Tree Physiology 33, 743–752
doi:10.1093/treephys/tpt049

Research paper

# Carbon dioxide emitted from live stems of tropical trees is several years old

Jan Muhr[1,6], Alon Angert[2], Robinson I. Negrón-Juárez[3], Waldemar Alegria Muñoz[4], Guido Kraemer[4], Jeffrey Q. Chambers[5] and Susan E. Trumbore[1]

[1]Department of Biogeochemical Processes, Max-Planck Institute for Biogeochemistry, Jena 07745, Germany; [2]The Institute of Earth Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel; [3]Ecology and Evolutionary Biology, Tulane University, 400 Lindy Boggs, New Orleans, LA 70118, USA; [4]Universidad Nacional de la Amazonía Peruana, Facultad de Ciencias Forestales, Calle Pevas 584, Iquitos, Peru; [5]Lawrence Berkeley National Laboratory, Climate Sciences Department, Cyclotron Rd MS 50-4037, Berkeley, CA 94720, USA

[6]Corresponding author (jmuhr@bgc-jena.mpg.de)

Received January 24, 2013; accepted June 24, 2013; published online July 25, 2013; handling Editor Daniel Epron

Storage carbon (C) pools are often assumed to contribute to respiration and growth when assimilation is insufficient to meet the current C demand. However, little is known of the age of stored C and the degree to which it supports respiration in general. We used bomb radiocarbon ($^{14}C$) measurements to determine the mean age of carbon in $CO_2$ emitted from and within stems of three tropical tree species in Peru. Carbon pools fixed >1 year previously contributed to stem $CO_2$ efflux in all trees investigated, in both dry and wet seasons. The average age, i.e., the time elapsed since original fixation of $CO_2$ from the atmosphere by the plant to its loss from the stem, ranged from 0 to 6 years. The average age of $CO_2$ sampled 5-cm deep within the stems ranged from 2 to 6 years for two of the three species, while $CO_2$ in the stem of the third tree species was fixed from 14 to >20 years previously. Given the consistency of $^{14}C$ values observed for individuals within each species, it is unlikely that decomposition is the source of the older $CO_2$. Our results are in accordance with other studies that have demonstrated the contribution of storage reserves to the construction of stem wood and root respiration in temperate and boreal forests. We postulate the high $^{14}C$ values observed in stem $CO_2$ efflux and stem-internal $CO_2$ result from respiration of storage C pools within the tree. The observed age differences between emitted and stem-internal $CO_2$ indicate an age gradient for sources of $CO_2$ within the tree: $CO_2$ produced in the outer region of the stem is younger, originating from more recent assimilates, whereas the $CO_2$ found deeper within the stem is older, fueled by several-year-old C pools. The $CO_2$ emitted at the stem–atmosphere interface represents a mixture of young and old $CO_2$. These observations were independent of season, even during a time of severe regional drought. Therefore, we postulate that the use of storage C for respiration occurs on a regular basis challenging the assumption that storage pools serve as substrates for respiration only during times of limited assimilation.

*Keywords*: bomb radiocarbon $^{14}C$, non-structural carbohydrates, storage carbon pools, tree respiration

## Introduction

Trees allocate carbon (C) to a number of different processes including growth, respiration, reproduction and storage (Chapin et al. 1990). Storage, in this context, is the sum of any non-structural carbohydrates (NSC) pools within the plant that can be remobilized for other processes, e.g., respiration or growth (i.e., transformation into structural carbohydrates). By definition, storage includes not only reserve NSC pools but also recycling, for example, of defense compounds. Although storage in trees has been investigated for a long time, our understanding of when and to what extent storage NSC pools

are mobilized still is limited, and storage NSC pools are often not accounted for in ecosystem-level C budgets (Chapin et al. 1990, Le Roux et al. 2001, Körner 2003, Trumbore 2006, Litton et al. 2007). It is a common assumption, reflected in many of the current models of tree growth, that trees preferentially use recently assimilated C for growth and respiration (Le Roux et al. 2001). From this perspective, storage C is used only when current assimilation becomes insufficient to meet current C demand (Sala et al. 2010). This perspective has been questioned based on recent findings using continuous in situ $^{13}C$ labeling (Keel et al. 2006), which indicate the use of storage reserves during the growth of new tissues, but not the degree to which these contribute to plant respiration fluxes. Kuptz et al. (2011) also used $^{13}C$ labeling to demonstrate that respired $CO_2$ originates partly from current assimilation and partly from pre-label NSC storage pools. These studies showed the use of pre-label NSC in studies that ranged from weeks up to 4 years. However, it is impossible to make any prediction about whether storage C considerably older than this exists and to what degree it is used as a substrate for respiration. Further, all measurements to date are in temperate forest trees, with no data from tropical forests. Improved understanding of C turnover in trees requires better information on the age of storage reserves (Richardson et al. 2012) and how and when these reserves are used to support plant respiration.

'Bomb-radiocarbon', i.e., radiocarbon ($^{14}C$) produced during atmospheric testing of thermonuclear weapons during the early 1960s, provides an excellent tool for measuring the age of C in trees on an annual to decadal timescale. Before 1950, $^{14}C$ was present in the atmosphere at approximately constant levels for several hundred years ('pre-bomb $^{14}C$'). In the late 1950s and early 1960s, atmospheric thermonuclear weapon tests nearly doubled the abundance of $^{14}C$ in the atmosphere. The period of atmospheric tests was relatively short, as they were banned in 1964, and hence could be described as an unintentional pulse-labeling of the atmosphere and the global C cycle. Following the nuclear-test-ban treaty, atmospheric $^{14}C$ levels have decreased every year due to net uptake of excess 'bomb' $^{14}C$ into oceanic and terrestrial C pools and due to the emission of $^{14}C$-free $CO_2$ from the combustion of fossil fuels (Levin and Kromer 2004). In the southern hemisphere, the peak $^{14}C$ was attenuated and occurred a year later than that of the northern hemisphere (Hua and Barbetti 2004) (Figure 1).

The fixation of C from the atmosphere by plants results in an automatic labeling of each year's photosynthetic products with a distinct $^{14}C$ signature. Radiocarbon data are reported to remove the effects of mass-dependent fractionation by using the measured $^{13}C$ signature and the assumption that $^{14}C$ is fractionated twice as much as $^{13}C$ (Stuiver and Polach 1977). Hence, the reported $^{14}C$ signatures measured in the same year are identical for $CO_2$ and the photosynthetic products fixed from it. The current annual rate of decline in atmospheric $\Delta^{14}CO_2$ and, consequently, the $\Delta^{14}C$ of photosynthetic products is ~4–5‰ per year, larger than the current precision of $^{14}C$ measurements of 2–3‰. Consequently, we are able to determine the year when C was assimilated between the mid-1960s and today by measuring the $^{14}C$ signature of a sample and comparing it to the atmospheric record (Levin and Kromer 2004, Currie et al. 2011).

