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Invited review: Part of an invited issue on carbon allocation

Pulse-labelling trees to study carbon allocation dynamics: a review of methods, current knowledge and future prospects

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Pulse-labelling of trees with stable or radioactive carbon (C) isotopes offers the unique opportunity to trace the fate of labelled CO_2 into the tree and its release to the soil and the atmosphere. Thus, pulse-labelling enables the quantification of C partitioning in forests and the assessment of the role of partitioning in tree growth, resource acquisition and C sequestration. However, this is associated with challenges as regards the choice of a tracer, the methods of tracing labelled C in tree and soil compartments and the quantitative analysis of C dynamics. Based on data from 47 studies, the rate of transfer differs between broadleaved and coniferous species and decreases as temperature and soil water content decrease. Labelled C is rapidly transferred belowground—within a few days or less—and this transfer is slowed down by drought. Half-lives of labelled C in phloem sap (transfer pool) and in mature leaves (source organs) are short, while those of sink organs (growing tissues, seasonal storage) are longer. ^{13}C measurements in respiratory efflux at high temporal resolution provide the best estimate of the mean residence times of C in respiratory substrate pools, and the best basis for compartmental modelling. Seasonal C dynamics and allocation patterns indicate that sink strength variations are important drivers for C fluxes. We propose a conceptual model for temperate and boreal trees, which considers the use of recently assimilated C versus stored C. We recommend best practices for designing and analysing pulse-labelling experiments, and identify several topics which we consider of prime importance for future research on C allocation in trees: (i) whole-tree C source–sink relations, (ii) C allocation to secondary metabolism, (iii) responses to environmental change, (iv) effects of seasonality versus phenology in and across biomes, and (v) carbon–nitrogen interactions. Substantial progress is expected from emerging technologies, but the largest challenge remains to carry out in situ whole-tree labelling experiments on mature trees to improve our understanding of the environmental and physiological controls on C allocation.

Keywords: carbon isotope, forest, partitioning, residence time, transfer time.

Introduction

Carbon (C) allocation is an important determinant of the C budget of forests and its response to changing environmental conditions. It affects tree growth (competition between aboveground and belowground C sinks), the acquisition of resources (light, nutrients and water) that often limit forest productivity and C sequestration in both standing biomass and soil organic matter (Litton et al. 2007). Carbon allocation results from several processes (Cannell and Dewar 1994); and the term 'allocation' is thus often used to describe many different aspects of plant and ecosystem physiology, including patterns in live biomass, the flux of C to a particular plant compartment and the distribution of flux as a fraction of gross photosynthesis (Litton et al. 2007).

Carbon allocation has been estimated for decades with C mass-balance approaches, which typically combine measurements of standing biomass with measurements of respiratory CO_2 efflux. For example, belowground C flux, including growth and respiration of roots and mycorrhiza, as well as exudates, can be estimated as the cumulative soil CO_2 efflux minus C input from aboveground litter plus changes in C stored in roots, in the forest floor and in the soil (Giardina and Ryan 2002). The aboveground C flux is often inferred from annual changes in aboveground biomass derived from allometric relationships and from measurements of respiration of aboveground organs that are further scaled to the stand level (Ryan et al. 1996). Alternatively, the total aboveground C flux can be computed as the difference between gross primary productivity inferred from eddy flux measurements of net ecosystem CO_2 exchange and belowground C flux estimates (Navarro et al. 2008). While these approaches have proven to be fruitful for quantifying the whole ecosystem C budget, uncertainties remain about the contribution of different above- and belowground C fluxes to ecosystem respiration, which is the major efflux of C from the biosphere to the atmosphere, and to C sequestration within the ecosystem. Budget-based mass-balance approaches also provide limited insight into the short-term dynamics of C allocation that are critical for understanding the mechanisms underlying the annual patterns. One of the most important unanswered questions is the role of environmental drivers versus phenology on changes in C allocation patterns at seasonal scales and how these two factors are affected by climate change. Given the major importance of belowground C allocation for soil processes, we also need a quantitative mechanism for the coupling of belowground processes with canopy C assimilation. Recent technological developments offer promising approaches to answer these important questions by directly tracing C fluxes with high temporal resolution.

Fluctuations of photosynthetic C isotope discrimination can be used to trace the origin and fate of C into metabolites and respired CO_2 at various temporal and spatial scales in plants and ecosystems (see Dawson et al. 2002), but the low signal (a few per mil) makes resolving transfer rates and time lags difficult. Nevertheless, this approach has been used to investigate C transfer in trees and between forests and the atmosphere (Ekblad and Högberg 2001, Bowling et al. 2002, Knohl et al. 2005, Brandes et al. 2006, Keitel et al. 2006, Kodama et al. 2008, Marron et al. 2009, Wingate et al. 2010). Correlations between the isotope composition of respired CO_2 and either climatic drivers or online measurements of daily photosynthetic C isotope discrimination can constrain transfer rates of C from the foliage to different C pools in the ecosystem (Wingate et al. 2010, Brüggemann et al. 2011). Because the strength of the natural signal is weak, precise estimates of transfer rates and time lags are hampered by post-photosynthetic fractionation (both biological and physical like those occurring during CO_2 transport through soil pores), by mixing of several C sources (recently assimilated versus stored C) and by mixing of autotrophic and heterotrophic components of soil CO_2 efflux (McDowell et al. 2004, Kodama et al. 2008, Wingate et al. 2010, Salmon et al. 2011). Such mixing and fractionation effects can cause up to 12‰ short-term variation in C isotope composition of plant and of soil-respired CO_2 (Werner and Gessler 2011), sometimes causing a complete loss of the photosynthetic isotopic signal from the canopy to the soil (Kodama et al. 2008).

The more promising technique for understanding C allocation is to artificially alter the C isotope content of assimilated C using stable (^{13}C) CO_2 or radioactive (^{14}C and ^{11}C) CO_2 as short pulses or over long periods. In these labelling experiments, partitioning of photosynthesis products to sinks can be estimated as the amount of labelled C retained in a compartment or lost by respiration, exudation or volatile organic compound emissions, relative to the amount of labelled C assimilated by the plant. In addition, allocation may also refer to the partitioning of labelled C among several C-containing compounds in a given organ, for example between structural and non-structural C (Kagawa et al. 2005, 2006*b*), or between storage compounds like starch and metabolites with high turnover (Vizoso et al. 2008). Because accretion of labelled C in a compartment results from the net flux of labelled C into versus out of this compartment, the term allocation refers therefore to both the dynamics and the amount of labelled C retrieved in a compartment (Figure 1). Following Litton et al. (2007), we here use the term partitioning as a quantitative estimate of the fraction of labelled C supplied to a tree that is allocated to any given compartment.

Carbon isotope labelling experiments have been implemented for decades on potted trees (Lippu 1994, 1998, Maillard et al. 1994, Andersen and Rygiewicz 1995, Rouhier et al. 1996) or at the branch level for field-grown trees (Schneider and Schmitz 1989, Hansen and Beck 1990, Lacointe et al. 2004, Kagawa et al. 2005, Nogués et al. 2006,

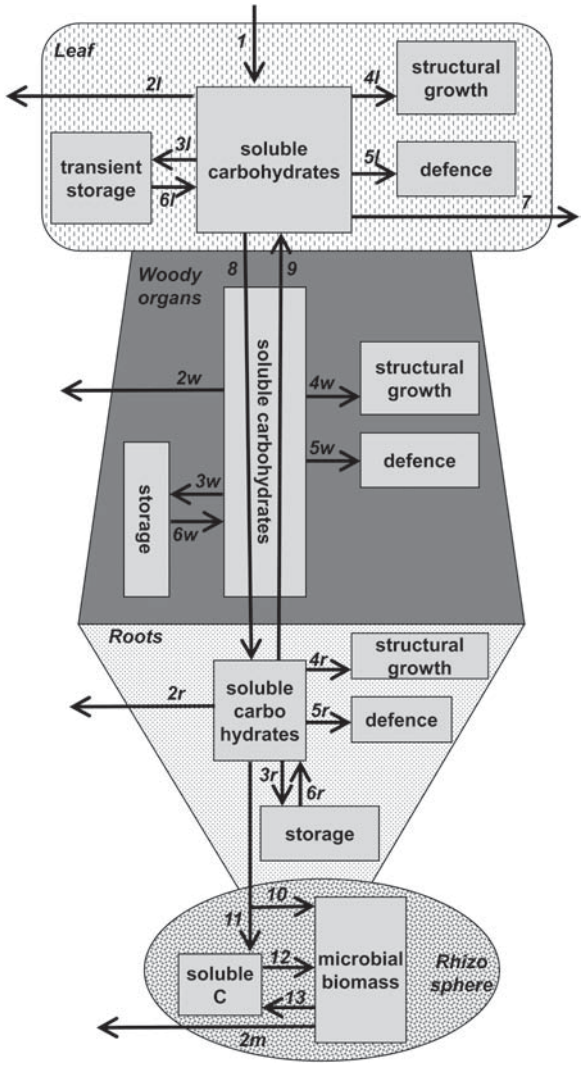

Figure 1. Schematic representation of the fate of labelled C in a tree. The assimilated labelled C [1] fills a pool of soluble carbohydrates that is further allocated to respiration [2], storage [3], structural growth [4] and defence compounds [5]. The soluble carbohydrate pools are also filled by remobilization from reserves [6]. The emission of organic compounds also potentially occurs [7]. Soluble sugars are transferred both basipetally [8] from leaves to the other organs or acropetally from storage tissues in perennial organs to new shoots [9]. Labelled C is also transferred to the rhizosphere, either indirectly via hyphae of mycorrhiza [10] or directly via exudation [11]. It can be transferred into the microbial biomass [12] and further recycled owing to the high turnover rate of microbes [13]. The letters l, w, r and m indicate the compartment (respectively leaf, woody organs, roots and microbes).

Keel et al. 2007). But until recently, field labelling experiments have been restricted to short-stature, homogeneous vegetation, such as crops and grasslands (Warembourg and Paul. 1973, Gregory and Atwell 1991, Bélanger et al. 1992, Ostle et al. 2000, Johnson et al. 2002, Bahn et al. 2009, Gamnitzer et al. 2009, Lattanzi et al. 2012). In situ whole-tree labelling experiments are still in their infancy (Carbone et al. 2007, Högberg et al. 2008, 2010, Plain et al. 2009, Andersen et al. 2010, Endrulat et al. 2010, Dannoura et al. 2011, Epron et al. 2011, Grams et al. 2011, Kuptz et al. 2011, Ritter et al. 2011, Keel et al. 2012) and mostly done on small-sized trees. However, only such experiments offer the opportunity to better understand the dynamic changes in C allocation in trees across seasons, influenced by species phenology and environmental conditions.

