Global Change Biology

Global Change Biology (2010) **16**, 3386–3406, doi: 10.1111/j.1365-2486.2010.02179.x

REVIEW

# Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls

YAKOV KUZYAKOV* and OLGA GAVRICHKOVA†

*Department of Agroecosystem Research, University of Bayreuth, BayCEER, 95440 Bayreuth, Germany, †CNR, Institute of Agro-Environmental and Forest Biology (IBAF), 05010 Porano, Italy*

## Abstract

$CO_2$ efflux from soil depends on the availability of organic substances respired by roots and microorganisms. Therefore, photosynthetic activity supplying carbohydrates from leaves to roots and rhizosphere is a key driver of soil $CO_2$. This fact has been overlooked in most soil $CO_2$ studies because temperature variations are highly correlated with solar radiation and mask the direct effect of photosynthesis on substrate availability in soil. This review highlights the importance of photosynthesis for rhizosphere processes and evaluates the time lag between carbon (C) assimilation and $CO_2$ release from soil. Mechanisms and processes contributing to the lag were evaluated. We compared the advantages and shortcomings of four main approaches used to estimate this time lag: (1) interruption of assimilate flow from leaves into the roots and rhizosphere, and analysis of the decrease of $CO_2$ efflux from soil, (2) time series analysis (TSA) of $CO_2$ fluxes from soil and photosynthesis proxies, (3) analysis of natural $\delta^{13}C$ variation in $CO_2$ with photosynthesis-related parameters or $\delta^{13}C$ in the phloem and leaves, and (4) pulse labeling of plants in artificial $^{14}CO_2$ or $^{13}CO_2$ atmosphere with subsequent tracing of $^{14}C$ or $^{13}C$ in $CO_2$ efflux from soil. We concluded that pulse labeling is the most advantageous approach. It allows clear evaluation not only of the time lag, but also of the label dynamics in soil $CO_2$, and helps estimate the mean residence time of recently assimilated C in various above- and belowground C pools. The impossibility of tracing the phloem pressure–concentration waves by labeling approach may be overcome by its combination with approaches based on TSA of $CO_2$ fluxes and its $\delta^{13}C$ with photosynthesis proxies. Numerous studies showed that the time lag for grasses is about $12.5 \pm 7.5$ (SD) h. The time lag for mature trees was much longer (~4–5 days). Tree height slightly affected the lag, with increasing delay of 0.1 day $m^{-1}$. By evaluating bottle-neck processes responsible for the time lag, we conclude that, for trees, the transport of assimilates in phloem is the rate-limiting step. However, it was not possible to predict the lag based on the phloem transport rates reported in the literature. We conclude that studies of $CO_2$ fluxes from soil, especially in ecosystems with a high contribution of root-derived $CO_2$, should consider photosynthesis as one of the main drivers of C fluxes. This calls for incorporating photosynthesis in soil C turnover models.

*Keywords:* $^{14}C$ and $^{13}C$ labeling, C cycle, $CO_2$ partitioning, delay, FACE, natural $^{13}C$ abundance, phloem transport, photosynthetic active radiation, priming effect, rhizosphere processes, soil respiration, time series analysis

*Received 16 July 2009 and accepted 28 November 2009*

## Introduction

Long-term changes of climate parameters, i.e., trend of mean temperature and precipitation, lead to slow adaptation of ecosystems. Short-term extreme events such as heat waves (Breda *et al*., 2006; Rennenberg *et al*., 2006), cooling (Kreyling *et al*., 2008; Matzner & Borken, 2008), prolonged drought (Hopkins & Del Prado, 2007; Borken & Matzner, 2009) may have much stronger impacts on pools and/or fluxes in ecosystems compared with long-term trends. Such extreme conditions may lead to very strong ecosystem disturbances, requiring recovery on a scale of years to decades. In contrast, short-term small variations of climatic drivers have less pronounced effects on ecosystem functioning than extreme events. However, the frequency of small variations is much higher, leading to repeated fluctuations of processes with considerable impact on ecosystems. Three key factors complicate the evaluation of the drivers responsible for such small fluctuations:

(1) The amplitude of fluctuation of climatic drivers is usually much higher than the subsequent fluctuation of the state and/or processes in ecosystems. This is due to the buffering effect of stable ecosystem components and compensation through oppositely directed processes.
(2) The effect of one climatic driver could be easily masked by another one because their fluctuations

Correspondence: Yakov Kuzyakov, tel. + 49 921 552292, fax + 49 921 552315, e-mail: kuzyakov@uni-bayreuth.de

are often simultaneous and could be similar in direction and magnitude.

(3) The response of ecosystem processes to a change is often delayed. Frequently, at the time of the response the fluctuation of the climatic driver has already ceased or even changed its direction. The delay is connected with a number of subsequent chain processes.

In this review, we focus on the $CO_2$ efflux from soil in the context of points 2 and 3 above. We chose soil $CO_2$ efflux because: (1) it is the end product of mineralization of organic substances and therefore reflects processes of carbon (C) turnover. (2) Fluxes of C are a direct proxy of the energy passed through biota. (3) Most terrestrial C is sequestered in soils. Accordingly, small changes in $CO_2$ efflux from soil over long periods may accumulate to strong changes in atmospheric $CO_2$ concentration. Finally, (4) $CO_2$ is the main component of green house gases, and exact knowledge of the main drivers of $CO_2$ efflux from soil is a prerequisite for modeling the processes responsible for atmospheric $CO_2$ changes. This explains why many studies have been and will be devoted to $CO_2$ efflux from soils of various ecosystems.

### *Sources of $CO_2$ efflux from soil*

Five main sources contribute to total soil $CO_2$ efflux (Fig. 1, modified after Kuzyakov, 2006): (1) microbial decomposition of soil organic matter (SOM), frequently referred to as 'basal respiration', (2) microbial decomposition of SOM affected by recent input of rhizodeposits or/and fresh plant residues (termed 'priming effect'), (3) microbial decomposition of dead plant (shoot and root) remains, (4) microbial decomposition of rhizodeposits of living roots, referred to as 'rhizomicrobial respiration', and (5) root respiration.

The contribution of individual sources to the total $CO_2$ efflux varies strongly in different ecosystems, depending on biotic factors (plant community, development stage, relative contribution of coarse and fine roots) and abiotic factors (climatic drivers, soil conditions, sampling period, management type). Review of 50 studies on partitioning of soil respiration showed that the contribution of root and rhizomicrobial respiration (root-derived $CO_2$) to total $CO_2$ efflux from soil varied (range: 10%–90%) among studies and ecosystems (Hanson *et al*., 2000). The lower values were found in nonforest ecosystems. Contribution of root-derived $CO_2$ to total $CO_2$ efflux from soil increased with the increase of annual soil $CO_2$ efflux (Subke *et al*., 2006). Stand age had no effect on the root-derived $CO_2$ to SOM-derived $CO_2$ ratio. Boreal forests were characterized by a higher contribution of SOM-derived $CO_2$ than temperate and tropical ones (Subke *et al*., 2006).

Several studies revealed that the contribution of root-derived $CO_2$ decreases in dormant periods compared with the growing season (Dörr & Münnich, 1986; Rochette & Flanagan, 1997). Seasonal changes in root biomass and root-specific activity should therefore be considered. Fine roots proved to be metabolically more active and to have higher specific respiration rates than coarse roots (Larionova *et al*., 2003). This indirectly confirms that photosynthesis affect $CO_2$ efflux from soil especially through growing roots. A direct confirmation of the link between photosynthesis and $CO_2$ efflux is the effect of productivity on soil respiration as summarized

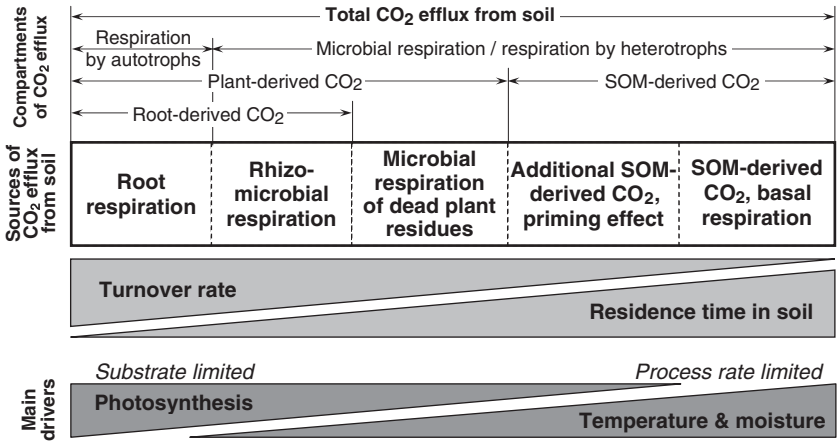


**Fig. 1** Five main biogenic sources of $CO_2$ efflux from soil, ordered according the turnover rates and mean residence times of carbon (C) in soil. The sources and compartments of the $CO_2$ efflux consider C pools with different turnover rates and mean residence time (MRT), the localization of C pools and the agents of $CO_2$ production (Kuzyakov, 2006, changed). The limiting factors and the dependence of individual $CO_2$ sources on photosynthesis and soil temperature is presented in the bottom.

for 18 forest ecosystems (Janssens *et al*., 2001) and various ecosystems around the world (Raich & Tufekcioglu, 2000). However, the question is on which time scales does photosynthesis control the $CO_2$ efflux from soil?

Partitioning total $CO_2$ efflux into root-derived and SOM-derived $CO_2$ has received considerable attention because differential responses of these components to environmental change have profound implications for the soil and ecosystem C balance (Subke *et al*., 2006). As the five $CO_2$ sources have partly different drivers, it is insufficient to simulate $CO_2$ efflux based solely on soil temperature and moisture (as is done in most models). Quantifying the main drivers for individual $CO_2$ sources (Fig. 1) will improve our understanding of seasonal, interannual and diurnal variability of $CO_2$ efflux from soils. This is a necessary prerequisite for successful prediction of C fluxes in ecosystems. In this review, we identify the *main drivers for the short-term processes* in the C cycle contributing to the total $CO_2$ efflux from soils.

