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RESEARCH ARTICLE

COMPARISON OF THE RELATIVE TURNOVER RATE OF 14C-LABELLED SENESCING ROOTS AND MYCORRHIZAL HYPHAE IN SOIL

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ABSTRACT

A significant proportion of C fixed in net primary production by plants is allotted below-ground to mycorrhizal fungi. Consequently, mycorrhizal hyphal death is likely to contribute substantially to the inputs of organic C to soil. Despite this, our knowledge of the relative turnover rate of hyphal necromass and its relative contribution to overall soil organic matter cycling remains poor. Here we studied the mineralisation of senescing, isotopically-labelled extraradical hyphae growing in association with the plant *Cistus monspeliensis* L.. Plants were grown in compartmentalized rhizoboxes and labelled with $^{14}CO_2$ for 5 hours. Two days after ^{14}C-labelling, the above-ground biomass of the plant was removed. Subsequently, $^{14}CO_2$ evolved from the (1) root + mycorrhizal (R+M), and (2) mycorrhizal-only (M) compartments of the rhizoboxes were measured over time. Roots and associated soil lost substantial amounts of $^{14}CO_2$ within 1 week of shoot removal, but thereafter $^{14}CO_2$ losses remained relatively slow until the end of the study period. After 8 months, the amount of ^{14}C loss from the R+M and M compartments was 33% and 48% of the initial amount of ^{14}C present in the soil. ^{14}C mineralisation from soils was best described by a single exponential plus asymptote kinetic model. From the model we estimated that the proportion of labile ^{14}C was greater in the M compartment but the C turned over slower ($t_{½}$= 23 d) in comparison to the ^{14}C in the R+M soil ($t_{½}$ = 11 d). The model also predicted that a greater proportion of the ^{14}C in the root + mycorrhizal treatment was highly recalcitrant in comparison to the root-free treatment. Our results suggest that both roots and mycorrhizal hyphae significantly contribute to below-ground C storage. A quantitative estimate of the relative contribution of root and mycorrhizal hyphae to soil, however, can only be made once the live standing biomass and longevity of both roots and mycorrhizal hyphae in soil are known. Further studies are also required to elucidate changes in hyphal chemistry during their decomposition to determine which forms of fungal-derived C are most persistent.

Key words: Senescing Hyphae, Hyphal Necromass, Rhizoboxes, ^{14}C Mineralisation, Soil C.

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INTRODUCTION

Mycorrhizal associations with plants are almost ubiquitous and they represent a key pool of below-ground carbon (C) in all terrestrial ecosystems (Godbold *et al*., 2006). These fungal symbionts form various types of associations with roots which can be classified based on their different morphological structures (e.g. arbuscular mycorrhizas (AM), ectomycorrhizas (EM), and ericoid mycorrhizas (EcM); Lukac and Godbold, 2011). In all types, the fungal symbiont produces an extensive hyphal (extraradical or extramatrical) mycelium to explore the soil and acquire nutrients, however, the hyphae in this network are thought to be somewhat ephemeral (Smith and Read, 1997).

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In Swedish forests, the total EM fungal biomass has been estimated to be between 0.7-0.9 t ha^{-1}, where *ca.* 80% of the biomass is contributed by the extraradical mycelium (Wallander *et al*., 2001). Although many previous studies have focused on measuring hyphal growth rates and the amount of standing mycorrhizal biomass in soil, they have tended to overlook the subsequent rate of hyphal death and the turnover of this hyphal necromass. Mycorrhizal hyphae in soil are thought to be short lived (e.g. AM hyphal turnover rate of 5-6 days; Staddon *et al*., 2003), however, the factors controlling the production rate of mycorrhizal necromass and its ultimate contribution to the formation of stable soil organic matter is lacking. Knowledge of this, however, remains fundamental to the accurate modelling of C flow and storage within terrestrial ecosystems (Drigo *et al*., 2012). In forest ecosystems around the world, typically 25-63% of the C plant's net C fixed is translocated below-ground, consequently roots and mycorrhizal hyphae play as an important conduit for C movement into soil (Litton *et al*., 2007).

