Biogeosciences Discuss., 8, 921–940, 2011
www.biogeosciences-discuss.net/8/921/2011/
doi:10.5194/bgd-8-921-2011

Biogeosciences Discussions

Rapid transfer of photosynthetic carbon through the plant-soil system in differently managed grasslands

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Received: 22 January 2011 – Accepted: 31 January 2011 – Published: 2 February 2011

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Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Plant-soil interactions are central to short-term carbon (C) cycling through the rapid transfer of recently assimilated C from plant roots to soil biota. In grassland ecosystems, changes in C cycling are likely to be influenced by land use and management that changes vegetation and the associated soil microbial communities. Here we tested whether changes in grassland vegetation composition resulting from management for plant diversity influences short-term rates of C assimilation, retention and transfer from plants to soil microbes. To do this, we used an in situ ^{13}C-CO_2pulse-labeling approach to measure differential C uptake among different plant species and the transfer of the plant-derived ^{13}C to key groups of soil microbiota across selected treatments of a long-term plant diversity grassland restoration experiment. Results showed that plant taxa differed markedly in the rate of ^{13}C assimilation and retention: uptake was greatest and retention lowest in *Ranunculus repens*, and assimilation was least and retained longest in mosses. Incorporation of recent plant-derived ^{13}C was maximal in all microbial phosopholipid fatty acid (PLFA) markers at 24 h after labeling. The greatest incorporation of ^{13}C was in the PLFA 16:1ω5, a marker for arbuscular mycorrhizal fungi (AMF), while after one week most ^{13}C was retained in the PLFA 18:2ω6,9 which is indicative of assimilation of plant-derived ^{13}C by saprophytic fungi. Our results of ^{13}C assimilation, transfer and retention within plant species and soil microbes were consistent across management treatments. Overall, our findings suggest that changes in vegetation and soil microbial composition resulting from differences in long-term grassland management will affect short-term cycling of photosynthetic C, but that restoration management does not alter the short-term C uptake and transfer within plant species and within key groups of soil microbes. Moreover, across all treatments we found that plant-derived C is rapidly transferred specifically to AMF and decomposer fungi, indicating their consistent key role in the cycling of recent plant derived C.

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1 Introduction

Considerable quantities of carbon (C) are stored in terrestrial vegetation with even more in soils (Ostle et al., 2009a), but this C is vulnerable to losses due to respiration, leaching via soil water, and erosion (Lal, 2004; Smith et al., 2007; Quinton et al., 2010). The main factors that determine rates of C loss via soil respiration are thought to be soil temperature and moisture (Davidson and Janssens, 2006). However, the underlying process of C mineralization is primarily governed by the activity of soil biota which are very responsive to plant C inputs and the transfer of recent photosynthetic C to soil via roots and their exudates (Olsson and Johnson, 2005; Ostle et al., 2007; Kuzyakov, 2010). In general, our understanding of the short-term transfer of C between plants and soil biota remains limited, although it is widely recognized that this interaction plays a key role in the C cycle and soil C sequestration (Ostle et al., 2009b; Bardgett et al., 2009; Paterson et al., 2009).

Vegetation in itself represents a substantial pool of terrestrial C. However, plant taxa can differ strongly in the rate at which they assimilate and lose C by respiration, exudation and tissue turnover, and hence in their effect on the soil C balance (Dorrepaal, 2007; De Deyn et al., 2008; Paterson et al., 2009). For example, dominance of plants with high C assimilation rates may not be beneficial for ecosystem C sequestration if these plants also experience fast rates of C loss, and/or if they promote the activity of decomposer organisms through the inputs of exudates to soil. In contrast, dominance of communities by slow-growing plants can be beneficial for ecosystem C sequestration because dead tissue of these plants generally decomposes more slowly than that of faster-growing plant species as it is a poor food source for soil microbes.

Living vascular plants provide recent assimilated C to soil biota via their roots. The primary consumers of this new plant C are bacteria, mycorrhizal fungi and saprophytic fungi. These groups of soil biota play different roles in C cycling because of divergence in the speed of assimilation of plant-derived C, their spectrum of C sources and their average C:N stoichiometry (van der Heijden et al., 2008; De Deyn et al., 2008; Strickland

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and Rousk, 2010). In particular, mycorrhizal fungi are responsive to plant C assimilation and allocation to roots because of their symbiotic nature (Johnson et al., 2002; Olsson and Johnson, 2005). More generally, bacteria and fungi may differentially affect C cycling, so that ecosystem management that promotes fungi over bacteria, such as the cessation of fertiliser application (Bardgett and McAlister, 1999; Smith et al., 2008), is expected to promote soil C sequestration (Six et al., 2006; Strickland and Rousk, 2010).