Recent development of novel tools to trace labelled C in respired CO_2 by isotope ratio infrared spectroscopy (IRIS) at a high frequency has enabled precise quantification of C residence times in short-lived storage pools and of transfer rates among plant compartments and between plants, soil and the atmosphere (Bahn et al. 2009, Plain et al. 2009, Barthel et al. 2011, Dannoura et al. 2011, Epron et al. 2011, Warren et al. 2012). Combining long-term tracer time courses with compartmental modelling techniques is promising to estimate the number and the half-life of C pools contributing to C partitioning in trees. Although information on half-lives, transfer rates and mixing pools is highly relevant to improve our understanding of allocation in tree–soil systems, reliable data are still scarce.

This review aims to (i) identify the experimental, methodological and analytical challenges to currently available pulse-labelling techniques and give recommendations on practices for designing future C pulse-labelling experiments, (ii) summarize results from pulse-labelling experiments on trees to synthesize current knowledge and elaborate emerging research questions and (iii) present the classical mathematical methods commonly used for characterizing the transfer of labelled C into different compartments and fluxes and highlight the potential of more mechanistic approaches, based on compartmental modelling of tracer time courses.

We synthesize data reported in 47 pulse-labelling studies on trees using ^{11}C, ^{13}C or ^{14}C as tracers under laboratory conditions and in the field (Table 1), in which the tracer was applied either to branches or to trees of different sizes by enclosing one or several trees in chambers or using free air C isotope enrichment systems. These studies were conducted on a limited number of species (mainly beech and different species of pine) from temperate (33 studies) and boreal (14 studies) forests, while trees from tropical ecosystems have not yet been assessed. These studies addressed several processes, including allocation to respiration, residence time, within-tree and belowground transfer of photosynthates, partitioning of labelled C among organs and the use of stored C (Figure 1). Long-term labelling experiments can elucidate processes that occur over the longer term, but they are not within the scope of this review, although we do mention important results from long-term labelling experiments wherever relevant.

The goals of C pulse-labelling experiments are typically to differentiate between environmental and biological controls on C transfer and allocation, and to elucidate the contributions of old (e.g., stored) and current assimilates to C sink activities.

Table 1. Allocation of assimilated labelled C to different components and processes in trees and soil, as studied by pulse-labelling of trees. Numbers in square brackets refer to the flux depicted in Figure 1. IsoFACE refers to a free air fumigation system. The study reporting ^{14}C pulse-labelling of tall trees was done in the frame of the EBIS project (see the text for details).

Flux	Methods	Type of tree	Species	References	Main finding
Respiration [2]	Pulse ^{14}C, whole tree	Seedlings	Pine	Hansen et al. (1996)	Respiration is a major sink of labelled C assimilated in winter
	Pulse ^{14}C, ecosystem	Shrub, <5 m	Deciduous perennial shrubs	Carbone and Trumbore (2007)	Three pools of labelled C with different half-lives contribute to respiration
	Pulse ^{13}C, crown	Tree, <15 m	Beech	Plain et al. (2009)	Two pools of labelled C with different half-lives contribute to trunk and soil CO_2 efflux
	Pulse ^{14}C, whole tree	Seedling	Pine, spruce and birch	Pumpanen et al. (2009)	Roots and rhizosphere respire 9–26% of assimilated C
	IsoFACE	Tree, >15 m	Beech, spruce	Andersen et al. (2010)	Recent photosynthates contribute differently to root respiration in the two species
	IsoFACE	Tree, >15 m	Beech, spruce	Ritter et al. (2011)	O_3 exposure reduces allocation of recent assimilates to respiration in beech
	Pulse ^{13}C, crown	Tree, <15 m	Beech, oak, pine	Epron et al. (2011)	Allocation to soil respiration varies seasonally depending on species
Storage [3]	Pulse ^{13}C, crown	Tree, < 1 m	Chestnut	Mordacq et al. (1986)	Storage occurs in stump in late summer
	Pulse ^{14}C, branch	Tree, <5 m	Pine	Hansen and Beck (1994)	In autumn, recent photosynthates are transiently stored as soluble C and supply root growth in winter
	Pulse ^{14}C, whole tree	Seedlings	Pine	Hansen et al. (1996)	Starch builds up in winter in root
	Pulse ^{13}C and ^{14}C, branch	Tree, <1 m	Walnut	Lacointe et al. (2004)	Storage is an active sink, not a buffer for excess C
	Pulse ^{13}C, branch	Tree, >15 m	Broadleaved species	Keel et al. (2007)	Recent C mixes fast with older C in some species, depending on the nature of storage compounds
	IsoFACE	Tree, >15 m	Beech, spruce	Kuptz et al. (2011)	Storage is an important sink at the end of summer in beech
	Pulse ^{13}C, ecosystem	Tree, <5 m	Pine	Keel et al. (2012)	Allocation and residence time are different in fine roots and root tips
Structural growth [4]	Pulse ^{13}C, crown	Tree, <1 m	Chestnut	Mordacq et al. (1986)	Aboveground growth is the main sink of recent C in early summer
	Pulse ^{14}C, branch	Tree, <5 m	Pine	Hansen and Beck (1994)	Recent photosynthates of previous year needles supply sprouting at bud break and secondary growth in summer
	Pulse ^{14}C, whole tree	Seedling	Pine	Lippu (1994)	Carbon allocation to root is high before and after the period of intensive shoot growth
	Pulse ^{14}C, whole tree	Tree, <5 m	Poplar	Horwath et al. (1994)	Allocation shifts between aboveground in early summer and belowground in late summer
	Pulse ^{13}C, crown	Tree, <1 m	Larch	Kagawa et al. (2006*a*)	Allocation shifts between aboveground in early summer and belowground in late summer
	Pulse ^{13}C, branch	Tree, >15 m	Beech, hornbeam	Hoch and Keel (2006)	Infructescence photosynthesis contributes to fruit development in hornbeam, not in beech

(*continued*)

Table 1. (Continued)

Flux	Methods	Type of tree	Species	References	Main finding
	Pulse ^{14}C, whole tree	Seedling	Pine, spruce and birch	Pumpanen et al. (2009)	Root (and ectomycorrhiza) growth uses 13–21% of recently assimilated C
	Pulse ^{13}C, whole tree	Tree, <1 m	Fir	Endrulat et al. (2010)	Carbon allocation to cellulose in roots is independent of fine root diameter
	Pulse ^{13}C, branch	Tree, >15 m	Oak, beech, lime tree	Keel and Schädel (2010)	Expanding leaves are strong sink of recent photosynthates, especially in lime tree
	Pulse ^{1}C, whole tree	Cuttings	Birch	Kasurinen et al. (2012)	O_3 shifts C allocation from above to belowground
Remobilization from reserve [6]	Pulse ^{14}C, branch	Tree, <5 m	Pine	Hansen and Beck (1990)	Early wood formation partly relies on stored C
	Pulse ^{14}C, whole tree	Seedling	Pine	Lippu (1998)	Stored C supports root respiration in winter
	Pulse ^{13}C & ^{14}C, branch	Tree, <1 m	Walnut	Lacointe et al. 2004	Stored C supports sprouting in spring
	Pulse ^{13}C, branch	Tree, <15 m	Beech	Nogués et al. (2006)	Stored C contributes to leaf respiration in a proportion varying seasonally
	Pulse ^{13}C, whole tree	Tree, <1 m	Larch	Kagawa et al. (2006*a*)	Half C in new needles is derived from storage
	Pulse ^{14}C, ecosystem	Tree, <5 m	Spruce	Carbone et al. (2007)	Rhizosphere respiration relies more on stored C than on recent photosynthate
	Pulse ^{13}C, whole tree	Seedling	Oak	Vizoso et al. (2008)	40–85% of C in new leaves derives from stored C
	Pulse ^{14}C, EBIS	Tree, >15 m	Broadleaved species	Gaudinski et al. (2009)	50–60% of fine root growth is supported by stored C
	Pulse ^{13}C, whole tree	Tree, <1 m	Fir	Endrulat et al. (2010)	Stored C supports new root growth in next spring
	Pulse ^{14}C, whole tree	Seedling	Acacia	Schutz et al. (2009)	Root starch supports shoot growth after fire-induced stem death
	IsoFACE	Tree, >15 m	Beech, spruce	Kuptz et al. (2011)	Trunk respiration is mostly supplied by stored C in spruce all year round, but only in spring in beech
	IsoFACE	Tree, >15 m	Beech	Grams et al. (2011)	Remobilized C contributes significantly to phloem sugars in summer
BVOC emission [7]	Pulse ^{13}C, whole tree	Seedlings	Pine, spruce, larch, birch	Ghirardo et al. (2010)	The fraction of BVOC emissions originating from recent photosynthates or from stored C varies among species
Basipetal phloem transport [8]	Pulse ^{11}C, leaf	Seedling	Ash, elm, spruce, pine	Thompson et al. (1979)	Transfer rate is higher in broadleaved than in coniferous species
	Pulse ^{11}C, leaf	Seedling	Ash, rowan	Jahnke et al. (1998)	Differences in anatomy account for difference in transfer rate between the two species
	Pulse ^{13}C, branch	Tree, <5 m	Japanese cedar	Kagawa et al. (2005)	Sieve cell connections change seasonally
	Pulse ^{13}C, branch	Tree, <5 m	Larch	Kagawa et al. (2006*b*)	Spiral translocation of photosynthate occurs in this species
	Pulse ^{13}C, ecosystem	Tree, <5 m	Pine	Högberg et al. (2008)	Half-life of labelled C in phloem sap is short (1.3 day)
	Pulse ^{13}C, whole tree	Sapling	Beech	Ruehr et al. (2009)	Transfer rate is reduced by drought

(*continued*)

Table 1. (Continued)

Flux	Methods	Type of tree	Species	References	Main finding
	Pulse ^{13}C, crown	Tree, <15 m	Beech, oak, pine	Dannoura et al. (2011)	Transfer rate is higher in broadleaved than in coniferous species
	Pulse ^{13}C, whole tree	Sapling	Beech	Barthel et al. (2011)	Transfer rate is reduced by drought
	Pulse ^{13}C, whole tree	Tree, <15 m	Pine	Warren et al. (2012)	Transfer rate is not affected by shading
Acropetal phloem transport [9]	Pulse ^{14}C, whole tree	Tree, <1 m	Larch	Schneider and Schmitz (1989)	Labelled C moves acropetally during sprouting
Transfer to soil biota [10–13]	Pulse ^{14}C, whole tree	Seedling	Pine	Andersen and Rygiewicz (1995)	O_3 decreases C transfer to the fungal symbiont
	Pulse ^{13}C and ^{14}C, whole tree	Seedling	Birch and fir	Simard et al. (1997*a*, 1997*b*)	Bidirectional transfer of C occurs between seedlings
	Pulse ^{14}C, tree	Seedling	Pine	Heinonsalo et al. (2010)	The sink strength depends on the root-associated mycorrhizal fungal symbiont
	Pulse ^{14}C, tree	Tree, <1 m	Aspen	Mikan et al. (2000)	Elevated CO_2 increases transfer of labelled C to microbial biomass
	Pulse ^{14}C, tree	Seedling	Pine	Leake et al. (2001)	Mycorrhizal mycelia allocate C to fine roots foraging for nutrients
	Pulse ^{13}C, ecosystem	Tree, <5 m	Pine	Högberg et al. (2008)	Recent C is rapidly found in microbial cytoplasm
	Pulse ^{13}C and ^{14}C, tree	Seedling, in the field	Fir	Teste et al. (2010)	Transfer of C between seedlings exists but it is small and it depends on disturbance
	Pulse ^{13}C, ecosystem	Tree, <5 m	Pine	Högberg et al. (2010)	Transfer to soil microbes is higher in late than in early summer and is reduced by nitrogen fertilization
	Pulse ^{13}C, crown	Tree, <15 m	Beech, oak, pine	Epron et al. (2011)	Recent C is rapidly found in ectomycorrhiza and microbial cytoplasm

BVOC = biogenic volatile organic compounds.