Several studies have also reported that between 15-28% of net primary production is specifically allocated to the mycorrhizal network (Vogt *et al*, 1982; Finlay, 2008). In accordance with this, Högberg *et al*. (2001) demonstrated via tree girdling studies that the flux of newly assimilated C is a major driver of soil respiration and that decreased by 37% within 5 days after severing the supply of C from the shoots to the root system and associated mycorrhizas. The fate of mycorrhizally-derived C after it has entered the soil, however, is complex as it can undergo a range of fates including: (1) use as a C substrate by the soil microbial community, (2) physical and chemical protection within (stable) soil organic matter, (3) loss as soluble C during leaching or surface runoff, (4) small amounts of loss from the soil surface as volatile organic compounds. In forest ecosystems, most of this mycorrhizal necromass-C is believed to be rapidly lost from soil via microbial breakdown processes (i.e. respiration) with only a small proportion of the necromass-C becoming protected in stable (recalcitrant) soil organic matter (Janssens *et al*., 2001). Hyphal necromass adds a mixture of labile and relatively recalcitrant compounds to the soil, with the recalcitrant fractions mainly contributing towards the replenishment of soil organic matter (Drigo *et al*., 2012). However, rates of hyphal decomposition are expected to vary due to a range of factors relating to hyphal tissue chemistry (e.g. N and melanin concentrations), soil physical properties (e.g. texture, moisture and temperature) and the size, composition and activity of the decomposer community (Koide and Malcom, 2009; Fernandez and Koide, 2014). Further, Wilkinson *et al*. (2011) have reported that mycorrhizal species richness may also be important in regulating the hyphal decomposition process.

Most previous mycorrhizal necromass decomposition studies have been relatively short in duration (28-35 days) and have used hyphae sourced largely from fungi grown in artificial media within the laboratory (Olsson and Johnson, 2005;Koide and Malcom, 2009; Wilkinson *et al*., 2011; Drigo *et al*., 2012). According to some studies, hyphal necromass is expected to have a long mean residence time in soil due to the presence of chemically complex substrates such as chitin, melanin and glomalin which are relatively resistant to enzymatic attack (Bloomfield and Alexander, 1967; Ekblad *et al*., 1998; Treseder and Allen, 2000). For example, it has been reported that the AM exoglycoprotein, glomalin, can reside in soil for up to 42 years (Rillig *et al*., 2001). Additionally, glomalin along with the roots and mycorrhizal hyphae have been suggested as a key stabilisation mechanism in soil organic matter via the promotion of soil aggregation (Wu *et al*., 2014). This suggests that more long term studies are needed to elucidate the fate of mycorrhizal-derived C in soil.

Significant progress has been made in the development of methods to measure the production, standing biomass and turnover of mycorrhizal hyphae in soil including the use of mycelial in-growth bags, chemical markers combined with molecular or isotopic markers and large scale manipulations such as trenching and girdling (Ekblad *et al*., 2013). Pulse labelling plants (and their associated symbionts) with ^{13}C or ^{14}C with subsequent measurement of the evolution of $^{13}CO_2$ or $^{14}CO_2$ from soil has also been widely used to measure the turnover and decomposition of root and mycelial necromass. However, these isotopic approaches rarely enable us to understand the microbial nature of the decomposition process and individual contributions of roots and mycorrhizae to the total evolved $^{13}CO_2$ or $^{14}CO_2$. Additionally, there is a risk of uneven isotopic labelling into various components of the fungal biomass such as respiratory substrates and structural cell materials (Dawson *et al*., 2002). Nonetheless, recent studies have successfully used this approach to trace the incorporation of mycorrhizal-derived C into free living heterotrophic soil fungi (Wilkinson *et al*., 2011; Drigo *et al*., 2012; Wallander *et al*., 2013). Here we use ^{14}C labelling to study the contribution of senescing hyphae to the persistence of C in soil under controlled conditions.

MATERIALS AND METHODS

Soil characteristics: Soil was obtained from a temperate agricultural site at the Henfaes Experimental Station located in Abergwyngregyn, Gwynedd, North Wales, UK (53°14' N, 04°01' W). The soil is sandy loam textured and classified as a Eutric Cambisol (FAO) or Dystric Eutrudepts (US Soil Taxonomy) (Glanville *et al*., 2012).The mean annual soil temperature at 10 cm is 11°C and annual rainfall is 1250 mm. Soil was collected from the top layer 0-10 cm depth (Ah horizon) and placed in gas-permeable plastic bags and transferred to the laboratory. Soil was allowed to pass through a 2 mm sieve to remove stones, plant roots and earthworms and basic soil characteristics were determined as follows. Field-moist soil (10 g) was oven-dried (105°C, 16 h) to determine moisture content. Soil pH and electrical conductivity (EC) were determined in a 1:2 w/v soil: deionised water extracts (250 rev min^{-1}, 1 h). The supernatant from the extract was subsequently analysed for nitrate using the vanadate method of Miranda *et al*. (2001) ammonium using the salicylate-nitroprusside- hypochlorite procedure of Mulvaney *et al*. (1996) and free amino acids by the fluorometric OPAME procedure of Jones *et al*. (2002). Soil general properties are presented in Table 1.

Table 1. General properties of soil used in the mineralisation studies. Values represent means. Value presented in parenthesis indicates standard error mean; *n* = 3.