The rate of assimilation, transfer and retention of recent photosynthetic assimilated C in ecosystems thus depends on the community composition of plants and/or of soil microbes (Dorrepaal, 2007; Ward et al., 2009; Woodin et al., 2009; Paterson et al., 2009), but also on the activity of the plant and microbial taxa which can be altered by ecosystem management (Treonis et al., 2004; Hill et al., 2007; Denef et al., 2009). In a long-term grassland restoration experiment Smith et al. (2003, 2008) showed that cessation of mineral fertiliser use and the seeding of target plant species increased plant species richness and the abundance of soil fungi, compared to soil bacteria. These restoration treatments also enhanced the rate of soil C and N accumulation (De Deyn et al., 2011), suggesting potential management impacts on short-term C fluxes through plants and soil biota.

In this study we explored the effects of plant taxa and management treatments aimed at restoring plant species diversity (i.e. cessation of fertilser use and seeding) on the rate of C assimilation, retention and transfer to soil microbes in a temperate grassland. This was achieved in a long-term diversity manipulation experiment in northern England (Smith et al., 2003, 2008) using an in situ ^{13}C-CO_2 pulse-chase labeling technique (Ostle et al., 2003, 2007). We predicted that C flow would be faster in fertilised plots and in plants with high growth rates (Ward et al., 2009). We also expected mycorrhizal fungi to show rapid assimilation of plant C (Johnson et al., 2002) but less so in fertilised plots (Bradley et al., 2006; Denef et al., 2009), with longer retention of plant C in fungi than in shorter-lived bacteria (Ostle et al., 2003; Rousk and Baath, 2007).

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2 Material and methods

2.1 Experimental design

Measurements were made in four treatments arranged in three blocks in a long-term multi-factorial grassland restoration experiment (Smith et al., 2008). We selected treatments which have had the most significant impact on vegetation composition with the greatest increases in plant species diversity: i.e. the cessation of NPK fertiliser application and the addition of seed mixtures (of *Ranunculus bulbosus*, *Lotus corniculatus*, *Briza media* and *Geranium sylvaticum*), and their full factorial combinations (i.e. stopping fertiliser application without seed addition, continued fertiliser application with seed addition, and continued fertiliser application without seed addition). Fertiliser addition was done yearly in early May (25 kg N and 12.5 kg P and K per ha) (Smith et al., 2003, 2008).

2.2 $^{13}CO_2$ pulse labeling and ^{13}C enrichment in vegetation and soil microbes

To investigate the fate of recently plant assimilated C in plant and soil microbes a $^{13}CO_2$ pulse-chase assay was performed according to the method of Ostle et al. (2003). In brief, in each treatment plot vegetation was exposed to air in which ambient CO_2 was replaced by ^{13}C labeled CO_2 (99 atom % ^{13}C enriched) at 370 ppm within a transparent acrylic chamber at flow rates of 6 l per minute and maintained for 6 daylight h on 31 August 2006.

Plant and soil samplings were made immediately before $^{13}CO_2$ labeling and 2, 24 and 48 h, and 1 and 3 weeks after labeling. At each sampling, shoot material from each of six different species within the chamber were sampled. The selected species were common grass, forb, legume and moss species across the treatments: *Anthoxanthum odoratum* and *Festuca rubra* (grasses), *Ranunculus repens* and *Rumex acetosa* (forbs), *Trifolium repens* (legume) and *Brachythesium rutabulum* (moss). Vegetation was sampled by snipping 2 cm long leaf tips of young undamaged leaves, a leaflet of