We specifically investigate (i) how fast the assimilated C is transferred belowground, how the rate of C transfer differs among species and how this transfer rate responds to environmental factors; (ii) how residence times of labelled C differ among tree compartments depending on their source/sink status and on the respective season; (iii) how climate and environmental stresses influence C allocation to different sink organs and whether we are able to disentangle the effects of phenology from those mediated by environmental factors; and (iv) how stored versus current assimilates contribute to growth and respiration demands in sink organs, owing to the role of C storage in perennial species.

Pulse-labelling of trees

Pulse-labelling of trees with labelled CO_2 allows C fluxes to be traced in the tree–soil system. Different forms of labelled C were applied to branches, potted trees or field-grown trees of different sizes (Table 1). We address whether these methods are adequate to investigate short-term C allocation patterns and based on past and current works, we recommend best practices for designing future C pulse-labelling experiments.

Carbon isotopes as tracers

Labelling plants with isotopically enriched CO_2 has been used for decades to study the fate of C into photosynthetic products in leaves. One historical example is the experiments that were conducted in the 1950s by Calvin and co-workers leading to the discovery of the photosynthetic C reduction cycle (Calvin 1961). ^{14}C (half-life of 5730 years) was initially used because tools were available for tracing the radioactivity in the labelled compounds (autoradiography, liquid scintillation (LS) spectrometry and accelerator mass spectrometry (AMS)). The precision of the LS counter technique is adequate for studies

conducted in mesocosms in the laboratory where the β-radiation emissions resulting from the decay of ^{14}C in the label are high enough to detect, but remain under the safety limits. More recently, the development of AMS has enabled the use of much lower amounts of radioactivity opening the doors for in situ labelling experiments (Carbone et al. 2007, Carbone and Trumbore 2007), despite substantially higher costs per analysed sample compared with LS. Large releases of $^{14}CO_2$ from a hazardous waste incinerator have unintentionally pulse-labelled a mature deciduous forest (EBIS project, http://ebis.ornl.gov/) and ^{14}C was successfully used to infer residence time and turnover of C in various forest ecosystem compartments, e.g., roots (Joslin et al. 2006, Gaudinski et al. 2009). Another radioactive C isotope, ^{11}C, with very short half-life (about 20.4 min), has proven to be a useful tracer for studying the response of translocation of photosynthates in small plants to fast changes in environmental conditions or to source–sink manipulations. The advantage of ^{11}C is that it allows multiple labelling of the same plants at very short time intervals (Roeb and Britz 1991, Thorpe et al. 2011). However, ^{11}C has been rarely used and only for tree seedlings because the need to produce $^{11}CO_2$ and detect ^{11}C restricts its use to specifically equipped laboratories (Thompson et al. 1979, Jahnke et al. 1998, 2009).

For pulse-labelling trees in the field, one advantage of $^{14}CO_2$ compared with $^{13}CO_2$ is that very small amounts of $^{14}CO_2$ can be supplied to the foliage because of the high sensitivity of AMS and the very weak environmental background for ^{14}C compared with ^{13}C in the atmosphere and in the plant–soil system. In contrast, when trees are pulse-labelled with $^{13}CO_2$, either the CO_2 concentration has to be raised above the ambient level or the CO_2 concentration in the labelling chamber has to be decreased prior to adding the label. Nevertheless, because of the high cost of sample preparation and of AMS analyses of ^{14}C, using $^{13}CO_2$ for pulse-labelling is much more cost effective than using $^{14}CO_2$, and laws restrict the field use of the radioactive isotopes in many countries. The use of $^{13}CO_2$ for studying the fate of C in the plant–soil system built upon the lack of restrictions of its use, and the increased availability of stable isotope ratio mass spectrometry since the early 1990s.

Dual labelling with both ^{13}C and ^{14}C is a powerful approach for studying C flux and turnover in pools that differ in turnover rates: the low cost of ^{13}C analysis allows frequent samplings for pools with high turnover rates and ^{14}C can be used for infrequent samplings of pools with slower turnover rates (Lacointe et al. 2004, Carbone and Trumbore 2007). Dual labelling with ^{13}C and ^{14}C can also be used to study bi-directional transfer of C, e.g., between tree seedlings (Simard et al. 1997*b*, Teste et al. 2010).

Potential and limitations of the pulse-labelling approaches

The pulse-labelling approach is appropriate for studying how fast and where C fixed in photosynthesis moves into the tree and to the soil, especially if the main interest is in the seasonal changes in C allocation dynamics (Table 1). The newly assimilated C is predominantly allocated to active metabolic sinks. These sinks can also attract C remobilized from storage compartments (Paterson et al. 2009). Depending on when the labelling is done and until when the labelled C is further traced, each of these sources can be studied.

Furthermore, pulse-labelling can also estimate the mean residence time and the half-life time of C in compartments. The mean residence time corresponds to the C stock to C flux ratio and it is not an intrinsic property of a compartment. Pools with long residence time have either a large C stock or low exchange rates (or both). Long-term labelling approaches are more appropriate to estimate residence times in pools that turnover slowly.

Long-term labelling experiments have so far been restricted to young, small potted trees because of the high cost of continuously flushing the labelling chamber with isotopically enriched CO_2 (Maillard et al. 1994, Dyckmans and Flessa 2001, Dijkstra and Cheng 2007, Guérard et al. 2007, Esperschütz et al. 2009*b*, Palacio et al. 2011). The free air CO_2 enrichment (FACE) studies, which were designed to study the effects of elevated CO_2 on ecosystems (Palmroth et al. 2006, Millard et al. 2007) gave opportunities to follow the fate of assimilated C over longer times because industrial CO_2 is ^{13}C depleted compared with ambient CO_2 (Keel et al. 2006, von Felten et al. 2007, Bader et al. 2009). However, elevated CO_2 may shift C partitioning towards belowground biomass compartments, and thus bias estimates of C allocation. Such CO_2 effects are still unclear and may depend on the genetically determined development and growth of the species, the developmental stage of the tree and the availability of resources other than C at the site (Körner 2006, Dieleman et al. 2010). In addition, labelling starts when the CO_2 treatment begins, therefore any dynamics revealed by the 'tracer' corresponds to a transient system that is adapting its growth, allocation and storage patterns in response to elevated CO_2, and thus limiting inferences regarding mechanisms for ambient, elevated or slowly changing CO_2 concentrations.

Free air systems (IsoFACE) have been specifically developed for labelling small trees in the field (Talhelm et al. 2007) but also tall trees in situ (Grams et al. 2011) for several days or weeks. Although the labelling duration is shorter than typically used in long-term labelling experiments, trees are however exposed to above ambient CO_2 concentrations for longer periods than in classical pulse-labelling approaches. While this longer labelling period may be useful for estimating half-lives in pools that turn over slowly and bypasses the effects of diurnal allocation changes, it is of limited value for studying the rapid dynamics of C transfer among pools or compartments.

An alternative pulse-labelling approach based on the injection of dissolved ^{13}C-carbonate into the xylem was recently

applied on small cedar trees (Powers and Marshall 2011). This technique might become a cheap and easy option for pulse-labelling big trees in remote areas. Xylem-delivered carbonates are thought to provide CO_2 to the leaves where it will be fixed by photosynthesis and transported into the other plant compartments. However, labelled organic C from non-photosynthetic, anaplerotic CO_2 fixation in the trunk might lead to misinterpretations of assimilate transport and transport velocities.

Implementing setups for pulse-labelling

Because of their simplicity, closed systems have been used in most pulse-labelling experiments with trees. A suitable labelling chamber, whatever its size, should be airtight to limit the amount of labelled CO_2 lost and not taken up by the foliage. Since CO_2 and water vapour concentrations inside the chamber will change rapidly over time because of leaf photosynthesis and transpiration, they should be monitored. Furthermore, it is strongly advised to regulate both air temperature and air humidity inside the chamber using air-conditioning devices (Högberg et al. 2008, Plain et al. 2009), since high air temperatures, potentially damaging the photosynthetic apparatus, are expected in closed chambers under high solar radiation. This is one of the reasons why the labelling duration should be as short as possible and, if the cooling capacity is insufficient, should be done quite early in the morning. However, allocation patterns to different foliar C pools (e.g., transient starch storage, respiration, VOC emission) may change over the course of the day and thus need to be considered for data interpretation.

While the injection of $^{13}CO_2$ tracer can be regulated by mass flow controllers to maintain CO_2 concentrations within the chamber at ambient level, additional unlabelled CO_2 should be injected into the chamber when using $^{14}CO_2$ to compensate for photosynthetic uptake, maintaining CO_2 concentrations at the ambient level. In both cases, mixing of air is needed to enable a uniform distribution of labelled CO_2 inside the chamber (Carbone et al. 2007).

Using open systems could solve many of the problems described above: ambient CO_2 concentrations would not change, enrichment would be constant and chambers would not need to be air-tight, although the consumption of labelled CO_2 would be significantly larger. At optimized air flow, changes in air humidity and temperature inside labelling chambers would be minimal, even under high irradiance. But until now, open systems have been used only in grasslands (Lattanzi et al. 2012) or for long-term labelling of potted, small trees (Dyckmans and Flessa 2001, Esperschütz et al. 2009*b*, Palacio et al. 2011), although in principle, they are suitable for short-term labelling of several large chambers operating simultaneously on large trees, depending only on the size of the air-generating unit.

In IsoFACE systems, trees are not enclosed in chambers. However, even using 99% $^{13}CO_2$, the enrichment of the plant that can be achieved is relatively low, while the pulse duration needs to be quite long (5 days in Talhelm et al. 2007). Thus, the costs for labelled $^{13}CO_2$ are clearly restricting the use of this approach for taller trees. Although costs could be significantly reduced using ^{13}C-depleted CO_2 from fossil fuels, the labelling signal would be relatively small compared with that of enriched $^{13}CO_2$ and again require long labelling periods (Grams et al. 2011).

