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Short-term natural δ^{13}C and δ^{18}O variations in pools and fluxes in a beech forest: the transfer of isotopic signal from recent photosynthates to soil respired CO$_2$

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Abstract. The fate of photosynthetic products within the plant-soil continuum determines how long the reduced carbon resides within the ecosystem and when it returns back to the atmosphere in the form of respiratory CO$_2$. We have tested the possibility of measuring natural variation in δ^{13}C and δ^{18}O to disentangle the potential times needed to transfer carbohydrates produced by photosynthesis down to trunk, roots and, in general, to belowground up to its further release in the form of soil respiration into the atmosphere in a beech (*Fagus sylvatica*) forest. We have measured the variation in stable carbon and oxygen isotope compositions in plant material and in soil respired CO$_2$ every three hours for three consecutive days. Possible steps and different signs of post-photosynthetic fractionation during carbon translocation were also identified. A 12 h-periodicity was observed for variation in δ^{13}C in soluble sugars in the top crown leaves and it can be explained by starch day/night dynamics in synthesis and breakdown and by stomatal limitations under elevated vapour pressure deficits. Photosynthetic products were transported down the trunk and mixed with older carbon pools, therefore causing the dampening of the δ^{13}C signal variation. The strongest periodicity of 24 h was found in δ^{13}C in soil respiration indicating changes in root contribution to the total CO$_2$ efflux. Other non-biological causes like diffusion fractionation and advection induced by gas withdrawn from the measurement chamber complicate data interpretation on this step of C transfer path. Nevertheless, it was possible to identify the speed of carbohydrates' translocation from the point of assimilation to the trunk breast height because leaf-imprinted enrichment of δ^{18}O in soluble sugars

was less modified along the downward transport and was well related to environmental parameters potentially linked to stomatal conductance. The speed of carbohydrates translocation from the site of assimilation to the trunk at breast height was estimated to be in the range of 0.3–0.4 m h^{-1}.

1 Introduction

Tracing the fate of carbon (C) within a plant-soil continuum is relevant for understanding the C source-sink relationships and the ecosystem C budget. External and internal factors affect C allocation patterns. C assimilated by photosynthesis is transported toward and resides in different sink organs depending on vegetative stage, environmental conditions and other factors (Gavrichkova and Kuzyakov, 2010; Brueggermann et al., 2011). Thus, the time needed for the assimilated C to return back to the atmosphere as respiratory CO$_2$ may vary widely. Among various methods to study the time-lag between C assimilation and respiration, one of the most commonly used is the pulse labeling of plants in artificial atmosphere (^{14}CO$_2$ or ^{13}CO$_2$). However, up to now this technique has been mainly applied to stands not higher than 8–10 m due to the complicated and expensive experimental set-up (Högberg et al., 2008; Plain et al., 2009; Dannoura et al., 2011). Recently, the advantages of plant ^{13}C and ^{18}O natural abundances and its variation in quantifying the velocity of carbohydrates translocation have been recognized for mature stands, as no limit of plant age and height exists for the application of this method (e.g. Bowling et al., 2002; Wingate et al., 2010). The level of naturally occurring discrimination against ^{13}C in leaves during photosynthesis depends on a number of plant internal and environmental

factors which affect stomatal conductance and Rubisco photosynthetic capacity (Farquhar et al., 1982). These result in a continuous variation of $\delta^{13}C$ signature in the formed organic matter. Such variations could be detectable on the subsequent stages of C translocation, therefore acting as a natural labeling. To trace the fate of C, the analyses of the time series of $\delta^{13}C$ signature in the interested pools and fluxes is performed, together with the environmental variables potentially responsible for the observed $\delta^{13}C$ changes (e.g. Kodama et al., 2008; Wingate et al., 2010). Additionally, ^{18}O composition of transported carbohydrates is closely related to that of leaf water in which they are formed and shares the dependence on stomatal conductance with ^{13}C of the formed organic matter (Farquhar et al., 1998; Yakir, 1998). It is therefore possible to follow changes in the diurnal evaporation enrichment of the leaf water in leaf and phloem organic matter at different stages of the carbohydrates transport path and account for the velocity of phloem carbohydrates transport (Keitel et al., 2003; Barnard et al., 2007).

However, the way the isotope natural abundance method has been often implemented in previous studies does not permit the issue of time-lags in C translocation to be completely solved. Firstly, many studies were focused on the seasonal variation of $\delta^{13}C$ in ecosystem respiration (Bowling et al., 2002; McDowell et al., 2004; Knohl et al., 2005). Ecosystem respiration (R_E) integrates two major sources of CO_2 with different $\delta^{13}C$ signatures: aboveground and belowground respiration, the relative importance of which varies between different ecosystems and changes during the growing season within the same stand (Law et al., 1999; Mortazavi et al., 2005; Unger et al., 2010). These two main contributors of C are separated by the phloem path through which the C has to be transported. They therefore differ in the timing of evolution of CO_2 with a certain isotopic signal determined by particular leaf level photosynthetic discrimination. Consequently, the time lags between CO_2 assimilation and R_E calculated in such experiments are hardly comparable because often the C pool, mostly contributing to ecosystem respiratory fluxes, and therefore responsible for the measured $\delta^{13}C$ values, is not known. Secondly, some recent studies suggest that the residence time of C fixed within a specific pool varies during the growing season (Plain et al., 2009; Wingate et al., 2010) and, therefore, the lag time elapsed between its assimilation and its return to the atmosphere by respiratory process is also variable. Low frequency measurements of $\delta^{13}C$ signature in respiration fluxes (both, ecosystem and soil respiration) are not suitable to cover this variability in C residence time and therefore, this often results in the absence of correlation between assimilation and respiration or multiple peaks in correlation strength, due to changes in the timing of C translocation over the growing season (e.g. Bowling et al., 2002).

Frequent sampling of plant material and respiration fluxes was performed in several studies on mature tree stands in the field (Brandes et al., 2006; Gessler et al., 2007; Barnard et

al., 2007; Kodama et al., 2008; Rascher et al., 2010). Diurnal variation of $\delta^{13}C$ and $\delta^{18}O$ signature in recently fixed organic matter associated with leaf level gas exchange and oscillation in starch content during the day/night cycle has been reported in these papers. These approaches gave the opportunity to trace short-term diurnal changes in sink organs and, applying time series analyses, to calculate the velocity of C translocation via the phloem. The results reported by several authors for the C translocation velocity is in the range of 0.3–1 m h^{-1} for mature tree stands (Gessler et al., 2004; Keitel et al., 2003; Barbour et al., 2005; Barnard et al., 2007; Rascher et al., 2010). However, in some cases it was not possible to clearly identify the time of translocation or zero lags were reported (Gessler et al., 2007; Kodama et al., 2008). Evidences for post-photosynthetic ^{13}C isotope fractionation on different steps of carbohydrates metabolism and transport have been observed. Contrasting results have been reported on ^{13}C isotopic fractionation during respiration in heterotrophic tissue. Fractionation favouring ^{13}C enriched substrates, leaving behind depleted components have been observed in some experiments (Cernusak et al., 2001; Brandes et al., 2006; Gessler et al., 2007; Kodama et al., 2008), whereas other authors have reported evidences of an enrichment of the organic matter along the C translocation path (Damesin and Lelarge, 2003; Gessler et al., 2004, 2009; Wingate et al., 2010). Such changes in $\delta^{13}C$ during translocation of organic material are not considered, for example, when the isotope approach is applied for partitioning net CO_2 ecosystem exchange (e.g. Bowling et al., 2001; Knohl and Buchmann, 2005) into ecosystem respiration and gross primary production, leading to potentially biased results. Furthermore, the extent to which the initial photosynthetic isotope discrimination signal is altered by post-photosynthetic fractionation, mixing and exchange with other storage C pools along the C translocation path strongly affects the reliability of phloem sap and soil respiration $\delta^{13}C$ as tracers of short- and long-term changes in leaf level discrimination (Gessler et al., 2007; Kodama et al., 2008; Unger et al., 2010; Wingate et al., 2010). The amplitude of leaf-level diurnal variation of ^{18}O in the formed organic matter is generally larger than that of ^{13}C (e.g. Barnard et al., 2007) and therefore given the possibility to maintain its initially imprinted isotopic signal during phloem transport and allocation.

