

# Tree species' influences on soil carbon dynamics revealed with natural abundance $^{13}\text{C}$ techniques

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## Abstract

**Background and aims** The carbon (C) sequestration potential of land-use practices is increasingly important. Trees sequester atmospheric C into biomass and above and belowground litter but may also prime the decomposition of soil organic matter (SOM). We compared the influence of *Acer pseudoplatanus* (Sycamore) and *Larix x. eurolepis* (Hybrid Larch) on soil C decomposition. **Methods** We used natural abundance  $^{13}\text{C}$  to partition soil-surface  $\text{CO}_2$  efflux into root and SOM sources.  $\text{CO}_2$  was sampled from incubated root-free soil and from live tree roots using in-situ chambers. Combined surface efflux  $\delta^{13}\text{CO}_2$  was measured using dynamic chambers and cavity-ringdown spectroscopy. **Results** Under Sycamore,  $\text{CO}_2$  emissions were dominated (80–90 %) by root respiration. SOM contributed 10–20 % with a mean residence time of centuries. Under Larch, 24–33 % of total  $\text{CO}_2$  efflux was root respiration, the remainder originating from an SOM pool with a turnover time of decades. Total soil C stocks were similar between the two plot types. Root-respired  $\delta^{13}\text{CO}_2$  was consistently different by *c.* 2 ‰ between the species. **Conclusions** The decomposition rate of soil C and its mean residence time are markedly different under the

two tree species. Species differences in root-respired  $\delta^{13}\text{CO}_2$  may reflect plant C allocation or respiratory fractionation.

**Keywords** Tree species differences · Stable isotope partitioning · Chamber · Soil organic matter SOM turnover · Soil carbon mean residence time · Soil respiration · Carbon dioxide emissions · Root respiration · Mycorrhizal strategy · Microbial decomposition · Natural abundance  $^{13}\text{C}$  ·  $\delta^{13}\text{CO}_2$  · *Acer pseudoplatanus* · *Larix eurolepis*

## Introduction

The release of  $\text{CO}_2$  to the atmosphere from microbial decomposition of C in soil organic matter (SOM) is one of the largest fluxes in the global C cycle. It is balanced by primary productivity: plants sequester C from the atmosphere and convert a portion of it to biomass, and add to soil C stocks via aboveground litter and belowground rhizodeposits. It is largely the balance between these two processes that determines whether an ecosystem is a net source of, or sink for, C. The rate at which soil C is decomposed is affected by numerous factors including climate, land-use practices and vegetation type (Raich and Schlesinger 1992, West and Post 2002; Bond-Lamberty and Thomson 2010, Powlson et al. 2011). Managing these fluxes so as to enhance soil C storage presents an opportunity to partly offset rising atmospheric  $\text{CO}_2$  concentrations (Lal 2010).

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Plants can influence soil C cycling by various mechanisms but their interaction with soil occurs predominantly via rhizosphere activity and microbiota, and litterfall. Rhizosphere effects on SOM decomposition rate (the ‘priming effect’; Kuzyakov et al. 2000) have been investigated extensively in laboratory studies and field experiments (with techniques such as trenching and isotope labelling; Kuzyakov 2006). Such effects can be positive or negative and are strongly influenced by many factors including N availability and soil clay content (Drake et al. 2012; Kuzyakov 2002; Sulman et al. 2014). However, measuring priming effects in the field remains technically challenging and it is uncertain to what extent these experimental findings apply to the undisturbed field situation (Kuzyakov 2006).

Among tree species, specific differences in productivity and allometry (Chen et al. 2014), litter chemistry, quantity and quality (Pérez-Harguindeguy et al. 2000), quantity and composition of root exudates (Michalet et al. 2013), associated microfauna (Ayres et al. 2009) and mycorrhizal type (Cornelissen et al. 2001; Phillips et al. 2013) determine the makeup of the free-living soil microbial community and have a decisive influence on the chemical properties of the soil. In forests, one of the main factors associated with differences in soil microbial community is whether trees are coniferous or broadleaved (Prescott and Grayston 2013). It is possible that different tree species will have disparate influences on the decomposition rate of SOM and therefore on the long-term accumulation of soil C stocks.

However, measuring the rate of soil C decomposition is not easy and this hinders the identification (and employment) of land-use strategies that augment soil C storage. Changes in total soil C content can be assessed with long-term field studies but SOM turnover typically occurs over timescales of tens or hundreds of years (Kuzyakov 2006) and it may be decades before significant changes in soil C stock are detectable. Moreover, the stability of soil C is dependent on many factors, such as chemical composition, microbial processing, the extent of biochemical ‘protection’ and soil mineral content (Dungait et al. 2012; Schmidt et al. 2011; Six et al. 2002), so simple increases in bulk soil C content do not necessarily imply long-term stability of the SOM pool. In the field, direct measurement of the CO<sub>2</sub> produced from microbial decomposition of SOM is complicated by the proximate respiration of plant roots. One method of separating root and SOM-derived CO<sub>2</sub> is to use differences in the natural abundance  $\delta^{13}\text{C}$  content of these

components (Graham et al. 2012; Millard et al. 2010; Unger et al. 2010). This technique provides an almost immediate measure of the CO<sub>2</sub> efflux attributable to SOM decomposition ( $R_{\text{H}}$ ) and to autotrophically-fuelled root and rhizosphere respiration ( $R_{\text{A}}$ ). It can be employed in the field to partition the total CO<sub>2</sub> efflux from undisturbed ground ( $R_{\text{S}}$ ), thus overcoming some of the drawbacks of other techniques which physically separate roots and soil (Kuzyakov 2006).