### *Importance of photosynthesis for C turnover in the rhizosphere and $CO_2$ fluxes*

$CO_2$ efflux from soil is an integrative respiration of roots and microorganisms decomposing organic substances at different rates. The efflux intensity generally depends on two factors: (1) substrate availability for microorganisms and roots, and (2) decomposition rates of the substrates or respiration rates (Parton *et al*., 1987; Taylor *et al*., 1989; Trumbore *et al*., 1990; Schimel *et al*., 1994; Schulze *et al*., 2000). The other factors such as temperature, water and $O_2$ availability, microbial community structure or enzyme activity, etc. affect $CO_2$ efflux indirectly – through substrate availability and/or decomposition rates. Many studies document that the second factor (decomposition and respiration rates) is strongly affected by temperature, and a $Q_{10}$ function is used to describe this effect (Raich & Schlesinger, 1992; Raich *et al*., 2002). However, defining the classical effect of temperature on rates of decomposition processes with the $Q_{10}$ function has recently been challenged (Davidson *et al*., 2006; Davidson & Janssens, 2006). This is because $Q_{10}$ is based on chemical reactions, whereas biochemical reactions driven by enzymes correspond to Michaelis–Menten kinetics and therefore depend on maximal enzyme capacity ($V_{max}$) as well as enzyme affinity to the substrate ($K_m$).

The importance of the first factor – substrate availability for microorganisms – is frequently neglected, assuming that temperature (Lindroth *et al*., 1998; Granier *et al*., 2000) and moisture (Davidson *et al*., 1998) are the main $CO_2$ drivers. In models of the global C balance, soil respiration is often represented as being driven by a single abiotic factor such as temperature (McGuire *et al*., 1992). However, in most terrestrial ecosystems *substrate availability* (and not temperature) *limits the microbial activity* and thus the $CO_2$ efflux from soil. Considering the broad variety of substrates in soil, their availability ranges from hardly available polymers with nonregular structure bound on clays and sesquioxides with a mean residence time (MRT) of hundreds of years (Theng *et al*., 1992; Trumbore, 1997; Rethemeyer *et al*., 2004) up to free low molecular weight organic substances (LMWOS) with an MRT of hours (Jones *et al*., 2003; Fischer *et al*., 2009) or even minutes (Fischer & Kuzyakov, 2010). The contribution of hardly decomposable polymers to the efflux is generally low. In contrast, despite the low content in soil of easily available substances (Fischer *et al*., 2007) with an MRT of hours to a few days, their contribution to the $CO_2$ efflux is very high. This mainly reflects high turnover of LMWOS due to their fast production and fast decomposition.

There are three main sources of LMWOS in soil: (1) microbial decomposition of plant and microbial residues, (2) microbial decomposition of SOM, and (3) rhizodeposits of living roots including exudates, mucilage, root hairs, sloughed-off rhizodermal cells and mycorrhizal hyphens. The release of exudates is most directly coupled with photosynthesis (Haller & Stolp, 1985; Flores *et al*., 1996; Merbach *et al*., 1999; Kuzyakov & Cheng, 2001, 2004; Dilkes *et al*., 2004; Murray *et al*., 2004; Thornton *et al*., 2004). The most abundant components of root exudates – soluble sugars (Merbach *et al*., 1999; Kuzyakov *et al*., 2003) – are rapidly utilized by mycorrhiza and microorganisms in the rhizosphere and thus contribute to root-derived $CO_2$. Accordingly, rhizomicrobial respiration, as one of the $CO_2$ sources, is strongly controlled by photosynthesis (Fig. 1).

The other $CO_2$ source that is tight temporal coupled to photosynthesis is root respiration. Increasing evidence suggests that the supply of assimilates from photosynthetically active plant organs significantly affects root respiration (Xu *et al*., 2008; Subke *et al*., 2009) and contributes to the $CO_2$ efflux from soil. In fact, *temperature changes are closely linked to solar irradiation and often mask the effect of photosynthesis on root-derived $CO_2$*. When root and microbial respiration were measured separately over a short period during which soil temperature dropped by 6 °C, microbial respiration decreased while root-derived $CO_2$ remained insensitive to temperature changes (Bhupinderpal-Singh *et al*., 2003).

Most of the energy derived from respiration is used for growth and maintenance (Hansen & Jensen, 1977; Veen, 1981; Amthor, 1994; Desrochers *et al*., 2002). The sensitivity of root respiration to soil temperature is

determined mainly by the maintenance respiration that is highly temperature dependent (Sprugel & Benecke, 1991). In late autumn and winter in the absence of plant growth, root respiration is reduced to the level of maintenance (Desrochers *et al*., 2002; Wieser & Bahn, 2004). During this period, respiration rates undergo immediate changes with soil temperature by a direct effect on enzymatic activity, soil water and nutrient availability. During the growing season, however, maintenance respiration can be limited by the inputs of assimilates from aboveground (Hunt & Loomis, 1979). Growth respiration is associated with production of new plant and mycorrhizal hyphens material. It is unaffected by temperature, depending mostly on the C supply from aboveground (Penning de Vries *et al*., 1974; Desrochers *et al*., 2002).

We therefore conclude from the above-mentioned five components of total $CO_2$ efflux from soil that root respiration and rhizomicrobial respiration (both termed root-derived $CO_2$) are the two $CO_2$ sources that are very closely linked to the supply of assimilates from aboveground. These two types of respiration therefore depend directly on photosynthesis (Fig. 1).

In many ecosystems, the contribution of root-derived $CO_2$ is quite high, reaching up to 90% of total soil $CO_2$ (Hanson *et al*., 2000). Accordingly, total soil $CO_2$ could also be highly affected by the canopy photosynthetic activity. Any factors that affect photosynthesis or substrate supply to roots and rhizosphere microorganisms – such as irradiation, water stress, nutritional status, human and herbivore activity – could thus be important determinants of root-derived $CO_2$ efflux from soil. The failure to consider these aboveground processes may lead to erroneous interpretations of belowground processes related to C turnover (Paterson, 2003) and of data on soil respiration. One of the difficulties in linking photosynthesis with belowground processes and $CO_2$ fluxes from the soil is time lag between them.

This review therefore: (1) identifies the processes responsible for the time lag between photosynthesis and $CO_2$ efflux, (2) evaluates approaches estimating the time lag between photosynthesis and the $CO_2$ efflux, (3) evaluates the time lag between photosynthesis and the efflux for trees and grasses, and (4) evaluates biotic and abiotic factors controlling the time lag.

## Processes responsible for the time lag between photosynthesis and $CO_2$ efflux from soil

### *Photosynthesis and release of organics into the rhizosphere*

Coupling plant photosynthetic activity with belowground C turnover may occur through two mechanisms (Fig. 2): (a) direct transport of assimilates from leaves through phloem to the roots, with subsequent utilization for root respiration or release to the soil, and (b) indirect physicochemical effect on root activity through pressure–concentration waves – increase of turgor in the phloem (Thompson & Holbrook, 2003; Thompson, 2006; Davidson & Holbrook, 2009; Mencuccini & Hölttä, 2010)[1].

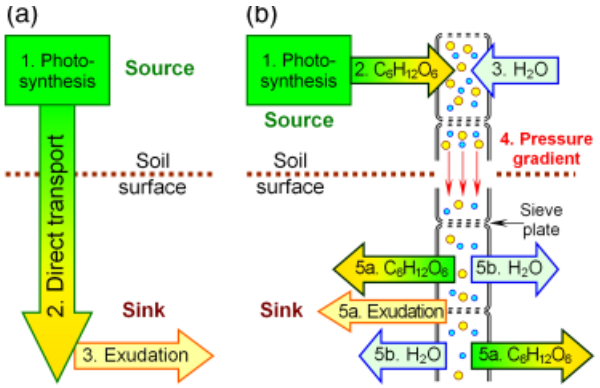


**Fig. 2** Increased photosynthesis leads to two response mechanisms of release of soluble organic substances (mentioned as 'Exudation') and transport of assimilates from leaves through stem and roots to the rhizosphere: (a) direct transport of molecules and (b) indirect response of the release of soluble organics from roots by phloem loading and pressure-concentration waves. See text for description of the processes 1–5.

In the first mechanism (Fig. 2a) – direct transport – the photosynthates built in the leaf are actively loaded into the phloem and are transported to the root. There, the photosynthates are unloaded and utilized (see next section). Phloem flow is driven by gradients in hydrostatic pressure: solutes, by diffusion or active transport into and out of the sieve elements of the phloem, decrease the osmotic pressure from leaf to root, creating further hydrostatic pressure gradients (Nobel, 2005). By this mechanism, the molecules assimilated in leaves at time $t_1$ will be allocated to roots at time $t_2$. The time interval between $t_1$ and $t_2$ should be directly predictable by phloem transport rates, which can vary from 0.2 to $2\,m\,h^{-1}$ but typically range from 0.5 to $1.0\,m\,h^{-1}$ (Zimmermann & Braun, 1971; Ekblad & Högberg, 2001; Keitel *et al*., 2003; Barnard *et al*., 2007). Changes in xylem water potential could influence the rate of phloem translocation. Therefore, during moderate water stress or rapid transpiration, for example, phloem transport generally decreases (Ruehr *et al*., 2009). The rates can also vary between species and growth stages (Thompson & Holbrook, 2003; Nobel, 2005).

[1]The study of Mencuccini & Hölttä (2010) released after our review was already accepted. Therefore, it was not possible to consider in details their results.

The second mechanism – indirect response – is connected with phloem loading or unloading and subsequent concentration and turgor changes (Münch, 1930; Fig. 2b). When new assimilates are actively loaded into phloem (identically with the first mechanism; Process No. 2 on Fig. 2b), the osmotic pressure in the phloem increases, water moves inside the cells and the turgor rises (No. 3). The local turgor increases the pressure in the whole phloem and form a pressure gradient between phloem in leaves and phloem in roots (No. 4). Accordingly, the pressure wave rather than molecules is transferred belowground: the soluble organics already present in the roots are then released together with water into the rhizosphere (No. 5a and 5b, Fig. 2). In the second mechanism, the organic molecules assimilated in the leaf at time $t_1$ are not the same as those released into the rhizosphere at time $t_2$. Pressure–concentration waves move several orders of magnitude faster than the solution and molecules themselves (Thompson & Holbrook, 2004; Thompson, 2005; Davidson & Holbrook, 2009). However, the rate will exceed the sap flow only when the osmotic pressure is high relative to the turgor differences between the two ends of the phloem (Phillips & Dungan, 1993; Hölttä *et al.*, 2009). This is possible when the conductivity of the phloem elements and the solute concentration are high (Thompson & Holbrook, 2004; Thompson, 2006; Mencuccini & Hölttä, 2010). The last two parameters are highly variable among species (Thompson & Holbrook, 2003).

### *Bottle-neck processes responsible for time lag*

Evaluating the time lag between photosynthesis and $CO_2$ efflux from soil requires identifying the chain of processes responsible for this delay. Recently assimilated C, after being transported from aboveground to belowground, may be variously utilized (Fig. 3). Here, we describe only fast processes (rates of minutes to a few days) because the longer processes (i.e., decomposition of plant residues or SOM) cannot reflect the changes of photosynthesis in the $CO_2$ efflux (Mencuccini & Hölttä, 2010).