Whole-tree pulse-labelling

For the practical reasons mentioned above, pulse-labelling experiments have largely been restricted to seedlings and small trees ex situ until recently (Table 1). However, C allocation rules are thought to change with tree age and to be influenced by neighbouring trees (Litton et al. 2007, Poorter et al. 2012), requiring in situ labelling of trees. For several decades, in situ labelling was restricted to individual branches, ever since the pioneering work on mature white oak trees (McLaughlin et al. 1979) and orchard trees (Hansen 1970). The main limitation of branch labelling is the mixing of labelled carbohydrates with a large amount of unlabelled carbohydrates coming from the remaining branches of the crown. Recovery of labelled C at the whole-tree level or in the soil is therefore compromised. Despite its limitation, branch labelling was useful to study the transition from heterotrophy to autotrophy after leaf emergence (Keel and Schädel 2010), branch autonomy (Lacointe et al. 2004) and the mixing of old and new C pools in the branch tissues (Keel et al. 2007), and to reveal spiral translocation paths in the stem of larch saplings (Kagawa et al. 2006*b*).

Until recently, whole-tree labelling in the field was restricted to short coniferous trees in the boreal forest (Kagawa et al. 2006*a*, Carbone et al. 2007, Högberg et al. 2008, 2010, Keel et al. 2012) or to woody shrubs in semi-arid ecosystems (Carbone and Trumbore 2007). More recently, the crowns of 10 m tall trees (Plain et al. 2009, Dannoura et al. 2011, Epron et al. 2011) were successfully pulse-labelled. Two options have been proposed for labelling whole trees in the field: large canopy chambers covering 10–50 m^2 of soil including a small 'forest ecosystem patch' with several short trees (Carbone et al. 2007, Högberg et al. 2008) and chambers in which the entire crown of a single tree is enclosed while excluding the soil surface (Plain et al. 2009). Crown chambers allow labelling of trees only one by one and require manpower when moving them from tree to tree, limiting the number of replicates. Moreover, trees are often labelled on different days under different environmental conditions (Dannoura et al. 2011, Epron et al. 2011). In contrast, canopy chambers allow labelling of several trees at the same time (which are in fact pseudo-replicates), although being restricted to short trees. A disadvantage

of canopy chambers is back-diffusion, i.e., labelled CO_2 enters the soil pores during the labelling and diffuses back out several hours later, potentially confounding recovery of labelled CO_2 in belowground respiration (Subke et al. 2009). However, back diffusion of labelled CO_2 can be monitored on root-free collars inside the chamber. Furthermore, labelled CO_2 entering soil pores may be assimilated by the soil microbes (Miltner et al. 2004) or even by the roots through the anaplerotic pathway (Ford et al. 2007, Gessler et al. 2009). A similar artefact could also be expected for bark, into which labelled CO_2 might diffuse during labelling and there be assimilated by bark photosynthesis or anaplerotic reactions.

The duration of the pulse should be as short as possible for a precise determination of time lags between the start of the labelling and the first appearance of labelled compounds in sink compartments or respiratory efflux (Figure 2). The shape of the curve is affected by the duration of the labelling, and this impact is amplified by the radial velocity profile in the sieve tubes of the phloem due to friction along the tube wall (Dannoura et al. 2011) and by the mixing of labelled and unlabelled C. For this reason, we strongly recommend using a constant labelling duration among treatments and replicates, if the main objective is to study the rate of transfer and residence times of C in labile pools. On the other hand, if the main objective is to study allocation among plant compartments, using the same amount of labelled substrate for each tree is defensible, even if it may change the duration of the labelling depending on the photosynthetic activity of the tree (Epron et al. 2011).

The amount of label should be high enough, especially in the field, to be detected in compartments where it will be diluted by a large pool of unlabelled C. This is more challenging with $^{13}CO_2$ than with $^{14}CO_2$ because of their respective atmospheric backgrounds and detection limits (isotope ratio mass spectrometry (IRMS) versus AMS). Thus, a trade-off exists between the length of the labelling period and the level of enrichment used, since exposing trees to CO_2 concentrations well above ambient levels should be avoided. Increasing the amount of labelled CO_2 without increasing the total chamber CO_2 concentrations could be done by reducing chamber CO_2 concentrations prior to labelling either by removing air inside the chamber (Keel et al. 2007), scrubbing CO_2 with soda lime (Ruehr et al. 2009) or waiting for reductions in CO_2 concentrations through photosynthesis (Plain et al. 2009).

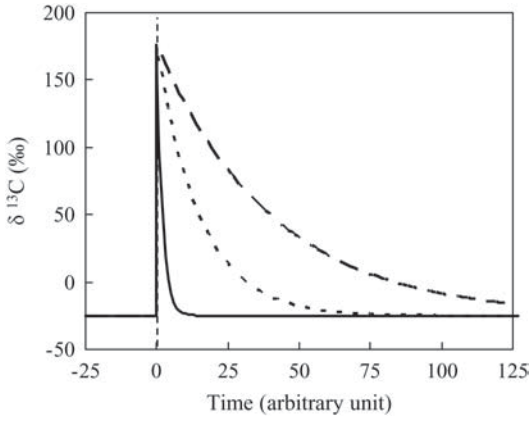

Figure 2. Schematic diagram showing the exponential, first-order kinetics of a tracer (^{13}C) in any plant compartment with a mean residence time of 2 ($t_{1/2} = 1.4$, continuous line), 15 ($t_{1/2} = 10.4$, dotted line) or 40 ($t_{1/2} = 27.6$, dashed line) time units, as typically observed in pulse-labelling experiments. The time unit on the *x*-axis is arbitrary. The labelled CO_2 is introduced at time 0 (vertical dashed bar) as a pulse. No time lag is considered between labelling and tracer recovery.

Monitoring the amount of labelled C delivered to the tree

While the isotope composition of CO_2 within the labelling chamber can be known a posteriori by taking air samples from the headspace frequently and analysing these samples, monitoring total CO_2 concentration during the labelling is more challenging because the new generation of infrared gas analysers (IRGAs) is quite specific to $^{12}CO_2$ and rather insensitive to $^{13}CO_2$ and $^{14}CO_2$ (Tohjima et al. 2009). While this is not an issue for $^{14}CO_2$ labelling because the amount of $^{14}CO_2$ remains low compared with the amount of $^{12}CO_2$, the problem is more serious for $^{13}CO_2$ labelling, since $^{13}CO_2$ is typically the major isotopologue of CO_2 in the chamber during labelling. One option is to use an old generation IRGA and accurately measure total CO_2 concentrations. Another option is using two IRGAs with different $^{13}CO_2$ sensitivities. A further option is to use a dual IRGA that measures both $^{12}CO_2$ and $^{13}CO_2$ concentrations within the same concentration range (Plain et al. 2009). Isotope ratio infrared spectrometers are not necessarily suitable for measuring high $^{13}CO_2$ concentration in the labelling chamber, unless the air stream coming from the chamber is strongly diluted with $^{13}CO_2$-free air before entering the IRIS.

Tracing the labelled C

The goal of pulse-labelling experiments is to trace the labelled C within the tree–soil system. Technologies are developing fast but sampling issues remain the Achilles heel of these experiments, especially under field conditions.

Expression of isotope composition

While the isotope composition at natural abundance levels is often expressed as a ratio relative to an international reference standard ($\delta^{13}C$, $\Delta^{14}C$), the isotope composition of an enriched compartment can be better expressed as percent atom excess (Dawson et al. 2002). This value is defined as the relative abundance of the heavier isotope in a labelled compartment exceeding the natural isotope abundance in the same unlabelled compartment.

$$\text{Percent atom excess} = (Ab_L - Ab_{UL}) \times 100$$

$$\text{with} \quad Ab = \frac{^{H}C}{^{12}C + ^{H}C}$$

Ab is the relative abundance of the heavy isotope in the labelled (L) and unlabelled (UL) compartments and ^{H}C is either ^{13}C or ^{14}C. For ^{13}C, Ab can be calculated directly from the concentration of both isotopologues (e.g., measured by IRIS) or derived from the δ values (IRMS) and the isotope ratio of the international reference standard (R_{ref}):

$$Ab = \frac{\left((\delta^{13}C)/(1000) + 1\right) \times R_{ref}}{\left[\left((\delta^{13}C)/(1000) + 1\right) \times R_{ref}\right] + 1}$$

Labelled C in the CO_2 efflux

Several approaches are commonly used for tracing labelled CO_2 in the respiratory efflux: IRMS or IRIS are used for ^{13}C and LS or AMS for ^{14}C. When using laboratory IRMS facilities, CO_2 efflux is monitored using closed-loop chamber systems and the air is sampled at different times after closing the chamber (i.e., at different CO_2 concentration in the chamber). The C isotope composition of the respiratory CO_2 can be deduced from the Keeling-plot intercept (Högberg et al. 2008, Ruehr et al. 2009). The main limitation of this approach is the low frequency of sampling and the analysis costs. Mobile IRMS facilities and flow through chambers allow continuous measurement of $\delta^{13}C$ of CO_2 in the respiratory efflux on site, thus increasing the measurement frequency (Schnyder et al. 2004, Subke et al. 2009). More recently, IRIS has offered the opportunity to sharply increase the measurement frequency at reduced costs (Bahn et al. 2009, Marron et al. 2009, Plain et al. 2009). The frequency of the measurements depends on the number of chambers connected to the device, but typically 10 chambers can be measured within 1 h or less. Several IRIS technologies are now available including tuneable diode laser systems (e.g., TGA 100 A, Campbell Scientific, Logan, UT, USA), quantum cascade laser systems (e.g., CO_2 Isotope Trace Gas Monitor, Aerodyne, Billerica, MA, USA), cavity ring down spectroscopy (e.g., G2131, Picarro, Santa Clara, CA, USA), off axis integrated cavity output spectroscopy (e.g., CCIA-36, Los Gatos Research, Mountain View, CA, USA) or Fourier transform infrared spectroscopy (e.g., Vector 22, Bruker Optics, Ettlingen, Germany), all presenting advantages and disadvantages that are not within the scope of this review. Calibration is an important issue whatever technology is used because a wide range of C isotope compositions is expected. In contrast to IRMS, IRIS does not measure the isotope ratios but the mixing ratios of individual isotopologues (e.g., $^{12}CO_2$ and $^{13}CO_2$). The working standards should therefore bracket the expected mixing ratios of both isotopologues.