Understanding the effect of different tree species on microbial decomposition of soil C stocks is fundamental to exploiting and managing the C capture capacity of forests. The aim of this study was to use natural abundance  $^{13}\text{C}$  partitioning to compare the rate of microbial respiration of SOM in two different tree species: hybrid Larch (*Larix x. eurolepsis*; a fast-growing and robust deciduous conifer with high value timber due to its resistance to repeated wet/drying cycles) and Sycamore (*Acer pseudoplatanus*; broadleaved deciduous, also fast-growing and producing valuable, easily-worked timber). Our mature forest stands were adjacent to each other and had the same initial soil characteristics, endured the same climate and were of the same age. This meant that we could compare the effects of tree species on soil C cycling in a relatively controlled field setting.

## Methods

The field site was at the James Hutton Institute, Glesnaugh Research Station in north-east Scotland (national grid ref NO665 788; Lat. 56° 53' 58" N, Long. 2° 33' 01" W) where adjacent experimental plantations of Sycamore (*Acer pseudoplatanus*) and Hybrid Larch (*Larix x. eurolepsis*) were established on pasture in 1988 at a plantation density of 2500 trees ha<sup>-1</sup>. The area of each plot is approximately 0.1 ha and both plots are first rotation and uncropped. Tree height is c. 8–10 m in the Sycamore and c. 10–12 m in the Larch. Sycamore associates mostly (although not exclusively) with arbuscular mycorrhizal fungi (Harley and Harley 1987) which access inorganic nutrients mostly from recent plant litter, while Larch associates only with ectomycorrhizal fungi which can liberate nutrients from soil organic matter via the secretion of fungus-derived enzymes. Microclimate conditions are different between the plots because the Larch has a denser canopy; the soil surface here receives less rainfall, wind and sun. The soil is a freely drained cambisol of the Strichen

Association. The experimental site is described in more detail by Sibbald et al. (2001). In August and December 2014 we sampled soil-surface CO<sub>2</sub> efflux and root-respired CO<sub>2</sub> frequently over a 24 h period. The  $\delta^{13}\text{CO}_2$  of SOM-derived CO<sub>2</sub> was sampled in September 2014.

In each plot, soil-surface CO<sub>2</sub> efflux ( $R_S$ ) was measured at four locations using dynamic throughflow chambers (Midwood et al. 2008; Midwood and Millard 2011). Chambers were placed directly on the soil surface (collars were not used (see Snell et al. 2014)) and the chamber edge was embedded a few mm into the soil; chambers were then weighted to ensure that a good seal with the ground was maintained. Any surface litter was removed before placing the chambers. The [CO<sub>2</sub>] inside the chambers was controlled at 400  $\mu\text{mol mol}^{-1}$  by automated regulation of the inflow of CO<sub>2</sub>-free air and outflow of sample air. Once [CO<sub>2</sub>] had stabilised, CO<sub>2</sub> flux was calculated from the chamber area and the flow rate of gas through the chamber. Four chambers were controlled by a management system comprised of pumps, mass flow controllers, IRGA, datalogger and laptop. Sample air from the chambers was analysed in the field by wavelength-scanned, cavity ringdown spectroscopy (CRDS) (Picarro G1101-i; Picarro Inc., Santa Clara, CA, USA). The chambers were sampled sequentially for approximately 10 min each, this allows time for the isotope analysis to stabilise. Three minutes of stable data were extracted to calculate the sample mean  $\delta^{13}\text{CO}_2$ . This setup of four chambers, management system and CRDS was replicated in the Sycamore and Larch plots allowing simultaneous measurements to be made. To account for analytical drift and to ensure comparability between the two analysers, air from a gas cylinder of known [CO<sub>2</sub>] and  $\delta^{13}\text{CO}_2$  (535  $\mu\text{mol mol}^{-1}$ ; -34 ‰) was analysed by both CRDS instruments every 40 min throughout the sampling periods to act as a reference. These data were used to correct the CRDS sample analyses to a standard value, defined by IRMS analysis of the same cylinder gas. CRDS sample analyses were also corrected for [CO<sub>2</sub>]-dependent, non-linearity of the CRDS instruments (Snell et al. 2014).

Root-respired CO<sub>2</sub> ( $R_A$ ) was sampled from live roots in situ using three purpose-built chambers in each plot. For a full description of the chambers see Snell et al. (2015). Briefly, the soil from a  $\sim 30 \times 30$  cm area was carefully excavated from around the tree roots, a chamber was then installed around a layer of roots and

flushed with CO<sub>2</sub>-free air to remove any atmospheric air, ensuring that any CO<sub>2</sub> that accumulated in the chamber was derived from root-respiration. Chamber headspace gas was sampled every 4–5 h into triplicate evacuated exetainers and following each sampling the chambers were again flushed with CO<sub>2</sub>-free air. A correction factor of -1.4 ‰ was applied to the  $\delta^{13}\text{CO}_2$  measurement of root chamber samples to account for unavoidable contamination of atmospheric air when sampling from chambers at ambient pressure into evacuated exetainers (Snell et al. 2015). In August, chambers were installed in the morning and sampling commenced later that day; in December they were installed one day prior to sampling and were flushed with CO<sub>2</sub>-free air before sampling began. Respiration rate of the root sample in the chamber can be estimated from the known chamber volume and the rate of CO<sub>2</sub> accumulation. Samples were returned to the James Hutton Institute, Aberdeen for analysis by isotope ratio mass spectrometry (IRMS) (Thermo Delta Plus<sup>XP</sup>, interfaced to a Gas Bench II (Thermo, Bremen, Germany) and PAL Autosampler (CYC Analytics, Zwingen, Switzerland)) within 5 days.

