Biogeosciences Discuss., 8, 2183–2233, 2011
www.biogeosciences-discuss.net/8/2183/2011/
doi:10.5194/bgd-8-2183-2011

Biogeosciences Discussions

Diel variations in the carbon isotope composition of respired CO_2 and associated carbon sources: a review of dynamics and mechanisms

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Received: 15 February 2011 – Accepted: 21 February 2011 – Published: 4 March 2011

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Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Recent advances have improved our methodological approaches and theoretical understanding of post-photosynthetic carbon isotope fractionation. Nevertheless we still lack a clear picture of the origin of short-term variability in $\delta^{13}C$ of respired CO_2 ($\delta^{13}C_{res}$) and organic carbon fractions on a diel basis. However, closing this knowledge gap is essential for the application of stable isotope approaches for partitioning ecosystem respiration, tracing carbon flow through plants and ecosystems and disentangling key physiological processes in carbon metabolism of plants. In this review we examine the short-term dynamics in $\delta^{13}C_{res}$ and putative substrate pools at the plant, soil and ecosystem scales and develop mechanistic explanations for diel $\delta^{13}C_{res}$ dynamics at each scale. Maximum reported variation in diel $\delta^{13}C_{res}$ is 4.0, 5.4 and 14.8‰ in trunks, roots and leaves of different species and 12.5 and 8.1‰ at the soil and ecosystem scale in different biomes. Temporal variation in post-photosynthetic fractionation related to changes in carbon allocation to different metabolic pathways is the most plausible mechanistic explanation for observed diel dynamics in $\delta^{13}C_{res}$. In addition, mixing of component fluxes with different temporal dynamics and isotopic compositions add to the $\delta^{13}C_{res}$ variation on the soil and ecosystem level. Understanding short-term variations in $\delta^{13}C_{res}$ is particularly important for ecosystem studies, since $\delta^{13}C_{res}$ contains information on the fate of respiratory substrates, and may, therefore, provide a non-intrusive way to identify changes in carbon allocation patterns.

1 Introduction

Stable carbon isotopes have become an important tool to advance our understanding in carbon cycle processes on different temporal and spatial scales. As carbon travels from the atmosphere through plants and is respired back to the atmosphere by leaf, stems, roots and soil there are many processes which alter the carbon isotope ratio of the heavier ^{13}C to the lighter ^{12}C isotope (generally expressed against an international standard as δ-notation in ‰). Thus, the $\delta^{13}C$ isotope signature of dark-respired

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$CO_2\delta^{13}C_{res}$) is widely used tool for tracing carbon flow through plants and ecosystems (e.g. Knohl et al., 2005; Kodama et al., 2008), partitioning ecosystem respiration (e.g. Bowling et al., 2001; Unger et al., 2010a), and disentangling key physiological processes on the plant and stand levels (e.g. Yakir and Sternberg, 2000; Gessler et al., 2009a). Photosynthetic carbon assimilation in C_3-plants heavily discriminates against ^{13}C, with the $\delta^{13}C$ ratio of assimilated carbon being related to the ratio of leaf intercellular and ambient CO_2 concentration (Farquhar et al., 1982). Photosynthetic discrimination leaves an imprint on $\delta^{13}C$ of newly produced assimilates and respired CO_2, which are widely used to characterize environmental effects on the physiology of photosynthesis. In addition, post-photosynthetic fractionation processes in enzyme reactions of metabolic pathways downstream of photosynthetic carbon fixation can alter the isotopic signature of the organic matter among organs and chemical compound classes and also affect $\delta^{13}C$ of respired CO_2 (for a review see Ghashghaie et al., 2003). However, marked diel variations of dark-respired $\delta^{13}C_{res}$, which occur within minutes to hours over the 24 h cycle, have only lately gained scientific attention (e.g. Barbour et al., 2007; Werner et al., 2007; Wingate et al., 2010). Ignoring these short-term variations in $\delta^{13}C_{res}$ might weaken the power of isotope approaches for disentangling plant and ecosystem processes.

In spite of recent insights into the origin of $\delta^{13}C$ of different carbon pools (see reviews of Badeck et al., 2005; Bowling et al., 2008; Cernusak et al., 2009), we still lack a clear picture of the physiological mechanisms resulting in isotopic fractionation in metabolic processes downstream of photosynthesis and their implication for diel variation in $\delta^{13}C$ of different organic carbon fractions and respired CO_2.

Here, we provide a survey of marked short-term dynamics in respired $\delta^{13}C_{res}$ and putative substrate pools at the plant, soil and ecosystem scale. We have limited this review to exclusive cover publications evaluating diel (24 h) dynamics in $\delta^{13}C_{res}$ and providing mechanistic explanations. The mechanistic understanding is a prerequisite for disentangling physiological and environmental information encoded in short-term variations of $\delta^{13}C$ in both plant organic matter and respired CO_2.

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Compared to our progress in understanding fractionation mechanics in general and dark respiratory fractionation in particularly, the recognition of diurnal dynamics in $\delta^{13}C_{res}$ was slow. This was largely attributed to methodological constrains hindering high-time resolved analysis of $\delta^{13}C_{res}$. Recently technological advances opened new frontiers to assess the isotopic signature of respired CO_2 at time scales from minutes to hours over the day course, which will be shortly surveyed in the next section (for detailed methodological descriptions see Sect. 3 in companion paper by Werner et al., 2011).

2 New methodological developments in high time-resolved measurements of $\delta^{13}C_{res}$

First attempts to measure $\delta^{13}C_{res}$ were made with gas-exchange systems coupled to isotope ratio mass spectrometers (IRMS), allowing $\delta^{13}C$ analysis of CO_2 respired by leaves, roots or whole plants in an enclosure (normally a cuvette or phytotron). Alternatively, detached leaves, roots or soil have been incubated in small vials (e.g. exetainer). If flushed with CO_2-free air the $\delta^{13}C_{res}$ can be measured directly within 3 min on a gas bench-IRMS (in-tube incubation technique, Werner et al., 2007). High precision IRMS enables "on-line"-measurements, where an open gas-exchange system is directly coupled to the IRMS e.g. via an open-split and a GC-column for CO_2 separation, yielding a time resolution of ca. 5 min (e.g. Schnyder et al., 2003; Klumpp et al., 2005; Werner et al., 2007). Fully continuous monitoring of $\delta^{13}C_{res}$ can be achieved with new optical laser spectroscopy, e.g. tuneable diode laser spectroscopy (TDLS; e.g. Bowling et al., 2003) or cavity ring down spectroscopy (CRDS; e.g. Wahl et al., 2006), which continuously measure $^{12}CO_2$ and $^{13}CO_2$ concentrations in the gas stream (e.g. Barbour et al., 2007) in e.g. gas exchange chambers (e.g. Kodama et al., 2011) or in ecosystem height profiles (e.g. Wingate et al., 2010). The temporal resolution and precision depends on the integration-time and instrument (e.g. 0.25‰ at 1 s and about 0.08‰ at 30 min integration time for $\delta^{13}C$ and $\delta^{18}O$ in CO_2 with a TDLS; Barthel et al., 2010).

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High temporal resolution measurements of $\delta^{13}C_{res}$ determined in non-equilibrated closed chambers (e.g. Maunoury et al., 2007; Kodama et al., 2008) might, however, be affected by changes in transport fractionation as the CO_2 concentration in the chamber increases and could thus introduce errors under particular conditions (Ubierna et al., 2009) which has created particular concern for $\delta^{13}C$ measurements of soil respiration (e.g. Nickerson and Risk, 2009). Open dynamic chamber techniques, which can be applied with optical laser spectroscopy (e.g. Bahn et al., 2009) and continuous measurements of $\delta^{13}C$ in CO_2 over soil profiles (cf. Kayler et al., 2008, 2010) can, however, overcome these potential problems. Thus there are currently at least three independent techniques which yield accurate measurement of diel dynamics in $\delta^{13}C_{res}$, when specific instrument precautions are taking into account. Given the fact that that observed ranges in $\delta^{13}C_{res}$ exceed by far the variation, which may be caused by instrumental noise or non-equilibrium conditions, we have now gained a solid piece of data on short-term (minutes to day) variation in respired $\delta^{13}C_{res}$.

Determination of respiratory substrate $\delta^{13}C$ signatures, which are needed to understand the origins of variation, is not possible at the same high temporal resolution as measurements of $\delta^{13}C_{res}$. Even though hyphenated gas chromatographic (GC) and liquid chromatographic (LC) IRMS techniques have enabled us to assess compound specific $\delta^{13}C$ in organic substrates, destructive sampling and extraction prevents continuous measurements. Moreover, when interpreting data of the isotopic composition of carbohydrates, starch and other fast-turnover compounds, potential artefacts related to the extraction procedures have to be taken into account (Richter et al., 2009). In spite of these problems, more and more data for $\delta^{13}C$ of respiratory substrates is now available at a temporal resolution of a few hours. This information is a first step towards understanding the mechanisms of variations in diel dynamics in $\delta^{13}C_{res}$ of different plant organs and ecosystem compound which are summarized in the next section.

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3 Observed short-term variations in $\delta^{13}C$ of respired CO_2

Significant diel variations of $\delta^{13}C_{res}$ occur in plant leaves, stems and roots (Table 1) as well as in soil and ecosystem respiration (Table 2). Examples for Scots pine (soil and trunk) and bread wheat (roots and shoots) are shown in Fig. 1.

The largest diel variations in dark-respired $\delta^{13}C_{res}$ of up to 11.5‰ occurred in leaves (Table 1). A significant increase in $\delta^{13}C_{res}$ during the photoperiod and a subsequent decrease in the dark were found in a variety of drought-adapted trees and shrubs (e.g. Hymus et al., 2005; Prater et al., 2006; Sun et al., 2009, 2010; Werner et al., 2009; Unger et al., 2010a; Rascher et al., 2010) and in wheat (Kodama et al., 2011; Fig. 1b). An exceptionally high variation of 14.8‰ was found in hydroponically grown *Halimium* sp. (Wegener et al., 2010, Table 1). Only in 2007 it was recognized that different plant functional groups expressed systematic differences in the magnitude of $\delta^{13}C_{res}$ diel variability (Werner et al., 2007): the largest diel variations in $\delta^{13}C_{res}$ were found in some Mediterranean evergreens, shrubs and aromatic herbaceous species, while non-significant diel variations occurred in fast-growing herbs, grasses and some temperate trees (Priault et al., 2009). Furthermore, considerable variation has been observed in response to changing environmental conditions (Table 1, see discussion below).

Plant stems and tree trunks (see Fig. 1a) also exhibited marked diel variations in emitted $\delta^{13}CO_2$ (up to 4‰), sometimes associated with marked seasonal differences (e.g. in *Quercus petraea*; Maunoury et al., 2007). In contrast to leaves, where highest $\delta^{13}C_{res}$ values were often observed at the end of the light period, trunk $\delta^{13}C_{res}$ was most enriched at night (e.g. in *Pinus sylvestris*; Kodama et al., 2008 and *Ricinus communis*; Gessler et al., 2009b).

