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Importance of short-term dynamics in carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$) in a Mediterranean oak woodland and linkage to environmental factors

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Summary

• Temporal dynamics in carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$) were evaluated on hourly, daily and annual timescales in a Mediterranean woodland. Emphasis was given to the periods of transition from wet to dry season and vice versa, when the system turns from a net carbon sink to a source. The constancy of nocturnal $\delta^{13}C_R$ was tested.

• The relationship between $\delta^{13}C_R$ (determined through Keeling plots) and environmental factors was evaluated through time-lag analysis.

• $\delta^{13}C_R$ exhibited high annual variation (> 7‰). During the transition periods, $\delta^{13}C_R$ correlated significantly with factors influencing photosynthetic discrimination, soil respiration, and whole-canopy conductance. Time-lags differed between below- and above-ground variables, and between seasons. A shift in regression parameters with environmental factors indicated seasonal differences in ecosystem responsiveness (e.g. temperature acclimation). $\delta^{13}C_R$ exhibited substantial nocturnal enrichment (> 4‰) from dusk to dawn.

• These data indicate pronounced short-term dynamics in $\delta^{13}C_R$ at hourly to daily timescales and a modulated response to environmental drivers. Substantial short-term changes in nocturnal $\delta^{13}C_R$ may have important implications for the sampling protocols of nocturnal Keeling plots.

Key words: carbon isotope discrimination, carbon isotope ratio ($\delta^{13}C$), ecosystem respiration, Keeling plot, Mediterranean woodland, stable isotope, time-lag, vapour pressure deficit (VPD).

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Introduction

Global atmospheric CO_2 concentration – a major greenhouse gas – has been steadily increasing for decades (e.g. Keeling *et al.*, 1995). A full account of ecosystem carbon balance, necessary to predict long-term trends in carbon sequestration, requires the quantification of gas exchange between the terrestrial biosphere and atmosphere (e.g. Canadell *et al.*, 2000; Schulze *et al.*, 2000). Ecosystem respiration is a major component of the C balance that needs a better understanding (Valentini *et al.*, 2000; Reichstein *et al.*, 2002).

In recent years the temporal behaviour of the carbon source/sink strengths of terrestrial ecosystems has been measured in many ecosystems based on micrometeorological

techniques such as eddy-covariance (Janssens *et al.*, 2001). Stable isotopes provide an independent way to examine the opposing fluxes and the biological and physical processes involved in ecosystem CO_2 exchange (Flanagan & Ehleringer, 1998) and can be used to quantify individual flux component processes (Lloyd & Farquhar, 1994; Yakir & Sternberg, 2000). The large difference between the isotope composition of respiratory and tropospheric CO_2 is now frequently used to partition net ecosystem fluxes into their components (Keeling, 1958).

The 'Keeling plot' approach is based on a two-source mixing model and has been successfully used to estimate $\delta^{13}C$ of ecosystem respiration in various studies across different biomes (Flanagan & Varney, 1995; Buchmann *et al.*, 1996, 1997; Lloyd *et al.*, 1996; Hemming *et al.*, 2005). At the ecosystem scale, these signatures can be used to partition net CO_2 fluxes into photosynthetic and respiratory components (Yakir & Wang, 1996; Bowling *et al.*, 2001; Ogée *et al.*, 2003), as well as soil from leaf respiration (Mortazavi & Chanton, 2002), or even estimate ecosystem-level water use efficiency (Ponton *et al.*, 2006).

Understanding the driving factors of ecosystem-respired $\delta^{13}CO_2$ ($\delta^{13}C_R$) is important for applications of isotope-based models of the global carbon budget, as well as for understanding ecosystem-level variation in isotopic discrimination (McDowell *et al.*, 2004a). Whereas a solid foundation exists for our understanding of carbon isotope discrimination (Δ) at the leaf scale (Farquhar *et al.*, 1989; for a review see Brugnoli & Farquhar, 2000), our theoretical and empirical understanding of the temporal variability in $\delta^{13}C_R$ at the ecosystem level is comparatively weak.

So far, most global inversion models assume a constant $\delta^{13}C_R$, as relatively little variation in $\delta^{13}C_R$ was observed in earlier work (Flanagan *et al.*, 1996; Buchmann *et al.*, 1998). As discussed by McDowell *et al.* (2004b), such constancy in $\delta^{13}C_R$ might be the result of several factors: (i) a lack of variation or an insensitivity to changes in environmental variables; (ii) a balancing effect of driving variables on carbon isotope discrimination (i.e. if both, stomatal conductance and assimilation rise in proportion causing a constancy in ci/ca); (iii) a decoupling of Δ and $\delta^{13}C_R$; or (iv) maybe even a lack of data. Recently, however, significant variations in $\delta^{13}C_R$ between seasons were documented (e.g. up to 8‰, see McDowell *et al.*, 2004a; Lai *et al.*, 2005; Ponton *et al.*, 2006).

These variations in $\delta^{13}C_R$ have been linked to environmental factors controlling isotope discrimination at the leaf level, indicating a strong linkage between carbon assimilation and ecosystem respiration (Ekblad & Högberg, 2001; Bowling *et al.*, 2002). The correlation of vapour pressure deficit (VPD) or air humidity with $\delta^{13}C_R$ was time-lagged for several days, that is the humidity conditions several days earlier explained most of the variations in $\delta^{13}C_R$. This has generally been interpreted as the transport time of assimilates from the foliage to the bulk of the respiring tissue (Bowling *et al.*, 2002). A similar multiple-day lag between assimilation and soil respiration has been observed in a girdling experiment by Högberg *et al.* (2001).

Recently, water balance was proposed as a common factor regulating $\delta^{13}C_R$. Fessenden & Ehleringer (2003), Lai *et al.* (2005) and Ponton *et al.* (2006) observed significant negative relationships between $\delta^{13}C_R$ and soil water content (SWC). Ometto *et al.* (2002) found that $\delta^{13}C_R$ was coupled to monthly precipitation. Bowling *et al.* (2002) found that $\delta^{13}C_R$ was positively related to VPD with a time-lag of several days (see also Knohl *et al.*, 2005; Mortazavi *et al.*, 2005). McDowell *et al.* (2004a) observed a strong correlation between $\delta^{13}C_R$ and soil temperature during drought: SWC and VPD were the most important climate variables influencing $\delta^{13}C_R$ in a Douglas fir, whereas a pine forest exhibited a significant but weak link to canopy conductance. Fessenden & Ehleringer (2002) found differences with stand age, which were consistent with the hypothesis that a decrease in stomatal conductance associated with a decrease in hydraulic conductance leads to an increase in diffusional limitation in older coniferous trees.

These studies suggest that the isotopic signature of the respiration flux may be markedly linked to recent meteorological events. However, more field observations are required to characterize short-term effects of temperature and water availability on $\delta^{13}C_R$. Mediterranean ecosystems with a pronounced seasonality in water and temperature regimes might provide useful insights into identifying the driving factors for variation in $\delta^{13}C_R$ and its link to net ecosystem productivity. The transition from the productive spring period into summer drought is often rapid (within a few weeks), when high temperature and low water potentials limit plant productivity.

This study compares the temporal variability of $\delta^{13}C_R$ at a high sample frequency explicitly in the transition periods from wet to dry season and vice versa in an open Mediterranean oak woodland (ISOFLUX project). These transition periods are of special relevance because timing and length of these events strongly determine the period when the system turns from a net carbon sink into a net source. These rapid changes in environmental parameters and physiological responses provide excellent periods to evaluate their effect on the isotopic imprint of ecosystem respiration ($\delta^{13}C_R$) and the correlation with driving environmental factors.

We assessed three objectives: first, to evaluate the temporal variability of the isotopic composition of ecosystem respiration ($\delta^{13}C_R$) during short- (24 h), medium- (2 wk) and long-term (annual) timescales; second, to explore the relationships between $\delta^{13}C_R$ and several environmental variables during periods of rapid transition (i.e. from wet to dry season and vice versa); and third, to investigate potential mechanisms causing short-term nocturnal variations in $\delta^{13}C_R$. The latter results may have important implications for the sampling protocols commonly used for night-time gradients of CO_2 concentration and $\delta^{13}CO_2$ and Keeling-plot analysis.

Materials and Methods

Field site and climatic conditions

The site is located in the centre of the Portuguese Alentejo at 'Herdade da Alfarrobeira', a rural district 12 km south of Évora (38°32′26.549″N, 8°00′01.424″W, elevation 264 m). The stand is a characteristic Mediterranean, savannah-type evergreen oak woodland (31.6% tree crown cover, leaf area index (LAI) of 0.55) in a very homogeneous landscape, which shows the signs of a typical silvo-pastoral system. The plant community is composed of canopy forming *Quercus ilex* ssp. *ballota* (syn. *Q. rotundifolia* Lam.) in mixture with *Quercus suber* L. and a grass layer dominated by herbaceous annuals, some drought deciduous graminea and a few shrubs (*Cistus* sp.). Soils are incipient derived from granitic rock. The climate is subhumid with a precipitation/potential evapotranspiration ratio (P/ETP) of 0.5–0.65, with hot dry summers and mild wet winters. Mean annual temperature (1961–90) is 15.5°C (mean max. and min. temperatures are 21.5 and 9.2°C, respectively) and mean annual precipitation is 669 mm (1961–90). The average number of days with precipitation above 1 mm is 76.