After photosynthesis in leaves (No. 1, Fig. 3) the assimilated C is loaded into the phloem and transported belowground (No. 2) by one of two ways described in the previous section (Fig. 2). After reaching the roots, the assimilate flow is partitioned for various processes including: C incorporation for growth of new root tissue (omitted in Fig. 3 because it is not connected with $CO_2$ fluxes), root respiration (No. 3a), and release of soluble organics from roots to the mycorrhizal fungi (4a), or from roots into the rhizosphere (3b) with subsequent uptake by microbial biomass (4b). Mycorrhizal fungi

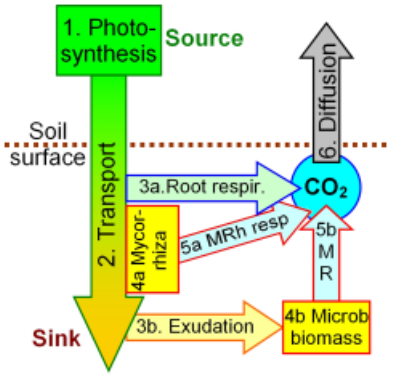


**Fig. 3** Chain of the main short-term processes contributing to the time lag between photosynthesis and $CO_2$ efflux from soil (see also Table 1).

Abbreviations: 1. Photosynthesis, photosynthesis in leaves; 2. Transport, transport of assimilated carbon (C) belowground; 3a. Root respir., root respiration; 3b Exudation, release of soluble organics from roots into rhizosphere; 4a Mycorrhiza, release of soluble organics from roots into mycorrhizal fungi; 4b Microb biomass, microbial biomass in rhizosphere; 5a MRh resp, respiration of mycorrhizal fungi; 5b MR, respiration of rhizosphere microorganisms; 6. Diffusion, diffusion of $CO_2$ from soil to surface. Note that only short-term processes are presented here. Such longer processes as utilization of assimilates for cell wall construction or temporary storage with later remobilization are not presented. Note that important part of $CO_2$ respired by roots will be not released into the soil, but will be transported through xylem (Aubrey & Teskey, 2009). This process is not shown here.

(5a) and rhizosphere microorganisms (5b) respire $CO_2$ which, together with $CO_2$ from root respiration (3a), diffuses to the soil surface (No. 6, Fig. 3) and back to the atmosphere. Which of these short-term processes may be the bottle-neck of the chain and thus mainly responsible for the time lag?

The first process – $CO_2$ assimilation by photosynthesis – is fast: $CO_2$ uptake occurs within seconds (Anten, 1995; Taiz & Zeiger, 2002) (Table 1). The second process – transport through the phloem – strongly depends on the distance between the locations of assimilation and utilization or release of organics by roots. This transport is clearly much longer for trees than for grasses. The rates of phloem transport average 1 m $h^{-1}$ but strongly vary between species and even within a single plant in response to changes in environmental conditions (Boersma *et al.*, 1991; Nobel, 2005; Plain *et al.*, 2009). Root respiration is also very fast: the utilization of recent assimilates for respiration starts immediately

**Table 1** Main short-term processes contributing to the time lag between photosynthesis and $CO_2$ efflux from soil, their typical process duration and affecting environmental factors (see also Fig. 2)

| Process (see Fig. 2) | Typical duration* | Factors decreasing the process duration† |
|---|---|---|
| 1. Assimilation by photosynthesis | $n \cdot$(min) | VPD ↓ , temperature ↑ , $CO_2$ ↑ , PAR ↑ |
| 2. Transport in phloem | $10n \cdot$(min) (grasses)<br>$n \cdot$(days) (trees) | VPD ↑ , temperature ↑ , water potential in xylem ↑ , osmotic pressure gradient ↑ |
| 3a. Root respiration | $n \cdot$(min) | Root age ↓ , N content ↑ , soil temperature ↑ , $H_2O$ ↑ , photosynthesis ↑ |
| 3b. Exudation (+ other rhizodeposition) | $n \cdot$(h) | N, P, K content ↑ , defoliation ↑ |
| 4a. Uptake by mycorrhiza | $n \cdot$(min) | Temperature ↑ |
| 4b. Uptake by microbial biomass | $n \cdot$(min) | SOM content ↑ , $H_2O$ ↑ , distance from roots ↓ |
| 5a. Respiration of mycorrhiza | $n \cdot$(min) | Photosynthesis ↑ Temperature ↑ |
| 5b. Respiration of microbial biomass | $n \cdot$(min) | N content ↑ , SOM content ↑ , soil temperature ↑ , $H_2O$ ↑ |
| 6. Diffusion | $n \cdot$(min) | $H_2O$ ↑ , clay content ↑ , SOM ↓ , temperature ↑ |

*$n$ is the number between 1 and 9; min = minutes.
†Decrease ( ↓ ) or increase ( ↑ ) of the factors below lead to decrease of the process duration ( = accelerate the process rates).
↓ means: the decrease of the factor contribute to the decrease of the duration of process mentioned in column 1; ↑ means: the increase of the factor contribute to the decrease of the duration of process mentioned in column 1.

after allocation (Cheng *et al*., 1993; Horwath *et al*., 1994; Kuzyakov *et al*., 1999, 2001) and is nearly completed within several days (Carbone & Trumbore, 2007). The rates of assimilate transport to mycorrhizal fungi are comparable with those of phloem transport (Kucey & Paul, 1982; Moyano *et al*., 2007, 2008). Because the distance between the root and mycorrhizal hyphae is very short, this delay is negligible. The exudation of organic compounds from root cells into the rhizosphere (No. 3b, Fig. 3) is partly passive, involving diffusion through cell membranes and partly active secretion. The permeability of plasmalemma for the main exudate compounds (sugars, carboxylic acids and amino acids) is very low. This maintains the concentration gradient between cell interior and exterior at about two orders of magnitude (Darrah, 1993; Jones *et al*., 2004).

Despite the very short distance from root surface to the soil, the low permeability of cell membranes strongly prolongs exudation; it is not an immediate process after allocation of assimilates to the roots. The other relevant rhizodeposition processes, such as the sloughing-off of cells or the death of root hairs and finest roots, as well as dying-off mycorrhizal hyphens need much more time compared with exudation – at least days and requires specific enzymes to utilize the C present in these more recalcitrant substrates (Kuzyakov & Domanski, 2002; Högberg & Read, 2006; Paterson *et al*., 2009). Although C transfer from roots to mycorrhiza involves active transport processes, the subsequent release of organics (similar to exudation) into the soil or saprophytic microorganisms needs more time. Exudate uptake by microbial biomass (not explicitly presented on Fig. 3) is also a fast process, usually completed within minutes (Hill *et al*., 2008; Schneckenberger *et al*., 2008; Blagodatskaya *et al*., 2009). It is, however, influenced by the time needed for exudate diffusion from root or mycorrhizal surface to microorganisms (Darrah, 1991). Further utilization of easily available organics by microorganisms takes only minutes, as it was shown after adding to soil substrates such as glucose (Blagodatsky *et al*., 2000; Hill *et al*., 2008), amino acids (Fokin *et al*., 1993; Jones & Hodge, 1999; Jones & Shannon, 1999) or low molecular weight carboxylic acids (Fischer *et al*., 2009). The final step that influences the time of the efflux of assimilated $CO_2$ into the atmosphere is $CO_2$ diffusion through the soil profile (No. 6, Fig. 3). In soils with neutral and alkali pH, however, this final step may be delayed by $CO_2$ dissolution in soil water.

The delay associated with $CO_2$ diffusion depends on the depth of the $CO_2$ production and on the $CO_2$ diffusivity in the soil (Mencuccini & Hölttä, 2010). The latter depends on molecular diffusivity in the free atmosphere, which is stable at a constant temperature and pressure, and on the soil porosity, which is the sum of soil volumetric air and water content (Moldrup *et al*., 1999; Tang *et al*., 2005a, b; Stoy *et al*., 2007). Volumetric air content changes with soil moisture significantly influencing the magnitude of soil $CO_2$ diffusivity. Even a moderate increase in soil moisture considerably decreases $CO_2$ diffusivity and thus increases the time until the $CO_2$ appears aboveground. For example, the time needed for $CO_2$ diffusion from a depth of 30 cm will change from 0.6 to 1.2 days if the volumetric soil water

content rises from 0.2 to 0.3 $m^3 m^{-3}$ (assuming soil porosity to be 0.6 and diffusivity in the free atmosphere to be 0.14 $cm^2 s^{-1}$). Note that diurnal variations of soil temperature and atmospheric pressure lead to the advection-diffusion processes. Finally, macropores strongly accelerate diffusion or even $CO_2$ mass flow from the soil to the atmosphere. Therefore, $CO_2$ appears on the soil surface faster than predicted solely by diffusion rates. As most roots lie in the upper 20 cm, the diffusion time is comparatively short. Importantly, many studies conducted under controlled conditions used forced air circulation, so that in such studies the time for $CO_2$ flow can be neglected.

Comparison of individual processes rates (Table 1) indicates that three of them may be the bottle-neck: (1) the transport of assimilates in phloem, (2) exudation from roots and (3) $CO_2$ diffusion from soil (for field experiments). Considering plant heights ranging within decimeters for grasses and meters up to tens of meters for trees, we conclude that assimilate transport in phloem is the most limiting step for trees. This is especially true for coniferous trees because phloem transport rates in conifers are much slower than in angiosperms (Kozlowski, 1992; Becker *et al*., 1999; Pumpanen *et al*., 2009). The liming steps for grasses (and many crops) remain unclear. Beside phloem transport, the exudation from roots may be limiting.

When, considering possible effects of exudation on the time lag, two other processes that parallel exudation should be kept in mind: root respiration and respiration by mycorrhizal fungi are responsible at least for the first appearance of assimilated $CO_2$ aboveground (Warembourg & Billes, 1979; Kuzyakov *et al*., 1999, 2002; Johnson *et al*., 2002; Moyano *et al*., 2007, 2008).

## Approaches to studying the time lag

Based on the literature review, we found four approaches suitable to estimate the time lag between photosynthesis and $CO_2$ efflux from soil:

1. *Interruption of assimilate flow* from leaves into the roots, mycorrhizal fungi and rhizosphere, and analysis of the decrease of $CO_2$ efflux (abbreviated as *Interruption*).
2. *Time series analysis (TSA) of $CO_2$ fluxes* from soil and photosynthesis parameters (*TSA of $CO_2$*):
   a. analyses of *total $CO_2$ fluxes* (*TSA of total $CO_2$*),
   b. analyses of *root-derived $CO_2$* obtained by $CO_2$ partitioning (*TSA of root-$CO_2$*).
3. Analysis of natural $\delta^{13}C$ variation *in $CO_2$* efflux with photosynthesis-related parameters or $\delta^{13}C$ in the phloem and leaves ($\delta^{13}C$ *of $CO_2$*).
4. *Pulse labeling* of plants in artificial $^{14}CO_2$ or $^{13}CO_2$ atmosphere with subsequent tracing of $^{14}C$ or $^{13}C$ in $CO_2$ efflux from soil (*Labeling*).

