PARTITIONING ECOSYSTEM SCALE CARBON FLUXES INTO PHOTOSYNTHETIC AND RESPIRATORY COMPONENTS WITH EMPHASIS ON DYNAMICS IN $\delta^{13}CO_2$.

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INDEX OF SYMBOLS AND ABBREVIATIONS

symbol	description	unit
$[CO_2]$	CO_2-concentration	[ppm]
Δ	photosynthetic discrimination	[‰]
ΔT	temperature difference	[°C]
$\delta^{13}C$	carbon isotope composition (VPDB-Standard)	[‰]
$\delta^{13}C_C$	carbon isotope composition of canopy respiration	[‰]
$\delta^{13}C_r$	carbon isotope composition of root respiration	[‰]
$\delta^{13}C_R$	carbon isotope composition of ecosystem respiration	[‰]
$\delta^{13}Cres$	carbon isotope composition of ecosystem component respiration (e.g. soil, roots, foliage)	[‰]
$\delta^{13}C_S$	carbon isotope composition of soil respiration	[‰]
$\delta^{13}C_{SMO}$	carbon isotope composition of soil microorganisms respiration	[‰]
$\delta^{13}C_T$	carbon isotope composition of tree respiration	[‰]
$\delta^{13}C_U$	carbon isotope composition of understory respiration	[‰]
ρ	density of air	$[kg \cdot m^{-3}]$
γ	psychrometric constant	$[kPa \cdot K^{-1}]$
λ	latent heat of vaporization	$[J \cdot kg^{-1}]$
A_S	sapwood area	$[m^2]$
A_L	leaf area	$[m^2]$
c_e	molar fraction of CO_2 entering the chamber	$[mmol\ mol^{-1}]$
c_o	molar fraction of CO_2 leaving the chamber	$[mmol\ mol^{-1}]$
c_P	specific heat of air at constant pressure	$[J \cdot kg^{-1}\ K^{-1}]$
D	= VPD	
f	CO_2-flux per m^2 ground	$[\mu mol\ m^{-2}\ s^{-1}]$
f_C	canopy CO_2-flux	$[\mu mol\ m^{-2}\ s^{-1}]$
f_r	root CO_2-flux	$[\mu mol\ m^{-2}\ s^{-1}]$
f_S	soil CO_2-flux	$[\mu mol\ m^{-2}\ s^{-1}]$
Fs	flux of a scalar unit	$[kg\ m^{-2}\ s^{-1}]$
f_{SMO}	soil microorganisms CO_2-flux	$[\mu mol\ m^{-2}\ s^{-1}]$
f_T	tree CO_2-flux	$[\mu mol\ m^{-2}\ s^{-1}]$
f_U	understory CO_2-flux	$[\mu mol\ m^{-2}\ s^{-1}]$

G_c	canopy conductance	[mmol m^{-2} s^{-1}]
GPP	gross primary productivity	[µmol m^{-2} s^{-1}]
G_s	stomatal conductance	[mmol m^{-2} s^{-1}]
IRGA	infrared gas analyzer	
IRMS	isotope ratio mass spectrometer	
JCO_2	CO_2-flux	[µmol m^{-2} s^{-1}]
J_S	sap flux	[mm h^{-1}]
LAI	leaf area index	[m^2 m^{-2}]
NEE	net ecosystem exchange	[µmol m^{-2} s^{-1}]
NPP	net primary productivity	[µmol m^{-2} s^{-1}]
PDH	pyruvate-dehydrogenase	
PEP-CO	phosphoenolpyruvate-carboxylase	
R^2	regression coefficient	
R_{eco}	ecosystem respiration flux	[µmol m^{-2} s^{-1}]
RN	net radiation	[W m^{-2}]
RQ	respiratory quotient	
R_{soil}	soil respiration flux	[µmol m^{-2} s^{-1}]
RUBISCO	ribulose-1,5-bisphosphat-carboxylase-oxygenase	
SM (SWC)	soil moisture (soil water content)	[m^3 m^{-3}]
SMO	soil microorganisms	
SOM	soil organic matter	
SOM-C	carbon from soil organic matter	
SWC	= SM	
SWP	soil water potential	[MPa]
T_{air}	air temperature	[°C]
T_{soil}	soil temperature	[°C]
u_e	molar airstream entering the chamber	[mol l^{-1}]
w	vertical wind speed	[m s^{-1}]
w_e	molar fraction of water entering the chamber	[mmol mol^{-1}]
w_o	molar fraction of water leaving the chamber	[mmol mol^{-1}]
WP_{PD}	predawn leaf water potential	[MPa]
WP_{MD}	midday leaf water potential	[MPa]
VPD (*D*)	vapor pressure deficit	[hPa; kPa]
VPDBee	*Vienna Pee Dee Belemnite* (international standard for C isotope analyses)	

GLOSSARY

dark respiration	autotrophic respiration in darkness (no photorespiration)
ecoflux model	flux mass balance at the ecosystem scale (**section 2.7**, **equ. 5**)
eco-isoflux model	isotopic flux mass balance at the ecosystem scale (**section 2.7**, **equ. 6**)
ecosystem productivity	amount and rate of production in an ecosystem over a given time periode
ecosystem respiration	amount of CO_2 respired by the ecosystem
eddy covariance	statistical tool to analyze high frequency wind and scalar atmospheric data series, yielding values of fluxes (e.g. of CO_2) at the ecosystem level (see **section 2.2**)
exetainer	12 mL glass vial capped with a piercable septum for air sampling
gap filling	statistical method to close data gaps in *eddy covariance* data sets
Intube-incubation	method to measure $\delta^{13}C_{res}$ of ecosystem components (foliage, roots, soils) using exetainers for incubation of the respiring material and subsequent sampling of CO_2 (see **section 2.5**)
keeling plot	estimation of $\delta^{13}C_R$ by a regression model between $\delta^{13}C$ and $[CO_2]$ (see **section 2.4**)
Mitra	name of the study site
net ecosystem exchange	net rate of carbon exchange between atmosphere and ecosystem (negative values - sink for CO_2 (release smaller than accumulation), positive values - source for CO_2 (release higher than accumulation)
net primary productivity	net rate at which an ecosystem accumulates energy or biomass, excluding the energy it uses for the process of respiration, while gross primary productivity (GPP) includes respiration
respiratory quotient	ratio of CO_2 produced to O_2 consumed by dark respiration
soilflux model	flux mass balance at the soil scale (**section 2.7**, first part of **equ. 5**)
soil-isoflux model	flux mass balance at the soil scale (**section 2.7**, **equ. 7**)
time-lag	delay between photosynthetic fixation of carbon and its release by respiration, explained as travelling time of fixed carbon from leaf to roots (Ekblad & Högberg, 2001; Bowling *et al*., 2002)

SUMMARY

Predicting large-scale effects of global warming on ecosystem productivity requires a process based understanding of ecosystem carbon sequestration and is currently a major objective of ecological research. Terrestrial carbon exchange is determined by the balance of inputs from photosynthetic fixation, storage in various pools (e.g. plant biomass, soil carbon) and loss of carbon by autotrophic and heterotrophic respiration. This partitioning study yielded valuable new insights into the carbon dynamics of a Mediterranean ecosystem. I combined straight-forward stable isotope methodology with carbon flux measurements at both soil and ecosystem scales to quantify the contribution of component fluxes (trees, understory vegetation, roots and soil microorganisms) to the ecosystem carbon balance.

The main goals of the study were:

(***i***) to evaluate temporal dynamics in isotopic composition of ecosystem respiration ($\delta^{13}C_R$) and respired CO_2 from ecosystem components ($\delta^{13}C_{res}$) on hourly, daily and annual time scales in a Mediterranean woodland and to assess the key climatic factors driving carbon fluxes and their isotopic compositions (**chapter 3, 4, 6**).

- $\delta^{13}C_R$ and $\delta^{13}C_{res}$ varied substantially on both seasonal and diurnal time scales. This study showed that these variations are partially linked to each other and that seasonal changes in $\delta^{13}C_R$ and $\delta^{13}C_{res}$ can be attributed to environmental drivers of photosynthetic discrimination (e.g. VPD, radiation, T_{air}). The rapid diurnal dynamics in $\delta^{13}C_{res}$ are mainly due to short-term changes in substrates as well as post-photosynthetic fractionation in the dark respiratory pathways, while diurnal variation in $\delta^{13}C_R$ result from shifts in the relative contribution of different ecosystem components to ecosystem respiration.
- Substantial short-term variation in soil and ecosystem respiration and their isotopic compositions after precipitation events during drought can be attributed to the *Birch* effect. $\delta^{13}CO_2$ indicated that the *Birch* effect is caused by hypo-osmotic stress of soil microorganisms in response to rewetting.

(***ii***) to quantify the component fluxes and to evaluate their impact on ecosystem scale carbon balance. Two models were applied: first, a flux based ecosystem scale mass balance approach (*ecoflux*, **chapter 5, 6**) for flux separation and second, an isotopic mass balance

(*eco-isoflux*, **chapter 6**) to asses the impact of isotopic composition of respired CO_2 from different components on $\delta^{13}C_R$ and $\delta^{13}C_{res}$ of soil.

- The straight-forward *ecoflux* model allowed separation of diurnal and nocturnal carbon fluxes of the major ecosystem components from NEE, giving very new insights into ecosystem carbon cycle dynamics that are not available from *eddy covariance* data alone.
- Flux partitioning revealed that drought sensitive understory species play the most important role determining the source/sink behavior of the ecosystem in the beginning of summer, while soil microorganisms dominate ecosystem carbon exchange during drought.
- The new *eco-isoflux* model revealed reasonable results at the soil scale while at the ecosystem scale results deviated partially from those, obtained by the *ecoflux* model, mainly because of uncertainties in $\delta^{13}C_R$ calculations. However, both models yielded similar results when $\delta^{13}C_R$ was modeled from the component fluxes.

The results presented here significantly contribute to a better understanding of ecosystem carbon exchange and its dependence on environmental conditions. Both *ecoflux* and *eco-isoflux* model approaches provide the possibility to directly access the different biotic responses in carbon fluxes to changes in abiotic drivers. Thus, they can be recommended for the use at larger spatial and temporal scales in order to achieve an accomplished comprehension of ecosystem and soil carbon dynamics.

ZUSAMMENFASSUNG

Ein umfassendes Verständnis der Prozesse, die der Kohlenstoffaufnahme von Ökosystemen zugrunde liegen, ist eine der wichtigsten Voraussetzungen für fundierte Prognosen des Einflusses der globalen Erwärmung auf die Ökosystemproduktivität. Der Kohlenstoffhaushalt terrestrischer Ökosysteme resultiert aus dem Zusammenspiel von C-Aufnahme durch Photosynthese, C-Speicherung in verschiedenen Reservoirs (z.B. Pflanzenbiomasse, Boden) und C-Verlust durch autotrophe und heterotrophe Atmungsprozesse. Diese Studie zeigt relevante neue Einsichten in den Kohlenstoffkreislauf eines mediterranen Ökosystems. Die Kombination von Kohlenstoffflüssen mit stabilen Isotopen ermöglichte eine genaue Quantifizierung der Beiträge verschiedener Ökosystemkomponenten (Baumschicht, Krautschicht, Bodenmikroorganismen und Wurzeln) zum Kohlenstoffkreislauf.

Die Hauptziele dieser Arbeit waren:

(***i***) die Beurteilung der Variabilität im Isotopenverhältnis der Ökosystematmung ($\delta^{13}C_R$) und der Atmung der einzelnen Ökosystemkomponenten ($\delta^{13}C_{res}$) auf verschiedenen Zeitskalen in einem mediterranen Steineichenwald sowie die Erfassung der klimatischen Schlüsselfaktoren, die für diese Variationen verantwortlich sind (**Kapitel 3, 4, 6**).

- Es konnten starke Variationen sowohl in $\delta^{13}C_R$ als auch in $\delta^{13}C_{res}$ auf allen zeitlichen Skalen verzeichnet werden. Diese Studie zeigt, dass saisonale Änderungen in $\delta^{13}C_R$ und $\delta^{13}C_{res}$ verschiedenen, die photosynthetische Isotopendiskriminierung beeinflussenden Klimafaktoren unterlagen (z.B. VPD, Strahlung, Temperatur). Die Variationen im Tagesverlauf von $\delta^{13}C_{res}$ konnten kurzfristigen Änderungen im Atmungssubstrat sowie post-photosynthetischen Isotopendiskriminierungsprozessen zugeordnet werden, während Kurzzeitvariationen in $\delta^{13}C_R$ eher durch Veränderungen im Beitrag der einzelnen Ökosystemkomponenten zur Gesamtatmungsbilanz verursacht wurden.
- Starke Kurzzeitvariationen in Boden- und Ökosystemrespiration sowie ihrer Isotopenzusammensetzung nach Regenfällen während der mediterranen Sommerdürre sind auf den *Birch*-Effekt zurückzuführen. $\delta^{13}CO_2$-Messungen zeigten, dass diesem Effekt wahrscheinlich hypoosmotischer Stress der Bodenmikroorganismen durch den rapiden Anstieg im Bodenwasserpotential zugrunde liegt.