There is limited information on diel dynamics in root $\delta^{13}C_{res}$ lending a non-uniform picture: only slight variations in $\delta^{13}C_{res}$ (<2‰) occurred in herbaceous and shrubby species under controlled conditions (Gessler et al., 2009b; Wegener et al., 2010).

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Under natural conditions, however, root $\delta^{13}C_{res}$ showed a clear diel cycle in wheat (5.4‰, Kodama et al., 2011; Fig. 1b) and in a Mediterranean herb, diel $\delta^{13}C_{res}$ variations increased from 2.4‰ to 4.6‰ during increasing drought (Unger et al., 2010a). In *Acacia longifolia* and *Pinus pinaster* a slight increase at the end of the light period of ca. 2‰ was observed in the field also under drought conditions (Rascher et al., 2010).

Both ecosystem and soil respiration derive from multiple sources the latter comprising heterotrophic and autotrophic rhizosphere respiration. To stress this origin from multiple sources we term the isotopic composition of CO_2 emitted from the soil or whole ecosystems $\delta^{13}C_R$. Diel variations in soil $\delta^{13}C_R$ (0.5–5.8‰, Table 2) have been reported in grasslands (Dudziak and Halas, 1996; Bahn et al., 2009), forests (Kodama et al., 2008; Fig. 1a, Marron et al., 2009), Mediterranean woodlands (Maseyk et al., 2009; Unger et al., 2010a, b; Tu and Dawson, 2011), and agricultural systems (Kodama et al., 2011), while non-significant diel variations were detected in a boreal forest (Betson et al., 2007) (Table 2). A highly variable range in soil $\delta^{13}C_R$ of 0.3–12.5‰ occurred in an experimental garden with deciduous trees (Moyes et al., 2010).

The information on dynamics of ecosystem respiration ($\delta^{13}C_R$ assessed by Keeling-plot approaches) presents again a very heterogeneous picture: while Ogée et al. (2003) and Schnyder et al. (2004) found only minor nocturnal variation of $\delta^{13}C_R$ (<3‰), others report that nocturnal ecosystem $\delta^{13}C_R$ presented the largest variation among different respiratory components (Kodama et al., 2008; Unger et al., 2010a). Nocturnal variations in $\delta^{13}C_R$ were 6.4‰ in a grassland (Bowling et al., 2003), 4.2–8.1‰ in a Mediterranean woodland (Werner et al., 2006; Unger et al., 2010a), 6.1‰ in a *Pinus sylvestris* stand (Kodama et al., 2008), 2.6–3.6‰ in a subalpine forest (Bowling et al., 2005; Riveros-Iregui et al., 2011), and 3.8‰ a beech-dominated deciduous forest (24 h-cycle, Knohl et al., 2005).

The overview in this section clearly demonstrates that the short-term variations in $\delta^{13}C$ of respired CO_2 do not follow a straightforward pattern and differ between organs, species, ecosystem compartments and ecosystems. This indicates the necessity to understand the processes responsible for the observed patterns and differences

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among systems. Accordingly we will now focus on the potential mechanisms driving these short-term dynamics.

4 Mechanisms

The potential mechanisms, which may drive the diel variations in $\delta^{13}C_{res}/\delta^{13}C_R$ on the plant, soil and ecosystem level can be summarised in three main groups:

M1: substrate driven variations: short-term variations in the carbon isotopic signature of the major respiratory substrate (i.e. sugars or water soluble organic matter) and/or switches between substrates with different carbon isotope composition drive plant $\delta^{13}C_{res}$.

M2: fractionation driven variations: changes in respiratory fractionation in different metabolic pathways over the diel course determine plant $\delta^{13}C_{res}$.

M3: flux ratio driven variations: temporal variability in the contribution of component fluxes with distinct isotopic signatures to composite fluxes (e.g. soil and ecosystem respiration) drive variations in $\delta^{13}C_R$.

These three mechanisms are not mutually exclusive and a combination of these can and most likely occurs. In the following we will explore step by step whether the three main mechanisms can explain the observed short-term variability in $\delta^{13}C_{res}$ and $\delta^{13}C_R$. The complexity of the different processes on the plant level is indicated in Fig. 2 and summarized in Table 3.

4.1 Substrate driven variations (M1)

It is well established that different mechanisms and processes can induce diel variations in $\delta^{13}C$ of primary assimilates in leaves and during transport to heterotrophic plant tissues; thereby potentially inducing short-term variation in $\delta^{13}C_{res}$ in leaves,

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stems and root. When we, as a first approximation, assume that respiration is fed by only *one major respiratory substrate pool* (i.e. new soluble sugars of current photosynthesis) with a homogenous $\delta^{13}C$ (i.e. all substrate molecules share a comparable $\delta^{13}C$ at a given time) the following mechanisms (M1.1 to M1.4) related to carbon assimilation and transport could potentially drive diel variability in $\delta^{13}C_{res}$ of leaves, stems and root.

M1.1: photosynthetic carbon isotope discrimination (Farquhar et al., 1982), which determines the $\delta^{13}C$ of primary respiratory substrate, varies over the diurnal course (e.g. Gessler et al., 2007; Wingate et al., 2010) as a result of changes in light intensity, air temperature, vapour pressure deficit (VPD) and other environmental factors, which affect assimilation, stomatal (g_s) and mesophyll conductance (g_m) as well as photorespiration and dark respiration (see Fig. 2; M1.1), reviewed by Brugnoli and Farquhar, 2000). While we can precisely predict changes in carbon discrimination and variations in $\delta^{13}C$ of fresh assimilates in response to changes in VPD, light and temperature, much less is known on the isotopic effects of mesophyll CO_2 conductance (g_m), photorespiration and dark respiration throughout the day (e.g. Warren and Adams, 2006; Wingate et al., 2007; Lanigan et al., 2008). There have been recent insights that there is active regulation of internal CO_2 conductance through aquaporins, which are transporting CO_2 across plasma membranes (Hanba et al., 2004; Flexas et al., 2008). This might allow fast diel adjustment of mesophyll conductance to meet photosynthetic requirements (Flexas et al., 2007), but so far measurements of diurnal dynamics in g_m are often constrained by methodological issues (Pons et al., 2009).

Overall, photosynthetic discrimination alone cannot explain the strong day-night variations in $\delta^{13}C_{res}$ (and respiratory substrate) as it is active only during daylight. In addition, sugar $\delta^{13}C$ values at night are far more positive than predicted by photosynthetic discrimination alone (Gessler et al., 2008) and thus post-photosynthetic processes must be taken into account in order to fully explain observed diel variations of $\delta^{13}C_{res}$.

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M1.2: post-photosynthetic carbon isotope fractionation related to transitory starch metabolism. Starch accumulation during daylight and remobilization at night alter the isotope signal of leaf and phloem-exported sugars on the diel scale (Tcherkez et al., 2004; Gessler et al., 2008; see Fig. 2; M1.2). During the day, the synthesis of transitory starch is either under plant internal control to adapt the storage C supply to environmental conditions (Zeeman et al., 2007) or occurs mainly when the utilisation of newly produced triose-phosphates from the chloroplast becomes rate limiting to carbon assimilation (Beck and Ziegler, 1989). Transitory starch synthesis favours ^{13}C during fructose production in the chloroplast by aldolase (Gleixner and Schmidt, 1997). As a consequence, ^{13}C-depleted triose phosphates are exported from the chloroplast, which are used for sucrose production during the light period. During the night the ^{13}C-enriched transitory starch is used for sucrose synthesis. As a result, a ~4‰ $\delta^{13}C$ oscillation between light- and dark-exported sucrose has been predicted and observed (Ghashghaie et al., 2001; Tcherkez et al., 2004; Gessler et al., 2008, 2009a).

However, these variations in the fast-turnover organic matter pool in leaves had a much lower day-night amplitude than the observed diel changes in respired $\delta^{13}CO_2$ (Brandes et al., 2006, 2007; Gessler et al., 2007, 2008; Kodama et al., 2008; Werner et al., 2009, see Table 1) and were also phase-shifted compared to $\delta^{13}C_{res}$ (Kodama et al., 2008, see also Fig. 1). Furthermore, opposing trends in diel variation of $\delta^{13}C_{res}$ and $\delta^{13}C$ of the leaf sugars and phloem sugars (Gessler et al., 2007, 2009b) occurred as shown for leaves of *R. communis* in Fig. 3a. Others found no significant diel variations in leaf soluble sugars or water soluble organic matter (WSOM) (Hymus et al., 2005; Sun et al., 2009; Werner et al., 2009; Wegener et al., 2010; Rascher et al., 2010) but still strong variations in $\delta^{13}C_{res}$ (Fig. 3b–c), indicating that diel variations in leaf $\delta^{13}C_{res}$ cannot be solely explained by changes in the isotopic signature of the substrate.

M1.3: isotope effects during carbon transport: during phloem transport sugars are continuously released from the phloem but a major part is retrieved again into the

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sieve tubes (Van Bel, 2003). This process might be responsible for the intermixing of sucrose molecules with different metabolic histories and residence times (Brandes et al., 2006). As a result, the diel variations in $\delta^{13}C$ originating from starch accumulation and breakdown are dampened with increasing transport distance along the stem in basipetal direction (Fig. 2; M1.3).

Consequently, the diel cycle of $\delta^{13}C$ in organic matter in trunks and stems of trees is mainly dependent on the position along the trunk (Gessler et al., 2007) and dampening as well as time lags have been observed (Keitel et al., 2003; Brandes et al., 2006, 2007). At the tree trunk base often no diel variation in phloem sugar $\delta^{13}C$ was present (Gessler et al., 2007; Kodama et al., 2008; Betson et al., 2007; Rascher et al., 2010) whereas strong diel variations in $\delta^{13}C_{res}$ are generally observed (Kodama et al., 2008; Maunoury et al., 2007). They thus cannot be explained by variation in substrate $\delta^{13}C$ (Fig. 3b for trunks *P. sylvestris*).

In roots there are generally only very low or non-significant short-term variations in $\delta^{13}C$ of sugars or WSOM (Göttlicher et al., 2006; Wegener et al., 2010; Kodama et al., 2011). The lack of short-term variations in roots (Fig. 3d) is highly plausible, given the mixing of sugars with different residence times during phloem transport into the roots. The only exception we are aware of is *Ricinus communis*, where $\delta^{13}C$ of root sugars varied by approx 3.7‰ within 24 h (Gessler et al., 2009b), which also explained 72% ($p < 0.01$) of the diel variation of root $\delta^{13}C_{res}$. In contrast, in field-grown plants, root $\delta^{13}C_{res}$ showed a clear diel variation (Table 1, Unger et al., 2010a), even without significant variations in the respiratory substrate (Kodama et al., 2011; Fig. 1b).

In conclusion, we have a conceptual framework for explaining the observed short-term variations in $\delta^{13}C$ of sugars and other fast turn-over carbon compounds (Fig. 2). However, $\delta^{13}C$ variations of new assimilates are too small or uncorrelated to explain $\delta^{13}C_{res}$ dynamics (Fig. 3), and thus cannot be solely responsible for the diel variations in $\delta^{13}C_{res}$, when one major respiratory pool consisting of one compound class is assumed to fuel respiration. Another aspect of substrate induced variations (M1) might be related to the use of different respiratory substrates.