Soil parameter	Measured quantity
Water content (% of field moist soil weight)	26.2 (0.1)
pH (1:2 H_2O)	5.40 (0.02)
EC (1:2 H_2O μS cm^{-1})	169 (4)
Available NO_3^- (mg N kg^{-1}soil)	10.2 (0.1)
Available NH_4^+ (mg N kg^{-1} soil)	0.12 (0.01)
Free amino acids (mg C kg^{-1} soil)	1.30 (0.48)

Table 2. List of endo and ectomycorrhizal fungi present in TNC Mycorr^{Multi} inoculum (The Nutrient Company, 2014)

Endomycorrhizae (12 cfu g^{-1})	Ectomycorrhizae (5000 cfu g^{-1})
Glomus clarum	*Rhizopogon amylpogon*
Glomus intraradices	*Rhizopogon fulvigleba*
Glomus mosseae	*Rhizopogon rubescans*
Glomus deserticola	*Rhizopogon villosuli*
Glomus monosporus	*Laccaria laccata*
Glomus brasilianum	*Pisolithus tinctorius*
Glomus aggregatum	*Scleroderma sp*
Gigaspora margareta	

Growing and inoculating the plants in the rhizoboxes: Rhizoboxes were used to study the ^{14}C mineralisation rates of extraradical mycorrhizal hyphae (Fig. 1). Each rhizobox (170 × 160 × 30 mm) consisted of two compartments (*i.e.*, a root + mycorrhizal compartment, and mycorrhizal-only compartment, abbreviated as R+M and M respectively).

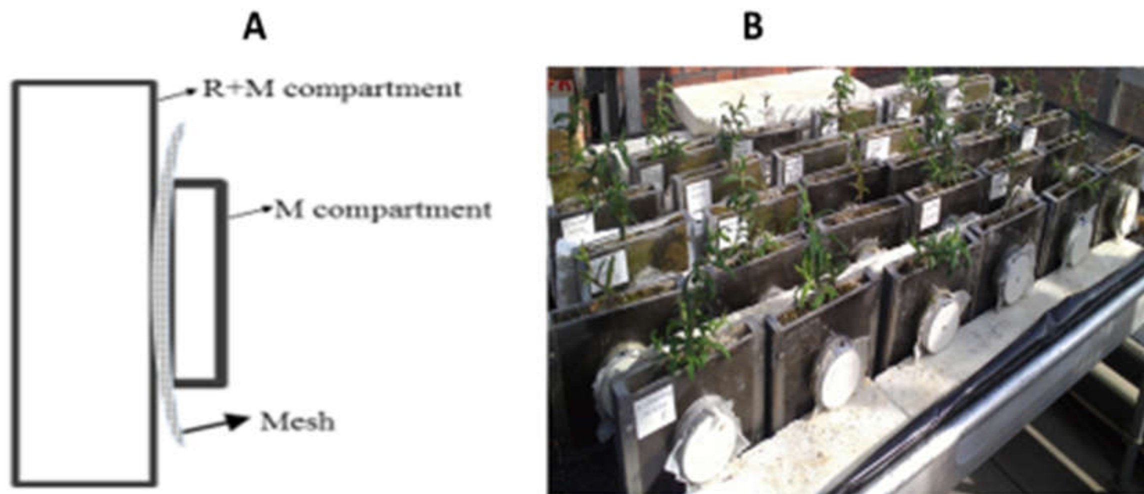

Fig 1. Schematic representation of a rhizobox (Panel A) and *C. monspeliensis* L. plants growing in the rhizoboxes (Panel B)