(***ii***) die Quantifizierung der Kohlenstoffflüsse der einzelnen Ökosystemkomponenten, um ihren Einfluss auf die Nettokohlenstoffbilanz zu prüfen. Dazu wurden zwei Modelle angewendet. Das erste Modell basiert auf einem Massenbalance-Konzept der Ökosystemflüsse (*ecoflux*, **Kapitel 5, 6)** und diente dazu, die C-Flüsse der einzelnen Ökosystemkomponenten aufzutrennen. Das zweite Modell kombiniert stabile Isotope mit den Ökosystemfüssen (*eco-isoflux*, **Kapitel 6**) um einen besseren Einblick in das Zusammenwirken von $\delta^{13}CO_2$ der verschiedenen Respirationsquellen auf der Boden- und der Ökosystemebene zu gewährleisten.

- Das innovative *ecoflux* Modell erlaubte eine Auftrennung des Nettoökosystemflusses in die respiratorischen und photosynthetischen Beiträge seiner Hauptkomponenten, was neue Einsichten in den Kohlenstoffkreislauf gewährte, die aus den Nettoflüssen nicht ersichtlich waren.
- Dies zeigte, dass im späten Frühling die dürreempfindliche Krautschicht eine Schlüsselrolle bei der Quellen/Senken Bilanz des untersuchten Ökosystems spielt, während heterotrophe Bodenmikroorganismen den Hauptbeitrag zur Kohlenstoffbilanz während des Sommers leisten.
- Das neuartige *eco-isoflux* Modell offenbarte gute Ergebnisse auf der Bodenebene, während die Ergebnisse auf der Ökosystemebene teilweise vom *ecoflux* Modell abwichen. Dies war hauptsächlich auf Regressionsunsicherheiten bei der Berechnung von $\delta^{13}C_R$ zurückzuführen. Allerdings waren beide Modelle im Einklang, wenn $\delta^{13}C_R$ aus $\delta^{13}C_{res}$ der Ökosystemkomponenten modelliert wurde.

Die in dieser Arbeit erzielten Ergebnisse liefern einen signifikanten, neuen Beitrag zum besseren Verständnis der Ökosystemkohlenstoffbilanz und ihrer Abhängigkeit von biotischen und abiotischen Umweltfaktoren. Beide Modelle ermöglichen eine direkte Quantifizierung der unterschiedlichen Reaktionen einzelner Flüsse innerhalb des Kohlenstoffkreislaufs auf Änderungen der abiotischen Umweltbedingungen. Sie können potentiell auf größeren zeitlichen und räumlichen Ebenen eingesetzt werden, wo sie von großer Relevanz bei der Aufklärung von Kohlenstoffdynamiken auf der Ökosystemebene sein könnten.

CHAPTER 1

GENERAL INTRODUCTION

1.1 MEDITERRANEAN ECOSYSTEMS UNDER GLOBAL CHANGE

All western coasts of the continents between 32° to 40° latitude of both hemispheres are subject to the Mediterranean climate. Typical for ecosystems in this climatic zone is a precipitation maximum during the winter alongside a hot and dry period during the summer (e.g. Aschmann, 1973; Mooney, 1978; Walter, 1990). With 3.25 x 10^6 km^2 Mediterranean vegetation covers only 2.3% of the terrestrial surface on earth (Rambal, 1999). Nevertheless, Mediterranean ecosystems have large ecological, hydrological and landscape-protecting relevance (Tirone *et al*., 2003) and their high species diversity constitutes a valuable natural reserve of worldwide significance (Davis & Richardson, 1995).

Main trials for Mediterranean plants apart from summer drought, which constitutes a period of high evaporative demand and low soil water availability during most of the potential growing period (Tenhunen *et al*., 1990), are chilling during the relatively cold winters (Di Castri, 1981; Nahal, 1981) and the temporal unpredictability of rainfalls (Joffre *et al*., 1999). Such environmental constraints restrict the main growing period in these regions to the springtime (Baptista & Rodrigues, 1991), when rain falls are strong though short and irregular, lowering their effectiveness for plant growth (Nahal, 1981).

The natural vegetation form is the *macchia* formation characterized by a plant community of evergreen shrubs with sclerophyllous long-life foliage (Quezel, 1977) and the *garrigue* formation degenerated from the *macchia* by anthropogenic influences such as cattle pasture and fire (Tomaselli, 1981; Baptista & Rodrigues, 1991). With increasing population numbers and land use change these natural habitats, however, nowadays for the most part have vanished and large areas of the European Mediterranean Basin are covered by man-made ecosystems called *dehesa* in Spain and *montado* in Portugal. These oak-grass savannahs are abundant on the southwestern Iberian Peninsula (Joffre *et al*., 1999) and have been reported to amount to 8.5 Gha, with biomass of 50Gt and a total productivity of 6 Gt y^{-1} in the Mediterranean basin (Whittaker & Likens, 1975). Hence, they make up a large part of annual carbon balance and knowledge about the behavior of

these ecosystems confronted with new environmental trials is of utmost importance for future ecosystem management.

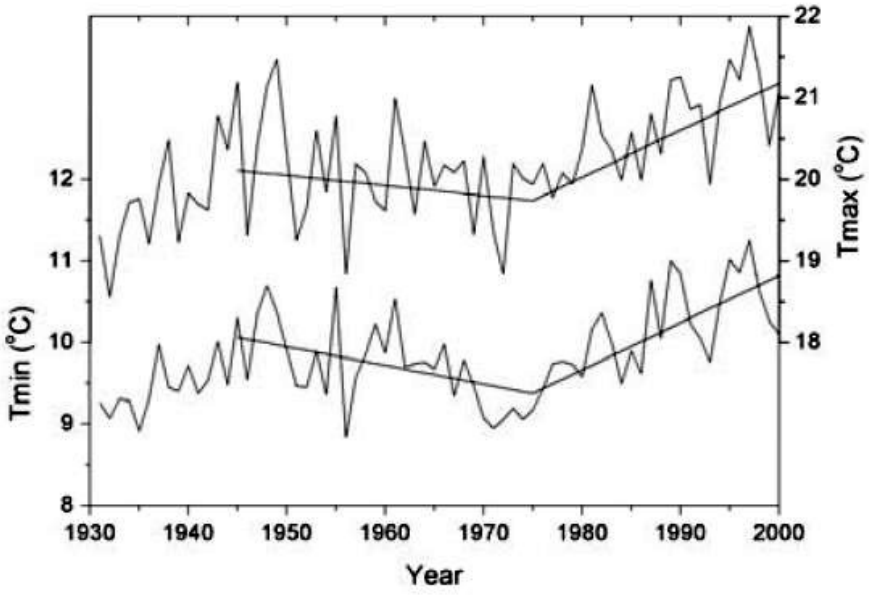

Fig. 1.1 Evolution of mean minimum (bottom curve, left axis) and mean maximum (top curve, right axis) air temperatures from 1930 to 2000; average for the mainland of Portugal, from Miranda *et al*. (2002).

Atmospheric carbon dioxide concentration has been increasing by about 25% since the industrial revolution, and is still rising by about 1.8 ppm per year (Watson *et al*., 1990; Prentice *et al*., 2001; IPCC, 2007). Currently, rapid changes occur in the planets climate system, which are mainly attributed to the anthropogenic emissions of CO_2 and other greenhouse gases (Keeling *et al*., 1995) that alter global radiation balance, commonly known as the *anthropogenic green house effect* (IPCC, 1996). Mediterranean ecosystems are especially endangered by this development. Steadily increasing air temperatures (Miranda *et al*., 2002; **Fig. 1.1**) have large effects on biodiversity and structure of Mediterranean ecosystems (Peñuelas *et al*., 2004; IPCC, 2007). The most hazardous consequence of climate change in the Mediterranean is a change in onset and length of summer drought (Ciais *et al*., 2005; Granier *et al*., 2007; Pereira *et al*., 2007). Climate model simulations for Portugal predict a three to four times increase in annual number of days with air temperatures above 35°C for the end of the century (Miranda *et al*., 2002; **Fig. 1.2**).

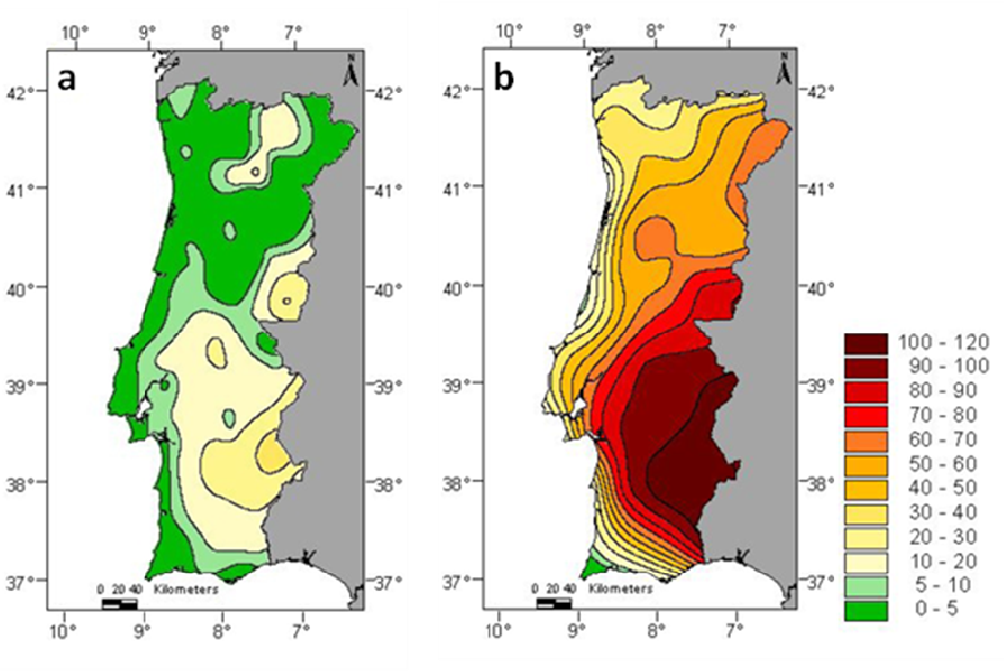

Fig. 1.2 Number of days per year with maximum temperatures above 35°C in Portugal for (**a**) 1961-1990 (climatology) and (**b**) HadRM GGa2 model simulation (2080-2100), adapted from Miranda *et al*. (2002).

Given these predictions, seasonal droughts will increase significantly in both length and severity, resulting in declining gross primary productivity and increasing CO_2-emission from respiration (Pereira *et al*., 2007) and enhanced forest fire frequency (Davis & Michealson, 1995). Such progresses would back-feed rising atmospheric CO_2-concentrations, although Mediterranean ecosystems would not significantly enhance global climate change due to their small global land cover (Reichstein, 2001).

Regional however, consequences of prolonged drought periods would accelerate desertification, erosion and land degradation processes (e.g. Hollis, 1992; Herrera *et al*., 1993; Naveh, 1995; Peñuelas *et al*., 2004), resulting in high ecological and socioeconomical harm. First effects of climate change on Mediterranean ecosystems are already detectable in decreased precipitation, especially during the main growing period in spring (Hofrichter, 2002). Summer droughts in recent years (2003 and 2005; driest episode in the last 140 years; Pereira *et al*., 2007; **Fig. 1.3**) where as severe as never before (Ciais *et al*., 2005; Granier *et al*., 2007; Pereira *et al*., 2007). Apart from the dangers of great losses in biodiversity and land use options, mitigation of drought-induced effects in Mediterranean countries is expected to produce high economical costs (Watson *et al*., 1995). Therefore, future predictions of climate change impacts on

ecosystem functioning are of special relevance in areas endangered by desertification and land degradation, such as the Mediterranean basin.