Continuous records of CO_2 and H_2O fluxes and climate variables were taken on a 28-m-high metal tower (at the Mitra site of the CARBOEUROPE-IP consortium) equipped with sonic anemometer (Gill R3, Gill Instruments, Lymington, Hampshire, England) and gas analyser (LI-6262, LI-COR, Lincoln, NE, USA). Weather conditions were continuously recorded by a solar-powered meteorological station (data-logger CR10X, Campbell Scientific, Logan, UT, USA), with a Q7 REBS net radiometer (Campbell Scientific), aspirated psychrometer H301 (Vector Instruments, Rhyl, Denbighshire, UK) and a rainfall recorder (Casella, Bedford, UK). Air temperatures (T_{air}), relative humidity, wind speed, net radiation (Rn) and precipitation were measured in 10 s intervals and were automatically stored as half-hourly and daily means/totals.

Soil parameters (see also Otieno *et al.*, 2006) were measured in 5 min intervals and recorded half-hourly (data-logger DL2e, Delta-T Devices Ltd, Cambridge, UK) at a nearby located field site at 10, 15, 20, 30 cm for soil temperature (T_{soil}, thermistor M841, Siemens, Munich, Germany), at 10, 20 and 30 cm depth for soil moisture (soil moisture probe Echo2, Decagon, Woodland, CA, USA) and at 40 and 100 cm depth for soil water potential (equitensiometer EQ 15, Ecomatik GmbH, Dachau, Germany).

Diurnal courses of leaf gas exchange (LI-6400 open-flow gas exchange system, LI-Cor, Lincoln, NE, USA), plant water potentials (pressure chamber, Manofrigido, Portugal), and soil respiration (PP-System EGM2 Soil Respiration System with SRC-1 chamber; PP-Systems, Amesbury, MA, USA) were recorded on 2–4 d at the beginning and towards the end of each intense sampling period. Measurements were conducted every 2–3 h on marked branches or plots for leaf and soil measurements, respectively. Leaf gas exchange and water potentials were measured on five *Q. ilex* trees in at least three sun-exposed leaves per tree. Soil respiration was measured on three to five plots with three measurements per plot. Standard deviations were calculated through error propagation procedure.

Sampling and isotopic analysis of organic matter

Isotope composition of organic material of different ecosystem components was analysed regularly in 2003. Five to 10 south-facing sun leaves of five marked trees (*Q. ilex*) and three to five leaves of five individuals of the main grass species (*Triticum aestivum*) were collected once per month. Only fully mature leaves from the latest growth period were used. Soil was collected at three depths (surface, 10 and 20 cm) through soil cores, sieved and roots removed. All material was oven-dried at 60°C for 48 h and milled to fine powder for carbon isotopic analysis. The isotopic composition of organic matter was determined at the University of Bielefeld. Sample preparation was performed in an elemental analyser (Elementar, Hanau, or EuroVector, Hekatech, Wegberg, Germany) where the samples are automatically combusted to water and CO_2 and analysed in a continuous-flow isotope ratio mass spectrometer (IsoPrime, GV Instuments, Manchester, UK). Samples were measured against IAEA-CH-4 and IAEA-CH-6 (International Atomic Energy Agency, Vienna, Austria). A cross-calibrated laboratory standard was measured every nine samples to verify any drift. Craig correction was applied. Values are reported relative to VPDBee, and repeated measurements precision was 0.05‰.

Sampling and isotopic analysis of atmospheric CO_2

We used small-volume (12 ml) soda glass vials (Exetainer, Labco, High Wycombe, UK) capped with pierceable septa for atmospheric air sampling, which have been tested to achieve stable ^{13}C isotope signals for 72 h and stable ^{18}O ratios for 24 h (C. Werner *et al.*, unpublished; see also Knohl *et al.*, 2005). Air was collected at nine different heights: 24, 20, 16, 12, 8, 4, 2, 1 and 0.5 m (the heights between 16 and 24 m above the tree canopy). Air was pumped through Dekarbon tubing (25 m length, inner diameter 6.9 mm; Sertoflex, Serto Jacob, Fuldabrück, Germany), which was fixed at the tower. Air was pumped at 10 l min^{-1} (pump Capex V2X, Charles Austen Pumps, Byfleet, Surrey, England) through the tubing until stable CO_2 concentrations were reached and subsequently flushed through the Exetainer vials (with two needles) for 1 min, which was determined to be twice the time required to reach stable CO_2 concentrations after full exchange of the air in the vial. Air leaving the vials was passed through an infrared gas analyser (BINOS 100 4P, Rosemount Analytical, Hanau, Germany; precision for $CO_2 \pm 1$ ppm), calibrated against known CO_2 concentrations in the field before each sampling, to monitor CO_2 concentrations in the vial. Samples

were repeatedly collected from the top to the bottom, resulting in two to three samples per height. Sample collection from all heights was completed within 30 min. Samples for Keeling-plot analysis were collected at noon and midnight (once a month in 2003, seasonally in 2004), on a daily basis during the field campaigns in spring and autumn, and at 2 h time intervals day and night during intensive 24 h sampling periods. Daytime Keeling plots could not often be calculated because of the high vertical mixing of the air in this open oak stand, resulting in insufficient CO_2 concentration gradients. Therefore, these data are not shown. Additionally, five Exetainer vials of known $\delta^{13}C$ and CO_2 concentration were filled in the field during each sample date and used as an external reference. Samples were brought to the laboratory (LIE, ICAT, Lisbon, Portugal) and analysed within 12–20 h after sample collection. Isotopic analysis was performed in a stable isotope ratio mass spectrometer (IsoPrime, GV Instruments, Manchester, UK) operating in continuous-flow method coupled to a Gilson/GV Multiflow prep-system (GV Instruments), which allowed the high automatic throughput of sample vials and high-precision chromatographic separation and TCD detection of N_2, CO_2 and H_2O for selective admission of CO_2 to the mass spectrometer. Craig corrections are applied as standard corrections for CO_2 analysis. In addition to external reference gas collected in the field, vials from a bottle of known isotopic composition in the laboratory were measured to verify any drift. Peak height of atmospheric samples was approximately 1.8–4 nA. Repeated measurement precision was < 0.1‰.

Mass balance approach

The Keeling-plot approach (a two-source mixing model) was used to assess the isotopic composition of ecosystem respired CO_2. Assuming that both source CO_2 and background CO_2 remain constant during the sampling period, the isotopic signature of ecosystem respiration ($\delta^{13}C_R$) can be calculated as the *y*-intercept of a linear regression of $\delta^{13}C$ vs the inverse of the CO_2 mixing ratio obtained from vertical profiles solving the following isotopic mass balance equation (Keeling, 1958):

$$\delta^{13}C_a = c_T(\delta^{13}C_T - \delta^{13}C_R)(1/c_a) + \delta^{13}C_R$$

($\delta^{13}C$, isotopic composition; *c*, CO_2 concentrations [CO_2] of the mixing ratios). The subscripts indicate sample air from several heights above and within the canopy (a), tropospheric air (T) and air respired from the ecosystem (R).

Following current practice (Pataki *et al.*, 2003), geometric mean (model II) regressions were calculated and uncertainties in the Keeling-plot intercepts were expressed as standard errors of the intercept estimated from ordinary least square (OLS, model I) regressions. To remove outliers, residual analyses were performed. Data points were removed from the regression when the residual of an individual data point was higher than three times the standard deviation (as proposed by Pataki *et al.*, 2003). Regressions were rejected when not significant ($\alpha = 0.01$). Statistical and regression analyses were performed using Statistica (StatSoft, Tulsa, OK, USA) and JMP (SAS Institute Inc., Cary, NC, USA).

Sap flow and canopy conductance

Sap flow density was continuously measured in eight *Q. ilex* trees by the Granier method (Granier, 1985, 1987). One sensor (UP GmbH, Cottbus, Germany) was radially inserted in the south-facing xylem of each tree. Each sensor consists of a pair of 20-mm-long probes inserted in the tree stem at breast height and vertically separated at 15 cm. The upper probe was heated to constant power, whereas the lower probe was unheated and remained at trunk temperature. Sensors were connected to CR10X data-loggers (Campbell Scientific, Shepshed, UK), scanning temperature differences between probes (ΔT) every 10 s and recording 10 min averages. Sap flux density was calculated from 10 min ΔT values and the absolute maximum temperature difference between probes (ΔT max) over 10 d periods – see Granier (1985) for further details. Sapwood conductive thickness was estimated from the sap flow radial profile obtained by the heat field deformation method (Nadezhdina *et al.*, 1998) in two of the sampled *Q. ilex* trees. Results showed that only the outer 30% of the trunk radius was effective conductive sapwood. Nevertheless, this sapwood depth always exceeded the Granier probe length. When compared with the radial sap flux profile, the average sap flow density measured by the Granier probes proved to be a good estimate of the average sap flow density over the entire conductive area. A similar finding was obtained by David *et al.* (2004) when data from 20-mm-long Granier probes were compared with the radial sap flux profile of a *Q. ilex* tree measured by the heat pulse method (Cohen, 1994). Therefore, tree sap flow was computed as the product of sap flow density (measured by the Granier method) and the sapwood conductive area of each tree. Tree sap flow values were expressed per unit of crown-projected area (kg m^{-2} s^{-1} or mm d^{-1} or mm h^{-1}).