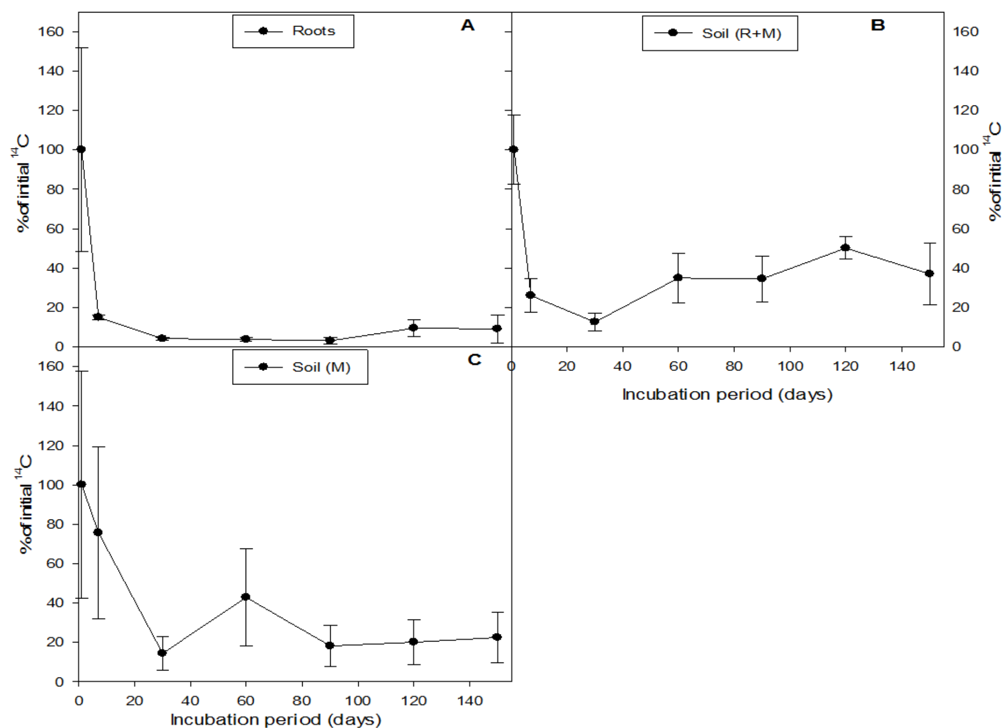

Fig. 2. Temporal changes to ^{14}C content in (A) senescing roots, (B) soil (R+M compartment), and (C) soil (M-only compartment). The values presented are percentage of ^{14}C remaining relative to that present at the start of the incubation. Values represent means. Error bars indicate standard error mean; *n* = 3

The compartments were separated from each other by a 30 µm nylon mesh (Normesh, Lancashire, UK) that allows free passage of water and mycorrhizal mycelia between the compartments but not plant roots (Ren *et al*., 2013). Each rhizobox was filled with 750 g of field-moist soil. A seedling of *Cistus monspeliensis* L. was transplanted into the soil and the rhizoboxes subsequently transferred to a greenhouse (temperature 18-26°C) where they were maintained with a weekly watering regime until they were ^{14}C-labelled. In nature, genus *Cistus* may form both ecto- and endo-mycorrhizal associations with over 200 fungal species belonging to 40 genera (Smith and Read, 1997; Comandini *et al*., 2006). In this study, we chose a broad spectrum mycorrhizal inoculum to inoculate the plants. Hence after 12 weeks, when the root system was fully developed, each rhizobox was inoculated with 5 g of fungal inoculum (TNC Mycorr^{Multi}. The Nutrient Company, Rochdale, UK) consisting of a mixture of both arbuscular and ecto mycorrhizal species (Table 2). The inoculum was added directly into the plant root zone.

^{14}C-labelling of plants in the rhizoboxes: Three months after inoculating the rhizoboxes with mycorrhizal fungi, plants were labelled with ^{14}C in closed acrylic chambers (410 × 410 × 500 mm). $^{14}CO_2$ was generated by adding 1 ml of acetic acid in to a microcentrifuge tube containing 200µl of 3 MBq $NaH^{14}CO_3$ for 5 hours to allow photoassimilation. The chambers were sealed and a small internal fan was provided to facilitate the equal distribution of generated $^{14}CO_2$ to all the rhizoboxes (Farrar *et al*., 2012). 48 hours after the labelling, plant tops were removed by severing the stems at the soil surface. This short-term labelling approach was chosen as earlier labelling studies have reported that maximum ^{14}C dilution below-ground

occurs within 72 h (Wu *et al*., 2002). After shoot removal, the rhizoboxes were transferred to the laboratory for further analysis.

^{14}C mineralisation from roots and extra-radical mycorrhizal hyphae: Rhizoboxes were destructively sampled after 1 day. Each rhizobox compartment was carefully split apart; first roots were removed and then soils in both compartments (R+M and M) were separated. At sampling time, 20 g of fresh soil from each compartment was placed into a 50 cm^3 polypropylene tube and $^{14}CO_2$ evolution measured by placing a 1 M NaOH trap (1 ml) in the polypropylene tube (Jan *et al*., 2009). NaOH traps were changed after 1, 3, 6 and 24 h and then at 7, 14, 30, 60, 90, 120, 150, 180 and 240 days. At each sampling point the amount of $^{14}CO_2$ captured was determined using a Wallac 1409 liquid scintillation counter (EG and G, Milton Keynes, UK) using ScintSafe 3 scintillation fluid (Fisher Scientific, UK). The roots that were separated from the rhizoboxes were washed gently to remove any adhering soil and dried at 80°C overnight. Similarly the remaining soils from both the compartments were dried overnight at 105°C. The ^{14}C content of the dried roots and soils were determined using a OX-400 Biological Sample Oxidizer (RJ Harvey Instrument Corp., Hillsdale, NJ) by collecting the $^{14}CO_2$ evolved in Oxosol scintillation fluid (National Diagnostics, Hessle, UK) and measuring the amount of ^{14}C using a Wallac 1409 liquid scintillation counter (EG and G, Milton Keynes, UK).

^{14}C in the soluble pool in soil: The amount of ^{14}C substrate in the soil soluble pool was determined using a cold (1°C) 0.5 M K_2SO_4 extraction (Rousk and Jones, 2010). Briefly, 3 g of fresh soil from each rhizobox compartment was subsampled into 50 cm^3 polypropylene tubes at each sampling point. These were placed on an orbital shaker for 15 minutes at 200 rev min^{-1} after adding 15 ml of cold (1˚C) 0.5 M K_2SO_4. After shaking, samples were centrifuged at 15,000 g for 10 minutes and the ^{14}C content of the supernatant solution determined as described previously. A similar extraction procedure was repeated at the end of the incubation period (240 days).