Labelled C recovered in trees

In order to properly trace the labelled C into the bulk material of trees the sampling scheme must allow capturing both the label peak and the long lasting tail of the recovery kinetics. Since an exponential decay of the isotope signal might be expected, it is advisable to take samples frequently at the beginning of the experiment—starting immediately after labelling—and to reduce the sampling rate progressively following a geometric law. Studying allocation will require sampling of foliage, twigs, branches, trunk cores as well as coarse and fine roots. Soil samples are needed for the analysis of labelled C in microbial biomass and soil organisms, in group-specific membrane-bound fatty acids or putatively in nucleic acids.

Special care should be taken into account for the spatial heterogeneity, especially for fine roots retrieved from soil cores (Endrulat et al. 2010). Two main points need to be considered when sampling fine roots in the field: dead fine roots should be disregarded and the living roots sampled should only belong to the labelled trees. The labelled trees can be isolated for at least several months before labelling by digging a trench around the tree. The trench needs to be lined with a polyethylene foil and refilled with soil. This approach ensures that all roots and root exudates within the known soil volume originate from the isolated tree and that all active roots of the labelled tree are contained inside this trenched area (e.g., Plain et al. 2009). However, regrowth of roots to re-establish the root-to-shoot ratio might take much longer depending on the intensity of the disturbance. The size of the delimited area should be large enough to avoid any water stress resulting from a reduction of the size of the root system. This may not be possible with species that spread their roots far from the trunk or with deep-rooting species. The problem of sampling roots of unlabelled trees is also avoided when using large chambers that cover e.g., 50 m^2 or more of soil, instead of single crown chambers. Högberg et al. (2008) assessed the influence of unlabelled trees by labelling the soil in the studied central area of the plots with ^{15}N, and traced the ^{15}N label in the tree canopies inside and just outside the plots, assuming that the transfer of N in acropetal direction was most likely associated with the transfer of C in the reverse direction. A more laborious option is to genetically identify the target tree species from root fragments (Endrulat et al. 2010).

Plant organs are composed of various C pools of different ages and with different residence times, and it is worthwhile to study each pool individually to assess the dynamics of each

pool and the rates of exchange between the pools. It is at least interesting to isolate the soluble fraction that contains a mixture of sugars, organic acids and amino acids (Brandes et al. 2006, Ruehr et al. 2009), since starch and structural C have different turnover rates. It is also an option to collect phloem sap samples either directly by making shallow incision into the bark (bleeding techniques) in suitable species, by exudation of soluble compounds from small pieces of bark tissues punched from the trunk or by using aphids as sap collectors (Yoneyama et al. 1997, Gessler et al. 2004, Dannoura et al. 2011).

Labelled C recovered in soil microorganisms

Because recent photosynthates are rapidly transferred to the soil microbial compartments (Högberg and Read 2006, Högberg et al. 2010), either directly through the mycorrhizal network or through root exudation, labelled C is expected to be retrieved in soil soluble organic matter and in the microbial biomass. Soluble organic C can be extracted with a weak saline solution (typically K_2SO_4) from fresh soil samples. Microbial C can also be extracted from fresh soil samples that have previously been fumigated with chloroform to disrupt the microbial membranes (Vance et al. 1987). The difference in labelled C between fumigated and non-fumigated soil samples is supposed to be the microbial C released from microorganisms that have been destroyed by the fumigation (Rangel-Castro et al. 2005*b*, Ruehr et al. 2009, Epron et al. 2011). This approach does not discriminate between fungi, bacteria and Archaea. The ^{13}C transferred to the mycorrhizal hyphae network can be retrieved in soil cores surrounded by an appropriate nylon mesh (Johnson et al. 2002, Epron et al. 2011). Molecular approaches to trace the ^{13}C or ^{14}C in nucleic acids (Ostle et al. 2003, Rangel-Castro et al. 2005*a*), amino-sugars or fatty acids (Treonis et al. 2004, Amelung et al. 2008, Denef et al. 2009, Esperschütz et al. 2009*b*, Högberg et al. 2010) are promising to identify the microbial groups that strongly respond to tree photosynthesis. They are, however, challenging in the field because of the dilution of the tracer within the large soil C pool (Griffiths et al. 2004). While pulse-labelling experiments are normally sufficient for tracing ^{13}C from plants to phospholipid fatty acids of microbial groups, longer labelling periods might be required for probing ^{13}C in microbial DNA and RNA for the identification of functional or taxonomic groups (Neufeld et al. 2007) in forest soil.

Dynamics of photosynthates in tree and soil compartments

Tracing labelled C after pulse-labelling a tree allows quantifying at least three important aspects of whole-plant C metabolism: the rate of transfer of C substrates between compartments, the residence time of C in these compartments and the number of kinetically distinct pools in a metabolic network. Robust hypotheses accounting for the effects of environmental factors on—and species differences in—the rate of transfer have already emerged. An increase in the measurement frequency of labelled C in the CO_2 efflux combined with compartmental analysis of tracer time courses is promising for characterizing the half-life and the relative importance of the different substrates that fuel respiration.

The pattern of recovery: what a shape can tell

The shape of the recovery of labelled C in a compartment or in the respiratory efflux has a few distinct phases (Figure 2). Tracer appearance is typically delayed relative to the labelling pulse (time lag). Then, the amount of labelled C increases until a maximum is reached (peak), and subsequently decreases more or less exponentially until eventually no tracer is left (new isotopic equilibrium).

The time lag can vary from a few minutes to several hours or days. This, of course, depends on the distance between the source leaves and the observed sink. It also depends on the rate of transport of C within the plant and into the soil. Recent studies show that transport rates vary depending on species, phenology and environmental conditions (Ruehr et al. 2009, Dannoura et al. 2011, Epron et al. 2011).

The shape of tracer time courses reflects both (i) the extent to which labelled C has mixed with unlabelled C pools before arriving in the sampled compartment (i.e., the number and turnover rate of those mixing pools), and (ii) the turnover rate of the observed compartment itself. Thus, time courses characterized by sharp and rapid increases and decreases are indicative of pools closely associated to (mainly fed by) current assimilates. As the label is transferred along the translocation path, for instance, from the top of the tree to belowground sinks (Dannoura et al. 2011), the shape of the tracer time course is changing. The often observed slower rates of increase, the lower and broader peaks, and the slower rates of decrease reflect the fact that the tracer is mixing with more and more pools as it travels downwards.

A clear record of the shape of the peak requires a high measurement frequency, in particular before and after the maximum when rates of change are fast. For compartments close to C assimilation, such as soluble carbohydrates in photosynthetic leaves, this implies that sampling should ideally start during labelling. It also requires a sustained sampling effort throughout the exponential decrease, so that all acting pools can be detected. For tissues with a slow turnover rate, such as the structural biomass of roots, sampling should thus last for several months (although not necessarily at high frequency).

Of course, the actual values of tracer content are strongly affected by the duration and enrichment of the labelling pulse. Therefore, direct comparisons of tracer contents among

treatments, or even among individuals within a given treatment, are often meaningless. Yet, the parameters that can be derived from the shape of the tracer timecourse—i.e., the time lag, the time to peak, the rate of exponential decrease and the time to new isotopic equilibrium—would be invariant.

Variations in the rate of C transfer among species

The rate of C transfer (flux [8] in Figure 1) is often calculated from the time lag between C uptake (i.e., labelling time) and the recovery of the label in the CO_2 efflux in the soil, or from differences in time lags between the start of the labelling and the appearance of ^{13}C in CO_2 efflux measured at different positions along the trunk or along a coarse root (Dannoura et al. 2011). The rate of C transfer in the tree as well as between the canopy and the soil varies greatly among different labelling experiments and numerous factors may account for this large range of variation.

One source of variation that explains differences in time lags is undoubtedly the time resolution of measurements. The time lag between uptake and recovery also depends on the size of the tree, i.e., on the distance between the source and the sink, and on the species (Figure 3a). There are indeed clear species differences with coniferous species exhibiting on average 10 times lower rates of label transfer than broadleaved species (Thompson et al. 1979, Schneider and Schmitz 1989, Jahnke et al. 1998, Högberg et al. 2008, Plain et al. 2009, Dannoura et al. 2011, Table 2). Pulse label experiments thus confirm observations based on the transfer of weather-induced change in photosynthetic isotope discrimination signals (Kuzyakov and Gavrichkova 2010, Mencuccini and Hölttä 2010) and most likely reflect differences in phloem anatomy (Jensen et al. 2012). When the rate of transfer to the trunk is estimated with the same method for trees of similar size, the relation between the rate of C transfer and the time lag of peak $^{13}CO_2$ efflux from the soil differs markedly between broadleaved species (oak and beech) and pine (Figure 3b). Differences in soil macroporosity were unlikely to explain the differences in the rate of transfer in this study. The results suggests a longer retention time in the pine foliage or the roots that may be due to strong mixing of new, labelled C with old unlabelled C.

Responses of the rate of transfer to environmental factors

The velocity itself might also be size dependent, with higher rates of transfer in tall compared with small trees. For instance, ^{13}C label in soil microbial biomass was detected one day after labelling in beech saplings of 0.5 m height (Ruehr et al. 2009) as well as in 10 m tall beech trees (Epron et al. 2011). This finding is substantiated by the observation that in beech the velocity of C transfer in the trunk is positively related to the diameter of the tree (Dannoura et al. 2011). The velocity of C transport also varies within a tree, with higher speed at the top of the trunk than at the bottom.

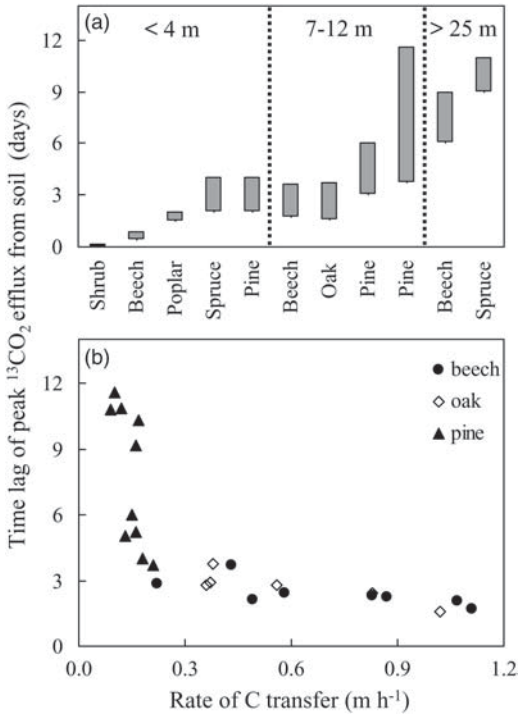

Figure 3. Time lag of peak $^{13}CO_2$ efflux from soil (or coarse roots). (a) For different tree species and sizes. The size of the box reflects both the seasonal variability and uncertainties due to low measurement frequency (data from Horwath et al. 1994, Carbone et al. 2007, Carbone and Trumbore 2007, Högberg et al. 2008, 2010, Barthel et al. 2011, Epron et al. 2011, Kuptz et al. 2011, Warren et al. 2012). (b) Relationships between the lag before labelled C peaks in soil CO_2 efflux (Epron et al. 2011) and the rate of C transfer in the trunk (Dannoura et al. 2011) in 10 m tall beech (closed circles), oak (open diamonds) and pine (closed triangles) trees pulse-labelled at different times of the year.

