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Technical Note: A combined soil/canopy chamber system for tracing $\delta^{13}C$ in soil respiration after a $^{13}CO_2$ canopy pulse labelling

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Abstract

In this study we present a combined soil/canopy chamber system that allows the investigation of carbon flow through the atmosphere-plant-soil system via a $^{13}CO_2$ canopy labelling approach – especially when using short vegetation such as tree saplings. The developed chamber system clearly separates soil and canopy compartment in order to (a) prevent physical diffusion of ^{13}C tracer into the soil chamber during a $^{13}CO_2$ canopy pulse labelling (b) study stable isotope processes in soil and canopy individually and independently. In combination with novel laser spectrometry, measuring CO_2 (Aerodyne Research Inc.) and H_2O (Los Gatos Research Inc.) isotopologue mixing ratios at a rate of 1 Hz, we were able to trace the label transport from leaves to roots in small beech saplings (*Fagus sylvatica* L.) without interference due to contamination of the soil matrix and/or canopy re-labelling via tracer returning from soil respiration. A very tight coupling between above- (photosynthesis) and belowground (soil respiration) processes was found, where newly assimilated carbon fixed from the $^{13}CO_2$ atmosphere re-appeared in soil respiration 2 h after it has been photosynthetically fixed. We were able to demonstrate that leaf metabolism acts on substrate for soil respiration on a diurnal timescale, with input of fresh photosynthates during daytime and starch remobilisation during nighttime. Long-term fluctuations in the $\delta^{13}C$ of soil respiration, as observed under reduced water availability, could not be described by any biological or instrumental mechanism, as they did occur in an atypical ca. 15 hourly rhythm – potential mechanisms driving these fluctuations are hypothesized.

1 Introduction

Understanding the carbon cycle in terrestrial ecosystems has become increasingly important under the aspect of climate change as terrestrial ecosystems contribute – via photosynthesis and respiration – to the regulation of atmospheric CO_2 concentrations. Hence, whether an ecosystem functions as a net carbon source or sink to the

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atmosphere depends on the relative strength of photosynthesis and ecosystem respiration. The largest component of ecosystem respiration, and thus the largest CO_2 source from terrestrial ecosystems to the atmosphere, is soil respiration. Soil respiration contributes, for instance, for up to 50% of the total flux in Canadian boreal coniferous ecosystems (Lavigne et al., 1997) and for up to 70% across European forest ecosystems (Janssens et al., 2001). Recent studies show that the magnitude of soil respiration is, apart from abiotic drivers, like soil moisture and soil temperature, much dependent on the supply of recent assimilates (Högberg et al., 2001; Tang et al., 2005; Wertin and Teskey, 2008), since heterotrohic (microbial) and autotrophic (root-rhizoshzere) belowground activity is strongly linked to plant metabolism, photosynthesis and litterfall (Ryan and Law, 2005). Root-rhizosphere respiration accounts for ca. 50% of total soil respiration (Law et al., 2001; Rühr and Buchmann, 2010) and encompasses a major proportion of the plants carbon budget. Approximately 10–50% of daily carbohydrate production in plants is spent on root respiration depending on environmental conditions and species (Van der Werf et al., 1992). The strong links between above- and belowground processes complicate the overall understanding of terrestrial ecosystem carbon cycling and its integration into climate models. In the past, carbon isotope (^{14}C, ^{13}C) methods have proved to be a powerful tool to trace the carbon flow through the atmosphere-plant-soil system and, furthermore, to understand its source-sink relationships (Carbone and Trumbore, 2007; Carbone et al., 2007; Högberg et al., 2008; Talhelm et al., 2007). ^{13}C natural abundance studies showed that mainly changes in recent aboveground weather conditions such as air temperature, vapour pressure deficit, air relative humidity or precipitation explain variations in the $\delta^{13}C$ of soil respiration, indicating a direct, but time-delayed link of photosynthesis and soil respiration (Ekblad and Högberg, 2001; Ekblad et al., 2005; Bowling et al., 2002; Barbour et al., 2005; Knohl et al., 2005; Kodama et al., 2008). Furthermore, carbon isotope pulse chase labelling experiments also showed that photosynthesis is directly linked to root-rhizoshpere respiration (Bahn et al., 2009; Plain et al., 2009; Salmon 2009; Ruehr et al., 2009), determining its $\delta^{13}C$ signal ($\delta^{13}C_{SR}$) even on a diurnal timescale

(Bahn et al., 2009). Studies on carbon transfer in plants using ^{13}C enriched tracer techniques were, however, often constricted since the soil matrix has been contaminated while labelling, thus blurring the signal – particularly within the first few hours after label application (Bahn et al., 2009; Högberg et al., 2008). Subke et al. (2009) show a confounding influence of physical isotopic CO_2-tracer return from the soil matrix and argue that the interpretation of data is prone to erroneous conclusions during the initial period following the pulse due to physical diffusion of tracer into soil pores. Consequently, soil return fluxes are overestimated under these conditions. Only a recent study on tall, 20-year-old beech trees in a French forest was able, by enclosure of the entire canopy, to assess accurately transfer/residence times through the plant without contaminating the soil matrix (Plain et al., 2009). However, in short vegetations such as grasses or tree saplings the transport of fresh assimilates into the roots may occur within a few hours. Therefore, an isolated canopy labelling without contaminating the soil, combined with high time resolution measurements, is necessary in order to investigate the speed link between assimilation and root respiration in such short vegetation. In this study we present a combined soil/canopy chamber system, which was designed for laboratory experiments on small tree saplings, ensuring a gas-tight separation between canopy and soil when applying a ^{13}C canopy pulse label. A clear separation between canopy and soil prevents any contamination of the soil matrix with tracer material and, similarly important, avoids potential canopy re-labelling via ^{13}C re-respired from soil respiration. These two aspects enable to clearly identify the initial biotic $\delta^{13}C$ soil respiration tracer return from a single $^{13}CO_2$ canopy pulse label and further, to assess its diurnal variations. In order to detect such fast and dynamic processes like diurnal variations in the $\delta^{13}C_{SR}$ signal stable isotope laser spectroscopy technology was deployed. It allows to perform continuous real-time measurements of CO_2 isotopologues with an accuracy close to conventional mass spectrometry methods (0.25‰). Particularly in ecosystem research it permits to investigate stable isotope processes which have been previously limited for their high time resolution requirements. Hitherto, only a few studies have applied on-line laser spectrometry for the assessment of soil respiration processes