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M1.4: a switch between respiratory sources of different storage pools or substrate types including, soluble sugar, starch, lipids or amino acids, or stored and fresh assimilates with different isotopic signature could account for variation in $\delta^{13}C_{res}$ (Tcherkez et al., 2003; Nogués et al., 2004; Fig. 2; M1.4).

Leaf respiration uses several carbon sources with different isotopic characteristics and residence times (Schnyder et al., 2003; Lehmeier et al., 2008, 2010). However, in spite of differences in $\delta^{13}C$ between glucose, fructose and sucrose, mass-balance calculations taking into consideration measured diel changes in pool sizes and $\delta^{13}C$ signatures could only explain 1.1‰ variation in *Halimium halimifolium* even though observed diel $\delta^{13}C_{res}$ variation was 8.9‰. The amount of explainable variation was even less in four other species (Werner et al., 2009).

The effect of switches between substrate classes (e.g. from sugars to lipids) on $\delta^{13}C_{res}$ has been shown experimentally during plant starvation under continuous dark (up to 10‰ shift, Tcherkez et al., 2003) and may play a role under natural conditions in the case of severe stress, like wilting or senescence (Unger et al., 2010a). However, a complete shift between different respiratory substrates during the day seems rather unlikely for healthy plants under ambient conditions (Hymus et al., 2005). One exception might be a transient shift in utilization organic acid pools, which accumulated in the light and are rapidly decarboxylated upon darkening.

M1.5: light enhanced dark respiration (LEDR) is the transient increase in respiration upon darkening in a photosynthesis-dependent manner (Azcon-Bieto and Osmond, 1983; Atkin et al., 1998). Light-acclimated leaves released strongly ^{13}C-enriched CO_2 as compared to potential substrates in the first 5–10 min after darkening followed by a rapid decline in $\delta^{13}C_{res}$ (Barbour et al., 2007; Werner et al., 2007). Both the extent of enrichment and the subsequent ^{13}C-depletion augment during the light period (Fig. 4, Werner et al., 2009). In *Ricinus communis* LEDR-dependent ^{13}C-enrichment was fully explained with the accumulation of ^{13}C-enriched malate in the light and rapid malate decarboxylation just after darkening (Gessler et al., 2009b, see Fig. 2; M1.5).

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In the light, both glycolysis and particularly the Krebs cycle (KC) are strongly inhibited and thus malate fixed via phosphenolpyruvatecarboxylase (PEPc) can accumulate (see Fig. 5a). However, the malic enzyme is most certainly associated with an isotope effect. If we assume a dynamic Rayleigh process (see Gessler et al., 2009b) $\delta^{13}C_{res}$ would be more depleted immediately after darkening while getting more enriched as the malate pool declines (Werner et al., 2009). Indeed, such a transient increase is sometimes observed during the first 5–10 min upon darkening (Fig. 4, red arrows); however, a rapid decline from positive $\delta^{13}C_{res}$ values is generally observed during light-dark transitions (Fig. 4, Barbour et al., 2007; Werner et al., 2007, 2009), which could indicate several overlaying processes such as a partitioning of malate between the malic enzyme and mitochondrial malate dehydrogenase. LEDR is a transient effect and under natural conditions it has been shown to occur at dusk after sunny days (Barbour et al., 2011). Based on current knowledge, the short duration of LEDR cannot explain continuous nocturnal dynamics in $\delta^{13}C_{res}$ (e.g. Sun et al., 2009, 2010; Unger et al., 2010a). Furthermore, a diurnal increase in $\delta^{13}C_{res}$ can also be observed after the first transient LEDR effect (e.g. Fig. 4, grey arrow) indicating processes in addition to LEDR are influencing $\delta^{13}C_{res}$.

In conclusion, it is unlikely that diel variations of respired $\delta^{13}CO_2$ can be entirely explained by $\delta^{13}C$ variation in a single substrate or by a switch between substrates. In autotrophic tissues at least part of the day-night differences in $\delta^{13}C_{res}$ might be attributed to LEDR (Kodama et al., 2011) but these do not apply for non-photosynthetic tissues. As a consequence fractionation driven variations (M2) should be focussed on.

4.2 Fractionation driven variations (M2)

Since $\delta^{13}C_{res}$ markedly deviates from substrate $\delta^{13}C$, we have to assume that diel variation in $\delta^{13}C_{res}$ may be affected by carbon isotope fractionation during respiration. The following mechanism might be involved:

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M2.1: fragmentation fractionation and enzyme related isotope effects: the often observed $\delta^{13}C_{res}$ enrichment above the organic source is assumed to originate from the fragmentation of the substrate molecule due to heterogeneous isotope distribution (Tcherkez et al., 2003, 2004; see Figs. 2f, 5). There is a non-homogeneous distribution of $\delta^{13}C$ within the glucose molecule where C-3 and C-4 are ^{13}C-enriched compared to other positions due to fractionation in the aldolase reaction (Rossmann et al., 1991; Gleixner and Schmidt, 1997; Hobbie and Werner, 2004). During glycolysis, C-1 of pyruvate derived from enriched C-3 and C-4 of glucose is decarboxylated by pyruvate dehydrogenase (PDH). Consequently, the PDH reaction releases ^{13}C-enriched CO_2, whereas the remaining molecule enters the Krebs Cycle (KC) which releases in turn ^{13}C-depleted CO_2 – compared with the mean $\delta^{13}C$ of the original glucose molecule (see Fig. 5). Any change in the relative contribution of CO_2 decarboxylated in the KC versus by PDH to total CO_2 production may thus cause variations in $\delta^{13}C_{res}$. Furthermore, kinetic and equilibrium fractionation in glycolysis and KC may also occur and it is most likely a mixed influence of fragmentation fractionation and enzymatic isotope effects related to metabolic flux rates (e.g. see Fig. 5) which together drive $\delta^{13}C_{res}$ variations.

The isotope effect derived from fragmentation fractionation depends on the extent of intramolecular ^{13}C variation, which was determined as 6‰ for glucose in yeast (by stepwise biochemical degradation, Rossmann et al., 1991). Newer NMR data for sucrose show a larger intramolecular range of 13.3‰ (Gilbert et al., 2009) with the C-4 position being slightly more enriched (−17‰) compared to the data from Rossmann et al. (1991: −18.7‰). Nevertheless, assuming a complete isomerisation reaction between glyceraldehyd-3-phosphate and dehydroxyacetonphosphate, the isotopic signature within the pyruvate molecule would be similar. However, new emerging NMR data indicate that the heterogeneous ^{13}C distribution in carbohydrates may vary among species and be related to environmental conditions (Gilbert et al., 2011), which may add to species specific differences in $\delta^{13}C_{res}$.

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Taking the data from Rossmann et al. (1991; Fig. 5), the potential variation in $\delta^{13}C_{res}$ due to fragmentation fractionation can be calculated: if only pyruvate decarboxylation by PDH is assumed (i.e. when the KC cycle is fully inhibited in the light) $\delta^{13}C_{res}$ of C1 of −21‰ is released (Fig. 5b), whereas the complete decarboxylation of the glucose molecules in KC produces $\delta^{13}C_{res}$ of the substrate with −25‰ (Fig. 5a). Thus the shift from 0 to 100% decarboxylation in the KC produces an isotope shift of 4‰ (illustrated in Fig. 6 for 0 or 100% carbon flow into KC decarboxylation). However, the KC is also an important source for amino-acid biosynthesis, providing carbon skeletons for glutamic and aspartic acid (notably amino acids which are strongly enriched in ^{13}C, Hayes, 2001). If pyruvate is not fully respired, both equilibrium and kinetic isotope effects occur in the KC leading to a depletion of $\delta^{13}C_{res}$ (Tcherkez and Farquhar, 2005). The depletion in $\delta^{13}C_{res}$ depends on the carbon partitioning into KC and the effective enzymatic fractionations, which are dependent on the flux rate. The theoretical effects can be calculated by the following equation:

$$\delta^{13}C_{res} = f_1 \times \delta^{13}C - 1 + f_2 \times (\delta^{13}C - 2 + \varepsilon_{eff(CS)}) + f_2 \times (\delta^{13}C - 3 + \varepsilon_{eff(KG)})/(f_1 + 2f_2) \quad (1)$$

with f_{1-3} being the carbon flux and $\delta^{13}C1–3$ the isotopic composition of the carbon molecules at the C1 to C3 positions of pyruvate, and α denotes the fractionation factor and ε denotes the isotope effect of the enzymes in the KC of the citrate synthase (ε_{CS}) and the α-ketoglutarate dehydrogenase (ε_{KG}) and ε_{eff} is the effective enzyme fractionation which is dependent on the carbon flow in the KC by:

$$\varepsilon_{eff} = \frac{\alpha}{1 + \varepsilon \cdot f} - 1 \quad (2)$$

Figure 6 exemplifies the theoretical effect of varying carbon flux rates f (from 0–100%) through the PDH (f_1) and KC ($f_{2,3}$) considering fractionation by (i) citrate synthase (ε_{CS} ~23‰) and (ii) α-ketoglutarate dehydrogenase (ε_{KG} ~23‰).

If the carbon flow into the KC is low (e.g. 5%), fractionation is high and the CO_2 released in KC enzymatic reactions will be strongly depleted in ^{13}C (−48.9‰). However

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this has little effect on the overall $\delta^{13}C_{res}$ as it constitutes only a small fraction which is mixing with the enriched $\delta^{13}CO_2$ released by PDH (−21‰). Inversely, if the carbon flow into KC decarboxylation is high (e.g. 95%) the effective fractionation diminishes, and $\delta^{13}C$ in respired CO_2 approaches −25‰. However, Fig. 6 clearly illustrates that the largest decrease (∼9‰) occurs at intermediate mixing ratios (at 50% in the given example), when the CO_2 release from KC decarboxylation is still relatively depleted (38.6‰) and constitutes two-thirds of the overall CO_2 evolved (due to two decarboxylation steps in the KC) so that the total $\delta^{13}C_{res}$ decreases to −29.8‰ (Werner, 2010).

Moreover, potential fractionation can also occur in the PDH reaction, which would further deplete the Acetyl-CoA at the C-2 position if the reaction is incomplete (Melzer and Schmidt, 1987, effect indicated on the z-axis, Fig. 6). However, the latter might be less relevant in vivo as the reaction is generally considered to be fast and the regulation of the carbon flow through the PDH may be determined solely by substrate availability (Voet and Voet, 1995).