Data and statistical analysis: The ^{14}C content of the dried roots and soil and the ^{14}C mineralisation data from both R+M and M compartments was plotted using Sigma plot v12.3 (Systas Software Inc., Chicago, IL). ^{14}C mineralisation rates were calculated for soils in R+M and M compartment based on the initial ^{14}C content (day 1) and expressed as percentages. ^{14}C mineralisation data was best fitted to a single exponential kinetic equation with asymptote (Farrar *et al*., 2012; Glanville *et al*., 2012).

$Y = S + [A \times \exp^{(-kt)}]$(equation 1)

Where Y is the amount of ^{14}C remaining in the soil, S is the asymptotic value equating to the amount of ^{14}C that is unavailable to soil microbes, A represents the amount of ^{14}C mineralised by the soil microbes and k is the decay constant corresponding to the total amount of bioavailable ^{14}C present; t is incubation time. Substrate half-life ($t_{½}$) was calculated using equation (2).

$t_{1/2} = \ln(2)/k$ (equation 2)

All other statistical procedures were carried out using SPSSv20.0 (SPSS Inc., Chicago, IL) by one way ANOVA with PostHoc Least Significant Difference (LSD) test. We accepted $P \leq 0.05$ as an indication of statistical significance.

RESULTS

Temporal changes of ^{14}C content in the senescing roots and soil: The first sampling was done 1 day after removal of the above-ground plant components from the rhizoboxes (i.e. 3 days after ^{14}C labelling).Therefore, we consider the ^{14}C content in roots and soil at the first sampling point to represent the baseline to which all other values are compared (i.e. 100% of the ^{14}C present at the start of the experiment). Overall, there was a high variability in the initial amount of ^{14}C present in the soil among the replicates (Fig. 2B, C), which is presumably due to the differences in the relative abundance of root and mycorrhizal hyphal biomass in the rhizoboxes. As expected, the ^{14}C content of the senescing roots themselves was ca. 170 and 3000 times greater in comparison to the soil in the R+M and M compartments respectively (values not presented). The background ^{14}C values for unlabelled soils from this site were below detection limits. The total amount of ^{14}C retained in the soil within the R+M compartment showed a rapid decline over time followed by a more stable phase (Fig. 2B). Approximately 74% of ^{14}C in the R+M compartment soil was lost during the first 7 days. Conversely, ^{14}C content in the soil from the mycorrhizal-only compartment dropped rapidly and then remained broadly stable throughout the remainder of the study period (Fig. 2C). Roots lost ca. 85% of their ^{14}C content within the first 7 days of our study (2A).

^{14}C mineralisation from the root and mycorrhizal soils: Overall, 33.3 and 47.8% of the initial ^{14}C present in the soil was mineralised from the R+M and M treatments respectively during the 8 month incubation period (Fig. 3A). During the first hour, ^{14}C mineralisation from the soil in the M compartment (1.09%) was ca. 3-fold higher in comparison to that produced from the R+M compartment (0.3%). However, after 24 h, the rate of ^{14}C mineralisation from the two treatments was not significantly different ($P \geq 0.05$; $n = 3$). The rate of ^{14}C mineralisation then increased until day 30 after which rates of $^{14}CO_2$ evolution were low. Soil in the R+M compartment lost ca. 22.7% of its initial ^{14}C during the first 30 days (Fig. 3B), followed by an additional ca. 10.5% during the next 210 days. In comparison, soil from the M compartment lost ca. 27.2% during the first 30 days (Fig. 3B) and another 20.7% during the subsequent 210 days (3A).

Modelling the kinetics of ^{14}C loss from soil: A single exponential decay with asymptote model fitted well to the ^{14}C experimental mineralisation data from both soil compartments (Table 3). This is consistent with the model reported earlier by Glanville *et al*. (2012) which allows attribution of ^{14}C to two distinct ^{14}C pathways, consisting of a rapidly (labile) mineralizable C pool (Pool *A*) and a less bioavailable (recalcitrant) C pool (Pool *S*). Overall, the model allocated more C to the recalcitrant C pool (Pool *S*) in the R+M treatment than in the M-only treatment. In contrast, in the M-only treatment equal approximately equal amounts of C were allocated to both the labile (Pool *A*) and recalcitrant C pool (Pool *S*). The rate constant (k) describing the turnover of Pool *A* was low in both treatments from which half-lives for this pool can be estimated at 11.1 d and 23.3 d in the R+M and M-only treatments respectively.

