PHYSIOLOGICAL ECOLOGY - ORIGINAL PAPER

Temporal dynamics of the carbon isotope composition in a *Pinus sylvestris* stand: from newly assimilated organic carbon to respired carbon dioxide

Naomi Kodama · Romain L. Barnard · Yann Salmon · Christopher Weston ·
Juan Pedro Ferrio · Jutta Holst · Roland A. Werner · Matthias Saurer ·
Heinz Rennenberg · Nina Buchmann · Arthur Gessler

Abstract The ^{13}C isotopic signature (C stable isotope ratio; δ^{13}C) of CO_2 respired from forest ecosystems and their particular compartments are known to be influenced by temporal changes in environmental conditions affecting C isotope fractionation during photosynthesis. Whereas most studies have assessed temporal variation in δ^{13}C of ecosystem-respired CO_2 on a day-to-day scale, not much information is available on its diel dynamics. We investigated environmental and physiological controls over potential temporal changes in δ^{13}C of respired CO_2 by following the short-term dynamics of the ^{13}C signature from newly assimilated organic matter pools in the needles, via phloem-transported organic matter in twigs and trunks, to trunk-, soil- and ecosystem-respired CO_2. We found a strong 24-h periodicity in δ^{13}C of organic matter in leaf and twig phloem sap, which was strongly dampened as carbohydrates were transported down the trunk. Periodicity reappeared in the δ^{13}C of trunk-respired CO_2, which seemed to originate from apparent respiratory fractionation rather than from changes in δ^{13}C of the organic substrate. The diel patterns of δ^{13}C in soil-respired CO_2 are partly explained by soil temperature and moisture and are probably due to changes in the relative contribution of heterotrophic and autotrophic CO_2 fluxes to total soil efflux in response to environmental conditions. Our study shows that direct relations between δ^{13}C of recent assimilates and respired CO_2 may not be present on a diel time scale, and other factors lead to short-term variations in δ^{13}C of ecosystem-emitted CO_2. On the one hand, these variations complicate ecosystem CO_2 flux partitioning, but on the other hand they provide new insights into metabolic processes underlying respiratory CO_2 emission.

Keywords Respiratory fractionation ·
Soil and trunk respiration · Phloem transport ·
Carbon stable isotope ratio · Trees and forest ecosystem

Communicated by Andrea Polle.

N. Kodama · J. P. Ferrio · H. Rennenberg
Institute of Forest Botany and Tree Physiology,
University of Freiburg, Georges-Koehler Allee 53/54,
79085 Freiburg, Germany

R. L. Barnard · Y. Salmon · R. A. Werner · N. Buchmann
Institute of Plant Sciences, ETH Zurich,
Universitaetsstrasse 2, 8092 Zurich, Switzerland

C. Weston · A. Gessler
School of Forest and Ecosystem Science,
University of Melbourne, Creswick, VIC 3363, Australia

J. Holst
Meteorological Institute, University of Freiburg,
Werthmannstr. 10, 79085 Freiburg, Germany

M. Saurer
Laboratory of Atmospheric Chemistry,
Paul Scherrer Institute, 5232 Villigen, Switzerland

A. Gessler (✉)
Core Facility Metabolomics, Centre for System Biology
(ZBSA), University of Freiburg, Habsburgerstr. 49,
79104 Freiburg, Germany
e-mail: arthur.gessler@sonne.uni-freiburg.de

Introduction

The feedback between processes in terrestrial ecosystems and the atmosphere is one of the largest uncertainties in understanding the global C cycling (IPCC 2007). CO_2 fixation (photosynthesis) and release (heterotrophic and autotrophic respiration) fluxes of terrestrial ecosystems

modify global atmospheric CO_2 concentrations and are in turn strongly influenced by climatic and soil conditions (e.g. Saxe et al. 1998; Nabuurs et al. 2003). Net ecosystem CO_2 exchange (NEE) alone, which is now routinely determined by eddy covariance analysis in numerous ecosystems worldwide (http://daac.ornl.gov/FLUXNET/), does not provide direct insights into differential effects of climatic and weather variations on photosynthesis and respiration fluxes. Any mechanistic understanding that allows the reliable prediction of changes in the CO_2 fluxes of terrestrial ecosystems under a changing climate will depend on understanding the variation in these component fluxes.

At the ecosystem level, the relative difference in C stable isotope ratios [δ^{13}C; expressed as deviation from the Pee Dee Belemnite (PDB) standard] has been used to partition NEE into photosynthetic and respiratory gross fluxes, with coupled eddy covariance, and isotopic approaches (e.g. Yakir and Wang 1996; Bowling et al. 2001; Ogée et al. 2003; Knohl and Buchmann 2005). In this approach, fluxes of CO_2 and $^{13}CO_2$ must be determined, as well as the C isotope signature of CO_2 in the canopy and of ecosystem-respired CO_2 ($\delta^{13}C_{R\ eco}$) (Bowling et al. 2001). $\delta^{13}C_{R\ eco}$ is measured during nighttime using a Keeling plot approach (Keeling 1958, 1961) and is generally assumed to be constant during the period of sampling and during the day–night cycle (Pataki et al. 2003). In addition, when interpreting $\delta^{13}C_{R\ eco}$, it is often implied that the δ^{13}C of CO_2 respired from different sources (autotrophic respiration of twigs, trunks and roots, soil heterotrophic respiration) is uniform or, if non-uniformity is taken into consideration, that the relative contribution of different sources to total ecosystem respiration does not change with time (Pataki et al. 2003). Finally, short-term variations in $\delta^{13}C_{R\ eco}$, and thus the uncertainty of nighttime Keeling plot values, become a considerable source of error in isoflux approaches, especially when the isotopic disequilibrium between respiratory and assimilatory CO_2 fluxes is large (Ogée et al. 2004). Recent studies, however, have shown highly variable ^{13}C signatures of respired CO_2 from different plant organs and at the ecosystem scale over short time periods. Knohl et al. (2005) observed variations in $\delta^{13}C_{R\ eco}$ of up to 3.8‰ within 24 h in a beech-dominated deciduous forest. Comparable or even larger variations were observed in CO_2 emitted from a Mediterranean oak woodland during 1 night at the ecosystem level (4.2‰; Werner et al. 2006), from leaves of Quercus ilex (8‰; Werner et al. 2007) and from European beech trunks over a diel course (3‰; Maunoury et al. 2007).

One potential source of such observed variations might be related to temporal changes in the δ^{13}C of the respired organic substrates. However, we still lack a clear

understanding of the influence of short-term variations in δ^{13}C of respiration substrates on δ^{13}C of plant-, soil- and ecosystem-respired CO_2. It is known that $\delta^{13}C_{R\ eco}$ can reflect recent environmental conditions and be linked (with time lags) to environmental conditions affecting C isotope fractionation during photosynthesis (Lancaster 1990; Bowling et al. 2002; Scartazza et al. 2004; Knohl et al. 2005), which in turn is determined by the ratio of the leaf internal to external CO_2 concentration (c_i/c_a; Farquhar et al. 1982). Only recently have additional post-carboxylation discrimination steps been identified in downstream metabolic pathways of plants (Ghashghaie et al. 2001; Damesin and Lelarge 2003) and associated with transport and other processes (Badeck et al. 2005; Brandes et al. 2006). Such post-carboxylation discrimination can additionally influence the δ^{13}C of organic matter pools with a fast turn-over and might consequently affect the δ^{13}C of CO_2 respired from these C pools. In particular, diel (24-h day-night) patterns of transitory starch accumulation and remobilisation (Tcherkez et al. 2004; Gottlicher et al. 2006) can result in day–night variations in δ^{13}C of leaf-exported sugars of up to 2.5‰ under field conditions (Gessler et al. 2007a). Such changes in the potential substrate are expected to affect the δ^{13}C of CO_2 from trunk respiration. Moreover, since soil respiration is largely driven by recent photosynthates (Högberg et al. 2001; Ekblad and Högberg 2001; Carbone and Tumbore 2007; Johnsen et al. 2007), the ^{13}C signature of phloem-transported sugars could also influence the δ^{13}C of soil CO_2 efflux over a diel cycle. Transport of assimilates to the roots and transfer to the rhizospheric microbial community might result in time-lagged correlations between the δ^{13}C of phloem-transported sugars and of soil-respired CO_2.

Another source of temporal variation for δ^{13}C of respired CO_2 is C isotopic fractionation during respiration. Post-carboxylation fractionation is responsible for differences in the intra-molecular distribution of ^{13}C in hexose phosphates, and subsequent fragmentation of the molecule (i.e. fragmentation fractionation) during respiration can result in respired CO_2 being more enriched in ^{13}C relative to the substrate of respiration (Tcherkez et al. 2004). Several field experiments have shown variable respiratory C isotope fractionation in tree leaves over a diel course (Hymus et al. 2005; Werner et al. 2007), which can result in changes in the δ^{13}C of respired CO_2, independently of the C isotope composition of the organic substrate.

In the present study, we followed the short-term dynamics of δ^{13}C from newly assimilated water-soluble organic matter pools in needles, to phloem-transported organic matter in twigs and trunks, and finally to trunk-, soil- and ecosystem-respired CO_2 in a Scots pine forest with a 3-h sampling resolution over a 4-day period. In order to investigate environmental and physiological controls of

changes in $\delta^{13}C$ of respired CO_2, we: (1) assessed diel variations in $\delta^{13}C$ for CO_2 emitted from trunks and soil and their relationships to $\delta^{13}C_{R\ eco}$, and (2) tested whether such variations were related to comparable (but potentially time-shifted) temporal patterns of $\delta^{13}C$ of the organic substrate, or if other processes such as respiratory C isotope fractionation were involved.

