

## ***Interactive comment on “An approach to the investigation of CO<sub>2</sub> uptake by soil microorganisms” by K. M. Hart et al.***

### **Anonymous Referee #2**

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This paper describes a study in which the autotrophic CO<sub>2</sub> uptake by soil microorganisms was investigated and quantified through the use of a CO<sub>2</sub> and temperature controlled incubation chamber. The study demonstrated the incorporation of CO<sub>2</sub> into soil in the absence of plants or light, and under favorable conditions for chemoautotrophy, and provided proof of microbial involvement in this C uptake by employing a <sup>13</sup>C tracer and assessing its incorporation into microbial cell membrane lipids. The study confirms what has been observed already by others (e.g. Miltner et al. 2004) in terms of providing evidence of microbial uptake of CO<sub>2</sub> by autotrophic processes. What is rather unique in this study is the quantification of the contribution to C inputs by autotrophic processes, at least under most favorable and controlled conditions. While the topic falls within the scope of Biogeosciences and the information provided may be of interest to its scientific audience, the paper suffers from a number of flaws.

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1) The chamber failed to maintain a constant CO<sub>2</sub> concentration due to a serious leak. Although the authors were able to account for this leak in their calculations of CO<sub>2</sub> uptake, the impact of this leak on the outcomes of this study cannot be overlooked (a decline of 500 ppmv over a 52h time period is a 50% loss of the targeted CO<sub>2</sub> concentration in the chamber . . .). This is of particular concern since the main objective of this paper, which distinguishes itself from other papers that have investigated this process, was to quantify CO<sub>2</sub> uptake by soil autotrophic processes. Also, looking at Fig. 5, the CO<sub>2</sub> loss from the blank incubation appears rather similar in rate and amount when compared to some of the soil incubations (A, B and C), once past the first 12-15 h. In this figure, it is also not clear to me why the increase in CO<sub>2</sub> upon injection (when concentrations dropped below 950 ppmv) was so different across the 3 replicate soil incubations and across the different time points. The authors attribute these different trends across the triplicate soil samples to differences in heterotrophic activity and impacts of physical pre-treatment (pg. 9251-9252), although it looks to me more like an issue with the operation of the chamber and a non-consistent leak for the different incubations.

2) The authors assume autotrophic CO<sub>2</sub> uptake to be similar for bare soil (what they call basal respiration) and planted or algae covered soil, and used the basal respiration data to assess the uptake by plants and algae. I am not convinced that this assumption is correct and would argue that in the presence of plants, autotrophic uptake may be reduced, as facultative heterotrophic microbes may switch to heterotrophic processes.

3) The materials and methods section is far too long and contains too much detail and repetition. For example, the section describing the different soils (section 2.1) could be greatly shortened by just mentioning the type of analyses performed on all soils and then including all data in a table (classification, texture, %C and %N, etc.).

4) There are a lot of figures and tables, and several that could be taken out or combined into a smaller set of figures and tables. Table 1 demonstrates the leak in the chamber. To my opinion, this really affects the credibility of the study. . . Table 2 could

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be described in the method section in the text. Table 3 is OK, but not clear what the blank represents (I assume initial soil before incubation?). Table 4 should also include statistical analysis to help understand the trends in the amounts of the different fatty acids over time. Figure 2 can be removed. Figure 3 and 4 can be combined in one graph (two lines). Figure 10 can be removed.

5) The methods description is hard to follow and needs to be improved. It is not always clear which soils were used in which incubations, what analyses were replicated (i.e. on the same soil), what was considered a blank or control (terms are used without good description; e.g. blank is sometimes used for empty chamber or for non- $^{13}\text{C}$  labeled soil incubations. . .). There is also a lot of repetition which makes this section rather long. The Results section also needs improvement in terms of its structure. It would make more sense to report the observations in a Results section and then synthesize in a concise discussion, focusing on the most important findings, and placed in a bigger context in which the relevance of this work and its finding becomes clear. An additional and important implication of these findings, which would be worth addressing in this paper, to my opinion, is related to study of rhizodeposit C processing by the soil microbial community. This is often done by  $^{13}\text{CO}_2$  pulse-labeling of plants and following this tracer into the microbial biomass, without acknowledging the potential contribution of inorganic C uptake to the  $^{13}\text{C}$  enrichment of the microbial biomass constituents.

6) No statistical analyses were performed, mostly due to a lack of replication of incubations on the same soil. The only replication that was done was on the 'Abbeyside soil' for the  $\text{CO}_2$  monitoring and fatty acid analysis (quantification and  $^{13}\text{C}$  enrichment), but no statistical analysis was performed beyond reporting the standard deviation. From the figures (Fig. 5), it can be concluded that there was a clear  $\text{CO}_2$  uptake happening (though I'm still unsure of how much of this  $\text{CO}_2$  loss is due to soil uptake and not due to the leak) without much statistical analysis needed. But for the other soils, replication would have been good to include. Also for the interpretation of the change in fatty acid

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concentrations over time (pg. 9258, ln. 6), statistical analyses is required.

7) The addition of the electron donor was done to provide favorable conditions for chemoautotrophic processes. To know if this is something that is realistic or not for field applications, it would be good if some figures were provided of common sulfur-based fertilizer addition rates in agricultural systems.

8) Can the authors explain the reasoning behind their choice of working with slurries? Was this done to reduce the infiltration of CO<sub>2</sub> into the soil pore space, which could be otherwise wrongly interpreted as CO<sub>2</sub> uptake? I'm just curious, as this also may cause changes in the microbial functioning.

9) I am confused about the several unidentified FAME peaks following the C18:0. Since a GC-MS was used for peak identification, I would expect to see a FAME ID associated with these peaks. The authors mention on pg. 9258, ln. 23 that there were no fatty acids > C20, though it was not indicated what the chain length was of those unidentified peaks in Fig. 10 – this information should be easily obtained from the mass spec outputs. Also, on pg. 9258, ln. 26, the authors mention that C18:1w9 belongs both to fungi and gram-positive bacteria. The latter should be gram-negative bacteria (mono-unsaturated PLFAs are characteristic of gram-negative bacteria).

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