The easiest and most direct approach to determine the age of C substrates being respired by a tree is to measure the resulting $CO_2$. The amount of $CO_2$ emitted from tree stems can account for up to 16% of the C taken up by gross primary production in a forest (Ryan et al. 1997, Chambers et al. 2004, Litton et al. 2007). Although there is an ongoing discussion concerning the origin of $CO_2$ emitted from tree stems (Teskey et al. 2008), it is assumed to predominantly originate from respiratory processes that occur within rather than outside the tree. Hence, its isotopic signature will reflect the average C sources, including NSC pools, the tree uses for respiration. Thus, by measuring $\Delta^{14}C$, i.e., the ratio of $^{14}C$ : $^{12}C$ in $CO_2$ emitted from a tree stem, corrected for mass-dependent isotopic fractionation and expressed as the deviation in parts per thousand relative to a pre-industrial wood standard, we have a tool for detecting the use of C reserves that have been stored for a year or more in the trees. Carbon that has been assimilated and respired by the tree in the same year will have the same $\Delta^{14}C$ in both respired and atmospheric $CO_2$. In other words, the difference between the $\Delta^{14}C$ of the respired $CO_2$ and atmospheric $CO_2$, defined here as $\Delta\Delta^{14}C$, would be zero. The use of C from older storage pools would be reflected in $\Delta\Delta^{14}C$ values >0, since C assimilated in the past (1965 to today) has a higher $\Delta^{14}C$ than the current atmosphere.

In 2010, the Amazon experienced an extensive drought (Lewis et al. 2011, Marengo et al. 2011). During this anomalous dry season and the subsequent wet season, we collected radiocarbon in $CO_2$ emitted from and in the stems of three species of trees growing in a forest reserve near Iquitos, Peru. We hypothesized that storage reserves in these mature trees would be old enough (>1 year) to detect their use for respiration, and further hypothesized that the contribution of $CO_2$ derived from older C reserves to respiration would be greatest during the dry season.

## Materials and methods

### *Site description*

The study was carried out at the Center for Research and Forest Learning (CIEFOR, 3°49′53.8″S, 73°22′28.2″W) of the National University of the Peruvian Amazon (UNAP) in the community of Puerto Almendras, located 16 km southwest of the city of Iquitos, Peru. The study site was located within

1300 ha of forested area managed by the UNAP Faculty of Forest Engineering (FCF). Average canopy height in the area is around 30 m. More than 500 tree species are found within the area, and the more common species include *Hymenolobium pulcherrimum* Ducke (Mari Mari), *Tachigali paniculata* Aublet (Tangarana), *Simarouba amara* Aublet (Marupa), *Euterpe precatoria* C. Martius (Huasai) and *Guarea glabra* M. Vahl (Requia).

Meteorological information is available from the Meteorological and Hydrological National Service of Peru (SENAMHI), which has a meteorological station at CIEFOR (www.senamhi.gob.pe). They report an average annual rainfall of 2979 mm for the years 1971–2000. A dry season with reduced precipitation typically occurs between May to October. In 2010, the region was affected by the widespread drought in the Amazon basin (Lewis et al. 2011), and rainfall deficits were observed from May 2010 to February 2011, with anomalously low rainfall in August (43 mm) and September (102 mm), corresponding to 20 and 41% of average rainfall for these months, respectively. Our measurements were carried out in the first week of October 2010 (dry season) and in April 2011 (the following wet season).

For this study, we selected three trees per species of three common tree species: *H. pulcherrimum*, *T. paniculata* and *S. amara*. *Hymenolobium pulcherrimum* and *T. paniculata* are both members of the family Fabaceae, whereas *S. amara* belongs to the family Simaroubaceae. The three species differ in wood density, with *H. pulcherrimum* (0.53 g $ml^{-1}$) and *T. paniculata* (0.65 g $ml^{-1}$) having higher wood densities than *S. amara* (0.35 g $ml^{-1}$) (Chambers et al. 2004). For the measurements, we installed chambers and in-stem probes at heights between ca. 1.3 to 2 m. Data about tree diameter and estimated height can be found in Table 1.

### *Installation of stem chambers and sampling of emitted $CO_2$*

We used chambers based on a design developed by Ubierna et al. (2009*b*). Chambers were built from polypropylene (PP) T-pieces (Ostendorf HTRE DN 110, outer diameter 11 cm) that come with a threaded lid for closing the center aperture. The other two openings (the ones facing each other) were welded shut with PP disks. The T-piece was then cut longitudinally, thus removing a segment of the tube opposite to the threaded lid and creating an opening along the whole length of the tubing (length 27.2 cm, width 7.0). The chambers were individually fit to the shape of the tree trunk at the exact spot of installation and kept in place by two sets of lashing straps. To provide a gas-tight seal, we applied hot glue all along the contact surface of the chamber and the stem. Chambers were tested for leaks by measuring [$CO_2$] inside the chamber with a portable infrared gas analyzer system featuring a Li-820 (LI-COR Environmental - GmbH, Bad Homburg, Germany) while blowing respiratory air through a piece of tubing on all potential leaky spots, a method that is both easy and effective due to high [$CO_2$] in respiratory air. On the detection of a leak, more hot glue was applied to the spot, and the procedure was repeated until the chambers were leak-free. When not in use for sampling, the chambers' openings were covered with a lid made from stainless-steel mesh, both enabling ventilation and preventing insect infestation. For measurements, the chambers were closed with a specially equipped lid fitted with gas-sampling ports.

In October 2010, we used evacuated 1 l glass flasks, equipped with two O-ring valves (Louwers Glastechniek en Technisch Keramiek BV, Hapert, The Netherlands, 12 mm OD, 9 mm ID). The flasks were connected to the lid of the chamber by two sequential capillaries (flow-restricting capillary: length

Table 1. Diameter, estimated height and radiocarbon data for all nine measured trees. Radiocarbon measurements are given separately for samples of $CO_2$ emitted from the tree stems into the atmosphere ('$CO_2$ efflux') and for $CO_2$ sampled from within the stem at a depth of 5 cm ('Instem $CO_2$'). Sampling was carried out at two different dates, once during the dry and during the wet season. Where samples were lost and data are not available, this is indicated by 'n.a.'. Ages were estimated based on the difference ($\Delta\Delta^{14}C$) between the sample $\Delta^{14}C$ and the atmospheric $\Delta^{14}C$ (41.9‰) as shown in Figure 1.