Short-term diurnal variation of $\delta^{13}C$ and $\delta^{18}C$ in source C pools (leaves), transport pools (phloem), final sinks (shoot, bark, root) and respiration up to date were studied on conifers (*Pinus sylvestrs*: Brandes et al., 2006; Barnard et al., 2007; Kodama et al., 2008; *Pinus Pinaster*: Rascher et al., 2010; Wingate et al., 2010) and in evergreen broadleaved trees (*Eucaliptus delegatensis:* Gessler et al., 2007; *Acacia longifolia*: Rascher et al., 2010). To our knowledge, deciduous broadleaved species remain a big plant group not covered by experimental investigations.

The present study aims (i) to assess short-term variation in $\delta^{13}C$ and $\delta^{18}O$ signature of various pools and fluxes along

the carbohydrates' transfer route within a plant-soil system in a *Fagus sylvatica* forest; (ii) to check if the $\delta^{13}C$ and $\delta^{18}O$ changes during carbohydrate formation are subsequently imprinted in the successive stages of their translocation; (iii) to identify steps causing possible ^{13}C and ^{18}O fractionation process during downward carbohydrates' translocation; (iv) to quantify the speed of organic C translocation within the plant-soil system using both $\delta^{18}O$ and $\delta^{13}C$ natural variations.

2 Materials and methods

2.1 Experimental site

The experiment was conducted at the end of August 2009 in the European beech (*Fagus sylvatica*) forest at the Long Term Ecological Research (LTER) site of Collelongo – Selva Piana (Abruzzo, Italy, 41°50′58 N, 13°35′17″ E, 1550 m a.s.l.). The site is equipped with an eddy-covariance flux tower since 1993–1994 (see for site details Valentini et al., 1996; Scartazza et al., 2004). The stand density is 885 trees ha^{-1} and the average height of trees at the site is 21.5 m. Trees near to the flux tower were chosen for leaf material collection and were slightly higher (∼24 m) than average; the mean tree age at the time of sampling was around 118 yr. Effective LAI was regularly measured with LAI-2000 (Li-Cor Biosciences, USA) and in 2009, at season's peak, was 6.03±0.16 m^2 m^{-2}. The soil is developed on calcareous bedrocks and is classified as humic alisol (FAO classification) with the depth varying between 40 and 100 cm. The climate at the site is Mountain-Mediterranean with an average annual temperature of 6.9 °C and average annual precipitation of 1230 mm. Meteorological measurements are taken from the scaffold tower of 26 m height and cover the whole canopy profile. Eddy-covariance water vapor and CO_2 fluxes are measured 8–10 m above the canopy with the EUROFLUX set-up (Aubinet et al., 2000). In 2009, the Collelongo site was part of CarboEurope-IP where gap filling and flux partitioning (GPP-R_E) was calculated with a standard procedure used for all FluxNet sites (Reichstein et al., 2005; Papale et al., 2006). During the period covered by this paper, the gap filling was very limited and data coverage was close to 90 %.

2.2 Sampling

Plant material and soil air were collected every 3 h in the course of three days: from 20 to 22 August. Four target trees accessible from the scaffold tower were chosen for the leaf sampling. To avoid excessive disturbance of the trees near the tower, sampling of phloem exudates and soil air was performed on other representative individuals with similar morphological characteristics within the main footprint of the eddy covariance measurements.

2.3 Extraction of leaf soluble sugars and isotopic analyses

At each sampling occasion five leaves (further treated as one single sample) were collected from four target trees at three canopy heights: from the top (approx. 24 m), middle (approx. 19 m) and base crown (approx. 15 m) layers. The leaves were immediately frozen into liquid N and conserved at −80 °C until further analyses. Each sample was divided into two subsamples. One subsample was used to extract soluble sugars for further isotopic analyses as in Brugnoli et al. (1998). Briefly, this subsample was reduced to a fine powder, suspended in demineralised water and shaken for 1 h. After 5 min of centrifugation at 12 000 g, the supernatant was filtered through ion exchange columns filled with ion exchange resins (Dowex-50 and Dowex-1) to remove all the charged compounds. Extracts were freeze-dried and the remaining leaf soluble sugar fraction (LSS) was analysed for its ^{13}C signature on IRMS (Isoprime, Cheadle, UK) coupled to an elemental analyser (NA1500, Carlo Erba, Milan, Italy). The remaining leaf bulk material after LSS extraction was also freeze-dried and analysed for its isotope composition. Additionally, oxygen isotopic composition ($\delta^{18}O$) was analysed in LSS extracted from the top-canopy leaves. For these purposes LSS subsamples were placed into silver capsules and subjected to pyrolysis thermochemical decomposition on a pyrolitic unit (Euro Pyr-OH, Euro Vector Instruments & Software, Milan, Italy) coupled to the IRMS. The repeated measurement precision (SD, $n = 10$) of laboratory standard was ±0.3 ‰ for $\delta^{18}O$ and ±0.05 ‰ for $\delta^{13}C$.

The second subsample of fresh leaf material was treated separately and analysed for starch and soluble sugars (glucose, fructose and sucrose) contents as in Antognozzi et al. (1996). Briefly, carbohydrate concentrations were determined enzymatically by conversion of sugars, including also glucose originated from the starch hydrolysis, to the units of Glucose-6-Phosphate and by further spectrophotometric determination of NADH quantity in the reaction products of Glucose-6-Phosphate reduction with NAD$^+$ in the presence of Glucose-6P-dehydrogenase. The readings were performed in dual-wavelength mode (340–405 nm) in an Anthos 2001 plate reader (Anthos Labtec Instruments, Salzburg, Austria).

Leaves collected from each single tree were analysed separately; the results for each individual tree were averaged for each single height layer.

2.4 Extraction of phloem soluble sugars

Organic material transported with the phloem flow was extracted by phloem exudation technique as described by Schneider et al. (1996) and Gessler et al. (2004).

In brief, pieces of bark 1 cm in diameter were collected to a depth of wood from the stem breast height of four selected trees. After washing with doubly demineralised water the bark-phloem pieces were placed in 12 ml vials containing

2 ml of pure demineralized water and left for 5 h exudation. After centrifugation at 12 000 g for 5 min, the supernatant was freeze-dried and the remaining organic material was analysed for its δ^{13}C signature on EA-IRMS (Elemental analyser coupled to an isotope ratio mass spectrometer as described above). Additionally, the extracted material was analysed for oxygen isotopic composition (δ^{18}O) as described above.

Sucrose was found to be the predominant component of the beech sap (ex. Gessler et al., 2004). We therefore assume that the organic material extracted from the phloem was almost entirely composed of sugars, in particular sucrose and further define it as phloem soluble sugars (PSS).

2.5 δ^{13}C in soil respiration

Four static opaque soil chambers were constructed (ground surface 434 cm^2, volume 7 l) for air sample collection. At the time of measurements, the chambers were gently placed onto the soil in the previously prepared deepening of approx. 0.5 cm deep and maintained stable for the time of measurement. Replicate gas samples of 12 ml were drawn with a syringe and stored in pre-evacuated glass flasks with cup and rubber septa (all Labco Limited, UK) of the same volume for subsequent isotopic analyses. Samples were taken with a 3 min frequency from time 0 to 12 min after chamber closure. Between two subsequent samplings the chambers were left open. To prevent any possible exchange of air between the flask and the atmosphere, the cup of each flask was sealed with hot wax. Gas samples were analysed for CO_2 concentration and isotopic composition within a week after the collection on an IRMS (Isoprime, Cheadle, UK) coupled to a Multiflow (Isoprime, Cheadle, UK). The δ^{13}C isotopic signature of the source of respiratory CO_2 (soil respiration), was calculated using the Keeling plot approach (Keeling, 1958, 1961), which allows to separate the δ^{13}C of background atmospheric CO_2 from the δ^{13}C of CO_2 respired from the soil (root-derived and microbial-derived). Calibration curves between known CO_2 concentrations and the peak area on the mass 44 estimated by IRMS were constructed. The CO_2 concentration in the collected flasks was determined from the obtained calibration curves. The uncertainty of the mole concentration determination was 5 %.