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3 sub-leaves for *Trifolium repens*, and the top 2 cm of green bryophyte *Brachythesium rutabulum*. Samples were put in individual eppendorf tubes and immediately frozen at −20 °C. Soil samples were collected by taking a single core (3.4 cm diameter, 10 cm deep), from which subsamples were collected from the soil layer at 3 to 5 cm depth to standardise sampling. Roots were removed with tweezers and root-free soil was immediately frozen and freeze-dried. The coring holes were refilled immediately with soil cores from the same plot, but from outside the pulse chamber. Enrichment of ^{13}C in plant aboveground tissues was determined using freeze-dried and finely ground plant material. This was weighed into tin cups, and the samples were analysed for total C and $^{13}C/^{12}C$ isotopic ratio using a Flash EA 1112 Series elemental analyser (Thermo Electron Corporation, Bremen, Germany) coupled with a Delta^{plus} Advantage isotope ratio mass spectrometer (IRMS, Thermo Finnigan, Bremen, Germany). We expressed enrichment of ^{13}C in plants as ^{13}C atom% excess with atom% excess = atom% enriched sample – atom% background sample (i.e. before labeling), in which atom% = $[R_{sample}/(R_{sample} + 1)] \times 100$ and $R_{sample} = {}^{13}C/{}^{12}C$ ratio measured by IRMS (Leake et al., 2006).

Phosopholipid fatty acid (PLFA) markers in soils from samplings 2, 24, 48 h and one week were extracted from freeze-dried ground soil using the protocol described in Harrison and Bardgett (2010). To determine the $^{13}C/^{12}C$ isotopic ratio of individual PLFAs the extracts were measured on a GC-C-IRMS (Thermo Finnigan, Bremen, Germany). Isotopic enrichment in PLFAs was expressed as $\delta^{13}C$ values, with $\delta^{13}C(‰) = [(R_{sample} - R_{reference})/(R_{reference})] \times 10^3$ where $R_{reference}$ is the $^{13}C/^{12}C$ ratio of reference material calibrated to the Vienna Pee Dee Belemnite scale. The PLFA 16:1ω5 was used as an indicator of AMF abundance (Olsson, 1999; Chung et al., 2007); 18:2ω6,9 and 18:1ω9 were used as markers for saprophytic fungi; 10Me18:0 was used for actinomycetes; 16:1ω7c, 18:1ω7 and 19:0cy were used as markers for Gram-negative bacteria; and i15:0 and a15:0 were used for Gram-positive bacteria (Patra et al., 2008).

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2.3 Plant biomass

Plant community total biomass and the standing biomass of grasses, forbs, legumes and mosses in the area of each labeling chamber (40 cm diameter, 20 cm height; 1257 cm^2) was determined by clipping the vegetation 1 cm above the soil surface 3 weeks after the pulse label (21 September). Vegetation was sorted, dried for 48 h at 70 °C, and weighed. Vegetation was sampled again in a similar way in February.

2.4 Data analysis

The effect of management treatments (i.e. fertiliser use and seeding) and time since the pulse on ^{13}C enrichment in plant tissue (or in signature PLFAs) was tested per plant species (or per signature PLFA) across sampling times using Repeated Measures ANOVA with time as repeat, fertiliser use, seeding and their interaction as fixed factors and block as a random factor. The effect of plant species identity (or PLFA identity) on ^{13}C enrichment in plant tissue (or in signature PLFA) was tested per sampling time. For the samples collected 2 and 24 h and one week after the ^{13}C-CO_2 pulse we used General Linear Models (GLM) with fertiliser use, seeding, plant species (or signature PLFAs) and their interactions as fixed factors and block as a random factor. Enrichment in plant tissue collected 48 h and 3 weeks after the pulse was analysed using non-parametric Kruskal-Wallis ANOVA with plant species as predicting factor, because of unequal variances between plant species. Restoration management effects on the total dry weight of the plant community in September and February was tested by GLM with fertiliser use, seeding, and their interaction as fixed factors and block as a random factor. Treatment effects on the dry weights of the different plant groups (grasses, forbs including legumes and moss) in September and February were tested using repeated measures ANOVA with plant group as repeat and with the aforementioned predictor variables. Differences between levels of significant factors were tested using Tukey HSD (parametric) and Mann-Whitney U (non-parametric) post-hoc tests. Enrichment

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values were log $(1 + x)$ transformed prior to analysis in order to obtain equal variances between species.

3 Results

3.1 Management effects on plant biomass and C flux

Total standing plant biomass in September and February did not differ across management treatments of seeding ($F_{1,6} = 0.18$, $P > 0.05$) or fertiliser use ($F_{1,6} = 0.29$, $P > 0.05$) (Fig. 1), but plant biomass distribution was markedly affected (interaction fertiliser use x plant group September: $F_{2,12} = 10.18$, $P = 0.003$; February: $F_{2,12} = 18.28$, $P < 0.001$). Moss biomass was promoted in the absence of mineral fertiliser (September: $F_{1,6} = 25.35$, $P = 0.002$; February: $F_{1,6} = 13.77$, $P = 0.01$) and contributed most to the dry weight of the plant community in the February winter sampling (Fig. 1).