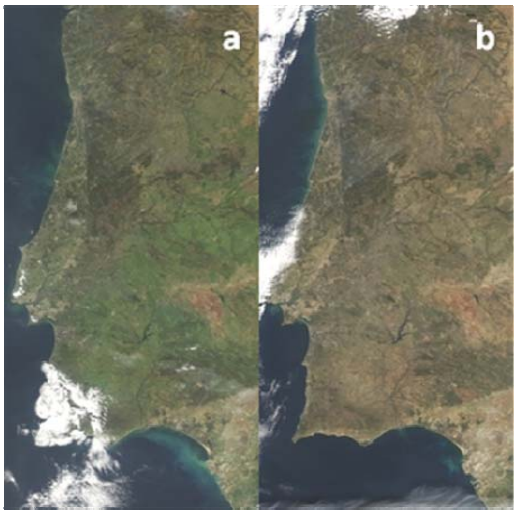

Fig. 1.3 Drought effects visible in satellite photographs of Portugal in (**a**) February 2004 and (**b**) February 2005 (from: http://earthobservatory.nasa.gov/NaturalHazards/natural_hazardsv2.php3?img_id=12757)

1.2 MEASURING AND MODELING ECOSYSTEM SCALE CARBON FLUXES

The global carbon cycle consists of carbon pools in atmosphere, oceans, biomass and lime stone exchanging carbon over a variety of processes (**Fig. 1.4**).

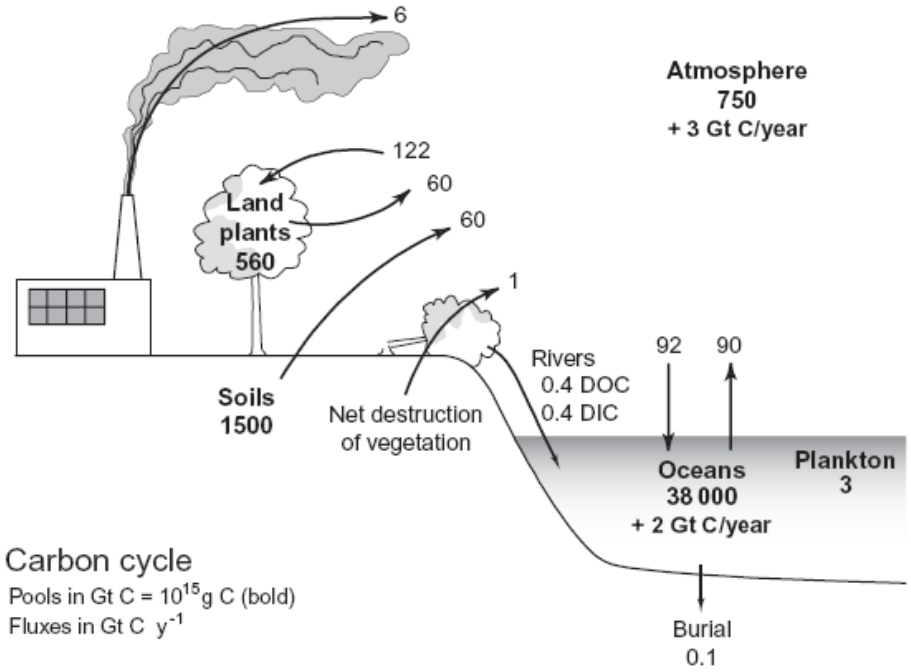

Fig. 1.4 The global carbon cycle (after Schlesinger, 1997).

In terrestrial ecosystems carbon balance is controlled by inputs via photosynthetic fixation, storage in various pools (e.g. plant biomass, soil carbon) and loss of carbon by autotrophic and heterotrophic respiration as well as non respiratory processes such as leaching, physical demineralization of inorganic soil carbon, erosion and forest fires (e.g. Schulze *et al*., 2000; Law *et al*., 2002; Trumbore, 2006). Through there direct effect on atmospheric CO_2-concentrations (by photosynthetic assimilation and respiratory CO_2-release) terrestrial ecosystems are considered to be important regulators of global climate (Reichstein, 2001). They have the potential to act as long-term carbon *sinks* (positive net carbon balance) or as carbon *sources* (negative net carbon balance) and thus, can counterbalance or increase global warming, respectively. The current assumption is that terrestrial ecosystems accumulate 1.1 Pg C y^{-1}.(IPCC, 2001). Cox *et al*. (2000) predicts a drastic turn in global carbon balance within the next 50 years, when more and more terrestrial ecosystems will turn into carbon sources. For future climate change prognostics it is therefore, essential to assess the consequences of temperature increase on the net carbon balance in ecosystems all over the world.

Worldwide effort is made to quantify the contribution of terrestrial ecosystems to global carbon balance. Net carbon fluxes are therefore, monitored in a wide range of terrestrial ecosystems using *eddy covariance* technique (e.g. Janssens *et al*., 2001; for a thorough explanation see **section 2.2**), a micrometeorological method, particularly suitable to account for carbon source/sink strength from local to regional scales (Baldocchi *et al*., 2001). Assessment of carbon fluxes by *eddy covariance* integrates complex processes (Valentini *et al*., 2003) and yields high time resolved data sets for empirical modeling. Within the recent years effort was made to build up networks to monitor net ecosystem exchange (NEE) and regional flux networks such as CARBOEUROFLUX, AMERIFLUX or ASIAFLUX have created the global network FLUXNET (Baldocchi *et al*., 2001). These networks aim to combine regional scale data to continental and even global carbon flux models and to predict the impact of future climate scenarios on the global carbon budget (e.g. Papale & Valentini, 2003; Reichstein *et al*., 2003). However, there is a general shortcoming concerning *eddy covariance*. Since it only measures net carbon fluxes it is difficult to achieve an understanding of biological processes causing changes in these fluxes, which is necessary for restoring ecosystem management. For example, a decrease in NEE could be caused by increased ecosystem respiration (R_{eco}), decreased gross ecosystem exchange (GEE) or a combination of both.

Partitioning NEE into assimilatory and respiratory compounds has become an important tool to describe sources and sinks of carbon and a range of methods to measure or model the opposing fluxes emerged within the recent years. One way to achieve a partitioning between those fluxes is to apply temperature corrections on nighttime NEE to model ecosystem respiration during daytime (R_{eco}) which then can be used to partition GEE from daytime fluxes (e.g. Aubinet *et al*., 2000; Reichstein *et al*., 2002b; Reichstein *et al*., 2005). Other approaches using stable isotopes in combination with *eddy covariance* measurements (e.g. Bowling *et al*., 2001; Ogée *et al*., 2003; Knohl & Buchmann, 2005), take advantage of the isotopic disequilibrium between ecosystem respiration (as derived by nighttime *keeling plots*) and canopy assimilation (as derived by the *Farquhar model* (Farquhar *et al*., 1989) applied to the canopy).

Still, achieving a separation between the main ecosystem carbon fluxes is not enough for a thorough process-based understanding of ecosystem scale carbon sequestration, which is the result of complex interactions of numerous C-pools with different dependencies on environmental variables and multi-temporal and -spatial dynamics (e.g. Schimel *et al*., 2001; Law *et al*., 2002; Valentini *et al*., 2003). Therefore, future predictions of carbon cycle processes on the ecosystem scale and consequent equilibrating ecosystem management are difficult to achieve with empirical models which rely on rather simplistic physical assumptions (dependencies on moisture, temperature etc.) disregarding complex interactions of biological processes. Although some studies regarding annual carbon budget have focused on flux partitioning by measuring carbon fluxes of ecosystem components (e.g. soil, foliage, roots) alongside NEE (e.g. Goulden *et al*., 1996; Lavigne *et al*., 1997; Law *et al*., 1999; Law *et al*., 2001a; Davidson *et al*., 2006a), a thorough separation of all major carbon fluxes in response to changing environmental conditions has not been achieved until present. Thus, our understanding of many small-scale processes and their impact at ecosystem scale carbon dynamics (e.g. exchange mechanisms between carbon pools or factors controlling carbon sequestration and -release) is still poor. It remains a challenge to disentangle these processes and their environmental drivers, a knowledge which is crucial for correct carbon balance predictions under climatic change.

1.3 ISOTOPIC IMPRINTS ON THE CARBON CYCLE

Through their irregular distribution between different C-pools during carbon exchange processes, stable carbon isotopes provide an independent tool to acquire information about the carbon cycle and its drivers on a wide range of temporal and spatial scales (Dawson *et al*., 2002). The heavy carbon isotope ^{13}C is abundant in nature with only 1.11%, whereas the lighter isotope ^{12}C makes up the major part of total global carbon (98.89%; Fritz & Fontes, 1980). Being heavier in mass, ^{13}C is allocated differentially from ^{12}C during many biochemical and physical carbon exchange processes, an occurrence generally referred to as *isotope effect*. We distinguish *kinetic* (uneven distribution during kinetic reactions or diffusion) and *equilibrium effects* (uneven distribution during phase changes). The most important isotope effect for carbon cycle research is probably photosynthetic discrimination against ^{13}C by the enzyme RUBISCO, which preferentially uses $^{12}CO_2$ as substrate, whereas PEP-CO of C4 plants is an unselective acceptor (Bender, 1968, 1971; Smith & Epstein, 1971). This effect causes that the entire carbon fixed by C3-plants, and therefore, the majority of organic carbon compounds as well as CO_2 derived from these by respiration and biomass burning, is significantly depleted in ^{13}C (~ -25‰) as compared to atmospheric CO_2 (~ -8‰; Keeling & Whorf, 2002). Since photosynthetic discrimination is related to the ratio of leaf internal to leaf external [CO_2] (ci/ca; Farquhar *et al*., 1982), the isotopic composition of organic compounds and respired CO_2 yields information about the environmental conditions, when CO_2-accumulation occurred (Farquhar *et al*., 1989). Analyzing the isotopic composition ($\delta^{13}C$) of bulk leaf material, starch and cellulose provides a long-term integrated measure of photosynthetic discrimination, reflecting the time-period of foliage development, whereas soluble sugars and leaf respired CO_2 carry the signature of recent photosynthetic activity. The imprint of photosynthetic discrimination on assimilated carbon was shown to reappear in respired carbon of soil and ecosystems with a time-lag ranging from hours to days (e.g. Ekblad & Högberg, 2001; Bowling *et al*., 2002; Mortazavi *et al*., 2005; Werner *et al*., 2006). This time-lag has generally been interpreted as the transport time of assimilates from the foliage to the bulk of the respiring tissue (e.g. Bowling *et al*., 2002). Recently, other ^{13}C fractionation processes than photosynthetic discrimination have become evident, which occur post-photosynthetic due to fragmentation of hexose-molecules with heterogeneous distribution of ^{13}C. These

fractionation processes are thus, attributable to neither *kinetic* nor *equilibrium* effects and were terminated *fragmentation fractionation* (Tcherkez *et al*., 2004). This effect changes the isotopic signature between substrate and product in many biochemical reactions, e.g. during transport and allocation of carbon within the plant (e.g. during starch break-down for phloem-loading, Tcherkez *et al*., 2004) and within the dark respiratory pathways of leaves (Tcherkez *et al*., 2003; for a review see Ghashghaie *et al*., 2003 and Werner *et al*., 2007a). During decarboxylation of pyruvate to Acetyl-CoA by pyruvate dehydrogenasis (PDH) the enriched C-atoms of pyruvate are respired. Acetyl-CoA, which is fed into biosynthesis and Krebs cycle pathways consists of the depleted C-atoms of the pyruvate (Fig. **1.5**). Hence, the interplay of PDH reaction, Krebs cycle and biosynthesis of Acetyl-CoA to fatty acids will, besides the imprint of photosynthetic discrimination, determine the isotopic signature of overall respired CO_2.

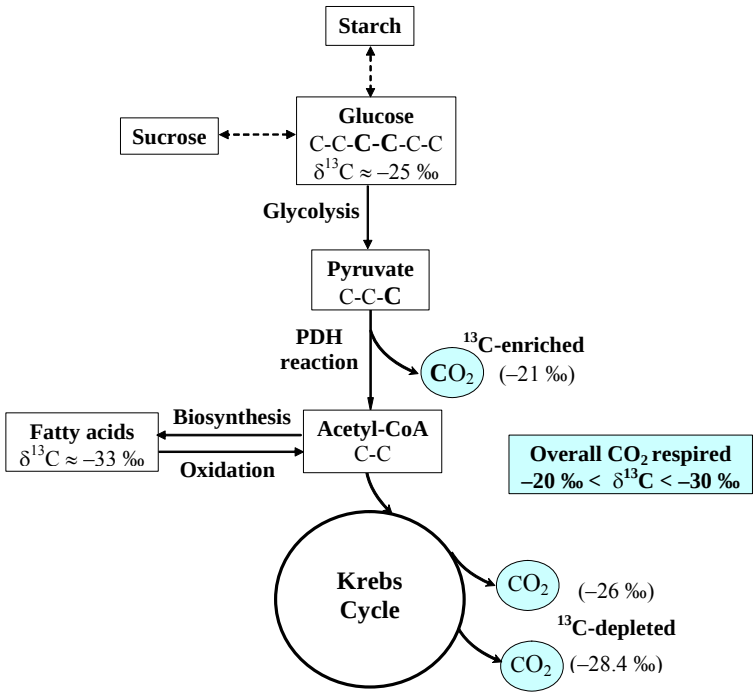

Fig. 1.5 Respiratory metabolic "pattern" explaining the $\delta^{13}C$ of respired CO_2 in the dark. $\delta^{13}C$ values are those measured in French bean leaves by Tcherkez *et al*. (2003) except those of CO_2 in parentheses, which are derived from positional $\delta^{13}C$ values in natural glucose as given by Rossmann, Butzenlechner & Schmidt (1991). Carbon atoms C-3 and C-4 of glucose and thus, C-1 of pyruvate which is decarboxylated during pyruvate dehydrogenase (PDH) reaction are ^{13}C-enriched (bold letters: **C**). Other carbon atoms of glucose forming acetyl-CoA, which are then decarboxylated in the Krebs cycle are ^{13}C-depleted, from Werner *et al*. (2007a).