Below we describe the principles, advantages and shortcomings of each approach (see also Table S1).

### *Interruption of assimilate flow from leaves into roots*

*Principle:* The method is based on the instantaneous interruption of the flow of photosynthates from above- to belowground plant parts. The effect of recent assimilate supply on the root-derived $CO_2$ and the time lag is analyzed by the decrease of the $CO_2$ efflux from soil after the interruption (Fig. 4, top). Two parameters can be distinguished: (1) the time lag until significant changes of $CO_2$ efflux (Lag on Fig. 4), and the period to the maximal decrease of efflux. After the maximal decrease is achieved, the remaining part of the total efflux is not directly linked with photosynthesis. This remainder originates from SOM or litter decomposition. The decrease rate of initial flux allows the calculation of the utilization rate ($k$) of assimilates stored in roots [tan($k$); Fig. 4, top].

For tree stands, the common interruption is trenching (Buchmann, 2000; Ross *et al*., 2001; Lee *et al*., 2003) or girdling (Högberg *et al*., 2001; Bhupinderpal-Singh *et al*., 2003; Olsson *et al*., 2005). For grassland ecosystems, defoliation (clipping, cutting, grazing, mowing) and shading is used to inhibit the flow of assimilates to belowground (Craine *et al*., 1999; Wan & Luo, 2003; Bahn *et al*., 2006; Zhou *et al*., 2007). These methods were reviewed in detail earlier (Kuzyakov, 2006).

An innovative approach for interruption of belowground C allocation is based on the physiological girdling of trees by chilling the stems at a certain height with cold-block systems (Johnsen *et al*., 2007). In contrast to the earlier suggested 'destructive girdling' (Högberg *et al*., 2001), this physiological girdling does not kill trees and allows recovery to the initial state of $CO_2$ fluxes after chilling ceases. Physiological girdling therefore allows studying the phenology effects on the C allocation patterns in the same stands. Conversely, frequent measurements of soil $CO_2$ recovery after chilling provide additional information on the importance of aboveground C inputs for belowground processes.

Concerning the two mechanisms of the response of $CO_2$ efflux on photosynthesis (Fig. 2), all interruption approaches clearly help identify the first one, based on the direct transport of molecules. The absence or decrease of photosynthesis, however, decreases phloem loading. We therefore assume that the effect of pressure-gradient waves can also be traced by interruption approaches.

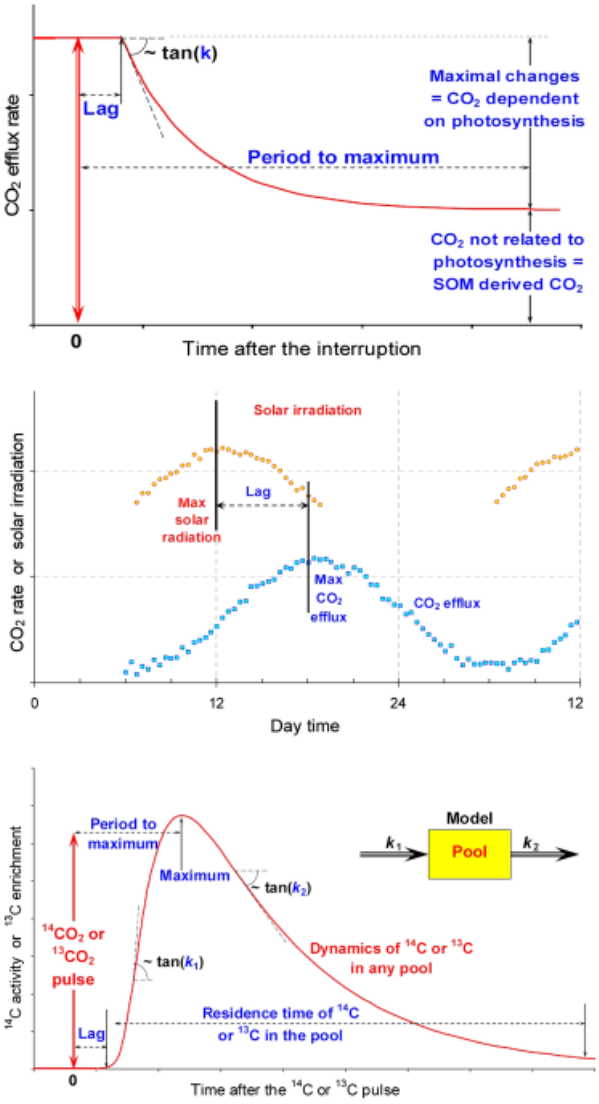


**Fig. 4** Identification of time lag between solar radiation and $CO_2$ efflux from soil by three approaches: Interruption (top), Time series analysis (middle), Labeling (bottom). Top: Parameters of the dynamics of $CO_2$ efflux from soil after interrupting assimilate flow to the roots: (1) lag period, (2) maximal changes of $CO_2$ efflux as response to interrupting assimilate flow, (3) period to maximum, and (4) rough estimation of the flux rates $k$ responsible for decrease of $CO_2$ efflux as tangent of the curve at the start of decrease (the angles should be in radians). The interruption was started at time '0' – vertical double line. Bottom: parameters of the dynamics of the label ($^{14}C$ or $^{13}C$) in any pool after the pulse labeling: (1) lag period, (2) maximum label in pool, (3) period to maximum, (4) residence time of label in pool, and (5) rough estimation of flux rates $k_1$ and $k_2$ as tangent of the curve at the inflection points (the angles should be in radians). The label was added as pulse at time '0' – vertical double line.

*Advantages:* The method is simple, cheap and requires no expensive and laborious analyses. No measurements of photosynthesis or related parameters are necessary. In different variations, the Interruption approach is suitable for all ecosystems.

*Disadvantages:* All variants of this approach (except shading and physiological girdling) are destructive and irreversible. The destructive procedures kill the plants or plant parts and create large amounts of root debris including mycorrhizal mycelium (Högberg *et al.*, 2001; Bhupinderpal-Singh *et al.*, 2003; Olsson *et al.*, 2005). Thus, the interruption of assimilate flows into the roots and rhizosphere is partly compensated or even over-compensated by decaying roots. This decomposition may mask the time lag between the cessation of C transport and decrease of $CO_2$ efflux. The carbohydrate reserves in roots could also sustain root metabolism, blocking a drop in respiration after the interruption. This is especially true for most pasture plants, which are perennial and whose well-developed roots serve as a C storage to sustain new growth in spring or after grazing (Johansson, 1993; Paterson & Sim, 1999; Bahn *et al.*, 2006; Zhou *et al.*, 2007).

For grasslands, fewer studies have concentrated on the response of single components of $CO_2$ efflux (root and microbial respiration) to clipping (Craine *et al.*, 1999; Bahn *et al.*, 2006; Zhou *et al.*, 2007). Generally, the total $CO_2$ efflux from soil was analyzed (Bremer *et al.*, 1998; Wan & Luo, 2003; Cao *et al.*, 2004). This complicates the interpretation because both root- and microbial-derived $CO_2$ respond to a similar degree to changes in photosynthetic C supply (Craine *et al.*, 1999; Bahn *et al.*, 2006; Zhou *et al.*, 2007).

Overall, we conclude that nondestructive methods for interrupting assimilate flow (shading, physiological girdling) are promising approaches for future work.

### *TSA of $CO_2$ fluxes from soil and related photosynthesis parameters*

This group of approaches integrates methods based on analyzing variation of the components of $CO_2$ efflux in relation to variation of photosynthesis parameters (Fig. 4, middle).

*TSA of total $CO_2$ fluxes. Principle:* High-resolution measurements of total $CO_2$ efflux from soil are related to changes of climatic parameters directly affecting leaf $CO_2$ exchange – photosynthetically active radiation (PAR), vapor pressure deficit (VPD) – or with proxies of photosynthesis like gross primary production (GPP). The PAR is the parameter directly affecting photosynthesis intensity. To calculate the VPD, the primary data must include air temperature and vapor

pressure (or humidity). To obtain GPP with high temporal resolution, eddy covariance measurements are used (Foken, 2008). The GPP is calculated as the difference between net ecosystem exchange (NEE) and total ecosystem respiration (TER). TER is calculated by the algorithm (Reichstein *et al*., 2005) that derives a short-term temperature sensitivity of TER from eddy covariance data based on the exponential regression model (Lloyd & Taylor, 1994).

The correlation of $CO_2$ efflux with photosynthesis parameters can be done by TSA (i.e., cross-correlation, Fourier analysis, seasonal decomposition) (Fig. 4, middle). This allows evaluation of the time lag, changes of amplitude, and (if relevant) changes in the frequency of variations (Gorbenko & Panikov, 1989; Tang *et al*., 2005a, b; Liu *et al*., 2006). This approach enables to decouple soil $CO_2$ efflux from temperature. Such studies often yield different $CO_2$ fluxes under the same temperature during one day (Tang *et al*., 2005a, b; Bahn *et al*., 2008; Vargas & Allen, 2008; Gavrichkova, 2009). This is a clear proof that temperature is a weak and indirect indicator of diurnal variation in soil respiration. Further development of the TSA technique is a wavelet coherence analysis on continuous data of soil $CO_2$ production (Vargas *et al*., 2010). The advantage of wavelet analysis over other TSA is that the window size is not fixed; it varies as a function of frequency and overcomes the problems of nonstationarity in time series.

*Advantages:* The TSA of total $CO_2$ fluxes approach enables studying the changes in the time lag during the growing season. This advantage is important compared with all other approaches because the time lag may change depending on the plant development and on environmental parameters (e.g., soil moisture). It also helps assess the amplitude of variation of $CO_2$ fluxes in response to photosynthesis changes. With modifications, the method can be adapted to evaluate the time lag between photosynthesis and $CO_2$ production at various soil depths (Tang *et al*., 2005a, b). This indirectly allows evaluation of assimilate transport rates within roots. The TSA also accounts for the accelerating effect of pressure–concentration waves on root exudation process.