| Tree species | # | Diameter (m) | Estimated height (m) | $CO_2$ efflux (chambers) | | | | | | In-stem $CO_2$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Dry season | | | Wet season | | | Dry season | | | Wet season | | |
| | | | | $\Delta^{14}CO_2$ (‰) | $\Delta\Delta^{14}C$ (‰) | Age of C (years) | $\Delta^{14}CO_2$ (‰) | $\Delta\Delta^{14}C$ (‰) | Age of C (years) | $\Delta^{14}CO_2$ (‰) | $\Delta\Delta^{14}C$ (‰) | Age of C (years) | $\Delta^{14}CO_2$ (‰) | $\Delta\Delta^{14}C$ (‰) | Age of C (years) |
| *S. amara* | 1 | 0.33 | 15 | 48.3 | 6.3 | 2 | 48.5 | 6.5 | 2 | 52.0 | 10.0 | 2 | 53.0 | 11.0 | 3 |
| | 2 | 0.43 | 18 | 48.7 | 6.7 | 2 | 52.7 | 10.7 | 3 | 70.2 | 28.2 | 6 | n.a. | n.a. | n.a. |
| | 3 | 0.38 | 16 | 52.3 | 10.3 | 3 | 64.6 | 22.6 | 5 | 50.6 | 8.6 | 2 | 57.0 | 15.0 | 4 |
| *T. paniculata* | 1 | 0.71 | 23 | 50.3 | 8.3 | 2 | 48.2 | 6.2 | 2 | 57.5 | 15.5 | 4 | n.a. | n.a. | n.a. |
| | 2 | 0.42 | 20 | 52.1 | 10.1 | 2 | 45.4 | 3.4 | Zero | 53.8 | 11.8 | 3 | 61.9 | 19.9 | 5 |
| | 3 | 0.88 | 23 | 50.5 | 8.5 | 2 | 49.5 | 7.5 | 2 | 60.9 | 18.9 | 4 | n.a. | n.a. | n.a. |
| *H. pulcherrimum* | 1 | 1.34 | 25 | n.a. | n.a. | n.a. | 55.9 | 13.9 | 3 | 124.1 | 82.1 | 16 | 132.0 | 90.0 | 17 |
| | 2 | 0.39 | 18 | 59.7 | 17.7 | 4 | 52.3 | 10.3 | 3 | 159.3 | 117.3 | 20 | 116.0 | 74.0 | 14 |
| | 3 | 1.13 | 30 | 62.7 | 20.7 | 5 | 67.1 | 25.1 | 6 | 151.0 | 109.0 | 19 | 187.4 | 145.4 | 23 |

8 cm, ID 0.17 mm; extension capillary: length 2 m, ID 1.00 mm). The chambers were incubated for several hours to increase [$CO_2$] in them before sampling. The flasks were opened, with the small-diameter capillary restricting the flow from the chamber to the flask and expanding the filling time of the flasks to ca. 20 min. To avoid a sudden rapid drop of pressure inside the chamber, atmospheric air was allowed to enter the chamber through a 300 ml plastic trap filled with soda lime (AnalaR NORMAPUR, VWR International BVBA, Leuven, Belgium) that removed atmospheric $CO_2$ from the filled air. After sampling, the flasks were closed and sent to the WM Keck Carbon Cycle Accelerator Mass Spectrometry laboratory at the University of California, Irvine (UCI), USA, for further processing. One gas sample (from the *H. pulcherrimum* chamber taken in the dry season) was lost during shipment when the flask broke.

In April 2011, we used a simpler procedure. A smaller flask (volume of 45 ml, not evacuated, but filled with local atmospheric air, and equipped with one 12 mm O-ring valve) was directly connected to the lid and opened as soon as the chamber was closed. The flask was left in place for several days, equilibrating with the gas inside the chamber. At the end of the incubation period, the flasks were closed and sent to the Max-Planck-Institute of Biogeochemistry (MPI-BGC) in Jena, Germany, for further processing.

### *Installation of in-stem probes and sampling of internal $CO_2$*

For sampling gas from inside the stem, we have slightly modified the design of Ubierna et al. (2009*b*). After the removal of the bark, we drilled a 5-cm deep hole (12 mm diameter) into the stem, and then hammered in a stainless-steel tube with an outer diameter (12.7 mm) that slightly exceeded that of the hole. After the installation of the tube, we applied hot glue around the edges of the drill hole to seal the wound against possible leaking or infection by microorganisms or insects. As described in Angert et al. (2012), we connected the sampling flasks (with volumes of 45 or 12.5 ml during first and second samplings, respectively) filled with air at atmospheric pressure directly to the stainless-steel tube using rubber tubing to secure an air-tight seal. The flasks, which had a valve with an O-ring seal (Louwers H.V. glass valves, 12 mm OD, 9 mm ID), were left open for 10 days, then sealed by closing the valve and were removed. This approach is simpler than the original design of Ubierna et al. (2009*b*), which required injecting acidified water into an equilibration volume to replace air removed, while simultaneously collecting the sample with a syringe. Stem gas was sampled once during the dry and once during the wet season.

### *Measurement of $\Delta^{14}C$*

All gas samples for measuring $\Delta^{14}CO_2$ were cryogenically purified and converted to graphite targets using the modified sealed tube zinc reduction method described by Xu et al. (2007). Samples from the 1-l flasks were purified and graphitized at UCI, whereas samples from the smaller flasks (45 and 12.5 ml) were purified and graphitized at the MPI-BGC. All graphitized samples were analyzed by the Keck Carbon Cycle AMS facility at UCI with a precision and accuracy of 2–3‰ (Xu et al. 2007). Radiocarbon data are expressed as $\Delta^{14}C$, which is the per mil deviation from the $^{14}C/^{12}C$ ratio of oxalic acid standard in 1950. The sample $^{14}C/^{12}C$ ratio has been corrected to a $\delta^{13}C$ value of –25‰ to account for any mass-dependent fractionation effects (Stuiver and Polach 1977). Thus, our $\Delta^{14}C$ values can be directly compared with the record of $^{14}C$ in atmospheric $CO_2$ for the southern hemisphere. Note that as a byproduct of the extraction process the total amount of C in each flask is quantified, effectively allowing calculation of the [$CO_2$] of the sample.

### *$^{14}C$-based estimates of $CO_2$ age*

To estimate the mean time elapsed between the time C was fixed and when it was respired, we used the atmospheric curve to estimate the year when the $^{14}C$ of $CO_2$ (and newly fixed C) had the same value as the C in our respiration sample (Figure 1). We sampled the atmospheric air using pre-evacuated 1-l glass flasks to get an atmospheric reference for the time of our measurement campaign (Figure 1). The flasks