In general, it has to be considered that, both the KC and to a lesser extend also the mitochondrial PDH are down-regulated in the light (Budde and Randall, 1990; Tcherkez et al., 2009). Inversely, in the dark the reaction can be assumed to go to completion resulting in little net isotope effect. Thus, fragmentation fractionation within the mitochondria may only play a minor role, and is probably only relevant during transitory stages (e.g. during up-regulation upon darkening). However, there are other metabolic branching points within the cell which could lead to fractionation effects and carbon partitioning along different pathways has to be considered:

M2.2: variations of fluxes in the metabolic pathways: the relative carbon fluxes involved in metabolic pathways may change depending on the metabolic status of cells, tissues or plants. Day-night variations with relatively higher allocation of carbon to fatty acids (Pleite et al., 2008), isoprenoids (Loivamäki et al., 2007) and various other secondary compounds (Ayan et al., 2006) as well as the non-cyclic nature of the KC

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in the light (Tcherkez et al., 2009) are thus highly plausible triggers for changes in the relative contribution of PDH to KC derived CO_2 as described above (see Fig. 2; M2.2).

Increased activity of the oxidative pentose phosphate pathway (PPP), which decarboxylates the ^{13}C depleted C-1 position of glucose, can be significant in roots (Dieuaide-Noubhani et al., 1995; Bathellier et al., 2008, 2009). Thus, temporal changes in the partitioning of carbon originating from glucose-6-phosphate between glycolysis and PPP might also be responsible for diel variations in $\delta^{13}C_{res}$.

Marked differences between carbon allocation into different metabolic pathways, i.e. an increasing secondary metabolism when carbon accumulated throughout the day, could be related to differences between plant functional groups (Priault et al., 2009). A variety of secondary compounds including volatile isoprenoids, oxygenated VOCs, aromatics, and fatty acid oxidation products can be emitted by plants (e.g. Jardine et al., 2010a). The synthesis evolves the decarboxylation of the ^{13}C-enriched C-1 from pyruvate (or phosphenolpyruvate), leading to the biosynthesis of VOCs from the PDH bypass, 2-C-methyl-D-erythritol 4-phosphate, and mevalonic, shikimic, and fatty acid pathways (e.g. Jardine et al., 2010b). In contrast to the mitochondrial PDH, the plastidial PDH is activated by light-induced changes in the stroma (Tovar-Méndez et al., 2003) as it fuels Acetyl-CoA for fatty acid synthesis and secondary metabolism.

A pyruvate positional ^{13}C-labelling experiment provided direct evidence that diel changes in the relative activity of the PDH-reaction occurred in species with marked increase in $\delta^{13}C_{res}$ (Priault et al., 2009). Diel variations in $\delta^{13}C_{res}$ were related to an increased metabolic activity of the PDH probably due to an increase in carbon allocation to secondary metabolism, while carbon flow into KC remained at a constant low level (Priault et al., 2009; Wegener et al., 2010). Indeed, recently direct proof for the biosynthesis and emission of both volatile isoprenoids and oxygenated VOCs from C-2 labelled pyruvate has been gained (Jardine et al., 2010b). In contrast, an herb without significant diel variation in $\delta^{13}C_{res}$ and presumably low secondary metabolism had a stable, low activity of both PDH and KC activity throughout the day (Priault et al., 2009; Wegener et al., 2010).

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Considering mass-balance requirements, the release of highly enriched $\delta^{13}C_{res}$ could be counterbalanced by the emission of ^{13}C-depleted VOCs; otherwise a compensating effect on the $\delta^{13}C$ of leaf organic matter would have to occur. Interestingly, Wegener et al. (2010) observed that strong enrichment in leaf $\delta^{13}C_{res}$ above substrate was highly correlated with differences in autotrophic vs. heterotrophic tissue ^{13}C, i.e. species with high diel leaf $\delta^{13}C_{res}$ enrichment had larger ^{13}C-differences between leaf and root WSOM than species with lower diel leaf $\delta^{13}C_{res}$. Nevertheless, most leaves (particularly evergreen or longer-lived leaves) do not exhibit a progressive depletion once the leaf has matured (Eglin et al., 2009; Werner and Máguas 2010). Thus, a counterbalancing effect from the emission of VOCs depleted in ^{13}C might be a plausible explanation.

Moreover, a close positive correlation between respiration rate and respiratory fractionation over the diel course was observed for trunks of *P. sylvestris* and for shoots of *Triticum aestivum* (Kodama et al., 2008, 2011): with higher enrichment of $\delta^{13}C_{res}$ at low compared to high respiration rates. Furthermore, respiration rates increased linearly with temperature. As glycolysis (and thus decarboxylation of pyruvate) were less temperature-dependent than mitochondrial oxidation capacity and thus KC mediated CO_2 flux (cf. Berry and Raison 1981; Atkin et al., 2000), the relative contribution of CO_2 from glycolysis to total respiration might increase at lower temperatures (e.g. in the night) explaining the higher apparent fractionation at lower respiration rates. Thus, the fractionation hypothesis based on temporal variations in fragmentation fractionation during respiration (M2.1) due to changes in carbon fluxes through different metabolic pathways (M2.2) might offer a conclusive explanation for day-night variations of $\delta^{13}C_{res}$.

A particular case is the variation of carbon fluxes, which are directed in opposite directions:

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M2.3: re-fixation of CO_2 by PEPc causes a CO_2 flux in the direction opposite to the respiratory flux (Fig. 2; M2.3). PEPc discriminates against ^{13}C by ca. 2.2‰. Equilibrium dissolution of CO_2 into water concentrates $^{13}CO_2$ in the gas phase by 1.1‰, while the hydration equilibrium favours ^{13}C by 9‰, resulting in an overall discrimination of 5.7‰ against ^{12}C (Farquhar et al., 1989; Brugnoli and Farquhar, 2000). Thus PEPc activity causes the produced organic matter to be ^{13}C enriched whereas the remaining (non-fixed) CO_2 is relatively ^{13}C depleted. Thus (re)-fixation by PEPc can also alter the $\delta^{13}C$ of CO_2 emitted from a plant. Since both processes and the effective fractionations cannot be separated the isotopic difference between putative substrate and respired CO_2 is often referred to as apparent fractionation (e.g. Gessler et al., 2009b).

It is known that differences in PEPc activity among organs can cause differences in apparent respiratory fractionation and thus in $\delta^{13}C$ of respired CO_2 along the plant axis (Badeck et al., 2005). PEPc activity has been found in all plant organs (e.g. Hibberd and Quick, 2002; Berveiller and Damesin, 2008). PEPc activity may also be involved in diel variations in $\delta^{13}C_{res}$. The anaplerotic PEPc reaction in leaves of C_3-plants is activated in the light (Duff and Chollet, 1995) to replenish the carbon skeletons of the TCA used for biosynthesis. Theoretically, the increased PEPc activity during day might thus be directly responsible for ^{13}C enriched CO_2 emitted from light acclimated leaves. In roots and stems, however, Gessler et al. (2009b) did not find any relation between PEPc activity and $\delta^{13}C_{res}$. It is consequently unlikely that PEPc mediated re-fixation of CO_2 played a large role in observed diel variations in $\delta^{13}C$. We, however, need more information on diel variations in PEPc activity with simultaneous assessments of $\delta^{13}C_{res}$ from different species to draw more reliable conclusions.

In summary M2.1 and M2.3 can explain part of the variation in $\delta^{13}C$ over the diel course. However, they give no explanation for $\delta^{13}C_{res}$ values more positive than the $\delta^{13}C$ of the enriched position in glucose (−21‰ in our example above, or 4‰ above the mean glucose $\delta^{13}C$) and thus other co-occurring processes such as LEDR in leaves must also occur.

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At the soil and ecosystem scale, mixing of different respiratory fluxes varying over the diel course might also be involved in the diel pattern of $\delta^{13}C$ of emitted CO_2 and might even enhance the short-term variations.

4.3 Flux ratio driven variations (M3)

On the soil and ecosystem level the net respiration flux consists of several component fluxes and mixing between these fluxes with potentially different isotopic signatures and associated diel variation in both $\delta^{13}C_{res}$ and flux rates has to be considered for the explanation of temporal variations of $\delta^{13}C_R$. We have shown above that the diel patterns (i.e. the timing of maxima and minima) in $\delta^{13}C_{res}$ differ among respiratory fluxes from different plant tissues. Furthermore $\delta^{13}C$ of soil and plant respiration are not synchronous (e.g. Kodama et al., 2008; Unger et al., 2010a), and even soil and ecosystem respiration fluxes are partially phase-shifted with distinct diel patterns (e.g. Unger et al., 2009), so that strong temporal dynamics in the component fluxes and consequently $\delta^{13}C_R$ of the total flux have to be expected. There are several processes, which drive the diel variation in different component fluxes.

M3.1: effect of diel changes in abiotic drivers and physical factors on component fluxes: on the one hand different respiratory components (e.g. above and belowground respiratory sources) experience different amplitudes and phase-shifted diel variations due to changes in abiotic environmental factors (such as temperature, moisture and PPFD). On the other hand, respiratory sources differ in their responsiveness to these abiotic drivers, thus resulting in changes in the mixing-ratios of respiratory fluxes.

At the soil scale it is often reported that temperature and moisture are the main drivers for CO_2 flux (e.g. Davidson et al., 1998; Carbone et al., 2008; Paterson et al., 2009), which are both characterized by a marked diurnal cycle. Moreover, diurnal temperature changes are buffered and phase-shifted compared to air temperature with increasing soil depth. As a consequence, the resulting soil and ecosystem respiration flux consists of a temporally variable mixture of different component fluxes with different

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isotope signatures (e.g. Werner et al., 2006; Unger et al., 2010a). Many soils show a marked gradient in $\delta^{13}C$ of soil organic matter (SOM) within the soil profile (Ehleringer et al., 2000). Thus, diel changes in the contribution of CO_2 originating from different soil layers and thus from organic substrates with different $\delta^{13}C$ can induce diel variations of the net soil efflux $\delta^{13}C_R$. Maseyk et al. (2009) estimated that the depth-related enrichment in SOM and respired $\delta^{13}C$ could contribute to ~0.5‰ of the observed diel-scale variability in soil $\delta^{13}C_R$ through temperature driven shifts in the relative contribution of $\delta^{13}C_{res}$ from different soil depths.

Soil $\delta^{13}C_R$ can also be influenced by physical effects on soil CO_2 diffusivity (Stoy et al., 2007). The diffusive velocity of CO_2 through the soil pores is altered by the physical environment, such as porosity and soil moisture (Stoy et al., 2007), and thus diurnal changes during drying and wetting of upper soil layers may alter mixing ratios from different soil depths. Transient diffusive fractionation during non-steady state conditions could induce diel variation in soil $\delta^{13}C_R$, which seemed particularly large when soil respiratory fluxes were low but the variability (fluctuation) was high (Moyes et al., 2011).

A further aspect is the different responsiveness of respiratory components to variations in abiotic drivers. At the soil scale, CO_2 flux derives from two major components with different isotopic signatures: autotrophic and heterotrophic soil respiration, which are two fully distinct processes, controlled by different underlying factors (see Brüggemann et al. (2011) and literature therein for details), particularly regarding their temperature sensitivity. To date, published results yield a non-uniform picture: in some ecosystems autotrophic soil respiration was found to have a higher temperature sensitivity than heterotrophic soil respiration (e.g. Boone et al., 1998; Bhupinderpal-Singh et al., 2003). In these systems the proportional contribution of autotrophic respiration may therefore increase from the morning to the afternoon, thus producing diel variations in soil $\delta^{13}C_{res}$ (Carbone et al., 2008; Marron et al., 2009). In contrast, others (e.g. Bol et al., 2003; Hartley and Ineson, 2008; Vanhala et al., 2007) suggested that heterotrophic respiration with recalcitrant soil organic material as substrate was highly temperature sensitive.