(Bahn et al., 2009; Marron et al., 2009; Plain et al., 2009). Moreover, the only study, known to us, which used a similar chamber concept was found in Palta et al. (1997), which, however, did not use online laser spectrometry in their isotopic measurements. In this paper we aim to (1) present a combined chamber system, based on open-flow through technique, separating mechanically soil and canopy in order to study their isotopic gas-exchange processes individually (2) demonstrate the value of this chamber system for labelling experiments with short vegetation (tree saplings) (3) present real-time measurements of $\delta^{13}C_{SR}$ after a $^{13}CO_2$ canopy pulse labelling conducted with laser spectroscopy in order to detect fast and dynamic changes (4) show the fast and tight linkage between photosynthesis and soil respiration.

2 Methods

2.1 Experimental set-up and design

The study was conducted from 21 May 2009 to 24 June 2009 in a climate chamber, with controlled air humidity, air temperature and light conditions. Within the climate chamber, six combined soil/canopy chamber replicates were installed, each enclosing fully the canopy and soil compartment of one potted European beech sapling (*Fagus sylvatica* L.). A physical barrier between these compartments allowed the measurement of isotopic gas-exchange processes in canopy and soil separately on the same plant individual. Of a total of six beech trees, two samples originated from a different tree nursery (different provenance/soil; one contaminated during labelling) and were therefore excluded from the result section of this study. Over the duration of the entire experiment (5 weeks) half of the trees ($n = 2$) were not watered, which reduced the relative soil water content moderately down to 67% and 84% compared to field capacity. Within the climate chamber a diurnal cycle with a light period of 15 h was simulated, achieving PPFD values up to 600 µmol m^{-2} s^{-1}. The air temperature outside the canopy was set to 11 °C in order to balance the greenhouse effect in the canopy chambers caused by

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the heat radiated from the light bulbs. Similarly, humidity conditions outside the canopy chamber were kept at about 40% to prevent condensation within the canopy chamber due to the transpiration intensity of the plants. Air temperature inside the different canopy chambers varied between 13 °C and 25 °C and air humidity between 53% and 87%, depending on transpiration intensity, radiant heat of the light bulbs and total leaf area. Total leaf area was measured destructively after the experiment using a leaf area meter (LI-3050A/4; LI-COR Biosciences, Inc., Lincoln, NE, USA). Communication between PC, sensors as well as valves was done using network data acquisition/control modules (I-7000 series, ICP DAS Co, Hukou Township, Taiwan). All data have been continuously logged at a rate of 1.8 s using a custom written LabVIEW (National Instruments Corp., Austin, TX, USA) interface.

2.2 Steady-state through-flow chamber system

A combined soil-canopy chamber system (Fig. 1) was developed to conduct isotopic gas-exchange measurements on the same plant individuals, yet with a gas-tight physical separation between canopy- and soil-compartment. All chambers were constructed as steady-state through-flow chambers (open dynamic chamber) (Pumpanen et al., 2004; Livingston and Hutchinson, 1995). The soil compartment, a bottom closed cylinder consisting of PVC (ø=300/297 mm, l=250 mm, V=17.67 L), was designed to fully enclose the planting pot. In order to ensure adequate gas tightness the soil chamber was clamped between two square wooden boards, which could be tightened via threaded rods. Because of the canopy, the upper wooden board had a circular opening which could be closed with two semi-circle shaped PVC discs – leaving only a small opening (ø=35 mm) for the stem. In that way, physical separation between canopy and soil compartment was ensured. Finally, a top closed transparent acrylic glass cylinder (ø=300/292 mm, l=800 mm, V=56.54 L, Plexiglas®, KUS-Kunststofftechnik, Reckling-hausen, Germany) with two openings for inlet/outlet was mounted on top, serving as the canopy chamber. All potential leakage points, especially those between soil and