$\delta^{13}C$ of respired CO_2 has become an important indicator of carbon source for respiration or combustion. In example, anthropogenic originated CO_2 from geologically ancient carbon sources (e.g. petroleum byproducts) is generally very depleted in ^{13}C, with values typically ranging from -27 to -45‰ (Pataki *et al*., 2003b). Carbon respired from different ecosystem components, such as foliage from different heights, roots, stems or soils of different depths can have substantially different isotopic signatures (Knohl *et al*., 2005; Tu & Dawson, 2005). However, the lack of knowledge concerning isotope effects during carbon sequestration and the interplay of processes driving variation in $\delta^{13}C$ of respiration at the ecosystem scale is still large.

Isotopic composition of CO_2 respired by ecosystem components ($\delta^{13}C_{res}$) such as soil, roots and foliage can be measured with chamber and incubation methods (see **section 2.5**). Provided, that signatures of the different components are distinguishable, they might serve to trace back the origin of ecosystem scale respired CO_2 (R_{eco}) and its isotopic composition ($\delta^{13}C_R$). $\delta^{13}C_R$, as derived by *keeling plots* (a two-source mixing model describing mixing between atmospheric and ecosystem derived CO_2 (Keeling, 1958); for a thorough explanation see **section 2.4**) can potentially be applied to isotopic mass balance approaches (see **section 2.7**) for partitioning studies of the respiration flux at the ecosystem scale. $\delta^{13}C_R$ is known to vary seasonally with changes in climatic conditions, driving photosynthetic discrimination and respiratory activity (e.g. Bowling *et al*., 2001; 2002; Ponton *et al*., 2006; Werner *et al*., 2006; 2007a). Recent studies showed small (Ogée *et al*., 2003; Still *et al*., 2003; Schnyder *et al*., 2004) to large (up to 6‰, e.g. Bowling *et al*., 2003; Werner *et al*., 2006) variation in $\delta^{13}C_R$ even within a single night**.** However, there are various problems associated with the determination of $\delta^{13}C_R$, since the *keeling plot* method assumes a stable background signal during the sampling period as well as a sufficiently high CO_2-gradient (~30 to 50ppm) for reliable *keeling plot* calculations (Pataki *et al*., 2003a). In ecosystems with low respiratory activity (e.g. during Mediterranean summer drought) it can be difficult to capture these CO_2-gradients within a time frame of 30 min, where presumably no variation in the background signal occurs.

Measuring all major respiratory component fluxes and their respective isotopic signals does enable $\delta^{13}C_R$-estimates independently from the *keeling plot* approach, which could

be particularly useful in low productive ecosystems in seasonally dry climates such as the Mediterranean basin.

1.4 OBJECTIVES OF THIS WORK

This work aims at contributing to a better understanding of biotic and abiotic processes driving temporal and spatial dynamics in carbon fluxes of Mediterranean ecosystems, particularly endangered by global climate change.

Within recent years, stable isotopes have become increasingly important as tracers of carbon fluxes and indicators of environmental change. However, we are only beginning to understand the complex processes responsible for isotopic exchange between the carbon pools at the ecosystem scale.

Therefore, the main focus of this thesis is to disentangle drivers of ecosystems scale carbon dynamics (respiration and assimilation) during changing environmental conditions that can be expected from global climate models within the next century and to unravel the complex isotopic pattern of respiratory CO_2 from ecosystem and its main components (soils, trees, understory vegetation, roots).

These goals were achieved by:

- **first**, monitoring the variation in R_{eco} and its isotopic composition ($\delta^{13}C_R$) on different temporal scales and relating it to climatic parameters.
- **second**, investigating the influence of drought and precipitation pattern on ecosystem and soil respired $^{13}CO_2$.
- **third**, the application of a flux-based mass balance model (*ecoflux*) to partition ecosystem-scale assimilation and respiration fluxes into the fractions of their main component fluxes (soils, trees, understory vegetation, roots) during increasing temperatures and water deficit.
- **fourth**, the application and evaluation of a stable isotope model (*eco-isoflux*) at the ecosystem and soil scale.

1.5 OVERVIEW

Chapter 1 gives a general introduction and background information on recent scientific progress on the major concerns of the present work, (i) the impact of global climate change and drought on Mediterranean type ecosystems; (ii) the possibility to measure and model ecosystem scale carbon fluxes for development of future climate impact models and (iii) the use of stable carbon isotopes to gain a more process-based understanding of ecosystem scale carbon sequestration.

Chapter 2 presents a detailed description of the chosen Mediterranean *montado* field site *Herdade de Alfarrobeira*, near Évora, Portugal and species studied as well as a review of sampling techniques, monitoring methods and analyses used in this study to measure and model carbon fluxes and their respective isotopic signatures.

Chapter 3 (i) evaluates the variability in carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$) on different temporal scales (annual, seasonal, diurnal); (ii) presents a thorough analysis concerning the time-lags between photosynthesis and respiration at the ecosystem scale during periods of transition from the wet to the dry season, when the ecosystem turns from a net carbon sink into a source, and vice versa and (iii) highlights new implications for sampling protocols of $\delta^{13}C_R$.

Chapter 4 addresses the influence of rain pulse events during Mediterranean summer drought on both ecosystem and soil respiration and their isotopic composition, known as the *Birch* effect. Three data sets are presented, two with artificially induced rain pulse events in the beginning and the middle of summer and one with natural precipitation pulses at the end of the drought season. Here, (i) the effects of these rain pulses on ecosystem NEE are quantified and (ii) recent theories about the origin of the *Birch* effect are evaluated by analyses of $\delta^{13}C_R$ and $\delta^{13}C_{soil}$.

Chapter 5 deals with the influence of seasonal drought on NEE. Here, an ecosystem-scale mass balance model is used to partition NEE into ecosystem assimilation

and ecosystem respiration during a spring to summer transition period. Drought induced changes in the opposing fluxes are attributed to variation in component fluxes (trees, understory, soil microorganisms and roots). This partitioning approach disentangles changes in respiratory and photosynthetic ecosystem fluxes that are not apparent from the *eddy covariance* or the soil respiration data alone. The combination of both component fluxes (soil, roots understory) and *eddy covariance* data allows a new and improved understanding of factors controlling carbon sequestration during Mediterranean summer drought.

Chapter 6 regards the comparison of two different model approaches (i: flux partitioning - *ecoflux* and ii: stable isotope partitioning - *eco-isoflux*) to partition ecosystem respiration, the main determinant of carbon balance in most ecosystems, into its major component fluxes. Further, substantial diurnal and seasonal variation in $\delta^{13}C_{res}$ and $\delta^{13}C_R$ are discussed. Stable isotope partitioning might be a new and powerful tool to model $\delta^{13}C_R$ independently from the *keeling plot* approach. Its value for the analysis of processes altering ecosystem- and soil respiration and their respective isotopic compositions will be assessed.

Chapter 7 contains some general comments and concerns on the topics studied in the present work. Further, the main results of this study are discussed and some future perspectives are given.

CHAPTER 2

FIELD SITE CONDITIONS AND METHODS USED

2.1 THE FIELD SITE

The site (**Fig. 2.1**) is located in the centre of the Portuguese Alentejo at *Herdade da Alfarrobeira* henceforth called *Mitra* a rural district 12 km south of Évora, Portugal (38°32′26.549″N, 8°00′01.424″W, 264m a.s.l.).

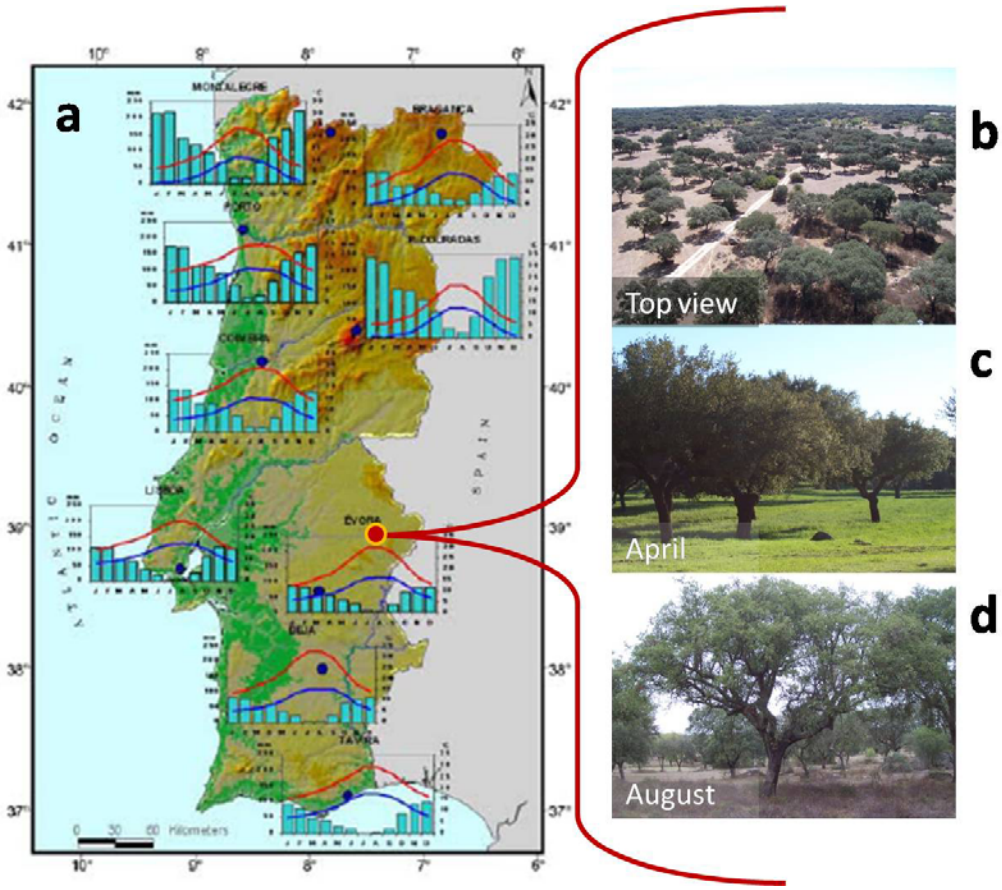

Fig. 2.1 (**a**) Map of Portugal with mean annual cycles of monthly temperature (min and max) and precipitation patterns in selected regions, for the period 1961-1990. Shading represents orography. The location of the study site is indicated by the red dot. (**b-d**) Photographs of the field site during different seasons, (**b,d**) during August 2005, (**c**) during April 2005, (**a**) redrawn from Miranda *et al.* (2002).

The stand is a characteristic Mediterranean savannah-type evergreen oak woodland (*montado*, with tree density of 30 ha^{-1}, 21% tree crown cover, leaf area index (LAI) of 0.55; Carreiras *et al.*, 2006) in a very homogeneous, slightly undulated landscape, which shows the signs of a typical silvo-pastoral system. The plant community is composed of sparse canopy forming *Quercus ilex* ssp. *ballota* L. (syn. *Q. rotundifolia* Lam.) in mixture with *Quercus suber* L. and

a grass layer dominated by herbaceous annuals (e.g. *Tuberaria guttata* (L.) Fourr.), some drought deciduous graminea and a few shrubs (e.g. *Cistus salvifolius* L.). The tree crowns have a wide and shallow shape, reflecting the traditional pruning performed to increase acorn production and shade for livestock (David *et al.*, 2006). In 2002 the entire area was ploughed to remove the shrub cover and wheat (*Triticum aestivum*) was sown, which was the dominant understory species in 2003. In the following years native plant species (complete species list in appendix) recovered and dominated the site. *Triticum* disappeared largely already in 2004.
Soils are incipient derived from granitic rock. The climate is Mediterranean with a precipitation/potential evapotranspiration ratio (P/ETP) of 0.5–0.65, with hot dry summers and mild wet winters. Mean annual temperature (1951–80) is 15.5°C (mean max. and min. temperatures are 21.5 and 9.2°C, respectively) and mean annual precipitation is 669 mm (1951–80).