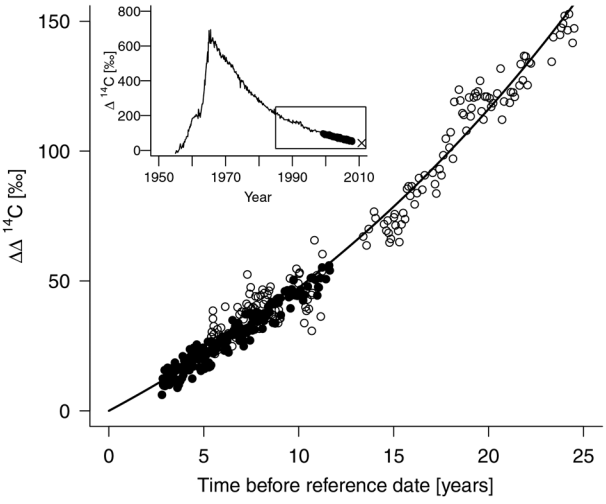


Figure 1. Reference $^{14}C$ data and regression for calculating the time elapsed between fixation and respiration based on the $\Delta\Delta^{14}C$ of a sample. The small inlay graph in the upper left shows the atmospheric $\Delta^{14}C$ for the southern hemisphere. Data are from Wellington, New Zealand (open symbols in the main graph, solid line in the inlay graph, Currie et al. (2011), for 1954–2005) and Graven et al. (2012) including data from Cape Matatula, Palmer Station, and the South Pole (closed symbols; data available for the years 1999–2007). The 'x' in the inlay graph indicates our mean reference measurement of atmospheric $^{14}C$-$CO_2$ during our field campaign in Peru. For calculating the time elapsed between fixation and respiration of any given sample, we fit a non-linear regression to the available data from all records for the period from 1985 until today as shown in the main graph (solid line).

were sent to the UCI for further processing as described above. The atmospheric $\Delta^{14}C$ values of previous years were taken from datasets from measurement stations in the southern hemisphere (Currie et al. 2011, Graven et al. 2012). The data were expressed relative to our reference data (reference date: 7 October 2010, reference $\Delta^{14}C$: 41.9 ± 1.4‰, $n = 2$), then we calculated a non-linear regression through the origin ($y = 104.9 \times (\exp(0.037 \times x) - 1)$). The inverse function was used to calculate the time elapsed between original fixation of C and the time of our measurement campaign. We refer to this as the mean 'age' of respired C. Samples that had a $\Delta\Delta^{14}C$ that was within ±6‰ (two times the measurement precision) of zero were assumed to have an age of zero. Note that a $\Delta\Delta^{14}C$ of 6‰ would be equivalent to an estimated age of 1.5 years, so by this definition we could only determine the age of samples with C fixed on an average of at least 1.5 years previously.

### Statistics

Owing to the low number of replicates, we used non-parametric tests for statistical analysis. For comparing data from the dry and the wet season within the same species, we used a Mann–Whitney *U* test. For comparing data from the three different tree species, we used the Kruskal–Wallis one-way analysis of variance. On detection of significant differences, we used the Mann–Whitney *U* test to further analyze the specific sample pairs for significant differences. All statistical analyses were performed in the R statistical environment (R Core Development Team v2.13.1).

## Results

### Results from chamber samples

All chamber samples contained a mixture of $CO_2$ derived from C fixed recently and several years previously (Table 1). The average time elapsed since fixation was estimated to be between 0 and 6 years. We found no significant differences in the mean age of the respired $CO_2$ between the dry and the wet season for any of the three tree species.

Differences in the sampling technique between dry and wet season resulted in differences in the absolute sample size (not shown) and $CO_2$ concentration (Figure 2) of the chamber samples, without affecting the measured $\Delta^{14}C$. During the wet season, the mean (±SD) $CO_2$ concentration in the chambers affixed to the *S. amara* trees was higher (3.7 ± 1.0%) than in the *T. paniculata* (2.5 ± 0.9%) and *H. pulcherrimum* (1.6 ± 0.4%) trees, but these differences were not significant (Kruskal–Wallis, $P = 0.49$).

### Results from in-stem samples

The $^{14}C$-based age estimates for the in-stem $CO_2$ samples again revealed a mixture of $CO_2$ derived from sources fixed recently and several years previously, with average ages between 2 and 23 years (Table 1). The oldest ages were found for the three *H. pulcherrimum* trees (between 14 and 23 years). The estimated ages of the in-stem samples tended to be higher than simultaneous chamber measurements on the same tree (Figure 3).

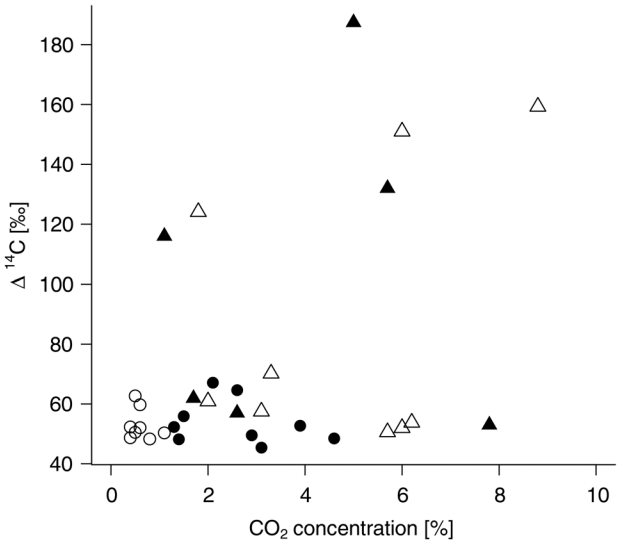


Figure 2. Measured $\Delta^{14}C$ values as a function of the $CO_2$ concentration in the sample. Data are given for samples taken from chambers during the dry (○) and the wet (●) seasons as well as for in-stem probes during the dry (△) and the wet (▲) seasons. Note that the in-stem samples from the *H. pulcherrimum* trees with their very high $\Delta^{14}C$ values clearly separate from all other samples.

In-stem samples had $CO_2$ concentrations ranging from 1.1 to 8.8% (Figure 2), thus always being considerably higher than the atmospheric $CO_2$ concentration (0.04%). We found no significant differences between the dry and wet season measurements for *S. amara* ($P = 1.0$), *T. paniculata* ($P = 0.5$) or *H. pulcherrimum* ($P = 0.4$). Owing to the differences in flask size, the total C content (data not shown) was greater in dry season samples than in wet season samples, but neither $CO_2$ concentration nor sample size affected the measured $\Delta^{14}C$ values. We found no systematic differences in $CO_2$ concentration between the three species.

