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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.

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We thank the reviewer for her/his constructive comments. We especially appreciate the referees recognition that only a small number of studies have attempted to track the C flow through the plant-soil system in-situ and for recognizing the novelty of our question of how grassland management practices for increasing plant diversity impact on plant-C transfer to soil microbial communities. In order to improve our manuscript the referee points raised three main issues: 1. The need to be more careful about statements on "C retention" 2. The need for more background about the field site (location, soil and vegetation properties) 3. The need to provide more details on the methodology of

PLFA identification and for data on their abundances

Below we provide an answer to each of these points, followed by answers to more specific comments.

1. We modified our statements with respect to restoration management impacts on 'C retention' in vegetation because we agree with the comments of the referee in that we did not track the %recovery of total 13C uptake per plant species in the different plant compartments and account for dilution effects. In our study, we could not capture the 13C recovery per plant species because the plants were growing in mixed swards and it was not possible to separate the species below-ground and destructively harvest a large part from the labeled area. Therefore, to address this point raised by the referee, we removed the word 'retention' from most parts of the text and changed several sections in our manuscript as follows:

In the discussion section (4.1) we changed 'Overall our results support the hypothesis that in the field plant species differ markedly in the rate of assimilation, retention and translocation of recently photosynthesised C to soil.' into 'Overall our results support the hypothesis that in the field plant species differ markedly in the rate of assimilation and translocation of recently photosynthesised C to soil.'

In the section (4.2) we changed 'Given that we found longer C retention in soil fungi than bacteria our results also suggests that grassland management that promotes soil fungi over bacteria could promote the retention of recent plant assimilate C in soil.' into 'Given that we found higher concentrations of 13C in soil fungi than bacteria, which remained high one week after the pulse, our results also suggests that grassland management that promotes soil fungi over bacteria could promote the retention of recent plant assimilate C in soil.'

In the conclusion (5) we changed 'Overall we found striking differences in assimilation and retention of 13C between grassland plants, irrespective of long-term plant diversity restoration management treatments.' into 'Overall we found large differences in assimi-

lation and maintenance of 13C concentration between grassland plants, irrespective of long-term plant diversity restoration management treatments.' Also, in the conclusion section we changed 'Plant derived 13C was rapidly and mostly transferred to AMF and saprophytic fungi, but saprophytic fungi retained the fresh C longest. Plant diversity restoration management did not directly affect the C assimilation or retention amongst plant taxa or groups of soil microbes, but can impact the fate of recent C by changing their abundances.' into: 'Plant derived 13C was rapidly and mostly transferred to AMF and saprophytic fungi, but saprophytic fungi showed the signature of the fresh C longest. Plant diversity restoration management did not directly affect the C assimilation and transfer amongst plant taxa or groups of soil microbes, but can impact the fate of recent C by changing their abundances.'

2. In response to the referees request for a better description of the experimental field site we have now provided more information about the field experiment. We now state the location, when the treatments started, the type of soil, organic matter and C and N content and pH, and also include a description of the vegetation composition of the plots that we used in our pulse-labeling study in the method section and Table 1.

In the method section we provide this information by including the text: 'Measurements were made in four treatments arranged in three blocks in a long-term (since 1990) multi-factorial grassland restoration experiment (Smith et al., 2008). The study site, Colt Park meadows, is located in North-West England in the Ingleborough National Nature Reserve (latitude 54° 12' N, longitude 2° 21' W) on Lolium perenne-Cynosorus cristatus grassland. The soil is a shallow brown earth over limestone of moderate-high residual fertility (15 mg P2O2 L-1), with 19% organic matter and a C% of 7.7 and N% of 0.75 and average pH of 5.5. All plots were grazed in autumn and spring and cut for hay on 21st of July since 1999.' We also included reference to the plot numbers of the plots that we selected for the pulse-labeling in relation to earlier published studies that present results from the same long-term field experiment. We included that information in the following text: 'The in situ pulse-labeling was performed simultaneously

in 12 plots, which correspond with the plots that did not receive seeding with Trifolium pratense in the study on soil C sequestration by De Deyn et al. (2011).'