Table 3. Modelled ^{14}C mineralisation dynamics for soil contained in the R+M and M-only compartments. *S* is the asymptotic value (recalcitrant ^{14}C pool poorly available to microbes); *A* represents the amount of labile ^{14}C available to soil microbes; *S* and *A* values are expressed as a total percentage of ^{14}C present at the start; *k* represents the decay constant value for Pool *A*; r^2 represents the fit of the kinetic model to the experimental data. Values represent mean. Valuepresented in parenthesis indicates standard error mean; *n* = 3.

Treatment	*S* (%)	*A* (%)	*k* (day^{-1})	r^2 value
R+M	69.5 (0.9)	29.0 (1.3)	0.062 (0.011)	0.973
M	54.3 (1.1)	42.6 (1.5)	0.030 (0.004)	0.985

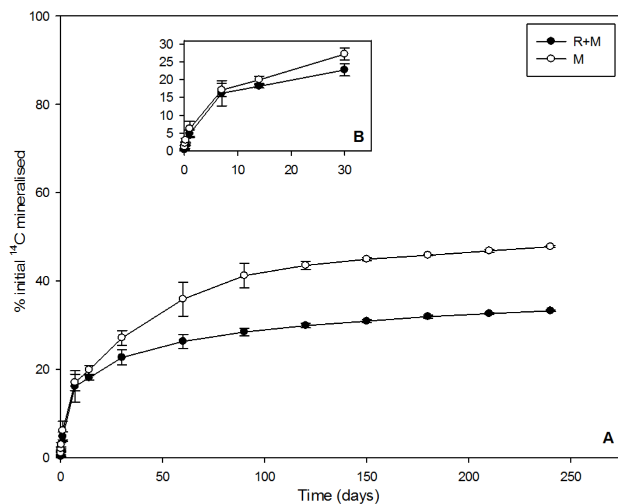

Fig. 3 ^{14}C mineralisation in soil containing either ^{14}C-labelled senescing roots and mycorrhizas or just ^{14}C-labelled mycorrhizas over a 240 day incubation (Panel A). The inset panel (Panel B) shows 1^{4}C mineralisation during the first 30 days of the experiment. R+M denotes soil from the Roots + Mycorrhizal compartment and M denotes soil from the Mycorrhizal-only compartment. Data points represent means. Error bars indicate standard error mean; *n* = 3

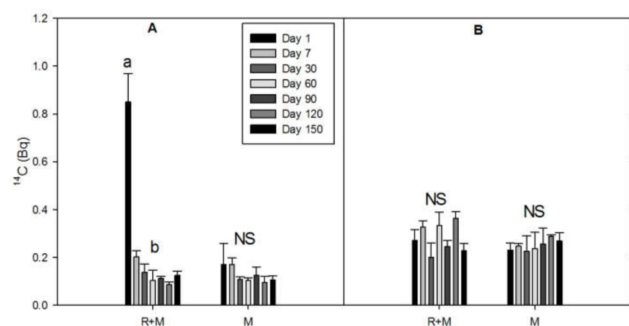

Fig. 4 Amount of ^{14}C present in the K_2SO_4 extractable soil pool (Panel A) at the time of each rhizobox was sampled and at the end of the study period (Panel B; 240 days). Values are mean. Error bars indicate standard error mean. Letters aand b indicate significant differences between different time points for each respective treatment (*P*< 0.05; *n* = 3), NS denoted no significant difference.

Soluble ^{14}C remaining in the soil: Based on the 0.5 M K_2SO_4 extraction, soluble ^{14}C in the soil was highest in the R+M treatment sampled on day 1. Recovery of ^{14}C rapidly decreased from 0.84 Bq to 0.2 Bq by day 7 and remained low until the end of the study. In contrast, in the M-only compartment ^{14}C recovery remained low throughout the study period (Fig. 4A). As expected, at the end of the study, ^{14}C recovery from the soil in the R+M and M compartments were not significantly different (Fig. 4B, *P*> 0.05; *n* = 3). However, ^{14}C recovery in the K_2SO_4 extract increased between the initial and final extraction for each incubation period, with the exception of the day 1 samples (difference between 4A and 4B for each sample). Fig. 4 Amount of ^{14}C present in the K_2SO_4 extractable soil pool (Panel A) at the time of each rhizobox was sampled and at the end of the study period (Panel B; 240 days). Values are mean. Error bars indicate standard error mean. Letters a and b indicate significant differences between different time points for each respective treatment (*P*< 0.05; *n* = 3), NS denoted no significant difference.

