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Biogeosciences Discussions

Interactive comment on "Rapid transfer of photosynthetic carbon through the plant-soil system in differently managed grasslands" *by* G. B. De Deyn et al.

Anonymous Referee #1

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General comments: This paper investigates how grassland restoration management (fertilization cessation and seeding) affects short-term plant C uptake and retention across different plant taxa, and plant-C transfer to and within the soil microbial community. This was investigated by conducting a 6h 13CO2 pulse-labeling experiment in the field and subsequent analysis of 13C enrichment of plant aboveground biomass and microbial PLFAs over time. Only a small number of studies have attempted to track the C flow through the plant-soil system in-situ and this particular study largely confirms what others have found – both in terms of the rapid transfer of plant-C to microbial, and in particular fungal, communities (e.g. Treonis et al., 2004; Denef et al., 2007; 2009) and the longer retention of C in saprotrophic fungal biomass (e.g. Treonis et

al., 2004). Also, the observed differences in plant-C allocation by functionally different plant species (vascular vs. mosses) confirm what has been found by others (e.g. Woodin et al., 2009), although I am not convinced that the authors can make proper statements concerning what they call 'C retention' from the analyses performed in this study (see further).

While some of the outcomes in this study were not all that new, the objectives of this study were rather novel, i.e. the impact of grassland management practices for increasing plant diversity on plant-C transfer to microbial communities, even though no differences were observed in terms of 13C enrichment of the plant biomass or signature PLFAs across treatments. Since the treatments were carefully selected for largest differences in species composition, I would have expected some information on how the species composition differed across the treatments; however, this was absent in the paper.

I also found it unfortunate that the study did not look at possible microbial community shifts (relative abundance of PLFAs) as a result of the different management practices, which could have been easily analyzed on the same PLFA extracts analyzed for 13C enrichment. An absent treatment effect in terms of 13C enrichment of the individual PLFAs does not necessarily mean that across the different treatments, microbial communities took up similar amounts of plant-derived C. If one treatment would have resulted, for example, in an increase in fungal biomass, an increase in PLFA-13C enrichment would not necessarily be observed, but their total 13C uptake would still be larger in absolute terms, and more fungal-derived C would cycle through (and potentially become sequestered in) the soil... If the authors do have this data available, I would encourage them to present this in a graph or table, or discuss this in the text (e.g. if no differences were observed).

This study is only one of few in-situ stable isotope probing studies designed for the specific purpose of evaluating the fate of plant-C through the plant-soil system. These studies are extremely valuable to improve our understanding of plant-biota interactions

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and their role in the C cycle. I therefore think that this paper will be valued by many readers of Biogeosciences. However, I do think the paper needs to be revised as I do have some concerns about some of the conclusions stated which, to my opinion, are not entirely supported by the data presented and would even require a different experimental approach or additional analyses. As mentioned earlier, the authors should be especially more careful when talking about differences in the 'retention' of newly assimilated C among the different plant species, as this cannot be evaluated solely based on 13C signatures in the small aboveground biomass sub-samples taken over time following pulse-labeling. The 13C decrease observed in grass and forb aboveground vegetation with time (fig. 3) is most likely a result of both C exports belowground through the plant, as well as dilution by non-labeled CO2 uptake during continued plant growth. Especially when harvesting only the relatively younger plant biomass at each sampling time, the contribution of this dilution could be substantial considering the observed rapid assimilation of CO2 into new biomass. A more acceptable approach would have been to look at the tracer recovery (% of total 13C uptake) in the different plant parts, including the belowground pools (roots, soil,. . .) as done for example by Woodin et al. (2009) after a certain time period. In order to compare across plant species, a different pulse-labeling experiment would be needed, where the labeling of one species is targeted in one chamber and the recovery of label over time in different plant fractions is quantified (again, cf. Woodin et al., 2009). Since this was not possible in the current experiment, I advise the authors to acknowledge this limitation in this study and to write their conclusions more carefully.

Another remark I have (but one that can be easily addressed) is the lack of information on the field experiment, site/soil properties, and the treatment effects on plant species diversity. The authors refer to the differences in vegetation composition and species diversity throughout the text (e.g. pg. 926, ln. 5 – basis for treatment selection at the field site), yet, no details were provided on how the different treatment plots differed in species diversity. Even though details of the experimental design can be found in the Smith et al. papers, a good description of the experimental plots selected for this

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study, and the species composition in each of the treatments would be useful (e.g. table or text description). Especially since the objective of this study, as stated in the abstract, was to evaluate if changes in vegetation composition alters short-term rates of C assimilation, retention and transfer from plants to soil microbes. The location and start of the field manipulation experiment should also be mentioned and some general soil characteristics (soil type, texture, pH, C and N content, etc.).