Canopy conductance (G_c) was calculated from sap flow data via the Penman–Monteith equation (Oren *et al.*, 2001). Air temperature was used rather than leaf temperature because of a lack of continuous leaf temperature data. Average daytime G_c was calculated based on hourly values from 08:00 to 18:00 h, which was tested to yield better correlations than restricting the data from 10:00 to 14:00 h.

Results

Annual variations in $\delta^{13}C_R$ and organic matter

The typical seasonality of Mediterranean climates for the study period is shown in Fig. 1. The annual mean temperature was similar in both years (15.8 and 15.9°C in 2003 and 2004,

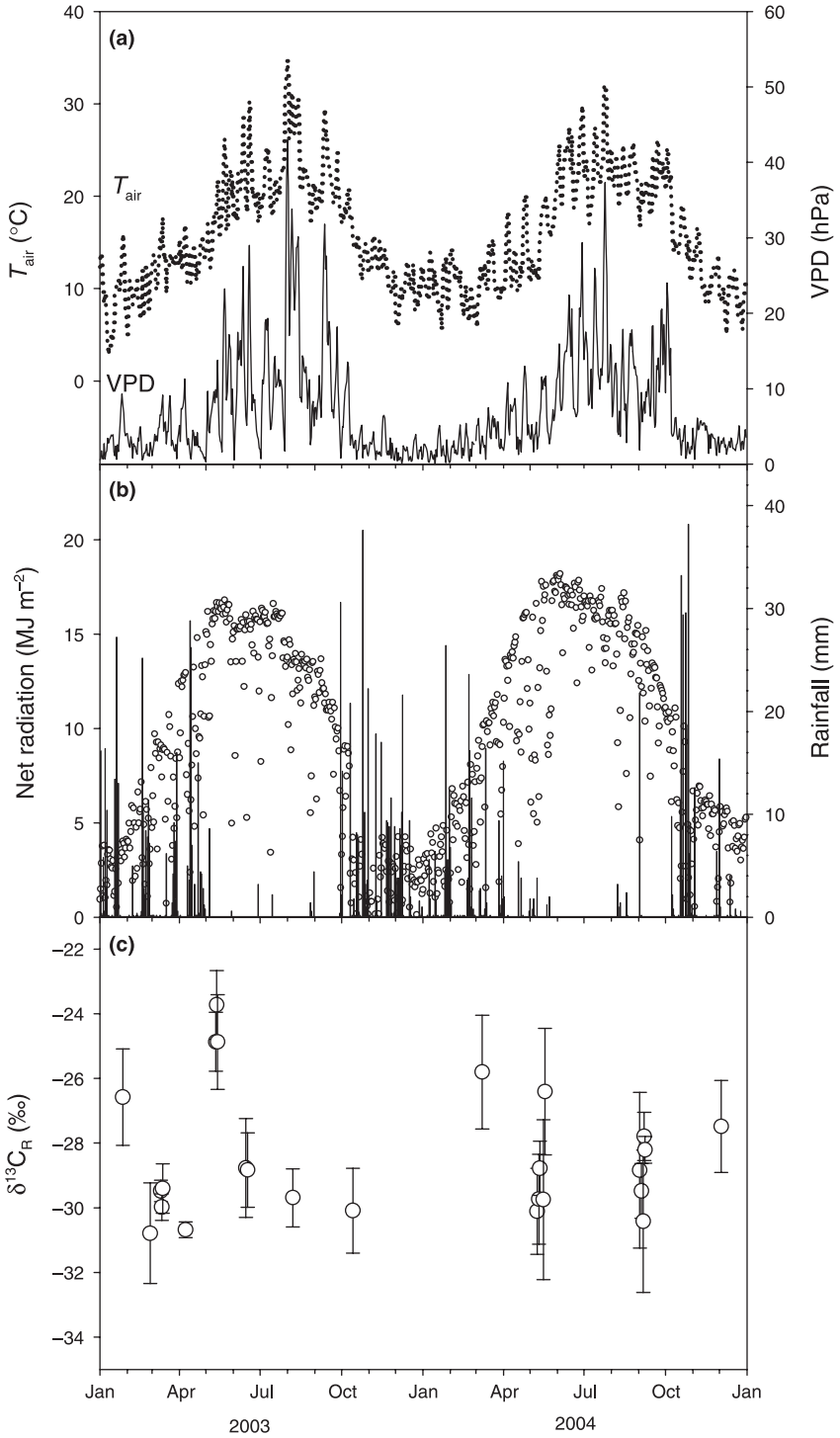

Fig. 1 Annual variation in climatic variables and carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$) in 2003 and 2004. (a) Daily means of air temperature (T_{air}, dotted line) and vapour pressure deficit (VPD, solid line); (b) total net radiation (circles) and daily rainfall (bars); (c) carbon isotope ratios of ecosystem-respired CO_2 ($\delta^{13}C_R$). Error bars represent standard errors.

respectively). During the first year (2003) precipitation was slightly above the long-term mean, with a cloudy period in midsummer, whereas the second year (2004) was considerably drier (706 and 488 mm precipitation in 2003 and 2004, respectively). A large isolated rain event occurred during late summer in 2004.

The ecosystem respiration varied with total annual rainfall and with the frequency and temporal distribution of rain events in both years (J. S. Pereira *et al.*, unpublished): ecosystem respiration was 98% of gross primary productivity (GPP) in the wetter 2003, and 96% of GPP in 2004 with lower rainfall. The seasonal variation in GPP followed the usual pattern of

Table 1 Temporal variation in carbon isotope ratios ($\delta^{13}C^a$) of bulk organic material of different ecosystem components in 2003

	Q. ilex leaves (‰)		Grass (‰)		Litter (‰)	Soil (‰)		
Month	Previous year	Current year	Leaves	Roots		Surface	5–10 cm	10–30 cm
March 03	−28.87 ± 0.72	–	−30.14 ± 1.04	−29.13 ± 1.05	−28.14 ± 0.57	−27.66 ± 0.07	−27.51 ± 0.34	−26.12 ± 0.48
April 03	−30.60 ± 0.82	–	−28.70 ± 0.26	−28.52 ± 0.5	−29.62 ± 0.35	−27.18 ± 0.17	−26.51 ± 0.20	−25.70 ± 0.67
May 03	−30.41 ± 0.64	−27.50 ± 0.23	−31.08 ± 0.72	−30.49 ± 0.89	−28.98 ± 0.56	−27.65 ± 0.40	−27.03 ± 0.19	−25.66 ± 0.10
June 03	−29.20 ± 0.51	−28.27 ± 0.70	–	–	−28.49 ± 0.49	−27.67 ± 0.26	−27.33 ± 0.34	nd
July 03	−29.37 ± 0.45	−28.43 ± 0.88	–	–	−28.53	−27.69 ± 0.15	−26.79 ± 0.33	nd
August 03	nd	−27.96 ± 0.46	–	–	−28.46 ± 0.26	−27.15 ± 0.25	−26.61 ± 0.43	−27.35 ± 0.43
October 03	nd	−28.57 ± 0.88	−28.27 ± 0.47	−27.13 ± 0,73	−28.57 ± 0.37	−27.84 ± 0.09	−27.43 ± 0.13	nd
November 03	nd	−28.76 ± 0.69	−30.46 ± 0.49	−30.85 ± 0.51	−28.46 ± 0.63	−28.92 ± 0.35	−27.02 ± 0.76	−25.83 ± 0.14
Mean	−29.64 ± 0.93	−28.20 ± 0.75	−29.73 ± 1.24	−29.17 ± 1.53	−28.53 ± 1.05	−27.69 ± 0.53	−27.03 ± 0.50	−26.15 ± 0.76

n = 4–5 ± SD.
nd, not determined.
[a]$\delta^{13}C$ of previous year and current year grown tree leaves (*Quercus ilex*), drought deciduous grass leaves and roots, litter and soil (surface, from 5 to 10 and 10 to 30 cm depth), for each month and annual mean in 2003.

the region, with a maximum in the spring and a minimum during summer drought. A secondary maximum occurred in autumn. Ecosystem respiration, however, reached a daily maximum following the first rain events at the end of summer or the beginning of autumn (data not shown). After the prolonged rainless season, during which soil biological activity declines, the first rains elicited large CO_2 efflux, which offset GPP. The effects of peak ecosystem respiration after the end of summer rains and the delay in GPP increase relative to respiration were reflected in the monthly carbon balance of October (data not shown). In 2003, ecosystem respiration represented 11% of the annual CO_2 efflux, leading to a net source of carbon of 40 g m^{-2} $month^{-1}$ (the annual net ecosystem exchange was −12 g m^{-2} $year^{-1}$). In the much drier autumn of 2004, October represented 6% of the annual ecosystem respiration, leading to a milder source of carbon of 13 g m^{-2} $month^{-1}$ (the annual net ecosystem exchange was −31 g m^{-2} $year^{-1}$).

The carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$) exhibited a large variation of > 7‰ but did not follow the seasonal changes in environmental variables (Fig. 1c). Day-to-day variation during intensive sampling campaigns in 2004 (> 5‰) was more than two-thirds the magnitude of total $\delta^{13}C_R$ variation observed at this site. Correlation analysis between the annual variation in $\delta^{13}C_R$ and potential driving environmental variables did not reveal significant relationships.