2.6   Calculations and statistical analyses

Canopy weighted δ^{13}C of LSS was calculated according to Scartazza et al. (2004):

$$\delta^{13}\text{C canopy} = \frac{\left(\delta^{13}\text{C}_t \cdot \text{LAI}_t \cdot S_t + \delta^{13}\text{C}_m \cdot \text{LAI}_m \cdot S_m + \delta^{13}\text{C}_b \cdot \text{LAI}_b \cdot S_b\right)}{\sum_t^b \text{LAI}_i \cdot S_i}$$

where LAI_i is the leaf area index at the respective height (t = top; m = middle, b = base), S_i is the concentration of the soluble sugars on the same height and δ^{13}C is the isotope

composition of the extracted LSS. Leaf sugars and starch content was analysed in samples collected on the second sampling day only, therefore δ^{13}C canopy data are available only for these sampling occasions.

All statistic analyses were performed using the software STATISTICA 7 (StatSoft). The significance of differences between δ^{13}C values measured in various pools and fluxes was estimated applying analyses of variances (ANOVA).

Time series analysis was performed to determine the time lags between the environmental and physiological variables of interest. For these purposes, the Pearson correlation coefficient was calculated between two respective time series and tested for the significance. The considered lags were from 0 to 30 h with the step of 3 h for the correlations between δ^{13}C and δ^{18}O in pools and fluxes. Lags up to 5 d with the time step of 30 min were tested for significance to assess the dependence of isotopic variation in pools on environmental parameters.

Spectral (Fourier) analysis of STATISTICA 7 was used to determine if there is any periodical component in the diurnal course of δ^{13}C and δ^{18}O in fluxes and pools. Such analysis aims to decompose a complex time series with cyclical components into a few underlying sine and cosine functions of particular wavelength. The "wavelength" of a sine or cosine function is expressed in terms of the number of cycles per unit time (frequency, ν). Analysis will identify the correlation (sine or cosine coefficient) of sine and cosine functions of different frequency with the observed data. A large correlation indicates a strong periodicity of the respective frequency in the data. To obtain a periodogram the squared coefficients for each frequency are summed. Computation of the Periodogram is the following:

$$P_k = (\text{sin coefficient}_k^2 + \text{cos coefficient}_k^2) \cdot \frac{N}{2}$$

where P_k is the periodogram value (dimensionless) at frequency ν_k and N is the overall length of the series. Obtained periodograms are corrected for series averages and trends (see also Brandes et al., 2006 for details). As a result, cycles of different lengths are identified in the time series of interest, which initially may have an aspect of simply random noise.

3   Results

3.1   Meteorological and eddy data

At the time of sampling, vapour pressure deficit (VPD) at 25 m height peaked around 14:00 LT and minimum values were registered during the night (Fig. 1a). VPD generally exceed 2 kPa in the time period from noon to 17:00 LT. The absolute maximum was 2.5 kPa and was reached between 14 and 15 o'clock on the second sampling day.

Maximum soil temperatures at 5 cm depth were reached in the evening around 20:00 LT with values close to 13.7 °C
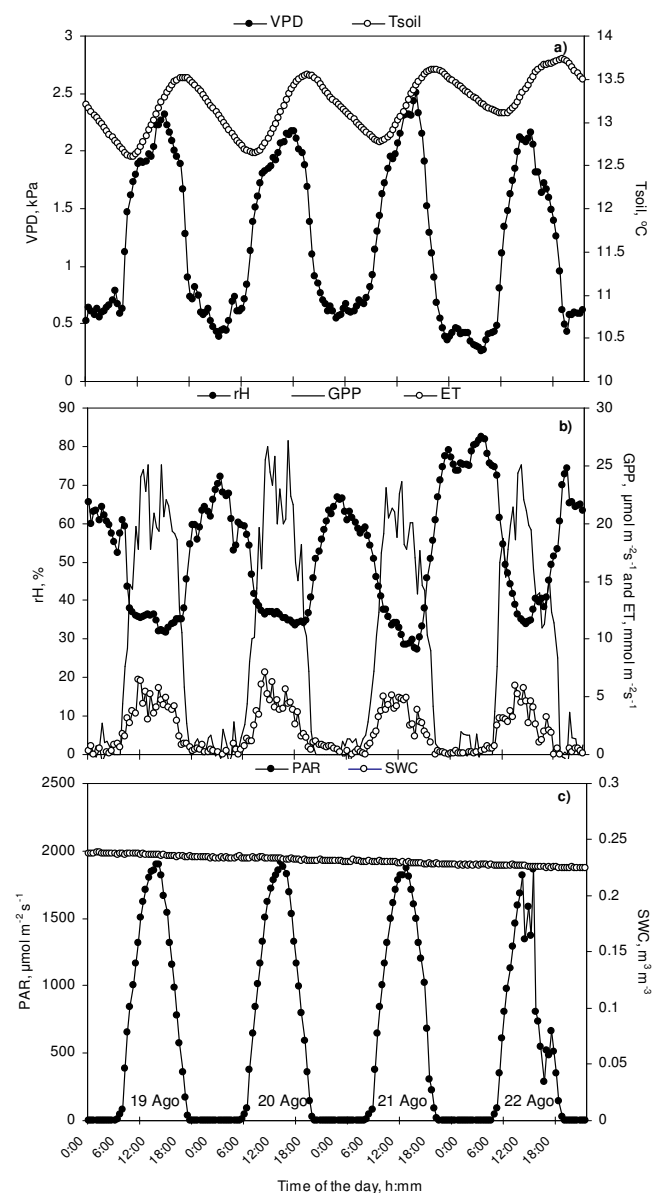
Fig. 1. Variation in **(a)** vapour pressure deficit (VPD, kPa) and soil temperature (Tsoil, °C) **(b)** gross primary production (GPP, μmol m^{-2} s^{-1}), evapotranspiration (ET, mmol m^{-2} s^{-1}) and air relative humidity (RH, %) **(c)** soil water content (SWC, m^3 m^{-3}) and photosynthetic active radiation (PAR, μmol m^{-2} s^{-1}) measured on the day before and during three days of sampling. VPD was calculated for 25 m height, PAR was measured at 27 m height, and GPP and ET – 10 m above the canopy, Tsoil and SWC correspond to a depth of 5 cm.

(Fig. 1a). Minimum temperatures were observed in the morning. Day/night soil temperature variation did not exceed 1 °C.

Air relative humidity was decreasing from the early morning to the afternoon hours and increasing further up to the maximum rates of 65–85 % in the late evening and night (Fig. 1b).

Gross primary production (GPP) derived from the eddy covariance measurements was approaching the values of approximately 25 μmol m^{-2} s^{-1} in the morning hours, decreasing slightly around a midday and on some days recovering in the afternoon (Fig. 1b). Timing of evapotranspiration (ET) maxima and minima were consistent with GPP variations, reaching 7 mmol m^{-2} s^{-1} during the morning hours (Fig. 1b).

Diurnal variations of soil water content (SWC) at 5 cm depth were barely detectable and always not statistically significant. During the period of measurements, a slight gradual decrease in SWC was registered: from 0.24 m^3 m^{-3} at the beginning to 0.22 m^3 m^{-3} at the end of the sampling period (Fig. 1c).

Maximum values of photosynthetic active radiation (PAR) exceeded 1800 μmol m^{-2} s^{-1}; with a slight dimming due to convective clouds during the central part of the day that was observed only on the last day of sampling (Fig. 1c).