2.2 PERMANENT MEASUREMENTS

Fluxes of CO_2 and water

Continuous records of CO_2- and H_2O-fluxes were taken by *eddy covariance* technique on a 28 m-high metal tower with a footprint of ~800m (**Fig. 2.2**, at the *Mitra* site of the CARBOEUROPE-IP consortium).

Eddy covariance is a micrometeorological method, first described independently by Swinbank (1951) and Obukhof (1951), to continuously measure net carbon exchange of whole regions in situ without disturbances of the environment. Vertical transport of energy, heat and mass is always the result of turbulences. Thus, measuring carbon and water fluxes requires the measurements of turbulence. Up and down drafts of a scalar unit (e.g. CO_2) in the turbulent current depend on the size of the turbulent elements (*eddies*). The basic theory of determining turbulent fluxes is therefore, given by the following equation:

$$F_S = \overline{\rho w\, S} \qquad \textbf{(1)}$$

where, F_S is the average scalar flux (ratio with that an air package of determined quality passes a given horizontal area), ρ is the density of air, w is the vertical wind speed and S is the concentration of the transported scalar unit. The over bar signifies the averaging over a given time period. Hence, continuous measurements of CO_2 and H_2O concentrations together with vertical wind speed at very high temporal resolution (10-20 Hz; Foken, 2003) is demanded to

account for all turbulent incidents. Further demands are a flat and homogeneous landscape and sufficiently high wind speed to ensure turbulent mixing.

Concentration of trace gases were measured with a gas analyzer (LI-6262, LI-COR, Lincoln, NE, USA; in January 2005 changed to LI-7000, LI-COR, Lincoln, NE, USA) and vertical wind speed was monitored with a sonic anemometer (Gill R3, Gill Instruments, Lymington, Hampshire, England), which uses high time-resolved ultrasound technique to reflect turbulences. For carbon flux partitioning between understory and tree canopy I used data from a small *eddy covariance* system at a height of 2,5m (3D sonic anemometer, 1210R3, Gill Instruments Ltd., Lymington, UK; IRGA. LI-7500, LI-COR, Lincoln, NE, USA) installed at a nearby field site with similar conditions and understory species composition.

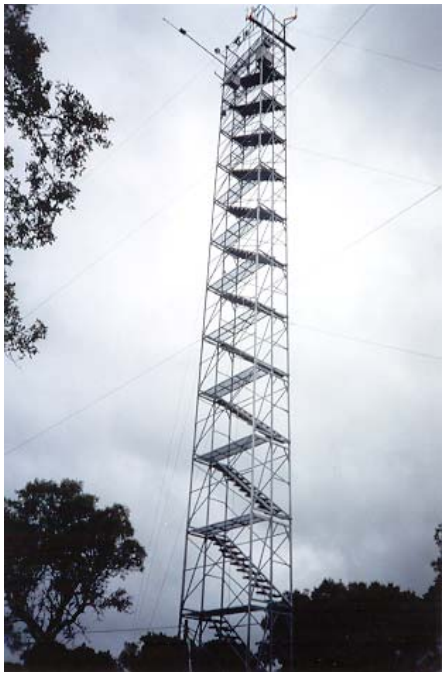

Fig. 2.2 *Eddyflux* tower installed at the *Mitra* site, Portugal.

The raw data from the *eddy covariance* measurements were processed off-line using the software *Eddyflux* (Meteotools, Jena, Germany). Following the Carboeurope-IP recommendations a planar fit coordinate rotation (Wilczak *et al.*, 2001) for wind components was performed. The CO_2-fluxes were determined, on a half-hourly basis (block averaging). A time-lag for each averaging period was determined in order to maximise the covariance between vertical wind velocity and carbon dioxide signal from the gas analyser. The fluxes were corrected for the damping loss of the closed-path analyser at high frequencies, according to Eugster and Senn (1995). In general, the correction factors varied between 1.05 and 1.30. A

CO_2-storage term, calculated for one point measurement as in Greco and Baldocchi (1996), was added to the estimated carbon flux. In the grassland this storage term was negligible.
The quality of all primary data was guaranteed by a routine of equipment calibration and, for meteorological data, a comparison with data from close stations. To exclude non-representative 30 min measurements of carbon dioxide flux, the following screening criteria were applied: fluxes were removed if the mean vertical velocity deviation to zero was higher than what would be considered as normal for the site, following the same principle as in Rebmann *et al.* (2005); fluxes were excluded if the high frequency spikes replaced or the absolute limits violations exceeded 1% of the total records of any of the three components of wind velocity and/or CO_2-concentration.
Total data gaps during the whole study period, due to missing and rejected data, were about 57% and 42% for *Mitra* and grassland, respectively. Gap filling and flux-partitioning methods proposed by Reichstein *et al.* (2005) were used to fill data gaps and to separate the net ecosystem exchange (NEE) into gross primary production (GPP) and ecosystem respiration (R_{eco}), respectively.

Microclimatic measurements

Weather conditions were continuously recorded by a solar powered meteorological station (data-logger CR10X, Campbell Scientific, Logan, Utah, USA), with Q7 REBS net radiometer (Campbell Scientific, Logan, Utah, USA), aspirated psychrometer H301 (Vector Instruments, Denbigshire, UK) and a rainfall recorder (Casella, Bedford, UK). Air temperatures (T_{air}), wind speed, net radiation (RN) and precipitation were measured in 10s intervals and were automatically stored as half-hourly and daily means/ totals. Vapor pressure deficit (VPD) was calculated from dry and wet bulb temperatures of the aspirated psychrometer.

Example for Mitra (2003/2004)

An example for climate conditions and carbon balance in the studied ecosystem (*Mitra*) is given in **Fig. 2.3**.

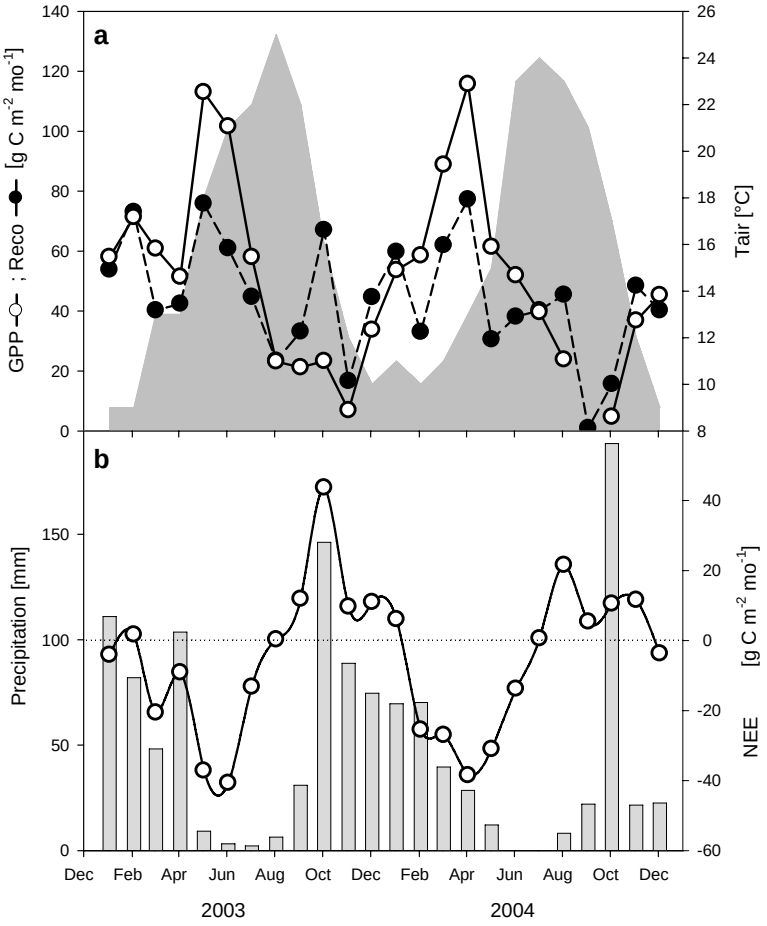

Fig. 2.3 Monthly means of (**a**) ecosystem respiration (R_{eco}, black circles), gross primary production (GPP, white circles), air temperatures (shaded area), (**b**) rain fall (bars) and net ecosystem exchange (NEE, white circles) in *Mitra* during the years 2003 and 2004, redrawn from Werner *et al.*, 2007a.

Annual ecosystem respiration (R_{eco}) varied not as much with temperature as with total rainfall (**Fig. 2.3a, b**) and the frequency of rain events. The seasonal variation in gross primary productivity (GPP) followed the usual pattern of the regional climate with a maximum in spring and a minimum during the summer. Although R_{eco} was high when GPP was high, it had a maximum impact on net carbon exchange (NEE, **Fig. 2.3b**) at the end of summer or autumn, following the first rains when photosynthesis was still low but pulses of CO_2-efflux followed each rainfall event (*Birch* effect, Jarvis *et al.*, 2007). The ecosystem was a net sink for carbon during the period between February and June and became a net source for carbon between July and January in both years. Annual carbon balance was negative in both years, though very low (~ -38g C m^{-2} y^{-1}; Pereira *et al.*, 2007), which means that the system is a very weak sink for carbon at the annual scale.

2.3 SAPFLOW AND CONDUCTANCE CALCULATIONS

Sapflow density was continuously measured in 8 *Q. ilex* trees by the Granier method (Granier, 1985, 1987). One sensor (UP GmbH, Cottbus, Germany) was radially inserted in south facing xylem of each tree. Each sensor consists of a pair of 20 mm long probes, inserted in tree stem at breast height 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-day periods (see Granier, 1985) for further details). Sapwood conductive thickness was estimated from the sapflow 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 to the radial sap flux profile, the average sapflow density measured by the Granier probes proved to be a good estimate of the average sapflow 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 to the radial sap flux profile of a *Q. ilex* tree measured by the heat pulse method (Cohen, 1994). Therefore, tree sapflow was computed as the product of sapflow density (measured by the Granier method) and the sapwood conductive area of each tree. Tree sapflow values were expressed per unit of crown-projected area (kg m^{-2} s^{-1} or mm day^{-1} or mm h^{-1}).

Canopy conductance (G_c) was calculated from sapflow data via a modification of the Penman-Monteith-equation (Monteith & Unsworth, 1990; see Oren *et al.*, 2001; **equ. 2**).

$$G_s = \frac{\gamma \times \lambda}{\rho \times c_p \times D} \times \frac{J_s \times A_s}{A_L} \qquad \textbf{(2)}$$

where G_S is the canopy stomatal conductance for water vapor (m s^{-1}), γ is the psychrometric constant (kPa·K^{-1}), λ is the latent heat of vaporization (J·kg^{-1}), ρ is the density of air (kg·m^{-3}), c_P is the specific heat of air at constant pressure (J·kg^{-1} K^{-1}), D is the vapor pressure deficit (kPa), J_S is the sapflux and A_S/A_L is the ratio of sapwood area to leaf area.

Air rather than leaf temperature was used due to a lack of continuous leaf temperature data. Average daytime G_c was calculated based on hourly values for from 8am to 6pm. G_c was expressed as whole tree conductance, since reliable LAI estimates for the sapflow-trees where not available.

2.4 THE *KEELING PLOT* APPROACH

Provided that turbulent mixing is not too strong a concentration gradient of CO_2 builds up in terrestrial ecosystems during night with height and time. CO_2-concentration close to the ground is higher due to strong respiratory influence and becomes smaller with height, because mixing with atmospheric CO_2 increases. Atmospheric and respired CO_2 have substantially different isotopic signatures. Therefore, $\delta^{13}C$ of and concentration of CO_2 during nighttime are strongly related (**Fig. 2.4c**). The *keeling plot* approach (a two-source mixing model) takes advantage of this relationship. Assuming that both source CO_2 and background CO_2 remain constant during the sampling period (< 30 min), 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):

$$\delta^{13}C_a = c_T (\delta^{13}C_T - \delta^{13}C_R) (1/c_a) + \delta^{13}C_R \quad \textbf{(3)}$$

where $\delta^{13}C$ denotes isotopic composition and c denotes 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).

The *y*-intercept of the regression is the value of y when x is zero. The intercept of a *keeling plot* (**Fig. 2.4a, b**) is the isotope ratio of respiration in the absence of dilution by atmospheric CO_2, and thus, can be assumed equal to isotopic composition from ecosytem respiration ($\delta^{13}C_R$).

