

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

K. Klumpp et al.

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1. 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 ¹³C 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.

-Abstract was rewritten with clear aims, a testable hypothesis and the importance of findings and their relevance. Details on methods have been deleted. The focus of the abstract is now more balanced between method development and application to the study of carbon turnover in grassland soils as affected by disturbance. 2. What is the

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significance of the delta 13 C values reported in the abstract?

-The labelling intensity (delta 13C) is mentioned in the abstract as this information is clearly important for potential users of the method. Most other 13C values have been deleted from the abstract. 3. Why is the fraction of soil organic matter greater than 0.2 mm important?

-This is now explained in the introduction as follows: Transformation of plant litter is a key process of the carbon and nutrient cycles, which drives C mineralization and C accumulation in the soil organic matter (Tateno and Chapin, 1997). Detrital carbon accumulation accounts for most of an ecosystem's capacity to store organic carbon belowground within a few years and is largely conditioned by the plant turnover rate (Cebrian & Duarte, 1995). Root litter transformation is an important determinant of the carbon cycle in grassland ecosystems, which is affected both by the root litter quality and by the rhizosphere activity (Personeni & Loiseau, 2004, 2005). Tateno, M. & Chapin, F.S. (1997) The logic of carbon and nitrogen interactions in terrestrial ecosystems. *Am. Naturalist*, 149, 723-744. Cebrian, J. & Duarte, C.M. (1995) Plant growth-rate dependence of detrital carbon storage in ecosystems. *Science*, 268, 1606-1608.

4. Why will the reader be interested in the full technical details of the air flow and 13C enrichment in the experimental chambers at this stage?

-Agreed most technical details have been deleted from abstract. 6. The findings appear limited to confirmation : -that by supplying air depleted in 13C results in plant tissues with reduced 13C enrichment, and -reducing 'disturbance by cutting' (this is not defined in the abstract) results in increased residence time of detrital carbon.

-We now state in the abstract that the labelling has depleted coarse soil organic matter by 8-10 per mil. To our knowledge, this is the first time that a long term labelling has succeeded in changing the isotope signature of soil organic matter (SOM). 7. The weaknesses in focus are to a large extent reflected in the main body of the paper.

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The main body has also been edited to reflect the focus stated in the abstract. 8. The introduction does not set out any explicit hypotheses that were to be tested.

-A testable hypothesis has been clearly stated on the role of disturbance for turnover of carbon in coarse soil organic fractions. 9. The aims are not very clearly articulated or justified. There are some factual errors or parts that need rephrasing. (see below) Specific comments 1. On page 4 lines 4-7 it is stated that the supply of ^{13}C depleted air can not be used to study C cycling under ambient CO_2 . This is unclear and incorrect.

-FACE experiments usually use ^{13}C depleted CO_2 to enrich the atmosphere with CO_2 . This means that no ^{13}C signature is available for ambient CO_2 controls in these experiments. We clarified this sentence. 2. 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}C depletion value needs to be contextualised- how does this compare to the ambient air ^{13}C signature?

-We edited Figure1 and the legend. We discussed the $\delta^{13}\text{C}$ of CO_2 injection (-34.7‰) in comparison to that of ambient air. 3. Page 6 line 12-13. Explain and justify the concentration of CO_2 used. Is 425 micromoles of CO_2 significantly higher than ambient concentrations? Why was this concentration chosen? -This concentration was close to ambient at our site. We explained this in the text.

4. Lines 16-18. I am very unsure about the rationale for pumping air through the soil. This will inevitably greatly increase the O_2 and decrease the 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

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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.