canopy, were additionally sealed with Terostat® (Henkel Teroson GmbH, Heidelberg, Germany). The chamber system was tested for its gas-tightness by measuring the empty canopy chamber when using a soil pot with a metal stick (as stem) in the soil chamber. This test showed no increase in CO_2 concentration in the canopy chamber confirming gas-tight separation of both chambers. During the labelling experiment, one chamber experienced a small leak between the soil/canopy interface resulting in a contamination of the soil matrix with labeled material (Fig. 2, open triangles). Even after flushing following the contamination, back diffusion of labeled material from the soil matrix prevented a clear interpretation of the initial phase after the canopy label. Inside the canopy chamber a small permanently running electrical fan (80×80 mm, 16.5 L/s, Multicomp, KDE1208PTV2.13.MS.A.GN) was installed, ensuring a well mixing of the canopy chamber air. The inlet of the soil chamber (ø=19/21 mm, l=250 mm) served also as the entrance point for the respective probes, measuring soil moisture (ECH$_2$O EC-5, Decagon Devices, Inc., Pullman, WA, USA), soil temperature (AD 592, Analog Devices, Inc., Norwood, MA, USA), relative humidity, air temperature (HygroClip® S3C03, rotronic AG, Bassersdorf, Switzerland), and leaf temperature (Thermocouple Type K, Omega Engineering Inc., Stamford, CT, USA). The wiring for the canopy chamber probes was threaded, along with the stem, through the opening between the semicircle shaped discs. Moreover, irrigation tubings were installed at each soil chamber allowing watering without deconstruction of the chambers. Both, soil and canopy chambers were permanently flushed via a vacuum pump (VTE 6 & VLT 15, Gardner Denver Inc., Quincy, IL, USA) at a rate of ca. 4 L/ min and ca. 5 L/ min, respectively, in order to maintain steady state conditions. From the flushing air stream, subsamples were drawn to the laser spectrometers at a rate of 500 mL/ min and analyzed for their respective isotopic composition and mixing ratios. The $\delta^{13}C$ values of soil respiration were calculated via an isotopic mass balance similar to Bahn et al. (2009):

$$\delta^{13}C_{SR} = \frac{\delta^{13}C_o[CO_2]_o - \delta^{13}C_i[CO_2]_i}{[CO_2]_o - [CO_2]_i} \quad (1)$$

where *o* refers to the chamber outlet and *i* to the chamber inlet. [CO_2] denotes CO_2 mixing ratios.

2.2.1 Canopy chamber transparency

The light intensity and the potential spectral change of visible light within the canopy chamber was tested with a photo spectrometer (USB4000, Ocean Optics, FL, USA) and a PAR sensor (LI-189; LI-COR Biosciences, Inc., Lincoln, NE, USA). The sensors were placed at the same position within the climate chamber and measured with and without the canopy chamber mounted on top. The light transmission was reduced by ca. 12%, probably due to light attenuation of the plexiglas® material and showed no change in the spectral composition.

2.3 Measurement principle and label application

Once per hour all 12 chambers (6×canopy and 6×soil) were consecutively measured, whereupon soil chambers were measured twice, giving a total of 18 chamber measurements per hour. The respective inlet and outlet measurements were 90 s each with a logging interval of 1.8 s, resulting in 50 logged values per inlet/outlet measurement. However, for steady-state reasons only the last 15 values of each inlet/outlet measurement were used for further calculations of mean and standard deviation. Before each chamber measurement (outlet), the well mixed climate chamber air was sampled as reference (inlet), leaving each chamber measurement framed with two reference measurements. Pulse labelling was achieved by connecting all canopy chambers in parallel in a closed loop (SERTOflex, Serto AG, Aadorf, Switzerland), where air was circulated at a rate of 32 L/ min (Fig. 2). In order to avoid contamination of the reference air, the labelling was done outside the climate chamber by directing a part of the loop out and back into the climate chamber. To generate $^{13}CO_2$ labeled air, 99% ^{13}C sodium carbonate (Na_2CO_3)(Cambridge Isotope Laboratories, Inc., Andover, MA, USA) was chemically converted to CO_2 via acidification with sulphuric acid (H_2SO_4) leaving just

CO_2 and H_2O as end-products. To accomplish that, a gas tight box equipped with a rubber seal was connected to the outside part of the loop. Inside the box two small beakers, containing approximately 4 g Na_2CO_3 each, were placed whereat H_2S_4 could be applied with a syringe, causing a label release into the air stream. In order to monitor and adjust the CO_2 concentration within the system a $^{13}CO_2$ sensitive IRGA (LI6262, LI-COR Biosciences, Inc., Lincoln, NE, USA) was also connected to the main loop. Within 30 min H_2SO_4 was added twice into the system which resulted in CO_2 concentration increase from 360 to 800 ppm. During the label period no condensation of the canopy chambers could be observed, although air humidity continuously increased due to transpiration. After the labeled air had been circulated for 30 min within the canopy system, the remaining label was flushed into the rooms' ventilation exhaust, leaving no labeled air inside the canopy chambers or the climate chamber itself. Flushing was done at a rate of 38 L/ min for 1 h until CO_2 mixing ratios in the canopy chambers returned to pre-labelling values of ca. 300 to 350 ppm. Within the flushing period, the label loop was disrupted at the canopy inlet in order to avoid condensation within the canopy chamber due to transpiration. That interference caused a slight disturbance of our gas-exchange data (Fig. 3). After flushing was completed, all tubings used for labelling were removed and original measurement setup restored. The label had been applied at 11:00 a.m. CET on 15 June 2009.

2.4 Instrumentation and calibration

A commercially available CO_2 isotope laser spectrometer (QCLAS-ISO, Aerodyne Research, Inc., Billerica, MA, USA) was mounted outside the climate chamber. By scanning across a small spectral window (near 2310 cm^{-1}) the quantification of the CO_2 isotopologues $^{12}CO_2$, $^{13}CO_2$, and $C^{18}O^{16}O$ is achieved. The spectrometer enables real-time simultaneous measurements of the isotopic ratios of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ in CO_2 as well as CO_2 mixing ratios in air. The pulsed quantum cascade laser spectrometer system operates via two optical multiple pass absorption cells, whose pressures