Table 1: Plant species richness (per 4 m2) and abundance cover (%) in relation to restoration management treatments. Management treatments are: C= no seed, no fertiliser ; S= with seed, no fertiliser; F= no seed, with fertiliser; SF= with seed, with fertiliser.seed addition. Values are means +/- 1 SE (N= 3), no number indicates absence of the species, SE of species abundance cover not included because many species occurred in only one plot.

3. In order to provide more information in the methods on the PLFA analysis, the extraction and identification of the PLFAs we changed the reference to the method to Bardgett et al. (1996), as suggested, and included following sentence in the method section: 'Individual PLFAs were separated by using a Trace Ultra GC (Thermo Finnigan, Bremen, Germany) with a J&W DB-5 capillary GC column (60 m $\times$ 0.25 mm id $\times$ 0.25 μm film thickness; Agilent Technologies Ltd, Berkshire, UK), and their identification based on retention times as compared to those of known standards (Supelco, Supelco UK, Poole, Dorset, UK).'.

Furthermore we included data on PLFA abundances across management treatments in the results section: 'Restoration management had no effect on the average abundances of soil bacteria, fungi, actinomycetes and AMF across the pulse-chase sampling campaign (fertiliser F1,42 = 0.46, P= 0.50; seed addition F1,42 = 0.10, P= 0.76), but abundances were distinct between the groups of soil microbes (Wilks Lambda F3,40 = 400.9, P< 0.0001). Generally bacteria dominated the microbial biomass in all treatments (average PLFA biomass 49.4 +/- 1.7 10-6 g per g soil dw) and saprophytic fungi were the second (average PLFA biomass 10.1 +/- 0.4 10-6 g per g soil dw) and AMF third (average PLFA biomass 3.5 +/- 0.1 10-6 g per g soil dw) most abundant group of soil microbes.'

We did not find significant differences between management treatments in PLFA abun-

dances of different groups of soil microbes. Nevertheless, earlier studies using the same plots did find significant differences in PLFA biomass of fungi, especially in response to the mineral fertiliser treatment (Smith et al. 2003, 2008). We have included in the discussion reasoning as to why these differences could have occurred as follows: 'The studies of Smith et al. (2003, 2008) were performed at the same experimental site, including the plots we used in the current study. Therefore, it is rather surprising that we did not find significant differences in PLFA abundances across management treatments. However, this discrepancy could be due to differences in the time of sampling between studies: Smith et al. (2003, 2008) sampled in July, while we sampled in September, and PLFA abundances, and especially those of fungi, are known to be very responsive to seasonality being most abundant in spring and least in autumn (Bardgett et al., 1999). Moreover, the direction and magnitude of effects of grassland management treatments on soil microbial communities, measured using PLFA, are known to vary across seasons (Bardgett et al., 1999), and may be attributed in part to the length of time since fertiliser has been applied. In our study, the longer recovery time for fungi, who tend to decline rapidly in response to fertiliser N application which is done at our site in May (Donnison et al., 2000ab; Smith et al., 2003, 2008), may have resulted in similar PLFA abundances of the soil microbes across treatments.'

Bardgett, R. D., Hobbs, P. J., and Frostegård, A.: Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland, Biol. Fertil. Soils, 22, 261-264, 1996.

Specific comments: - Since the increase in 13C 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 13C 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.

Reply: We do not have primary data on the 13C values of the PLFAs before the start of the pulse-labeling because we expected that those values would hardly differ for the values 2h after the 13C pulse so we put most effort in samplings after the labeling. According to the literature, natural abundance 13C values of soil microbial PLFAs in temperate grassland range from -22.3 to -34.4‰ (Denef et al., 2009) or -28.6 to -35.2‰ (Butler et al., 2003). The values obtained 2h after pulse labelling do indeed fit in that range, suggesting that no notable 13C enrichment of the PLFAs had yet occurred apart perhaps from a small increase in PLFA 16:1ω5. We therefore do think that the pattern of 13C enrichment is illustrated by the change in δ13C as compared to the δ13C at 2h after the 13C pulse. Given that we do not have primary data on natural abundance levels of the PLFAs in our experiment we prefer to retain our current presentation using δ13C values, a notation that is also often used in studies comparable to ours (Butler et al., 2003; Treonis et al., 2004; Chung et al., 2009; Denef et al., 2009).