The  $\delta^{13}\text{CO}_2$  of microbial respiration of SOM ( $R_H$ ) was estimated as described in Snell et al. (2014). Soil disturbance rapidly changes the microbially respired  $\delta^{13}\text{CO}_2$  (Zakharova et al. 2014) and this change can be modelled and regressed to estimate the pre-disturbance  $\delta^{13}\text{CO}_2$  (Snell et al. 2014). Over three consecutive days in September, soil was sampled from eight locations in the Larch plot and six in the Sycamore. Soil was excavated from an area approximately 25 × 25 cm and 20 cm deep, separated from roots by hand and placed into a gastight Tedlar bag. The air in the bag was repeatedly evacuated and replaced with CO<sub>2</sub>-free air to remove CO<sub>2</sub>. The soil was then left to incubate for a c. 15 min until soil-respired CO<sub>2</sub> had accumulated in the bag at a concentration adequate for isotopic analysis (at least 400  $\mu\text{mol mol}^{-1}$ ). The bag headspace gas was then sampled into triplicate evacuated exetainers and the flushing and incubation procedure was repeated. Each soil incubation was sampled in this way four or five times over a 90 min period. CO<sub>2</sub> samples were returned to the James Hutton Institute, Aberdeen, for carbon isotope analysis by IRMS within 24 h. For regression of the change in respired  $\delta^{13}\text{CO}_2$ , the first CO<sub>2</sub> sample from each soil incubation was removed from the dataset because these can be slightly contaminated with atmospheric air (Snell et al. 2014).

Soil-surface CO<sub>2</sub> efflux (R<sub>S</sub>) is partitioned into its autotrophic (root-derived, R<sub>A</sub>) and heterotrophic (SOM-derived, R<sub>H</sub>) components using the following equations

$$f_{RH} = \frac{\delta^{13}C_{RS} - \delta^{13}C_{RA}}{\delta^{13}C_{RH} - \delta^{13}C_{RA}}$$

and

$$f_{RA} = 1 - f_{RH}$$

where  $\delta^{13}C_{RS}$ ,  $\delta^{13}C_{RA}$  and  $\delta^{13}C_{RH}$  are the isotopic signatures of total soil-surface efflux, root respiration and SOM derived respiration;  $f_{RH}$  and  $f_{RA}$  are respectively the fractions of total soil-surface efflux attributable to SOM derived and root respiration. Heterotrophic flux is the product of  $f_{RH}$  and total soil-surface flux, R<sub>S</sub>. The uncertainty (variance) on fractional estimates of  $f_{RA}$  and  $f_{RH}$  contributions was estimated taking into account the variance of both the total efflux and end-member  $\delta^{13}CO_2$  measurements (Phillips and Gregg 2001). The standard error of the partitioned CO<sub>2</sub> flux rate estimates was then calculated from the estimates of  $f_{RA}$  (or  $f_{RH}$ ) and its variance, the variance in the total efflux rate, and the covariance between  $f$  and efflux rate (as measured across the seven, 3 to 4 h-long sampling periods within each plot and season).

Local soil temperature at ~7 cm depth was monitored at two or three locations in each plot by thermistors connected to the chamber management system. Climate and soil moisture were recorded at an automated weather and soil monitoring station approximately 1 km north of the site.

Soil C content was calculated from data collected by Beckert et al. (2015) in 2012, 24 years after tree plots were established. They sampled soil from the A (0–15 cm) and B (15–30 cm) horizons in three locations in each plot. Total soil carbon content (as a percentage of dry weight) was measured by an automated Dumas combustion procedure (Flash 2000 Elemental Analyser, Thermo Scientific, Bremen, Germany) at the James Hutton Institute, Aberdeen. Total C as tonnes of C per hectare was calculated from % C and soil bulk density, adjusted to account for the volume of stones. A and B horizon C content were then summed to calculate total C stock for each plot. According to the conventional propagation of error formula, the variances of the C measurements in each horizon ( $n = 3$ ) were used to calculate the uncertainties on the total C values.

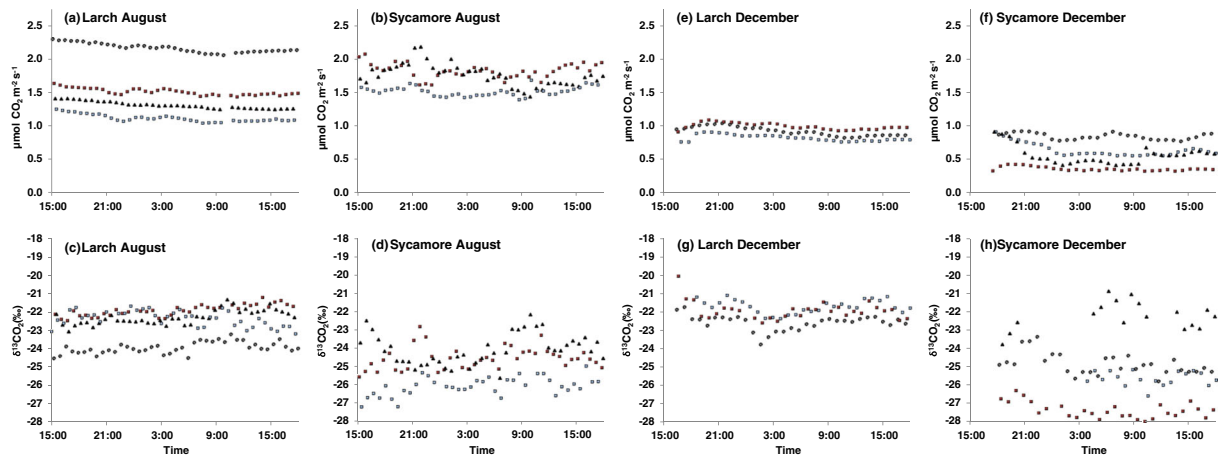
Soil CO<sub>2</sub> efflux rates and  $\delta^{13}CO_2$  values between plots and seasons were compared using T-tests. Total soil C content of Larch, Sycamore and pasture plots was assessed using AnOVA and post-hoc Tukey tests. These analyses, and regression of change in incubated SOM-respired  $\delta^{13}CO_2$  over time, were carried out using Microsoft Excel (Microsoft Office Enterprise 2007, Microsoft Corporation, Redmond, WA 98052, USA) or Minitab 17 (Minitab Inc, Coventry, UK).