*Disadvantages:* Expensive instrumentation for high-resolution measurements of soil $CO_2$ efflux and photosynthesis-related parameters is necessary. As the *total* $CO_2$ efflux is commonly measured, no information on $CO_2$ sources is provided. Thus, the changes of $CO_2$ efflux from sources highly dependent on photosynthesis (root and rhizomicrobial respiration) may be masked by the $CO_2$ fluxes from other sources independent of photosynthesis (decomposition of litter and SOM). The opposite variations of photosynthesis intensity and of factors responsible for decomposition of litter and SOM, such as soil temperature and moisture, may overshadow the effect of photosynthesis. Diurnal decoupling between temperature and $CO_2$ efflux often observed in such studies may be determined by factors other than photosynthesis (Bahn *et al*., 2008). Shifts in the phase and amplitude of soil temperature with depth or diurnal changes of moisture modify the $CO_2$ diffusion rates. This calls for monitoring the diurnal $CO_2$ production in the main rooting zone, together with associated soil temperature and moisture changes, to separate the confounding effects of biotic and abiotic factors.

*TSA of root-derived $CO_2$ efflux. Principle:* This approach is a further development of the previous one (TSA of total $CO_2$). In contrast to the ambiguity of correlation between photosynthesis parameters and the *total* $CO_2$ (previous approach), the correlation with *root-derived* $CO_2$ is analyzed. The delay between the photosynthesis and $CO_2$ efflux from the rhizosphere is estimated by a TSA identifying peaks in correlation strength between root-derived $CO_2$ and time-shifted photosynthesis parameters. The root-derived $CO_2$ is estimated indirectly by subtracting SOM-derived $CO_2$ from total $CO_2$ efflux from soil. In most studies, the root-derived $CO_2$ is estimated by the interruption approaches (i.e., trenching). However, instead of estimating the time lag based on the decrease of $CO_2$ efflux after interruption, a TSA is applied (Fig. 4, middle).

*Advantages:* The approach is cheap and widely applied in various ecosystems, providing an opportunity for between-ecosystem comparisons. Compared with the correlations with total $CO_2$ efflux, the approach based on root-derived $CO_2$ should be more precise. However, the uncertainties of $CO_2$ partitioning limit the precision of this approach (Kuzyakov, 2006).

The approach was successfully applied for crops, broad-leaf and needle-leaf forests (Moyano *et al*., 2007, 2008), where root-derived $CO_2$ was well correlated with the lagged GPP. The root exclusion technique with mesh cores impenetrable for roots was used to obtain the root-derived component of soil respiration. Respiration of excised grassland roots was related to 2 days lagged solar radiation (Fitter *et al*., 1998). Significant correlation between rhizosphere respiration and lagged gross ecosystem photosynthesis was observed by root-exclusion (Gaumont-Guay *et al*., 2008).

*Disadvantages:* The indirect measurement of the root-derived $CO_2$ by interruption approaches leads to various errors and limits its applicability. In particular, as root-derived $CO_2$ is calculated by the difference between total and SOM-derived $CO_2$, short-term variation of both fluxes will increase the variation of the estimated root-derived $CO_2$. The SOM-derived $CO_2$ may be affected by temperature (which is highly correlated with solar radiation), making the unbiased 'extraction' of

root-derived $CO_2$ from the total $CO_2$ questionable. Additional measurements of related photosynthesis parameters are necessary.

*Analysis of natural $\delta^{13}C$ variation in $CO_2$ efflux with photosynthesis-related parameters or $\delta^{13}C$ in the phloem and leaves*

*Principle:* Fractionation of C isotopes by photosynthesis is caused primarily by the carboxylating enzyme ribulose-1,5-biophosphate (RuBisCo), and by $CO_2$ diffusion from the atmosphere to the site of $CO_2$ fixation (Farquhar *et al*., 1989; Brugnoli & Farquhar, 2000). The contribution of each fractionation source to the final $\delta^{13}C$ of assimilates depends largely on the $c_i/c_a$ ratio, which is the ratio between the partial pressure of $CO_2$ inside and outside the leaf (works only for $C_3$ photosynthesis). $c_i/c_a$ is controlled by the stomata closer and aperture in response to environmental conditions (Farquhar *et al*., 1989). High light intensity boosts photosynthesis and strongly decreases the $c_i/c_a$, especially under water limitation. Numerous studies confirmed the linear relationship between $^{13}C$ discrimination and $c_i/c_a$. The $\delta^{13}C$ of the leaf soluble sugars synthesized in the diurnal course is highly correlated with the weighted average of $c_i/c_a$ over the entire day (Brugnoli *et al*., 1988). $\delta^{13}C$ and $\delta^{18}O$ in water-soluble organics transported in the phloem are also reliable indicators of the short-term changes in $c_i/c_a$ (Keitel *et al*., 2003; Barbour *et al*., 2005; Brandes *et al*., 2006; Barnard *et al*., 2007). These findings suggests that if root respiration and exudation are coupled to aboveground photosynthetic activity, then the variation of the $\delta^{13}C$ signature of root-derived $CO_2$ would reflect $\delta^{13}C$ changes of soluble organics in leaf or phloem sap. Accordingly, the $\delta^{13}C$ of root-derived $CO_2$ should correlate with time lag to environmental factors that affect $^{13}C$ discrimination during $CO_2$ fixation.

Variability in the $\delta^{13}C$ of soil and ecosystem respiration, to which soil respiration contributes the most (Janssens *et al*., 2001), was frequently investigated in response to changes in environmental factors such as PAR, VPD, air relative humidity and precipitation. Such studies were conducted for loblolly pine forest (Mortazavi *et al*., 2005), mixed coniferous boreal forest (Ekblad & Högberg, 2001), Norway spruce forest (Ekblad *et al*., 2005), mixed deciduous forest (Knohl *et al*., 2005), for various series of coniferous forests (Bowling *et al*., 2002), and for tropical forest (Ometto *et al*., 2002). $\delta^{13}C$ in ecosystem respiration is sensitive to changes in the $\delta^{13}C$ signature of phloem and leaf soluble organic material (Scartazza *et al*., 2004) and to variation in VPD (Bowling *et al*., 2002). Strong diurnal variation of $\delta^{18}O$ in water sampled from different parts of pine trees let conclude that the time lag between evaporation-controlling factors and $\delta^{18}O$ should be considered in studies of C transport, source-sink and C flux partitioning (Barnard *et al*., 2007). Alstad *et al*. (2007) found a strong correlation between $^{13}C$ in ecosystem respiration and environmental parameters (VPD, $T_{air}$, $T_{soil}$, photosynthetic photon flux) for boreal, coastal and deciduous forests. The strength, significance and the time when the maximum correlation was obtained, however, differed among the forest groups.

*Advantages:* The method involves no soil disturbance. The time lag is obtained by the direct relationship of $CO_2$ efflux with photosynthesis products and substrates for respiration, such as nonstructural C in leaves and phloem (Göttlicher *et al*., 2006; Xu *et al*., 2008).

*Disadvantages:* The first disadvantage is the applicability solely for plants with $C_3$ photosynthesis, because $^{13}C$ fractionation in $C_4$ photosynthesis depends much less on the $c_i/c_a$ ratio. The approach is suitable only for the direct transport of assimilates to the roots (mechanism 1, Fig. 2) and is unsuitable to trace the time lag induced by phloem pressure waves.

The approach based on natural $\delta^{13}C$ variation of $CO_2$ efflux is associated with several complications: various environmental parameters and processes modify the $\delta^{13}C$ of assimilates while they are utilized for structural C and are transported from the leaves to the roots. Potential mixing of various C pools with different metabolic histories during downward phloem transport was frequently reported (Keitel *et al*., 2003, 2006; Nobel, 2005; Brandes *et al*., 2006, 2007; Barnard *et al*., 2007; Kodama *et al*., 2008). Accordingly, $\delta^{13}C$ in trunk phloem sap integrates mean canopy $c_i/c_a$ over several days. Based on $^{14}C$ in nonstructural sugars and organic acids of rice after labeling in $^{14}CO_2$ atmosphere, $^{14}C$ in $CO_2$ was predicted with $R^2$ between 67% and 77% (Xu *et al*., 2008). However, such a high correlation was possible because of intensive $^{14}C$ flux through the plant after the pulse labeling and because other environmental parameters were constant.

Additional complications reflect the fact that $CO_2$ respired by roots and rhizosphere microorganisms differs in $\delta^{13}C$ signature (Bhupinderpal-Singh *et al*., 2003; Gessler *et al*., 2007). This means that the $\delta^{13}C$ variation of $CO_2$ released by microorganisms also contributes to temporal variation in $\delta^{13}C$ of root-derived $CO_2$; its elusive effect depends on the relative contribution of microbial $CO_2$ to total $CO_2$ efflux from soil. The $\delta^{13}C$ of microbial respiration is affected by seasonal availability of the substrates for microorganisms, which changes in response to variation of temperature, moisture and seasonal variation in productivity of above- and belowground litter (Ekblad *et al*., 2005).

Diurnal changes in $^{13}C$ fractionation could also occur by respiration as a consequence of fractionation by

postcarboxylation (Bowling *et al*., 2008): the released $CO_2$ originates either from relatively $^{13}C$-enriched ($C_3$ and $C_4$ in the glucose molecule) or depleted ($C_1$, $C_2$, $C_4$, $C_6$ in the glucose molecule) atoms of the same substrate molecule (Tcherkez *et al*., 2004; Hymus *et al*., 2005). The same process could also occur during root or microbial respiration, altering the $^{13}C$ signature at an uncertain rate independent of the C isotope composition of the substrate (Bowling *et al*., 2008).

### *Pulse labeling of plants in artificial $^{14}CO_2$ or $^{13}CO_2$ atmosphere*

*Principle:* Pulse labeling in $^{13}CO_2$ or $^{14}CO_2$ atmosphere and subsequently tracing the labeled C in the soil $CO_2$ is the most widely used technique to estimate the time lag between assimilation of $^{13}C$ or $^{14}C$ and their release from soil as $CO_2$. The labeling approach used since 1973 (Warembourg & Paul, 1973) was reviewed in detail earlier (Whipps, 1990; Kuzyakov & Domanski, 2000; Kuzyakov *et al*., 2001). The time necessary for downward transport of recent assimilates and their respiration by roots and microorganisms is assessed by analyzing the $^{13}CO_2$ or $^{14}CO_2$ in soil respiration several times during the chase period and then identifying the first appearance of the label and/or the maximum of labeled C in the $CO_2$ efflux (Fig. 4, bottom).