Butler, J. L., Williams, M. A., Bottomley, P. J., Myrold, D. D.: Microbial community dynamics associated with rhizosphere carbon flow, Appl. Env. Microbiol., 69, 6793-6800, 2003. Chung, H. G., Zak, D. R., Reich, P. B..: Microbial assimilation of new photosynthate is altered by plant species richness and nitrogen deposition, Biogeochem., 94, 233-242, 2009. Denef, K., Roobroeck, D., Wadu, M. C. W. M., Lootens, P., and Boeckx, P.: Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils, Soil Biol. Biochem., 41, 144-153, 2009. Treonis, A. M., Ostle, N. J., Stott, A. W., Primrose, R., Grayston, S. J., and Ineson, P.: Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs, Soil Biol. Biochem., 36, 533-537, 2004.

- 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, acknowledging the non-uniqueness of certain PLFAs.

Reply: As indicated by the referee, some PLFAs might be more of 'a signature' than other PLFAs. More specifically Zelles (1997) showed that the PLFA 18:1ω9 occurs predominantly in fungi, but also reported its presence in bacteria and actinomycetes, albeit in lower amounts. In studies on soil microbes, the PLFA 18:1ω9 is consistently used as fungal PLFA (Treonis et al., 2004; Chung et al., 2007; Denef et al., 2009), nevertheless we do think it is important to mention that also some bacteria and actinomycetes may contribute to the biomass of the PLFA 18:1ω9. To address this point of the referee, we now include the following text in the discussion: 'It is notable that the 13C signature in the PLFA 18:1ω9, which is often used as fungal PLFA (Treonis et al., 2004; Chung et al., 2007; Denef et al., 2009), showed a pattern more alike that found in bacteria than in the other PLFA indicative for saprophytic fungi. This result may be due to the fact that the PLFA 18:1ω9 is also found in bacteria (Zelles, 1997).' . Chung, H. G., Zak, D. R., Reich, P. B., and Ellsworth, D. S.: Plant species richness, elevated CO2, and atmospheric nitrogen deposition alter soil microbial community composition and function, Global Change Biol., 13, 980-989, 2007. Chung, H. G., Zak, D. R., Reich, P. B..: Microbial assimilation of new photosynthate is altered by plant species richness and nitrogen deposition, Biogeochem., 94, 233-242, 2009. Denef, K., Roobroeck, D., Wadu, M. C. W. M., Lootens, P., and Boeckx, P.: Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils, Soil Biol. Biochem., 41, 144-153, 2009. Treonis, A. M., Ostle, N. J., Stott, A. W., Primrose, R., Grayston, S. J., and Ineson, P.: Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs, Soil Biol. Biochem., 36, 533-537, 2004. Zelles, L.: (1997) Phospholipid fatty acid profiles in selected members of soil microbial communities, Chemosphere, 35, 275-294, 1997.

- 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).

Reply: In the material and method section we included the notion that the experimental treatments of mineral fertiliser use started in 1990, so sixteen years before our work on the study site. Time in years therefore seems not to be the issue, but seasonal effects and time since N application given the low dose of N applied may be a primary reason. In order to stress the potential influence of N fertilisation level on 13C incorporation and abundance of PLFA we reformulated the original text (P931, lines 6-12) into: 'The reason why we did not find effects of fertiliser use on 13C enrichment may due to the time elapsed since application fertiliser application in May with pulse labelling last day of August, and the modest addition rate (25 kg/ha 20:10:10 N:P:K) of the fertiliser. In the aforementioned studies of Bradley et al. (2006) and Denef et al. (2009) the levels of N applications that did cause significant shifts in soil PLFA abundances, irrespective of time since application, and their signature of new photosynthate-C were much higher: ranging from 225 to 450 kg N/ha/year, suggesting that the responses might also be strongly dose dependent.'