3.2 Leaf sugars

A gradual ^{13}C enrichment of leaf soluble sugars (LSS) was observed going from the base crown to the top leaf layers (Fig. 2a). The average ($\pm$SD) δ^{13}C values were -29.2 ± 0.72‰; -27.0 ± 0.82‰ and -25.0 ± 1.0‰ for the base, middle and top crown leaves ($p < 0.001$), respectively.

Trends for diurnal dynamic in δ^{13}C in LSS synthesized at the top of the crown could be observed, with different maximum levels during the night periods and midday hours and minimum (more negative) values in the morning and afternoon. Day/night variation in LSS δ^{13}C considering all samples measured in the top crown leaf layers was as high as 4.2‰ (1.4‰ variation among the means, Fig. 2).

No night enrichment in ^{13}C isotopic composition was observed in LSS synthesized at the middle and base crown while elevated δ^{13}C (more positive) could be still detected during the day hours (Fig. 2a).

Current assimilates at the top crown were on average ^{13}C enriched by 3‰ compared to total organic carbon analysed in the same leaf layer ($p < 0.001$) (Fig. 2a). As expected, diurnal variations in δ^{13}C of the bulk dry material were dampened in comparison to LSS at the respective height.

Oxygen isotopic composition of LSS sampled from the top crown over the three sampling days ranged between 34.2‰ and 37.4‰ among means (Fig. 2c). The most enriched values in ^{18}O were observed during the afternoon and night hours while the least enriched were reached in the early morning.

Diurnal dynamics in leaf starch content were analysed for the three crown heights (Fig. 3a). The highest starch content was observed at the top crown layer and it gradually decreased going down through the crown.

A clear diurnal periodicity in starch content could only be seen in the top crown leaves, with the maximum starch accumulation between 14:00 and 17:00 LT and minimum
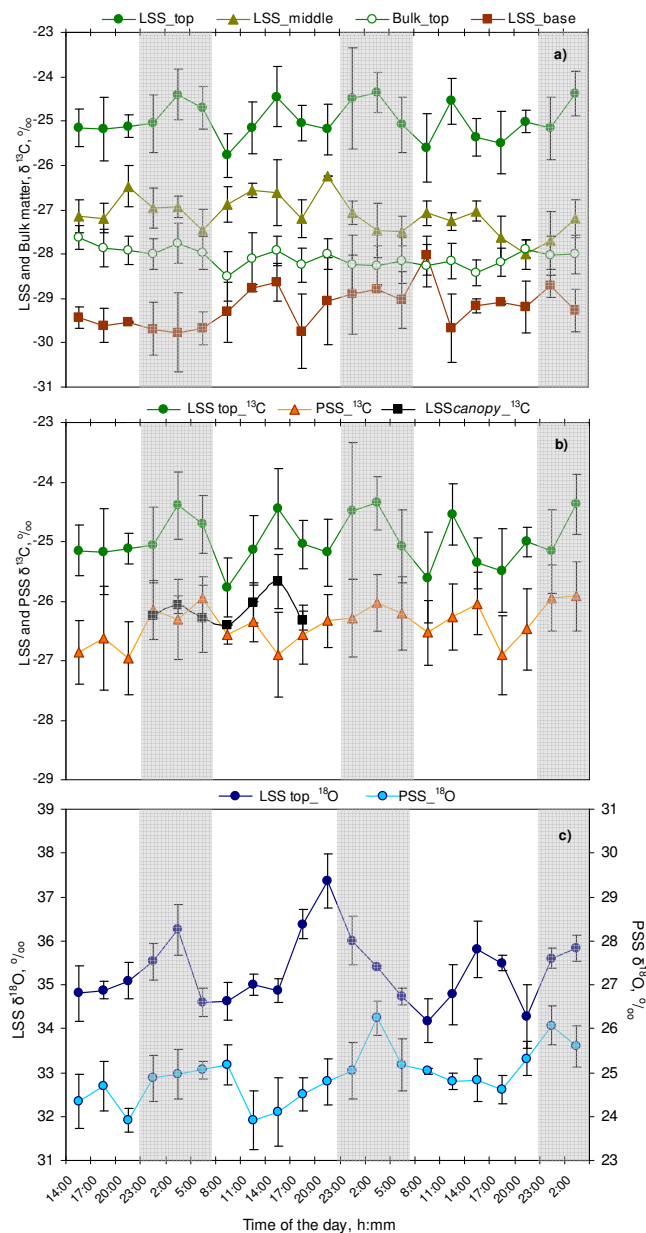
Fig. 2. (a) Dynamic of ^{13}C in leaf soluble sugars (LSS) of beech leaves sampled from the top, middle and base crown. Total ^{13}C in the bulk dry matter for the top crown leaves is also shown; (b) diurnal variation in δ^{13}C in soluble sugars in phloem exudates (PSS), sampled at breast height (1.3 m), in canopy weighted δ^{13}C (δ^{13}C canopy) and in LSS of the top crown leaves; (c) dynamic of δ^{18}O in LSS and PSS. White and grey hatching of the graph indicate day and night periods. Bars represent the standard error of mean ($n = 4$).

Fig. 3. 24-h time-course of (a) leaf starch content and (b) leaf sugars content (each point is a sum of sucrose, fructose and glucose) in the top, middle and base crown leaves of beech trees. Bars represent the standard error of mean ($n = 4$).

leaves, respectively. Leaf sugars content (glucose, fructose, sucrose) followed a diurnal dynamic similar to that observed for starch (Fig. 3b), with minimum concentrations measured in the early morning and maxima in the afternoon. The average daily LAI-weighted contribution of each individual leaf canopy layer to the overall sugar production was calculated to be 46 % in the top leaves, 46 % in the middle layer, and 8 % in the base crown leaves. Sucrose was the most abundant soluble sugar among the analysed ones, its relative contribution accounted to 95 %.

3.3 Phloem sugars

Phloem soluble sugars (PSS) were generally depleted in ^{13}C by 1.5 ‰ compared to LSS of the top sunlit leaves of the crown and enriched with respect to the LSS extracted from the middle and base crown ($p < 0.001$) (Fig. 2b). PSS δ^{13}C values were close instead to the canopy weighted δ^{13}C (by mean -26.39 ‰ and -26.14 ‰, respectively) and not significantly different from it in most sampling occasions. Canopy