A variation of the labeling approach used to estimate the time lag is the free-air $CO_2$ enrichment (FACE) approach developed to study the effect of elevated $CO_2$ on intact ecosystems under natural conditions. The added $CO_2$ is depleted in $\delta^{13}C$ compared with atmospheric $CO_2$, enabling its use it as a continuous $^{13}C$ label. Shifts in the $\delta^{13}C$ signature of soil $CO_2$ shortly after applying enriched $CO_2$ allows estimating the C translocation rate from above- to belowground (Andrews *et al*., 1999; Steinmann *et al*., 2004; Keel *et al*., 2006). However, the FACE approach is strongly limited by sensitivity because the differences between $\delta^{13}C$ available for plants under elevated $CO_2$ and atmospheric $CO_2$ differ by about 12–15‰. Additionally, the released root-derived $CO_2$ is diluted by SOM-derived $CO_2$ and C assimilated by plants before the FACE started; it will also be masked by high variation of $\delta^{13}C$ of $CO_2$ released from soil. This approach can be used only once – at the start of FACE.

*Advantages:* The appearance of $^{13}CO_2$ or $^{14}CO_2$ in the $CO_2$ efflux from soil is a clear parameter for that C that was assimilated at labeling (see below for abiotic $CO_2$ fluxes from soil). Labeling allows a clear separation of root-derived $CO_2$ from the total $CO_2$ efflux. It is not biased by any effects of temperature or moisture on decomposition of plant residues or SOM. Pulse labeling does not require disturbing the plants and soil structure. Performed under controlled conditions, it allows studying the variability of the time lag in response to changes of single abiotic or biotic factors (soil moisture, nutrient supply, defoliation, etc.). Beyond the time lag, parameters of C flux dynamics in various pools in plants and soil as well as in $CO_2$ can be analyzed by pulse labeling and chasing.

*Disadvantages:* Special equipment for chambers and analyses is necessary. A uniform distribution of the label within the plant cannot be achieved due to preferential allocation of the label to the active growth zones (Mehard, 1994; Thornton *et al*., 2004; Paterson *et al*., 2009). Labeling studies are most often conducted on crops, grasses or small trees under controlled conditions, which only partially reflect a plant's real growing environment. Only few labeling studies have been conducted on young trees under field conditions (Horwath *et al*., 1994; Högberg *et al*., 2008; Pumpanen *et al*., 2009; Subke *et al*., 2009; Plain *et al*., 2009). Studies in pots may alter the root–soil relationship and change the amount of C translocated belowground and respired as $CO_2$. *In situ* application is possible but the complications can compromise the interpretation. The label penetration into the soil pores during the labeling is one key difficulty of the *in situ* experiments (Leake *et al*., 2006; Bahn *et al*., 2009; Subke *et al*., 2009); this penetration could be erroneously interpreted as a fast respiration of the recently assimilated C. Despite various advantages for investigating time lags, this approach is suitable only to estimate the flow of that C that was assimilated by the labeling. It is unsuitable to estimate the accelerating effect of the pressure gradient waves on C release into the rhizosphere (Mencuccini & Hölttä, 2010).

*Theoretical dynamics of the label in any pool after pulse labeling.* Despite the above-mentioned disadvantages, we consider this approach as the most suitable to estimate the time lag between photosynthesis and $CO_2$ efflux from soil. This is because it is possible to study the dynamics of recently assimilated C in various plant and soil pools as well as in $CO_2$ efflux. Various parameters of the time lag can also be estimated.

In any pool described by influx and outflow, the post-pulse dynamics of the label in the pool depend on the rates of both flows (Fig. 4, bottom). Independent of the rate of influx ($k_1$) and outflow ($k_2$), the following steps are typical: (1) lag phase – no label is detectable in the pool, (2) fast accumulation of the label in the pool, (3) attainment of the maximum and (4) subsequent release of the label and its decrease down to the detection level (Fig. 4, bottom). The total period between the start until the end of detection of the label in the pool is the estimated residence time of the label in the pool. The duration of the lag phase depends

mainly on the number of pools passed by the label before it is released as $CO_2$ and the duration of the transport between the sites of photosynthesis and respiration. Note here that the shape of the curve presented in Fig. 4 strongly depends on the ratio between the rates of influx ($k_1$) and outflow ($k_2$). Similar dynamics of $^{14}C$ or $^{13}C$ are typical in the $CO_2$ efflux from soil after the pulse labeling of grasses (Cheng *et al*., 1993; Horwath *et al*., 1994; Kuzyakov *et al*., 1999, 2001; Warembourg & Estelrich, 2000; Bahn *et al*., 2009) and trees (Högberg *et al*., 2008; Subke *et al*., 2009; Plain *et al*., 2009).

Concerning the time lag, a key aspect is to identify which parameter of time lag was estimated in the respective study. It is important to distinguish between the two parameters: the first appearance of the label (corresponds to the lag phase) and maximum content of the label in $CO_2$ efflux from soil. Both together, however, were presented in only very few studies (Cheng *et al*., 1993; Horwath *et al*., 1994; Kuzyakov *et al*., 1999, 2001; Warembourg & Estelrich, 2000; Gavrichkova & Kuzyakov, 2008).

Other specific factors affecting the obtained lag are the sampling start and the sampling frequency. Starting the first $CO_2$ sampling too late (common in many studies) bears the risk of missing the first appearance of the label in $CO_2$. Therefore, the lag period measured by labeling studies is frequently overestimated. Note that the label dynamics schematically shown in Fig. 4 represent the flux only for an intermediate pool in a unidirectional sequence of processes chain. If the respective pool is involved in exchange with other pools (there are some bi- or multidirectional processes), the dynamics may have another shape, e.g., with more prolonged tail.

Comparing the advantages and shortcomings of the four approaches for estimation time lags we conclude that unbiased time lags and the links between photosynthesis and $CO_2$ efflux from soil can only be determined by combining (1) TSA of $CO_2$ fluxes (and its $\delta^{13}C$) from soil with photosynthesis proxies and (2) pulse labeling of plants in $^{13}CO_2$ atmosphere (on separate plots) with subsequently tracing $^{13}C$ in soil $CO_2$ under field conditions.

## Time lags between photosynthesis and $CO_2$ efflux from soil for trees and grasses

As noted above, the time lag depends on the transport from the leaves to roots (for root respiration), uptake by mycorrhizal fungi, and on the release of root exudates (for rhizomicrobial respiration) (Fig. 2; Table 1). As the transport distance differs strongly between trees and grasses, and different steps may be the bottle-neck for the process chain, we describe the time lag for woody and nonwoody vegetation separately.

### *Time lag for trees*

Nearly all studies on tree species were done under field conditions. The time lags estimated by various techniques and obtained from various forest stands were pulled together (Table S2) and plotted vs. stand average age (Fig. 5, top) or height (Fig. 5, bottom).

The time lags estimated by various approaches differed significantly ($P < 0.01$). The *post hoc* Tukey's HSD test showed that the time lag obtained by FACE and Interruption approaches were significantly longer than estimated by other approaches (Fig. 5, Table 2). This is connected with low sensitivity of FACE and high variability of $\delta^{13}C$ in respired $CO_2$. Because of strong overestimation of the time lag by FACE approach, these data were not included in the evaluations presented on Fig. 5.

Although different methods were used to obtain the time lags, certain trends are clear in respect to tree height and age. The lag showed a sharp asymptotic increase up to a plant age of 50 years (Fig. 5, top). For mature trees (>50 years) the lag stabilized at 4–5 days. However, if the time lag was related to plant height (Fig. 5, bottom) no clear stabilization was observed. After the tree height of about 10–15 m, the time lag increased with the rate of about 0.1 day $m^{-1}$. This underlines the importance of phloem transport rates for the time lag for trees.

The lag obtained by various approaches behaved differently (Fig. 5). Their magnitude was almost constant in time based on the analysis of $\delta^{13}C$ in $CO_2$. The values obtained by TSA of $CO_2$ and the labeling approach increased logarithmically with time, with lower lags stemming from the TSA of $CO_2$ approach. The trends obtained by Interruption and FACE were unclear due to lack of data points (FACE is not shown on Fig. 5).

### *Time lag for grasses and herbs*

The lag is much shorter for grasses (Table S3) than for trees. All studies registered the first appearance of the label in $CO_2$ efflux at the first sampling of the soil air. This first sampling was typically performed 30 min after completing the labeling. Note that in the experiments with grasses, the $CO_2$ sampling was generally done more frequently than for trees. The values for grasses are therefore more reliable. Almost all results for grasses and herbs were obtained using only one approach – pulse labeling of plants in $^{14}CO_2$ or $^{13}CO_2$ atmosphere. We summarize the lags obtained from different studies in relation to plant height (Fig. 6, top)

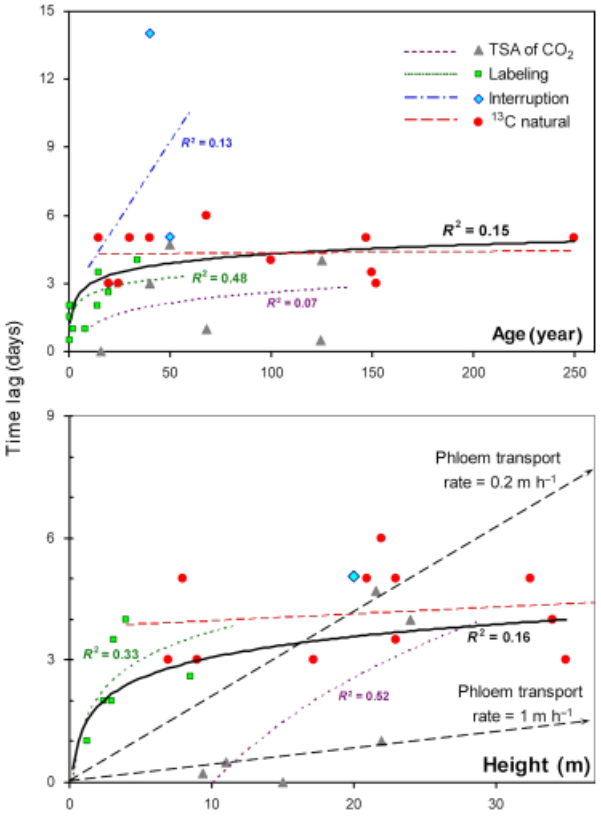


**Fig. 5** Time lag between photosynthetic carbon (C) uptake by leaves or needles of trees and its release as $CO_2$ through the roots and rhizosphere microorganisms vs. age (top) or height (bottom) of the tree stand. An average age was taken for multilevel stands. If the multiple lags had been reported, the first one observed was chosen to be plot on the graph. The color of each regression line corresponds to the color of the method it describes (see text for explanation of approaches of time lag estimation). The $R^2$ are presented only for values higher than 0.1. Black regression line integrates all the data. (Not all literature sources provided information on the parameters age and height, explaining the different number of points in both subfigures.) The two straight dashed arrows at the bottom subfigure show the change of the time lag assuming the commonly reported phloem transport rate of 1 m $h^{-1}$ and the slowest reported phloem transport rate of 0.2 m $h^{-1}$. The data points were taken from: Andrews *et al*. (1999), Bowling *et al*. (2002), Bhupinderpal-Singh *et al*. (2003), Baldocchi *et al*. (2006), Carbone *et al*. (2007), Ekblad & Högberg (2001), Gärdenäs (2000), Gaumont-Guay *et al*. (2008), Ekblad *et al*. (2005), Johnsen *et al*. (2007), Högberg *et al*. (2008), Horwath *et al*. (1994), Keel *et al*. (2006), Knohl *et al*. (2005), Liu *et al*. (2006), McDowell *et al*. (2004), Mikan *et al*. (2000), Mortazavi *et al*. (2005), Moyano *et al*. (2008), Olsson *et al*. (2005), Phillips & Fahey (2005), Plain *et al*. (2009), Pumpanen *et al*. (2009), Pypker *et al*. (2008), Steinmann *et al*. (2004), Subke *et al*. (2009), Tang *et al*. (2005a, b). (For detailed data see Table S2).

age (Fig. 6, bottom). Nonetheless, the data on grass height are poorly presented in the literature related to $CO_2$ fluxes (Table S3). The lags decreased up to a plant height of about 20 cm (Fig. 6, bottom), stabilizing at about 12 h. As the effect of plant height on time lag was checked only a single species – *Lolium perenne* (Kuzyakov *et al*., 1999, 2001; Kuzyakov & Domanski, 2002), the generalization for other grasses is not possible.