## Results

In August, mean CO<sub>2</sub> efflux from the soil-surface was similar between the Sycamore ( $1.71 \pm 0.096 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $n = 3$ ) and Larch ( $1.54 \pm 0.226 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $n = 4$ ) plots. In December, soil-surface efflux was significantly slower ( $P = 0.03$ , one-tailed t-test) in the Sycamore ( $0.60 \pm 0.102 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $n = 4$ ) than the Larch ( $0.89 \pm 0.050 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $n = 3$ ) (Fig. 1). Soil-surface efflux  $\delta^{13}CO_2$  was significantly different between the plots in both seasons ( $P = 0.027$  in August,  $P = 0.038$  in December, one-tailed t-tests), being 2–3 ‰ more depleted in the Sycamore plot (Fig. 1). Within tree species, soil-surface efflux  $\delta^{13}CO_2$  was similar between seasons. In the Larch plot it was  $-22.6 \text{ ‰}$  ( $\pm 0.434$ ) in August and  $-22.0 \text{ ‰}$  ( $\pm 0.253$ ) in December; in the Sycamore it was  $-24.6 \text{ ‰}$  ( $\pm 0.585$ ) in August and  $-24.9 \text{ ‰}$  ( $\pm 1.04$ ) in December (Fig. 1).

Root respired  $\delta^{13}CO_2$  was also similar between sampling seasons and different between the tree species (Fig. 2). Root-respired  $\delta^{13}CO_2$  of Larch was  $-27.4 \text{ ‰}$  ( $\pm 0.461$ ) in August and  $-27.6 \text{ ‰}$  ( $\pm 0.254$ ) in December; Sycamore was  $-25.4 \text{ ‰}$  ( $\pm 0.303$ ) in August and  $-25.5 \text{ ‰}$  ( $\pm 0.062$ ) in December. Differences between the plots were significant in both seasons ( $P = 0.020$  in August and  $P = 0.008$  in December). There were clear differences in root mass and morphology between the two tree species. Sycamore roots were denser and had large clusters of highly branched root tips; Larch roots were less abundant and straighter with less branching. Within the 24 h sampling periods, variation in root-respired  $\delta^{13}CO_2$  was significant only in the Larch plot in August ( $P < 0.001$ ).

Change in SOM  $\delta^{13}CO_2$  following excavation was best described by a logarithmic regression (Fig. 3), which gave a higher R<sup>2</sup> value (0.42) than linear regression models. When the data were separated by tree

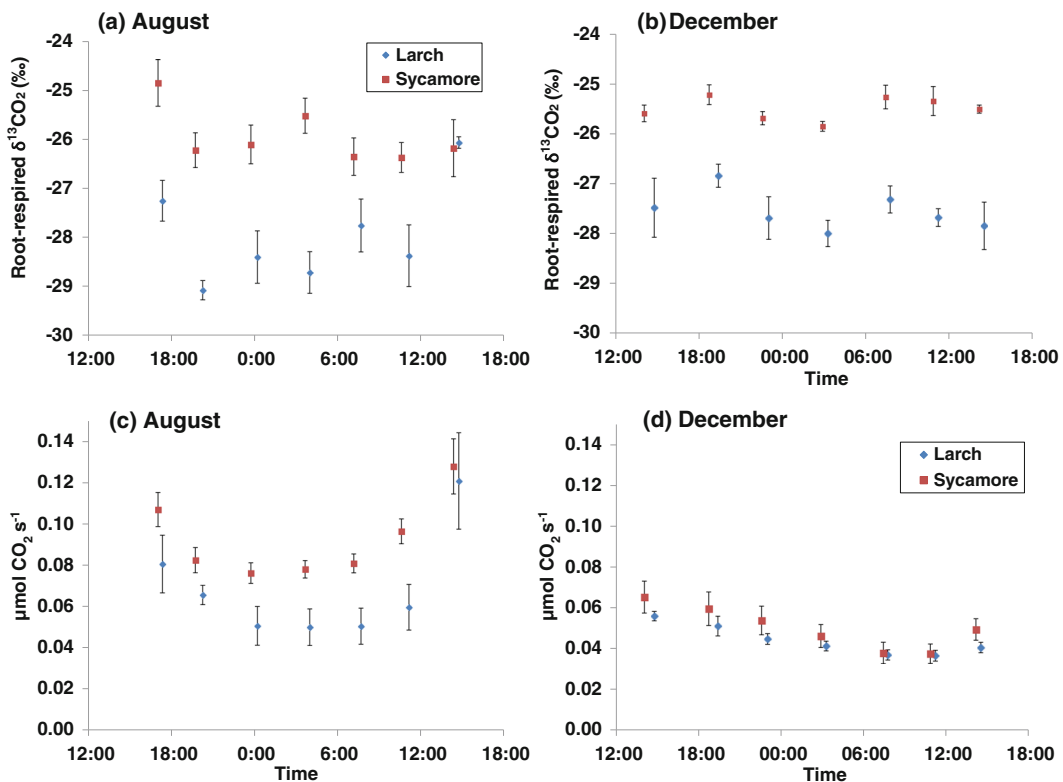


**Fig. 1** Soil surface CO<sub>2</sub> efflux rate (top panels **a, b, e & f**) and  $\delta^{13}\text{CO}_2$  (bottom panels **c, d, g & h**) in adjacent Sycamore and Larch plantations over 24 h in August (**a, b, c & d**) and December

(**e, f, g & h**). Different symbols represent the three or four locations sampled in each plot in each month

species the intercept estimates were not significantly different so all the data were combined to derive one SOM  $\delta^{13}\text{CO}_2$  end-member estimate for the site:

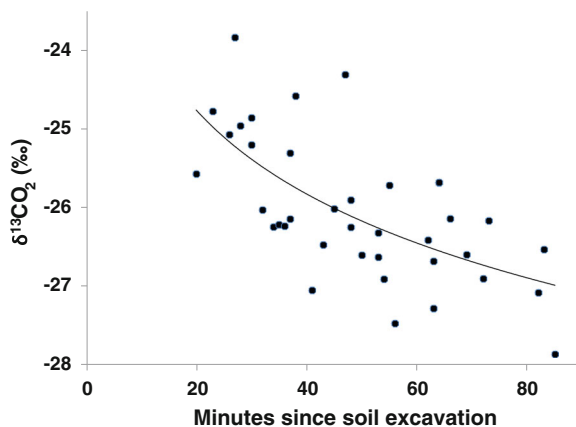
$-20.2 \pm 1.15 \text{ ‰}$  (Fig. 3). This estimate is close to that derived from a much larger dataset at a nearby site (Snell et al. 2014).