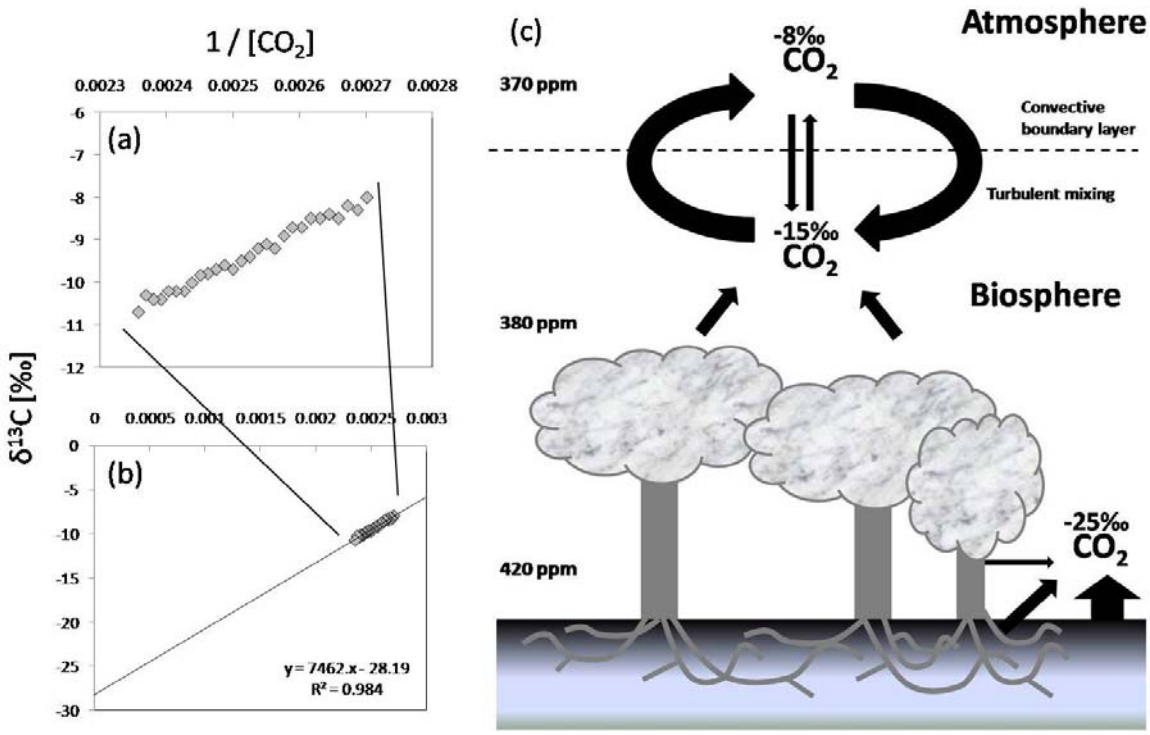

Fig. 2.4 Example for a *keeling plot* regression (**a,b**) and scheme demonstrating nocturnal ecosystem atmosphere carbon exchange processes and the relationship between $\delta^{13}C$ and CO_2-concentration (**c**).

Model I regressions (y on x; OLS) assume that x has no natural variability or measurement error. Therefore, Keeling plot intercepts will experience a bias, if this regression model is applied. Regarding this fact it has become current practice to calculate $\delta^{13}C_R$ with Model II regressions (y on x and x on y; Geometric Mean; Pataki *et al.*, 2003a). For two variables x and y, the slope of a GMR regression is the square root of the product of the OLS slope of y vs. x and the inverse of the OLS slope of x vs. y.

Following this equation Geometric mean (model II) intercepts were calculated from ordinary least square (OLS, model I) regression equations and uncertainties in the *keeling plot* intercepts were expressed as standard errors of the intercept estimated from the OLS 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.*, 2003a). Regressions were rejected when not significant (α=0.01). Statistical and regression analyses were performed using *Statistica* (StatSoft, Tulsa, OK, USA) and *JMP* (SAS Institute Inc., Cary, NC, USA).

2.5 GAS - SAMPLING AND ISOTOPIC ANALYSES

I 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 $\delta^{13}C$ and $\delta^{18}O$ isotope signals for 72 and 24 hours, respectively (see also Knohl, 2003; Werner *et al.*, 2007b).

Air collection for $\delta^{13}C_R$ measurements

A tubing system (Dekarbon tubing, 25 m length, inner diameter 6,9mm, Sertoflex, Serto Jacob, Fuldabrück, Germany) was fixed at 9 different heights: 24, 20, 16, 12, 8, 4, 2, 1, and 0.5 m (heights between 16 and 24 m above the tree canopy) at the *eddy flux* 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 analyzer (IRGA, BINOS 100 4P, Rosemount Analytical, Hanau, Germany; precision for CO_2 ±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. Daytime *keeling plots* could often not 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.

Air collection for $\delta^{13}C_{Res}$ measurements – Chamber *keeling plots*

CO_2-sampling for chamber *keeling plots* of soil respiration was done in a similar way using the same pump, IRGA and flushing equipment connected with Dekarbon tubing to a properly built, well ventilated and gas tight Plexiglas chamber (**Fig. 2.5b**, 17L) in a closed system. Climatic conditions inside the chamber were controlled with two thermocouples fixed at different heights and a quantum sensor, connected to a datalogger (CR10; Campbell Scientific, Inc., Utah). The chamber was tightly fitted on a metal ring (**Fig. 2.5a**), dug into the soil and sealed with therostat. Once the chamber was placed on the plot and CO_2-concentrations inside the air stream were rising, air was sampled every minute over 10 minutes, collecting 10 samples in total for one soil *keeling plot*. Soil *keeling plots* were

performed on intact soil patches (+ roots) and trenched patches (- roots). Trenching was achieved by solarization (sun generated heat treatment) with black plastic foils and trenching by wooden barriers or metal rings permanently buried (~20cm deep) at least three weeks before measurements were started.

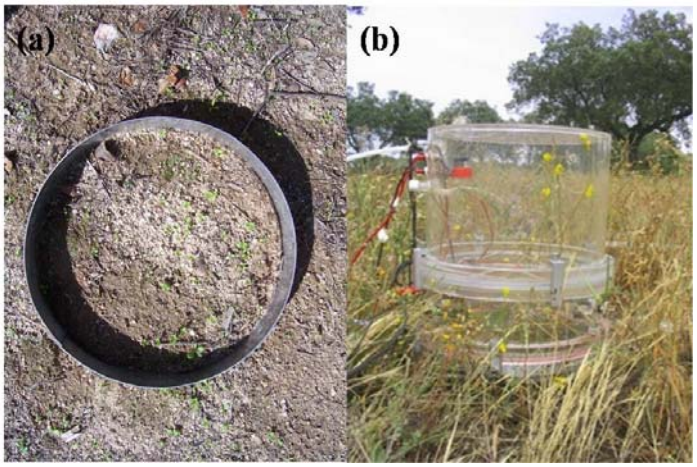

Fig. 2.5 Chamber system used to sample soil *keeling plots*; (**a**) metal ring inserted in the ground, (**b**) chamber placed on metal ring.

The here described Plexiglas chamber was occasionally used to monitor carbon fluxes of soil patches with and without vegetation. To that purpose it was set up as a cuvette in an open-system mode in combination with a Minicuvette system (CMS 400, Walz, Effeltrich, Germany, **Fig. 2.6**). Two pumps were necessary to pump air at a constant rate (800 ml min^{-1}) into and out of the chamber, since overpressure inside the chamber would have caused error by the escaping of air through the soil pores. The Minicuvette system was used as a pump and flow control for the reference and measuring gas streams. At the point of steady state between the CO_2-concentrations of reference and measure site CO_2-concentrations were recorded at the gas exits of both sites of the Minicuvette system with an external IRGA (Binos 100 4 P; Rosemount Analytical, Hanau, Germany) set in absolute mode. Calculation of chamber carbon fluxes was achieved using **equ. 4** with the usual temperature and air pressure corrections.

$$J_{CO_2} = \frac{u_e\,(1-w_e)}{s\,(1-w_o)}\,(c_e-c_o) - \frac{u_e\,(w_o-w_e)}{s\,(1-w_o)}\,c_e \qquad (4)$$

, where J_{CO_2} is the CO_2-flux in µmol m^{-2} s^{-1}, $\mathbf{u_e}$ ist the molar airstream into the chamber, $\mathbf{w_{e,o}}$ and $\mathbf{c_{e,o}}$ are the molar fractions of water and CO_2 in the airstream into and out of the chamber, respectively and **s** is the area of the soil patch.

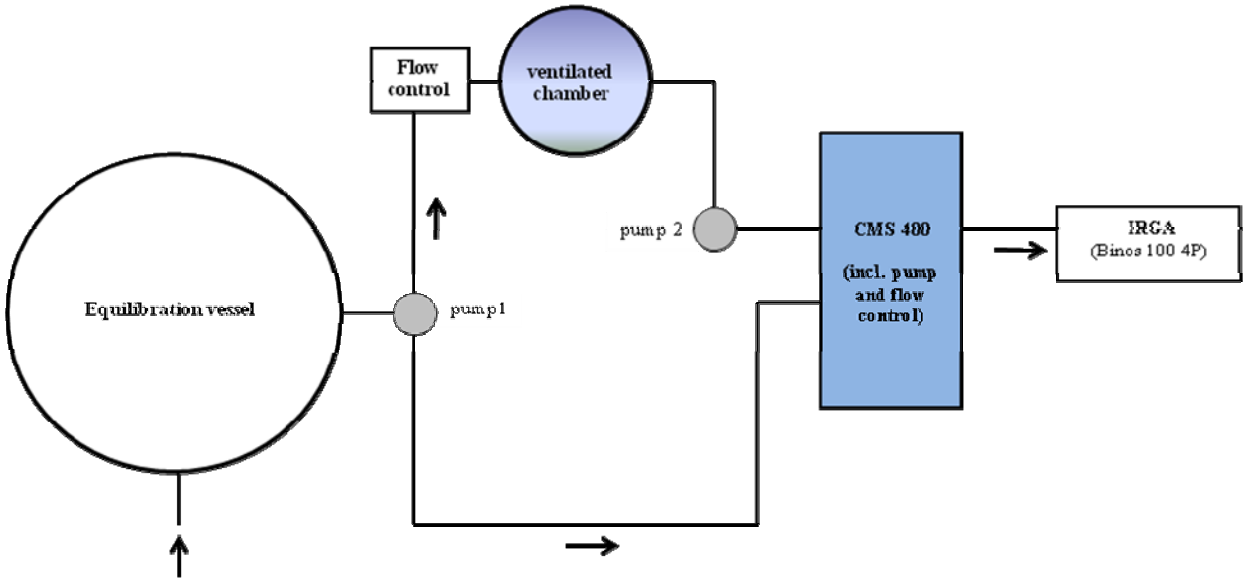

Fig. 2.6 Schematic description of the open chamber system set up to measure carbon exchange of soil patches with the above described ventilated Plexiglas chamber. Arrows indicate direction of air stream.

Air collection for $\delta^{13}C_{Res}$ measurements – *Intube-incubation* method

For collection of leaf and root respired CO_2 10 leaves of *Q. ilex* as well as ten leaves and ten root fragments of *T. guttata* were cut and immediately placed into exetainers which then were flushed with CO_2-free air (*Sodalime*) and left for incubation (incubation time here 10 min, varies depending on respiring material and IRMS-setup) in a temperature isolated plastic box (**Fig. 2.7**, see Werner *et al.*, 2007b).

Then, respired CO_2 was collected with a 25mL gas tight syringe (SGE International, Victoria, Australia) from the exetainer and injected by flushing into a second previously CO_2-free flushed exetainer, with rapid needle removal following injection. This method was chosen to

avoid outside air to enter through the rubber septum while injecting the needle to an evacuated vial. Tests revealed no significant differences in carbon isotopic composition between CO_2 before and after the transfer (SD < 0.15 ‰).