The temporal variation in $\delta^{13}C$ of organic material was small in all ecosystem components, in spite of pronounced differences between materials (Table 1). Leaves generally presented the most depleted source ($P < 0.001$), with grass leaves being slightly more enriched than current year tree leaves, but similar to previous year tree leaves. Grass organic matter exhibited the highest temporal variation, but grass leaves were on average slightly enriched compared with grass roots ($P < 0.05$). A marked ^{13}C gradient occurred from leaves to litter and soil ($P < 0.001$). Only tree leaves grown in 2003 were not depleted compared with litter ($P = 0.17$). The soil provided the most enriched ecosystem component, with a marked enrichment from the surface to 30 cm depth of approximately 2.5‰.

Seasonal variation in $\delta^{13}C_R$ in relation to environmental variables

To explore the variation during rapid climatic changes in the transition periods of the onset and end of summer drought, we conducted intensive 2-wk sampling periods with daily measurements of $\delta^{13}C_R$ in May and September 2004. Even though the criteria for reliable Keeling-plot analysis were not met on some nights because of windy conditions or low ecosystem activity, the remaining data indicate a clear trend in both periods (Fig. 2). A relatively cold and cloudy period at the beginning of May was followed by a large increase in maximum air temperature from 16 to 27°C and a consequent increase in VPD (Fig. 2a,c). This resulted in a pronounced enrichment in $\delta^{13}C_R$ from −30.1 to −26.4‰ (Fig. 2e).

During the summer drought, Keeling plots could not be determined because of a lack of ecosystem activity, and hence insufficient build-up of a CO_2 gradient at night. The first rainfall on September 2 (2004, Fig. 2d) induced a large CO_2 pulse with a peak in soil respiration of 8.6 μmol m^{-2} s^{-1}, declining to 3.6 μmol m^{-2} s^{-1} on September 6 (2004). Subsequently, a pronounced decline in $\delta^{13}C_R$ from −26.4 to −30.9‰ (Fig. 2f) occurred, which was of similar magnitude as the enrichment observed at the beginning of the drought period in May.

To explore the driving factors for the observed changes in $\delta^{13}C_R$ in both periods, correlation analysis with environmental variables and $\delta^{13}C_R$ were conducted. Shifted time series (up to 10 d) with different averaging periods (0–3 d) of environmental and physiological variables and $\delta^{13}C_R$ were tested.

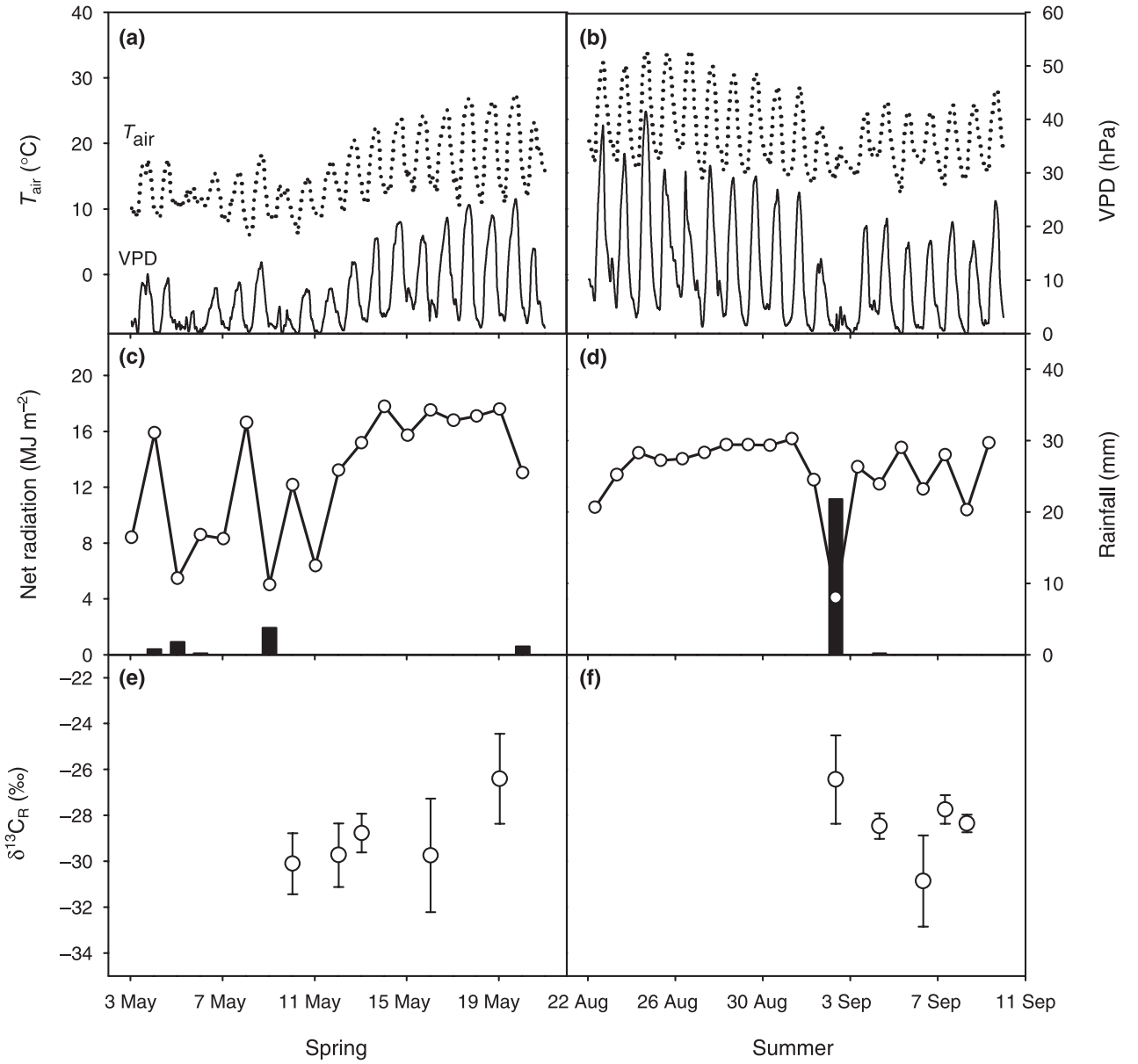

Fig. 2 Daily variation of climatic variables and carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$) during the transition periods in May and September 2004. Hourly means of air temperature (T_{air}, dotted line) and vapour pressure deficit (VPD, solid line) (a, b); daily totals of net radiation (solid line) and rainfall (bars) (c, d); and daily measurements of $\delta^{13}C_R$ (during fortnight periods) (e, f). Error bars represent the standard error.

Parameters for the best-fit regression are shown in Table 2. Highly significant regressions were found with variables affecting photosynthetic discrimination (VPD, Rn and T_{air}), soil activity (below-ground variables: T_{soil}, soil moisture and water potential), as well as sap flow and calculated canopy conductance (G_c). Only correlations with precipitation did not reveal significant relationships (data not shown). However, these regression analyses are only meaningful if all variables that affect $\delta^{13}C_R$ in a similar fashion (e.g. VPD, Rn and T_{air} through the effect on photosynthetic discrimination) reveal the same time-lag and averaging period. Therefore, we grouped the variables and tested for the best regression with a common time-lag and averaging period (Table 2, bottom). Since the time-lags varied only slightly within each variable group, highly significant regressions were still obtained, which explained 79–95% of the variation with above-ground variables. Only the below-ground variables yielded lower correlation coefficients because of non-significant regressions with soil moisture, which may have been caused by uncertainties in the measurements, probably as a result of poor contact between soil and sensors, especially during summer.

Different patterns can be observed (Table 2): variables affecting photosynthetic discrimination revealed longer time-lags (2 d longer) than soil variables, indicating that

Table 2 Summary of the correlation analysis for carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$) vs meteorological, edaphic and physiological variables for May and September

Best-fit regressions[a]	Lag (d)	Average (d)	Regression +/–	Regression type	r^2	*P*-level
May 2004						
VPD	5	2	+	Linear	0.95	< 0.001
Net radiation	5	2	+	Linear	0.89	< 0.001
Air temperature	6	3	+	Linear	0.87	< 0.01
Sap flow	4	1	+	Linear	0.99	< 0.05
G_c (whole tree)	3	1	–	Linear	0.92	< 0.05
Soil moisture	4	1	–	Log	0.75	< 0.05
Soil temperature	3	1	+	Linear	0.74	< 0.01
Soil water potential	3	1	–	Linear	0.94	< 0.001
September 2004						
VPD	3	2	+	Log	0.97	< 0.001
Net radiation	2	3	+	Log	0.86	< 0.01
Air temperature	3	2	+	Log	0.96	< 0.001
Sap flow	4	1	+	Log	0.70	< 0.001
G_c (whole tree)	3	1	–	Linear	0.90	< 0.001
Soil moisture	0	1	–	Linear	0.54	ns
Soil temperature	1	3	+	Linear	0.57	< 0.05
Soil water potential	0	3	–	Log	0.67	< 0.001
Best common regressions[b]						
May 2004						
Above-ground variables	5	2		Linear	0.95–0.79	< 0.001
Trees (sap flow, G_c)	4	1		Linear	0.99–0.60	< 0.05
Below-ground variables	3	1		Linear	0.94–0.50	< 0.05, ns[c]
September 2004						
Above-ground variables	3	2		Linear	0.94–0.86	< 0.001
Trees (sap flow, G_c)	3	2		Linear	0.44–0.30	< 0.01
Below-ground variables	1	1		Linear	0.52–0.34	< 0.05, ns[c]

[a]The regression that provided the best statistical fit is presented for each variable (best fit regressions). The number of days lagged and averaged; the regression sign and type, the correlation coefficient (r^2) and the significance level ($P < 0.05$, 0.001 and 0.001) of the regression are given.
[b]The regression parameters for a common time-lag and averaging period for each variable group (best common regressions) for above-ground variables (vapour pressure deficit (VPD), net radiation and air temperature), tree variables (sap flow, canopy conductance (G_c) and below-ground variables (soil moisture, water potential and temperature) are shown in the lower part of the table.
[c]For below-ground variables only, soil moisture yielded insignificant regressions.

changes in soil conditions have a faster and probably more direct effect on $\delta^{13}C_R$. Tree variables (sap flow, canopy conductance) showed an intermediate response. Highest correlation coefficients were always found for above-ground variables. Furthermore, differences among seasons were visible: during May longer time-lags occurred in all parameter groups, whereas after the rain pulse in September, $\delta^{13}C_R$ responded more rapidly to changes in environmental variables.

