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Interactive comment on “Autotrophic fixation of geogenic CO₂ by microorganisms contributes to soil organic matter formation and alters isotope signatures in a wetland mofette” by M. E. Nowak et al.

Anonymous Referee #1

Received and published: 28 September 2015

This manuscript used the differences in ¹³C and ¹⁴C discrimination of the geogenic CO₂ emitted at mofette soils and atmospheric CO₂ in reference soils to distinguish C fixed by plants from C fixed by autotrophic microorganisms. The results show that CO₂ 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. 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 $\delta^{13}\text{C}$ shift was caused

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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. Furthermore, I think it is inappropriate to classify acetogenic microorganisms, methanogenic microorganisms and chemo-lithoautotrophic microorganisms into different catalogs.

2. Page2 Line 13. Change “organisms” to “microorganisms”.
3. Page2 Line 14. Consider adding “carbon” before “observed reservoir effects” here.
4. Page3 Line 2-4. Many published papers concerned this issue. Specify the differences of your study from these published researches.
5. Page3 Line 30. “indicates”
6. Page4 Line 23-25. Do the cbbL genes encode Form I RbusiCO 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?
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?
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?
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

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sectioned into 0-5cm, 5-10cm, 10-20cm, 20-30cm and 30-40cm.

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

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

14. Page9 Line 4. The temperature may affect microbial activity. The incubation temperature was 10°C in the first labelling experiment and it was set to 12°C in the second labelling experiment. How do you set the temperature?

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

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

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).

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?

19. Page13 Line4-5. What about the radiocarbon and stable isotope concentrations of CO₂ sampled at different depth (5cm, 15cm, 25cm, 40 cm)?

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

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

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 $1.40E+08 \pm 1.69E+08$. These results did throw my doubt on their quantitative PCR.

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

BGD

12, C5812–C5815, 2015

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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.

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

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

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?

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