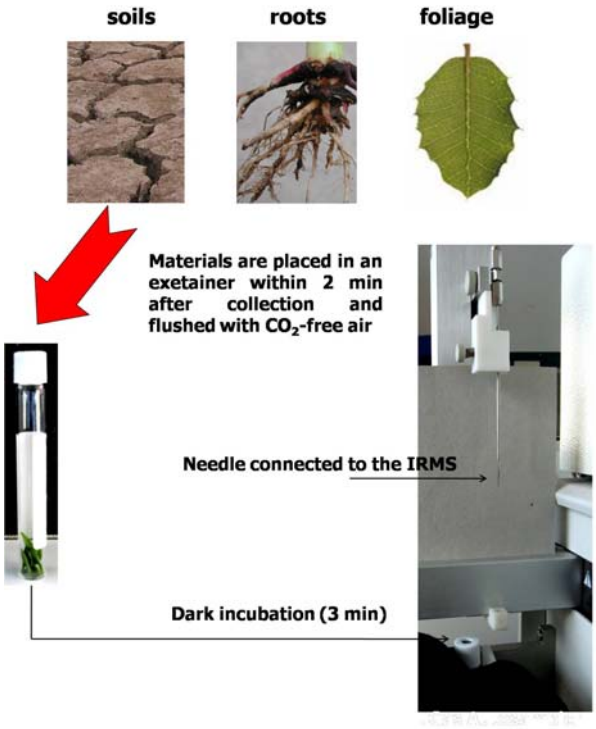

Fig. 2.7 Schematic description of *Intube* analysis method to assess $\delta^{13}C_{res}$.

Reference gases and isotopic analyses

Five exetainer of known $\delta^{13}CO_2$ and 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) and analyzed within 12-20h 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‰.

2.6 ORGANIC MATTER – SAMPLING AND ISOTOPIC ANALYSES

Isotope composition of organic material of different ecosystem components was analyzed regularly in 2003. Five to ten 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 cm and 20 cm) through soil cores, sieved and roots removed. All material was oven dried at 60°C for 48h and milled to fine powder for carbon isotopic analysis. The isotopic composition of organic matter was determined at University of Bielefeld. Sample preparation was performed in an elemental analyzer (Elementar, Hanau, or EuroVector, Hekateck, Germany) where the samples are automatically combusted to water and CO_2 and analyzed in a continuous-flow isotope ratio mass spectrometer (ISOPRIME, GV, 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‰.

2.7 MASS BALANCE APPROACHES FOR FLUX AND ISOFLUX PARTITIONING

For flux partitioning during nighttime mass balance equations were applied, assuming that the sum of component isotopic fluxes equals the ecosystem isotopic flux and that no more component fluxes than soil flux (rhizosphere and heterotrophic soil microorganisms) and canopy flux (foliage of trees and understory) contribute to the ecosystem flux.

ecoflux approach:

$$f_R = f_S + f_C = f_r + f_{SMO} + f_T + f_U \quad \textbf{(5)}$$

eco-isoflux approach:

$$\delta^{13}C_R * f_R = (\delta^{13}C_S * f_S) + (\delta^{13}C_C * f_C) = (\delta^{13}C_r * f_r) + (\delta^{13}C_{SMO} * f_{SMO}) + (\delta^{13}C_T * f_T) + (\delta^{13}C_U * f_U) \quad \textbf{(6)}$$

soil-isoflux approach:

$$\delta^{13}C_S * f_S = (\delta^{13}C_r * f_r) + (\delta^{13}C_{SMO} * f_{SMO}) \quad \textbf{(7)}$$

, where $\delta^{13}C$ denotes isotopic composition and *f* denotes CO_2-flux per m^2 ground. Subscripts indicate respired CO_2 from ecosystem (R), soil (S), canopy (C), roots (r), soil microorganisms (SMO) and from tree (T) and understory leaves (U). Respiration from non micorrhizal fungi and bacteria is included in heterotrophic soil respiration, whereas autotrophic soil respiration flux comprises root respiration and associated rhizospheric components (e.g. micorrhizal respiration). Here, I will refer to heterotrophic soil respiration as soil microorganism respiration flux (f_{SMO}) and to autotrophic soil respiration as root respiration flux (fr).

The different partitioning approaches will be referred to as *ecoflux*, *eco-isoflux* and *soil-isoflux* approach for **equ. 5, 6** and **7**, respectively.

CHAPTER 3

VARIATION IN CARBON ISOTOPE RATIOS OF ECOSYSTEM RESPIRATION ($\delta^{13}C_R$) AT DIFFERENT TEMPORAL SCALES IN A MEDITERRANEAN OAK FOREST

3.1 ABSTRACT

Temporal dynamics in $\delta^{13}C$ of ecosystem respiration ($\delta^{13}C_R$) were evaluated at hourly, daily and annual time scales 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 aboveground 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 dust to dawn.

These data indicate pronounced short-term dynamics in $\delta^{13}C_R$ at hourly to daily time scales 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*.

3.2 INTRODUCTION

Global atmospheric carbon dioxide 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 behavior of the carbon source/sink strengths of terrestrial ecosystems has been measured in many ecosystems based on micrometeorological techniques like *eddy covariance* (e.g. 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 (e.g. Flanagan & Varney, 1995; Lloyd *et al.*, 1996, Buchmann *et al.*, 1996, 1997; Hemming *et al.*, 2005). At the ecosystem scale, these signatures can be used to partition net CO_2-fluxes into photosynthetic and respiratory components (e.g. 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 due to several reasons: 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 c_i/c_a), 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).

This variation in $\delta^{13}C_R$ has been linked to environmental factors controlling isotope discrimination at the leaf-level, indicating a strong linkage between carbon assimilation and ecosystem respiration (e.g. Ekblad & Högberg, 2001; Bowling *et al.*, 2002). The correlation of vapor pressure deficit (VPD) or air humidity with $\delta^{13}C_R$ was time-lagged for several days, i.e. 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 (e.g. 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 and 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 and 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 to identify 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.

I followed three objectives: first, to evaluate the temporal variability of the isotopic composition of ecosystem respiration ($\delta^{13}C_R$) during short- (24h), medium- (2 weeks) and long-term (annual) time scales; 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); 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 nighttime gradients of CO_2-concentration and $\delta^{13}CO_2$ and *keeling plot* analysis.

3.3 MATERIALS AND METHODS

Throughout the years 2003 and 2004 variation in $\delta^{13}C_R$ was monitored on a monthly to seasonal basis at *Mitra* (see **section 2.1**). Additionally, two intensive field campaigns were conducted during May and September 2004, each within a period of 14 days.

Continuous records of micrometeorological measurements of CO_2- and H_2O-fluxes and climate variables (see **section 2.2**) as well as sapflow (see **section 2.3**) were taken.

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, California, 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, Nebraska, USA), plant water potentials (pressure chamber, Manofrigido, Portugal), and soil respiration (PP-System EGM2 Soil Respiration System with SRC-1 chamber, PP-Systems, Amesbury, Massachusetts, USA) were recorded on two to four days at the beginning and towards the end of each field campaign, to monitor changes in the physiological response with ongoing transition. Measurements were conducted every 2-3 hours on marked braches or

plots for leaf and soil measurements, respectively. Leaf gas exchange and water potentials were measured on five *Q. ilex* trees with 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).

Climate conditions in 2003 followed the typical pattern (mean T_{air}: 15.9°C; precipitation: 706 mm), whereas 2004 was drier with a longer dry season (mean T_{air}: 15.8°C; precipitation: 488 mm, dry period from April to October with a heavy single rain event on September 2).

Isotope composition of organic material of different ecosystem components was analyzed regularly in 2003 (see **section 2.6**).

Atmospheric CO_2 for *keeling plot* calculation (see **section 2.4**) was sampled and analyzed as described in **section 2.5**. 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 in 2-hour time intervals day and night during intensive 24h sampling periods. Daytime *keeling plots* could often not be calculated due to high vertical mixing of the air in this open oak stand resulting in insufficient CO_2-concentration gradients and are not shown.

Canopy conductance (G_c) was calculated from sapflow data, as described in **section 2.3**.

Statistical analyses of $\delta^{13}C$ data for organic matter were performed using *STATISTICA* 6.0 (StatSoft, Tulsa, USA). After ensuring normal distribution by Kolmogorov-Smirnov tests ANOVAs were performed, testing for significances with Duncans multiple range test. Regression analyses were performed using correlation matrices of *STATISTICA* 6.0 (StatSoft, Tulsa, USA). R^2-coefficients of Pearson product-moment correlations and significances ($p<0.05$) were computed. Comparison between regression lines was achieved by ANCOVA analyses using *JMP* (SAS Institute Inc., Cary, USA).

3.4 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. 3.1**.

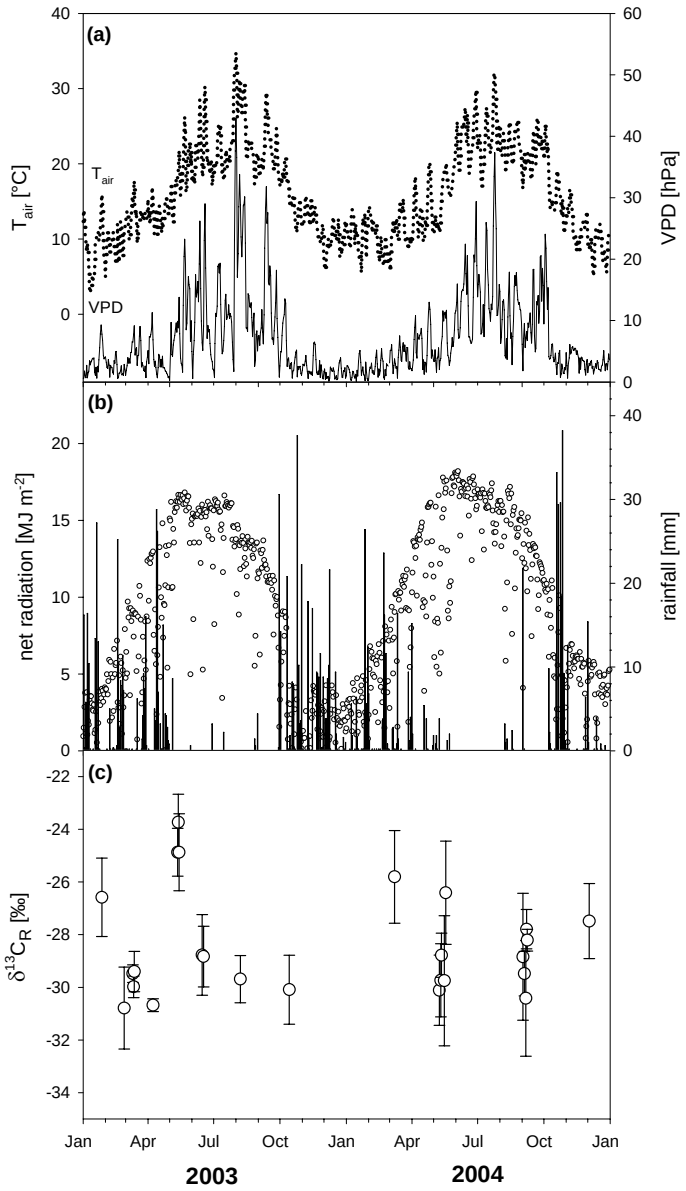

Fig. 3.1 Annual variation in climatic variables and carbon isotope ratios of ecosystem respiration ($\delta^{13}C_R$) in 2003 and 2004. Daily (**a**) means of air temperature (T_{air}, dotted line) and vapor pressure deficit (VPD, closed line); (**b**) total net radiation (circles) and daily rainfall (bars); (**c**) carbon isotope ratios of ecosystem respired CO_2 ($\delta^{13}C_R$). Error bars represent standard errors, from Werner *et al*. (2006).

The annual mean temperature was similar in both years. 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 488mm 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 (Pereira *et al*., 2007). The seasonal variation in GPP followed the usual pattern of the region with a maximum in the spring and minimum during summer drought. During the prolonged rainless season, soil biological activity declines. However, ecosystem respiration reached a maximum at the end of summer following the first rains (data not shown).

Tab. 3.1 Temporal variation in $\delta^{13}C$ of bulk organic material of different ecosystem components in 2003. $\delta^{13}C$ of previous year and current year grown tree leaves (*Quercus ilex*), drought deciduous grass leaves and roots, litter, and soil (surface, from 5-10 and 10-30 cm depth), for each month and annual mean in 2003; n= 4-5 ± SD; n.d. = not determined., from Werner *et al*. (2006)

Month	*Q. ilex* leaves (‰)		grass (‰)		litter (‰)	soil (‰)		
	previous /	current year	leaves	roots		surface	5-10cm	10-30cm
Mar 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
Apr 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	n.d.
July 03	-29.37±0.45	-28.43±0.88	-	-	-28.53	-27.69±0.15	-26.79±0.33	n.d.
Aug 03	n.d.	-27.96±0.46	-	-	-28.46±0.26	-27.15±0.25	-26.61±0.43	-27.35±0.43
Oct 03	n.d.	-28.57±0.88	-28.27±0.47	-27.13±0,73	-28.57±0.37	-27.84±0.09	-27.43±0.13	n.d.
Nov 03	n.d	-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

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. 3.1c**). 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 (**Tab. 3.1**). Leaves presented generally 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 to 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 to 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, I conducted intensive two-week 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 due to windy conditions or low ecosystem activity, the remaining data indicate a clear trend in both periods (**Fig. 3.2**). A relatively cold and cloudy period at the beginning of May was followed by a large increase in maximum air temperature from 16°C to 27°C and consequent increase in VPD (**Fig. 3.2a, c**). This resulted in a pronounced enrichment in $\delta^{13}C_R$ from -30.1 to -26.4‰ (**Fig. 3.2e**).