The rate of transfer is also strongly related to environmental conditions and is decreasing with lower temperature and soil water content (Ruehr et al. 2009, Barthel et al. 2011, Dannoura et al. 2011). In contrast, shade did not affect the rate of C transfer in pine (Warren et al. 2012). Changes in phloem sap viscosity and changes in the turgor pressure gradients between source and sink organs are thought to account for these changes. The changes in turgor gradients are mediated by tree transpiration, xylem water potential and by the activity of source and sink organs where phloem loading and unloading occur. However, the processes that control the sink activity and the feedbacks on source activity, and therefore the long distance transport of C in the tree, are still poorly understood at the whole-plant level.

Fast belowground C transfer

Whatever the species differences in transport velocity are, all results highlight a rather fast transfer of recent assimilates to the soil biota (fluxes [10-13] in Figure 1). The labelled C is

Table 2. Rates of C transfer in broadleaved and coniferous tree species, as estimated from pulse-labelling experiments.

	Species	Methods	References	Velocity (m h^{-1})
Broadleaved	Ash, elm	^{11}C (seedlings)	Thompson et al. (1979)	0.3–6.0
	Ash, rowan	^{11}C (seedlings)	Jahnke et al. (1998)	0.3–1.3
	Beech	^{13}C (10 m tall trees)	Plain et al. (2009)	1.0 (late summer)
	Beech, oak	^{13}C (10 m tall trees)	Dannoura et al. (2011)	0.2–1.2 (over the growing season)
	Beech	^{13}C (saplings)	Barthel et al. (2011)	0.4 (control)–0.1 (drought)
Coniferous	Spruce, pine	^{11}C (seedlings)	Thompson et al. (1979)	0.1
	Larch, boreal	^{14}C (saplings)	Schneider and Schmitz (1989)	0.1–0.2 (over the growing season)
	Pine (boreal)	^{13}C (2 m tall trees)	Högberg et al. (2008)	0.1 (late summer)
	Pine (temperate)	^{13}C (10 m tall trees)	Dannoura et al. (2011)	0.1–0.2 (over the year)

observed after a few days or less and detected almost at the same time in roots and in the microbial biomass (Högberg et al. 2008, Ruehr et al. 2009, Epron et al. 2011). The likely mechanism for this to happen is that labelled C is rapidly captured by symbionts and/or exuded by the mycorrhizal roots and taken up by soil microorganisms.

Among soil biota, fungi (especially ectomycorrhizal fungi) and Collembola were rapidly and strongly labelled, compared with bacteria, Acari and Enchytraeidae (Esperschütz et al. 2009*b*, Heinonsalo et al. 2010, Högberg et al. 2010). A fast transfer of labelled C was observed in litter patches, where growing mycorrhizal mycelia were an active C sink for pine seedlings (Leake et al. 2001). Labelled C transferred to the mycorrhizal network can further be shared among competing seedlings (Simard et al. 1997*a*, 1997*b*, Teste et al. 2010).

The challenge now is to quantify the importance of this flux for the metabolism of both plants and microorganisms (Grimoldi et al. 2006). Most probably, the transfer of labelled C from the plant is most relevant for microorganisms living in close proximity to root tips, and less important for those feeding on older pools of soil organic matter. Most interestingly, drought stress decreased (even cancelled) C transfer to soil microorganisms (Gorissen et al. 2004, Ruehr et al. 2009, Barthel et al. 2011). To what extent this finding is due to the lower C assimilation rate, to changes in C partitioning, or to changes in microorganism activity is not yet clear.

The residence time in tree and soil

The rate of decrease of the amount or the fraction of labelled C recovered in a compartment or a flux after the peak can often be described by an exponential function:

$$C(t) = C_0 \exp(-kt)$$

where t is the time after the peak, C_0 the quantity or fraction of labelled C at peak time, k is the rate constant of tracer loss and $C(t)$ the quantity or fraction of labelled C at time t (Figure 2). Assuming that the system is in a steady state (no change in size), that the process follows first-order kinetics (fluxes are directly proportional to pool sizes), that only one kinetic pool is acting, and that tracer disappearance is only governed by tracer efflux, the mean residence time (τ, the average time which C atoms reside in a reservoir) and the half-life ($t_{1/2}$, the time required to exchange 50% of the C atoms in a reservoir) can be calculated (Derrien and Amelung 2011) as

$$\tau = \frac{1}{k} \quad \text{and} \quad t_{1/2} = \frac{\ln(2)}{k}$$

Mean residence time and half-life are both important attributes of the C dynamics. It is therefore important that the caveats for their estimation are explicitly stated. For most studies, the assumption that the system is in steady state and follows first-order kinetics is only a good approximation. Moreover, the condition of a pool which only loses tracer is not realized for belowground compartments (roots, microbial biomass, soil), or for structural compounds, which typically exhibit bell-shaped patterns of label recovery (Endrulat et al. 2010, Epron et al. 2011). Furthermore, analysing a single physical compartment does not assure that the tracer is present in a single kinetic pool. For instance, leaves contain (at least) three kinetically contrasting pools: labile, transiently stored and structural compounds (Figure 1). Therefore, it is important that fairly complete records of tracer content are available (i.e., from the beginning of the pulse and for a chase period of adequate duration) so that the presence of slow pools can be detected and accounted for when estimating mean residence time and half-life.

Differences in half-lives between tree compartments

The half-life of labelled C in the phloem sap is similar between deciduous broadleaved species and evergreen coniferous species (Table 3). In pine, $t_{1/2}$ values in winter are more than twice those estimated for the growing season. The half-life in phloem sap is typically short, indicating its transport function from the canopy to sink organs (Högberg et al. 2008, Dannoura et al. 2011, Warren et al. 2012). For similar reasons, the half-life of labelled C in the soil solution is short (Esperschütz et al. 2009*a*) and the labelled C is sometimes undetectable due to a rapid absorption by the soil microorganisms (Epron et al. 2011). The amount of labelled C recovered in the mature foliage decreases

Table 3. Half-life of labelled C in mature leaves, phloem sap and respiratory substrate pools contributing to soil CO_2 efflux (assuming either one or two pools), as estimated from pulse-labelling experiments.

	Species	Methods	References	Half-life (days)
Phloem	Pine (boreal)	^{13}C (2 m tall trees)	Högberg et al. (2008)	1.3 (late summer)
	Pine (temperate)	^{13}C (7 m tall trees)	Warren et al. (2012)	4.7 (late summer)
	Pine (temperate)	^{13}C (10 m tall trees)	Dannoura et al. (2011)	0.4–3.4 (over the year)
	Beech	^{13}C (10 m tall trees)	Dannoura et al. (2011)	0.4–1.7 (over the growing season)
	Oak	^{13}C (10 m tall trees)	Dannoura et al. (2011)	0.9–1.3 (over the growing season)
Leaves	Pine (boreal)	^{13}C (2 m tall trees)	Högberg et al. (2008)	1.3 (late summer)
	Pine (temperate)	^{13}C (7 m tall trees)	Warren et al. (2012)	0.5 (late summer)
	Pine (temperate)	^{13}C (10 m tall trees)	Unpublished[1]	1.4–3.6 (over the year)
	Beech	^{13}C (10 m tall trees)	Unpublished[1]	1.3 (over the growing season)
	Beech	^{13}C (saplings)	Ruehr et al. (2009)	1.7 (control)–2.3 (drought),
Soil CO_2 efflux—1 pool	Poplar	^{14}C (3 m tall trees)	Horwath et al. (1994)	2.9–4.1 (over the growing season)
	Spruce (boreal)	^{14}C (<4 m tall trees)	Carbone et al. 2007	10 (late summer)
	Pine (boreal)	^{13}C (2 m tall trees)	Högberg et al. (2008)	1.5 (late summer)
	Beech	^{13}C (saplings)	Barthel et al. (2011)	0.8 (control)–1.7 (drought)
Soil CO_2 efflux—2 pools	Shrubs (semi-arid)	^{14}C (shrubs)	Carbone and Trumbore 2007	2.6–3.1 (over the growing season)
	Beech	^{13}C (10 m tall trees)	Epron et al. (2011)	4.8–13.1 (over the growing season)
	Oak	^{13}C (10 m tall trees)	Epron et al. (2011)	6.0–7.7 (over the growing season)
	Pine (temperate)	^{13}C (10 m tall trees)	Epron et al. (2011)	4.9–33.6 (over the year)

[1]Details about this experiment can be found in Dannoura et al. (2011).

rapidly after labelling, with a half-life of <2 days during the growing season (Högberg et al. 2008, Warren et al. 2012, Table 3). The half-life of labelled C was higher in drought-stressed beech saplings (Ruehr et al. 2009) or during winter in pine (Table 3), due to lower export rates.

Longer half-lives of labelled C are expected in growing sinks where the labelled C is not only used to fuel respiration or to be exported, but is allocated to structural and storage compounds. Young, immature pine needles exhibited a different pattern of recovery compared with mature needles with an increase in labelled C several days after labelling and a longer half-life. This pattern highlights the fact that these immature needles are strong C sinks and that they accumulate labelled C into structural biomass (unpublished). Labelled C in fine roots of fir had a half-life of several months independent of diameter class (Endrulat et al. 2010). In pine, labelled C in root tips exhibited shorter half-life than in fine roots (6 versus 15 months, Keel et al. 2012). A half-life of several years was even found in fine roots in a mixed hardwood forest after an accidental ecosystem labelling with $^{14}CO_2$ (Joslin et al. 2006).

When the tracer is continuously or repeatedly supplied to the tree, it can be traced over long time scales. This approach is more suitable than pulse-labelling for estimating the mean residence time of C in long-lived pools of organic matter both in trees (e.g., in tree rings, Palacio et al. 2011) and in the soil (Andrews et al. 1999, Allen et al. 2000, Paterson et al. 2009, Esperschütz et al. 2009*b*).

Half-life of labelled C in respiratory substrate pools

The decrease in labelled CO_2 in the respiratory flux may be used as a proxy for respiratory substrates. It should be kept in mind that several differently labelled substrates can contribute to respiration depending on the time of the day. The overall pattern of tracer dynamics in respired CO_2 is often best approximated by using two (or more) exponential functions. For beech, recovery patterns of labelled C in trunk and soil CO_2 efflux have been well described with two exponential functions with half-lives of 3.5 to 5 days for the first exponential function and of 16–18 days for the second (Plain et al. 2009). This pattern highlights the existence of a rapidly cycling, metabolically active C pool and much slower cycling pools of stored C (Carbone and Trumbore 2007, Mortazavi et al. 2009). These fast cycling C pools were found to contribute to 71% of soil CO_2 efflux in a pine plantation (Taneva et al. 2006).