Overall, management treatments had no significant effect on the ^{13}C enrichment of the different plant species at any sampling time (Fig. 2). However, across treatments, consistent differences in ^{13}C enrichment between plant species were detected. Two hours after the ^{13}C-CO_2 pulse all plants showed significant ^{13}C enrichment in their aboveground tissues, but ^{13}C enrichment was significantly higher ($F_{5,44} = 17.01$, $P < 0.001$) in vascular plants than in moss (Fig. 3). Across vascular plant species, *R. repens* showed the highest, *R. acetosa* and *T. repens* the lowest, and *A. odoratum* and *F. rubra* intermediate levels of ^{13}C enrichment across all treatments. By 24 h after the pulse labelling, ^{13}C enrichment in aboveground tissue had strongly declined in all vascular plant species, but there was still a significant effect of plant species ($F_{5,44} = 2.94$, $P < 0.05$) due to higher ^{13}C enrichment in *R. repens* than in moss across all treatments. Also, after 48 h (KW $H_{5,\ n=71} = 12.70$, $P < 0.05$) and one week ($F_{5,44} = 3.28$, $P < 0.05$) there was a plant species effect on ^{13}C enrichment: *R. repens* remained more enriched than moss and *F. rubra* after 48 h, and than moss 1 week after the pulse.

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Three weeks after the pulse labelling, there was no longer a plant species effect on the levels of ^{13}C enrichment (KW $H_{5,\ n=71} = 8.94$, $P = 0.11$) (Fig. 3).

3.2 Photosynthate C flux through soil microbes

Management treatments did not affect ^{13}C enrichment in any of the PLFAs, while time since pulse labelling strongly affected enrichment in all but the actinomycete specific PLFA 10Me18:0 (Table 1). All signature PLFAs were most enriched (i.e. showed the largest content of recently assimilated plant ^{13}C) 24 h after pulse labelling (Fig. 4). Across signature PLFAs, 16:1ω5 (AMF) and 18:2ω6,9 (saprophytic fungi) showed the largest incorporation of recent plant derived ^{13}C between 2 and 24 h after the pulse. At 48 h after labelling, ^{13}C enrichment was comparable to that at 24 h for most PLFAs, but was significantly reduced in the 18:1ω9 fungal PLFA. One week after the pulse, ^{13}C enrichment in PLFAs had declined to similar levels as at 2 h after pulse labelling the vegetation, except for the fungal PLFA 18:2ω6,9 which showed significantly higher enrichment then all other PLFAs ($F_{7,42} = 32.74$, $P < 0.0001$).

4 Discussion

4.1 Species and management effects on C flux

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.

In line with studies in peatland and arctic systems we found that mosses assimilated C at a much slower rate but retained it longer in their tissues as opposed to vascular plants (Dorrepaal, 2007; Woodin et al., 2009; Ward et al., 2009). Within the vascular plants forbs did not behave differently from grasses, but rather behaved in a species

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specific way: *R. repens* showed the highest initial C assimilation rate and *R. acetosa* and *T. repens* the lowest. This indicates that using a priori defined functional groups of grasses, forbs and legumes may not be the best way of aggregating plant groups in relation to short-term C cycling, supporting the suggestions of Wright et al. (2006) for the relationships between plant diversity and above- and belowground biomass production. In contrast to our expectation, under the assumption of overall increased plant photosynthetic activity with fertilisation given higher productivity (De Deyn et al., 2011), we did not find effects of fertiliser application on the C uptake, retention and transfer to microbes for any of the plant species. However, our results are in line with the study of Hill et al. (2007) where mineral N application also did not affect C assimilation rates in *Lolium perenne*, as opposed to the strong effect of elevated CO_2 levels. In their recent review Kuzyakov and Gavrichkova (2010) also indicate that ambient CO_2 concentration, temperature and photosynthetic active radiation rather then soil nutrient availability drive photosynthetic C assimilation. Overall it appears that the short-term assimilation and transfer of C by individual grassland species were not influenced by restoration management practices.