## Discussion

Age estimates based on radiocarbon measurements presented here provide clear evidence that C sources fixed prior to the year of sampling contribute to in-stem and emitted $CO_2$ in all three Peruvian rainforest tree species we sampled. The reproducibility among trees of the same species makes it unlikely that the old $CO_2$ is produced solely from decomposition in the stems. Indeed as discussed in more detail below, the ages of stored C reported in the literature are consistent with the mean ages of $CO_2$ we observed. The lack of seasonal variation means it is unlikely that the older $CO_2$ is produced in response to

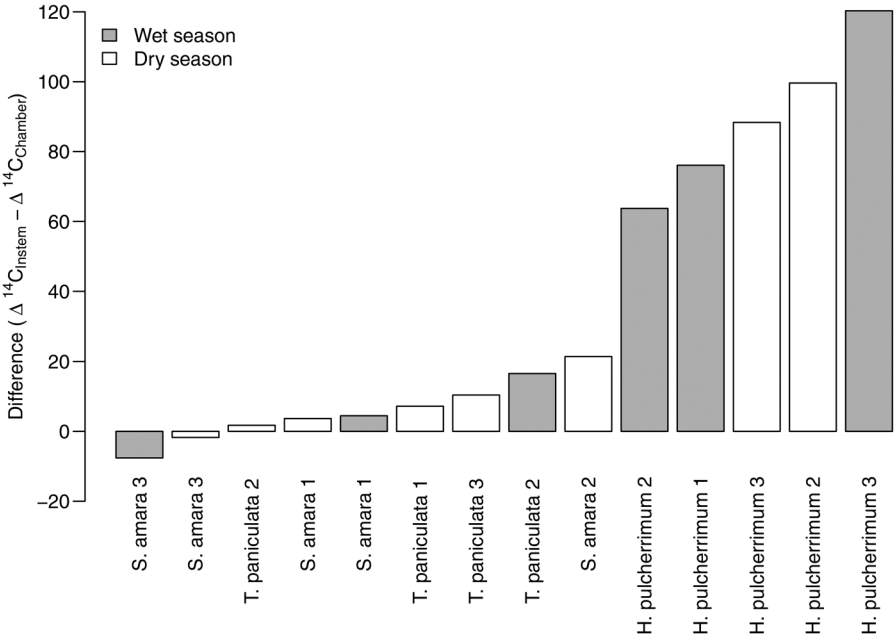


Figure 3. Each bar represents the difference between the $\Delta^{14}C$ of the in-stem vs. the chamber sample of a specific tree either during the dry (white) or the wet (grey) seasons.

seasonal stress, e.g., potential drought stress, even in an extreme drought year (2010). Rather, our results suggest that the investigated trees consistently rely on a mixture of recent assimilates and storage C.

There are few studies we are aware of that provide clear evidence for trees using storage C as a C source. Kuptz et al. (2011) and Nogués et al. (2006) both used $^{13}C$ labeling to determine the contribution of older C sources to $CO_2$ emitted from the tree stem. However, owing to methodological restrictions, these two studies could only conclude that the age of storage reserves pre-dated their label application, i.e., older than several days to weeks. Similarly, a $^{14}C$ pulse labeling experiment by Carbone et al. (2007) indicated that C assimilated during the labeling event still contributed to root and canopy respiration even 30 days post-labeling. Application of the bomb-radiocarbon approach to root-respired $CO_2$ from boreal forest black spruce trees revealed mean ages of several years (Czimczik et al. 2006, Schuur and Trumbore 2006), supporting our findings.

Other evidence for the use of storage reserves in trees comes from the age of newly grown plant tissues. Measurements of $^{14}C$ in newly grown roots from tropical (Trumbore et al. 2006) and temperate forests (Gaudinski et al. 2009) showed that they grew from C sources that were <2 years old. In contrast, Vargas et al. (2009) showed that newly grown fine roots in a forest recovering after a hurricane had $^{14}C$ signatures indicating that they were grown from C fixed between 4 and 11 years previously, providing clear evidence for the ability of trees to use several-year-old storage reserves of C to recover from disturbance. If we assume that the $CO_2$ respired during growth should have the same $^{14}C$ as the new growth itself, respired $CO_2$ in this system would have been expected to have mean ages of at least several years. Keel et al. (2006) reported isotope measurements from newly formed tree ring biomass in a mature temperate deciduous forest subjected to continuous fumigation with $^{13}C$-labeled $CO_2$. Even after 4 years of continuous labeling, they found ~9% of the C used to grow new tree rings came from unlabeled C pools. While this could indicate the use of C >4 years old for growth, the authors also indicated that it could have resulted from incomplete labeling of the trees.

Richardson et al. (2012) recently reported mean ages of roughly a decade for NSC (sugars and starch) extracted from the outermost 2 cm of tree stems, with maximum ages being as high as 31 and 24 years for starch and sugar, respectively. These surprisingly old NSC pools could serve as substrates for respiration and explain the presence of several-year-old $CO_2$ as found in our measurements. The similar ages found by Richardson et al. (2012) for the starch and the sugar pools were interpreted as evidence that they must regularly exchange C, further supporting the idea of trees' availability to remobilize C fixed years previously. In summary, a number of observations indicate that the C which is fixed for years previously contributes to tree growth as well as maintenance metabolism. The reported ages are in accordance with most of our measurements, but to our knowledge this is the first time that the contribution of C as old as 23 years to respired $CO_2$ has been shown.

What is the source of the $CO_2$ we measure? There is increasing evidence that the $CO_2$ in and emitted from stems is actively transported within the tree (Teskey et al. 2008). In a

related study (Angert et al. 2012) we measured the apparent respiration quotient (ARQ) (Table 2), or the relative decrease in $O_2$ compared with the increase in $CO_2$, in the same trees and at the same time as the $^{14}C$-$CO_2$ measurements. All trees consumed a larger amount of $O_2$ than the amount of $CO_2$ they emitted. Apparent respiration quotient values averaged 0.6 in the chambers, but were much lower (0.2–0.3) in the in-stem gases. Assuming that carbohydrates are the main respiratory substrates, we expect a respiration quotient close to 1.0. Because of the low solubility of $O_2$ compared with $CO_2$ in stem water, Angert et al. (2012) concluded that the low ARQ values indicate net transport of $CO_2$ out of the region underlying our chambers (Trumbore et al. 2013). Given the evidence for considerable $CO_2$ transport in stem water, we have to consider that some of the $CO_2$ we measured could have been respired elsewhere in the plant. At the same time, uptake of $O_2$ (being significantly less soluble in water than $CO_2$, thus reflecting predominantly local processes) is a good proxy for respiration occurring in the stem directly underlying the chamber (Angert et al. 2012), so at least part of the $CO_2$ we measured originates from local respiration. Possible sources of $CO_2$ potentially transported into the area beneath the chamber include (i) root-respired $CO_2$, (ii) $CO_2$ produced by microorganisms in the soil and (iii) $CO_2$ produced by microorganisms within the tree trunk.

Aubrey and Teskey (2009) used measurements of sap flow and of stem-internal [$CO_2$] to suggest that as much as 50% of all the $CO_2$ produced by roots is transported upward in the transpiration stream, where it might contribute to $CO_2$ efflux from above-ground tissues. While it is still unclear that this is important in tree species other than the ones studied by Aubrey and Teskey (2009), it is possible that a considerable amount of $CO_2$ in our samples could have originated from root respiration rather than local stem respiration. However, regardless whether the old $CO_2$ comes from root or stem respiration, both ultimately represent the respiration of live tree tissue and thus tree metabolism, hence the overall conclusion in both cases would be identical: trees metabolize C from several-year-old pools on a regular basis. For now, we have to assume that our samples consist of a mixture of $CO_2$ produced in the stem as well as in roots.