Other sections in the M&M also lack some important information. I agree that well-established methods do not need to be repeated if well described in other papers, but for the PLFA extraction and 13C-PLFA analysis, a short description would be useful. Reference was made to Harrison and Bardgett (2010), where no detailed method description was provided. The latter paper actually refers to Bardgett et al. (1996), which has a full method description. So, best to reference to this earlier paper. Furthermore, some details should be provided on how the identification of PLFAs was done (GC-MS? Or retention times compared to known standards ran on the GC-c-IRMS), and the GC specs (column, program, . . .).

Specific comments: - Since the increase in δ13C in the different PLFAs was not that high (fig. 4), in particular for the bacterial PLFAs, which stayed below -20 ‰ and the natural 13C abundance of different PLFAs can differ by a few parts per mils, I would be more interested in seeing the PLFA 13C enrichment (difference in δ13C between labeled and unlabeled PLFAs), as was done for the aboveground biomass 13C analysis (fig. 3). Were PLFAs extracted and analyzed by GC-c-IRMS in control soils? If so, make sure to indicate this in the text and use this to calculate your net 13C enrichment.

- The authors should also be careful with the term 'signature PLFA' as there are a few fungal PLFAs which have also been found in bacteria, e.g. 16:1w5 and 18:1w9. The high enrichment in 16:1w5 after 24h (much higher than all other bacterial PLFAs) would be a good argument for its use in this study as signature marker for AMF communities. However, the lower enrichment in 18:1w9 seems more in line with the lower bacterial C uptake rates. Perhaps the authors can make a note in the text somewhere,

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acknowledging the non-uniqueness of certain PLFAs.

- The lack of effect of N fertilization on C uptake by different microbial communities (in contrast to what has been found by others), may very well be a result of the low N addition rate in this study, as mentioned by the authors, but it would also be useful to know since when the N fertilizer cessation treatment has been going on (perhaps not long enough to see an effect?). The authors could also highlight the fact that the effects of N fertilization on fungal PLFA abundance observed by Bradley et al. (2007) was only significant at the high N addition rate. Bradley et al's mid-level N fertilization treatment (which was more in the range of what was applied here, i.e. 54 kg/ha/yr) did not show a significant effect. Denef et al. 2009 used much higher additions (225 and 450 kg/ha/yr).

- Pg. 930, ln. 25-29: Couldn't this also be explained by the fact that AMF appear to rapidly take up a large amount of new plant-C, which, in the period following pulse-labeling, is dominated by unlabeled C (hence the rapid decrease in 13C signature)? The saprotrophic fungi on the other hand, take up new plant-C also quite rapidly, but they might also use the 13C from decomposing root fragments and microbial biomass, explaining their continued 13C enrichment over time. . .

Technical comments: - Pg. 924, ln. 22-23: provide a reference. - Pg. 924, ln. 24: 'C flow' is a bit vague. Are you talking about the C uptake by the plants or the transfer of plant C belowground (i.e. to microbial communities)? Be more specific. - Pg. 926, ln. 9-10: Shouldn't this also include the 'no fertilizer, with seed' treatment? - Pg. 926, ln. 16: how big were the chambers? - Pg. 926, ln. 20: Soil samplings were also done before pulse-labeling. However, it seems as if no analyses were performed on these control soil samples. Correct? Why were these samples not analyzed for 13C PLFA? PLFAs do differ in their 13C natural abundance and enrichment should be expressed relative to this control 13C level. - Pg. 926, ln. 24: Refer to the Olsson papers. Chung et al., 2007 did not provide proof of the 16:1w5 as an indicator of AMF abundance. - Pg. 928, ln. 9-10: Indicate also the positive effect of fertilizer addition on grasses in September.

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- Table 1: Title should indicate that ANOVA results are shown for treatment effects (seeding (S), fertilizer (F)) and interactions (SxF), and sampling time (R1). Explain all abbreviations (S, F, R1). - Fig. 2: Include the exact treatment combination in the legend (i.e., no seed, no fertilizer; with seed, no fertilizer; no seed, with fertilizer; with seed, with fertilizer) - Fig. 3: The figure and legend present 6 different plant species. The figure caption only mentions 5. Include the legume. Perhaps group the different species per type in the legend (grass, forb, legume, moss). Indicate that data was averaged across treatment in the figure caption. - Fig. 4: 18:1w7 is indicated as a signature PLFA for gram-positive bacteria. This should be gram-negative bacteria (as correctly stated in the M&M) and have a solid line in the figure.

Interactive comment on Biogeosciences Discuss., 8, 921, 2011.