In line with our expectation ^{13}C enrichment of soil microbes was rapid and peaked 24 h after the pulse for AMF, but also in decomposer soil fungi and in gram negative and gram positive soil bacteria. The fast allocation of recent plant assimilated C to AMF and saprophytic soil fungi and the poor response of actinomycetes is in line with previous studies (Johnson et al., 2002; Treonis et al., 2004; Denef et al., 2009). However, we did not find a time lag between the response of saprophytic fungi and bacteria (Olsson and Johnson, 2005; Denef et al., 2007). Although ^{13}C enrichment in fungi and bacteria initially peaked at similar sampling times, with the enrichment being particularly high in AMF, one week after the pulse enrichment remained specifically high in saprophytic fungi, indicating longer retention times in this group of soil microbes, as was found by Treonis et al. (2004). This result could be explained by the generally longer lifespan of fungi as opposed to bacteria (Ostle et al., 2003; Rousk and Baath, 2007), but it is less clear why the signal declined as quickly in AMF; perhaps selective grazing by

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fungal-feeding soil fauna (Johnson et al., 2005) or relative large loss of C from AMF via C rich excretions (Johnson et al., 2002; Rillig, 2004) played a role.

Management treatments of fertiliser application have been shown to suppress the allocation of recent photosynthetic C to AMF, and more generally to all fungal PLFA biomarkers (Bradley et al., 2006; Denef et al., 2009). In our study we did not find such a response as ^{13}C enrichment in soil microbial PLFAs were consistent across management treatments. The reason why we did not find effects of fertiliser use on ^{13}C enrichment may due to the time elapsed since application fertiliser application in April with pulse labelling in August, and the modest addition rate (25 kg ha^{-1} 20:10:10 N:P:K) of the fertiliser. In the aforementioned studies N applications were much higher (ranging from 225 to 450 kg Nvha yr^{-1}), suggesting that the responses might be dose dependent.

4.2 Indirect management effects on C flux and storage

Restoration management treatments had a striking effect on the biomass of the different plant groups. Specifically the cessation of fertiliser use promoted moss and forbs and reduced grass biomass, but in such a way that total aboveground biomass was not affected. Given the long retention of photosynthate C in mosses and their high C:N ratio (Ward et al., 2007) this shift in vegetation composition has the potential to enhance C sequestration in vegetation in unfertilised treatments (De Deyn et al., 2011). These results also indicate that mosses might play a key role in the sequestration of C in grasslands, especially during times when vascular plant growth ceases. 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. Smith et al. (2003, 2008) showed that this can be achieved by ceasing fertiliser use and by the promotion of specific plant species by seeding.

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5 Conclusions

Overall we found striking differences in assimilitation and retention of ^{13}C between grassland plants, irrespective of long-term plant diversity restoration management treatments. Vascular and non-vascular plants showed most divergent responses, while responses of vascular plants were not linked to a priori "functional groups" of grasses and forbs. Plant derived ^{13}C 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. Together, our findings suggests the rapid assimilation, turnover and transfer of C from plants to microbes is common across plant species and that this short-term C cycling is unaffected by management change.

Acknowledgements. This project was funded by a grant from the Biotechnology and Biological Sciences Research Council (BBSRC) Agri-Food Committee, awarded to R. D. B. and N. O. G. B. D. D. was in part supported by ALW grant 865.05.002. The set-up and maintenance of the field experiments were funded by the UK Department for Environment, Food and Rural Affairs (Defra), as part of the species-rich grassland research programme, and the DIGFOR project led by R. D. B. We are grateful to Val Brown and Richard Brand-Hardy for supporting us in this programme. We thank Niall P. McNamara and Will Mallot for help with pulse-labelling at the field site, Roger Smith for establishing the biodiversity restoration experiment at Colt Park and Paul Evans (Natural England) for maintaining the field site.

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Table 1. Repeated measures ANOVA results across sampling time **(R1)** for ^{13}C enrichment in soil microbial PLFAs. Results of interactions between restoration factors and R1 and of block not shown as none were significant, apart from a small block effect for PLFA 18:2ω6,9 ($F_{2,5} = 10.97$, $P < 0.05$). Statistical significant factors in bold.