Plant age (Fig. 6, top) had contrasting effects on the lag. Only few studies dealt with plants younger than 25 DAS (days after sowing). At this grass age, the lag apparently peaks (up to 36 h; Warembourg & Estelrich, 2000; Gavrichkova & Kuzyakov, 2008). This effect could be species-specific because it was observed in corn and bromegrass, but not confirmed for wheat (Dilkes *et al*., 2004). From plant age between 20 and 60 DAS, the lag constantly increased, reaching a second peak of about 15–20 h. Further experiments are needed to cover the gap in the time lag, especially for very young seedlings (<40 DAS). Figure 6 includes only the results from studies conducted under controlled conditions, where forced air circulation is frequently used. The duration of $CO_2$ diffusion from the location of $CO_2$ production up to the soil surface can be thus neglected. Under field conditions or in experiments without forced air circulation, the diffusion of $CO_2$ to the surface may slightly prolong the lag.

The field experiments did not provide information on plant age and height at the time of the measurements: the data were restricted to the growth season and/or the phenology (Table S3). The plants grown under controlled conditions showed a shorter delay ($11.5 \pm 1.5$ h, $P < 0.001$, Table S4) between the photosynthetic C uptake and its release from soil compared with plants grown under field conditions ($22 \pm 4$ h). This may reflect $CO_2$ diffusion to the surface.

## Factors affecting the time lag

### *Transport distance: effect of plant height*

The preliminary conclusion on the effect of the distance between the source of C fixation (photosynthetically active leaves) and the respiratory sink (roots and rhizosphere microorganisms) on the duration of C downward translocation could be drawn based on the difference between the time lags for trees (Table S2; Fig. 5) and grasses (Table S3; Fig. 6). The distribution of the lag (as a maximum of labeled $CO_2$ efflux) for grasses and herbs showed a maximum of about 12.5 h (Fig. S1, bottom) and for trees of about 2.85 days (Fig. S1, top). The time lag for trees corresponds to an average C transport rate of 0.22 m $h^{-1}$. This rate is within the lower range of reported phloem C flux rates of 0.2–2 m $h^{-1}$ (Ekblad & Högberg, 2001; Keitel *et al*., 2003; Nobel, 2005; Barnard *et al*., 2007; Plain *et al*., 2009). However, predicting lags based on reported phloem C flux rates would be too imprecise.

**Table 2** Mean time lags (upper line, bold; in days) and *P*-values for significance of differences in the time lags estimated by various approaches

| | Approach: | | | | |
|---|---|---|---|---|---|
| Approach* | Interruption | TSA of $CO_2$ | $\delta^{13}C$ of $CO_2$ | Labeling | FACE |
| Mean | 7.33 ± 3.38 | 1.57 ± 0.77 | 3.56 ± 0.31 | 1.84 ± 0.35 | 14.00 ± 7.00 |
| 1. Interruption | *P* values† | | | | |
| 2. TSA of $CO_2$ | **0.029** | | | | |
| 3. $\delta^{13}C$ of $CO_2$ | 0.197 | 0.492 | | | |
| 4. Labeling | **0.027** | 0.999 | 0.496 | | |
| 5. Labeling (FACE) | 0.071 | **0.000** | **0.000** | **0.000** | |

Results of one-way ANOVA: *post hoc* Tukey's HSD test.
*See extended description of the approaches in text.
†*P*-values showing significant (<0.05) differences between the approaches are presented in bold.
TSA, time series analysis; FACE, free air $CO_2$ enrichment.

The effect of the source to sink distance on the time lag is more complex. For grasses, the time lag generally decreased, whereas for trees it constantly increased with plant height (Figs. 5, bottom and 6, top). The absence of a lag, (zero) or relatively short lags (<1 day), in some studies with trees (Scartazza *et al*., 2004; Tang *et al*., 2005a, b; Baldocchi *et al*., 2006; Liu *et al*., 2006; Gaumont-Guay *et al*., 2008) could be explained by the sieve tube's capacity for rapid transmission of pressure–concentration waves in response to local changes in osmotic pressure. Time lags much longer than those calculated from the phloem transport rate were observed by tracing labeled photosynthetic C from the tree canopy to roots (Högberg *et al*., 2008; Plain *et al*., 2009). This suggests that factors other than aboveground pathway length affect longer time lags in trees. These factors may include transitory storage and further remobilization of the stored carbohydrates in shoot and roots (Gessler *et al*., 2008; Bahn *et al*., 2009; Mencuccini & Hölttä, 2010) or main root depth contributing to the diffusion of $CO_2$ to the surface (Warembourg & Paul, 1973; Stoy *et al*., 2007).

### *Effect of root depth*

Vertical distribution of roots within the soil profile (Jackson *et al*., 1996) as well as variation in soil moisture and temperature (Hirsch *et al*., 2004; Jassal *et al*., 2004, 2005) affect $CO_2$ diffusion to the surface. The belowground extension of roots differs significantly between plants as well as within the same plant functional type. The quantity of C translocated to roots at different depths and the specific root activity affect the aboveground appearance of the $CO_2$. Using pulse labeling in $^{14}CO_2$ and sampling the soil air and roots at different depths Warembourg & Paul (1973) studied the time required for recent assimilate translocation from foliage to the roots at increasing depths. Maximum $^{14}C$ in soil $CO_2$ appeared 24 h after labeling at 15 and 35 cm depth. At 60 cm, however, 100 h were required. The relative contribution of $^{14}C$ to the total $CO_2$ efflux was higher for the roots in the upper soil layers, determining the time of the overall $^{14}CO_2$ maximum aboveground (Warembourg & Paul, 1973). The diffusion time from lower soil horizons contributes to the delay in the aboveground appearance. Thus, the location of the main root zone is one of the reasons for the different lags under field vs. controlled conditions, and among plant species with different rooting depths.

### *Effects of plant physiology and growth stage*

The average lag for gymnosperms (3.9 ± 0.66 days, $P<0.05$, Table S5) was significantly longer than in angiosperms (1.94 ± 0.51 days), confirming the differences in phloem cell structure and transport rates (Kozlowski, 1992). From an evolutionary standpoint, angiosperms developed sieve tubes for faster transport of assimilates via phloem. Pulse labeling yielded lags for angiosperms that were substantially shorter and differently shaped (depending on tree height) compared with gymnosperms (Fig. 7). For a 4 m tall gymnosperm the lag corresponded to a phloem C translocation velocity of 1 m $day^{-1}$ (Carbone *et al*., 2007), whereas for the angiosperm of the same height the expected velocity is twice as fast and the lag thus much shorter. However, labeling experiments were conducted mainly on young trees (Phillips & Fahey, 2005; Högberg *et al*., 2008; Subke *et al*., 2009) or on tree seedlings (Horwath *et al*., 1994), and only few labeling studies were done on mature trees (Carbone *et al*., 2007; Plain *et al*., 2009).

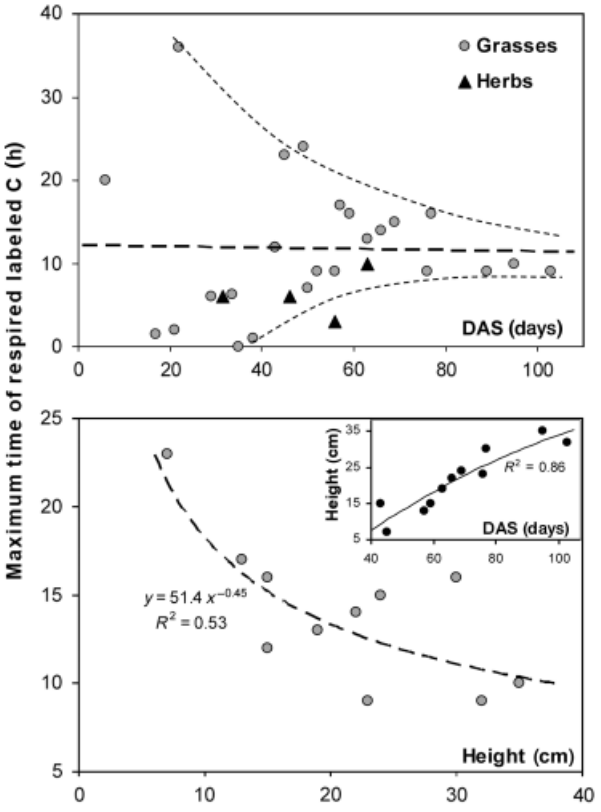


**Fig. 6** Time period between photosynthetic carbon (C) uptake by labeling and maximum C release as $CO_2$ from the soil vs. age of grasses (in days after sowing, DAS) (top), and plant height (cm) (bottom). Two dashed curves (top) show the upper and the lower limits of the time lag. The inset at the bottom shows the correlation between plant age (in days after sowing, DAS) and plant height. The data for bottom figure are taken mainly from Kuzyakov *et al*. (1999). The data points were taken from: Cheng *et al*. (1993), Dilkes *et al*. (2004), Domanski *et al*. (2001), Gavrichkova & Kuzyakov (2008, 2010), Gregory & Atwell (1991), Hawkes *et al*. (2008), Heinemeyer *et al*. (2006), Kucey & Paul (1982), Kuzyakov *et al*. (1999, 2001), Kuzyakov & Cheng (2001), Kuzyakov & Domanski (2002), Nguyen *et al*. (1999), Warembourg & Estelrich (2000), Xu *et al*. (2008). (For detailed data see Table S3).