Materials and methods

Study site and experimental setup

The study was conducted at the Hartheim long-term study site of the Meteorological Institute of Freiburg, a pine plantation in the southern upper Rhine valley, Germany (47°56′N, 7°36′E, elevation 201 m). The forest was mostly planted with Scots pine (*Pinus sylvestris* L.) in 1963, with only a few patches of Austrian pine (*Pinus nigra* L.). All measurements were made in *P. sylvestris* plots where stand density was 800 trees ha^{-1}. Most of the tree foliage was located between 11 m and the top of the canopy at approximately 15 m. The overstorey leaf area index (LAI) was 1.47 m^2 m^{-2}. The dominant plant species of the dense understorey layer were the grass *Brachypodium pinnatum*, and the sedges *Carex alba* and *Carex flacca*. In addition, shrubs and deciduous tree species (in particular *Ligustrum vulgare* and *Ulmus minor*) heterogeneously covered 60% of the ground area in the understorey. A detailed description of the experimental site and management is given by Mayer et al. (2000) and Brandes et al. (2006, 2007). Pine tree mean diameter at breast height was 0.18 m. Projected trunk area amounted to 0.89 m^2 (trunk area) m^{-2} (ground area), calculated from the mean trunk radius and the mean trunk height (below the crown) assuming the trunk to be a cylinder, and multiplied by tree density (800 trees ha^{-1}). The forest grows on a Calcaric Regosol, the soil textural class is sandy silt.

The measurement campaign took place from 1200 hours on 6 June 2005 to 0600 hours on 10 June 2005 (all times are expressed as Central European time, with a 0- to 24-h notation). Samples were taken and gas exchange and CO_2 measurements were performed every 3 h throughout this period, at 1200, 1500, 1800, 2100, 2400, 0300, 0600 and 0900 hours. Three adjacent dominant or co-dominant individuals of *P. sylvestris* were selected within reach of a truck-mounted hydraulic lift, and used as replicates for leaf-based measurements. Similar additional trees of comparable size, located in the close vicinity, were selected for trunk CO_2 efflux measurements. Soil CO_2 efflux measurements were carried out in a *P. sylvestris* patch that was free of understorey vegetation, within 3 m from the trees used for trunk CO_2 efflux measurements.

Micrometeorology and stand transpiration

Meteorological parameters were determined continuously at a measurement tower. Air temperature and humidity were measured using a psychrometer according to Frankenberger, at 12- and 2-m height, and water vapour pressure deficit (VPD) of the air was calculated from these data. Precipitation (Hellmann rain gauge) and photosynthetically active radiation (PAR; determined by Li-190sz-sensors; Licor, Lincoln, Neb.) were determined above the canopy. Volumetric soil water content was measured in the upper 0.3 m using a CS615 water content reflectometer (Campbell Scientific, North Logan, Utah), soil temperature was measured at 0.03 m depth using a Pt100 probe. Data were recorded every 30 s and averaged over 30-min periods.

Xylem sap flow densities (in l m^{-2} sapwood area s^{-1}) were measured every 5 min on 12 trees using Granier-style probes (Granier 1985, 1987), and averaged over 30-min periods. Stand xylem sapwood area (in m^2 m^{-2}) was determined, and radial variations in sap flow within trees were accounted for as described in detail by Brandes et al. (2007). Overstorey stand transpiration (in l m^{-2} ground area s^{-1}) was calculated by multiplying xylem sap flow density by stand xylem sapwood area (Granier et al. 1996).

Plant material collection

At each measurement time, three twigs per tree were sampled from the sunlit upper third of the crown (approximately 14–15 m) and used for collection of needles and twig phloem. The crown of the pine trees at the stand examined is short and sparse. Based on the work of Brandes et al. (2006) that found no intra-canopy gradients in $\delta^{13}C$ and gas exchange parameters, each tree crown was treated as a single unit and the sampled twigs were assumed to adequately represent the whole crown. Needle water-soluble organic matter was extracted separately from current growing season (N) needles and the previous growing season ($N-1$) needles (see below). In addition, twig bark samples (at the first proximal position without live needles) and trunk bark samples from 1.5-m height were collected, the phellem (cork) was removed and the inner bark used for phloem extraction. Bark samples (approximately 150 mg) were taken from the twig bark with a scalpel, and from the trunk bark with a core borer (13 mm diameter) as described by Gessler et al. (2004).

Needle water-soluble and phloem exudate organic matter

Needle water-soluble organic matter, which was assumed to be representative of the newly assimilated organic

matter, was extracted as follows. Immediately after harvesting, N and $N-1$ needles were frozen in liquid N_2, before being freeze-dried. The samples were homogenised and 50-mg aliquots were incubated for 60 min at 5°C in 1-ml demineralised water, heated at 100°C for 1 min to precipitate proteins, and centrifuged (12,000g for 5 min) at 5°C. The supernatant was used for isotope analysis (see below).

Phloem exudate organic matter, which was assumed to be representative of transported sugars, was extracted as described by Schneider et al. (1996) and Gessler et al. (2004). Immediately after collection, bark samples were washed with demineralised water and placed in 6-ml vials containing 2 ml of demineralised water and left at room temperature for exudation for 5 h. The exudation solution was used for isotopic analysis (see below).

Atmospheric CO_2 collection

Atmospheric air samples were taken every 3 h at ground level, from below the canopy, mid-canopy and above the canopy, at 0.02-, 9-, 12- and 19-m height, respectively, using automated air samplers (ASA; Theis et al. 2004). In brief, for each air sample, air was pumped (1 l min^{-1}) from the relevant level using Synflex 1300 tubing (Saint-Gobain Performance Plastics, Compagnie de Saint-Gobain, Nanterre), through one of the thirty-three 300-ml glass vials of the air sampler. The vials were filled successively, each vial being flushed for 7 min before closing the valves at each end of it. Time programming and switching of the multiport valves (Valco multiport ST valve, EMT4ST12MWE; VICI, USA) of the ASA were controlled by a data logger (CR10X; Campbell Scientific). The CO_2 concentration in the airflow was recorded at a 10-Hz frequency with a closed path infrared gas analyser (Li-840; Li-Cor). The samples were analysed for $\delta^{13}C$ within 12 h (see below). For each measurement time, we determined ($\delta^{13}C_{R\ eco}$ based on the CO_2 concentration and $\delta^{13}C$ in the air samples collected from the four heights in the canopy, using a profile-based so-called Keeling plot approach, i.e. a two end-members mixing model in which the CO_2 emitted by the ecosystem mixes in the background atmospheric CO_2 (Keeling 1958, 1961). We used a linear regression (model I), following the recommendations of Zobitz et al. (2006), and present only the regressions with $R^2 > 0.9$.

Collection of CO_2 respired from trunks and soils

Measurements of efflux rate and $\delta^{13}C$ of CO_2 respired from trunks were carried out according to Damesin et al. (2005). Clear perspex chambers (surface area 300 cm^2, volume 4.5 l, shielded from direct sunlight by paper-backed aluminium foil) were sealed to the trunks of three trees at 1.5-m height, using Terostat VII (Henkel Teroson, Heidelberg). We selected trunk sections without visible lichens or algae present on the bark. The chambers remained fixed with a strap to the trees throughout the campaign. Between measurements, the chambers were left open to the surrounding air. During gas sampling times, they were sealed airtight and an internal fan ensured thorough mixing of air. Efflux rate and $\delta^{13}C$ of CO_2 respired from soils were determined according to Ekblad and Högberg (2000). Three opaque PVC chambers (ground surface 434 cm^2, volume 10.8 l) were gently inserted 5 mm into the soil and maintained stable with a 2 kg weight.

CO_2 efflux rates from trunks and soils were calculated at each sampling time from the linear increase in CO_2 concentration within the first 2 min after closing the chambers, measured continuously with a closed path infrared gas analyser (Licor 6400; Li-Cor). At each sampling time, five air samples were taken from each chamber using a plastic 12-ml syringe, transferred to CO_2-free Ar-flushed sample vials (Exetainer; Labco, High Wycombe, UK) and analysed for $\delta^{13}C$ (see below). The first sample was taken within 2 min after closing the chamber, and the four following samples were taken at intervals of approximately 75 μmol mol^{-1} as the CO_2 concentration rose inside the chambers. The isotopic signature of trunk and soil CO_2 efflux was determined from the CO_2 concentration and $\delta^{13}C$ in the air samples using a Keeling plot approach as described above. Keeling plots with an $R^2 < 0.9$ were discarded. To detect any storage effect on the samples, additional sample vials were filled with standard gas samples with known isotopic signature from a gas cylinder (400 μmol CO_2 mol^{-1} in N_2) at each measurement time. In agreement with the observation of Gessler et al. (2007a), there was no significant change in $\delta^{13}C$ of the standard gas within the maximum storage period of the samples (7 days).

In order to assess the influence of soil plus trunk respiration on $\delta^{13}C_{R\ eco}$, we calculated flux-weighted averages for $\delta^{13}C$ of CO_2 respired by trunks and soil together. To standardise soil and trunk CO_2 fluxes, we calculated trunk respiration rate per squared metre ground area by using the projected trunk area of 0.89 m^2 m^{-2}. Soil- and trunk-respired CO_2 $\delta^{13}C$ values were then weighted by their respective ground area-based CO_2 fluxes, assuming $\delta^{13}C$ of trunk-respired CO_2 did not change with height, as previously shown for pine trees from the same stand (Brandes et al. 2006).