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Additionally, it has been suggested that growth respiration might be temperature insensitive while maintenance respirations might exhibit large temperature sensitivity (Kuzyakov and Gavrichkova, 2010). If growth and maintenance respiration differ in $\delta^{13}C_{res}$ due to differences in respiratory substrates (see M1) or respiratory fractionation (see M2) any change in temperature will lead to changes in $\delta^{13}C_R$.

Moreover, recently it has been questioned whether soil respiration is mainly driven by environmental factors such as soil temperature and moisture (Liu et al., 2006; Vargas and Allen, 2008; Kuzyakov and Gavrichkova, 2010) as opposed to biotic factors. There is evidence that soil respiration can be partially decoupled from soil temperature, probably because of the impact of recent photosynthates as substrates for root and (myco)rhizosphere respiration. Thus substrate-driven changes through the input of labile carbon compounds needs to be considered as a driving factor causing short-term variations in $\delta^{13}C_R$.

M3.2: substrate driven changes in component flux rates due to different responsiveness to input of recent assimilates: assuming that soil and ecosystem respiration rates are strongly influenced by photosynthetic assimilate supply to the soil (cf. Ekblad and Högberg, 2001, recent reviews by Davidson et al., 2006 and Paterson et al., 2009; Högberg and Read, 2006; Trumbore, 2006; Bahn et al., 2009) photosynthesis should influence $\delta^{13}C_R$ in two ways. First the isotopic signature of the labile carbon transferred from the canopy to roots and rhizosphere should be imprinted on the CO_2 respired from mycorrhizal roots and associated rhizosphere microorganisms. In addition, the ratio of heterotrophic to autotrophic contributions to respiratory fluxes is most likely altered. Soil and ecosystem $\delta^{13}C_R$ are indeed often well correlated with environmental factors driving changes in photosynthetic discrimination during the preceding days (e.g. Ekblad and Högberg, 2001; Werner et al., 2006). The rapid transfer of photosynthates to roots, root exudates and subsequent respiration in the rhizosphere has been demonstrated by ^{13}C labelling experiments (e.g. Carbone and Trumbore, 2007; Högberg et al., 2008; Bahn et al., 2009; Subke et al., 2009). Bahn et al. (2009) showed that in a grassland recent plant-assimilates were respired in the soil from the

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late morning hours onwards, whereas previous day assimilates were the substrate during the night and early morning hours. Moreover, there are new indications, suggesting a tight and rapid coupling between the onset of photosynthetic activity during the light period and increased C-supply to rhizosphere respiration (Mencuccini and Hölttä, 2010; Kuzyakov and Gavrichkova, 2010), which could be mediated by pressure-gradient waves. This mechanism could enable a tight coupling between phloem sugar loading with new assimilates and root-released exudates which would circumvent the time-lags associated with basipetal transport ways (Mencuccini and Hölttä, 2010).

Thus, the autotrophic soil flux is likely more dynamic over the diel cycle than heterotrophic respiration resulting in diel variations in soil $\delta^{13}C_R$ (e.g. Carbone et al., 2008).

As the soil flux constitutes a large proportion of total ecosystem respiratory flux in many ecosystems (e.g. Davidson et al., 2006) it may markedly contribute to diel variations in ecosystem $\delta^{13}C_R$. At the ecosystem level, an additional factor is the fact that respiration of leaves is strongly inhibited in the light (Tcherkez et al., 2008) but may exhibit a marked increase with very positive $\delta^{13}C_{res}$ (LEDR) at the beginning of the dark period (Barbour et al., 2011). A peak in enriched $\delta^{13}C_{res}$ after sunset (duration of 60–100 min) was correlated with the light intensity on the prevailing day, showing higher enrichment during sunny compared to cloudy days (Barbour et al., 2011), thus confirming the patterns observed in leaves (Prater et al., 2006; Priault et al., 2009). Indeed, markedly ^{13}C-enriched CO_2 has been measured in tree crowns at night (Mortazavi et al., 2006). Unger et al. (2010a) have shown in an isotopic-mass balance approach how different ecosystem components can vary in flux rates and $\delta^{13}C_{res}$ over short-term scales, with marked impacts on ecosystem $\delta^{13}C_R$. Given the high short-term dynamics of multiple sources in an ecosystem, it needs to be critically reassessed whether a simple two-source mixing model for Keeling-plots can adequately describe processes at the ecosystem scale. Tu and Dawson (2011) concluded that the ecosystem mass-balance could be closed only at predawn, when most ecosystem processes became relaxed and changes in fluxes were small.

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A further complication may arise if large portions of respired CO_2 are not released to the atmosphere but internally transported between organs as it has been suggested for xylem CO_2 (see Teskey et al., 2008). If a large portion of root respired CO_2 is transported via the xylem water inside the plant and subsequently re-fixed in stem and twigs and/or emitted via the stem to the atmosphere, it would add a further variable source coupled to diel changes in xylem flow delivering depleted $\delta^{13}C_{res}$ compared to atmospheric $\delta^{13}CO_2$. However, recent studies from Kodama et al. (2008) and Ubierna et al. (2009) showed that the influence of CO_2 from belowground – potentially transported with and stored in the xylem water – had only negligible influence on $\delta^{13}C_{res}$ of trunk respired CO_2. Aubrey and Teskey (2009) calculated that on a daily basis, the amount of CO_2 that moved upward from the root system into the stem via the xylem stream in a poplar plantation rivaled that which diffused from the soil surface to the atmosphere. If part of this CO_2 is released via trunk or twigs (or refixed via PEPc or stem photosynthesis), if it deviates in $\delta^{13}C$ from CO_2 produced in the above-ground tissues and if the contribution to trunk or stem efflux varies over the diel course temporal variations in $\delta^{13}C$ of ecosystem respired CO_2 would also result.

In summary, the mechanisms driving composite fluxes such as soil and ecosystem CO_2 fluxes are complex, since changes in the contribution of the relative flux rates of component fluxes with different isotopic signatures have to be taken into account. There is increasing recognition on close feed-backs between plant carbon assimilation and rhizosphere and soil respiration (see Brüggemann et al., 2011), but its impact on diel variations in $\delta^{13}C_R$ remain to be resolved. Variable contributions of different components fluxes, might at least partially explain the strong variations in $\delta^{13}C_R$ observed on the ecosystem level. We certainly need experiments targeted towards assessing the short-term variability of the isotopic fluxes from different ecosystem compartments and how they contribute to $\delta^{13}C$ of ecosystem respired CO_2. The emerging laser spectroscopic techniques which allow direct determination of $^{13}CO_2$ and $^{12}CO_2$ fluxes on the ecosystem level (Griffis et al., 2008) and within individual compartments (Wingate et al., 2010) will provide a powerful tool for such studies in the future.

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5 Conclusions

Our review suggests that direct relations between $\delta^{13}C$ of recent assimilates as the most probable respiratory substrates and respired CO_2 may not be present on a diel time scale and that other factors lead to short-term variations in $\delta^{13}C_{res}$ and in $\delta^{13}C_R$ of ecosystem-emitted CO_2. Temporal variation of respiratory fractionation due to temperature effects and changing allocation of carbon to metabolic pathways are highly plausible mechanisms that can explain diel patterns in $\delta^{13}C_{res}$. For leaves and other autotrophic organs, LEDR is an additional mechanism most probably responsible for the observed increase in $\delta^{13}C$ directly after sunset and upon initial darkening. Component fluxes with different and variable isotopic compositions and flux rates further complicate the interpretation of the respiratory isotope signal at the plant, soil and ecosystem scale. The quantification of component isofluxes at different scales including assessments of e.g. in vitro enzyme activities, transgenic PEPc knock-out and overexpressing lines and combined ^{13}C-labelling and natural abundance studies might all give deeper insights into the origin of short-term variations of respired CO_2 in future.

This is highly important since the carbon isotope composition of plant respired CO_2 contains information on the fate of respiratory substrates, and may, therefore, provide a non-intrusive way to identify changes in carbon allocation patterns over various scale levels.

Acknowledgements. We greatfully acknowledge financial support from SIBAE (Stable Isotopes and Biogeochemical Cycles in Terrestrial Ecosystems) COST action for publication and from the Deutsche Forschungsgemeinschaft (DFG, WE 2681/3-1, 5-1, 6-1, GE 1090/5-1, 7-1, 8-1 9-1). Thanks to M. Cuntz for contribution to calculations of Fig. 6.

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Table 1. Survey of diel variations in respired $\delta^{13}C_{res}$ of leaves, roots, trunks, (minimum, maximum and total range within 24 h) and the variation of the putative substrate (glucose – Glu, soluble sugars – SS, sucrose – Suc; water soluble organic mater – WSOM; bulk organic matter – OM). The species, growing conditions (field or controlled laboratory conditions – lab), environmental factors and references are given; ns – not significant; – not determined.