Table 2. Apparent respiratory quotient measured for chamber and in-stem samples. For chambers, data were measured during dry and wet season; for in-stem probes, data are available for two different dates in the dry season (modified from Angert et al. (2012)). Missing data are indicated as not available (n.a.).

| Tree species | # | ARQ | | | |
|---|---|---|---|---|---|
| | | Chambers | | In-stem | |
| | | Dry | Wet | Dry 1 | Dry 2 |
| *S. amara* | 1 | 0.23 | 0.90 | 0.13 | 0.16 |
| | 2 | 0.40 | 0.69 | 0.29 | 0.17 |
| | 3 | 0.75 | 0.71 | 0.88 | 0.77 |
| *T. paniculata* | 1 | 0.51 | 0.71 | 0.43 | 0.18 |
| | 2 | 0.53 | 0.47 | n.a. | 0.14 |
| | 3 | 0.80 | 0.61 | n.a. | 0.18 |
| *H. pulcherrimum* | 1 | 0.89 | 0.74 | n.a. | n.a. |
| | 2 | n.a. | 0.66 | n.a. | 0.19 |
| | 3 | 0.42 | 0.73 | n.a. | n.a. |

Unlike root-respired $CO_2$, $CO_2$ produced by microorganisms in the soil would have to be taken up into the root first before being transported upward with sap flow. Observations of $^{14}C$ in soil pore space $CO_2$ made in Brazilian tropical forests indicate values of $\Delta\Delta^{14}C$ that range from 10‰ near the surface, up to ~30‰ at several meters depth (Trumbore et al. 2006). While these values are consistent with some of our measurements, they cannot explain the extremely high $\Delta\Delta^{14}C$ values observed in *H. pulcherrimum* stems. The uptake of soil pore space $CO_2$ into the root might be restricted by root anatomical features, which—in the absence of aerenchyma—might limit the inflow of molecules other than water into the roots (Colmer 2003, De Simone et al. 2003). Labeling studies by Ford et al. (2007) and Ubierna et al. (2009*a*) concluded that the uptake of soil $CO_2$ by roots of mature forest trees likely is very small to irrelevant. In addition, the ARQ in the soil pore space adjacent to the trees we studied was measured (Angert et al. 2012). Net removal of $CO_2$ from the soil (i.e., more $CO_2$ being transported away relative to the less soluble $O_2$) would change the $CO_2$ : $O_2$ ratio and hence result in an ARQ < 1. Instead, the average ARQ (±SD, $n = 5$) measured in soil air was close to 1.0 (± 0.14) (Angert et al. 2012). In summary, it seems unlikely that $CO_2$ produced by soil microorganisms contributes substantial amounts of C to our samples.

Live trees can become infected by fungi which then decompose parts of the stem ('heart rot') and consequentially produce $CO_2$ that might add to the stem-internal $CO_2$ pool (Good et al. 1968). The $\Delta^{14}C$ of the $CO_2$ produced by such decomposition would depend on the $\Delta^{14}C$ of the decomposed tissue, which is given by the $\Delta^{14}C$ of the atmosphere (Figure 1) in the year of tissue formation (Hua and Barbetti 2004). Thus, the range of possible $\Delta^{14}C$ values associated with the decomposition of stem tissue ultimately depends on the age of the tree and the $\Delta^{14}C$ of $CO_2$ actually produced this way on the exact location of the site of decomposition within the tree. It seems unlikely that all nine trees investigated here are decomposing in exactly the same way, as is indicated by the agreement among trees within a given species. In particular, it is difficult to explain the consistently high $\Delta^{14}C$ values of the *H. pulcherrimum* trees. Interestingly, the three trees of this species differed widely in diameter, with one being considerably smaller than the other two (Table 1). For explaining the results with decomposition, we would nevertheless have to assume that all these three trees were affected by fungi in the areas of the stem with the same age, contributing comparable amounts of $CO_2$ to the

overall stem $CO_2$ pool, which we consider a highly unlikely scenario.

Comparison of our in-stem vs. chamber measurements indicates large differences in respired C age, suggesting that more recently fixed C is added between 5-cm depth and the stem surface in two of the three tree species (*H. pulcherrimum*, *T. paniculata*). We assume that the $CO_2$ produced in the outermost stem regions derives predominantly from recent assimilates (i.e., $\Delta\Delta^{14}C = 0$) transported in the phloem from the canopy source to the sink organs. As noted above, the $CO_2$ deeper within the tree stem ($\Delta\Delta^{14}C > 6‰$) could either originate from local mineralization of old storage pools, resulting from the transport of $CO_2$ respired elsewhere in the stem or roots, or possibly a mixture of both. Interestingly, the trees with the oldest $CO_2$ inside the stem (*H. pulcherrimum*) are also the trees with the highest wood density and slowest growth rate compared with the two other species investigated. Given observations by Richardson et al. (2012) that the mean age of NSC increases with depth in the stem, we would speculate that perhaps the constant 5-cm depth in-stem probes penetrated to older wood and therefore older NSC in the slowest growing tree, which might explain the greater age of $CO_2$ in *H. pulcherrimum* stems.

The concentration of $CO_2$ inside stems is much higher than the ambient air outside the stem, indicating that the diffusion of $CO_2$ must supply at least some of the $CO_2$ that accumulates in a chamber placed over the stem surface. Assuming that the accumulated $CO_2$ represents a mixture of two components: one with the isotopic signature of $CO_2$ measured with the in-stem probe and the second assumed to be derived from recent photosynthetic products (e.g., in phloem tissue), we can apply an isotope mixing model (Phillips and Gregg 2001) to calculate the relative contributions of each to the measured chamber $CO_2$ (Figure 4, Table 3). This calculation makes the following assumptions: (i) The age of $CO_2$ respired in the phloem and the surrounding tissue is zero, i.e., $CO_2$ produced there preferentially originates from the mineralization of assimilates that were photosynthesized very recently and thus have the same $\Delta^{14}C$ as atmospheric $CO_2$. (ii) The $\Delta^{14}C$ measured in the in-stem probes is a good approximation of the average isotopic signature of the older stem-internal pool, i.e., of the second source associated with the respiration of local storage pools. This calculation is only possible for trees where we have simultaneous measurements of the $\Delta^{14}C$ of internal and emitted $CO_2$, and where $\Delta^{14}C$ of the emitted $CO_2$ was bracketed by the $\Delta^{14}C$ of the assumed sources. Further, if the differences between the emitted and in-stem $^{14}C$ are small compared with the precision of $^{14}C$ measurements, it limits the ability to partition sources.