Isotope ratio mass spectrometry measurements

The $\delta^{13}C$ of phloem exudate organic matter and needle water-soluble organic matter was measured as follows:

150- and 100-µl aliquots were taken from the phloem exudation solutions and the needle water-soluble organic matter extracts, respectively. The aliquots were transferred to tin capsules and oven-dried at 60°C for 12 h. Chromosorb W (approximately 10 mg) was added to the tin capsules as sorbent for the liquid before drying. The samples contained on average approximately 400 µg organic C. Samples were combusted in an elemental analyser (NA 2500; CE Instruments, Milan) coupled to an isotope ratio mass spectrometer (Delta[plus]; Finnigan MAT, Bremen) by a Conflo II interface (Finnigan MAT). SD of repeated measurements ($n = 10$) of the laboratory standard was ± 0.1‰.

Exetainers filled with gas samples from soil and trunk CO_2 efflux were measured with a modified Finnigan Gasbench II periphery (Finnigan MAT) equipped with a custom-built cold trap coupled to an isotope ratio mass spectrometer (Delta[plus]XP; Finnigan MAT). The mean precision of the measurement of the laboratory standard was 0.25‰.

The ASA were connected to an isotope-ratio mass spectrometer (Delta[plus]XL; Finnigan MAT) via a gas concentration peripheral (Precon; Finnigan MAT). The switch-valves of the ASA were controlled by the system-software, while the ASA was connected to a He stream that flushed the gas to the Precon. After purification and cryogenic separation, the CO_2 of the air samples was led to the mass spectrometer for $\delta^{13}C$ analysis. Mean precision determined from repeated analysis of reference gas filled in the ASA was 0.2‰. Isotopic values are expressed in δ notation (‰ units), on the Vienna PDB scale.

Needle gas exchange

Needle gas exchange was determined under ambient light and temperature conditions in the upper third of the canopy by inserting small twigs (with their N and $N-1$ needles attached; three replicates per individual tree) in a conifer chamber connected to a portable gas exchange system (GFS3000; Walz, Effeltrich, Germany). Twigs that were next to the ones sampled for isotope analysis were chosen for gas exchange measurements. $N-1$ needles contributed on average approximately 65% of the total needle area in the chamber. Net CO_2 and H_2O exchange rates were measured, and stomatal conductance (g_s) was subsequently calculated according to von Caemmerer and Farquhar (1981). Separate values of leaf level assimilation rate (A) and c_i/c_a were calculated for N and $N-1$ needles (see Barnard et al. 2007 for details), assuming g_s and A of $N-1$ needles to amount to 53 and 61% of N needles (Beadle et al. 1985). The projected area of the N and $N-1$ needles that were inserted in the chamber was determined with a leaf area meter (ΔT Devices, Cambridge, UK), and three-

dimensional leaf area was calculated according to Luoma (1997).

Statistical analyses

Data analyses were performed using SPSS 10.05 (SPSS; Chicago, Ill.) and NCSS 2004 (Number Cruncher Statistical Systems, Kaysville, Utah). Correlations were calculated using bivariate correlation procedures. For multiple regression analyses, the independent variable subset selection was performed applying hierarchical forward switching.

In order to estimate potential time lags between two variables (X, Y), cross-correlation analyses were performed. The cross-correlation between X_t and Y_{t+k} is called the kth order cross-correlation of X and Y. The correlation coefficient (r_k) of the cross-correlation is calculated using the following equation (Kendall and Ord 2006):

$$r_k = \frac{\sum_{i=1}^{n-k} (X_i - \bar{X})(Y_{i+k} - \bar{Y})}{\sqrt{\sum_{i=1}^{n} (X_i - \bar{X})^2 \sum_{i=1}^{n} (Y_i - \bar{Y})^2}} \tag{1}$$

where $\bar{X} = \frac{1}{n}\sum_{i=1}^{n} X_i$ and $\bar{Y} = \frac{1}{n}\sum_{i=1}^{n} Y_i$. Cross-correlations were calculated for the single time points during the diel courses (8 sampling times per 24 h). A time lag of, e.g. $k = 1$, means a shift from (i) to ($i + 1$) of 3 h. For $k = 8$, the time lag is thus 24 h. For $k = 0$, r_k is equivalent to Pearson's correlation coefficient (r).

In order to assess whether the time course of $\delta^{13}C$ in organic matter and CO_2 contained a periodic component, a periodogram was calculated using the spectral analysis function of NCSS 2004 and corrected for series average and trend, according to Brandes et al. (2006) and Barnard et al. (2007).

Results

Meteorological variables and stand transpiration

With the exception of the first day, maximum daily PAR exceeded 1,700 µmol m^{-2} s^{-1} (Fig. 1a) during the sampling period. Mean day and night time air temperatures within the canopy at 12-m height were 14.4 and 9.3°C, respectively (Fig. 1b). The maximum air temperature of 20.6°C was reached in the afternoon on 10 June and the nocturnal minimum (3.4°C) in the early morning of the same day. Air temperature at 2-m height showed a comparable diel rhythm but was slightly lower at night. Mean soil temperature at 3-cm depth was 13.5°C, with a range between the evening maximum and morning minimum of 0.9–3.5°C. Light rainfall occurred during the night between

6 and 7 June (total of 1.88 mm rainfall above canopy, data not shown). Maximum daily VPD at 12-m height amounted to 8.9 hPa on 6 June and increased continuously until 10 June when a maximum value of 16.5 hPa was observed (Fig. 1c). Soil volumetric water content decreased constantly over the measurement period. The minimum daytime value was 20.6% on 6 July and 17.8% on 10 July.

Stand transpiration (for the overstorey trees) closely followed the temporal patterns of VPD at 12-m height. Daily sums of transpired water increased from 0.6 l m^{-2} day^{-1} on 6 June to 1.5 l m^{-2} day^{-1} on 10 June.

Needle, trunk and soil net CO_2 exchange

Needle net CO_2 exchange was calculated for both N and $N-1$ needle cohorts (Fig. 2a). The maximum daytime net photosynthesis rate (5.1 μmol m^{-2} s^{-1}) was measured for N needles on 8 June; the minimum was on 6 June when PAR was also lowest. Nighttime CO_2 efflux ranged between 0.1 and 0.5 μmol m^{-2} s^{-1} for both N and $N-1$ needles.

Fig. 2 Diel variations of **a** net CO_2 exchange (J_{CO_2}) of current year (N; *black squares*) and previous year needles ($N-1$; *white diamonds*) from the upper third of the crown (14–15 m) and **b** net respiration rates (R) of trunk at 1.5-m height (*grey squares*) and soil (*white triangles*). Note that positive values of J_{CO_2} denote net CO_2 uptake by needles, whereas positive values for R denote net CO_2 efflux to the atmosphere. Data are mean values ($\pm$SE in **b**, $n = 3$ in **a** and **b**). The *black bars at the bottom* of the figure indicate the nighttime period

Both trunk and soil respiration rates showed clear diel courses, with minimum rates in the dark period or shortly after sunrise (Fig. 2b). Maximum respiration rates were determined between 0900 hours and sunset. Particularly for trunk respiration rate, the amplitude of variation between diel maxima and minima increased with time. Soil respiration tended to decrease over the measurement period but daily mean values were not significantly different among days.

Diel courses of $\delta^{13}C$ in organic matter and CO_2

We found slight diel variations in $\delta^{13}C$ of water-soluble organic matter, in both N and $N-1$ needles at 14- to 15-m height (Fig. 3a). Water-soluble organic matter showed least negative $\delta^{13}C$ values at the end of the night, and minimum $\delta^{13}C$ values were observed during the light period. The diel range varied between 0.6 and 1.0‰ in N needles and between 0.4 and 0.7‰ in $N-1$ needles. $N-1$ needles were
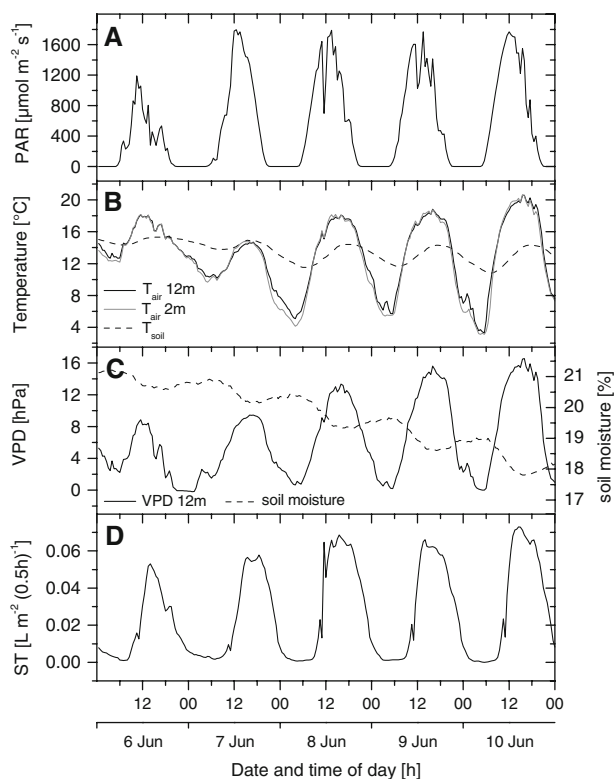

