

## ***Interactive comment on “Long-term steady state <sup>13</sup>C labelling to investigate carbon turnover in plant soil systems” by K. Klumpp et al.***

### **Anonymous Referee #2**

Received and published: 23 March 2007

This paper describes an experimental system in which carbon dioxide depleted in <sup>13</sup>C is supplied at near ambient concentrations, to grassland turf and soil monoliths under ambient light and temperature conditions for two years. The experimental system is employed to study plant and soil carbon pools in relation to frequency of grassland cutting.

The experimental manipulations are shown to alter the <sup>13</sup>C enrichment of the air, and vegetation, and to allow the carbon turnover and residence time in soil organic matter pools to be estimated.

Whilst the paper makes a significant contribution both in terms of method development and addition of new knowledge, the presentation, organisation, rationale and impor-

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tance of the findings could be made much clearer, and the English requires considerable minor editorial improvements.

The abstract presents a lot of detail regarding the methods (some of which appears excessive for an abstract), but does not provide a clear statement of the aims of the research, and does not explain the relevance or importance of the findings. There is a disjunction in emphasis- on the one hand the initial focus of the abstract is largely on the method development, but the actual findings from using the  $^{13}\text{C}$  labelling system are not clearly contextualised and their relevance or significance is not indicated- indeed the results are presented in the abstract almost as incidental findings. What is the significance of the delta  $^{13}\text{C}$  values reported in the abstract? Why is the fraction of soil organic matter greater than 0.2 mm important? Why will the reader be interested in the full technical details of the air flow and  $^{13}\text{C}$  enrichment in the experimental chambers at this stage?

Overall the abstract lacks a clear focus and the significance and importance of the findings are not clearly explained.

The findings appear limited to confirmation (1) that by supplying air depleted in  $^{13}\text{C}$  results in plant tissues with reduced  $^{13}\text{C}$  enrichment, and (2) reducing 'disturbance by cutting' (this is not defined in the abstract) results in increased residence time of detrital carbon.

The weaknesses in focus are to a large extent reflected in the main body of the paper. The introduction does not set out any explicit hypotheses that were to be tested. The aims are not very clearly articulated or justified.

There are some factual errors or parts that need rephrasing. On page 4 lines 4-7 it is stated that the supply of  $^{13}\text{C}$  depleted air can not be used to study C cycling under ambient  $\text{CO}_2$ . This is unclear and incorrect. If the  $\text{CO}_2$  concentration is maintained at ambient concentrations, but is supplied as  $^{13}\text{C}$  depleted  $\text{CO}_2$  (in a similar type of manipulation to that achieved in the present experiment) then this is perfectly possible.

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Similarly, line 10 on the same page, the statement about pulse labelling studies needs to be more precise- this refers only to  $^{13}\text{C}$  pulse labelling, as  $^{14}\text{C}$  pulse-labelling does permit long-term carbon residence-time studies.

Page 5 lines 1-4. Please note that several of the components of Fig. 1 do not have correct labels- the labels in the figure do not all match those in the legend. The section of text at the top of page 5 is poorly written. The delta  $-34.7$   $^{13}\text{C}$  depletion value needs to be contextualised- how does this compare to the ambient air  $^{13}\text{C}$  signature?

Page 6 line 12-13. Explain and justify the concentration of  $\text{CO}_2$  used. Is 425 micromoles of  $\text{CO}_2$  significantly higher than ambient concentrations? Why was this concentration chosen?

Lines 16-18. I am very unsure about the rationale for pumping air through the soil. This will inevitably greatly increase the  $\text{O}_2$  and decrease the  $\text{CO}_2$  concentration in the soil and is likely to cause major alteration to the normally slow diffusive pathways of air movement through soil which result in vertically structured variations in soil air composition (and associated microorganisms). See for example: Sheppard, S.K. Lloyd, D. 2002. Direct mass spectrometric measurement of gases in soil monoliths. *Journal of Microbiological Methods* 50, 175-188, which shows that in a well-drained grassland soil carbon dioxide enrichment, and oxygen depletion occur with increasing depth into the soil and at 6-8 cm depth methane production occurs as a result of the low oxygen supply rate. Pumping relatively oxygen-rich air through soil is likely to enhance microbial oxidation of soil organic matter, affect the methane producing microorganisms and may significantly alter rates of carbon turnover compared to soil without this artificial air flow. Whilst this effect should not bias the effects of above-ground cutting on below-ground carbon dynamics, it does raise the question as to whether the reported rates of carbon turnover in both treatments might be substantially altered compared to the situation in the field- and whether this represents a significant limitation in the experimental design.