Diel variation (max.) in respired $\delta^{13}C_{res}$ (‰)				Variation in substrate $\delta^{13}C$ (‰)			Species	Field/	Environ.	Reference
Min	Max	Range	Type	Min	Max	Range		lab	Factors	
Diel variation at the leaf scale*										
		6.4	SS			ns	*Quercus ilex*	field		Hymus et al. (2005)
		4.9				ns	*Quercus cerris*	field		
−31.0±0.6	−19.5±0.6	11.5					*Pinus elliotti*	field	April	Prater et al. (2006)
−27.6±0.5	−21.6±0.3	6.0	OM	−29.9±0.2	−29.1±0.1	0.7	*Pinus elliotti*	field	August	
−29.2±0.4	−21.9±0.3	7.3	OM	−30.4±0.1	−29.8±0.0	0.6	*Pinus elliotti*	field	April	
−26.7	−18.3	8.4					*Quercus ilex*	lab		Werner et al. (2007)
−26.0	−23.9	2.1 ns					*Tolpis barbarta*	lab		
−28.6±0.4	−25.7±0.2	2.9					*Pinus pinea*	lab		Priault et al. (2009)
−25.9±0.5	−18.6±0.8	7.3					*Quercus ilex*			
−28.8±0.2	−20.9±0.7	7.9					*Halimium halimifolium*	lab		
−23.9±0.8	−15.9±0.7	8.0					*Arbutus unedo*	lab		
−25.1±0.4	−23.7±0.1	1.4					*Ceratoma siliqua*	lab		
−30.2±0.5	−24.0±0.8	6.2					*Mentha piperita*	lab		
−30.5±1.1	−26.4±0.7	4.1					*Citrus hytrix*	lab		
−27.4±0.4	−20.9±0.6	6.5					*Rosmarinus officinalis*	lab		
−24.4±0.7	−21.1±0.4	3.3					*Ficus benjamina*	lab		
−24.6±0.6	−24.9±0.9	−0.3					*Tolpis barbata*	lab		
−28.5±0.1	−28.1±0.9	0.5					*Quercus petraea*	field		
−27.9±0.4	−24.1±0.4	3.9					*Sorbus cashmiriana*	field		
−28.1±0.9	−24.2±1.2	3.9					*Laurus sp*	field		
−27.7±0.4	−26.9±0.6	0.7					*Carpinus betulus*	field		
−28.9±0.7	−28.7±0.7	0.2					*Poa annua*	field		
−31.9±0.3	−32.2±0.2	−0.3					*Bellis perrenis*	field		
−31.7±0.6	−31.6±0.6	−0.3					*Trifolium pratensis*	field		
−28.5	−28.1	0.4	SS	−30.0	−30.4	−0.4	*Quercus petraea*	lab		Werner et al. (2009)
−27.5	−19.4	8.1	SS	−23.8	−23.6	0.2	*Quercus ilex*	lab		
−25.0	−24.4	0.6	SS	−30.4	−30.9	−0.5	*Tolpis barbata*	lab		
−29.6	−20.7	8.9	SS	−29.7	−28.7	1.0	*Halimium halimifolium*	lab		
−21.9±1.3	−14.7±0.5	7.2	WSOM	−26.9±1.4	−23.9±0.5	3.0	*Acacia longifolia*	forest	summer	Rascher et al. (2010)
−18.2±0.5	−15.0±0.5	3.2	WSOM	−23.6±0.6	−22.4±0.5	1.2	*Acacia longifolia*	dunes	summer	
−22.6±0.3	−17.9±0.1	4.7	WSOM	−26.4±0.3	−25.7±0.8	0.7	*Pinus pinaster*	forest	summer	
−24.5±0.8	−16.5±0.1	8.0	WSOM	−26.6±0.2	−25.3±0.5	1.2	*Pinus pinaster*	dunes	summer	
−20.2±1.2	−14.6±0.9		WSOM	−26.9±0.7	−25.7±1.0	–	*Acacia longifolia*	field	drought	Dubbert et al. (2011)
−22.6±1.2	−13.8±1.0		WSOM	−26.5±1	−25.4±0.8		*Rosmarinus officinalis*	field	drought	
−22.1±1.3	−15.9±2.0		WSOM	−28.2±1	−26.9±1.2	–	*Halimium halimifolium*	field	drought	
−29.9±0.9	−15.1±0.6	14.8	WSOM	−31.8±0.3	−30.2±0.2	ns	*Halimium halimifolium*	lab		Wegener et al. (2010)
−30.1±1.2	−23.5±0.4	6.6	WSOM	−30.0±0.3	−28.2±1.1	ns	*Melissa officinalis*	lab		
−26.2±0.8	−20.8±0.2	5.4	WSOM	−28.2±0.8	−27.0±0.7	ns	Salvia officinalis	lab		
−30.6±0.8	−27.2±1.1	3.4	WSOM	−30.0±0.9	−28.9±0.1	ns	*Oxalis triangularis*	lab		

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Table 1. Continued.

Diel variation (max.) in respired $\delta^{13}C_{res}$ (‰)				Variation in substrate $\delta^{13}C$ (‰)			Species	Field/	Environ.	Reference
Min	Max	Range	Type	Min	Max	Range		lab	factors	
−21.6±0.5	−18.3±0.1	3.3					*Quercus ilex*	field	spring	Unger et al. (2010a)
−22.6±0.2	−21.8±0.3	0.8					*Quercus ilex*	field	drought	
−21.6±0.3	−19.2±1.0	2.4					*Tuberaria guttata*	field	spring	
−28.5±0.6	−24.7±0.4	3.8					*Tuberaria guttata*	field	drought	
−21.7±0.9	−18.4±0.9	3.3±0.8					*Prosopis velutina*	Riparian	dry season	Sun et al. (2009)
−20.5±0.6	−17.7±0.9	2.8±0.7					*Prosopis velutina*	Upland	dry season	
−26.4±1.1	−21.3±1.2	5.1±1.1					*Prosopis velutina*	Riparian	wet season	
−24.7±1.4	−19.6±0.7	5.1±0.9					*Prosopis velutina*	Upland	wet season	
−25.0±1.0	−19.1±0.8	5.9	WSOM	−27.3±0.4	−26.8±0.7	ns	*Wheat shoots*	field	summer	Kodama et al. (2010)
−28.9±1.5	−27.4±0.4	1.5	SS	−31.8±0.6	−28.5±0.4	3.3	*Ricinus communis*	lab		Gessler et al. (2009)
Diel variation at the trunk/stem scale										
−26.8±0.4	−22.8±0.6	4.0	phloem	−26.4±1.3	−25.7±0.3	0.9	*Pinus silvestris*	field	summer	Kodama et al. (2008)
−32.1±0.8	−28.8±0.5	3.3	SS	−30.7	−27.7	3.0	*Ricinus communis*	lab		Gessler et al. (2009)
−24.2±0.4	−21.2±0.3	3.0±0.5	Suc	−24.6±0.2	−24.0±0.3	0.7±0.5	*Quercus patraea*	forest	April	Maunoury et al. (2007)
−25.9±0.4	−24.9±0.4	1.0±0.2		−24.0±0.5	−23.5±0.2	0.5±0.6			May	
−26.1±0.1	−25.2±0.4	0.9±0.3		−25.9±0.2	−25.1±0.4	0.8±0.5			June	
−24.8±0.2	−22.1±0.5	2.7±0.4		−26.6±0.0	−25.6±0.5	1.0±0.7			Nov	
Diel variation at the root scale										
−33.3±0.5	−30.5±0.2	2.8	SS	−31.4±0.4	−28.5±1.2	2.9	*Ricinus communis*	lab		Gessler et al. (2009)
−28.1±0.3	−22.7±1.8	5.4	WSOM	−24.7±0.8	−24.2±0.2	ns	*wheat*	field	summer	Kodama et al. (2010)
−27.3±0.7	−26.1±0.4	−1.2 ns	WSOM	−24.8±0.6	−23.8±0.6	ns	*Halimium halimifolium*	lab		Wegener et al. (2010)
−28.0±0.5	−24.6±0.7	−3.4*	WSOM	−27.4±0.1			*Melissa officinalis*	lab		
−27.5±0.4	−25.6±0.4	−1.96*	WSOM	−24.9±0.1			*Salvia officinalis*	lab		
−29.3±0.7	−28.6±0.7	−0.7 ns	WSOM	−28.8±0.9	−26.8±1.0	ns	*Oxalis triangularis*	lab		
−25.3±1.4	−20.2±1.8	5.1					*Acacia longifolia*	field	summer	Rascher et al. (2010)
−23.7±0.6	−21.4±0.9	2.6					*Pinus pinaster*	field	summer	
−19.0±1.9	−15.0±1.5	4.0	WSOM	−26.8±0.8	−23.9±0.7	2.9	*Acacia longifolia*	field	drought	Dubbert et al. (2011)
−21.4±1.8	−16.9±0.8	4.5	WSOM	−25.7±0.6	−25.0±0.6	0.7	*Rosmarinus officinalis*	field	drought	
−17.4±1.8	−16.3±1.9	1.1	WSOM	−26.8±0.9	−25.4±1	−1.4	*Halimium halimifolium*	field	drought	
−23.0±0.5	−20.6±0.4	2.4					*Tuberaria guttata*	field	spring	Unger et al. (2010a)
−25.6±0.2	−21.0±0.5	4.6					*Tuberaria guttata*		drought	

SS – soluble sugar; SStot – total soluble sugar fraction; WSOM – water soluble organic matter; SUC – sucrose; ns – not significant; * leaves were dark-adapted for 5–15 min before measurements.

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Table 2. Survey on nocturnal, diurnal and 24 h-variations in respired $\delta^{13}C_R$ of composite fluxes of soil and ecosystem respiration (minimum, maximum and total range) and the variation of the putative substrate (e.g. bulk soil OM). The ecosystem, environmental conditions and references are given. When several diel courses were available, the variation in the minimum, maximum and range over the measured period was given. ns – not significant; – not determined.

Noctural variation in respired $\delta^{13}C_R$ (‰)			Diurnal variation in respired $\delta^{13}C_R$ (‰)			Diel (24 h)	Substrate $\delta^{13}C$ (‰)		Ecosystem	Environ.	Reference/remarks
Min	Max	Range	Min	Max	Range	Range	Type	Range		factors	
Diel variation at the soil scale											
~ −22 – 20.5 ~ −25.5 ~ 21.5– −20.0	~ −21 ~ −20.5 ~ −16.0–17.5					1.1 4.0 4.0			Uncultivated grass field Field (winter wheat) Deciduous forest	 August	Dudziak and Halas (1996)
−26.1 ± 0.6	−23.6 ± 0.2	0.4–1.7	−25.0 ± 1.7	−22.4 ± 1.3	0.3–2.4	2.7			*Pinus silvestris* forest	summer	Kodama et al. (2008)
−29.3	−25.7	3.6	−29.7	−23.4	4.5	5.8			Wheat field		Kodama et al. (2010)[2]
−26.2 ± 1.8– −27.5 ± 1.1	−25.5 ± 1.1– −25.6 ± 0.7	0.7–2	−28.4 ± 0.6– −29.2 ± 0.6	−26.3 ± 0.9– −26.6 ± 0.8	1.8–2.8	2.9–3.6			Mediterranean oak forest	spring	Unger et al. (2010a)[3]
−26.9 ± 0.4– −27.8 ± 0.7	−23.4 ± 0.7– −24.3 ± 0.4	3.5	−28.4 ± 0.5– −29.3 ± 1.4	−24.0 ± 0.6– −27.0 ± 1.2	2.2–4.4	4.9–5.0			Mediterranean oak forest	drought	
−27.1				−24.8		2.6	SOM	−27.3–24.6	Mediterranean oak forest	April	Maseyk et al. (2009)
−26.3	–	ns				ns			boreal forest		Betson et al. (2007)
−27.3				−26.1		1.18			grassland		Bahn et al. (2009)[4]
−32.5	−28.3	4.3							boreal forest		Subke et al. (2009)
−27.84– −28.19	−27.04– −27.10	0.74–1.15	−27.98– −28.35	−26.12– −27.20	0.8–2.2	0.9–2.2			Beech-forest	July	Maron et al. (2009)
						0.3–12.5 0.4–10.6			Deciduous trees in exp. garden	untrenched trenched	Moyes et al. (2010)
Diel variation at the ecosystem scale											
−27.1 ± 0.3				−23.6*	−28.0 ± 0.3	~ 3	SOM	−28.0 ± 0.3	*Pinus pinaster* Aït.	drought	Ogée et al. (2003)[5]
−29.1 ± 0.4– −26.1 ± 0.3	−25.9 ± 0.2– −22.7 ± 0.8	1.8–6.4							grassland		Bowling et al. (2003)[6]
−29.4 ± 0.4	−27.4 ± 0.5	2.0							Sown grassland		Schnyder et al. (2004)[7]
						3.8			Mix deciduous forest		Knohl et al. (2005)
−27.0 ± 0.4	−21.7 ± 0.9	6.1							*Pinus silvestris* forest	summer	Kodama et al. (2008)
−29.2 ± 1.0 −31.1 ± 2.1	−26.7 ± 0.7 −26.9 ± 0.3	2.5 4.2							Mediterranean oak forest	May September	Werner et al. (2006)

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Table 2. Continued.