PLFA	Factor	*F*	*df*	*P*
16:1ω5	*S*	0.00	1,6	0.99
	F	0.74	1,6	0.42
	S × *F*	1.06	1,6	0.34
	R1	**19.63**	**3,18**	**<0.0001**
18:2ω6,9	*S*	0.06	1,5	0.82
	F	0.55	1,5	0.49
	S × *F*	2.45	1,5	0.18
	R1	**11.05**	**3,15**	**<0.001**
18:1ω9	*S*	0.26	1,5	0.63
	F	0.23	1,5	0.65
	S × *F*	4.35	1,5	0.09
	R1	**24.44**	**3,15**	**<0.0001**
18:1ω7	*S*	0.42	1,6	0.54
	F	1.69	1,6	0.24
	S × *F*	3.15	1,6	0.13
	R1	**27.00**	**2,12**	**<0.0001**
19:0cy	*S*	0.02	1,6	0.89
	F	0.45	1,6	0.53
	S × *F*	0.75	1,6	0.42
	R1	**193.1**	**3,18**	**<0.0001**
16:1ω7c	*S*	2.42	1,5	0.18
	F	0.51	1,5	0.51
	S × *F*	0.43	1,5	0.54
	R1	**44.43**	**3,15**	**<0.0001**
i15:0	*S*	0.24	1,6	0.64
	F	0.00	1,6	0.97
	S × *F*	0.31	1,6	0.60
	R1	**59.39**	**3,18**	**<0.0001**
a15:0	***S***	**7.36**	**1,6**	**0.035**
	F	0.02	1,6	0.90
	S × *F*	0.22	1,6	0.66
	R1	**59.55**	**3,18**	**<0.0001**
10Me18:0	*S*	0.56	1,5	0.47
	F	0.17	1,5	0.70
	S × *F*	1.54	1,5	0.27
	R1	**2.66**	**3,15**	**0.09**

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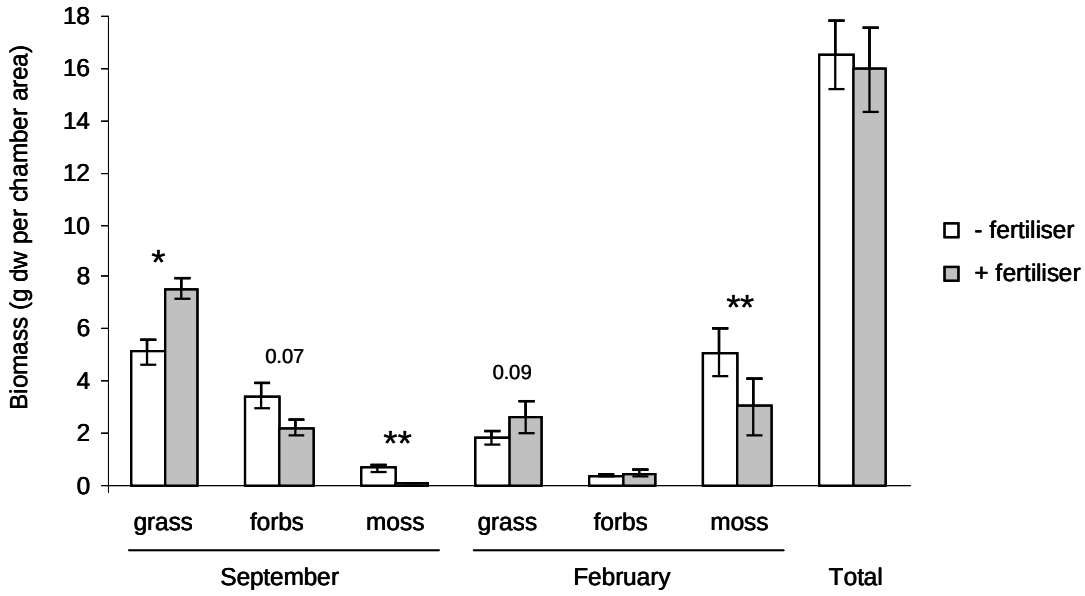

Fig. 1. Distribution of aboveground biomass over different plant groups in September and February in relation to fertiliser treatments. Values are means ±1 SE, significant differences between fertiliser treatments: * $P < 0.05$, ** $P < 0.01$.