and temperatures are continuously stabilized at 308 K and 7 kPa in order to maintain steady conditions during measurement. A detailed technical description of the CO_2 laser spectrometer system can be found in Tuzson et al. (2008) as well as in Nelson et al. (2008). Our instrument is equipped with an infrared detector cooled by liquid nitrogen. This was accomplished using an automated refilling device (liquid N_2 Micro-dosing system # 906, Norhof, Maarssen, The Netherlands). The self-made, fully automated calibration system and the automated liquid nitrogen refilling device reduced system servicing to a minimum. Calibration of the CO_2 laser system was done automatically every full hour for 6 min duration, using two calibration gases with known isotopic composition as well as mixing ratios. A third calibration gas was dynamically diluted with CO_2-free air in order to account for nonlinearity effects. The 1σ precision estimated from Allan variance plots is about 0.25‰ at 1 s and about 0.08‰ at 30 min averaging time for both $\delta^{13}C$ and $\delta^{18}O$ in CO_2. The long-term stability was assessed with repeated measurements of a quality control standard and resulted in an accuracy of about 0.25‰. This is close to what can be achieved with conventional mass spectrometers and adequate for ecophysiological research. Carbon isotopic compositions are presented as $\delta^{13}C = R_{sample}/R_{VPDB} - 1$, where R_{sample} is the sample $^{13}C/^{12}C$ ratio and R_{VPDB} is the $^{13}C/^{12}C$ ratio of the international Vienna-Pee Dee Belemnite (VPDB) standard. Concurrently to the CO_2 measurements, water vapour mixing ratios were measured using a water vapour isotope analyzer (WVIA, DLT-100, Los Gatos Research, Inc., Mountain View, CA, USA) which has been described in detail by Sturm and Knohl (2010). This instrument is capable for in-situ measurements of the water vapour isotopic compositition in air ($^{18}O/^{16}O$; $^{2}H/^{1}H$) and its respective mixing ratios. H_2O concentration was needed to calculate for stomatal conductance and leaf transpiration. For all connections between the chambers and instruments 6 mm synflex tubing (SERTOflex, Serto AG, Aadorf, Switzerland) as well as brass fittings (Swagelok, Solon, OH, USA) have been used.

2.5 Statistical analysis

For all statistical analyses and data processing the software R 2.8.1 (R Development Core Team, 2008) and MATLAB (The MathWorks, Inc.) were used. All equations used for the calculation of ecophysiological parameters are listed in Appendix A. For spectral analysis data were gap-filled by linear interpolation and fitted by a spline fit with a cut-off period of 8 days. The frequency components of the residuals of the spline fit were then calculated using a fast Fourier transform (FFT).

3 Results and discussion

3.1 Ecophysiological parameters

The hourly online measurements of CO_2 and H_2O gas exchange, their isotopoloques and environmental parameters in the canopy and soil chamber allow continuous calculation of the most relevant ecophysiological parameters. Figure 4 presents three diurnal cycle measurements during the days of label application showing differences between the various samples for photosynthesis, transpiration, isotopic leaf discrimination and soil respiration rate. All parameters seem not be influenced by the soil watering regime, since values are in the same range. Although soil water content was reduced in two samples we did not observe an apparent change in photosynthesis or transpiration compared to the preceding measurement period, which indicates a non-sufficient water stress.

3.2 Variations in $\delta^{13}C_{SR}$ after canopy labelling

3.2.1 Initial phase after labelling without contamination

The natural abundance values for $\delta^{13}C_{SR}$ varied from −26.5‰ to −25.5‰ between the replicates before the label was applied to the canopy (mean of 3 days before label,

see Table 1). During the application of the $^{13}CO_2$ label to the canopy no change in the $\delta^{13}C_{SR}$ signal was observed, which indicates that our chamber system was sufficiently gas-tight (Fig. 5) and thus no contamination of the soil matrix with labeled material occurred (compare Fig. 2). Evidently, the later observed enrichment in $\delta^{13}C_{SR}$ originates exclusively from recent assimilates which had been photosynthetically fixed and transported down the phloem to the roots. Considering the findings of Subke et al. (2009), where the abiotic $^{13}CO_2$ efflux from soil pores remained significant for up to 48 h after labelling, such an initial increase of $\delta^{13}C_{SR}$ related to assimilate transport, as found here, could not have been detected if the soil had been contaminated with tracer material. Within 2 h after label application a simultaneous increase in $\delta^{13}C_{SR}$ could be observed in all replicates.

3.2.2 Diurnal variations

The progression of the $\delta^{13}C_{SR}$ curves, however, differed in intensity, timing and shape between the four samples. The first peak in $\delta^{13}C_{SR}$ was reached after 590 to 718 min after label application, depending on the transpiration rate per leaf area of the respective canopy ($R^2 = 0.99$, $n = 4$, $p \leq 0.01$). The higher the transpiration rate the faster the first label peak was reached, what probably relates to xylem pressure, indirectly causing different phloem transport velocities. A similar root-rhizosphere respiration dependence on transpiration rate was also reported for a semi-arid *Pinus ponderosa* Doug. Ex Laws. forest (Irvine et al., 2005). Interestingly, such significant relationship could neither be found for photosynthetic rate nor stomatal conductance. However, the calcuation of stomatal conductance relies on a single leaf temperature measurement, which potentially provides an additional error source, whereas transpiration calculations integrate over the entire canopy. Furthermore, a strong diurnal cycle in $\delta^{13}C_{SR}$ was found during the first 24 h after label application. As mentioned above, the delay of the first $\delta^{13}C_{SR}$ peak relates most likely to the time needed for export and transfer of labeled assimilates from leaves to roots via the phloem. Therefore, it was assumed, that