The relative contribution of the two sources to total $CO_2$ emissions varies widely between the different trees (Table 3). The *H. pulcherrimum* trees, which had the largest difference between in-stem and chamber $^{14}C$-$CO_2$, had the most consistent results, with storage C contributing between 15 and 19% of the total emitted $CO_2$. For the other tree species, the sources were not as isotopically distinct and the estimates vary over a

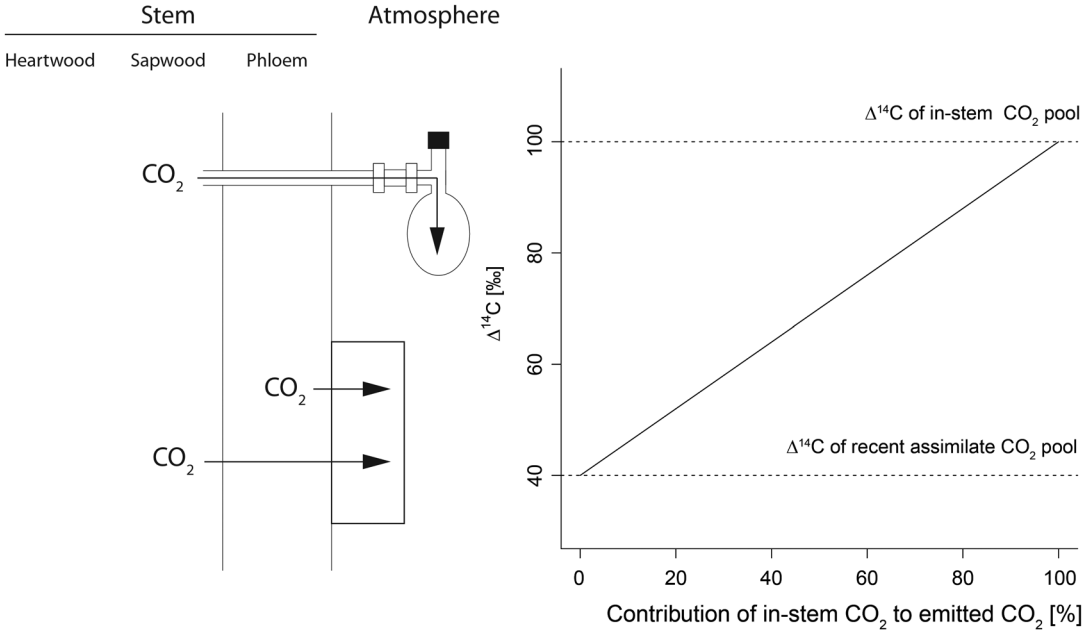


Figure 4. Illustration of the application of the two-source mixing model by Phillips and Gregg (2001) for calculating the relative contribution of a young and an old pool to the total $CO_2$ efflux as presented in Table 3. The left side illustrates sampling differences in the in-stem probes vs. the chambers, while the right side illustrates how the isotopic signature of a mixture sample would change depending on the relative contribution of both sources (here given as the percentage of the mixture originating from the older in-stem $CO_2$ pool).

Table 3. Relative contribution (*f*) of $CO_2$ from a hypothetically young C pool associated with the metabolizing of assimilates transported in the phloem, and an older pool located deeper within the stem, associated to metabolizing of storage C or transported $CO_2$. Relative contributions were calculated by applying a two-source mixing model as described by Phillips and Gregg (2001) with error propagation. Calculation was done for all available pairs of simultaneous measurement of $\Delta^{14}C$ of emitted $CO_2$ and stem-internal $CO_2$, as long as the conditions for the application of the mixing model were met (see the text). Missing data are indicated as not available (n.a.).

| Tree species | # | Dry season | | Wet season | |
|---|---|---|---|---|---|
| | | *f*(young) ± SE | *f*(old) ± SE | *f*(young) ± SE | *f*(old) ± SE |
| *S. amara* | 1 | 0.37 ± 0.26 | 0.63 ± 0.26 | 0.41 ± 0.27 | 0.59 ± 0.27 |
| | 2 | 0.76 ± 0.07 | 0.24 ± 0.07 | n.a. | n.a. |
| | 3 | n.a. | n.a. | n.a. | n.a. |
| *T. paniculata* | 1 | 0.46 ± 0.16 | 0.54 ± 0.16 | n.a. | n.a. |
| | 2 | 0.14 ± 0.25 | 0.86 ± 0.25 | 0.83 ± 0.13 | 0.17 ± 0.13 |
| | 3 | 0.55 ± 0.12 | 0.45 ± 0.12 | n.a. | n.a. |
| *H. pulcherrimum* | 1 | n.a. | n.a. | 0.85 ± 0.03 | 0.15 ± 0.03 |
| | 2 | 0.85 ± 0.02 | 0.15 ± 0.02 | 0.86 ± 0.04 | 0.14 ± 0.04 |
| | 3 | 0.81 ± 0.02 | 0.19 ± 0.02 | 0.83 ± 0.02 | 0.17 ± 0.02 |

wide range and with much larger calculated standard errors. With reference to Richardson et al. (2012), these findings could be interpreted as a fast cycling young pool, which is characterized by a relatively high contribution to the overall flux, and a slower cycling old pool, which contributes less to the overall flux. It should be noted that the application of a two-source mixing model is based on the implicit assumption that there is no difference between the two $CO_2$ pools with regard to the main transport pathways away from the site of production. With regard to the possibility of $CO_2$ being transported away along with the transpiration stream, this assumption is probably not correct. It is far more likely that much of the $CO_2$ produced in the outer regions of the stem diffuses out of the stem into the atmosphere, while a relatively bigger portion of the $CO_2$ produced deeper within the stem is transported along with the transpiration stream. Thus, our mixing model calculation would likely underestimate the contribution of the internal $CO_2$ pool, and thus the role of storage C pools, to the overall tree $CO_2$ efflux.

In summary, our findings provide clear evidence for the use of several-year-old storage C as a substrate for respiration in tree metabolism. This implies that the assumption of a typical mean turnover time of the assimilate pool that supplies substrates for respiration in trees of >1 year has to be reconsidered, and suggests that these trees make use of C assimilated in previous years on a regular basis. If this can be verified for more trees in other ecosystems, the use of storage reserves can provide a buffer and a potential mechanism for the survival of periods when C assimilation is reduced. Given the question of whether elevated $CO_2$ concentrations in the atmosphere have influenced the allocation to NSC pools (Körner 2003), the dynamics and the amount of NSC reserves is an important factor for describing C balance in trees.

## Acknowledgments

We thank our colleagues from the W.M. Keck Carbon Cycle AMS Laboratory at the University of Irvine, California, especially Xiaomei Xu, for their invaluable help with measuring the $^{14}C$ samples.

## Conflict of interest

None declared.

## Funding

Research by A.A. and S.E.T. was partly funded by the GIF grant #1139/2011.

## References

Angert A, Muhr J, Juarez RN et al. (2012) Internal respiration of Amazon tree stems greatly exceeds external $CO_2$ efflux. Biogeosciences 9:4979–4991.

Aubrey DP, Teskey RO (2009) Root-derived $CO_2$ efflux via xylem stream rivals soil $CO_2$ efflux. New Phytol 184:35–40.