Interestingly, $\delta^{13}C_R$ responded to absolute changes in some environmental variables in a similar way during different seasons: for example, an offset of 5.2°C in the relationship of $\delta^{13}C_R$ and T_{air} occurred between May and September (Fig. 3b). This means that an increase of approx. 7°C in mean daytime air temperature in May was accompanied by a similar change in $\delta^{13}C_R$ as a decrease of 6.5°C in T_{air} in September. This occurred irrespectively of the absolute temperature, which was > 5°C higher in September (ranging from 13.6 to 20.7°C and from 19.4 to 25.8°C in May and September, respectively). Similar offsets were observed for relationships of $\delta^{13}C_R$ with soil water potential, VPD and T_{soil}, with significant differences in the intercept ($P < 0.05$) but similar slopes ($P = 0.71–0.92$). Only the response to changes in Rn remained the same in both months ($P = 0.53$, 0.99 for slope and intercept, respectively; Fig. 3c).

These responses must be seen in the context of seasonal changes in the activity of different ecosystem components, such as the relative contribution of plant compared with soil respiration or seasonal physiological acclimation. Changes in ecophysiological parameters at the beginning and the end of each transition period in both May and September compared with environmental variables are shown in Fig. 4. Maximum sap flow rates reduced to one-third during drought, when compared with May (grey and open symbols, respectively, Fig. 4a). After the rain pulse in September, sap flow rates recovered to approximately one-half the rates reached in May under similar VPD (black symbols, Fig. 4a). The seasonal difference in leaf level response was even more pronounced: stomatal conductance (g_s) of sunlit leaves was high in May

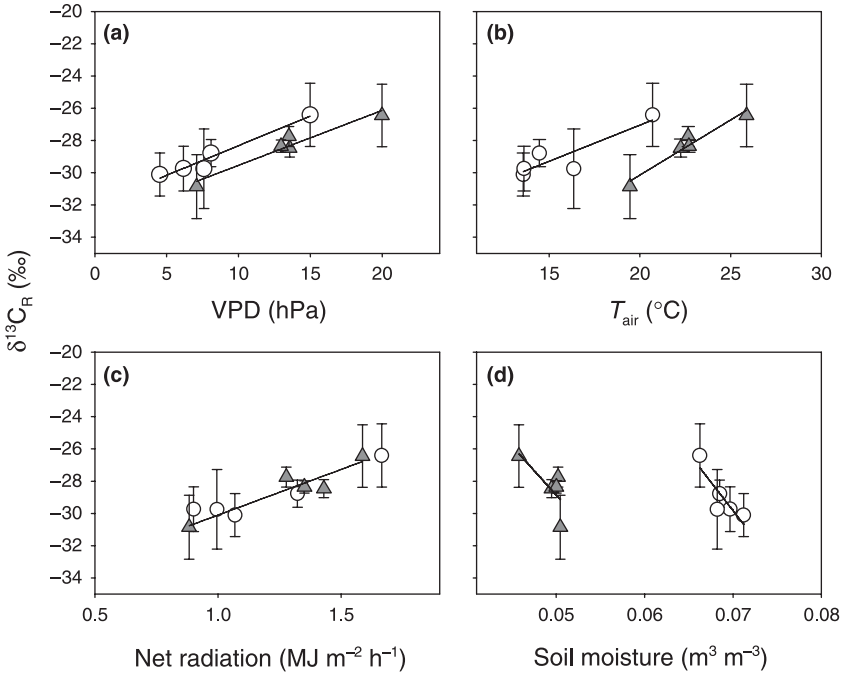

Fig. 3 Relationships between carbon isotope ratios of ecosystem-respired CO_2 ($\delta^{13}C_R$) and time-lagged daytime averages of climatic variables: vapour pressure deficit (VPD) (a), air temperature (T_{air}) (b), net radiation (c) and soil moisture (d) during May (open circles) and September (shaded triangles) 2004. Time-lags are according to best common regressions (see Table 2, lower section). Error bars represent standard errors.

(although strongly declining with increasing VPD) but was markedly reduced in September (Fig. 4c). Net photosynthetic rates were also much higher in May compared with September, but soil respiration rates exhibited a different response (Fig. 4e,f). Soil respiration rates ranged from about 1.9 to 2.4 μmol m^{-2} s^{-1} in May but were strongly reduced during drought. The same trend was found in respect to GPP and ecosystem respiration fluxes (as pointed out in the previous section). However, the rain pulse induced an immediate peak in soil respiration (highest rates in Fig. 4e,f) and subsequent higher respiration rates compared with May (black symbols, Fig. 4e,f). The temperature dependence of soil respiration was restored only after soil rewetting (Fig. 4f). This is a further indication that photosynthetic discrimination might have dominated $\delta^{13}C_R$ during spring, whereas changes in soil respiration might have triggered the response in $\delta^{13}C_R$ in September.

Diurnal changes in $\delta^{13}C_R$

During both periods in May and September 2004, 24 h high-frequency sampling was conducted to evaluate short-term dynamics in $\delta^{13}C_R$ (Fig. 5). Vertical mixing in the open canopy led to minimal vertical differences in [CO_2] and $\delta^{13}C$ during the day. After sunset, however, a large [CO_2] and ^{13}C-gradient built up, allowing statistically robust Keeling-plot analysis (Fig. 5c,d). Climatic conditions were very similar, with maximum T_{air} reaching approx. 26.5°C on both days. VPD was slightly higher in May compared with September (23.8 and 21.5 hPa, respectively), whereas soil temperature remained, on average, 2.8°C higher in September (Fig. 5a,b). During both nights, a large nocturnal shift in $\delta^{13}C_R$ was observed (> 4‰, Fig. 5g,h). On both occasions, enrichment at the beginning of the night was followed by stable ratios or a slight decline in the early morning hours. The range in isotopic signature of $\delta^{13}C_R$ was also very similar on both occasions, ranging from −30.6 to −26.7‰ and from −31.1‰ to −26.9‰ in May and September, respectively. This nocturnal shift reached nearly the same magnitude as the total $\delta^{13}C_R$ variation during the intense sampling campaigns in both periods (> 5‰, Fig. 2e,f) and reached more than half of the total annual variation observed at this site (> 7‰, Fig. 1c).

Discussion

Ecosystem respiration is a major component of the C balance that needs to be better understood. The stable isotope signatures of ecosystem-respired CO_2 can be used to understand and quantify the processes involved in ecosystem carbon fluxes. In spite of recent insights, we still lack a full mechanistic understanding of the variability in the isotopic signature of ecosystem respiration. This variation can be substantial even on a short-term scale. Here, we will discuss the dynamics in $\delta^{13}C_R$ found at different timescales, its linkage to environmental factors, and potential underlying mechanisms driving these dynamics.

Annual variation in $\delta^{13}C_R$ and organic ecosystem components

The $\delta^{13}C_R$ exhibited a high variability of 7‰ during the 2-year measuring period, which is similar or slightly lower than