the first peak in $\delta^{13}C_{SR}$ after label application corresponds in fact to the time of label fixation by the canopy. In order to relate the $\delta^{13}C_{SR}$ signal to the time of photosynthate production during label fixation, we shifted the data for the time-lag caused by phloem transfer (Table 1). When shifting the signal for this time-lag, it can be seen that light and hence photosynthesis drives directly the diurnal rhythm of $\delta^{13}C_{SR}$ (see inset Fig. 5). The data suggest that after the first $\delta^{13}C_{SR}$ peak caused by the canopy labelling, new, lighter, non-labeled assimilates dilute the carbon pool inside the plant, which in turn cause a depletion in the $\delta^{13}C_{SR}$ signal within the remaining daytime. Coincidental with nightfall, a second increase in the $^{13}C_{SR}$ was observed, probably relating to leaf starch metabolism. Transitory starch, synthesized in chloroplasts during the day, provides a steady and sufficient supply of carbon for growth, sucrose synthesis and respiration throughout the subsequent night (Zeeman et al., 2007), where degradation rate is dependent on the photoperiodic length. We hypothesize that transitory starch, which was labeled within the labelling time window, is re-mobilized at night thus enriching soil respiration. This is indirectly also substantiated with a model developed by Tcherkez et al. (2004), which proposes that sucrose, which is produced from starch breakdown during night, is isotopically heavier than sucrose produced in the light. Our results correspond also well to previous isotope natural abundance studies on plant diurnal cycles (Gessler et al., 2007; Kodama et al., 2008), which found a ^{13}C diurnal variation up to 2.5‰ in phloem excudates with enrichment during the night and depletion during the day. That was also coherent with transitory starch accumulation and re-mobilization (Gessler et al., 2007). Moreover, Bahn et al. (2009), who labeled a mountain meadow, found an enrichment of $\delta^{13}C_{SR}$ in the late night and early morning hours before the label started to decrease due to the influence of recently assimilated carbon. However, a re-labelling of the canopy during the daytime could not be excluded in their experiment. Controversially, a lack of diurnal patterns had been described by Betson et al. (2007) for a boreal *Picea abies* L. Karst. forest. However, very short nights in their experiment exerted probably too little forcing to drive a diurnal cycle in the $\delta^{13}C_{SR}$. The different intensities between the samples in $\delta^{13}C_{SR}$ in Fig. 5 are supposedly governed by

photosynthetic rate plus the leaf discrimination strength during label application. Also, increased carbon allocation to the roots in non-watered samples, as discussed by Palta et al. (1997) for *(*Triticum aestivum) L. cv. Kulin, could potentially cause different intensities. However, a direct dependency of $\delta^{13}C_{SR}$-intensity to single ecophysiological parameters like photosynthetic rate, stomatal conductance, leaf discrimination, leaf area or environmental parameters could not be found.

3.2.3 $\delta^{13}C_{SR}$ long-term fluctuations

As discussed in the previous section, recent photosynthetic activity and transitory starch accumulation/re-mobilisation are probable mechanisms which can explain the $\delta^{13}C_{SR}$ development for the first 24 h after the label had been applied (inset Fig. 5). After $\delta^{13}C_{SR}$ reached its maximum due to starch re-mobilisation, an exponential decay of $\delta^{13}C_{SR}$ was observed in all samples. The half-life of $\delta^{13}C_{SR}$, calculated from a fitted decay function, ranged from 1.1 d to 1.6 d between the samples (Table 1), where a faster decay rate was observed in the non-watered onces, thus indicating a faster pool turnover of the labeled fraction. Other studies show that the pool of non-structural plant compounds (such as phloem sugars) almost entirely supplies autotrophic respiration and that this pool is turned over very rapidly by new photosynthates with a half-life of ca. 2 to 2.6 days (Gamnitzer et al., 2009; Lehmeier et al., 2008). Besides a general decay trend in the $\delta^{13}C_{SR}$ signal periodical fluctuations could be observed in the non-watered samples (Fig. 5). A spectral analysis revealed that these long-term fluctuations in $\delta^{13}C_{SR}$ do not occur over diurnal cycles but in a atypical ca. 15 hourly rhythm (Fig. 6). As only two out of four samples showed these recurring cycles in $\delta^{13}C_{SR}$, instrumental fluctuations or periodic changes in climate chamber conditions could be excluded as an explaining mechanism. Biologically, a metabolic substrate switch, feeding respiration, as found in case of carbohydrate starvation in *Phaseolus vulgaris* by Tcherkez et al. (2003) is also improbable, since all labeled non-structural carbohydrates would have been used up and would therefore not available to drive an

iterated increase in $\delta^{13}C_{SR}$. Moreover, carbon which has already been incorporated into structural biomass is also protected from respiratory consumption. However, a repeated re-mobilisation/respiration of remaining labeled compounds, as suggested, for instance, by Bahn et al. (2008) cannot completely dismissed as a mechanism explaining long-term fluctuations in $\delta^{13}C_{SR}$ of the non-watered samples. The droughted conditions might have influenced phloem transport rate, phloem solute concentration and/or relative changes of heterotrophic (microbes) and autotrophic (roots) respiration fluxes, which then led to a deferred diurnality. Especially on the soil level might the water status indirectly affect soil respiration due to its influence on microbial biomass and/or dissolution of CO_2 in soil water. Another mechanism, which potentially explains long term fluctuations in $\delta^{13}C_{SR}$ could be a subsequent re-labelling of the canopy. Such a re-labelling could indirectly occur during daytime, as leaf respired CO_2 is also enriched in $\delta^{13}C$. Our data shows indeed an increase in $\delta^{13}C$ of canopy air after the labelling (see Δ in Fig. 4), which, however, decreases rapidly over the subsequent days in a similar fashion like $\delta^{13}C_{SR}$. If re-labelling would effectively occur, one would also expect 24 hourly fluctuations during the subsequent days in all samples as re-labelling is also driven by light and thus photosynthesis. Overall, spectral analysis revealed long-term fluctuations only for the non-watered samples. As the non-watered samples are also highest in label intensity and thus revealing more variation, one might argue that such fluctuations could also exist within the watered samples. In these samples might potential fluctuations, however, not be seized by spectral analysis as they are possibly too small. Further examination of underlying processes is required to solve whether soil moisture content, re-labelling or yet other processes drive the observed long-term fluctuations in $\delta^{13}C_{SR}$.

