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Biogeosciences Discussions

Interactive comment on "An approach to the investigation of CO_2 uptake by soil microorganisms" *by* K. M. Hart et al.

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We thank and welcome the comments from referee 2 in which the significance of the work was highlighted, although as acknowledged, soil autotrophic processes are already well known. We agree that the main significance of the work was the emphasis on CO2 quantification. We feel that currently in the literature there is a significant paucity in real-time data that makes any attempt at quantifying CO2 uptake by soil microorganisms. In this initial study we have attempted to break the ground work by presenting a method that could be applied to significant soil processes. To address the unavoidable leak and for demonstration purposes, 10 blank replicates were used to demonstrate its linearity and reproducibility (when no soil was present). It should have been emphasized in the manuscript that there is no precedence in the literature

for the quantification of CO2 uptake by soil chemoautotrophs. The flaws pointed out by referee 2 were in most cases unavoidable as we aim to discuss herein.

1) The leak as you mention was considerable, but only because of the high partial pressures involved. From the data in Table 1, it can be clearly observed that as the CO2 concentration decreases so does the leakage rate. Experiments involving CO2 at more normalized concentrations (450 – 600ppmv) would not be as subject to such extreme de-gassing as those observed in this particular work. This was not discussed in the paper (which is regrettable in hindsight) because we wanted to emphasize the experimental nature of the method. We are currently developing a mathematical model where we hope to accurately describe the leak at any time and concentration and therefore provide more precise quantification values.

That the CO2-blank data plot in Fig. 5 appears to be similar to the experimental soils after 12-15 h is indicative of the high de-gassing rate at 1000 ppmv CO2 and merely reflects the leak with subsequent re-injection of CO2 periodically. The adjacent experimental plots are clearly more dynamic and abrupt, but this is a matter of visual interpretation and we preferred to rely on the leak corrected calculations.

The issue in regards to the increases in CO2 after injection being so different is simply due to the sensitivity of the detector and the slight variations in gas volume injected by the onboard peristaltic pump. Each injection was time dependent (seconds) and therefore dynamic, leading to different volumes being introduced each time. Coupled with leakage and soil respiration the patterns for each soil replicate are different. The 10x replicate blanks showed the consistency of the chamber de-gassing and therefore we attribute the CO2 variances to gas fluctuations from the sample as it is subjected to partial pressure dependent leakage, soil respiration and a non-uniform gas introduction system.

2) The plant and algae incubations were carried out to act as a comparative control to assess the rate of sequestered CO2 (of a well understood system such as photosyn-

thesis) to the chemoautotrophic soil samples under investigation. No measurements of bacterial autotrophy were made during these photosynthesis incubations as we were simply concerned with the amount of CO2 removed by the photosynthesizers. We do however agree with referee 2, that soil microbial processes may bias certain results due to increased activity. Although (as referee 2 points out) it is more likely that facultative autotrophs would consume plant exudates at the expense of chemoautotrophy. This was not what we intended to show or claim to be apparent. Due to the potential for confusion we would be happy to remove the grass and algae data as it may detract, rather than enhance the work presented.

3) We agree and accept that the materials and methods section could be shortened. Although it would be difficult to decrease the experimental methods in regards to the GCMS, a reduction of the initial section describing the samples could easily be undertaken using a table. The description of the chamber and the incubation method could be reduced and we shall endeavor to provide a more concise explanation.

4) The reduction in tables and figures is welcome and will be executed. The inclusion of these figures was simply performed to provide as much transparency as possible while considering the online nature of the journal which allows for sufficient space. The comment on Table 3 in regards to the blank incubation was most pertinent and will also be addressed by clearly stating the sample conditions and providing a more obvious identification tag. The blank described here was of a soil exposed to 13CO2 over the prescribed period but crucially no chemical electron donor was applied to the soil. This triplicate experiment was used to demonstrate that the electron donor was the sole variable required to provide isotopic labeling after a short incubation time. We have failed to express to the reader the differences between samples by applying clear sample identification and therefore apologize for any confusion.

Statistical analyses are valuable additions to most data sets but these were not pursued to any degree for Table 4. The data was intended to show the abundance of labelled FAMEs after 13CO2 exposure. As the experiment was not intended to mimic

in situ activity it was considered prudent not to much emphasis this data to avoid overstatements about soil microbial processes. Figures 3 and 4 will be combined and the removal of Figures and tables is taken under advisement.

5) The methods section will be revised and all the samples shall be assigned a defined identification tag to ensure easy reference for the reader. We will particularly emphasis the difference betweens blank and control incubations.

The results section shall be condensed and speculative comments will be minimised. We welcome the suggestion by referee 2 that a possible applicability of the study would be to observe the incorporation of rhizodeposit organic carbon after plant mediated CO2 sequestration. The potential of the chamber is immense including the study of other chemoautotrophic groups such as the hydrogen oxidizers. These exciting projects could all be explored in the discussion with potential for demonstration once the above (and below) teething problems are eliminated.

6) The issue of statistical analyses shall be addressed in a revised version of the manuscript and the repetition of the 'other' soils may be required to provide more robust data. As referee 2 points out, uptake of CO2 was obvious without the need for extensive statistical analysis but nevertheless we now feel it must be added to the document to greatly strengthen the results (including the FA quantification).

7) The excellent suggestion of providing some figures describing sulphur-based fertilizer loading to soils could be readily incorporated into the manuscript as it demonstrates the applicability of the method for future experiments. Although, this suggestion was not considered by the authors previously, we have performed experiments where elemental sulphur was applied to agricultural soils (standard practice for acidification of land or as a sulphur nutrient fertilizer) and observed the δ13C enrichment of PLFAs, this work is currently under preparation.

8) The use of soil slurries was performed for these initial incubations to maximize the chance of detectable CO2 acquisition. This was important as we needed to deliver

nutrients, substrate and electron donor in a soluble form. We accept that the slurry method may possibly alter the microbial community structure but no less/more than the act of air drying and sieving (Petersen & Klug, 1994), a common practice in soil microbial studies. The use of a slurry, we feel, was justified in this instance as the experiment was intended to demonstrate that the procedure could be successful in isotopically labeling organisms involved in specific processes while also quantifying their impact on CO2 flux. We hope to extend the method in the future to better mimic in situ environments and thus better contribute to scientific knowledge.

9) Unidentified 'FAME' peaks were not disclosed in the manuscript as they were identified as being common plastizers from laboratory consumables during sample extraction, derivatisation etc. Phthalates are common contaminants in GCMS experiments being leached from plastics, especially when in contact with organic solvents and even aqueous solutions (Grosjean & Logan, 2007. Org. Geochem. 38:853-869; McDonald et al., 2008. Science 322:917).

The monounsaturated fatty acid C18:1ω9 has been demonstrated to be an indicator of gram-positive bacteria by Zelles (1999), Ruess & Chamberlain (2010), Zhilina et al. (2009)* amongst others. Although, this fatty acid is highly common and we fully acknowledge it is abundant in gram-negative species also. Therefore we intend to remove the comment from page 9258, line 26 indicating the association with gram-positives to avoid further confusion.

*Zelles 1999. Biol. Fert. Soil 29:111-129; Ruess & Chamberlain 2010. Soil Biol. Biochem. 42:1898-1910; Zhilina et al., 2009. Microbiol. 78(4):445-454.

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