**Fig. 2** Root-respired  $\delta^{13}\text{CO}_2$  from Sycamore (squares) and Larch (diamonds) trees measured over 24 h in **a** August and **b** December using purpose-built root chambers. Panels **c** and **d** show

concurrent CO<sub>2</sub> respiration rates of the root samples contained in the chambers. Points are means of three replicate root chambers and error bars are standard error





**Fig. 3**  $\delta^{13}\text{CO}_2$  of heterotrophic respiration of root-free soil as measured from 14 samples of incubated soil from the study site.  $\text{CO}_2$  was sampled from each soil incubation several times over 90 min. The fitted line is a logarithmic regression,  $y = -1.539 \ln(x) - 20.16$  of which  $R^2 = 0.425$ . The intercept ( $\pm$  standard error) of this line is the SOM end-member estimate for the site,  $-20.2 (\pm 1.15) \text{‰}$

Soil temperature and local moisture conditions were fairly constant during the August sampling period (Fig. 4). They were slightly more variable in December when soil temperature changed by  $1.4 \text{ °C}$  (Larch) and  $3 \text{ °C}$  (Sycamore), and overnight rain caused the soil moisture content to increase by c. 2 %. Temperature variability was more pronounced in the Sycamore plot where the canopy is less dense and conferred less shelter from wind and sun. Soil moisture was measured at 10 locations in each of the study plots on the 9th November and was significantly higher ( $P = 0.002$ ) in the Sycamore plot ( $54 \pm 2.4 \%$  ( $n = 9$ , one outlier removed)) than the Larch ( $42 \pm 2.2 \%$ ); this was in keeping with our observations that throughfall of rain was greater in the Sycamore plot than the Larch.

The partitioned  $\text{CO}_2$  efflux derived from SOM C was greatest in the Larch plot, comprising 67 % (August) and 76 % (December) of the total efflux. Autotrophically fuelled respiration dominated in the Sycamore plot, accounting for 83 % (August) and 89 % (December) of total respiration (Fig. 5).

Estimates of total soil C stock (Beckert et al. 2015) are  $69.9 \pm 4.3 \text{ t C ha}^{-1}$  in the Larch plot,  $88.7 \pm 4.4 \text{ t C ha}^{-1}$  in the Sycamore plot, and  $56.7 \pm 7.3 \text{ t C ha}^{-1}$  in an adjacent, unforested pasture plot (control). Across three replicate plots spread over several hectares at the same site, there were no significant differences in soil C stock between tree species; mean soil C stock values were  $82.0 \pm 4.29$  (Larch),  $76.6 \pm 4.36$  (Sycamore) and

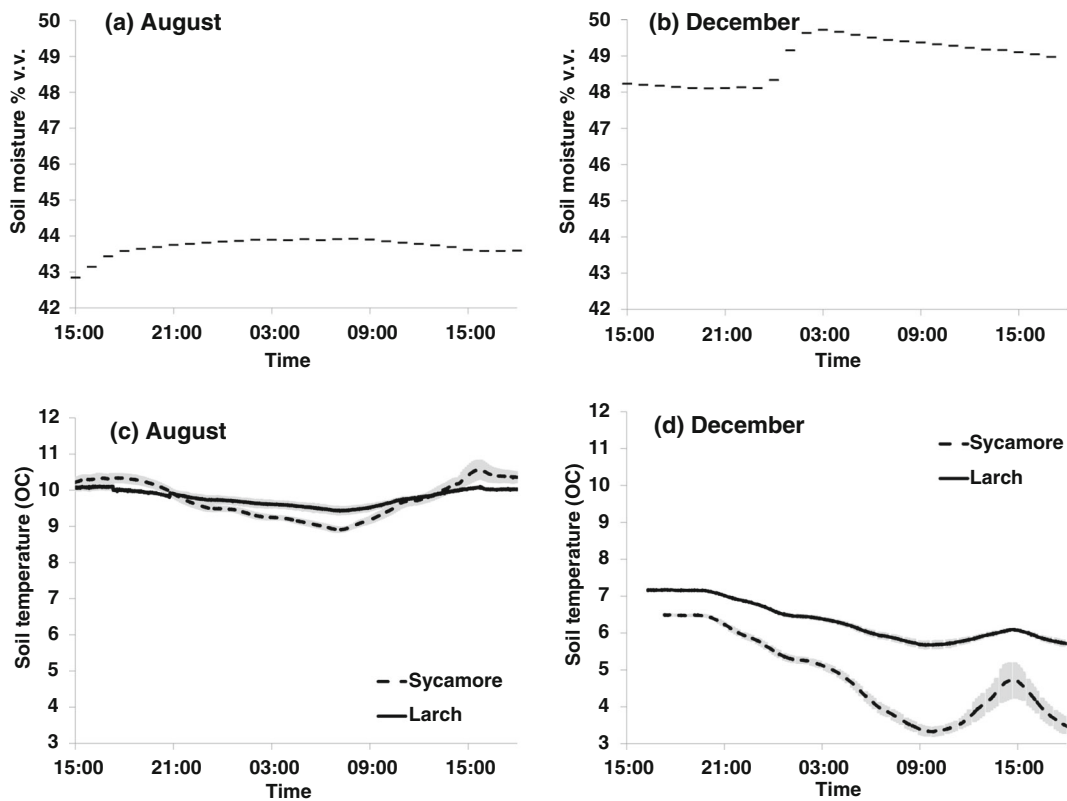
$65.7 \pm 5.88$  (pasture)  $\text{t C ha}^{-1}$  (Beckert et al. 2015). We used the soil C stock data and our measurements of heterotrophic respiration rate of SOM to estimate the mean residence times (MRTs) of the SOM C pool. These MRT estimates (which assume that the soil C pool is at steady-state) are of the order of decades in the Larch plot and centuries in the Sycamore plot (Table 1).