4 Conclusions

Our measurements show the importance of physically separating canopy and soil compartment in ^{13}C pulse labelling studies with short vegetation (e.g. tree sapling), where transport times of new assimilates occur within a few hours. When done correctly, we were able to avoid contamination of the soil matrix during the ^{13}C labelling period and also a subsequent re-labelling of the canopy due to enriched $\delta^{13}C_{SR}$. We conclude that this novel system is highly applicable for ecophysiological research questions, especially for pulse labelling experiments, which aim to study fast and dynamic processes within the atmosphere-plant-soil system. Without contamination of the soil matrix important steps of the post-label phase such as the time-lag to the first peak can be detected. The results show that leaf metabolism is unequivocally and tightly linked to belowground processes and modifies diurnally the source and supply of substrates utilized for root respiration. Fresh assimilates are provided to the root system within a very short time, depending on the transpiration rate of the canopy. Further, starch built up during the day is re-mobilized within the subsequent night and exported via the phloem down to the roots. Overall, a further quantitative understanding on the mechanisms controlling the link between above- and belowground processes is required to evaluate the carbon cycle in terrestrial ecosystems under changing environmental conditions and will be addressed in subsequent work.

Appendix A

Equations

$$E = \frac{F([H_2O]_o - [H_2O]_i)}{L_L(1 - [H_2O]_o)} \quad \text{(A1)}$$

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$$A = \frac{F([CO_2]_i - [CO_2]_o)}{L_L} - [CO_2]_o E \quad \text{(A2)}$$

$$SR = \frac{F([CO_2]_o - [CO_2]_i)}{L_S} \quad \text{(A3)}$$

$$\Delta = \frac{\xi(\delta^{13}C_o - \delta^{13}C_i)}{1000 + \delta^{13}C_o - \xi(\delta^{13}C_o - \delta^{13}C_i)} \times 1000 \quad \text{(A4)}$$

$$\xi = \frac{[CO_2]_i}{([CO_2]_i - [CO_2]_o)} \quad \text{(A5)}$$

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Table 1. Overview for various parameters: *A* (photosynthesis) [µmol $m^{-2}s^{-1}$], *E* (transpiration) [mmol $m^{-2}s^{-1}$], half-life of exponential decay [days], natural abundance of $\delta^{13}C_{SR}$ 3 days before label [], time-lag accounting for phloem transport [min], soil water content at label day compared to field capacity [%].

Sample N°	I	II	III	IV
A	3.34±0.27	3.58±0.13	4.49±0.12	5.25±0.13
E	0.39±0.02	0.50±0.01	0.51±0.01	0.74±0.01
half-life	1.64±0.05	1.15±0.02	1.31±0.03	1.07±0.02
$\delta^{13}C_{SR}$	−25.5±1.7	−26.3±2.0	−26.4±1.8	−25.9±1.8
time-lag	718	668	670	590
SWC	67	95	84	n.a.[a]

[a] Technical failure of probe.

Table 2. Abbreviation table of parameters used in equations and their corresponding units.

parameter	abbreviation	unit
ratio[a]	ξ	µmol/mol
discrimination	Δ	
photosynthesis	A	mol CO_2 m^{-2} s^{-1}
CO_2-intlet	$[CO_2]_i$	mol CO_2 mol air^{-1}
CO_2-outlet	$[CO_2]_o$	mol CO_2 mol air^{-1}
transpiration	E	mol H_2O m^{-2} s^{-1}
flow rate	F	mol air s^{-1}
H_2O-intlet	$[H_2O]_i$	mol H_2O mol air^{-1}
H_2O-outlet	$[H_2O]_o$	mol H_2O mol air^{-1}
leaf area	L_L	m^2
soil area	L_S	m^2
soil respiration rate	SR	mol m^{-2} s^{-1}

[a] Ratio of CO_2 entering the canopy chamber to rate of net CO_2 fixation by the leaves (Evans et al., 1981).