Fig. 1 Diel variation of **a** incident photosynthetically active radiation (*PAR*), **b** air temperature (T_{air}) at 12-m height (*solid black line*), at 2-m height (*solid grey line*) and in the soil (T_{soil}) at 0.03-m depth (*dashed line*), **c** atmospheric water vapour pressure deficit (*VPD*) at 12 m (*solid line*) and volumetric soil moisture (*dotted line*) and **d** stand transpiration (*ST*) rate of the overstorey pine trees
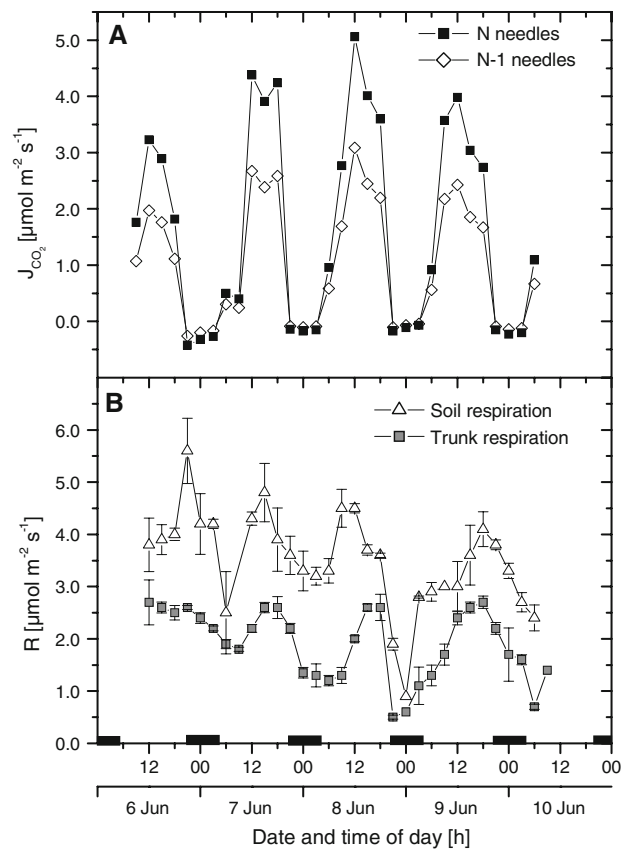
slightly, albeit not significantly, less depleted in ^{13}C compared to N needles. Over the measurement period, we identified no general trend for $\delta^{13}C$ in water-soluble organic matter.

The diel courses of $\delta^{13}C$ in twig phloem exudate organic matter were comparable to those of leaf water-soluble organic matter (Fig. 3b). The range between daytime maximum and nighttime minimum of $\delta^{13}C$ was between 0.6 and 0.9‰. Twig phloem exudate organic matter was significantly enriched in ^{13}C ($P < 0.05$) compared to leaf water-soluble organic matter of both N and $N-1$ needles during the first two day-night cycles, but not for the rest of the measurement period. Trunk phloem exudate organic matter was significantly enriched in ^{13}C compared to twig phloem exudate and needle

Fig. 3 Diel variations of $\delta^{13}C$ in **a** needle water-soluble organic matter (*OM*) [*leaf ws OM*; *N* needles (*black squares*), *N*−1 needles (*white diamonds*)], **b** bulk phloem exudate OM (*phloem OM*) at twig level (*black triangles*) and trunk level at 1.5-m height (*white squares*), **c** CO_2 respired from trunks at 1.5-m height (*grey squares*) and **d** CO_2 from soil (*white triangles*) and ecosystem (*black and white shaded circles*) respiration. Data are mean ± SE ($n = 3$). *Black bars* at the bottom of the figure indicate the nighttime period. Note the difference in scale of the *y*-axes between plots **a** and **b** vs. **c** and **d**. For other abbreviations, see Fig. 2

water-soluble organic matter ($P < 0.01$), but showed no clear diurnal pattern.

Mean R^2-values for soil and trunk Keeling plots were 0.971 ± 0.053 and 0.967 ± 0.059, respectively. The $\delta^{13}C$ of trunk-respired CO_2 showed clear diel patterns on the first 2 measurement days, with lowest $\delta^{13}C$ values in the evening (between 1800 and 2000 hours) and maximum $\delta^{13}C$ values in the morning between 0300 and 0600 hours (Fig. 3c). The maximum diel range of variation in $\delta^{13}C$ was 4.0‰. A morning peak for $\delta^{13}C$ was not observed on the 9 June but again on 10 June, when $\delta^{13}C$ in trunk-respired CO_2 increased from −25.7‰ at 0000 hours to −22.9‰ at 0600 hours. The $\delta^{13}C$ of soil-respired CO_2 showed a diel course that was slightly phase-shifted compared to trunk respiration. Minimum $\delta^{13}C$ values were generally observed around midnight and $\delta^{13}C$ maxima between 1200 and 1500 hours. Mean diel range of variation was 2.7‰.

The general lack of a CO_2 concentration gradient in the forest vertical profile during daytime, and the rain event at the beginning of the sampling period, resulted in poor regressions during daytime, thereby restricting most of our measurements of $\delta^{13}C$ of ecosystem-respired CO_2 to the last 3 nights of the campaign. The average and SD of the R^2 of the regressions used to determine the $\delta^{13}C_{R\ eco}$ data presented here was 0.993 ± 0.014. $\delta^{13}C_{R\ eco}$ varied by up to 6.1‰ within 9 h but showed no clear temporal pattern over the measurement period.

Periodicity of $\delta^{13}C$ in CO_2 and organic matter

Spectral data analysis indicated a clear 24-h periodicity [i.e. a maximum for $I(f)$ at a wavelength of approximately 8] of $\delta^{13}C$ in water-soluble organic matter from N and $N-1$ needles as well as in twig phloem exudate organic matter (Fig. 4a). In contrast, we observed no obvious periodicity for $\delta^{13}C$ in trunk phloem exudate organic matter, as indicated by the even distribution of $I(f)$. We found a 24-h periodicity for $\delta^{13}C$ in trunk and soil CO_2 efflux (Fig. 4b). Despite the common periodicity pattern for these two CO_2 sources (albeit phase shifted, cf. Fig. 3c, d), no significant correlation between them could be shown with any time lag between 0 and 12 h (Fig. 5). It might be assumed that CO_2 originating from belowground (thus carrying a C isotopic signature that is comparable to that of soil-emitted CO_2) and transported within the xylem stream contributes significantly to CO_2 emitted from stems (see review by Teskey et al. 2008). In that case, the changes in xylem flow rate should modify the relation between $\delta^{13}C$ of soil-emitted and trunk-emitted CO_2. However, a multiple regression analysis with xylem sap flow rate and $\delta^{13}C$ of soil respiration as independent factors could only explain 12% of the variation of trunk-emitted CO_2.
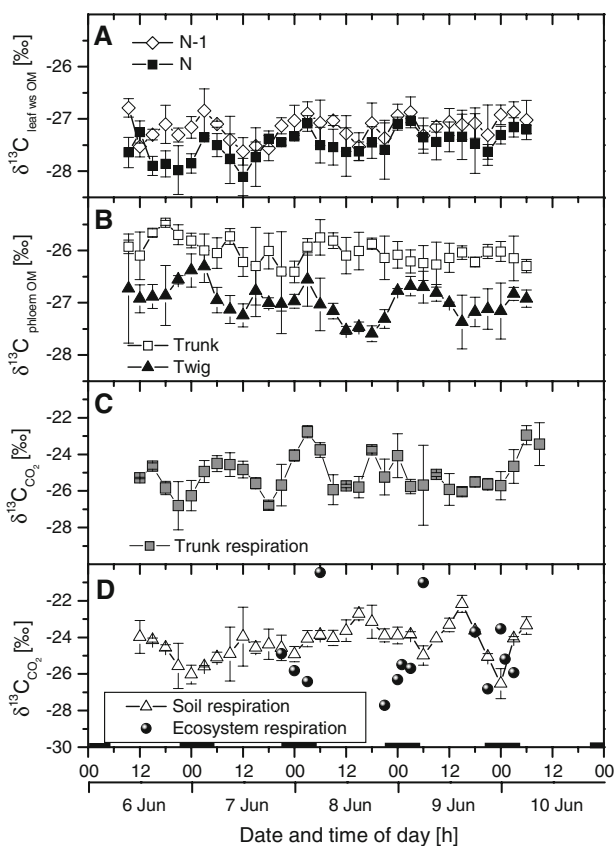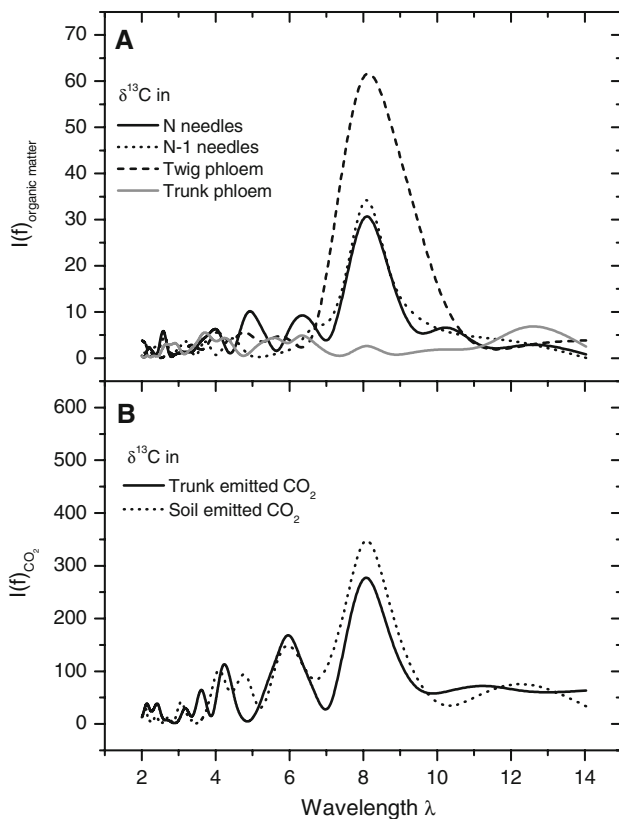
Fig. 4 Periodogram in the wavelength scale for the time series of $\delta^{13}C$ in OM pools (**a**) and in CO_2 respired by trunk and soil (**b**). **a** Periodogram of $\delta^{13}C$ of water-soluble OM in N needles (*solid line*) and $N-1$ needles (*dotted line*) as well as phloem exudate organic matter from twigs (*dashed line*) and trunk (*grey solid line*). **b** Periodogram of $\delta^{13}C$ in CO_2 respired by trunk (*solid line*) and soil (*dotted line*). A wavelength of 8 corresponds to a 24-h periodicity. Note that $I(f)$ is dimensionless. For abbreviations, see Figs. 2 and 3

$\delta^{13}C$ in CO_2 respired in relation to organic matter and environmental variables

In order to trace the fate of newly assimilated C within the trees and the whole ecosystem, we performed time series cross-correlation between the $\delta^{13}C$ values of the different organic matter pools and respiratory CO_2 along the C transport pathway from needle water-soluble organic matter to soil CO_2 efflux (Fig. 5). To estimate the influence of environmental (air and soil temperature, VPD, PAR, volumetric soil water content) and physiological parameters (transpiration rate, needle-level CO_2 exchange, as well as trunk and soil respiration rates) on $\delta^{13}C$ of soil- and trunk-respired CO_2, additional cross-correlation analyses were performed for these variables (Fig. 5). Applying multiple regression models did not improve the coefficient of determination, compared to single-variable models.