## Discussion

Rising atmospheric  $\text{CO}_2$  concentrations mean that it is imperative that the carbon sequestration potentials of land-use practices are maximised. Natural abundance stable isotope partitioning of the soil-surface  $\text{CO}_2$  efflux shows that heterotrophic decomposition of SOM is greater under Larch trees than Sycamore trees when these species are of similar age and grown under near-identical field conditions. Larch roots appear to prime the decomposition of SOM to a much greater extent than Sycamore roots. Although overall rates of soil-surface  $\text{CO}_2$  efflux were similar between the two plots, we measured a much greater contribution of autotrophically fuelled respiration (80–90 %) in the Sycamore plot, whereas in the Larch, two-thirds to three-quarters of the total  $\text{CO}_2$  efflux was attributable to SOM-derived carbon. In winter (after leaf and needle fall), root respiration was only slightly reduced and the difference in below-ground heterotrophic respiration rate between the two tree species was just as pronounced as in summer.

## Soil C stock

If all other soil C inputs and outputs (rhizodeposition, litterfall, DOC runoff etc.) were equal between the plots then a lower rate of SOM decomposition in the Sycamore plot would eventually lead to a measurable increase in the soil C content. Sycamore plot soil C stock was greater than that in the Larch plot (and adjacent unforested pasture) but these conclusions are based on spot measurements from our single study plots ( $n = 1$ ) and as such do not necessarily indicate that soil C stock in the Sycamore plot has increased over time. In a wider study of three replicate blocks of plots across the Glenshagh site, mean total soil C stocks are not significantly different between Larch, Sycamore and control plots (unforested pasture) (Beckert et al. 2015; Keith et al. 2014). So despite marked differences in our

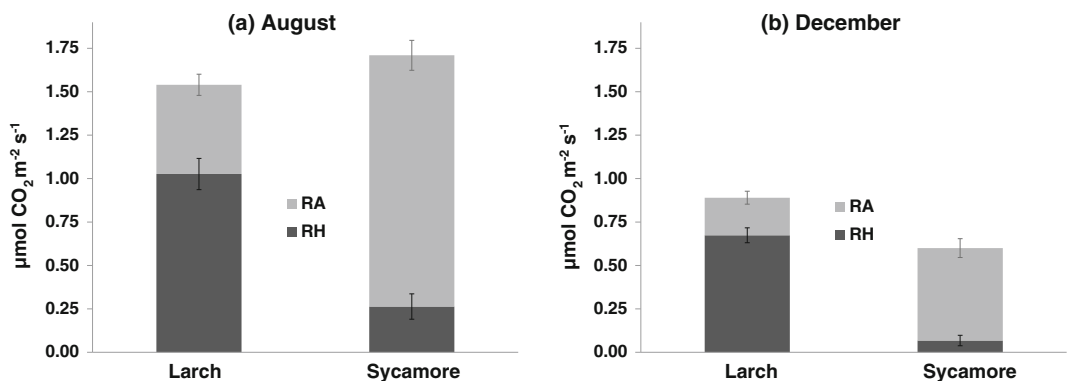


**Fig. 4** Soil moisture content (a, b) during the sampling periods, as measured at an automated monitoring station approximately 1 km north of the study site. Soil temperature at 7 cm depth (c, d) was

measured in the study plots at two (Larch) or three (Sycamore) locations; lines are means and grey shading is standard error

measurements of  $R_H$  between the plots we cannot conclude that these have caused a difference in overall soil C content. Possible reasons for this include 1) spatial variation in soil C content combined with low replicate number ( $n = 3$ ) obscuring the effect of tree type on soil C stock; 2) measurements of  $R_H$  in our study plots may not

necessarily be generalisable, and factors other than tree type (eg. DOC runoff, soil mineralogy) determine soil C content in some or all plots; 3) other C inputs/outputs (eg. litterfall, root turnover, DOC runoff) to the soil are different between the tree species and these counteract the difference in SOM decomposition rate.



**Fig. 5** Autotrophically fuelled ( $R_A$ , light grey bars) and SOM derived ( $R_H$ , dark grey bars) CO<sub>2</sub> efflux rates in adjacent Sycamore and Larch plots in a August and b December, estimated

using natural abundance <sup>13</sup>C partitioning of total soil-surface CO<sub>2</sub> efflux, as measured over 24 h periods. Error bars are standard error of the estimates

**Table 1** Estimated mean residence times of the SOM carbon pool based on our partitioned estimates of SOM-derived CO<sub>2</sub> efflux (R<sub>H</sub>) and measurements of the soil carbon stock in the A (5–15 cm depth) and B (35–45 cm) horizons in these plots (M. Beckert pers comm.). Estimates of mean residence time assume that the soil C

stock is unchanging. This assumption is concurrent with the findings of Beckert et al. (2015) that there is no difference in soil C stock between tree types and pasture control plots at the site (see Results)

	Larch		Sycamore	
	August	December	August	December
Partitioned SOM-derived (R <sub>H</sub> ) efflux (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	1.03	0.67	0.26	0.07
R <sub>H</sub> CO <sub>2</sub> efflux (t C ha <sup>-1</sup> y <sup>-1</sup> )	3.90	2.54	0.98	0.27
Mean total soil C stock (t C ha <sup>-1</sup> (± S.E.))	69.9 (±4.3)		88.7 (±4.4)	
Estimated residence time of SOM pool (years)	18	28	90	335
Mean estimated residence time of SOM pool (years)	23		213	