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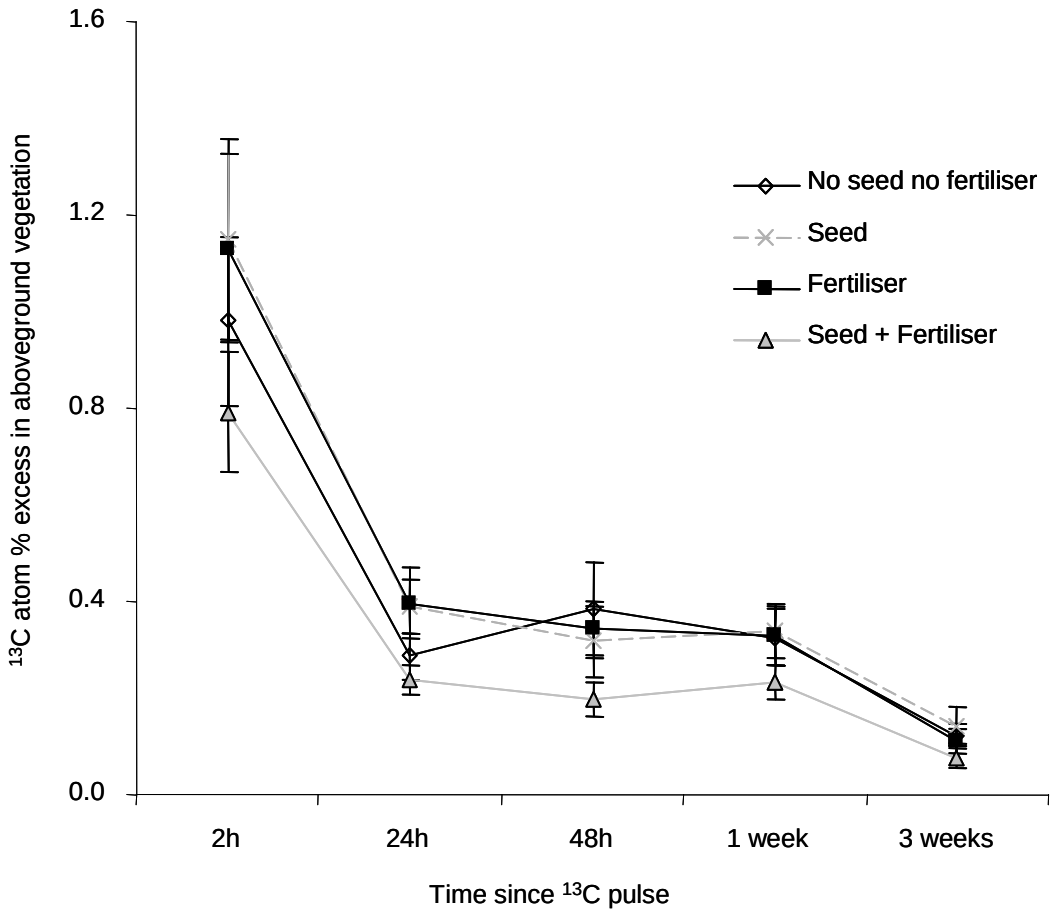

Fig. 2. ^{13}C atom% excess in leaf tissue across species in relation to restoration management over a 21-day $^{13}CO_2$ pulse chase period. Values are means ±1 SE.

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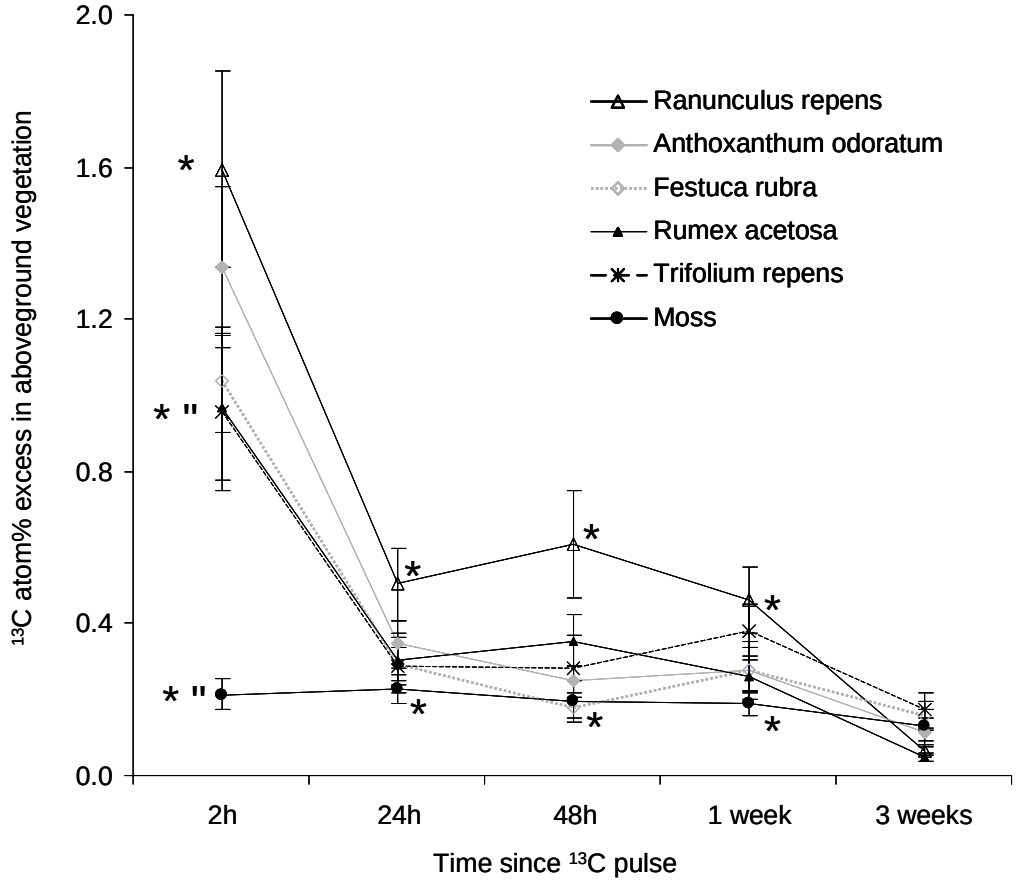