Twig phloem exudate organic matter was significantly correlated with water-soluble organic matter from both N

and $N-1$ needles (Fig. 5). The highest correlation coefficients were obtained when no time lag was assumed. There was, however, no correlation between twig and trunk phloem exudate organic matter or between $\delta^{13}C$ of soil- or trunk-respired CO_2 and $\delta^{13}C$ of trunk phloem exudate organic matter (no matter whether time lags were applied or not).

The $\delta^{13}C$ of trunk-respired CO_2 was negatively correlated to trunk respiration rate and to air temperature at 2-m height with no time lag, as well as to stand transpiration with a lag of 3 h (Fig. 5). Soil and trunk respiration rates were significantly positively correlated (assuming no time lag) with soil and air temperature, respectively. The $\delta^{13}C$ of soil-respired CO_2 showed significant correlations: with (1) volumetric soil water content (with no time lag), and (2) soil temperature with a time lag of 6 h (Fig. 5).

When separating day and night values for Pearson's correlation analysis, we found no changes in correlation coefficient for trunk respiration rate and $\delta^{13}C$ of trunk-respired CO_2. However, $\delta^{13}C$ of nighttime soil CO_2 efflux was significantly correlated to soil respiration rate ($R = -0.664$; $P = 0.018$) whereas during the daytime no such correlation was observed. When considering only daytime values, the correlation between the $\delta^{13}C$ of soil-respired CO_2 and soil water content was stronger ($R = -0.72$; $P < 0.001$) than for the analysis of the whole diel courses.

We found no significant correlation between $\delta^{13}C_{R\ eco}$ and any $\delta^{13}C$ value of the organic C pool or CO_2. In addition, there was neither a significant correlation of $\delta^{13}C_{R\ eco}$ with stand transpiration, needle-level CO_2 gas exchange, trunk and soil respiration rate, nor with any environmental parameter measured. The flux-weighted average $\delta^{13}C$ of trunk- and soil-respired CO_2 was not significantly correlated with $\delta^{13}C_{R\ eco}$.

In order to relate the apparent C isotope fractionation during trunk respiration to trunk respiration rate, we calculated the difference between $\delta^{13}C$ of trunk phloem exudate organic matter, the putative substrate for respiration and trunk-respired CO_2. We found a trend for the $\delta^{13}C$ of trunk-respired CO_2 to be strongly enriched in ^{13}C (by up to $>3\permil$) compared to $\delta^{13}C$ of phloem exudate organic matter when respiration rates were low, and to be comparable to $\delta^{13}C$ of phloem exudate organic matter when respiration rates were highest (Fig. 6). Since we assume phloem organic matter to be a potential substrate for soil respiration, we also calculated the difference between $\delta^{13}C$ of trunk phloem exudate organic matter and of soil-respired CO_2. We found, however, no significant relation between this difference and soil respiration rates (data not shown). In order to account for potential time lags between $\delta^{13}C$ of CO_2 and phloem organic matter due to the time for transport of phloem sugars belowground, we also correlated $\delta^{13}C_{trunk\ pholem\ OM} - \delta^{13}C_{CO_2\ soil}$ with R_{soil}, using $\delta^{13}C_{trunk}$

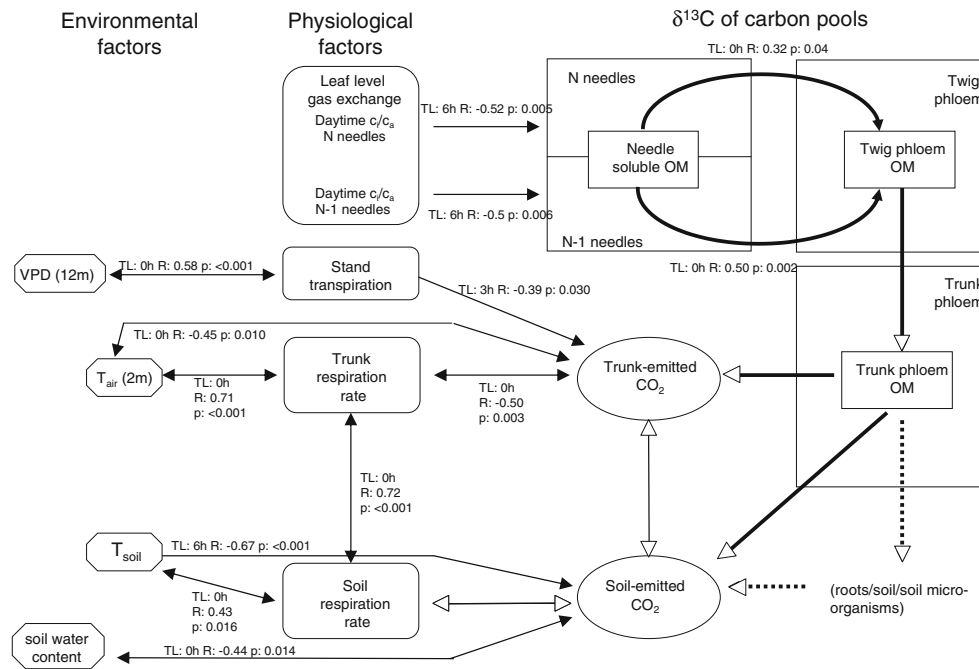**Fig. 5** Cross-correlations between $\delta^{13}C$ of C pools as C is transported from the needles to the trunk, and between trunk phloem exudate OM—as a putative source for respiration—and respired CO_2 (*right side*) as well as cross-correlations between $\delta^{13}C$ in CO_2 and physiological and environmental factors (*left side*). *Bold lines with one arrow head* indicate the assumed direction of C fluxes. *Thin lines with two arrow heads* indicate cross-correlations with a time lag of 0 h between different factors without a priori anticipating an effective direction. *Thin lines with one arrow head* indicate cross-correlations with time lags. Arrows point to the variable lagging behind. *White arrowheads* indicate missing significant correlation between two parameters. Only those environmental variables are shown which were significantly related to $\delta^{13}C$ of CO_2, OM or a physiological parameter. *TL* time lag, T_{air} (2 m) air temperature at 2-m height, VPD (12 m) VPD at 12-m height, T_{soil} soil temperature. For other abbreviations, see Figs. 1 and 3
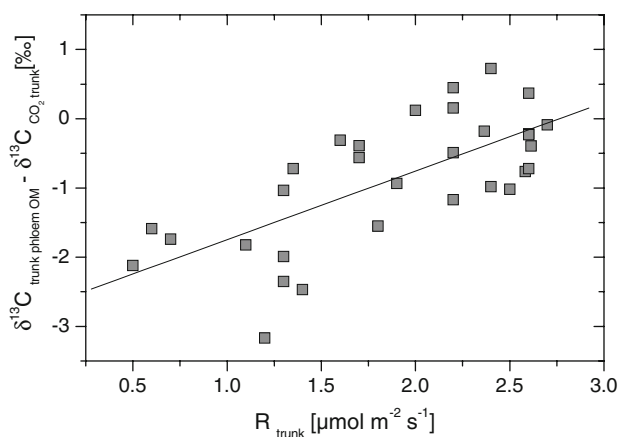

Fig. 6 Difference between $\delta^{13}C$ of trunk phloem OM ($\delta^{13}C_{trunk\ phloem\ OM}$) and of trunk-respired CO_2 ($\delta^{13}C_{CO_2\ trunk}$) plotted against trunk respiration rate (R_{trunk}). Data shown are mean values ($n = 3$). Regression line equation: $\delta^{13}C_{trunk\ phloem} - \delta^{13}C_{CO_2\ trunk}$ [‰] $= 0.98$ [‰ μ mol^{-1}m^{2}s] $\times R_{trunk}$[μ mol m^{-2}s^{-1}] $- 2.74$[‰] ($R^2 = 0.48$; $P < 0.001$)

$_{phloem\ OM}$ 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 h before the actually measured $\delta^{13}C_{CO_2\ soil}$. Independent of the time-lag applied there was no significant relation between

$\delta^{13}C_{trunk\ phloem\ OM} - \delta^{13}C_{CO_2\ trunk}$; and soil respiration rate (data not shown).

Discussion

We aimed to trace the fate of the isotopic signal of recently assimilated organic C during its transport from the canopy down the trunk and during respiratory conversion to CO_2 during the diel course. The main result was that the distinct diel variations in $\delta^{13}C$ observed in newly assimilated organic matter in the tree crown were strongly dampened during phloem transport to the trunk but that a 24-h periodicity was again observed in the $\delta^{13}C$ of trunk- and soil-respired CO_2.