Furthermore, estimates of the half-life of labelled C in the whole plant–soil system before being respired are higher when based on two exponential fits as compared with a single exponential fit (Table 3). Half-life of 1.5 days or 3–4 days was reported for boreal pines (Högberg et al. 2008) and temperate poplars (Horwath et al. 1994), respectively, when a single exponential decay function was fitted to the data, whereas it ranged from 5 to 13 days for beech, oak and pine during the growing season when a two-compartment model was used (Epron et al. 2011). Fitting a simple exponential function in such cases inevitably underestimates the half-life of C in these compartments. This highlights the need for more mechanistic approaches to assess residence times in complex systems.

Compartmental modelling of respiratory C pools

The availability of detailed time courses of both tracer content and CO_2 efflux rate allows the application of more refined

mathematical analyses. For instance, compartmental analyses have proven to be an effective and elegant tool to analyse the system supplying C substrates to respiration in ryegrass (Lehmeier et al. 2008, 2010*a*, 2010*b*). In this approach, conceptual models including fast turnover, metabolically active pools and storage pools (Figure 4, adapted from Epron et al. 2011) are translated into a set of differential equations that are fitted to the kinetics of labelled C to estimate the half-life of pools and their relative contribution to respiration. Compartmental modelling was used to account for the mixing of stored C and newly assimilated C after pulse-labelling of trees with ^{13}C (Epron et al. 2011). However, the situation encountered by trees in the field under fluctuating climatic conditions is rather complex and the limited numbers of pools (usually two or three) may not fully reflect physiological processes.

Carbon partitioning

For better understanding of processes underlying C allocation dynamics it is important to disentangle the effects of phenology from those mediated by environmental factors, which both affect sink and source activities. Because trees are perennial species, pulse-labelling experiments also provide insights into the contribution of old and current assimilates to growth and respiration in sink organs.

Scaling labelled contents to trees

The amount of labelled C recovered in one compartment is obtained by multiplying the percent atom excess of the heaviest isotope by the C content of the biomass of this compartment. Knowing the amount of labelled C allocated to fine roots is challenging: (i) because of the lateral and vertical extension of the root system it is difficult to obtain representative samples, (ii) because the tracer tends to concentrate in the finer, easier-to-lose, root tips and (ii) because of difficulties with sorting live and dead fine roots (Ruehr et al. 2009, Endrulat et al. 2010). Scaling the amount of labelled C recovered in respiration chambers to whole trees is also prone to large uncertainties. Trunk respiration can vary with diameter and position in the canopy (Ryan et al. 1996, Damesin et al. 2002). Also soil respiration is typically characterized by high spatial variability (Søe and Buchmann 2005, Ngao et al. 2012). Thus, C partitioning calculations that have been reported so far are likely associated with large uncertainties, but nevertheless provide important qualitative information.

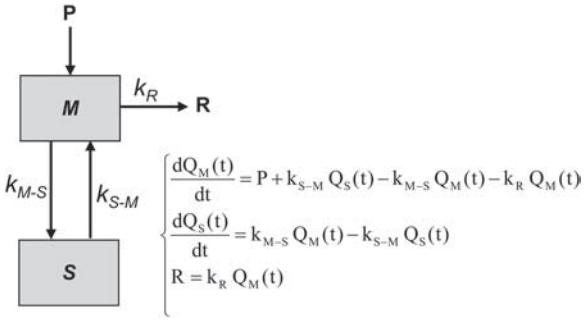

Figure 4. Typical compartmental model describing the kinetics of the labelled C recovered in respiration (R). The labelled C enters a metabolic pool (M) by photosynthesis (P), and a quantity of label (Q) is transferred and back-transferred to a storage pool (S) at rates following first-order kinetics (with rate constants *k*M_S and *k*S_M respectively). The label in the metabolic pool fuels respiration at a rate which also follows a first-order kinetics (*k*R). A lag, depending on the velocity of the phloem sap and on the path length, can be introduced between the uptake of the labelled C by the canopy and its arrival in the metabolic pool in the sink organ (not included in the equation).

Partitioning the total amount of labelled C assimilated

Because C partitioning refers to the fraction of labelled C in one compartment, the total amount of labelled C assimilated by the tree should be known. While it is possible to estimate the total amount of labelled C delivered to the tree (see above), it is less straightforward to quantify the amount that has been actually assimilated (flux [1] in Figure 1).

One option is to sum up the labelled C recovered in all compartments. This can be simplified by collecting leaves immediately after labelling when most of the tracer has not yet transported to other organs. The amount of labelled C in any compartment will thus be expressed relative to the initial amount recovered in the foliage (Keel et al. 2007, Plain et al. 2009, Endrulat et al. 2010). In order to account for the heterogeneity of leaf photosynthetic activity in the crown, a stratified sampling strategy is needed. Typically, the crown can be virtually separated into bottom, middle and upper parts, for which foliage can be sampled and analysed separately. In the case of evergreen species, the sampling design should also account for the different needle cohorts. The proportion of foliage mass belonging to each part of the crown should be known to upscale the C isotope composition to the crown level. In addition, a good mixing of air in the labelling chamber is a prerequisite to ensure that the heterogeneity in foliar C isotope composition reflects the heterogeneity of leaf photosynthetic activity. This approach provides a conservative estimate of the amount of labelled C taken up by the crown because respiratory losses and export of carbohydrates take place already during the labelling. Nonetheless, for herbaceous vegetation, Lattanzi et al. (2012) estimated that for labelling periods shorter than 6 h the underestimation is <10% of assimilated C. This is because the tracer mixes rapidly with several metabolic pools, and consequently is not respired or allocated immediately after its assimilation.

Another option to estimate the total amount of labelled C assimilated by the tree is to calculate uptake from photosynthesis measured with a canopy chamber before and after labelling, or from using data from a nearby eddy flux tower.

This approach should account for the $^{13}CO_2/^{12}CO_2$ mixing ratio during labelling and requires that that during labelling leakage is negligible.

Environmental controls of C allocation

The functional-balance concept predicts that C partitioning is tuned to maintain an optimal internal resource status, like a constant biomass C : N ratio (Reynolds and Chen 1996, Franklin et al. 2012). As expected from this concept, the amount of labelled C allocated belowground after a 1-year chase period following pulse-labelling was 60% lower in nitrogen (N)-fertilized plots compared with unfertilized plots in a boreal pine forest (Högberg et al. 2010). In contrast, N fertilization did not affect labelled C partitioning in potted young beech saplings (Dyckmans and Flessa 2001), but affected the shift in C allocation to belowground compartments that is observed in response to fumigation with elevated CO_2 concentration (Dyckmans and Flessa 2002). Elevated CO_2 concentration increased the amount of labelled C recovered in the microbial biomass in trembling aspen, suggesting that exudation was stimulated (Mikan et al. 2000).

Ozone was found to favour C allocation belowground at the expense of leaves in potted silver birch trees (Kasurinen et al. 2012) and allocation to the fungal symbiont in mycorrhizal pine seedlings (Andersen and Rygiewicz 1995). However, no clear patterns were observed in 60-year-old European beech and Norway spruce trees (Andersen et al. 2010). Allocation of photosynthates to the stem respiration (flux [2w] in Figure 1) decreased in beech exposed to O_3 while it increased in spruce (Ritter et al. 2011).

Disentangling the influence of seasonal variations in weather and species phenology

When interpreting seasonal patterns of C allocation it is important to disentangle the effects of phenology, as primarily driven by biological controls, and those directly caused by environmental factors. This is especially the case for seasonally variable parameters like temperature and soil water content.

Variations in sink strength are important drivers of the seasonal dynamics of C allocation patterns (Table 1, Lippu 1994). Bud break and sprouting (flux [4l] in Figure 1) in spring divert an important fraction of C assimilated by older foliage in evergreen species. Indeed, acropetal transport of labelled C (flux [9] in Figure 1) to the growing needles and shoots was reported for larch (Schneider and Schmitz 1989), and it was demonstrated that bud break and sprouting in spring was exclusively supplied by the recent photosynthates of previous year's needles in pine (Hansen and Beck 1994, Lippu 1998). Fruiting is another seasonally fluctuating C sink. This was nicely exemplified in beech and hornbeam for which almost all the labelled C assimilated by the fruit-bearing branches was allocated to their infructescences (Hoch and Keel 2006). Production of sporocarps by mycorrhizal fungi is also an important C sink (flux [10] in Figure 1) at the end of the growing season, which diverts recent assimilates in pine (Högberg et al. 2010). In boreal species, there is strong evidence that C allocation shifts from aboveground to belowground from the beginning to the end of the growing season (Lippu 1994, Kagawa et al. 2006*a*, Högberg et al. 2010), probably related to the delayed summer warming of the soil compared with the air.

At the whole-tree level, C allocation patterns are highly dynamic and change with the seasons (Figure 5), indicating that priorities among sink organs for recent assimilates exist at this time scale. Seasonal shifts in C allocation may also be expected in tropical species encountering a transition between wet and dry seasons. We are not aware of any labelling experiments addressing this question so far.

Seasonal changes in allocation to storage

An important flux of labelled C into storage pools (flux [3] in Figure 1) typically occurs at the end of the growing season (Hansen et al. 1996, Lacointe et al. 2004, Kuptz et al. 2011). It is still unclear as to whether C reserves are a passive pool or an actively regulated sink (Sala et al. 2012). For example, 80% of the newly assimilated C was allocated to aboveground growth in July (leaves and stems) while the same proportion was stored in the stump when coppiced chestnuts were labelled in October (Mordacq et al. 1986). A similar seasonal shift in C allocation was reported for young poplar trees (Horwath et al. 1994). In contrast, results obtained in the field on 20-year-old beech showed that C allocation to soil CO_2 efflux, and to respiration and biomass of fine roots and microbes, peaked in summer (Epron et al. 2011) and thus did not compete with C allocation to storage that occurred at the end of the summer (Kuptz et al. 2011). Seasonal shifts in C allocation belowground were not observed in spruce, sessile oak and maritime pine (Epron et al. 2011, Kuptz et al. 2011). The discrepancy among species may reflect differences in the contribution of stored carbohydrates that might buffer the seasonal changes in the partitioning of newly fixed C in some temperate species (Epron et al. 2011, Figure 5).