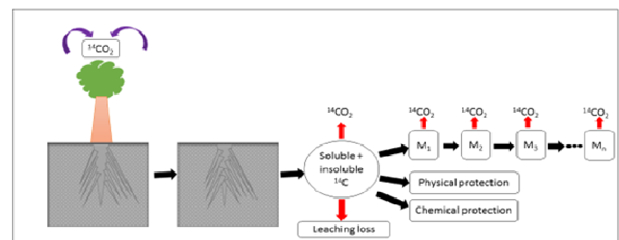

Fig. 5. Schematic diagram showing the fate of ^{14}C that entered into soil in R+M and M-only compartments. M_1, M_2, M_3 and M_n are microbes in soil food chain

DISCUSSION

Mycorrhizal hyphae contribute a significant amount of C to below-ground C flow (Godbold *et al*., 2006). However, determining the overall contribution of hyphae to total soil C and their relative persistence under field conditions is notoriously difficult due to their connectivity with the roots. In this study we have separated the hyphae from roots by growing plants in rhizoboxes with 2 compartments. Whilst plants are intact, total soil ^{14}C in the R+M compartment originates from root exudates and mycorrhizal hyphae. After removal of the plant tops, ^{14}C released from senescing roots and mycorrhizal hyphae could also contribute to soil organic matter. As roots were removed from the R+M compartment most of the contribution is assumed to be only through hyphal necromass. However, many fine roots and root hairs would remain in the soil and contribute to the total soil ^{14}C. Soil ^{14}C in the M-only compartment is obtained from hyphal exudation followed by senescing hyphae and/or hyphal necromass only. Moreover, based on our visual observations the M-only compartment was not highly colonized by mycorrhiza. Therefore, it is likely that the relative amounts of mycorrhiza in the R+M and M compartments are different.

^{14}C mineralisation from roots: Plant tops were removed 48 hours after ^{14}C labelling, therefore, it is obvious that a significant fraction of the assimilated ^{14}C may have been lost in the form of plant respiration during the first 48 hours, which we did not measure (Högberg *et al*., 2001). However, once the plant tops were removed, the supply of photosynthates to the roots would cease. Consequently, roots utilise the available C reserves within them before they die (Bingham and Rees, 2008). Root ^{14}C content declined in a biphasic manner. The rapid decrease in ^{14}C content during the first 7 days is mainly attributed to the loss of C reserves such as carbohydrates, proteins and fatty acids primarily due to root respiration. However, we do not know the exact time of root death in our study. The plants were labelled with ^{14}C for a relatively short period (5 hours) in this study; consequently, we hypothesise

that ^{14}C dilution was somewhat limited to the soluble fractions such as carbohydrates, amino acids, and proteins. Although, we did not measure the ^{14}C dilution into each plant component, the amount of ^{14}C loss (ca. 85% of total ^{14}C content) during the first 7 days supports this. This is due to the fact that, root biomass normally consists only 35-46% of soluble compounds such as carbohydrates, amino acids, proteins (Venkata *et al*., 2017), which are likely to be used as respiratory substrates during first 7 days (Brouquisse *et al*., 1991; Bingham and Rees, 2008). Moreover, our previous studies have shown that root soluble components lost only ca. 50% of their initial ^{14}C during the first 7 days (Venkata *et al*., 2016), although, we cannot directly compare both studies due to differences in methodology. Additionally, ^{14}C loss in the present study is due to autotrophic respiration (at least for first few days, before roots die), whereas in other study it is due to heterotrophic respiration. Nonetheless, this further emphasises the uneven distribution of ^{14}C into soluble and structural plant components. Further, ^{14}C content of roots in rhizoboxes sampled from day 7 onwards is broadly stable until the end of the study. This suggests only a minor fraction of ^{14}C has entered into relatively recalcitrant fractions such as structural components. Therefore, results from this study have probably underestimated the persistence time of root ^{14}C in soil thus, further detailed studies are required.

^{14}C mineralisation from soil : The ^{14}C mineralised from soil in the M-only compartment is a result of hyphal respiration and soil microbial respiration that are using hyphal exudates (Scheublin *et al*., 2010). Although, earlier studies suggests hyphae can live up to 5-6 days (Staddon *et al*., 2003), we did not measure their time of death in our study. Accordingly, in R+M compartment ^{14}C mineralisation occurs due to root (before they were removed from rhizoboxes), hyphae and soil microbial respiration. However, due to the practical difficulty in removing all the fine roots and root hairs, inevitably we have measured the $^{14}CO_2$ from the leftover senescing fine roots, and root hairs. This may have contributed to the overall ^{14}C loss from the soil in R+M compartment.

Overall, only 33 and 48% of initial ^{14}C was mineralised from R+M and M compartment soils respectively during the study period. This suggests that even after 240 days, substantial amounts of ^{14}C remain in the soil. Our results contradict those of Malik and Haider (1982), where 48-52% of ^{14}C from fungal (whole) mycelium was mineralised during 7 weeks with varied ^{14}C mineralisation rates among the mycelial components (cell wall, cytoplasm, and melanins). Similarly, Drigo *et al*. (2012) have reported that 48% of the ^{13}C was lost within 28 days in soils amended with *Pisolithus* mycelial necromass grown on artificial media under laboratory conditions. This variation with the earlier studies in terms of ^{14}C mineralisation rates from decomposing hyphal necromass is presumably due to the factors such as mycorrhizal species composition, soil characteristics and experimental conditions. In the present study we have inoculated the soil in rhizoboxes with a commercially formulated inoculant consisting of spores of several species including both ecto and arbuscular mycorrhizas (Table 2). However, we made no attempt to test their survival and colonising ability within the root system and their hyphal chemistry. Further, some of the mycelia could also be formed from the mycorrhizae and saprotrophic fungi that are native to that soil (Wallander *et al*., 2001), but we did not measured their relative abundance. The contribution of mycorrhizae and saprophytic fungi can be distinguished using PLFA or molecular markers in future studies (Olsson, 1999).