Diel variations in $\delta^{13}C$ of water-soluble organic matter were dampened during transport from the canopy to the trunk base

Soluble organic matter of both needle age classes, as well as twig phloem exudate organic matter, showed clear diel variations. The highest $\delta^{13}C$ values were observed during the dark period and the most negative values during the day

(Fig. 3). Part of this variation was explained by c_i/c_a during the time of the day when net assimilation occurred. Cross-correlation analyses exhibited a 6-h time lag between c_i/c_a and $\delta^{13}C$ (Fig. 5) that is consistent with results of a previous study tracing $\delta^{18}O$ at the same site (Barnard et al. 2007). These authors showed that the turnover time of leaf sugars caused the $\delta^{18}O$ signature of leaf water to be imprinted on the leaf sugars with a comparable time lag. However, c_i/c_a alone could explain only part (approximately 25%) of the changes in $\delta^{13}C$ of needle water-soluble organic matter, for two reasons. First, variation of c_i/c_a in the morning and evening, when light intensity and assimilation rate are low, may result in c_i/c_a measurements that are not representative of the longer time period that the $\delta^{13}C$ values of leaf sugars integrate over (Gessler et al. 2007a). Second, the diel patterns of $\delta^{13}C$ of needle and phloem sugars have been found to depend strongly on post-carboxylation fractionation (Brandes et al. 2006), with more negative $\delta^{13}C$ values during the day when starch is synthesised and more positive values during the night when ^{13}C-enriched starch is remobilised (Gleixner and Schmidt 1997; Tcherkez et al. 2004). In addition, it has to be acknowledged that leaf water-soluble organic matter as extracted here does not only consist of sugars but also of organic acids, amino acids and other compounds—a fact that could additionally complicate the interpretation of isotopic data. However, Brandes et al. (2006) showed that soluble organic matter and sugars in *P. sylvestris* needles had a similar ^{13}C signature.

As organic matter was transported from the canopy down the trunk to the C sinks in the tree, the 24-h periodicity of $\delta^{13}C$ disappeared almost completely (Fig. 4a). As a consequence, no correlation between twig and trunk phloem exudate was observed (Fig. 5). The lack of a day-night periodicity in $\delta^{13}C$ of trunk phloem organic matter of *Pinus sylvestris* is in agreement with results from Betson et al. (2007) in *Picea abies* and from Gessler et al. (2007a) in *Eucalyptus delegatensis*. The dampening of the diel $\delta^{13}C$ variations in phloem-transported organic matter at the trunk base observed here can be explained by a mixing of various C pools with different metabolic histories during phloem transport down the trunk (Brandes et al. 2006). This hypothesis is supported by findings of Keitel et al. (2003, 2006) and Brandes et al. (2007), showing that $\delta^{13}C$ in trunk phloem sap organic matter integrates mean canopy c_i/c_a over several days in European beech and Scots pine, respectively.

In contrast to trunk phloem organic matter, the $\delta^{13}C$ of trunk-respired CO_2 showed a clear diel periodicity

Our study showed large diel variations of up to 4‰ in the $\delta^{13}C$ of trunk-respired CO_2, while no comparable variations were observed in the $\delta^{13}C$ of phloem-transported organic C. Two possible hypotheses could explain this result. First, the substrate respired could change over time, switching from phloem sugars to another C source. A shortage of readily available carbohydrates is typically required for either the remobilisation of sugars from storage starch or for organic compounds other than carbohydrates (especially fatty acids; Tcherkez et al. 2003) to be respired. Insufficient C supply by phloem transport is very unlikely since phloem exudate organic C content did not change during the day-night cycle (data not shown). A switch from carbohydrates to fatty acids was also not likely to occur during our measurement period, since fatty acids were found to play only a minor role in C storage in Scots pine in the summer (Fischer and Holl 1991). A significant and temporally varying contribution of xylem sugars with a $\delta^{13}C$ signature that is potentially different from that of phloem sugars is also an unlikely reason for the variation in $\delta^{13}C$ of CO_2. Gessler et al (2007b) showed that xylem-transported sugars matched well in their (oxygen) isotope signature with phloem sugars, pointing to xylem sugars to originate from direct phloem-to-xylem exchange. As a consequence, different $\delta^{13}C$ values in the two pools are unlikely. In addition, newly developing xylem tissue has the same isotopic composition as phloem sugars (Cernusak et al. 2005), thus indicating the phloem pool as the ultimate C source for trunk tissues. Second, changes in the respiratory C isotope fractionation could occur over time. Such changes have been documented in leaves (Hymus et al. 2005; Prater et al. 2006; Werner et al. 2007) and trunks (Maunoury et al. 2007) of trees over a diel cycle. Our study showed a close correlation between the $\delta^{13}C$ of trunk-respired CO_2 and both respiration rate and air temperature. This is consistent with other studies that addressed the controls of the ^{13}C signature of respired CO_2 (Tcherkez et al. 2003; Maunoury et al. 2007) over longer time spans. Moreover, we found that apparent C isotope fractionation (calculated as $\delta^{13}C_{trunk\ phloem\ OM} - \delta^{13}C_{CO_2\ trunk}$; cf. Fig. 6) was highest (i.e. discrimination as calculated in Fig. 6 was the most negative) when respiration rate—which in turn was driven by temperature—was low and vice versa. Hymus et al. (2005) explained observed diel variations in $\delta^{13}C$ of CO_2 respired from leaves of two oak species by a shift between the relative contributions of CO_2 released from decarboxylation of pyruvate (originating from glycolysis) and CO_2 derived from the citrate cycle, without a change in the organic substrate for respiration. This is a consequence of fragmentation fractionation: the CO_2 released by the decarboxylation of pyruvate originates from the relatively ^{13}C-enriched C-3 and C-4 atoms of a glucose molecule, whereas the CO_2 emitted from citrate cycle decarboxylation reactions originates from the C-1, C-2, C-5 and C-6 atoms, which are relatively ^{13}C-depleted compared to

glucose (Rossmann et al. 1991; Tcherkez et al. 2003). Glycolysis (and thus decarboxylation of pyruvate) is less temperature-dependent than mitochondrial oxidation (citrate cycle) (Berry and Raison 1981; Atkin et al. 2000) and, thus, the relative contribution of CO_2 from glycolysis to total respiration is likely to increase at lower temperatures and subsequent lower respiration rates, resulting in higher apparent isotope fractionation. In contrast, at higher temperatures, a relatively higher contribution of citrate cycle-derived CO_2 to total respiration would not only result in increased respiration rates but also in apparent fractionation approaching zero when complete oxidation of the substrate is assumed (for a review see Ghashghaie et al. 2003). In conclusion, the diel pattern of $\delta^{13}C$ of trunk-respired CO_2 that we observed does not seem to be due to changes in the ^{13}C signature of the respired organic substrate, but most probably due to changes in respiratory C isotope fragmentation fractionation over the diel course.

The $\delta^{13}C$ of soil-respired CO_2 showed diel variations that are related to temperature and humidity

The $\delta^{13}C$ in CO_2 respired from soil displayed variations with a 24-h periodicity (Fig. 4), that were comparable to trunk respiration (albeit with a time shift; Fig. 3). Despite that common periodicity, $\delta^{13}C$ of trunk- and soil-respired CO_2 were not correlated (Fig. 5), as the range of variation of $\delta^{13}C$ was not consistent between the two CO_2 sources and overall trends were different over the period of observation. In addition, changes in xylem flow rate did not modify the relation between $\delta^{13}C$ of soil- and trunk-emitted CO_2. Thus, we conclude that $\delta^{13}C$ of trunk-respired CO_2 is not mainly determined by belowground-produced and xylem-transported CO_2, in contrast to observations on other species (Teskey and McGuire 2005). However, our measurement of soil-respired CO_2 integrates different CO_2 sources, therefore and despite the lack of correlation between $\delta^{13}C$ in soil and trunk-emitted CO_2, we cannot rule out the possibility that one (or several) of these sources (e.g. root or rhizosphere respiration) contribute(s) to stem CO_2 emission.

Our findings of diel variations in flux rate and $\delta^{13}C$ of soil respiration differ from the recent results of Betson et al. (2007), who measured no diel cycle for soil CO_2 efflux rate and ^{13}C signature in a boreal forest. These authors suggested that, in their study, the short nights (2–4 h) could prevent intensive exhaustion of photosynthate supply to the roots from the canopy, which could otherwise influence rate and $\delta^{13}C$ of soil CO_2 efflux. However, studies of root carbohydrate contents in adult coniferous and deciduous trees in temperate forests gave no indications of nighttime sugar depletion in roots (Gessler et al. 2002), hence we do not assume variations in carbohydrate supply to be a major

factor controlling short-term changes in the $\delta^{13}C$ of soil-respired CO_2 in our ecosystem. In contrast to Betson et al. (2007), we observed no correlation between $\delta^{13}C$ of trunk phloem-transported organic matter and soil-respired CO_2, and consequently cannot conclude that a direct coupling between these two pools occurs over the diel course.