Page 8 line 5 'Artificial urine was applied' specify the volume and concentration and

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chemical composition.

Page 8 line 19. How much leaf tissue was sampled on each occasion? How was it ensured that these samples were representative? How exactly were they sampled? Previous studies have clearly demonstrated that small samples comprising leaf-tips are often not representative of the  $^{13}\text{C}$  signatures of the whole plant- older tissues have different signatures to new tissues. Small samples, particularly of mixed-species grass turfs present particular sampling problems.

Line 25- How much tissue was sampled? How was it ensured that this was representative?

Page 9 lines 3-15. There is insufficient explanation or justification of the fractionations that were carried out. What was the aim of the size fractionation? Why were these particular fractions selected? Are there issues about possible loss of water-soluble organic matter from some of these fractions resulting from wet-sieving?

Page 10 lines 24-25. More details are needed of the surface on which the enclosures were placed. Were these surfaces gas-impermeable? How many replicates were studied?

Page 11 line 7. 'not causing leaks' this is poorly expressed.

Lines 8-12 The seasonal cycle in atmospheric  $\text{CO}_2$  concentration is a global phenomenon, and is not exclusively an 'urban' issue, although the effect may be greatest in urban areas.

Lines 19-20. this is unclear. Is 'indoors air' actually chamber air.

Page 13 Lines 21-22. If you have measured the difference between root and shoot  $^{13}\text{C}$  signatures in previous reports from this study please make this clear- at present it appears that the differences in enrichment are assumed from work done by others in other situations that may not be directly applicable here.

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Page 14 line 7-9. Mycorrhizas may have a major role in some of the root traits and carbon residence time. These associations have been entirely overlooked in the present study. Page 16 lines 2-8. The role of mycorrhizas and soil microorganisms supported by root exudates needs also to be considered: see- Rangel-Castro , J.I., Prosser, J.I., Ostle, N., Scrimgeour, C.M., Killham, K., Meharg, A.A. 2005a. Flux and turnover of fixed carbon in soil microbial biomass of limed and unlimed plots of an upland grassland ecosystem. *Environmental Microbiology* 7, 544-552, and related papers.

Line 14 and 15. The work presented in this paper is unable to provide information on the chronology of new carbon inputs. It is most likely that rhizodeposition actually occurs before carbon is released from root and rhizome turnover- mycorrhizal fungal mycelia show peak flux of labelled C in pulse-labelling experiments in advance of labelled C peaking in the roots, and C release from roots to microorganisms occurs more rapidly than C release from root turnover (death and root grazing).

Line 18-22 The comments on soil organisms are valid but there is no evidence here to support the assertion that in this study soil fauna are playing a significant role in decomposition and organic matter transport deeper into the soil. I agree that it is likely, but the study here provides no data or evidence to support this. This needs to be rephrased to make clear the conjecture rather than proof.

Page 16 Lines 23- 25, through to Page 17 line 4. This appears largely speculative- I do not see the supporting evidence for several of these statements.

Page 17 The conclusion is focussed exclusively on the labelling system methodology and its possible potentials. The omission of any main conclusions or implications from the findings of the measurements of  $^{13}\text{C}$  enrichment of plant and soil samples raises the question as to whether these findings are of any interest. It re-emphasises the points I have made regarding the abstract. Is this a method paper or is it a paper reporting important findings that have important bearing on effects of management on plant-soil carbon fluxes and turnover? What are the main aims? What are the main

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findings? What is their significance?

Figure 1 correct the legend for English, and components labelled differently in the figure and legend. Figure 3 is unintelligible as the same symbols have been used for 2 different variables.

Overall, this paper presents an interesting method and some potentially interesting findings, but the presentation is poor and the impact of the work will be very limited unless the authors can effectively deal with the issues raised. The authors need to deal with the issues relating to the possible effects of pumping air through soil on the carbon fluxes and decomposition rates.

A much clearer rationale for the work needs to be explained and the methods, approaches and aims properly contextualised and justified. The abstract needs to have less emphasis on technical details of the methods and more emphasis on the reasons for the study and the importance and significance of the findings.

I have not copy-edited the English, but this needs attention throughout.

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Interactive comment on Biogeosciences Discuss., 4, 797, 2007.

**BGD**

4, S172–S177, 2007

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