Noctural variation in respired $\delta^{13}C_R$ (‰)			Diurnal variation in respired $\delta^{13}C_R$ (‰)			Diel (24 h)	Substrate $\delta^{13}C$ (‰)		Ecosystem	Environ.	Reference/remarks
Min	Max	Range	Min	Max	Range	Range	Type	Range		factors	
Diel variation at the ecosystem scale											
−26.9 ± 1.5– −29.7 ± 0.8	−23.4 ± 0.8– −26.1 ± 1.9	3.5–3.6							Mediterranean oak forest	spring	Unger et al. (2010a)[3]
−27.9 ± 1.0– −28.2 ± 2.2	−20.1 ± 1.6– −24.0 ± 0.4	3.9–8.1							Mediterranean oak forest	drought	
~ −28.2	−25.2			−25.2	−23.3	~ 0.6 – 5			Subalpine forest		Bowling et al. (2005)
−27.3 ± 0.6	−23.7 ± 0.7	3.6							Subalpine forest	2006	Riveros-Iregui et al.
−26.9 ± 0.3	−24.3 ± 0.6	2.6							Subalpine forest	2007	(2011)
−27.3 ± 0.5	−24.3 ± 0.5	3.0							Subalpine forest	2008	

SOM – Soil organic; ns – not significant

[1] atmospheric $\delta^{13}CO_2$ above the canopy,

[2] smoothed data, measured with TDL,

[3] 30 min-Keeling plot intercepts measured every 2-h ± standard error for the intercept,

[4] mean values of 20-min measurements pooled over three plots and 13–16 days (within a four week period),

[5] night, all levels together, each time treated separately; day above the canopy,

[6] hourly Keeling plot intercepts ± standard error for the intercept,

[7] reports 1-hourly means ± SE of Keeling plot intercepts measured during the nights of 20 and 21 July 2004, on a grass-clover mixture (managed pasture) sown in 1999.

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Table 3. Potential mechanisms causing diel variation in $\delta^{13}C_{res}$ at the plant scale.

Substrate driven variations in $\delta^{13}C_{res}$	
M1.1:	Photosynthetic discrimination and potential effects on the diel patterns of $\delta^{13}C$ of assimilates
M1.2:	Post-photosynthetic carbon isotope fractionation during transitory starch accumulation
M1.3:	Isotope effects during basipetal transport: dampening of the diel variations in $\delta^{13}C$ of phloem sugars
M1.4:	Switch between respiratory sources with different isotopic signatures
M1.5:	Light enhanced dark respiration (LEDR) after light-dark transition during decarboxylation of a malate pool
Fractionation driven variations in $\delta^{13}C_{res}$	
M2.1:	Fragmentation fractionation (i.e. fractionation associated with the fragmentation of molecules with non-statistical intramolecular carbon isotope distribution) and enzyme related effects
M2.2:	Fractionation due to variations of fluxes in different metabolic pathways
M2.3:	Refixation of CO_2 by PEPc

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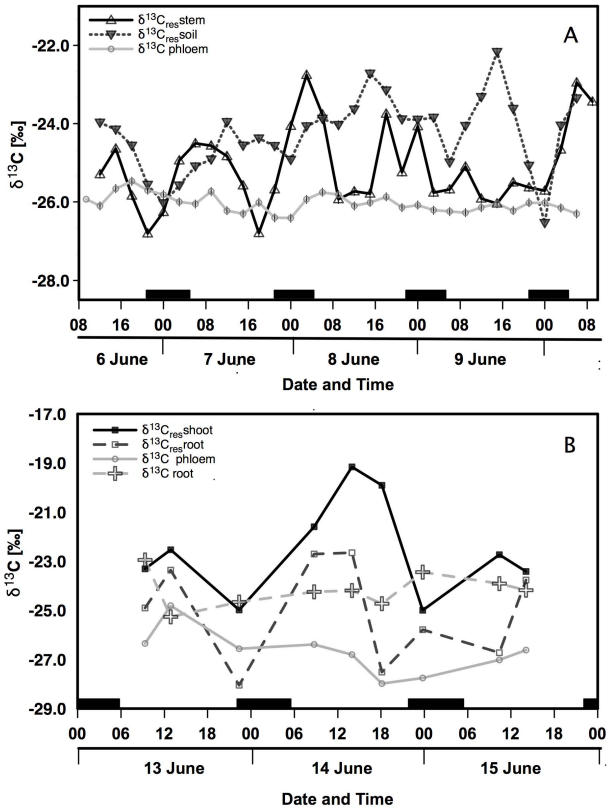

Fig. 1. Examples for diel variations in $\delta^{13}C_{res}$ and in $\delta^{13}C$ of putative respiratory substrates. **(A)** shows soil and stem $\delta^{13}C_{res}$ from a *Pinus sylvestris* forest compared to $\delta^{13}C$ of phloem exudates (Kodama et al., 2008). **(B)** shows diel variations in shoot and root $\delta^{13}C_{res}$ as compared to $\delta^{13}C$ in phloem exudate and root water soluble organic matter in *Triticum aestivum* (Kodama et al., 2011).

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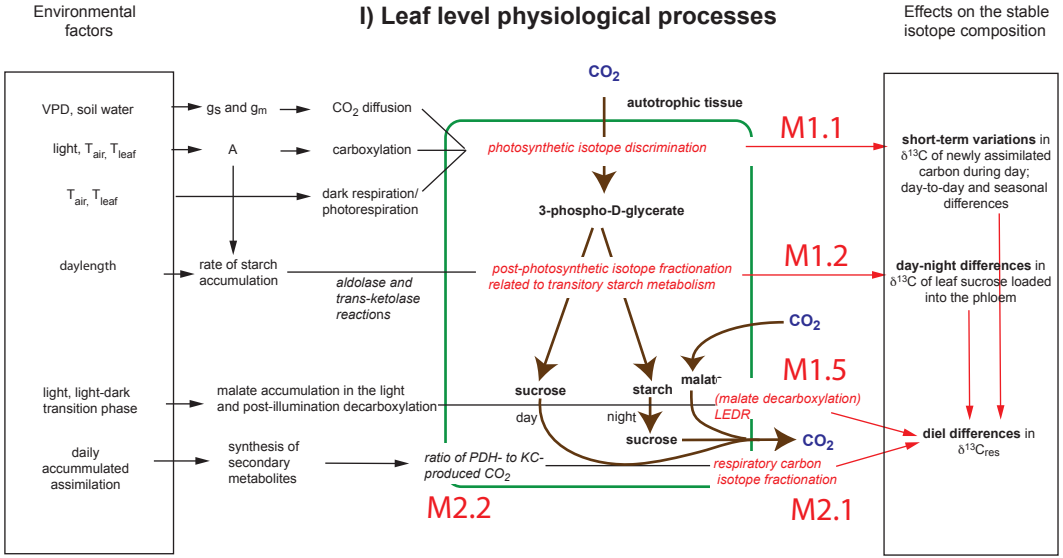

Fig. 2.

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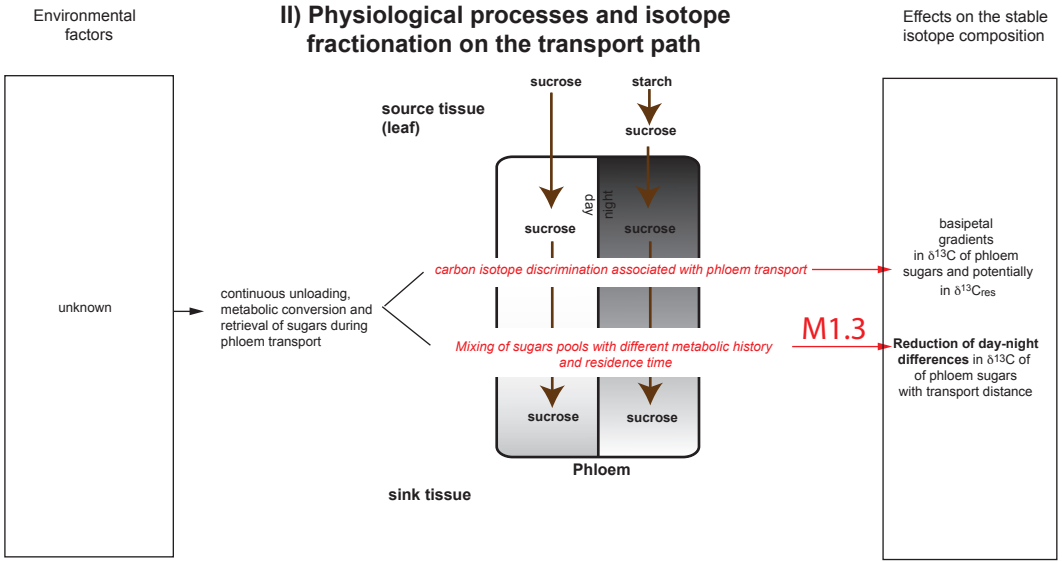

Fig. 2.

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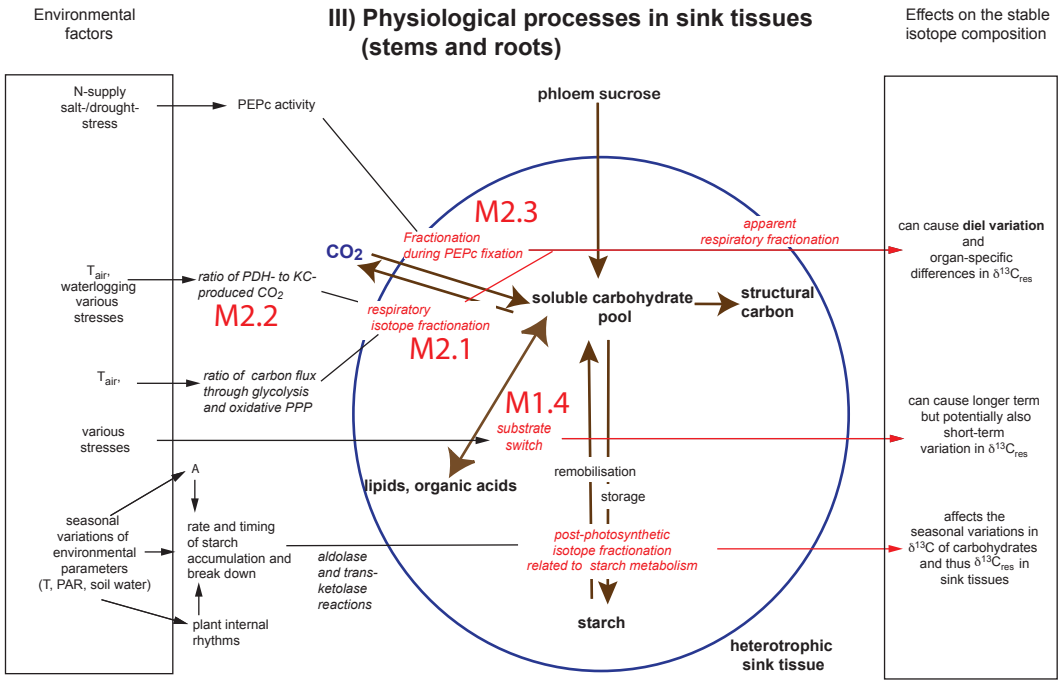

Fig. 2. Caption on next page.