Fig. 3. ^{13}C atom % excess in leaf tissue from co-occurring species in mixed grassland (two forb, two grass and a moss species) over a 21-day $^{13}CO_2$ pulse chase period. Values are means and means sharing the same symbol are significantly different at the respective time after labelling ($P < 0.05$).

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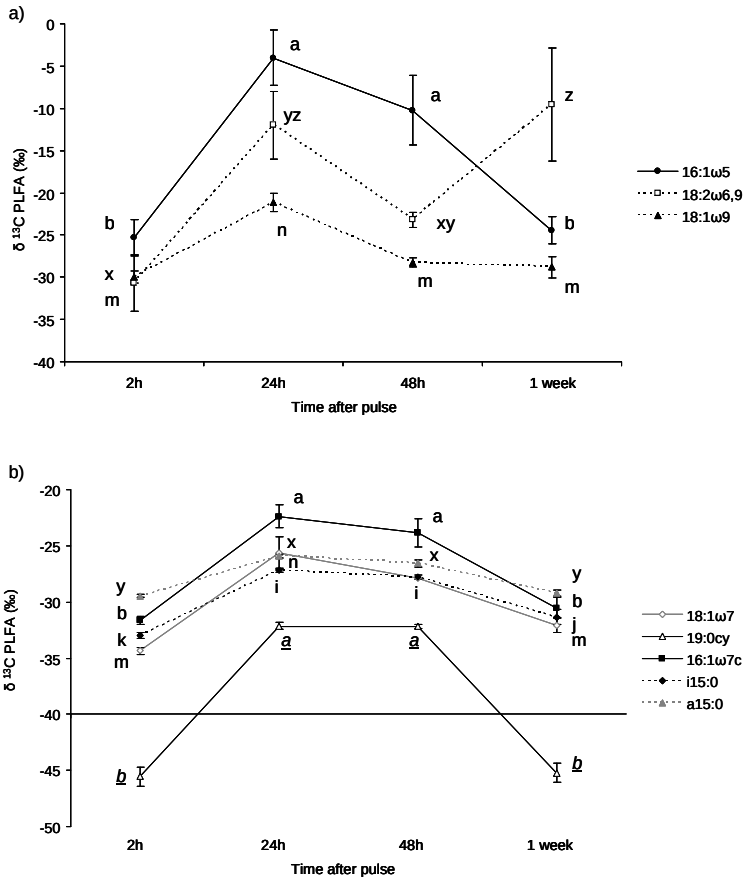

Fig. 4. ^{13}C enrichment over a 1-week $^{13}CO_2$ pulse chase period in **(a)** fungal PLFAs and **(b)** bacterial PLFAs. Values are means ±1 SE and means of the same PLFA not sharing the same letter are significantly different between the respective times ($P < 0.05$). Solid line indicates AMF **(a)** or gram negative bacteria **(b)**, dotted line indicate saprophytic fungi **(a)** or gram positive bacteria **(b)**.

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