In our study, the $\delta^{13}C$ of soil CO_2 efflux was correlated with soil temperature (6 h time-lag) and with soil moisture (no lag). Such changes in soil temperature and water content likely affect heterotrophic and autotrophic soil respiration differently (Boone et al. 1998; Bhupinderpal et al. 2003; Ekblad et al. 2005; Heinemeyer et al. 2007). In addition, several studies have shown that CO_2 respired by soil microorganisms and plant roots differs in its ^{13}C signature (e.g. Bhupinderpal et al. 2003; Gessler et al. 2007a). As a consequence, the changes in the $\delta^{13}C$ of soil-respired CO_2 that we measured over the diel course could be due to changes in the relative contributions of heterotrophic and autotrophic CO_2 fluxes to total soil efflux in response to environmental conditions. Multiple regression models for $\delta^{13}C$ of soil CO_2 considering concerted effects of soil water content, soil and air temperature, soil respiration rate and $\delta^{13}C$ of trunk phloem organic matter did not explain significantly more of the variability than the single regressions presented in Fig. 5, suggesting more complex interactions and additional possible drivers at play.

Although we acknowledge that the organic matter transported in the trunk phloem is not the direct organic source for soil respiration, we have to assume that phloem-transported sugars supply roots and soil microorganisms with rapidly metabolisable C. We hypothesise that in addition to direct environmental effects, changes in the substrates respired by both the soil microbial community and the roots may result in changes in the $\delta^{13}C$ of soil CO_2 efflux. In particular, changes in soil water content can alter substrate diffusion and availability for the soil microbial community, thereby influencing the $\delta^{13}C$ of heterotrophic respiration. As an additional factor, changes in soil moisture also affect the transport time of gas-phase soil CO_2 (Stoy et al. 2007), thus potentially altering the time lag between belowground respiration and CO_2 emission from the soil surface. In addition, as explained for trunk respiration, we might not only have to consider a shift in the relative contribution of heterotrophic and autotrophic respiration during the diel course, but changes in fragmentation fractionation during respiration of both soil microorganisms and roots.

$\delta^{13}C_{R\,eco}$ displayed much higher short-term variations than the $\delta^{13}C$ of trunk- and soil-respired CO_2

The 6.1‰ maximum variation of $\delta^{13}C_{R\,eco}$ that we measured within 1 night is comparable with observations in

other ecosystems (Bowling et al. 2003: 6.4‰, grassland; Knohl et al. 2005: 3.8‰, mixed deciduous forest; Werner et al. 2006: 4.2‰, oak woodland).

Although we calculated the partitioning of NEE into its respiratory and assimilatory components (based on isotopes and eddy covariance), the isotopic disequilibrium between canopy assimilation and ecosystem respiration was too small (approximately 1‰) to warrant satisfactory results (data not shown). As a consequence, we could not quantify the potential error that highly variable $\delta^{13}C_{R\,eco}$ introduces in NEE partitioning. Ogée et al. (2004) showed that when the isotopic disequilibrium is small, the large uncertainty in flux partitioning is not reduced when $\delta^{13}C_{R\,eco}$ is estimated more accurately. Under conditions of stronger isotopic disequilibrium, however, a consideration of the variability of $\delta^{13}C_{R\,eco}$ is likely to improve the accuracy of the isoflux approach (Ogée et al. 2004).

The variability of $\delta^{13}C_{R\,eco}$ determined in our study exceeded that of $\delta^{13}C$ of trunk- and soil-respired CO_2 (4 and 2.7‰, respectively). In addition, the flux-weighted average $\delta^{13}C$ of trunk- and soil-respired CO_2 could not explain variations in $\delta^{13}C_{R\,eco}$. These results point towards a major contribution of $\delta^{13}C$ of foliage respiration to the diel variation in $\delta^{13}C_{R\,eco}$ over the measurement campaign in our pine forest. These findings are consistent with those of Mortazavi et al. (2005) who observed that in a 17.2-m-tall evenly aged loblolly pine plantation, $\delta^{13}C_{R\,eco}$ was determined by the ^{13}C signature of foliage-respired CO_2, in stark contrast to a mixed broadleaf forest where $\delta^{13}C_{R\,eco}$ was clearly dominated by the ^{13}C signature of forest floor CO_2 efflux.

To estimate the relative contribution of foliage respiration to ecosystem respiration, we scaled up leaf-level respiration (Fig. 2) to a ground area basis (µmol m^{-2} ground area s^{-1}), assuming an overstorey LAI of 1.47 m^2 m^{-2} (Brandes et al. 2006) and a 50% contribution of both needle age classes (Barnard et al. 2007), and related this to ground area-based soil and trunk respiration fluxes. We acknowledge that this extrapolation might introduce some scaling error, and that the respiration of understorey species, which was not examined for $\delta^{13}C$ in respired CO_2 in our study, might also contribute to the observed variation in $\delta^{13}C_{R\,eco}$, and therefore consider this contribution of foliage respiration only a rough estimation. Based on this calculation, nighttime foliage respiration might have contributed to between 20 and 62% of total ground area-based respiration (soil respiration + trunk respiration + needle respiration) during our measurement period. C isotope discrimination of foliar respiration and consequently the $\delta^{13}C$ of leaf-respired CO_2 can vary by up to 8‰ in other tree species during the diel course (Hymus et al. 2005; Werner et al. 2007), and thus displays an even higher variability than that of $\delta^{13}C$ of trunk- and soil-respired CO_2

observed in the present study. Thus, the large variations of $\delta^{13}C_{R\,eco}$ can be explained by highly variable contributions of foliar respiration to total ecosystem respiration, combined with strong diel changes in $\delta^{13}C$ of leaf-respired CO_2.

References

Atkin OK, Edwards EJ, Loveys BR (2000) Response of root respiration to changes in temperature and its relevance to global warming. New Phytol 147:141–154

Badeck FW, Tcherkez G, Nogues S, Piel C, Ghashghaie J (2005) Post-photosynthetic fractionation of stable carbon isotopes between plant organs—a widespread phenomenon. Rapid Commun Mass Spectrom 19:1381–1391

Barnard RL, Salmon Y, Kodama N, Sörgel K, Holst J, Rennenberg H, Gessler A, Buchmann N (2007) Evaporative enrichment and time lags between $\delta^{18}O$ of leaf water and organic pools in a pine stand. Plant Cell Environ 30:539–550

Beadle CL, Neilson RE, Talbot H, Jarvis PG (1985) Stomatal conductance and photosynthesis in a mature Scots pine forest. 1. Diurnal, seasonal and spatial variations in shoots. J Appl Ecol 22:557–571

Berry JA, Raison JK (1981) Responses of macrophytes to temperature. In: Lange OL, Nobel PS, Osmond B, Ziegler H (eds) Physiological plant ecology. I. Responses to the physical environment. Springer, Berlin, pp 277–338

Betson NR, Göttlicher SG, Hall M, Wallin G, Richter A, Högberg P (2007) No diurnal variation in rate or carbon isotope composition of soil respiration in a boreal forest. Tree Physiol 27:749–756

Bhupinderpal S, Nordgren A, Lofvenius MO, Högberg MN, Mellander PE, Hogberg P (2003) Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. Plant Cell Environ 26:1287–1296

Boone RD, Nadelhoffer KJ, Canary JD, Kaye JP (1998) Roots exert a strong influence on the temperature sensitivity of soil respiration. Nature 396:570–572

Bowling DR, Tans PP, Monson RK (2001) Partitioning net ecosystem carbon exchange with isotopic fluxes of CO_2. Global Change Biol 7:127–145

Bowling DR, McDowell NG, Bond BJ, Law BE, Ehleringer JR (2002) ^{13}C content of ecosystem respiration is linked to precipitation and vapor pressure deficit. Oecologia 131:113–124

Bowling DR, Sargent SD, Tanner BD, Ehleringer JR (2003) Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem-atmosphere CO_2 exchange. Agric For Meteorol 118:1–19

Brandes E, Kodama N, Whittaker K, Weston C, Rennenberg H, Keitel C, Adams MA, Gessler A (2006) Short-term variation in the isotopic composition of organic matter allocated from the leaves

to the stem of *Pinus sylvestris*: effects of photosynthetic and postphotosynthetic carbon isotope fractionation. Global Change Biol 12:1922–1939

Brandes E, Wenninger J, Koeniger P, Schindler D, Rennenberg H, Leibundgut C, Mayer H, Gessler A (2007) Assessing environmental and physiological controls over water relations in a Scots pine (*Pinus sylvestris* L.) stand through analyses of stable isotope composition of water and organic matter. Plant Cell Environ 30:113–127

Carbone MS, Trumbore SE (2007) Contribution of new photosynthetic assimilates to respiration by perennial grasses and shrubs: residence times and allocation patterns. New Phytol 176:124–135

Cernusak LA, Farquhar GD, Pate JS (2005) Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus globulus*. Tree Physiol 25:129–146

Damesin C, Lelarge C (2003) Carbon isotope composition of current-year shoots from *Fagus sylvatica* in relation to growth, respiration and use of reserves. Plant Cell Environ 26:207–219

Damesin C, Barbaroux C, Berveiller D, Lelarge C, Chaves M, Maguas C, Maia R, Pontailler JY (2005) The carbon isotope composition of CO_2 respired by trunks: comparison of four sampling methods. Rapid Commun Mass Spectrom 19:369–374

Ekblad A, Högberg P (2000) Analysis of $\delta^{13}C$ of CO_2 distinguishes between microbial respiration of added C_4-sucrose and other soil respiration in a C_3-ecosystem. Plant Soil 219:197–209

Ekblad A, Högberg P (2001) Natural abundance of ^{13}C in CO_2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration. Oecologia 127:305–308

Ekblad A, Boström B, Holm A, Comstedt D (2005) Forest soil respiration rate and $\delta^{13}C$ is regulated by recent above ground weather conditions. Oecologia 143:136–142

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the inter-cellular carbon-dioxide concentration in leaves. Aust J Plant Physiol 9:121–137

Fischer C, Holl W (1991) Food reserves of Scots pine (*Pinus sylvestris* L.). 1. Seasonal changes in the carbohydrate and fat reserves of pine needles. Trees Struc Funct 5:187–195