### Carbon cycling

R<sub>A</sub> and R<sub>H</sub> differed markedly between the tree species and the associated mean residence times of soil C differed by an order of magnitude. Possible causes of the C cycling differences between the two tree species are discussed here. That R<sub>A</sub> flux under Sycamore was greater than under Larch is in keeping with the greater abundance of roots and the greater proportion of fine roots and tips in the Sycamore; fine roots respire relatively more than larger roots per unit biomass (Chen et al. 2009; Marsden et al. 2008). If rhizosphere priming effects were similar between the tree species then we would expect greater root mass to also be associated with a greater amount of rhizosphere-primed heterotrophic respiration in the Sycamore plot. However, we measured a higher rate of R<sub>H</sub> under Larch and we hypothesise that this was due to the different mycorrhizal associations of the two species.

Sycamore roots associate with arbuscular mycorrhizal (AM) fungi and Larch with ectomycorrhizal (ECM) fungi (Churchland and Grayston 2014). The (positive) rhizosphere effect on soil microbial activity and SOM decomposition is usually greater for ECM tree species than for AM tree species (Dijkstra and Cheng 2007; Phillips and Fahey 2006). ECM fungi have broader and more vigorous enzymatic abilities, producing proteolytic and lignolytic enzymes that enable them to decompose SOM, including the more recalcitrant fractions (Brzostek and Finzi 2011; Churchland and Grayston 2014), thereby increasing the availability of labile, low molecular weight organic fractions such as amino acids (Brzostek et al. 2013). In comparison, AM

fungi are less able to degrade SOM and instead produce enzymes that access inorganic N forms while encouraging rapid organic matter decomposition by priming the activity of free-living saprotrophs (Brzostek et al. 2013; Cheng et al. 2012). Consequently the lighter fraction of soil (which represents more recent plant material and younger SOM) is decomposed more quickly in AM deciduous sites, while the heavier fraction of soil (older SOM that is more thoroughly stabilised by being bound to mineral surfaces) accounts for more of the respired CO<sub>2</sub> in ECM, coniferous sites (Crow et al. 2006). A synthesis of these ECM/AM differences is proposed by Phillips et al. (2013) whereby AM species, such as Sycamore trees, host a fast cycling, largely inorganic, soil ‘nutrient economy’ in which plant litter is rapidly mineralised with little concurrent interaction with the older SOM pool. By contrast, in the organic nutrient economy of ECM dominated systems, such as those of Larch, fungal and plant litter decomposes slowly on average and organic N and P are continually mined from the SOM pool (Cornelissen et al. 2001; Phillips et al. 2013).

At our site, large quantities of first order roots and tips in the Sycamore plot meant that there was a large volume of fungal rhizosphere to fuel the rapid breakdown of recent plant-derived organic matter (such as rhizodeposits and above-ground litter). The δ<sup>13</sup>C<sub>CO<sub>2</sub></sub> of mineralised plant organic material would have been similar to that of root respiration since both were derived from recent plant photosynthate. A different situation occurs under Larch where there was a significant standing stock of litter (Beckert et al. 2015) decomposing slowly into the SOM carbon pool, whence it is



remobilised by ECM fungi which have the enzymatic capability to access nutrients (N and P) in SOM. C mineralised from this SOM pool has a  $\delta^{13}\text{C}$  signature distinct from that of Larch root respiration.

So in this instance, where organic matter is cycled rapidly under Sycamore trees, the natural abundance stable isotope partitioning approach has apportioned total soil  $\text{CO}_2$  efflux in this plot according to the age of the carbon being respired, rather than its root-respired or organic matter origin. Therefore, our estimate of  $R_A$  in the Sycamore plot probably includes  $\text{CO}_2$  respired from the decomposition of plant material, which takes place rapidly in this plot. This will not be the case under Larch, where organic matter decomposes much more slowly and consequently has a  $\delta^{13}\text{C}$  signature distinct from that of root respiration. One of the challenges of trying to quantify C transitions within plant-soil systems is that often there is not a clear demarcation between C pools (Kuzyakov 2006).

Other factors dependent on tree species are also likely to influence C cycling. For example, the depth distribution of soil C varies between the two tree species (both in our study plots and across the site). Under Sycamore there is more C in the B horizon while under Larch there is more in the surface soil (Beckert et al. 2015). This might be a result of tree-species-dependent faunal bioturbation (Reich et al. (2005) found that soil under *Acer pseudoplatanus* supported the greatest earthworm biomass of 14 tree species while *Larix decidua* supported almost none), or different rooting profiles of the two tree species, which would deposit carbon at different depths. Differences may also arise due to abiotic soil conditions of temperature and moisture which are determined by the amount of shade and rain throughfall provided by the different tree canopies.

Because litter type (its 'recalcitrance' or chemical quality), does not definitively determine decomposition rates (Schmidt et al. 2011; Six et al. 2002), and because our two study plots are of the same soil type, it is likely that the differences we observed in soil C cycles were strongly influenced by tree species effects such as those discussed above rather than by litter quality or soil properties. Such differences in soil C cycling characteristics may result in soil C stores that are not only different in quantity and distribution in the soil profile, but also in their physical and chemical properties, which could then determine their susceptibility to decomposition under future changes in land-use or climate.