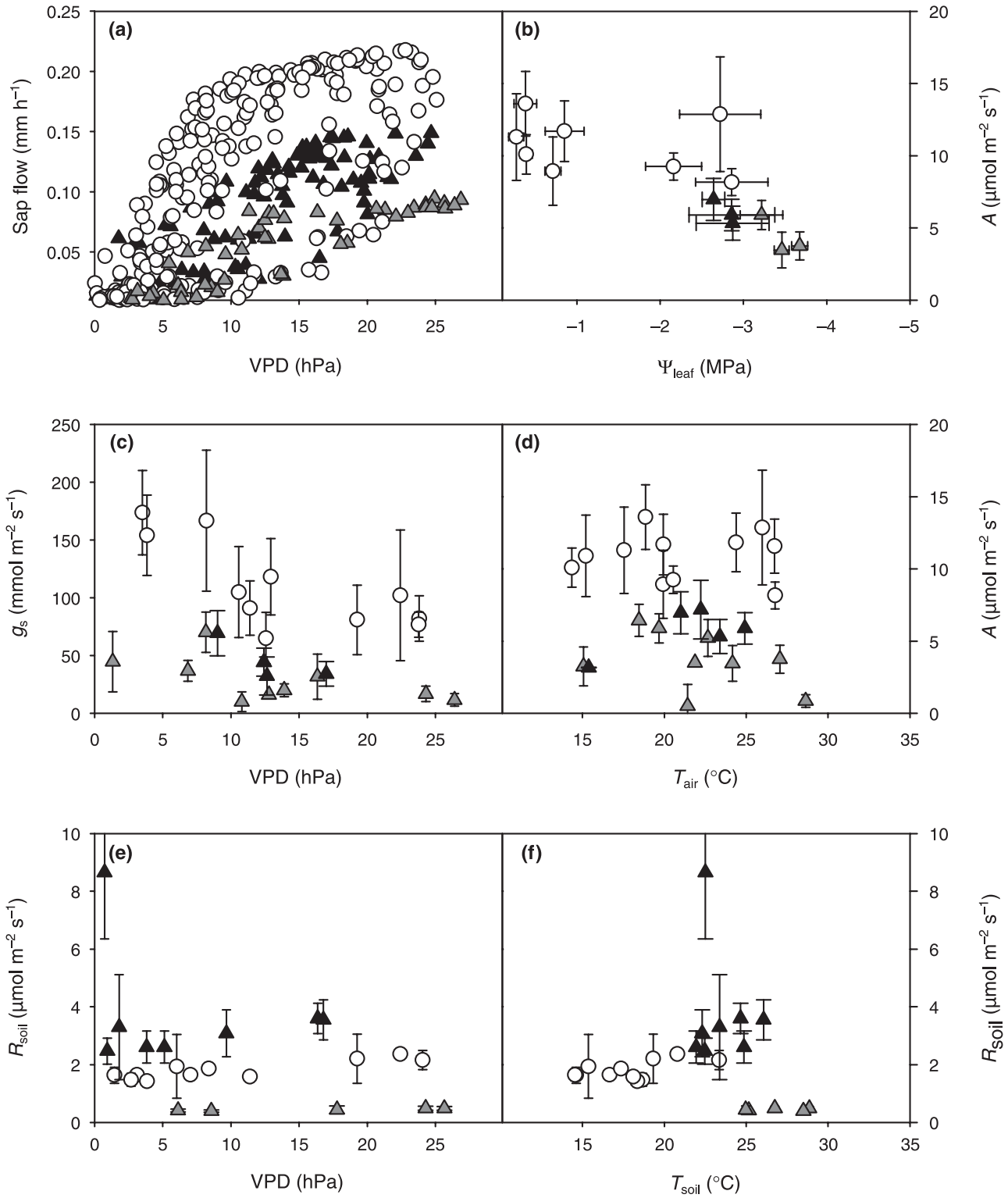

Fig. 4 Changes in ecophysiological parameters vs environmental conditions during the intense sampling periods of May (open circles) and September before (grey triangles) and after the rain event (black triangles). Relationship between vapour pressure deficit (VPD) and sap flow (a), stomatal conductance (g_s) of sunlit leaves (c), and soil respiration rates (R_{soil}) (e); relationships between leaf net assimilation rate (A) and leaf water potential (Ψ_{leaf}) (b) and air temperature (T_{air}) (d); and between soil respiration and soil temperature (T_{soil}) (f). Outliers in (e) and (f) are soil respiration rates immediately after the rain pulse. Values were plotted from diurnal courses during daylight hours at the beginning and at the end of each period. For details see the Materials and Methods section; $n = 3–15 \pm$ SD.

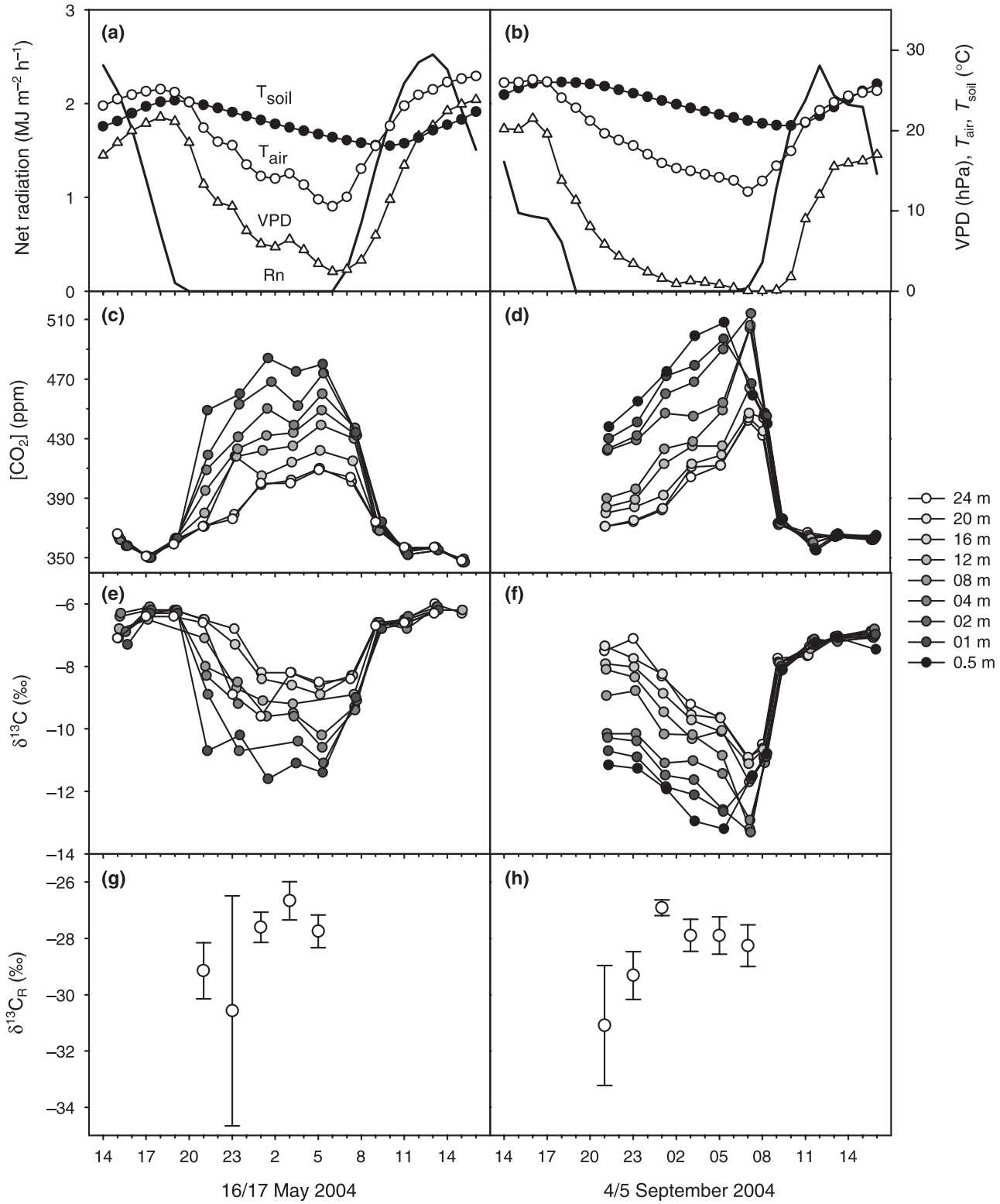

Fig. 5 Diurnal variation of climatic variables and carbon isotope ratios of ecosystem-respired CO_2 ($\delta^{13}C_R$) during 24 h cycles in May and September 2004. Hourly means of air temperature (T_{air}, open circles), soil temperature (T_{soil}, closed circles), vapour pressure deficit (VPD, open triangles) and net radiation (Rn, solid line) (a, b); 2-hourly measurements of CO_2 concentrations (c, d) and carbon isotope ratios (e, f) of atmospheric air at different heights (24 m to 0.5 m) represented by different shaded circles from open to black, respectively; and nocturnal variation of $\delta^{13}C_R$ (g, h). Error bars represent standard errors.

the maximum variation reported by Bowling *et al.* (2002) and McDowell *et al.* (2004a, for forests with marked seasonality in precipitation). However, it exceeded twice the variation in $\delta^{13}C_R$ found in temperate forests (Fessenden & Ehleringer, 2003; Knohl *et al.*, 2005). In spite of the pronounced seasonality of the Mediterranean climate, no annual pattern with enriched $\delta^{13}C_R$-signatures during drought (Pataki *et al.*, 2003; McDowell *et al.*, 2004a; Lai *et al.*, 2005) was detectable.

The magnitude of variation observed during intense sampling periods in 2004 exceeded two-thirds of the total range in $\delta^{13}C_R$ observed at this site. This might indicate short-term changes in environmental conditions as driving factors stimulating changes in $\delta^{13}C_R$ rather than seasonal variations. Owing to the pronounced seasonal acclimation of Mediterranean vegetation (Werner *et al.*, 1999, 2002), the system might express a differential response to similar environmental stimuli during different seasons. This is reflected in the different responsiveness (shorter time-lags between environmental drivers and $\delta^{13}C_R$) and offsets in the correlation between environmental variables and $\delta^{13}C_R$ (Fig. 3) in summer compared with spring (as will be discussed in detail later). It indicates that the influence of environmental factors on $\delta^{13}C_R$ is not constant, but varies throughout the year, probably with changes in carbon allocation, tissue metabolism, drought adaptations or seasonal changes in the fractional contribution of different ecosystem components to overall respiration.