We also addressed the aspect of management effects on PLFA abundances in our response to the referees 3rd main comment above.

- 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.

Reply: We agree with the referee that it is possible that especially in AMF a dilution effect occurred so that the 13C signature decreased, while saprofytes may have recycled the 13C released by other soil organisms. We now address this important point in the discussion by including the text: 'Moreover, it may well be that the 13C signal in AMF became rapidly diluted by the inflow of unlabeled fresh C via the plant roots soon after the 13C pulse. In contrast, the high levels of 13C in the saprophytic fungi one week after the pulse could in part be due to recycling of 13C released by other soil biota upon their death.'.

Technical comments: - Pg. 924, ln. 22-23: provide a reference. Reply: We are not sure which extra reference the referee is thinking of as we provided four references, two to the long-term restoration experiment and two to the in situ pulse-labeling method. Hence we did not include an extra reference but we did include more information on the location and treatments, as requested in the overall evaluation of our manuscript.

- 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. Reply: In order to state our prediction more specific we changed 'C flow' into 'C uptake by the plants and transfer of this C to soil microbes'.

- Pg. 926, ln. 9-10: Shouldn't this also include the 'no fertilizer, with seed' treatment? Reply: The referee likely is pointing at Pg. 925 and is indeed right that the treatment no fertiliser, with seed should also have been included. We corrected this and in our revision we added the lacking treatment combination.

- Pg. 926, ln. 16: how big were the chambers? Reply: We included the size of the chamber (40 cm diameter, 20 cm height; 1257 cm2) in this section of the manuscript and removed it where we provided this information in our original manuscript (Pg. 927 ln. 3-4).

- 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. Reply: The referee is right that soil samples from before the pulse-labeling were not analysed for 13C PLFA. We provided a more extensive reply in our response to the first specific comment posed above.

- 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. Reply: We removed the references to Chung et al. and maintained the reference to Olsson.

- Pg. 928, ln. 9-10: Indicate also the positive effect of fertilizer addition on grasses in September. Reply: We included the response of the grass biomass by including the sentence: 'In contrast to the moss response the continued application of mineral fertiliser promoted the grass biomass in September (Fig. 1).'

- 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). Reply: In the title of Table 1 (now renumbered as Table 2) we explained the abbreviations we used for the treatment factors (S= seed addition treatment and F= fertiliser use treatment and their interaction S x F) and time factor (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) Reply: We included the exact management treatments in the legend: Fig. 2. 13C atom % excess in leaf tissue across species in relation to restoration management over a 21-day 13CO2 pulse chase period. Management treatments are: no seed, no fertiliser; with seed, no fertiliser; no seed, with fertiliser; with seed, with fertiliser. Values are means + 1 SE (N= 3).

- 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. Reply: The referee is right that we overlooked to include the sixth species, the legume T. repens, in the legend. We have reworded the legend and included the letters F, G, L after the plant species names in the figure to indicate the group (forb, grass or legume) they belong to. The new legend is Fig. 3. 13C atom % excess in leaf tissue from co-occurring species in mixed grassland (two forb F, two grass G, one legume L and a moss species) over a 21-day 13CO2 pulse-chase period. Values are means and means sharing the same symbol are significantly different at the respective time after labeling ($P < 0.05$).

- 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. Reply: In figure 4b we did indicate the PLFA 18:1w7 as signature for gram-negative bacteria and used a solid line in the original figure, so we retained the figure as it was. Perhaps the line appeared dotted but is not dotted in the printed version of the referee? There should be three solid and two dotted lines in Figure 4b.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/8/C687/2011/bgd-8-C687-2011-supplement.pdf

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