values during the night and early morning hours. Such a trend was less pronounced for the middle crown leaves and completely absent in the lower crown. A fraction as high as 58 % of the daily average LAI-weighted starch content was formed in the top crown leaves, while approximately 39 % and 3 % originated from the middle and the base crown
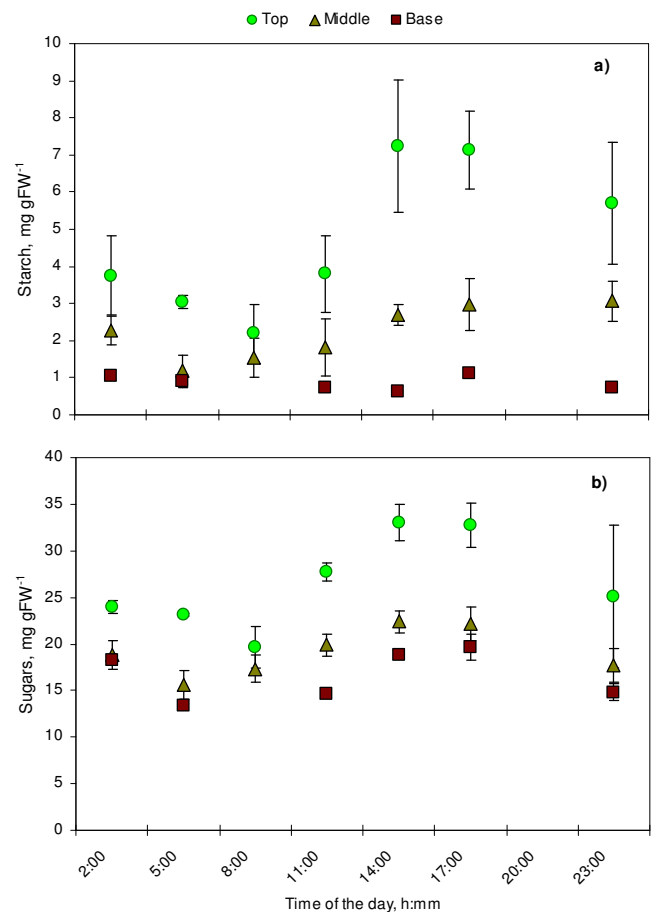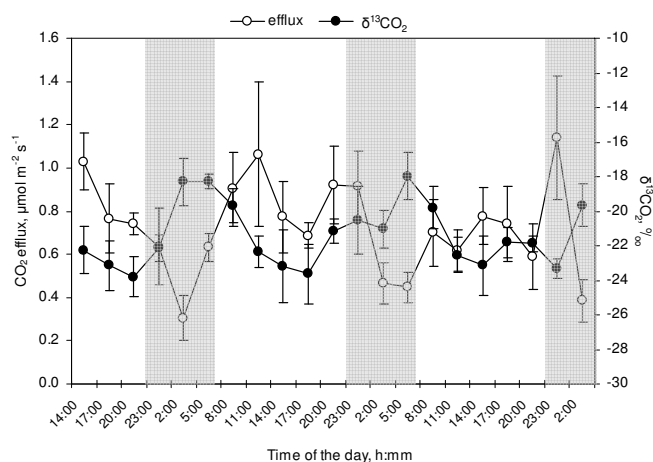
Fig. 4. ^{13}C of the soil CO_2 and soil respiration rates, obtained with Keeling plot technique. White and grey hatching of the graph indicate day and night periods. Bars represent the standard error of the mean ($n = 4$).

weighted $\delta^{13}C$ had an intermediate magnitude between top and middle crown LSS $\delta^{13}C$, its day/night undulations were similar to those of top crown LSS but considerably dampened (Fig. 2b).

Diurnal variation in ^{13}C of PSS was also almost absent: the oscillations between the night maximum and day minimum were much smaller than for LSS in top crown being approximately 1‰ by the mean.

PSS were significantly depleted in ^{18}O in comparison to LSS ($p < 0.001$) and diurnal range was from 26.2 to 23.9‰ among means. The most enriched values were observed during the night, and the most depleted – in day time (Fig. 2c).

3.4 Soil respiration

The mean R^2 for soil Keeling plots was 0.95 and the intercepts of regressions with R^2 lower than 0.9 were excluded from the analyses.

The soil CO_2 soil efflux was significantly enriched in ^{13}C compared to both phloem and leaf soluble sugars during the night; comparable but still more positive $\delta^{13}C$ values of soil respired CO_2 were observed during the day (Fig. 4)

Diurnal variation in $\delta^{13}C$ of soil respired CO_2 was more evident than in soluble sugar extracted from plant material, with day – night variation of about 5.8‰ on average.

3.5 Time lagged correlations

The time required for translocation of recently fixed carbohydrates from leaves along the trunk reaching the level of breast height were estimated from the highest correlation coefficient between phloem $\delta^{13}C$ and $\delta^{18}O$ and the time-lagged values of top crown soluble sugars $\delta^{13}C$ and $\delta^{18}O$, respectively. The highest and only significant correlation in the

case of $\delta^{13}C$ was observed for a 24 h time-lag (Table 1). PSS $\delta^{18}O$ was related significantly to 6 h and 27 h lagged LSS $\delta^{18}O$ (Table 1).

The $\delta^{13}C$ of the soil respired CO_2 showed the strongest correlation with 3 h and 24 h lagged value of $\delta^{13}C$ in PSS (Table 1). A high correlation coefficient was found between soil CO_2 $\delta^{13}C$ and the 27 h lagged $\delta^{13}C$ value of leaf soluble material (Table 1).

$\delta^{18}O$ of LSS was correlated with a lag of 5–8 h to PAR, GPP, RH and VPD (Table 1). Lower, but still significant correlation was observed with almost all parameters up to three days prior to sampling (not shown). Stable climatic conditions in the adjacent days during the sampling period resulted in similar ^{18}O fractionation patterns and therefore high correlation with environmental factors of different days. Both whole $\delta^{18}O$ dataset and day-time values were tested for time-lagged correlations. Accumulation and night export of ^{18}O enriched carbohydrates may result in decoupling of LSS ^{18}O from water ^{18}O signature at night. $\delta^{18}O$ of PSS was better related to PAR and GPP with ~3.6 d lag and to VPD and RH with a variable lag of 2.6–3.6 d, which correspond to a transfer velocity of 0.3–0.4 m h^{-1}. Since isotopic variation of C in PSS was not pronounced, it was only related to RH with the time lag of ~2.7 d. $\delta^{13}C$ in LSS responds almost immediately to changes in VPD and PAR. Night $\delta^{13}C$ values, which are more likely decoupled from environmental drivers and depend mostly on starch cycle, were excluded from the correlation analyses.

A strong diurnal periodicity was observed in $\delta^{13}C$ of soil-respired CO_2 (Fig. 5). The highest periodogram units correspond to a cycle step of 24 h. An additional periodicity was registered with a 10 h cycle, but the latter was much less pronounced. Periodical signals in $\delta^{13}C$ were completely dampened in PSS (Fig. 5) and in LSS in the middle and base crow layers (not shown). LSS $\delta^{13}C$ from the top leaves had a small increase in periodogram units with 10–12 h cycle step, similar in shape to the soil-respired CO_2 dynamic. To verify if the 12 h diurnal cycle in top crown LSS $\delta^{13}C$ was significant, we have tested separately the data obtained from each replicate tree for periodicity. Diurnal $\delta^{13}C$ variation, with increase in periodicity units close to 12th h cycle step was observed in the leaves of three from four replicate trees (data not shown). No clear diurnal variation in $\delta^{13}C$ of the top crown leaves was found only for one tree. A ~24 h periodicity was found for $\delta^{18}O$ in LSS and PSS.

4 Discussion

4.1 $\delta^{13}C$ in LSS

Daily variation in $\delta^{13}C$ observed in the LSS of top crown leaves supports other findings on the diurnal periodicity of ^{13}C signature in recently fixed sugars (Brandes et al., 2006; Gessler et al., 2007; Kodama et al., 2008). Day/night

Table 1. Parameters of correlation (lag, corresponding to the highest correlation (in days and hours), significance of correlation and Pearson correlation coefficient (R)) between isotopic composition (δ^{18}O and δ^{13}C) in pools (top crown and phloem sugars) and fluxes (soil respired CO_2) and physiological and environmental factors. PAR-photosynthetic active radiation, RH – relative humidity of the air, VPD – vapour pressure deficit, GPP – gross primary production. Not corr – not correlated.