Besides seasonal changes in allocation to storage compartments in perennial organs, transient storage also occurs at a daily time scale, especially in the foliage (fluxes [3l and 6l] in Figure 1). For example, it was shown that respiration of beech leaves was supplied by a mixture of current and stored carbohydrates (Nogués et al. 2006). In some species, the emission of biogenic volatile organic compounds (BVOC, flux [7] in Figure 1) is an additional sink of recently assimilated C, which is mostly active in summer when light and temperature are high (Loreto et al. 1996). However, the fraction of BVOC emissions originating from recent photosynthates or from stored C may vary among species (Ghirardo et al. 2010). In

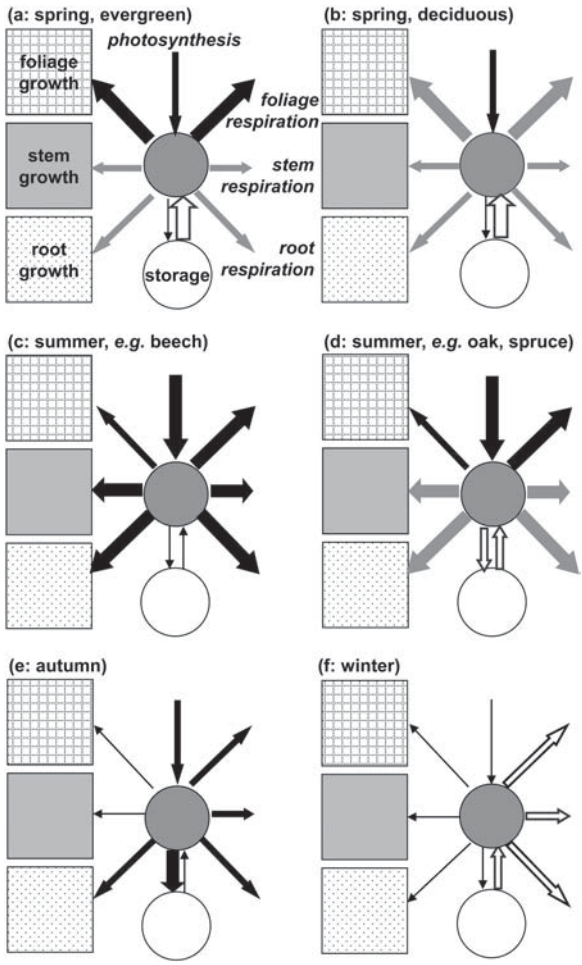

Figure 5. Conceptual representation of seasonal changes in C allocation in temperate and boreal trees, accounting for the use of recently assimilated C versus stored C. Black arrows indicate fluxes of recently assimilated C, white arrows fluxes of C remobilized from storage and grey arrows a mixture of both. The width of arrows indicates sink or source strength. During spring, sprouting (foliage growth and foliage respiration) is an important sink for new C in evergreen species (a) while storage remobilization largely contributes to sprouting in deciduous species (b). Stem growth, root growth, stem respiration and root (and associated symbionts) respiration are mainly sustained by storage remobilization. In summer, foliage growth almost stops in many species. Stem and root growth and respiration are major sinks of C and are either (c) mainly fuelled by new C (e.g., beech) or (d) by a mixture of old and new C (e.g., spruce, oak). In autumn, storage build-up is the major sink of recent photosynthate (e). During winter (f), photosynthesis and growth are either low (evergreen species) or zero (deciduous species), and respiration is fully sustained by storage remobilization.

view of the diurnal changes of the use of new C for storage in starch, respiration or exportation, future studies could address the question of how the timing of labelling may influence the fate of C.

Contribution of stored C to growth and respiration

When a pulse of labelled C is provided to a tree, the recently assimilated C has an isotopic composition that differs from the isotope signature of the unlabelled stored C. Pulse-labelling experiments were thus useful to disentangle the contribution of new (flux [1] in Figure 1) versus old/stored C [6] to growth [4] or to respiration [2]. While the contribution of recently assimilated and stored C to trunk and coarse root respiration was almost constant in spruce and was dominated by old carbohydrates, it changed seasonally in 60-year-old beech trees (Kuptz et al. 2011). Trunk and coarse root respiration are dominated by stored C when the C demand to sustain shoot growth uses most of the recently assimilated C (Figure 5).

Daily oscillations of the isotope signal after pulse-labelling in trunk CO_2 efflux were ascribed to diurnal changes in the C sources fuelling respiration, i.e., remobilization of labelled starch during the night versus use of unlabelled newly assimilated C during daytime (Plain et al. 2009, Barthel et al. 2011). In trees, however, diurnal changes in C sources could be confounded by internal CO_2 transfer if, for instance, CO_2 produced belowground affects the composition of trunk CO_2 efflux. Departures of root-produced CO_2 in the transpiration stream that is contributing to trunk CO_2 efflux have indeed been reported on several occasions (Teskey and McGuire 2007, Aubrey and Teskey 2009, Grossiord et al. 2012).

Sprouting in deciduous broadleaved species relies more or less on C that has been assimilated during the previous year and stored overwinter, questioning the concept of C autonomy of branches in spring (Lacointe et al. 2004, Keel et al. 2007). The leaves of 3-year-old potted beech were composed of 50–70% newly assimilated C, highlighting a significant contribution of stored C, especially for the initial synthesis of acid detergent-fibre lignin (Dyckmans et al. 2002). This was also observed in a deciduous coniferous species (larch) for which about half the C in new needles was derived from stored C (Kagawa et al. 2006*a*), and the contrast between evergreen and deciduous coniferous species was demonstrated by comparing larch and pine in a FACE system (von Felten et al. 2007). Labelled C that was stored over winter was also found in new fine roots that grew in the spring (Endrulat et al. 2010). Similarly, it was shown in a FACE experiment that fine root and mycorrhiza growth in scrub oaks were partly supported by C stored in rhizomes (Langley et al. 2002), in agreement with a large-scale ^{14}C labelling showing that stored C accounted for 55% of new root growth in a mixed hardwood forest (Gaudinski et al. 2009).

Wood formation results also from a mixture of recent and stored C in a proportion that varies among species, the relative contribution of stored C being greater in ring-porous than in diffuse-porous species (Keel et al. 2006, Palacio et al. 2011). This difference is thought to be related to the difference in their ability to restore hydraulic conductivity after winter embolism (Barbaroux and Bréda 2002). A different contribution of old and current assimilates to early wood and late wood formation was found in larch, early wood being more dependent on stored C than late wood (Kagawa et al.

2006*b*). Diametric growth in summer might also compete with shoot growth in polycyclic species like birch, relying thus more on stored C than shoot elongation (Palacio et al. 2011). Scots pine seedlings used preferentially stored C to synthesize secondary metabolites in stems when inoculated with a bark-beetle-associated fungus (Guérard et al. 2007). There are large sets of intra-annual records of the natural abundance of C isotope composition in tree rings (Barbour et al. 2002, Helle and Schleser 2004, Offermann et al. 2011), which allow qualitative assessment of the contribution of stored starch-derived versus recently assimilated C during the growing season. Combining ^{13}C labelling with such natural abundance approaches might give additional insights into the seasonal variation of storage and remobilization and C resource use in trees.

Sources of 'new' versus 'old' C

In most of the above-mentioned studies, labelled C is referred to as 'new C' and interpreted as derived from 'current photosynthesis' or 'recent assimilates' while unlabelled C is referred as 'old C' and interpreted as derived from 'stores' or 'reserves'. This is convenient but not strictly valid, as there is no invariant relationship between labelled and unlabelled substrates and the sources supplying a sink (Farrar and Gunn 1998, Lattanzi et al. 2005). After its assimilation, the tracer starts to mix within the plant metabolic network, moving through several physical and biochemical compartments. Thus, while part of the tracer goes directly to the sink (flux [8] in Figure 1), part of it is incorporated in transient stores (e.g., chloroplastic starch, flux [3l]), amino acids, proteins, and longer-term stores [3w and 3r]. When mobilized [6], these pools supply labelled C. This also holds for unlabelled C, which is not completely derived from stores but is also present in labile pools, which are closely related to current photosynthesis. Therefore, inferences based on a simplified definition of 'new' versus 'old' C require caution and, ideally, need to be informed by additional knowledge of the studied system.

Conclusion

Pulse-labelling experiments have provided unique and highly valuable information on the transfer and allocation of C in trees. They have revealed a fast transfer of assimilated C from the foliage to belowground, with species-specific velocities, which are likely due to phloem anatomy. Experiments have also highlighted the seasonality of allocation patterns as affected by environmental and endogenous controls on transfer rates and activities of sink organs. Growth and respiration rely both on recent photosynthates and on stored C, in a proportion that depends on the season and that varies among species. Environmental constraints (ozone, drought) and differences in resource availability (soil water, soil fertility and light) have an impact on C allocation within the tree and on soil biota, and can strongly affect the rate of C translocation.

This review has identified several open research questions which should be of high priority on the research agenda. (i) *Source–sink relations*: shading tree crowns to manipulate source activity (Warren et al. 2012) and varying fruit load (Palmer 1992) or tapping intensity (Chantuma et al. 2009) to manipulate sink demand, combined with labelling experiments, will offer promising perspectives to better understand biological controls on C allocation to storage organs and potentially differentiate between sink- versus source-driven controls. (ii) *C allocation to secondary metabolism*: insect and pathogen attacks play a major role in drought and heat wave-induced tree mortality (McDowell et al. 2011); however, patterns of C allocation to defence compounds (flux [5] in Figure 1) have not yet been considered. (iii) *Response of C allocation to environmental change*: combining labelling and ecosystem manipulation approaches like fertilization, water exclusion or warming experiments will be useful for disentangling the effects of environmental factors versus phenology, both being confounded when addressing seasonal variations of allocation patterns. (iv) *Seasonality versus phenology effects in and across different biomes*: there is a particular need for pulse-labelling experiments on tropical trees. They play a major role in the global C cycle (Malhi 2010) and have a distinct phenology compared with temperate and boreal tree species. (v) *C–N interactions*: dual C and N labelling experiments will not only deepen our understanding of the coupling of C and N dynamics in trees (Millard and Grelet 2010), but will also improve the parameterization of coupled biogeochemical cycles in forest C balance models (Chapin et al. 2009).

New tools like compound-specific isotope analysis (CSIA; Meier-Augenstein 1999, Godin et al. 2007), proton-transfer reaction mass spectrometry (PTR-MS; Ghirardo et al. 2010) or nano-scale secondary ion mass spectrometry (nano-SIMS; Herrmann et al. 2007, Hatton et al. 2012), which have become available only relatively recently, will permit tracing isotopes into specific C compounds and will enable micro-scale localization of labelled C in tree tissues and in the soil. This will facilitate the study of a range of topics, including the within-tree translocation of C, the exudation of organic C compounds by the rhizosphere or their emission to the atmosphere. Besides pulse-labelling, long-term labelling of trees in the field will improve our knowledge on residence times and turnover rates of C in long-lived pools of organic matter in perennial tree organs and in soils, both crucial for C sequestration (Körner 2006).

Finally it should be noted that much of the available knowledge has been gained in pulse-labelling experiments using relatively small trees, while in situ whole-tree labelling experiments are still scarce although urgently needed to improve our understanding of environmental and physiological controls on C allocation.

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Conflict of interest

None declared.

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