Similarly, $\delta^{13}C$ of organic material of different ecosystem components was a poor proxy for the isotopic content of respiratory fluxes (see also McDowell *et al.*, 2004b; Knohl *et al.*, 2005). Substantial differences in $\delta^{13}C$ of various carbon pools were found, with increasing enrichment from leaf to litter and soil. In spite of high variability, $\delta^{13}C$ of grass leaves was, on average, 0.55‰ more depleted than that of roots ($P < 0.05$), similar to findings by Badeck *et al.* (2005). They concluded that significant postphotosynthetic fractionation processes might be responsible for differences in the carbon isotope composition of different organs. In agreement with other studies, $\delta^{13}C$ of the soil organic matter was the most enriched ecosystem compartment, with increasingly enriched isotope signatures with depth (Buchmann *et al.*, 1997; Bowling *et al.*, 2002; Hemming *et al.*, 2005; for a thorough discussion, see Ehleringer *et al.*, 2000).

Relationships between $\delta^{13}C_R$ and environmental factors during transition periods

Unlike the annual cycle, the short transition periods from high productivity in spring to summer drought, and the reverse in late summer, provided excellent opportunities to evaluate the impact of driving environmental factors on the isotopic imprint of $\delta^{13}C_R$. During these rapid changes in Mediterranean climate conditions, pronounced isotopic disequilibrium can be expected, as carbon isotope discrimination will strongly change with the onset or end of drought.

Time-lagged relationships of $\delta^{13}C_R$ occurred with atmospheric factors (VPD, T_{air}, Rn), soil variables (e.g. soil moisture, T_{soil}), as well as with sap flow and calculated canopy conductance. The time-lagged correlations of $\delta^{13}C_R$ and environmental drivers of carbon discrimination have generally been interpreted as the time from carbon fixation, assimilate transport to roots, and subsequent release through root respiration and exudates (Ekblad & Högberg, 2001; Bowling *et al.*, 2002). However, this is only a reasonable assumption if all environmental factors affecting carbon isotope discrimination (VPD, T_{air}, Rn) reveal a common time-lagged response. Such common time-lags and averaging periods were found for each group of variables, which still explained 50–99% of the variation. Only below-ground variables and tree water relations in September revealed correlation coefficients ($r^2 = 0.33–0.52$). These data confirm that ecosystem respiration is closely linked to recent assimilates, as the relationship of $\delta^{13}C_R$ to VPD, T_{air}, Rn, and canopy conductance explained 86–95% of the variation. Time-lags of 4–5 d with VPD found in May are in accordance with what was found by Bowling *et al.* (2002), Ekblad *et al.* (2005) and Knohl *et al.* (2005).

However, we also found a strong relationship of $\delta^{13}C_R$ with below-ground variables (T_{soil} and moisture), indicating a second important component of $\delta^{13}C_R$ in this system. Similar results were reported by McDowell *et al.* (2004a), suggesting a significant role of below-ground respiration on ecosystem-scale $\delta^{13}C_R$. Soil water content has often been interpreted as a driving factor for vegetation response on $\delta^{13}C_R$ through its effect on hydraulic conductance and transpiration. We have considered soil water potential and moisture as factors affecting below-ground processes, since, first, during summer the oak trees in the study area rely on deeper water sources than the measured upper 20–40 cm (David *et al.*, 2004), whereas sap flow-based canopy conductance provides a better direct indicator of overall stomatal response. Second, soil respiration is strongly determined by water availability and provides an important component in this open woodland especially during summer when the herbaceous understorey has vanished.

Time-lags were shorter (1–2 d) for below- than above-ground variables, probably indicating that the proportion of $\delta^{13}C_R$ released from heterotrophic soil respiration responded faster to changes in edaphic conditions. Heterotrophic soil respiration relies on a mixture of fast- and slower-turnover carbon pools that might follow different dynamics than root respiration. Högberg *et al.* (2001) and Bhupinderpal-Singh *et al.*, 2003) showed that root respiration contributed 56 and 65% to total soil respiration during the first and second summers of a girdling experiment, respectively. Others found a somewhat lower response to girdling (31–44%, Scott-Denton *et al.*, 2006). Bowling *et al.* (2003b) reported that soil respiration may even be as high as 80% of the total respiratory flux, but this may vary among ecosystems or with soil moisture (Mortazavi & Chanton, 2002; Mortazavi *et al.*, 2005). Different sensitivity of the heterotrophic and rhizosphere

respiration to seasonal drought was found (Scott-Denton *et al.*, 2006). It can be argued that soil temperature might influence both root and heterotrophic respiration in a similar fashion. However, Bhupinderpal-Singh *et al.* (2003) found no variation in root respiration in response to a cold period, in spite of a decline in heterotrophic soil respiration. Similar conclusions were drawn by Knohl & Buchmann (2005), who found a strong response of respiratory isoflux (F_R) to soil temperature. However, the inverse (higher temperature sensitivity of autotrophic root respiration) has also been shown in a trenching experiment (Boone *et al.*, 1998).

If the isotopic composition of CO_2 released from heterotrophic respiration differs from that of rhizosphere respiration, a proportional change of both processes as a consequence of different response times to environmental stimuli will lead to an isotopic shift in soil-released CO_2 and, subsequently, $\delta^{13}C_R$. Interestingly, a shorter time-lag for the response in $\delta^{13}C$ of CO_2 respired from soil as compared with the ecosystem was also found by McDowell *et al.* (2004b). The lag periods reported by these authors were 1.1 and 4.9 d for $\delta^{13}C_{R\ soil}$ and $\delta^{13}C_R$, respectively, which are very similar to those reported in the current study. Hence, the different time-lags for above- and below-ground variables indicate that the resultant $\delta^{13}C_R$ must always be seen as the outcome of processes that can be controlled at different rates and dynamics (Högberg *et al.*, 2001).

Low ecosystem respiration rates during summer drought often impeded Keeling-plot analysis even during wind-still nights. The strong isolated rain event at the end of summer 2004 resulted in a pulse-like increase in soil respiration (Fig. 4), and time-lagged responses were 1–2 d shorter in September than in May. Below-ground parameters responded immediately (1 d time-lag), which could be mediated by the rapid response of soil microbial respiration after the rain pulse (Irvine & Law, 2002). Soil respiration rates were higher after the rain pulse as compared with May (Fig. 4f) and ecosystem respiration reached the highest annual rates after the first autumn rains (J. S. Pereira *et al.*, unpublished). This is an indication that photosynthetic carbon fixation and subsequent release through respiration might have dominated the ecosystem response in May, whereas soil respiratory processes might have triggered changes in $\delta^{13}C_R$ in September. It has been observed in many ecosystems that upon rewetting there is a sudden 'burst' of mineralization and CO_2 release – the Birch effect (Rey *et al.*, 2005; Jarvis *et al.*, 2006), which can bee seen in Fig. 4(f). The amount of carbon returned to the atmosphere in this way can reduce significantly the annual net carbon gain by the forests (Pereira *et al.*, 2004; Jarvis *et al.*, 2006). First autumn rains in Mediterranean ecosystems may provide an excess of mineralization in the soil during the early phases of the wet cycle (Rey *et al.*, 2005). By that time there are no herbaceous plants to utilize the nutrients released, thus leading to the loss of carbon and nitrogen from the soil pools (Jarvis *et al.*, 2006). In such cases, late summer or first autumn rains may often have a negative effect on plant productivity (Pereira *et al.*, 2004). The deep-rooted trees, in particular, cannot use current rainfall until water reaches deeper soil horizons and can be partly decoupled from the top soil water content, which was reflected by the slow stomatal response after rainfall (e.g. Fig. 4b,c). It is further unlikely that carbon isotope discrimination changed during the days before the rain event, and sap flow progressively increased during the following 3 d (data not shown). Given the 3 d time-lag, photosynthetic activity would only be expected to influence $\delta^{13}C_R$ 4 d after the rain pulse (interestingly, these data points are offset compared with the preceding days; Fig. 2). Still the correlation of $\delta^{13}C_R$ with VPD and T_{air} was high ($r^2 = 0.94$ and 0.86, respectively), even though discrimination might not have been the primary source of variation during the first days. This indicates that these lag analyses need to be interpreted with care, as we are not evaluating independent variables but all environmental variables are interrelated (in this case, the rain event was associated with a large drop in VPD, Rn and temperature).

Furthermore, important information can be obtained by comparing the regressions obtained for each season. Interestingly, the slopes of the regressions between $\delta^{13}C_R$ and air temperature were not significantly different between May and September ($P = 0.92$), but the regressions were offset by 5.2°C, hence a relative temperature shift resulted in a similar isotopic shift (from approx. −26.4 to −30.5‰; Fig. 3b), in spite of different absolute temperatures. This may be explained by large seasonal temperature acclimation of maximum photosynthetic capacity during the Mediterranean summer reported for these species (Tenhunen *et al.*, 1990; Larcher, 2000). A similar offset (hence, nonsignificant slopes, but significantly different intercepts) was found for T_{soil}, VPD, soil water potential and sap flow. Only the correlation of $\delta^{13}C_R$ with net radiation was equal for both seasons. Hence, the driving factor seems to be the rate of change rather than the absolute values of environmental parameters, which would also explain the lack of annual pattern of $\delta^{13}C_R$. To our knowledge this is the first report that demonstrates different time-lags in different seasons. These changes contain valuable information on ecosystem responsiveness and acclimation.