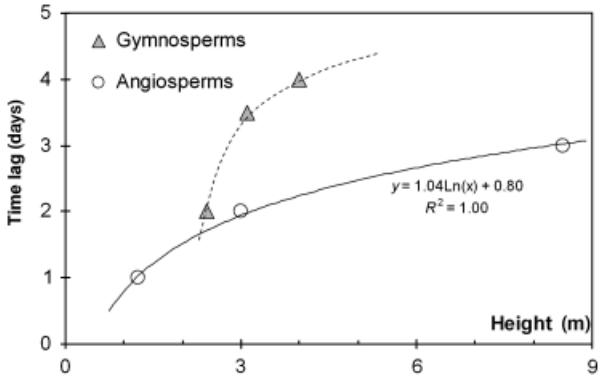


**Fig. 7** Time between photosynthetic carbon (C) uptake by labeling and maximum C release as $CO_2$ from the soil vs. plant height for angiosperms and gymnosperms. Data from labeling studies: Horwath *et al*. (1994); Phillips & Fahey (2005), Carbone *et al*. (2007), Högberg *et al*. (2008), Plain *et al*. (2009), Subke *et al*. (2009).

The time lag may change during ontogenesis because the C demand in different plant organs changes with plant development. The C fixed by shoots may be respired, used to produce new shoot material, temporarily stored, or allocated belowground into roots and from them to soil organisms (Leake *et al*., 2006). The stronger the C demand in the sink (which depends on sink size, its growth rate, metabolic activity, and respiration rate), the larger and faster the C supply from the source (Warembourg & Estelrich, 2001). During the growth period, usually early spring, storage organs could act as sugar sources; the many growing areas are sugar sinks. After the growth period, when the meristems are dormant, the mature leaves are sources, and storage organs are sinks. Thus, a higher delay in belowground C translocation in some periods of plant phenology could be attributed to the allocation preference of new C not to roots, but to shoots for development of leaves or reproduction organs.

In respect to time lag, only few experiments have been conducted on various growing stages. Labeling *Lolium perenne* at eight growing stages revealed that young grasses transport C downward and respire it more slowly than older plants (Kuzyakov *et al*., 1999). This trend was confirmed by pulse labeling two growing stages of *Zea mays* (Gavrichkova & Kuzyakov, 2010). Pooling more results reveals that this is true only for grasses between 60 and 100 DAS.

Studies on time lag changes during the growing season are scarce. Combination of continuous data of $CO_2$ efflux from soil with GPP in oak-grass savannah showed that the lag between C assimilation and efflux changes during the growing season from 7 h up to 12 h (Tang *et al*., 2005a, b). Cisneros-Dosal *et al*. (2006) suggested that a source of C respired by tree roots in a temperate forest may shift from stored C pools in early spring to recent photosynthates in summer. This confirms that the relative importance of roots as a C sink changes during the growing season in both grasses and trees. Labeling the canopies of poplar trees in $^{14}CO_2$ atmosphere showed that 50% of the $^{14}C$ was allocated belowground in September, but only 20% in July (Horwath *et al*., 1994). Despite the changes in allocation of assimilates, the lags did not differ. For different vegetation types Vargas *et al*. (2010) demonstrated that PAR regulates soil $CO_2$ fluxes at a 1-day periodicity, but this effect was not stable with time. Additional field studies are necessary to determine the effect of phenology on

the lag. Interpreting the translocation rate of recent assimilates belowground requires not only the lag duration, but also information on phenology. This means that time lag studies that correlate $\delta^{13}C$ or $CO_2$ with changes in GPP, VPD or other photosynthesis-related parameters (Ekblad & Högberg, 2001; Bowling *et al*., 2002; Fessenden & Ehleringer, 2003; Mortazavi *et al*., 2005; Moyano *et al*., 2007, 2008) – giving a single time lag for the entire season – should be specified for individual growth periods.

### *Effects of environmental conditions*

Even though phloem transport rates alone are insufficient to predict the time lag, environmental factors that affect transport rates have an effect. This mainly involves phloem loading: high osmotic pressure in the phloem increases the rates. Thus, intensive photosynthesis and water availability in soil accelerate assimilate transport. Water deficits strongly decrease the rates (Shelagh & Milburn, 1973; Nobel, 2005; Ruehr *et al*., 2009), delaying the appearance of assimilates in the rhizosphere. The sugar transport rate in the phloem is limited by solution viscosity (Hölttä *et al*., 2009). The reduced transpiration result in the decreased phloem transport rates. Accordingly, Plain *et al*. (2009) found that a 10 °C temperature drop decreased assimilate transport by nearly five times. This definitely prolongs the time lag between photosynthesis and $CO_2$ efflux from soil.

Beyond the above-mentioned factors affecting the time lag, we attempted to evaluate other variables such as the amount of assimilated C allocated belowground, availability and form of N in soil, plant species etc. At this stage, however, the lack of data hinders finding a clear connection between lags and other biotic and abiotic factors.

## Conclusions and outlook

Based on the review of time lags between C assimilation and the release of $CO_2$ from soil, we conclude that there is a close link between photosynthesis and rhizosphere processes. This link is direct, in contrast to the indirect connection between temperature and the $CO_2$ efflux from soil. Photosynthesis affects this efflux by supplying the roots and rhizosphere microorganisms with easily available recent photosynthates. The importance of photosynthesis for rhizosphere processes and $CO_2$ efflux can easily be overlooked because the link is masked by a high correlation of solar radiation (direct proxy of photosynthesis intensity) with temperature. An additional complication is the chain of processes occurring during photosynthates transport and utilization in roots and rhizosphere.

Only few studies have considered photosynthesis as a direct driver of the belowground processes. Despite highly variable time lags between aboveground C assimilation and subsequent $CO_2$ efflux from soil, we found lags for grasses to be about $12.5 \pm 7.5$ (SD) h. However, this conclusion was based mainly on studies under controlled conditions. The time lag for mature forest trees is about 4–5 days and is slightly affected by tree height.

The revealed dependence of $CO_2$ flux on photosynthesis provides new insights and perspectives. Firstly, primary production is an important driver of soil $CO_2$ fluxes not only on the annual time scale, but also on much shorter scale (hours, days). As stated by Paterson *et al*. (2009): 'The ultimate source of organic C to ecosystems is from a single process: photosynthesis' and our aim is to disentangle the relevance of the recent assimilates for the loop of C back to the atmosphere. Secondly, photosynthesis or even its proxies (PAR, VPD, GPP) should be important parameters in soil $CO_2$ flux studies.

Future investigations should focus more strongly on evaluating the link between soil $CO_2$ fluxes and rhizosphere processes. Evaluating the mechanisms responsible for C turnover in the rhizosphere are crucial for understanding, predicting and modeling $CO_2$ fluxes. The time lags are probably different for such rhizosphere processes as root respiration, rhizomicrobial respiration and rhizosphere priming effect, and are controlled by different physiological and environmental parameters. Lag studies, conducted at various periods of the vegetation season and under contrasting weather conditions, may help to disentangle the effects of individual drivers.

This review underlines that every approach for lag estimation has some shortcomings (Table S1). This calls for combining methodologies. In our opinion, unbiased time lags and the links between photosynthesis and $CO_2$ efflux can be determined by combining (1) TSA of $CO_2$ fluxes (and its $\delta^{13}C$) from soil with photosynthesis proxies and (2) pulse labeling of plants in $^{13}CO_2$ atmosphere (on separate plots) with subsequently tracing $^{13}C$ in soil $CO_2$. The recent and future development of Tunable Diode Lasers spectrometry and Quantum Cascade Lasers spectrometry will allow continuous and simultaneous on-site measurement of $CO_2$ and $^{13}CO_2$ (Bahn *et al*., 2009; Plain *et al*., 2009). This will strongly reduce the costs, accelerate analyses and promote approaches based on TSA of $^{13}CO_2$ fluxes and photosynthesis proxies.

This review strongly advocates that models of $CO_2$ efflux from soil should incorporate photosynthesis-related parameters as drivers. This would be the next significant step in the development of mechanistic

models, not only on the plot scale, but also on the landscape, regional and even global scale. We also expect that incorporating the time lag in the models will significantly increase their precision, especially on the time scale of hours and days. Such models would help predict not only total $CO_2$ efflux but also its $\delta^{13}C$. This is because various $CO_2$ sources (having various $\delta^{13}C$) are affected by different drivers (Fig. 1), not merely by soil temperature. Therefore, our last take-home message is: 'Stop correlating $CO_2$ efflux with temperature'. Such correlations are useless for at least of three reasons:

(1) Soil temperature is an *indirect* factor: it affects the decomposition rate of substances in soil, but not the presence of those available substances that are actually responsible for microbial activity, turnover and $CO_2$ fluxes.
(2) Variation of soil temperature is mainly driven by solar radiation, but the temperature response is strongly smoothed and delayed compared with the solar radiation.
(3) There is a *time lag* between changes of environmental parameters (e.g., temperature) and $CO_2$ efflux from soil. Therefore, simple correlations that fail to consider this time lag are useless on a short time scale (hours). Over the longer term (days – weeks) the correlations are insensitive to reflecting any mechanisms.

## Acknowledgements

We are very thankful to Sergey Blagodatsky for helpful comments on the first version of the manuscript and four anonymous reviewers for many valuable suggestions.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Basic principles, advantages and shortcomings of approaches for estimating time lags between photosynthesis and $CO_2$ efflux from soil.
**Table S2**. Literature data for forest stands on the time lag between photosynthesis and $CO_2$ efflux from soil.
**Table S3.** Literature data for grasses and herbs on the time lag between photosynthesis and $CO_2$ efflux from soil.
**Table S4.** The averaged time lags between photosynthesis and $CO_2$ efflux from soil for grasses grown under field or controlled conditions ($P < 0.001$).
**Table S5.** Averaged time lag between photosynthesis and $CO_2$ efflux from soil for gymnosperm and angiosperm trees ($P < 0.05$).
**Figure S1.** Distribution of time lags between photosynthetic C uptake and its release as $CO_2$ through the roots and rhizosphere for trees (top) and grasses (bottom). The red continuous lines show normal distribution and mean; the green dashed line for trees shows gamma distribution. Note different time lag scales for trees (days) and grasses (hours). The studies based on FACE are not considered. Note that here in contrast to Figs. 5 and 6, all data were bulked together and were not related to the height or age of the plants. Therefore, the means calculated here do not correspond to the asymptotic values calculated by regressions in Figs. 5 and 6.

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