| Lagged Variables	δ^{18}O_LSS	δ^{18}O_PSS	δ^{13}C_LSS	δ^{13}C_PSS	δ^{13}C_CO$_2$
PAR	6–8 h, $p < 0.05$, $R = 0.52$	3.5 d, $p < 0.001$, $R = 0.72$	2 h, $p < 0.05$, $R = 0.57$	not corr	
RH	6–7 h, $p < 0.05$, $R = -0.54$	2.5–3.5 d, $p < 0.001$, $R = -0.78$	2 d, $p < 0.05$, $R = -0.57$	2.7 d, $p < 0.05$, $R = -0.59$	
VPD	5–7 h, $p < 0.05$, $R = 0.57$	2.5–3.5 d, $p < 0.01$, $R = 0.70$	1 h, $p = 0,05$, $R = 0.49$	not corr	
GPP	7 h–1.3 d, $p < 0.05$, $R = 0.51$	3.5 d, $p < 0.001$, $R = 0.72$	1–3 h, $p < 0.05$, $R = 0.58$	not corr	
δ^{18}O_LSS	–	6 h and 27 h, $p < 0.01$, $R = 0.70$ and 0.73			
δ^{18}O_PSS		–			
δ^{13}C_LSS			–	24 h, $p < 0.01$, $R = 0.71$	6 h and 27 h, $p < 0.05$, $R = 0.53$, and 0.73
δ^{13}C_PSS				–	3 h and 24 h, $p < 0.05$, $R = 0.64$ and 0.60
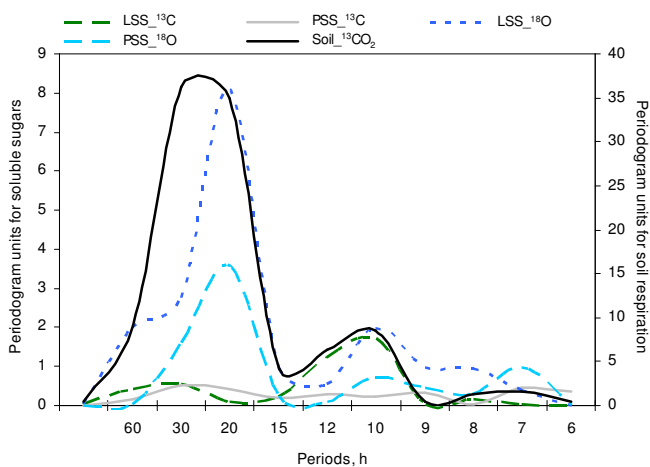
Fig. 5. Periodogram for the time series of ^{13}C and ^{18}O in recently fixed sugars in leaf and phloem, and in ^{13}C of soil respiration. Periods on the x-axes correspond to the number of hours necessary to complete one circle. Periodogram units on the y-axes are dimensionless.

differences were not high, but in the range of 1–2 ‰ usually reported for such type of observations. However, our data show a shorter cycle frequency with respect to the 24 h reported in the literature. In addition to the common ^{13}C

enrichment during the night already observed by these authors for LSS in different species such as *Pinus sylvestris* and *Eucaliptus delegatensis*, we have observed a midday ^{13}C enrichment which was not in phase with the diurnal variation in the starch synthesis/break-down (Figs. 2 and 5). The remobilization and break-down of the ^{13}C-enriched starch and the consequent formation of soluble sugars (sucrose, glucose and fructose) started around midnight (Fig. 3), as supported by a decrease in the starch content and by a parallel increase in ^{13}C enrichment of LSS (Fig. 2). Starch enrichment is the result of the isotopic fractionation during aldolase condensation reaction whereby isotopically enriched hexoses are formed and depleted triose-phosphates are left behind, given the origin to the light sugar molecules during the daytime (Gleixner and Schmidt, 1997; Tcherkez et al., 2004). The correlation between nighttime starch content and δ^{13}C in LSS was low but significant ($p < 0.05$, $R^2 = 0.40$).

The observed midday isotopic enrichment in LSS was probably explained by the especially dry conditions at Collelongo site with VPD values exceeding 2.0–2.5 kPa during the day. Furthermore, at this site, the wilting point derived from the water retention curve was 0.19 m^3 m^{-3} along the soil profile, values close to that and even lower at particular depth were measured during the present campaign. The measured VPD is certainly higher than in other experimental sites reported in the literature (e.g. Barbour et al., 2005; Barnard et

al., 2007; Kodama et al., 2008). Although stomatal conductance was not measured in the present experiment, it is quite likely that an increase in VPD led to partial stomatal closure, a decrease of c_i/c_a (CO_2 partial pressure in substomatal cavity to that of the ambient air) and to a subsequent ^{13}C enrichment of the newly synthesized carbohydrates. Flux data from the same dates indicate that GPP and evapotranspiration maxima were reached in the first part of the morning and during the subsequent hours the values were generally lower (Fig. 1b). This could be used as an indicator of midday depression of photosynthesis in response to partial stomatal closure.

The diurnal δ^{13}C cycle of the top crown leaves, even if not very pronounced, was completely absent in leaves from the base or middle crown layers (Fig. 2a). The lack of any significant daytime starch accumulation in the base crown leaves is in agreement with the absence of a nighttime ^{13}C enrichment of LSS. Although the middle crown leaves were accumulating starch in much lower quantities than leaves from the upper layer, diurnal differences in their starch contents were substantial (Fig. 3a).

The observed gradual ^{13}C enrichment of LSS from the base crown to the top is in agreement with our previous studies in the same site (Scartazza et al., 2004) and with other studies both on the same species and in other tree species (Buchmann et al., 1997; Göttlicher et al., 2006). On the other hand, contrasting results were reported for *Pinus sylvestris*, where no intracanopy variations in δ^{13}C of LSS were found with height (Brandes et al., 2006). Differences in LAI distribution in the canopy and canopy density among different species must be taken into account. The dense canopy in beech forest (LAI was 6.03 during the peak of the growing season) influences significantly the irradiance profile within the canopy and at different leaf layers, resulting in variation of air temperature, humidity and consequently c_i/c_a ratio. Differences in canopy density can also affect the extent of CO_2 recycling within the forest with potential effects on δ^{13}C, although air δ^{13}C within the canopy was found to have a minor effect on the δ^{13}C signature of LSS and leaf bulk material (Brooks et al., 1997; Buchmann et al., 2002; Scartazza et al., 2004; Göttlicher et al., 2006).

A significant variation in δ^{13}C was found not only in LSS among leaves from different heights, but also among LSS from different trees sampled at the same height as shown by the relatively large error bars (Fig. 2). However, a diurnal δ^{13}C variation with increase in periodicity units close to 12th h cycle step was observed in leaves of three from four replicate trees (data not shown). There were significant differences among individuals in the time the peaks were reached and in the range of the δ^{13}C variation, with some trees being constantly more positive in ^{13}C signature of LSS compared to others. Such differences may be related to slight changes in the radiation regime and exposition of the sampled leaves. In fact, at the top of the crown, fully exposed leaves were sampled but there were slight variation in their position with respect to adjacent branches which may have resulted in differences in the exposition time and shifts in the light curve, with consequent variations in the content of sugars and starch (Figs. 2 and 3). Similar results were reported in *Eucaliptus delegatensis*, where δ^{13}C measured in LSS sampled from certain leaves did not match with values predicted from c_i/c_a measured by gas exchange on the adjacent leaves (Gessler et al., 2007).

4.2 δ^{13}C in PSS

Phloem Soluble Sugars (PSS) sampled at breast height were depleted in ^{13}C compared to LSS extracted from the top crown, and their δ^{13}C was intermediate between that of LSS of top and middle crown leaves. These findings are in contrast with some previous results on the same and other species showing that PSS are enriched compared to the leaf organic matter and LSS at either of the layers (Hobbie and Werner, 2004; Cernusak et al., 2005; Scartazza et al., 2004; Brandes et al., 2006; Kodama et al., 2008; Wingate et al., 2010). Our LSS, being composed almost entirely of sugars, could be however more enriched than leaf water soluble organic matter used for comparison with PSS in other studies as they contain also other, relatively depleted ^{13}C substances (Bowling et al., 2008). On the other hand, a significant progressive ^{13}C depletion in PSS downward from the top to the lower part of the trunk in comparison to leaf organic matter was reported for *Acacia longifolia* (Rascher et al., 2010). To identify the crown layer exerting the highest influence on the δ^{13}C signature of PSS we have calculated the canopy weighted δ^{13}C according to Scartazza et al. (2004). The top crown leaves, even having a lower LAI than the middle layer, contributed significantly to the whole crown δ^{13}C (Fig. 2b) because of their 2–3 fold higher sugars and starch concentrations (Fig. 3). Diurnal variability in PSS δ^{13}C was similar in shape and magnitude to that of 24 h lagged canopy weighted δ^{13}C with slight night and midday enrichment and was also correlated significantly with 24 h lagged LSS δ^{13}C in the top crown. We therefore argue that more negative δ^{13}C of PSS could simply derive from the mixing of phloem sugars with isotopically depleted compounds formed in the lower crown layers, making leaf stratified sampling and determination of the ^{13}C signature in the formed organic matter in separate canopy layers important. Some authors have measured the ^{13}C enrichment in the CO_2 respired from heterotrophic tree tissues (roots and phloem), as compared to its putative substrates, leaving behind isotopically depleted organic material (Cernusak et al., 2001; Brandes et al., 2006; Gessler et al., 2007; Kodama et al., 2008; Rascher et al., 2010). In the present study, δ^{13}C of the trunk respiration was not measured, but the observed δ^{13}C signature of PSS could also suggest a fractionation favouring ^{13}C during phloem respiration. The contribution processes which were found to enrich the transported organic matter like fractionation during carbohydrates' loading (Hobbie and Werner, 2004; Cernusak et