-We agree pumping air through the soil compartment may affect microbes and alter carbon turnover. However, we have used a low air flow rate (3.5 L min⁻¹) which is unlikely to disturb the soil atmosphere. First, in contrast to Shepard et al. (2002) our soil is a well structured sandy loam (50 % sand) which has a large macroporosity. Second, we estimate from the soil texture and bulk density that the air filled pore space of the 3 monoliths varied between 60 and 90 L, which implies that only 4-6 % of the soil atmosphere was sampled each minute. Third, the CO₂ concentration at the outlet of the pump varied between 1000 and 2000 ppm, which is far above the concentration inside the shoot enclosure. Finally, we studied the soil organic carbon turnover in the top soil (0-10 cm), which was unlikely to be anaerobic even without air pumping. We have revised this section in the text to add this discussion. Moreover, we indicate the reason for using this method. In our experiment, air was pumped through the soil continuum to eliminated back-diffusion of CO₂ from the soil as small pressure head was maintained at the aboveground compartment by the open-flow system. The flow rate though the soil column was adjusted (with monoliths with bare soil) to suppresses diffusion of soil CO₂ in the above ground compartment without changing significantly soil respiration rates.

5. 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 ¹³C signatures of the whole plant- older tissues have different signatures to new tissues. Small samples,

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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?

-We sampled the last mature leaf lamina: i) to measure the delta ^{13}C signature of a fully labelled plant material and ii) to exclude possible effects of leaf senescence on the ^{13}C signature. This is now explained in the text. 6. 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 ?

-We have used standard methods to analyse soil organic matter fraction (see e.g. Six et al., 2001) by wet sieving. This method is appropriate to separate soil fractions in the litter continuum and has been widely tested and used with our soil (see Loiseau and Soussana, 1999; Personeni and Loiseau, 2004, 2005). In a previous study it was shown that coarse soil organic matter fractions have a faster turnover than finer fractions (Personeni and Loiseau, 2004). As shown by the results, the ^{13}C signature of the soil organic matter comprised between 0.05 and 0.2 mm varies only by a few per mils after two years of labelling, which shows that the ^{13}C labelling can best be applied to coarse fractions. Dissolved organic concentrations were small in the soil solution (see Klumpp et al., 2007) leading to carbon losses by leaching of 4-5 g C m⁻² yr⁻¹. Therefore, losses of water soluble carbohydrates from litter could be neglected. Personeni, E. & Loiseau, P.: How does the nature of living and dead roots affect the residence time of carbon in the root litter continuum? *Plant and Soil*, 267, 129-141, 2004. Personeni, E. & Loiseau, P.: Species strategy and N fluxes in grassland soil - A question of root litter quality or rhizosphere activity? *European J. Agr.*, 22, 217-229, 2005. Loiseau, P. and Soussana, J.F.: Elevated [CO₂], temperature increase and N supply effects on the accumulation of below-ground carbon in a temperate grassland ecosystem. *Plant and Soil*, 212, 123-134, 1999.

7. Page 10 lines 24-25. More details are needed of the surface on which the en-

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closures were placed. Were these surfaces gas-impermeable? How many replicates were studied?

-Measurements were carried out on two replicates, using a gas-tight surface (concrete). Details are now given in the text. 8. Page 13 Lines 21-22. If you have measured the difference between root and shoot ^{13}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.

-This was a result from another study, the sentence was revised. 9. 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.

-The objectives of the present MS was to show the feasibility of a new labelling method and to test the role of disturbance on the turnover of soil organic carbon in coarse soil fractions. Indeed there are highly complex biotic interactions in soils, such as the role of mycorrhizas and of soil micro-organisms supported by exudates. However, our study was not designed to target these processes, but rather to quantify carbon turnover. We have now included reference to an array of biotic interactions that can contribute to alter carbon turnover. 10. 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

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grazing).

-We agree, root exudates are released before litter decomposition is measurable. The chronology is not the subject of the paper. However, this section has been revised to indicate the likely sequence of events starting from rhizodeposition. 11. 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.

-In our experiment, the community structures of nematodes and earthworms were analysed. Both works are not published yet. However, we clarified that there is a possible implication of soil fauna but no evidence. 12. 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}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.

-We have fully revised the conclusion, which is now reflecting the balance of findings, including on the role of disturbance for soil carbon turnover.

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