment 6/7: We tried to improve the section according to the referees′ suggestion. cbbL encode for Form I RubisCO and we choose Form I genes encoding for subclasses 1A and 1C, because they can give us information weather CO2 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 CO2 fixing microorganisms.

Comment 8: Mofette 2 is approximately 500 m distant from mofette 1. The geochemical properties are very different, because both mofettes differ in size. This is also mentioned in the text (page 5, line 25). Mofette 1 is considerably smaller than mofette 2. Thus, plant litter input from the top is higher in mofette 1 compared to mofette 2, because plant growing at the rim of the structure can fall easier on the uncovered part in the centre of the mofette structure.

Comment 9 and 10: Dates from all three sampling campaigns were clarified. The sampling strategy was clarified. We took vegetation samples in the direct vicinity (rim) of both mofette structure, which was represented by Eriophorum vaginatum. Additionally we took samples from a transect from mofette 2, because mofette 2 represents an undisturbed hummock structure without disturbance of secondary exhalation structures, as it was the case for mofette 1. Here the vegetation changed with increasing distance from the central CO2 exhalation. This was clarified in the text. The goal of the transect sampling was to clarify, if δ13C and Δ14C values follow a linear trend with increasing CO2 concentrations. This was necessary to make predictions for mofette SOM, because mofette SOM is derived from plants which are exposed to fluctuating CO2 concentrations. In order to make predictions for mofette SOM from vegetation δ13C and Δ14C values, it is therefore necessary to test, weather plants follow a linear trend in δ13C and Δ14C values within a CO2 gradient, as well as the if the relationship between δ13C and Δ14C of plants is linear with increasing CO2 concentrations, or if fractionation of plants is influenced by elevated CO2 concentrations. The good correlation of δ13C and Δ14C, even with increasing CO2 concentrations proved that our used model is valid.

Comment 10: Table 1 shows the geochemical data from the sampling campaign conducted in September 2014, where we took samples for the second labelling experiment. The data given in table 1 represents the unlabelled T0 geochemical data from soils sampled for the second labelling experiment. We choose to use these data, to present δ13C isotope values with a higher spatial resolution, which gives a more de-

tailed insight into small scale changes 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 Δ14C values from the first sampling campaign into table 1, which has a broader depth resolution.

Comment 12: All stable isotope analyses were conducted in triplicates. For sampling campaign 1, we used a mixed homogenised sample that was comprised from three cores. This mixed 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 does represent the analytical uncertainty (see also figure caption 3A).

Comment 13: We removed the Oh layer because we wanted avoid CO2 uptake of phototrophic organisms like algae.

Comment 14: The given temperature for experiment 1 is a mistake in the text. Both experiments were conducted at 12°C. It is corrected in the text. The incubations were conducted in a dark incubation chamber, set at permanently at 12° C.

Comment 15: DNA extraction was performed in triple.

Comment 16: The exact protocol for amplification reactions and the used program was inserted in the text (page 11, line 22-30).

Comment 17: This is correct. We did not performed a t-test on radiocarbon data, because the error in Δ14C values represent only the analytical precision. This was corrected in the text.

Comment 18: The differences in C content, pH and C/N ratio are caused by permanently anoxic conditions in moffete soil compared to the reference soil and addition of plant material as well a microbial carbon, partly derived from autotrophic organisms. The reasons for the observed differences are also discussed in detail in section 4.1.

Comment 19: The radiocarbon and δ13C values of sampled CO2 is given in page 13,

line 27.

Comment 20: Ok

Comment 21: CFE means values obtained from the first experiment, where uptake rates were determined by CFE extractions and bulk refers to experiment 2, where 13CO2 incorporation was determined directly into bulk organic carbon, without extracting microbial biomass.

Comment 22: The high number of 16s RNA and cbbL 1C and 1A genes corresponds with low C/N ratios and high CO2 uptake rates. Thus, together with the geochemical data as well as activity measurements, the high number of 16S rRNA genes as well as genes encoding for RubisCO reflect a high number of microorganisms in these soils, which finally leads to a high contribution of microorganisms to SOM.

Comment 23: Processed OM refers to partly degraded OM. This was clarified in the text.

Comment 24: We know from the study from Beulig et al. (2014), that also 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., submitted). 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 endmember 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 setting the microbial isotope end-member in the isotope mass balance.

Comment 25: Ok

Comment 26: Yes, the axis title is wrong. The data points represent total number of cbbL and cbbM genes.

Comment 27: Ok it was changed in the text to “autotrophic organisms using the Calvin

Benson Cycle”.

Comment 28: Type I RubisCO is the dominant type, because it is most abundant in the mofette soil.

Interactive comment on Biogeosciences Discuss., 12, 14555, 2015.

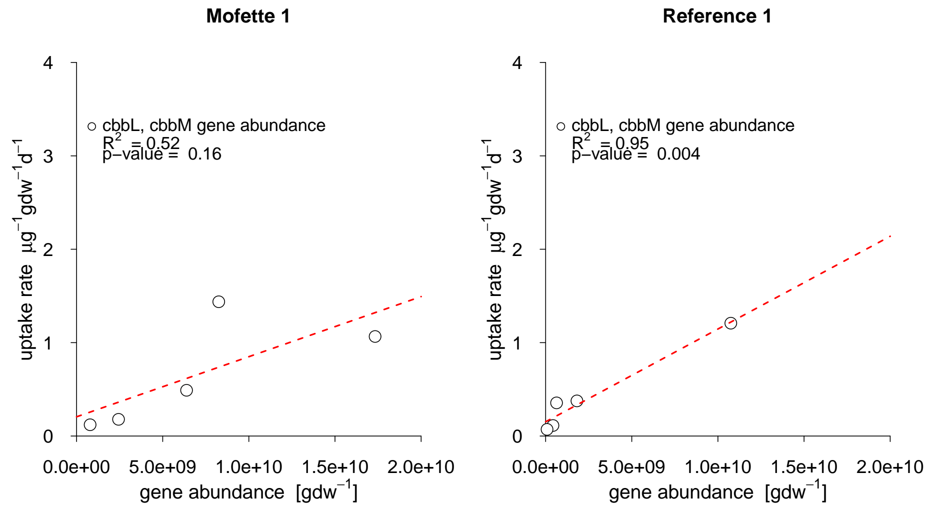

Fig. 1. Figure 4