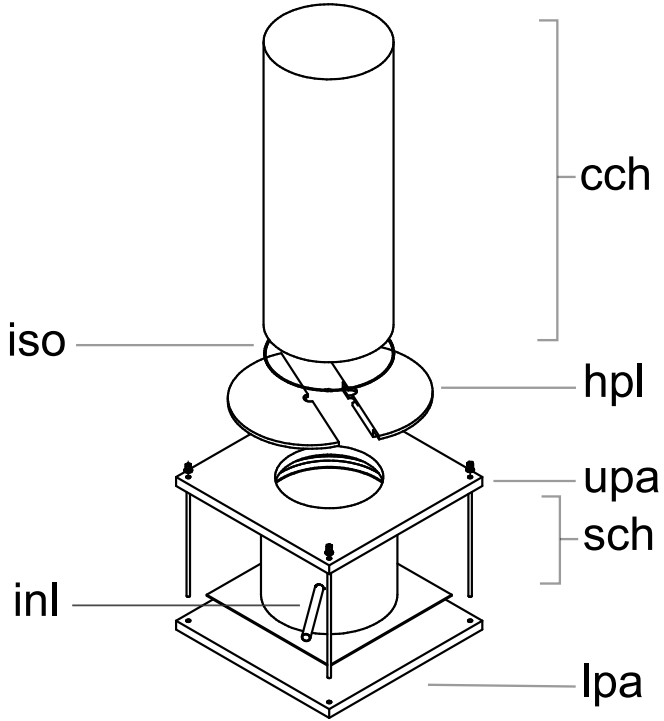

Figure 1. Explosion image of combined chamber setup for separate gas-exchange measurements of soil and canopy processes; designed for potted tree saplings; dimensions canopy chamber: ø=300/292 mm, l=800 mm, V=56.54 L; dimensions soil chamber: ø=300/297 mm, l=250 mm, V=17.67 L; cch=canopy chamber, hpl=semi-circle shaped discs, upa=upper panel, lpa=lower panel, sch=soil chamber, inl=inlet soil (ø=19/21 mm, l=250 mm), iso=isolation.

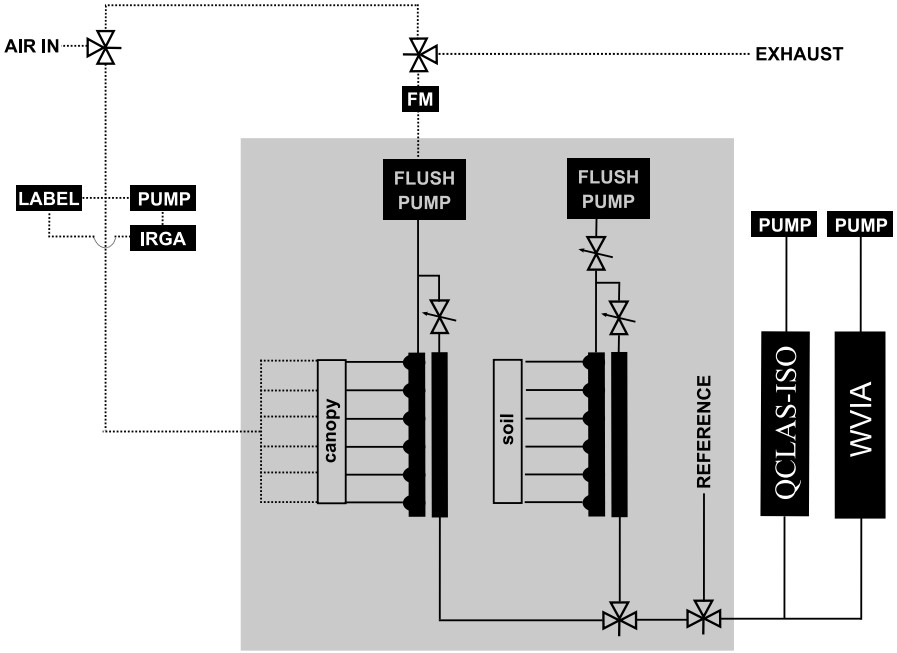

Figure 2. Setup flow scheme with solenoid valves; climate chamber (grey area); permanent setup (solid line); transient setup for label (dotted line), FM – flow meter, WVIA – water vapor isotope analyser, QCLAS-ISO – quantum cascade laser absorption spectroscopy.

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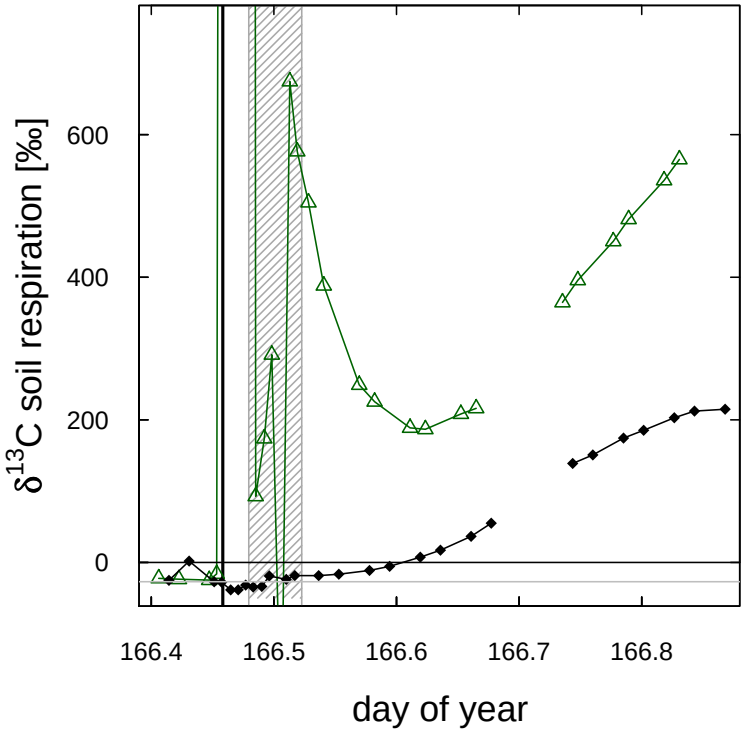

Figure 3. Evolving $\delta^{13}C_{SR}$ after label application to the canopy (solid black line) for the tracer contaminated sample (green triangles) and a gas-tight sample (black dots); flushing period (grey area), $\delta^{13}C_{SR}$ natural abundance (horizontal solid grey line).