## Root-respired $\delta^{13}\text{C}$

We saw strongly significant differences in root-respired  $\delta^{13}\text{C}$  of the two tree species. The  $\delta^{13}\text{C}$  of root respiration is not well studied and little is known about possible isotopic fractionation of carbon substrates during respiration (Werth and Kuzyakov 2010). The  $\delta^{13}\text{C}$  values of different plant tissues, cell types and metabolites can vary widely relative to that of atmospheric  $\text{CO}_2$ , being subject to numerous isotopically fractionating processes. The differences we saw in root-respired  $\delta^{13}\text{C}$  might be caused by species-specific differences in water use efficiency or the many physiological pathways that can cause fractionation of plant C (Lambers et al. 2008; Bowling et al. 2008). Or they may be due to the roots respiring different carbon substrates (i.e. recent photosynthate or starch reserves) that have distinct  $\delta^{13}\text{C}$  values (Bowling et al. 2008; Brüggemann et al. 2011). Rhizoplane (root surface) bacteria from Sycamore and Larch show distinct patterns of carbon utilisation, indicating that the carbon supplied to them by roots differs between the tree species (Grayston 2000). This suggests that root respiratory substrate type is a likely cause of the differences in root-respired  $\delta^{13}\text{C}$ , though it may be the case that bacterial carbon utilisation reflects only differences in exudate properties unrelated to root respiratory substrate.

Another possibility is that the isotope signature of root-respired  $\text{CO}_2$  is partly controlled by mycorrhizal requirements. ECM fungi influence the chemical composition of root exudates (Churchland and Grayston 2014; Johansson et al. 2009), and this could define the  $\delta^{13}\text{C}$  of carbon substrates remaining available for root respiration, thus making the  $\delta^{13}\text{C}$  values of root and hyphal respiration distinct. However, if this occurs it does not seem to be evident as differences in the  $\delta^{13}\text{C}$  of root and ECM hyphal material, which in general are  $^{13}\text{C}$ -enriched relative to needles or leaves by approximately the same degree, c. 2 ‰ (Bowling et al. 2008; Hogberg et al. 1999). Furthermore, in *Larix gmelinii*, ECM hyphal sheaths constitute over 30 % of root tip biomass and this amount decreases with increasing root order;  $\delta^{13}\text{C}$  however is unchanged across root orders indicating that the C isotope composition of roots and fungi is not different (Ouimette et al. 2013). This evidence indicates that ECM fungi are probably not the main determinants of the  $\delta^{13}\text{C}$  of root respiration.

AM fungal material can be 1.5 ‰ more  $^{13}\text{C}$ -depleted than root material (Staddon et al. 1999) so it is plausible that their respired  $\delta^{13}\text{CO}_2$  signatures are also different. In this case, the measured  $\delta^{13}\text{CO}_2$  of  $R_A$  would depend on whether hyphal respiration was included or excluded from the incubated root sample. AM fungal filaments probably became detached from our incubated root sample when soil was excavated, so their influence on autotrophically respired  $\delta^{13}\text{CO}_2$  will have been excluded from our measurements. Mycelial respiration can be significant, accounting for around a third of ‘root’ respiration (Söderström and Read 1987) or a quarter of total soil-surface  $\text{CO}_2$  efflux (Heinemeyer et al. 2012). If we assume firstly that respired  $\delta^{13}\text{CO}_2$  from roots and hyphae reflects that of bulk material and differs by 1.5 ‰ (Staddon et al. 1999), and secondly that hyphal respiration accounts for 30 % of ‘root’-respiration, then we can calculate that our measured  $\delta^{13}\text{CO}_2$  of root-respiration (hyphal respiration excluded) was more  $^{13}\text{C}$ -enriched by 0.45 ‰ than had it included hyphal respiration. This potential overestimation of sycamore root  $\delta^{13}\text{CO}_2$  by 0.45 ‰ does not account for the *c.* 2 ‰  $^{13}\text{C}$ -enrichment that we observed of Sycamore root respiration in relation to Larch. This indicates that factors other than mycorrhizal type probably contribute to tree species differences in root-respired  $\delta^{13}\text{CO}_2$ , a likely candidate being respiratory substrate as discussed above. An error of 0.45 ‰ would have decreased our estimates of  $fR_A$  by 0.08, making a minimal difference to our conclusions concerning either root-respired  $\delta^{13}\text{CO}_2$  or carbon cycling in these two plots.

Within species, there were short-term variations in root-respired  $\delta^{13}\text{CO}_2$  though differences were only significant in the Larch plot in August. These changes, and diurnal variation in the root respiration rate, had no discernible effect on concurrent soil-surface efflux or  $\delta^{13}\text{CO}_2$ . This indicates that short-term variations in root respiration are damped by the large reservoir of soil  $\text{CO}_2$  and that the soil-surface efflux does not necessarily reflect short-term changes in root respiration. For stable isotope partitioning, it may be important to accommodate variation in root-respired  $\delta^{13}\text{CO}_2$  by sampling at more than one time period in order to achieve a thoroughly representative mean value of  $\delta^{13}\text{CO}_2$ . The differences we observed between sampling times within

24 h periods give rise to variations in the estimate of  $fR_A$  of up to 0.32.

## Conclusions

Natural abundance stable isotope partitioning of soil-surface  $\text{CO}_2$  efflux can be used to quantify root and SOM-derived respiration in the field. Our findings indicate that under Larch trees there is decadal turnover of a more integrated pool of soil C as plant litter is decomposed to SOM and then mineralised to  $\text{CO}_2$ , while under Sycamore there is more rapid turnover of recent organic matter (leaf, root and hyphal litter) and correspondingly slow decomposition of an older SOM pool with a mean turnover time of centuries. However these differences were not apparent in the total soil C contents of plot types across the site. The  $\delta^{13}\text{CO}_2$  of different tree species root respiration is distinct and is variable over short timescales (hours) but is constant seasonally. The magnitude and isotopic composition of soil-surface  $\text{CO}_2$  efflux is not responsive to short-term variations in root respiration rate or  $\delta^{13}\text{CO}_2$ . If our findings are widely generalisable, they provide experimental evidence that co-existing tree species can have contrasting influences on soil carbon dynamics, but not necessarily on soil carbon stocks.

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