Nocturnal changes in $\delta^{13}C_R$

The high sampling frequency during the 24 h cycle revealed large nocturnal shifts in $\delta^{13}C_R$ (> 4‰), in both May and September (Fig. 5). Until recently, $\delta^{13}C_R$ was considered to remain constant during the night (Pataki *et al.*, 2003). To our knowledge, the only other study showing nocturnal changes in $\delta^{13}C_R$ is that of Bowling *et al.* (2003c). Ogée *et al.* (2003) did not find significant changes in $\delta^{13}C_R$ during one nocturnal cycle, similarly to Schnyder *et al.* (2004), who found nocturnal variations of $\delta^{13}C_R < 2$‰, concluding that

$\delta^{13}C_R$ remained relatively constant. However, Bowling *et al.* (2003c) found a shift of over 6‰ within a single night and they pointed out that this variation in magnitude was nearly as large as the seasonal range reported over a variety of environmental conditions from several years of sampling. Large nocturnal variations in $\delta^{13}C_R$ have been confirmed in three other 24 h time-series measured during the following year (changes in $\delta^{13}C_R$ ranging from 1.8 to 4.2‰, data not shown). Furthermore, nocturnal changes also occurred in soil-respired CO_2 (measured with a soil chamber in 2005) ranging from 1.1 to 4.0‰ (where seven out of 10 nights exhibited changes above 2.5‰; data not shown).

There are several possible reasons for these nocturnal shifts in $\delta^{13}C_R$, which may occur: (i) through changes in respiratory substrate; (ii) if diurnal changes in photosynthetic discrimination will translate into nocturnal variation of $\delta^{13}C$ of respired CO_2; (iii) if the respiratory signal from different ecosystem components (e.g. foliage, soil, roots) does not remain constant or the relative contribution of different respiratory fluxes changes (either through circadian rhythms or as a response to nocturnal changes in climatic variables); (iv) through fractionation in the respiratory pathways.

(i) Changes in respiratory substrate may occur if respiration early in the evening was from recent photosynthate and slowly changed to stored carbon substrate as the night progressed. Even secretion of root exudates could follow a circadian rhythm; however, to our knowledge there is no information on these processes.

(ii) Carbon isotope discrimination is not constant during the day, especially under Mediterranean climate conditions, where a rapid adjustment of photosynthesis and stomatal conductance mediates effective light utilization and avoidance of dehydration, for example during midday stomatal closure (Tenhunen *et al.*, 1984). Furthermore, estimated isotopic composition of the photosynthetic flux has shown high variation over the daylight period (Knohl & Buchmann, 2005). These dynamics, leading to different ^{13}C-enriched photosynthetic compounds, could translate into nocturnal changes in foliage respiration. Additionally, it could be reflected in root respiratory signals, mediated by the transport rate and amount of metabolites reaching the roots. It was shown that $\delta^{13}C_R$ may be related to the isotopic signature of the phloem sap on a seasonal basis (Scartazza *et al.*, 2004), and in one of two forests on a daily basis (Barbour *et al.*, 2005), but nothing is known about dynamics on an hourly basis. We evaluated this hypothesis, using lag analysis with hourly, instead of daily, data sets: indeed, nocturnal $\delta^{13}C_R$ values correlated well with changes in VPD, Rn T_{air}, and sap flow during the daylight period (e.g. the highest correlation was found 3 d + 12–14 h for September; $r^2 = 0.77–0.91$, data not shown). More data are needed for a rigorous statistical analysis, but this might be a first indication that diurnal dynamics in photosynthetic discrimination could drive nocturnal changes in $\delta^{13}C_R$.

(iii) It is well established that respiration rates are a function of temperature, and exhibit a pronounced diurnal/nocturnal cycle. During the night, leaf temperatures decrease over several hours (decreasing leaf respiration), whereas soil temperatures generally vary with a reduced amplitude (Fig. 5). This can change the fractional contribution of different respiratory sources (e.g. soil vs leaf respiration) to total ecosystem respiration. Similar conclusions have been drawn by Bowling *et al.* (2003b), examining large nocturnal variation in $\delta^{18}O$ of respired CO_2. Furthermore, as mentioned above, soil temperature and moisture might affect heterotrophic and autotrophic respiration differently (Boone *et al.*, 1998; Bhupinderpal-Singh *et al.*, 2003). Hence, the relative proportions of the different respiratory fluxes may change throughout a night, causing a shift in $\delta^{13}C_R$. Furthermore, there is increasing evidence that soil respiration might not simply be a function of temperature, but that direct effects of substrate supply, temperature and desiccation stress need to be separated from indirect effects of temperature and soil water content on substrate diffusion and availability (Davidson *et al.*, 2006), which could potentially result in different isotopic signals of the respired CO_2.

(iv) Recent work indicates that $\delta^{13}C$ of leaf respiration ($\delta^{13}C_{resp}$) can undergo quite dramatic diurnal changes of 5–10‰ in evergreen species under natural conditions (Hymus *et al.*, 2005; Prater *et al.*, 2006). Laboratory data indicate up to 8‰ diurnal enrichment during the light period and rapid depletion upon darkness for our oak species (N. Hasenbein & C. Werner, unpublished). Tcherkez *et al.* (2003) showed an increasing depletion upon continuous darkness and strong temperature dependence of $\delta^{13}C_{resp}$, which correlated well with RQ-values. This has been explained by apparent ^{13}C fractionation during leaf respiration (for a recent review, see Ghashghaie *et al.*, 2003). The current hypothesis is based on a shift in the ratio of CO_2 produced during initial carboxylation of pyruvate by pyruvate dehydrogenase, to the oxidation of actetyl-CoA in the Krebs cycle (Tcherkez *et al.*, 2003) and the nonstatistical distribution of ^{13}C in primary substrate (Schmidt & Gleixner, 1998). Under Mediterranean drought conditions, when growth has ceased and there is a low metabolic demand for respiratory products of the Krebs cycle (Rambal *et al.*, 2004), the synthesis of secondary compounds derived from ^{13}C-depleted acetyl-CoA, particularly lipids, could be favoured relative to the oxidation of these compounds (Hymus *et al.*, 2005), resulting in large diurnal/nocturnal variation in respired $\delta^{13}CO_2$.

So far, nothing is known about the short-term dynamics of root respiration, which might depend on the growth status of the root and investment into secondary compounds (e.g. for defence). Klumpp *et al.* (2005) have shown for plants under isotopic equilibrium that the ^{13}C-enriched respiration of the shoots was counterbalanced by ^{13}C-depleted respiration of the roots. The authors concluded that it might explain the conflicting results between leaf- and ecosystem-level ^{13}C

discrimination in respiration (Klumpp *et al.*, 2005). However, under natural conditions, it is unlikely that ecosystem gas exchange is at complete isotopic equilibrium.

We are probably only beginning to understand the large variation in isotopic signature of respiration (Ghashghaie *et al.*, 2003) and its impact on both temporal (e.g. Fig. 5) and spatial (Steinmann *et al.*, 2004) variation in ecosystem respiration.

Final remarks

While, in earlier works, $\delta^{13}C_R$ was considered to remain relatively constant on a monthly to seasonal basis, there is now increasing evidence of pronounced short-term dynamics in $\delta^{13}C_R$ in response to rapidly changing environmental variables.

In spite of the high annual variation in $\delta^{13}C_R$ (> 7‰), correlations with driving environmental factors were only significant during periods of rapid environmental changes. Different correlations of $\delta^{13}C_R$ and micrometeorological variables in May and September indicate a different responsiveness of the ecosystem (e.g. through temperature acclimation) during different seasons. Shorter time-lags (1–2 d) for below- than for above-ground variables might indicate a more direct response of $\delta^{13}C_R$ to changes in edaphic conditions, which seem to dominate the system response after the first rainfalls in September, whereas changes in photosynthetic discrimination with increasing drought might have dominated the $\delta^{13}C_R$ response in May.

Furthermore, large nocturnal variations in $\delta^{13}C_R$ point towards even faster dynamics on timescales of hours. Given our current knowledge of the strong link between $\delta^{13}C_R$ and environmental drivers and their well-known circadian cycles, we suggest that it is reasonable to expect nocturnal variations in $\delta^{13}C_R$ in response to variations in these driving factors. Indeed, based on this current knowledge, it seems difficult to justify the constancy in nocturnal $\delta^{13}C_R$, even though periods of stable $\delta^{13}C_R$ may occur, similar to what has been found for $\delta^{13}C_R$ on coarser timescales (McDowell *et al.*, 2004b).

This has large implications for the sampling protocols used to collect nocturnal Keeling-plot data, since timing of data collection will be decisive. The Keeling-plot method assumes either one respiratory source with a single isotopic composition or that the relative contributions of component fluxes that might differ in isotopic composition (such as foliar and soil respiration) do not change over the sampling period (Bowling *et al.*, 2003a). However, in many ecosystems, the range of CO_2 concentrations required for a reliable Keeling plot is difficult to capture in a short time period because of low activity of the systems, which is commonly overcome by extending the time of sampling over several hours until a sufficiently large gradient is reached (commonly 2–8 h; Pataki *et al.*, 2003). If these nocturnal shifts are frequent phenomena, and confirmed in other ecosystems, we might need to verify one of the basic assumptions for sampling nocturnal Keeling plots.

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