al., 2005; Gessler et al., 2007), enrichment during carbohydrate transport and exchange within the heterotrophic tissues (Damesin and Lelarge, 2003; Gessler et al., 2004), refixation of the respired CO_2 by PEPC (Gessler et al., 2009; Wingate et al., 2010) were probably less relevant in determining the PSS $\delta^{13}C$. Interestingly, $\delta^{13}C$ values of the phloem extracts measured at the same site in 2001 were enriched compared to LSS (Scartazza et al., 2004). These apparently conflicting results may be explained by differences in sampling techniques: Scartazza et al. (2004) was collecting PSS in a way that each sample was integrating carbohydrates transported over the whole day. This approach does not allow accounting for the diurnal variability in PSS $\delta^{13}C$; in addition they sampled leaves only once a day – before dusk, when the LSS are actually depleted.

All mentioned processes could be also responsible for the observed dampening of the day/night variation in PSS $\delta^{13}C$ with respect to that in the LSS of the top crown leaves (Fig. 5). The present results support previous reports showing that during carbohydrate transport from leaves to stem, their diurnal variation in $\delta^{13}C$ was actually dampened, with a smaller range in stem compared to leaves (Brandes et al., 2006; Gessler et al., 2007; Kodama et al., 2008). All these authors have concluded that phloem sap is continuously exchanging C with the surrounding tissues and, therefore, it integrates organic matter synthesized in different layers of the crown with considerable contribution also from storage compounds.

4.3 $\delta^{13}C$ in soil respiration

Among all pools and fluxes analysed for $\delta^{13}C$ dynamics, soil respiration was found to exhibit the most pronounced diurnal variations and it was the most enriched in ^{13}C (Figs. 4 and 5). Such enrichment of soil-respired CO_2 was also highlighted in other recent studies (Kodama et al., 2008; Marron et al., 2009; Rascher et al., 2010) and might find several different potential causes. The main complication in interpreting soil respiration data arises from the fact that soil cannot be treated as a single source but rather integrates different sources of CO_2 (Kuzyakov, 2006). Related to respiration substrate, the following sources of CO_2 could be identified: (1) root and rhizomicrobial respiration, whose substrate is mainly represented by carbohydrates transported from aboveground via the phloem flow and (2) microbial respiration in the root-free zone, which does not depend on recent photosynthetic supply of C, but rather on soil organic C.

Concerning root respiration, contrasting results about the fractionation associated with respiration in plant heterotrophic tissue were reported for different species and plant compartments. In some non-woody plants, measurements of $\delta^{13}C$ of root-respired CO_2 and of the putative respiratory substrate have indicated fractionation favouring ^{12}C, hence resulting in the ^{13}C depletion of root respired CO_2 with respect to the remaining organic matter (Klumpp et al., 2005;

Boström et al., 2007; Gessler et al., 2009). Re-assimilation of the respired CO_2 by PEP carboxylase, favouring ^{13}C was proposed to decrease the $\delta^{13}C$ of the remaining CO_2 pool. However, the prevailing dry summer conditions in Mediterranean ecosystems like that of this study may inhibit the activity of root and microbial PEP carboxylase, which uses dissolved HCO_3^- as substrate (Unger et al., 2010). Additionally, most field experiments on tree species like *Fagus sylvatica* (Damesin and Lelarge, 2003), *Eucalyptus delegatensis* (Gessler et al., 2007), *Pinus sylvestris* (Brandes et al., 2006), *Acer negundo* (Moyes et al., 2010), *Pinus Pinaster and Acacia longifolia* (Rascher et al., 2010), have demonstrated that CO_2 emitted from heterotrophic tissues is ^{13}C enriched compared to its putative C substrate. For example, the roots of *Acer negundo* were found to emit the CO_2 enriched by almost 10 ‰ with respect to the $\delta^{13}C$ of the root tissues (Moyes et al., 2010). This may also explain a significant enrichment of respiration, even if we consider that a depletion gradient of the soluble sugars found between leaves and phloem is maintained downwards so that root tissues are substantially depleted with respect to other C pools.

A dampened $\delta^{13}C$ diurnal signal in PSS with respect to the pronounced diurnal variation in soil respiration $\delta^{13}C$ (Fig. 5) suggests that other processes rather than leaf-imprinted C discrimination influence this step of the C transfer path. Soil CO_2 efflux in our site was negatively related to $\delta^{13}C$ of the evolved CO_2 (Fig. 4, $R^2 = 0.22$, $p < 0.05$). The most ^{13}C enriched CO_2 evolved from soil during the night and was associated with lower soil respiration rates. During the day, when higher respiration was generally observed, the $\delta^{13}C$ of the respired CO_2 was more depleted. Such variations could have several possible origins. The first – changes in the relative contribution of root and microbial respiration to the total CO_2 efflux over the diurnal course, which may explain both, nighttime significant isotope enrichment and the day/night variation in the CO_2 efflux rate. If this has taken place, then the ^{13}C enriched respiration at night is due to an increase in the relative contribution of microbial respiration to the total CO_2 efflux. In fact, dry summer conditions may result in redistribution of the microbial activity to the deeper soil layers, which are known to be characterized by an ^{13}C enriched SOM (Buchmann et al., 1997). Day/night variations in temperature and SWC were negligible, so that microbial respiration δ and efflux to a high probability should be constant. Root activity has been proposed to be sensitive to recent photosynthetic status on a short-term scale (Mencuccini and Höltta, 2010), resulting in low respiration rates when photosynthesis is declining or absent. Other authors have also proposed that changes in contribution of different sources to soil respiration over a day/night cycle may explain the diurnal ^{13}C patterns of soil respiration (Gessler et al., 2007; Kodama et al., 2008; Marron et al., 2009; Wingate et al., 2010). Many direct and indirect evidences support the hypothesis that the CO_2 produced during microorganisms activity in soil differs in ^{13}C signature from the CO_2 derived from root respiration

(Bhupinderpal et al., 2003; Gessler et al., 2007; Kodama et al., 2008; Moyer et al., 2010; Unger et al., 2010; Wingate et al., 2010).

In contrast, transient diffusive fractionation rather than changes in δ^{13}C of the CO_2 produced in soil was recently proposed to explain diurnal patterns of δ^{13}C in soil respiration (Moyen et al., 2010). The authors argued that day/night changes in the soil CO_2 production rate result in the gradual development of a new isotopic steady state as the diffusivities of the $^{12}CO_2$ and $^{13}CO_2$ are slightly different and lighter C left soil with initially higher rate followed then by the heavier one. Our data in general are consistent with the patterns reported by these authors (Fig. 7 in Moyes et al., 2010): day/night δ^{13}C variation measured in our site is among the highest reported in the literature (up to 11 ‰ within a single chamber and 6 ‰ by mean), whereas soil flux rates are very low and flux coefficient of variation is high, on the contrary ($\sim$0.3).

