

Interactive comment on “An approach to the investigation of CO₂ uptake by soil microorganisms” by K. M. Hart et al.

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In response to the criticism made by anonymous referee 1, we would like to thank you for the time you have taken to consider our manuscript. We begin by reiterating that the main emphasis of the manuscript is to demonstrate that a modified environmental chamber can be used to produce labelled biomaterial through incubation and provide real-time data to determine and quantify CO₂ flux, not to provide new insights of chemoautotrophy in soils in situ. To the best of our knowledge, the quantification of CO₂ uptake by soil chemoautotrophs has not been reported and this paper outlines the considerations, challenges and successes involved in such an approach. We will address the issues raised by referee 1 individually: Chamber leak: The environmental chamber was custom built to be air tight so as to avoid, as much as possible, CO₂

leakage. However, a small leakage of CO₂ is unavoidable, given the difficulty of accurately measuring atmospheric gases within enclosed spaces due to partial pressure effects. Therefore, the challenge is to be able to measure this leak and account for it in our CO₂ uptake calculations. Leakage rates were highly reproducible (a total of ten replicates were used) and the correction values generated (Table 1) acted as accurate correction rates. The rates generated in Table 1 clearly show the decreasing rate of de-gassing as the internal CO₂ concentrations fluctuate and hence, can easily be applied to detectable atmospheric fluxes. Variation in soil replicates: The variations observed for the three soil replicates are due to biological system heterogeneity that is not made homogeneous by sample preparation. Complex biological systems such as soil are unpredictable and the lag phase requirements of bacteria differ depending on many variables too numerous to predict for a large mixed culture as the one used here. The important outcome is that in three replicate soil slurries and allowing for a quantifiable gas leak we could quantify and monitor CO₂ uptake that did not occur in the blank experiments. In all cases GC-IRMS analysis confirmed that the labeled ¹³CO₂ had been incorporated into the biomass. The importance of the quantification was to show that positive results were obtained allowing for more complex methods of quantification to be developed in the near future.

20 mM thiosulfate The use of high concentrations of S₂O₃²⁻ can easily be justified as the study was a methodological approach to produce, quantify and detect isotopic labeling in a soil microbiological community within a climate controlled environment. We fully accept that the current data is not field relevant and this has already pointed out in the conclusions (Page 9259, lines 8-9). Further, it was not claimed anywhere in the text that chemoautotrophic microbes were important in soil CO₂ sequestration as the referee states (Interactive comment; Page C4207), but rather, the 'potential' to have an impact on CO₂ sequestration (page 9236, Lines 13-14) when one considers the volume of anthropogenic sulphur applied to land surfaces annually.

Fatty acid identification: Yes, the FA identifications shown in Fig. 10 have been iden-

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tified in error due to a mistake in assigning peaks from the GC chromatogram (in a revised version Fig. 10 will be removed). This silly oversight made by the authors is a regrettable mistake and has led to a full revision of the GCMS-IRMS data being carried out to ensure all the assignments and quantifications are correct. However, it must be pointed out that the GCMS-IRMS section was used primarily to show ^{13}C enrichment of biological material to support the hypothesis that CO_2 was sequestered into the soil via biological mediators and therefore the delta values were sufficient to show this. We do however agree that these errors present a limitation to the conclusions that can be drawn from the data and therefore will revise this area.

General comments made by Referee 1 on manuscript length and quality: The manuscript will be shortened largely in line with the comments of Referee 2. Referee 1 expresses serious concern with the overall quality of the manuscript. These concerns have been addressed above and argue that this is a sound approach to the study and quantification of CO_2 uptake by soil microbes that can be improved on in time. We are very happy to try to make the revised manuscript more readable but disagree that it was poorly written and find this comment very unhelpful. Previous peer reviewers have of course highlighted areas to improve on but none offered such a sweeping indictment and were generally positive.

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