Modelling of ^{14}C mineralisation: ^{14}C mineralisation from the soils in the R+M and M compartments could be well described by a single exponential decay model with asymptote (r^2> 0.97). This suggests that under experimental conditions described here, substrate ^{14}C acts in 2 fractions with microbial available and non-available pools (Glanville *et al*., 2012). The model implied that most of the available ^{14}C was mineralised within first 30 days with the remainder of the C being relatively stable in soil. However, we cannot calculate the turnover rates for the recalcitrant fraction as the timescales are insufficient. Here the first sampling was done 24 hours after the removal of the aboveground plant components. According to Malik and Haider (1982), 32% of the initial ^{14}C content of hyphal cytoplasm was mineralised in the first 24 hours. Therefore, we have probably missed the initial rapid ^{14}C mineralisation phase. Thus, inclusion of soil ^{14}C mineralisation data during first 24 hours would help in deriving a better model. Conversely, Fernandez and Koide (2014) used a single pool model while studying the decomposition of ectomycorrhizal fungal litter. The contradiction is presumably due to fungal species used, experimental methodology (measured based on accumulated biomass loss in litter bags), and duration (3 months) of the study.

Fate of ^{14}C in soil: While alive, ^{14}C entered into the soil primarily through root and mycorrhizal exudates and necromass after they die. The ^{14}C consists of both soluble and insoluble fractions (Malik and Haider, 1982). Soluble fraction comprises mainly low molecular ^{14}C compounds such as sugars, amino acids etc. that are labile in nature (Jones *et al*., 2004). Conversely, structural compounds are relatively recalcitrant in nature that includes insoluble fractions such as cell walls and melanin (van Hees *et al*., 2005). Although, we did not study the mineralisation of any individual compound in our study, earlier studies suggest glycogen in fungal necromass degrades faster than the lipids (Drigo *et al*., 2012). Hence, the initial ^{14}C mineralisation process (for first 30 days) can be attributed to the microbial mineralisation of the soluble compounds (Boddy *et al*., 2007). However, a fraction of microbially-available ^{14}C could also enter into various pools of microbial biomass or a series of several microbial communities in the soil food chain (Fig. 5), which could be a possible reason for increased ^{14}C recovery in our K_2SO_4 extractions in our study (Fig. 4). Under natural systems leaching could also represent a possible C loss pathway, however, in this study we watered the rhizoboxes in a controlled way to keep the leaching losses to a minimum and no losses by this method wer possible.

The long term persistence of ^{14}C in soil is presumably due to factors such as physico-chemical protection and also microbial turnover (Fig. 5). Physico-chemical protection of ^{14}C in soil is mainly due to formation of soil aggregates with crystalline and amorphous metal oxides and hydroxides (Si^{4+}, Fe^{3+}, Al^{3+}, Ca^{2+}) (Bronick and Lal, 2005). Here we did not measure the metallic cation concentrations in our study. Further, the study was conducted in a physically protected environment where soil disturbance in the microcosms was assumed to be minimal. Consequently, mesofaunal movement could be prevented, which would otherwise affect ^{14}C mineralisation rates significantly (Bradford *et al*., 2007). Therefore, results from our study warrant further research about the hyphal chemistry,

microbial relative abundance, ^{14}C flux through various microbial communities. Although the study was designed to quantify hyphal turnover and their contribution to the total below-ground C, certain flaws in the experimental design severely hampered our detailed understanding of changes in hyphal chemistry. Moreover, some of the fungal structural components are reported to stay in soil for years, whereas the length of our study is only 240 days. Therefore, we have presumably measured ^{14}C mineralisation of labile fractions and further longer duration studies are needed. Nonetheless, the results indicate that hyphal ^{14}C could persist for long times. This study therefore, provides a pathway for future studies in quantifying hyphal contribution to soil organic matter using soil chemical and molecular analytical tools.

Conclusion

Our investigation indicated that in comparison to roots, ^{14}C derived from extramatrical/extraradical hyphae persisted for longer in soil. During the 8 month incubation period only 33% and 48% of the initial ^{14}C present was lost as $^{14}CO2$ in the R+M and M treatments. However, longer duration studies are warranted to explore the detailed changes to hyphal chemistry and relative abundance of the decomposer community. This will enable us to understand the hyphal compounds that are actually contributed to the soil organic matter under long term conditions.

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