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Fig. 2. Physiological processes and isotope fractionations influencing the short-term variation of the carbon isotope signature of organic compounds and in respired CO_2 in leaves (I), on the transport pathway (II), and in the heterotrophic sink tissues (III) of plants. On the left side of the figure environmental factors potentially affecting carbon isotope fractionation processes are listed. In the middle of the figure the processes leading to an alteration of $\delta^{13}C$ are given in red. On the right side the effects on the carbon isotope composition of organic matter and respired CO_2 are described. The bold brown arrows denote the carbon flux through the plant. VPD, vapour pressure deficit; T_{air}, air temperature; T_{leaf}, leaf temperature PAR, photosynthetic active radiation; g_s and g_m, stomatal and mesophyll conductance, respectively; A, assimilation rates. Particular processes and mechanisms are denoted in detail in Table 3 (further information is given in the text): substrate driven variations in $\delta^{13}C_{res}$: M1.1: photosynthetic discrimination and potential effects on the diel patterns of $\delta^{13}C$ of assimilates; M1.2: post-photosynthetic carbon isotope fractionation during transitory starch accumulation; M1.3: dampening of the diel variations in $\delta^{13}C$ of phloem sugars during basipetal transport, M1.4: switch between respiratory substrates, M1.5: light enhanced dark respiration (LEDR); fractionation driven variations in $\delta^{13}C_{res}$: M2.1: fragmentation fractionation (i.e. fractionation associated with the fragmentation of molecules with non-statistical intramolecular carbon isotope distribution), M2.2: variations of fluxes in the metabolic pathways; M2.3: refixation of CO_2 by PEPC.

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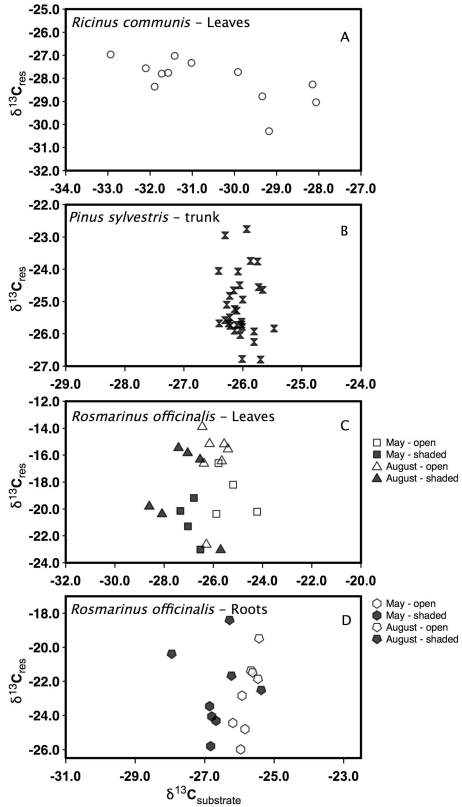

Fig. 3. $\delta^{13}C_{res}$ plotted against $\delta^{13}C$ of potential respiratory substrates during the diel course. **(A)** data for leaf emitted CO_2 and leaf soluble sugars in *R. communis* during a 24 h cycle. Each data point represents one individual plant at one time point. Samples were taken twice during the day (10:00; 15:30) and twice during the dark period (22:30; 03:30). Data are from Gessler et al. (2009b). **(B)** data for trunks of *P. sylvestris* taken from Kodama et al. (2008). As substrate for respiration we have chosen trunk phloem exudates from the same position where the CO_2 measurements were made. Data are from diel courses measured every 3 h over 4 days. **(C)** and **(D)** data from *Rosmariuns officinalis* of leaves and roots, respectively; of dark-respired $\delta^{13}C_{res}$ and WSOM measured every 2–3 h over the diurnal course in Portugal in May and August from Dubbert et al. (2011).

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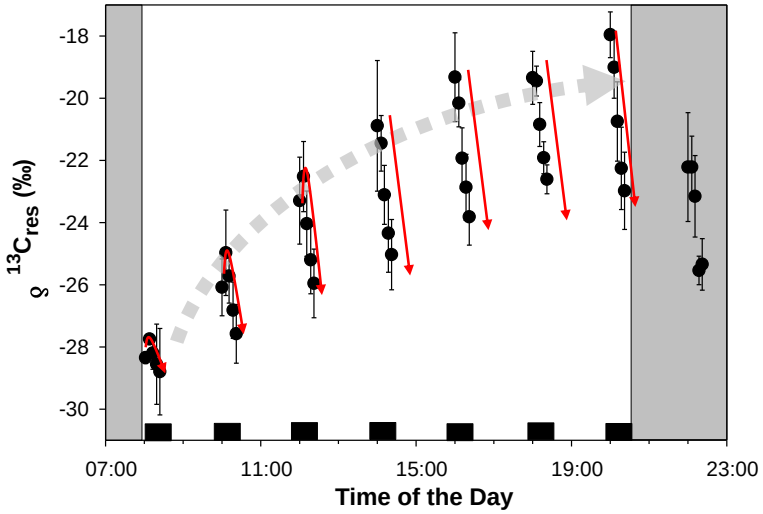

Fig. 4. Diel (grey dashed arrow) and short-term (red arrows) post-illumination changes of leaf dark respired $\delta^{13}CO_2$ ($\delta^{13}C_{res}$) during 25 min dark phases over the diurnal course (grey areas indicate the dark period). Black bars at the bottom of the figure represent the time during which the measured leaf was darkened, while the rest of the plant remained under the growth light conditions. Data are mean values of *Quercus ilex* leaves ($n = 3$, ±SE), reprinted from Werner et al. (2009).

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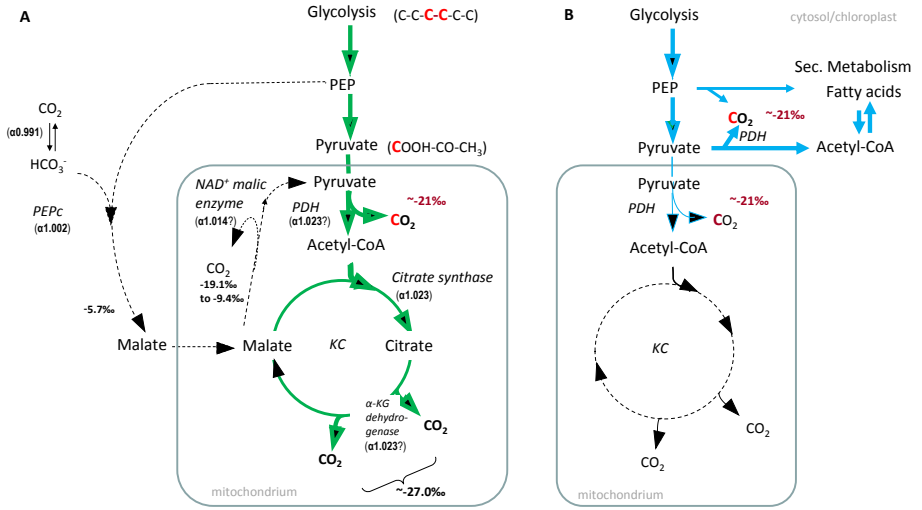

Fig. 5. Simplified metabolic scheme showing major fluxes of respiratory substrates (black arrows), isotopic compositions (‰) and fractionation factors (α) of key enzymes and processes that influence $\delta^{13}C_{res}$: C-1 of pyruvate which is decarboxylated during pyruvate dehydrogenase (PDH) reaction is ^{13}C-enriched (−21‰), while relatively depleted C-2 and C-3 (−27.0‰) which form acetyl-CoA enter the Krebs cycle (KC). Two distinct situations are indicated: **(A)** full decarboxylation in of the carbon molecules in the Krebs cycle (KC) or **(B)** high investment into secondary metabolism and fatty acid synthesis. Fractionation processes in the KC are exemplified by *citrate synthase* and α-ketoglutarate (α-KG) dehydrogenase ($\alpha = 1.023$). Further, the potential involvement of an enriched malate pool (−5.1‰) which is produced during *phospoenolpyruvate carboxylase* (PEPc) reaction with small kinetic enzyme fractionation against ^{13}C ($\alpha = 1.002$) and equilibrium fractionation against ^{12}C ($\alpha = 0.991$) during HCO_3^- equilibration is indicated in **(A)** (dashed line). *Malic enzyme* fractionates in favour of ^{12}C ($\alpha = 1.014$), the reaction following a Rayleigh distillation process after the light-dark transition. Adapted from Werner et al. (2009) and Gessler et al. (2009b); fractionation factors from: Melzer and Schmidt (1987); Rossmann et al. (1991); Tcherkez and Farquhar (2005); Barbour et al. (2007).

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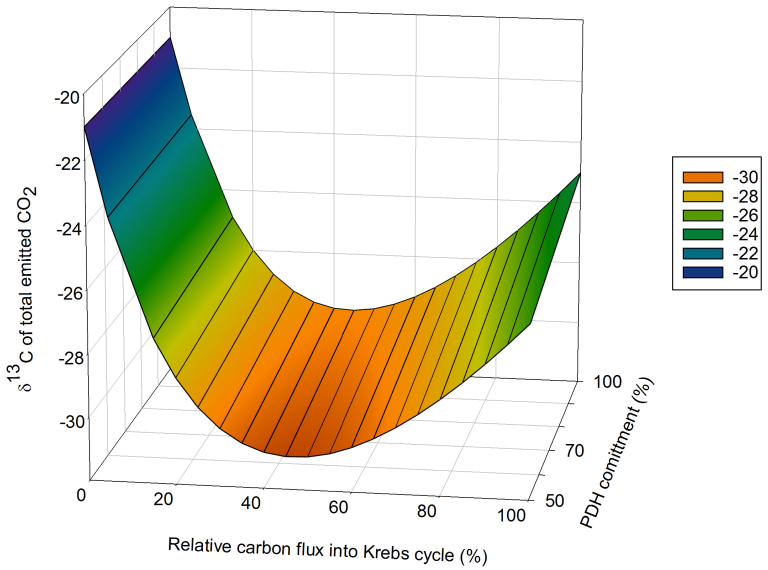

Fig. 6. Theoretical isotopic fractionation effects during decarboxylation of pyruvate. Fractionation effects were calculated assuming enzymatic fractionation in the Krebs cycle by citrate synthase and α-ketoglutarate dehydrogenase (of −23‰ see Fig. 5 for details) and varying carbon flow rates into Krebs cycle (0–100%). PDH could also potentially fractionate if the reaction is incomplete which does further deplete $\delta^{13}C_{res}$, which was tested assuming a commitment between 50–100% (z-axis), but occurrence of the latter processes in vivo is unknown.

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