Another non biological cause of significant enrichment and considerable day/night patterns in soil respiration could be advection induced by gas sampling process. Gas sample of 12 ml results in a pressure perturbation inside the chamber of $\sim$170 Pa each time the gas is withdrawn and causes a mass flow of air from the soil into the chamber. Xu et al. (2006) stated that removal of a small air sample from the chamber with a considerable ground surface and volume should not have a significant impact on respiration patterns. However, in that case they were referring to a 0.5 ml sample and a pressure perturbation of 10 Pa. If we assume that soil air close to the surface has the CO_2 concentration of 3000 μmol mol^{-1}, then the CO_2 concentration in the chamber due to the mass flow created by the 12 ml sample withdrawn will rise from initial 400 to 404 μmol mol^{-1}. This could be not a big problem at high CO_2 effluxes. However, in our case during the summer drought and consequently low respiration rates, this air could significantly influence respiration rates, δ^{13}C and also amplify day/night differences. Our soil chambers have characteristics similar to those used for δ evaluation of soil respiration with Keeling plot approach (volume range 6–10 l: Betson et al., 2006; Gessler et al., 2007; Kodama et al., 2008). However, no one has raised this sampling problem issue yet.

The unusually positive δ^{13}C values of soil CO_2 efflux could be explained not only by variation of its biological source and methodological constraints but also by the contribution of geological processes, like the dissolution of carbonates and exchange of this depleted C with soil CO_2 (Plan, 2005; Asano, 2007). The contact of the rock carbonates with the film of water necessary for solution process is however of a low probability under dry soil conditions.

All proposed explanations of δ^{13}C diurnal patterns (changes in relative contribution of root and microbial respiration with different δ^{13}C over diurnal cycle, diffusive fractionation and sampling induced errors) restrict the applica-

bility of soil respiration for disentangling of the time lags between photosynthesis and soil respiration.

4.4 δ^{18}O from leaves to the phloem

Diurnal variations in δ^{18}O of LSS and PSS were more pronounced than those of δ^{13}C measured in the same pools. Day/night changes in δ^{18}O were slightly dampened while sugars were transported from the site of assimilation downward, but not completely as in the case of δ^{13}C. This is because of the substantial day/night changes in leaf water δ^{18}O, which decreases in the evening when RH is high, tending to approach source water δ^{18}O during the night and increase during the day while the RH is low (Yakir, 1998; Cernusak et al., 2002; Barnard et al., 2007). Leaf water δ^{18}O dynamic is further imprinted in the δ^{18}O of the synthesized organic matter with certain fractionation factor. Periodicity close to 20–24 h was observed for both LSS and PSS δ^{18}O (Fig. 5). Apart from the daytime ^{18}O enrichment of LSS associated with transpiration process, some patterns for the night δ increase were also observed. This is probably associated with the night starch breakdown and release of the organic matter synthesized and equilibrated during the previous days and carrying the enriched ^{18}O signal of daytime water, or/and with the delay in reaching a new isotopic steady state with leaf water. It was reported that the time needed for the newly formed organic matter to reflect new isotopic conditions induced by environmental and consequently intercellular and leaf water chemical changes is in the range of 3–12 h (Barbour et al., 2000; Barnard et al., 2007). Maximum ^{18}O evaporation enrichment of leaf water itself could be also delayed in respect to changes in humidity rates by several hours because of slow mixing and non-equilibrium conditions (Yakir, 1998).

PSS were almost 10 ‰ depleted in ^{18}O respect to LSS. Basipetal gradient in ^{18}O along the trunk was also reported by Barnard et al. (2007) and Brandes et al. (2006). Continuous unloading and reloading of PSS into the phloem and involvement in the reactions with depleted xylem water may result in general depletion of the sugars along the trunk (Barnard et al., 2007). Refixation of the respired CO_2 by PEPC may also interact and modify the initially imprinted ^{18}O signal of sugars. A significant correlation between δ^{18}O in PSS and that in lagged LSS as well as distinct diurnal pattern in both of them make ^{18}O promising for an evaluation of carbohydrate transport velocity within the plant.

4.5 Time lags

Although spectral analysis did not detect a periodic signal for δ^{13}C variation in PSS (Fig. 5), this component was still significantly correlated with the 24 h lagged δ^{13}C in LSS (Table 1). This observation corresponds to the C translocation velocity of approximately 1 m h^{-1} from the sites of C assimilation to the tree trunk at breast height. It should be taken into

account that the experiment duration does not allow a reliable detection of lags longer than 1.5 days. In fact 1 m h^{-1} could be considered as a relatively fast translocation in comparison with the data reported in the literature for direct C transfer. We suppose that the lag is longer and observed 24 h could be its aliquot and result from similar environmental patterns between the adjacent days. A similar reason could be for the double lag, 6 h and 27 h, between LSS δ^{18}O and PSS δ^{18}O. The significant correlation found between δ^{13}C in soil respiration and the lagged δ^{13}C in PSS and LSS (Table 1) is more probable purely statistical and not causal as δ^{13}C variability due to photosynthesis discrimination was much lower than the one found for δ^{13}C in soil respiration.

The level of naturally occurring discrimination against ^{13}C during photosynthesis in leaves depends on the ratio of c_i/c_a (Farquhar et al., 1982). c_i/c_a is controlled by a number of environmental factors which affect stomatal conductance and by the activity of Rubisco. ^{18}O of leaf water shares the dependence on stomatal conductance with ^{13}C (Farquhar et al., 1998). Herewith, ^{18}O of assimilates carries the isotopic imprint of the water in which it was formed (Barbour et al., 2000). We have selected a number of environmental and internal parameters potentially linked to c_i/c_a and have tested their influence on the dynamic of ^{13}C and ^{18}O in leaf and phloem pool (Table 1).

δ^{18}O of LSS was negatively related with the lag of 5–8 h to RH and positively – to PAR, GPP, VPD (Table 1). Such a shift in maximum enrichment with respect to stomatal conductnace-related parameters is due to the already-discussed delay in reaching isotopic equilibrium with the leaf water and for leaf water – with humidity and transpiration changes. δ^{18}O of PSS, being very similar in variation patterns to lagged δ^{18}O of LSS were also correlated with the same factors. The highest correlation was shifted by 2.5–3.5 d. Interestingly, the relation of PSS δ^{18}O to tested parameters was much stronger than that of LSS δ^{18}O (see p- and R-values in Table 1). This is probably associated with different composition of phloem transported organic matter from that extracted from leaves, the first one having a greater exchange rate with water in which it was formed. Sucrose, as stated previously – the main component of transported organic matter – has no carbonyl groups for oxygen exchange with the surrounding water. However, intermediates involved in its synthesis in leaves, like triose phosphates, do have this and under high assimilation rates equilibrate completely with cytosolic water (Farquhar et al., 1998). Estimated velocity of carbohydrates' translocation from δ^{18}O dynamics is 0.3–0.4 m h^{-1}. This number is close to the one measured in such types of experiments and for plants with a similar height (Kuzyakov and Gavrichkova, 2010).

5 Conclusions

A strong correlation between δ^{13}C and δ^{18}O in leaf recent assimilates with that in sugars extracted from the phloem

at a breast height supports a tight link of belowground metabolism to C supply from aboveground and to recent photosynthetic activity. The main determinants of δ^{13}C signature in the recently fixed organic material in leaves were isotope effects during photosynthesis associated with leaf level gas exchange and starch day/night dynamic. Along the C transfer, δ^{13}C imprinted in these recently fixed carbohydrates, undergo substantial variations due to post-photosynthetic fractionation on various steps of translocation and due to further mixing with other pools of C carrying different isotopic signals. All these processes complicate the application of the natural abundance of ^{13}C for studying the fate of C within the plant-soil continuum. A significant improvement of the method will consist in the identification of the degree to which microbial respiration component overlap with root respiration δ^{13}C and in the ability to isolate these isofluxes. Sampling related issues and its influence on soil respiration δ^{13}C should be also addressed more carefully. δ^{18}O in recently fixed sugars at the leaf level is subjected to much more substantial diurnal variations than δ^{13}C. Leaf-imprinted oxygen isotopic signal is not completely dampened along sugar's downward transport, therefore making δ^{18}O promising for the identification of translocation velocities.

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