Carbone MS, Czimczik CI, McDuffee KE, Trumbore SE (2007) Allocation and residence time of photosynthetic products in a boreal forest using a low-level $^{14}C$ pulse-chase labeling technique. Global Chang Biol 13:466–477.

Chambers JQ, Higuchi N, Teixeira LM, dos Santos J, Laurance SG, Trumbore SE (2004) Response of tree biomass and wood litter to disturbance in a Central Amazon forest. Oecologia 141:596–611.

Chapin FS, Schulze ED, Mooney HA (1990) The ecology and economics of storage in plants. Annu Rev Ecol Syst 21:423–447.

Colmer TD (2003) Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant Cell Environ 26:17–36.

Currie KI, Brailsford G, Nichol S, Gomez A, Sparks R, Lassey KR, Riedel K (2011) Tropospheric $^{14}CO_2$ at Wellington, New Zealand: the world's longest record. Biogeochemistry 104:5–22.

Czimczik CI, Trumbore SE, Carbone MS, Winston GC (2006) Changing sources of soil respiration with time since fire in a boreal forest. Glob Chang Biol 12:957–971.

De Simone O, Haase K, Muller E, Junk WJ, Hartmann K, Schreiber L, Schmidt W (2003) Apoplasmic barriers and oxygen transport properties of hypodermal cell walls in roots from four Amazonian tree species. Plant Physiol 132:206–217.

Ford CR, Wurzburger N, Hendrick RL, Teskey RO (2007) Soil DIC uptake and fixation in *Pinus taeda* seedlings and its C contribution to plant tissues and ectomycorrhizal fungi. Tree Physiol 27:375–383.

Gaudinski JB, Torn MS, Riley WJ, Swanston C, Trumbore SE, Joslin JD, Majdi H, Dawson TE, Hanson PJ (2009) Use of stored carbon reserves in growth of temperate tree roots and leaf buds: analyses using radiocarbon measurements and modeling. Glob Chang Biol 15:992–1014.

Good HM, Basham JT, Kadzielawa SD (1968) Respiratory activity of fungal associations in zones of heart rot and stain in sugar maple. Can J Bot 46:27–36.

Graven HD, Guilderson TP, Keeling RF (2012) Observations of radiocarbon in $CO_2$ at seven global sampling sites in the Scripps flask network: analysis of spatial gradients and seasonal cycles. J Geophys Res 117:D02303.

Hua Q, Barbetti M (2004) Review of tropospheric bomb $^{14}C$ data for carbon cycle modeling and age calibration purposes. Radiocarbon 46:1273–1298.

Keel SG, Siegwolf RTW, Körner C (2006) Canopy $CO_2$ enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest. New Phytol 172:319–329.

Körner C (2003) Carbon limitation in trees. J Ecol 91:4–17.

Kuptz D, Fleischmann F, Matyssek R, Grams TEE (2011) Seasonal patterns of carbon allocation to respiratory pools in 60-yr-old deciduous (*Fagus sylvatica*) and evergreen (*Picea abies*) trees assessed via whole-tree stable carbon isotope labeling. New Phytol 191:160–172.

Le Roux X, Lacointe A, Escobar-Gutierrez A, Le Dizes S (2001) Carbon-based models of individual tree growth: a critical appraisal. Ann For Sci 58:469–506.

Levin I, Kromer B (2004) The tropospheric $^{14}CO_2$ level in mid-latitudes of the northern hemisphere (1959–2003). Radiocarbon 46:1261–1272.

Lewis SL, Brando PM, Phillips OL, van der Heijden GMF, Nepstad D (2011) The 2010 Amazon drought. Science 331:554.

Litton CM, Raich JW, Ryan MG (2007) Carbon allocation in forest ecosystems. Glob Chang Biol 13:2089–2109.

Marengo JA, Tomasella J, Alves LM, Soares WR, Rodriguez DA (2011) The drought of 2010 in the context of historical droughts in the Amazon region. Geophys Res Lett 38:L12703.

Nogués S, Damesin C, Tcherkez G, Maunoury F, Cornic G, Ghashghaie J (2006) $^{13}C/^{12}C$ isotope labelling to study leaf carbon respiration and allocation in twigs of field-grown beech trees. Rapid Commun Mass Spectrom 20:219–226.

Phillips DL, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. Oecologia 127:171–179.

Richardson AD, Carbone MS, Keenan TF, Czimczik CI, Hollinger DY, Murakami P, Schaberg PG, Xu X (2012) Seasonal dynamics and age of stemwood nonstructural carbohydrates in temperate forest trees. New Phytol 197:850–861.

Ryan MG, Lavigne MB, Gower ST (1997) Annual carbon cost of autotrophic respiration in boreal forest ecosystems in relation to species and climate. J Geophys Res 102:28871–28883.

Sala A, Piper F, Hoch G (2010) Physiological mechanisms of drought-induced tree mortality are far from being resolved. New Phytol 186:274–281.

Schuur EAG, Trumbore SE (2006) Partitioning sources of soil respiration in boreal black spruce forest using radiocarbon. Glob Chang Biol 12:165–176.

Stuiver M, Polach HA (1977) Discussion; reporting of $^{14}C$ data. Radiocarbon 19:355–363.

Teskey RO, Saveyn A, Steppe K, McGuire MA (2008) Origin, fate and significance of $CO_2$ in tree stems. New Phytol 177:17–32.

Trumbore SE (2006) Carbon respired by terrestrial ecosystems—recent progress and challenges. Glob Chang Biol 12:141–153.

Trumbore SE, Da Costa ES, Nepstad DC, Barbosa De Camargo P, Martinelli LA, Ray D, Restom T, Silver W (2006) Dynamics of fine root carbon in Amazonian tropical ecosystems and the contribution of roots to soil respiration. Glob Chang Biol 12:217–229.

Trumbore SE, Angert A, Kunert N, Muhr J, Chambers JQ (2013) What's the flux? Unraveling how $CO_2$ fluxes from trees reflect underlying physiological processes. New Phytol 197:353–355.

Ubierna N, Kumar AS, Cernusak LA, Pangle RE, Gag PJ, Marshall JD (2009*a*) Storage and transpiration have negligible effects on $\delta^{13}C$ of stem $CO_2$ efflux in large conifer trees. Tree Physiol 29:1563–1574.

Ubierna N, Marshall JD, Cernusak LA (2009*b*) A new method to measure carbon isotope composition of $CO_2$ respired by trees: stem $CO_2$ equilibration. Funct Ecol 23:1050–1058.

Vargas R, Trumbore SE, Allen MF (2009) Evidence of old carbon used to grow new fine roots in a tropical forest. New Phytol 182:710–718.

Xu X, Trumbore SE, Zheng S, Southon JR, McDuffee KE, Luttgen M, Liu JC (2007) Modifying a sealed tube zinc reduction method for preparation of AMS graphite targets: reducing background and attaining high precision. Nucl Instrum Meth B 259:320–329.