Gessler A, Kreuzwieser J, Dopatka T, Rennenberg H (2002) Diurnal courses of ammonium net uptake by the roots of adult beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees. Plant Soil 240:23–32

Gessler A, Rennenberg H, Keitel C (2004) Stable isotope composition of organic compounds transported in the phloem of European beech—evaluation of different methods of phloem sap collection and assessment of gradients in carbon isotope composition during leaf-to-stem transport. Plant Biol 6:721–729

Gessler A, Keitel C, Kodama N, Weston C, Winters AJ, Keith H, Grice K, Leuning R, Farquhar GD (2007a) $\delta^{13}C$ of organic matter transported from the leaves to the roots in *Eucalyptus delegatensis*: short-term variations and relation to respired CO_2. Funct Plant Biol 34:692–706

Gessler A, Peuke AD, Keitel C, Farquhar GD (2007b) Oxygen isotope enrichment of organic matter in Ricinus communis during the diel course and as affected by assimilate transport. New Phytol 174:600–613

Ghashghaie J, Duranceau M, Badeck FW, Cornic G, Adeline MT, Deléens E (2001) $\delta^{13}C$ of CO_2 respired in the dark in relation to $\delta^{13}C$ of leaf metabolites: comparison between *Nicotiana sylvestris* and *Helianthus annuus* under drought. Plant Cell Environ 24:505–515

Ghashghaie J, Badeck FW, Lanigan G, Nogués S, Tcherkez G, Deléens E, Cornic G, Griffiths H. (2003) Carbon isotope

discrimination during dark respiration and photorespiration in C_3 plants. Phytochem Rev 2:145–161

Gleixner G, Schmidt HL (1997) Carbon isotope effects on the fructose-1,6-bisphosphate aldolase reaction, origin for non-statistical ^{13}C distributions in carbohydrates. J Biol Chem 272:5382–5387

Göttlicher S, Knohl A, Wanek W, Buchmann N, Richter A (2006) Short-term changes in carbon isotope composition of soluble carbohydrates and starch: from canopy leaves to the root system. Rapid Commun Mass Spectrom 20:653–660

Granier A (1985) A new method of sap flow measurement in tree stems. Ann Sci For 42:193–200

Granier A (1987) Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. Tree Physiol 3:309–319

Granier A, Biron P, Breda N, Pontailler JY, Saugier B (1996) Transpiration of trees and forest stands: Short and longterm monitoring using sapflow methods. Global Change Biol 2:265–274

Heinemeyer A, Hartley IP, Evans SP, De la Fuente JAC, Ineson P (2007) Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. Global Change Biol 13:1786–1797

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Lofvenius M, Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789–792

Hymus GJ, Maseyk K, Valentini R, Yakir D (2005) Large daily variation in ^{13}C-enrichment of leaf-respired CO_2 in two *Quercus* forest canopies. New Phytol 167:377–384

IPCC (2007) Climate change 2007—The physical science basis. Contribution of working Group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, 996

Johnsen K, Maier C, Sanchez F, Anderson P, Butnor J, Waring R, Linder S (2007) Physiological girdling of pine trees via phloem chilling: proof of concept. Plant Cell Environ 30:128–134

Keeling CD (1958) The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochim Cosmochim Acta 13:322–334

Keeling CD (1961) The concentration and isotopic abundances of carbon dioxide in rural and marine air. Geochim Cosmochim Acta 24:277–298

Keitel C, Adams MA, Holst T, Matzarakis A, Mayer H, Rennenberg H, Gessler A (2003) Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L.). Plant Cell Environ 26:1157–1168

Keitel C, Matzarakis A, Rennenberg H, Gessler A (2006) Carbon isotope composition and oxygen isotope enrichment in phloem and total leaf organic matter of European beech (*Fagus sylvatica* L.) along a climate gradient. Plant Cell Environ 29:1492–1507

Kendall SM, Ord JK (2006) Time series. Oxford University Press, New York

Knohl A, Buchmann N (2005) Partitioning the net CO_2 flux of a deciduous forest into respiration and assimilation using stable carbon isotopes. Global Biogeochem Cycles 19

Knohl A, Werner RA, Brand WA, Buchmann N (2005) Short-term variations in $\delta^{13}C$ of ecosystem respiration reveals link between assimilation and respiration in a deciduous forest. Oecologia 142:70–82

Lancaster J (1990) ^{13}C fractionation in carbon dioxide emitting diurnally from soils and vegetation at ten sites on the North American continent. PhD Thesis, University of California, San Diego

Luoma S (1997) Geographical pattern in photosynthetic light response of *Pinus sylvestris* in Europe. Funct Ecol 11:273–281

Maunoury F, Berveiller D, Lelarge C, Pontailler JY, Vanbostal L, Damesin C (2007) Seasonal, daily and diurnal variations in the stable carbon isotope composition of carbon dioxide respired by tree trunks in a deciduous oak forest. Oecologia 151:268–279

Mayer H, Jaeger L, Matzarakis A, Fernbach G, Redepenning D (2000) Forstmeteorologische Messstelle Hartheim des Meteorologischen Instituts der Universität Freiburg. Ber Meteorol Inst Univ Freiburg 5:55–83

Mortazavi B, Chanton JP, Prater JL, Oishi AC, Oren R, Katul G (2005) Temporal variability in ^{13}C of respired CO_2 in a pine and a hardwood forest subject to similar climatic conditions. Oecologia 142:57–69

Nabuurs GJ, Schelhaas MJ, Mohren GMJ, Field CB (2003) Temporal evolution of the European forest sector carbon sink from 1950 to 1999. Global Change Biol 9:152–160

Ogée J, Peylin P, Ciais P, Bariac T, Brunet Y, Berbigier P, Roche C, Richard P, Bardoux G, Bonnefond JM (2003) Partitioning net ecosystem carbon exchange into net assimilation and respiration using $^{13}CO_2$ measurements: a cost-effective sampling strategy. Global Biogeochem Cycles 17

Ogée J, Peylin P, Cuntz M, Bariac T, Brunet Y, Berbigier P, Richard P, Ciais P (2004) Partitioning net ecosystem carbon exchange into net assimilation and respiration with canopy-scale isotopic measurements: an error propagation analysis with $^{13}CO_2$ and $CO^{18}O$ data. Global Biogeochem Cycles 18:GB2019

Pataki DE, Ehleringer JR, Flanagan LB, Yakir D, Bowling DR, Still CJ, Buchmann N, Kaplan JO, Berry JA (2003) The application and interpretation of Keeling plots in terrestrial carbon cycle research. Global Biogeochem Cycles 17

Prater JL, Mortazavi B, Chanton JP (2006) Diurnal variation of the δ^{13}C of pine needle respired CO_2 evolved in darkness. Plant Cell Environ 29:202–211

Rossmann A, Butzenlechner M, Schmidt HL (1991) Evidence for a nonstatistical carbon isotope distribution in natural glucose. Plant Physiol 96:609–614

Saxe H, Ellsworth DS, Heath J (1998) Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol 139:395–436

Scartazza A, Mata C, Matteucci G, Yakir D, Moscatello S, Brugnoli E (2004) Comparisons of delta C-13 of photosynthetic products and ecosystem respiratory CO_2 and their response to seasonal climate variability. Oecologia 140:340–351

Schneider S, Gessler A, Weber P, von Sengbusch D, Hanemann U, Rennenberg H (1996) Soluble N compounds in trees exposed to high loads of N: a comparison of spruce (Picea abies) and beech (Fagus sylvatica) grown under field conditions. New Phytol 134:103–114

Stoy PC, Palmroth S, Oishi AC, Siqueira MBS, Juang J-Y, Novick KA, Ward EJ, Katul GG, Oren R (2007). Are ecosystem carbon inputs and outputs coupled at short time scales? A case study from adjacent pine and hardwood forests using impulse-response analysis. Plant Cell Environ 30:700–710

Tcherkez G, Nogués S, Bleton J, Cornic G, Badeck F, Ghashghaie J (2003) Metabolic origin of carbon isotope composition of leaf dark-respired CO_2 in French bean. Plant Physiol 131:237–244

Tcherkez G, Farquhar G, Badeck F, Ghashghaie J (2004) Theoretical considerations about carbon isotope distribution in glucose of C_3 plants. Funct Plant Biol 31:857–877

Teskey RO, McGuire MA (2005) CO_2 transported in xylem sap affects CO_2 efflux from Liquidambar styraciflua and Platanus occidentalis stems, and contributes to observed wound respiration phenomena. Trees Struct Funct 19:357–362

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

Theis DE, Saurer M, Blum H, Frossard E, Siegwolf RTW (2004) A portable automated system for trace gas sampling in the field and stable isotope analysis in the laboratory. Rapid Commun Mass Spectrom 18:2106–2112

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas-exchange of leaves. Planta 153:376–387

Werner C, Unger S, Pereira JS, Maia R, David TS, Kurz-Besson C, David JS, Maguas C (2006) Importance of short-term dynamics in carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$) in a Mediterranean oak woodland and linkage to environmental factors. New Phytol 172:330–346

Werner C, Hasenbein N, Maia R, Beyschlag W, Maguas C (2007) Evaluating high time-resolved changes in carbon isotope ratio of respired CO_2 by a rapid in-tube incubation technique. Rapid Commun Mass Spectrom 21:1352–1360

Yakir D, Wang XF (1996) Fluxes of CO_2 and water between terrestrial vegetation and the atmosphere estimated from isotope measurements. Nature 380:515–517

Zobitz JM, Keener JP, Schnyder H, Bowling DR (2006) Sensitivity analysis and quantification of uncertainty for isotopic mixing relationships in carbon cycle research. Agric For Meteorol 136:56–75