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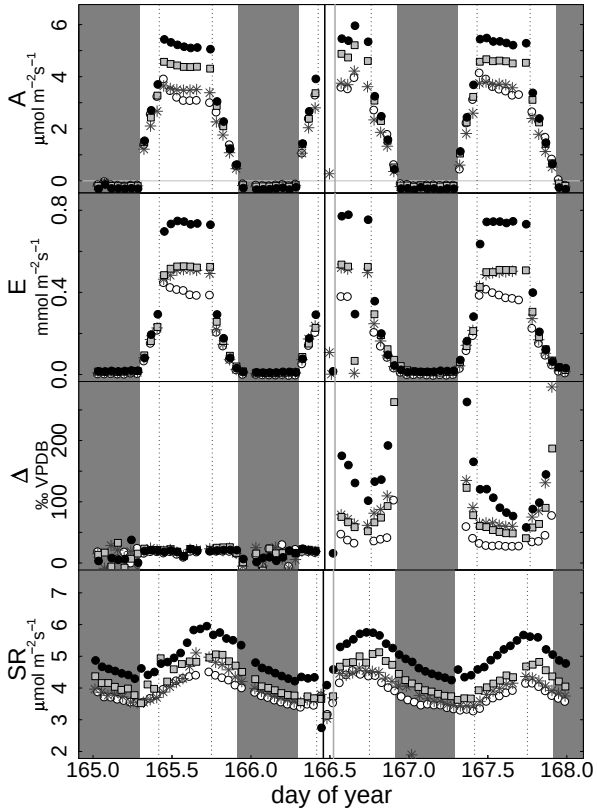

Figure 4. Diurnal cycles of ecophysiological parameters during days of label application: sample I (white circles, non-watered), sample II (grey stars, watered), sample III (grey squares, non-watered), sample IV (black circles, watered); **(a)** photosynthesis, **(b)** transpiration, **(c)** isotopic leaf discrimination **(d)** soil respiration rate; nighttime (grey areas); label application (vertical black line); end of flushing period (vertical grey line).

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$^{13}CO_2$ canopy pulse labelling using a combined chamber system

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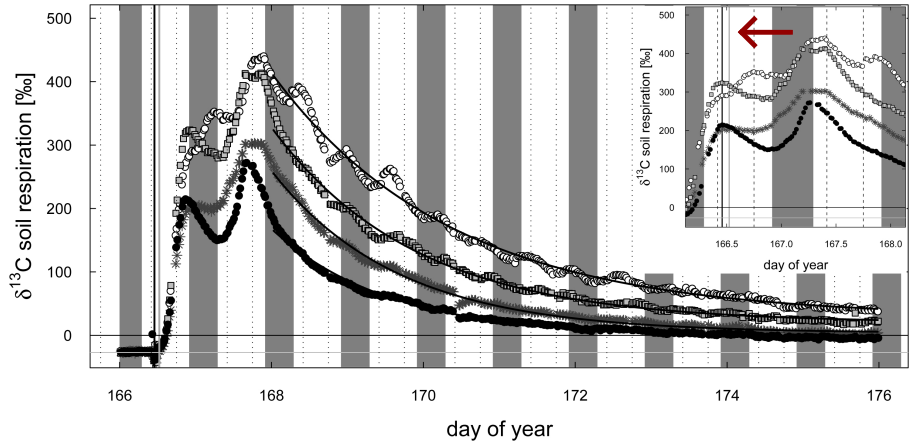

Figure 5. $\delta^{13}C_{SR}$ with exponential decay fit is shown for all replicates: sample I (white circles, non-watered), sample II (grey stars, watered), sample III (grey squares, non-watered), sample IV (black circles, watered). The inset shows the first 2 days after labelling shifted for the 1stpeak after labelling (red arrow); $\delta^{13}C_{SR}$ natural abundance (horizontal grey line); time of highest light intensity (vertical dotted lines); nighttime (grey areas); label application (vertical black line); end of flushing period (vertical grey line).

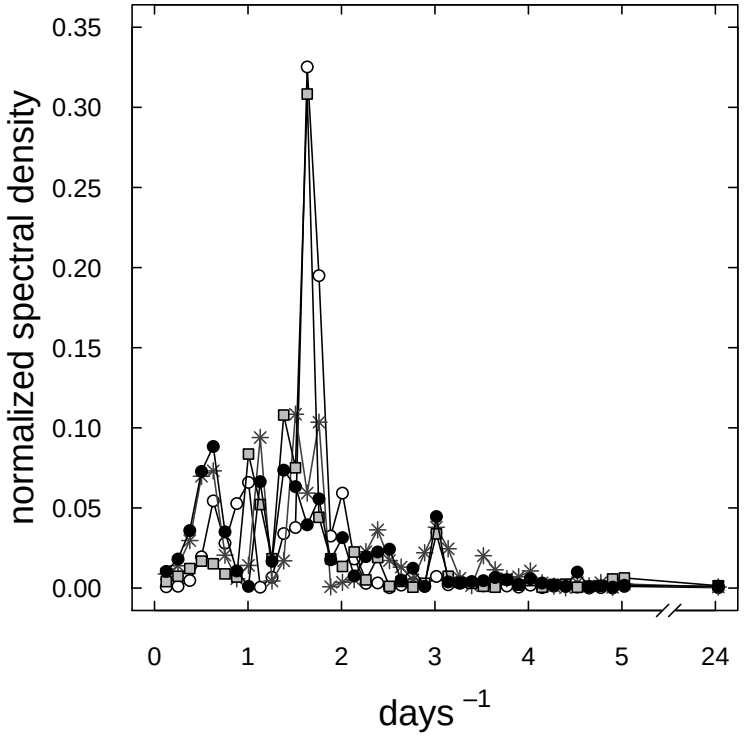

Figure 6. Power spectra gained from FFT for samples I–IV (empty symbols: non-watered, filled symbols: watered).

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