During the summer drought, *keeling plots* could not be determined due to a lack of ecosystem activity, and hence, insufficient build-up of a CO_2-gradient at night. The first rainfall on September 02, 2004 (**Fig. 3.2d**) induced a large CO_2-pulse with a peak in soil respiration of 8.6 $\mu mol\ m^{-2}\ s^{-1}$, declining to 3.6 $\mu mol\ m^{-2}\ s^{-1}$ on September 06, 2004. Subsequently a pronounced decline in $\delta^{13}C_R$ from -26.4 to -30.9‰ (**Fig. 3.2f**) occurred, which was of similar magnitude to 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 days) with different averaging periods (zero to three days) of environmental and physiological variables and $\delta^{13}C_R$ were tested. Parameters for the best-fit regression are shown in **Tab. 3.2**.

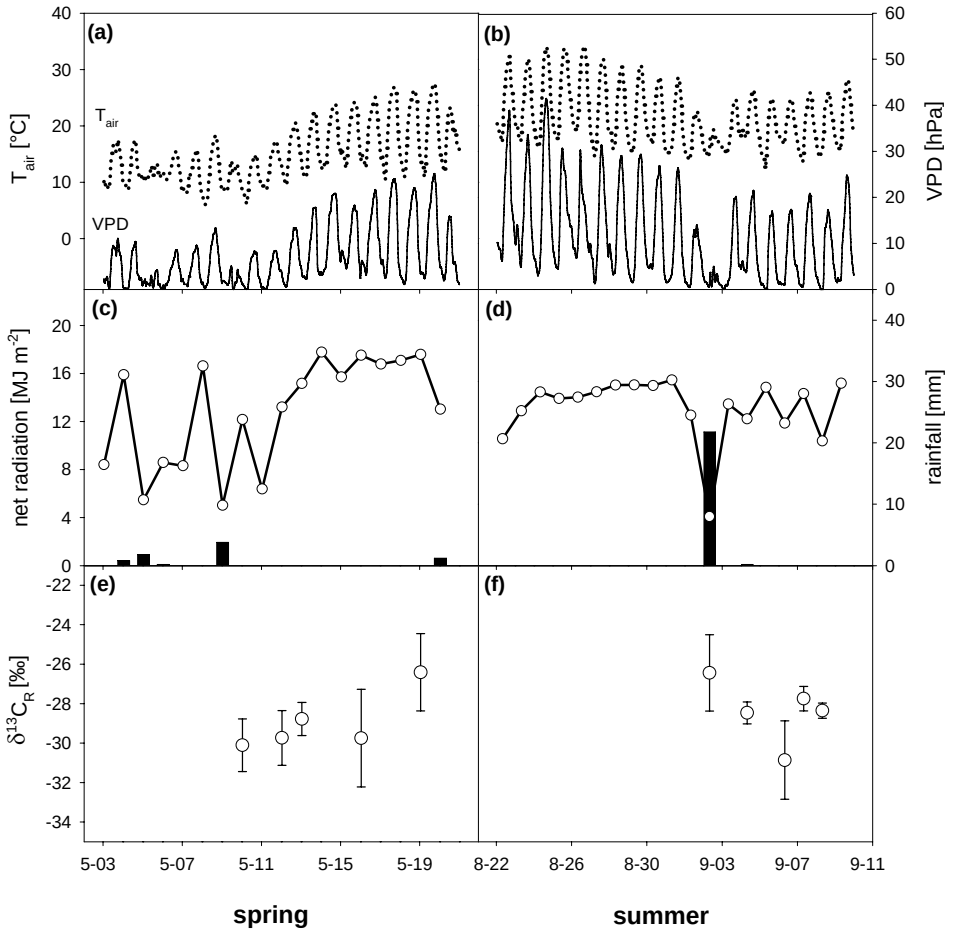

Fig. 3.2 Daily variation of climatic variables and $\delta^{13}C_R$ during the transition periods in May and September 2004. Hourly means of **a,b)** air temperature (T_{air}, dotted line) and vapor pressure deficit (VPD, closed line), **c,d)** daily totals of net radiation (closed line) and rainfall (bars) and **e,f)** daily measurements of $\delta^{13}C_R$ (during fortnight periods). Error bars represent the standard error, from Werner *et al*. (2006).

Highly significant regressions were found with variables affecting photosynthetic discrimination (VPD, Rn and T_{air}), soil activity (belowground variables: T_{soil}, soil moisture and water potential), as well as sapflow and calculated canopy conductance (Gc). Only correlations with precipitation did not reveal significant relationships (data not shown). However, these regression analyses are only meaningful, if all variables which affect $\delta^{13}C_R$ in a similar fashion (for example, VPD, Rn and T_{air} through the effect on photosynthetic discrimination) reveal the same time-lag and averaging period. Therefore, I grouped the variables and tested for the best regression with a common time-lag and averaging period (**Tab. 3.2**, bottom). Since the time-lags varied only slightly within each variable group, highly

Tab. 3.2 Summary of the correlation analysis for $\delta^{13}C_R$ vs. meteorological, edaphic and physiological variables for May and September 2005. Regression providing 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) are given. The regression parameters for a common time-lag and averaging period for each variable group (best common regressions) for above ground variables (VPD, net radiation and air temperature), tree variables (sapflow, canopy conductance (Gc)) and belowground variables (soil moisture, water potential and temperature) are shown in the lower part of the table. * for belowground variables only soil moisture yielded insignificant regressions, from Werner *et al*. (2006)

best fit regressions	lag (days)	average (days)	regression +/-	regression type	r^2	p-level
May 04						
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
sapflow	4	1	+	linear	0.99	< 0.05
Gc (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 04						
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
sapflow	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	n.s.
soil temperature	1	3	+	linear	0.57	< 0.05
soil water potential	0	3	-	log	0.67	< 0.001
best common regressions						
May 04						
above ground variables	5	2		linear	0.95-0.79	< 0.001
trees (sapflow, G_c)	4	1		linear	0.99-0.60	< 0.05
belowground variables	3	1		linear	0.94-0.50	< 0.05, n.s.*
September 04						
above ground variables	3	2		linear	0.94-0.86	< 0.001
trees (sapflow, G_c)	3	2		linear	0.44-0.30	< 0.01
belowground variables	1	1		linear	0.52-0.34	< 0.05, n.s.*

significant regressions were still obtained, which explained 79 to 95% of the variation with above ground variables. Only the belowground variables yielded lower correlation coefficients due to non-significant regressions with soil moisture, which may have been caused by uncertainties in the measurements probably due to poor contact between soil and sensors, especially during summer.

Different patterns can be observed (**Tab. 3.2**): variables affecting photosynthetic discrimination revealed longer time-lags (2 days longer) than soil variables, indicating that changes in soil conditions have a faster and probably more direct effect on $\delta^{13}C_R$. Tree variables (sapflow, canopy conductance) revealed 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.

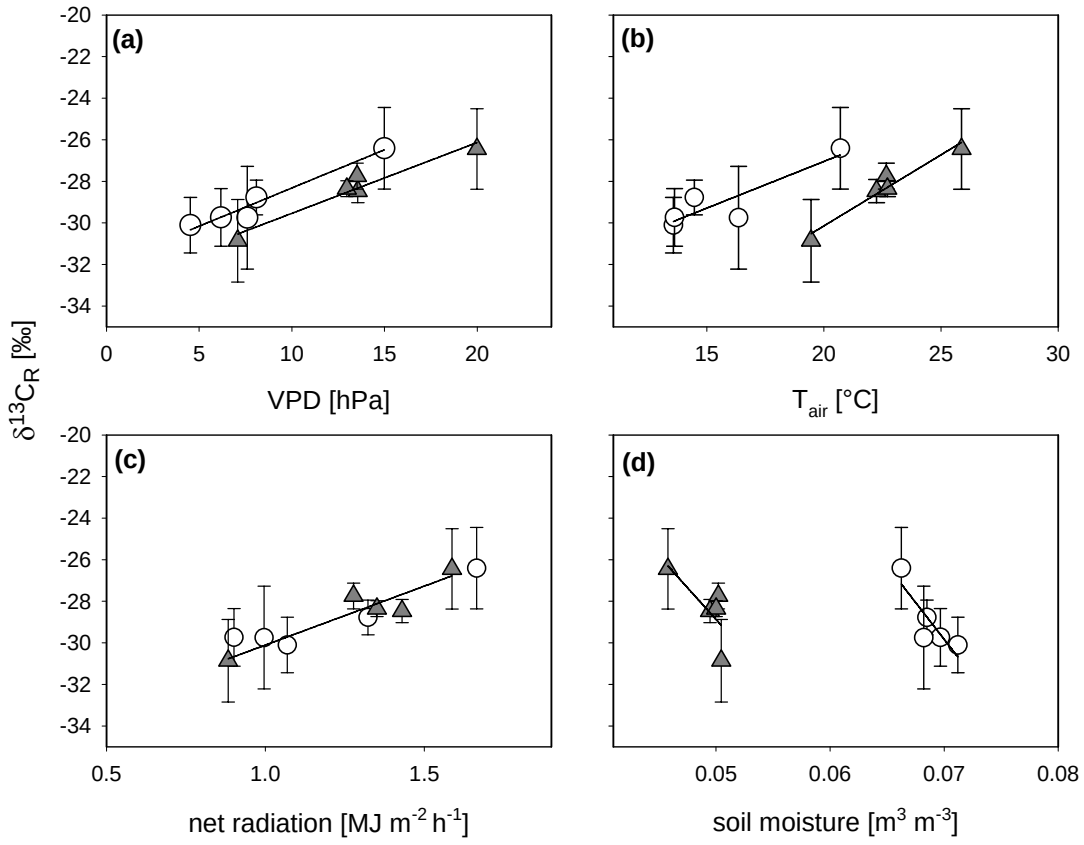

Fig. 3.3 Relationships between carbon isotope ratios of ecosystem respired CO_2 ($\delta^{13}C_R$) and time-lagged daytime averages of climatic variables: **a)** vapor pressure deficit (VPD), **b)** air temperature (T_{air}), **c)** net radiation and **d)** soil moisture during May (open circles) and September (shaded triangles) in 2004. Time-lags are according to best common regressions (see **Tab. 3.2** bottom). Error bars represent standard errors, from Werner *et al*. (2006)

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. 3.3b**). This means that an increase of ca. 7°C in mean daytime air temperature in May and a decrease of 6.5°C T_{air} in September was accompanied by a similar enrichment and depletion of $\delta^{13}C_R$ in May and September, respectively. 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. 3.3c**).

These responses have to be seen in context of seasonal changes in the activity of different ecosystem components, and, hence, the relative contribution of e.g. plant vs. soil respiration, as well as physiological acclimation during different seasons. Changes in ecophysiological parameters at the beginning and end of each transition period in May and September vs. environmental parameters are shown in **Fig. 3.4**. Sap flow rates were reduced to one third during drought as compared to May (grey and open symbols, respectively, **Fig. 3.4a**). After the rain pulse in September sap flow rates recovered to approx. half the rates reached in May under similar VPD (black symbols, **Fig. 3.4a**). The difference in leaf level response was even more pronounced: stomatal conductance (g_s) of sunlit leaves was high in May but strongly declining with increasing VPD, while gs was markedly reduced in September (**Fig. 3.4c**). Net photosynthetic rates were also much higher in May compared to September, while soil respiration rates exhibited a different response (**Fig. 3.4e, f**): soil respiration rates ranged about 1.9 to 2.4 $\mu mol\ m^{-2}\ s^{-1}$ in May but were strongly reduced during drought. The same trend was found in respect to NPP and ecosystem respiration fluxes (data not shown). However, the rain pulse induced an immediate peak in soil respiration (highest rates in **Fig. 3.4e, f**) and subsequent higher respiration rates compared to May (back symbols **Fig. 3.4e, f**). Only after rewetting the soil, the temperature dependence in soil respiration was restored (**Fig. 3.4f**). This is a further indication, that photosynthetic discrimination might have dominated the ecosystems respiration during spring, whereas changes soil respiration might have triggered the response in $\delta^{13}C_R$ in September.

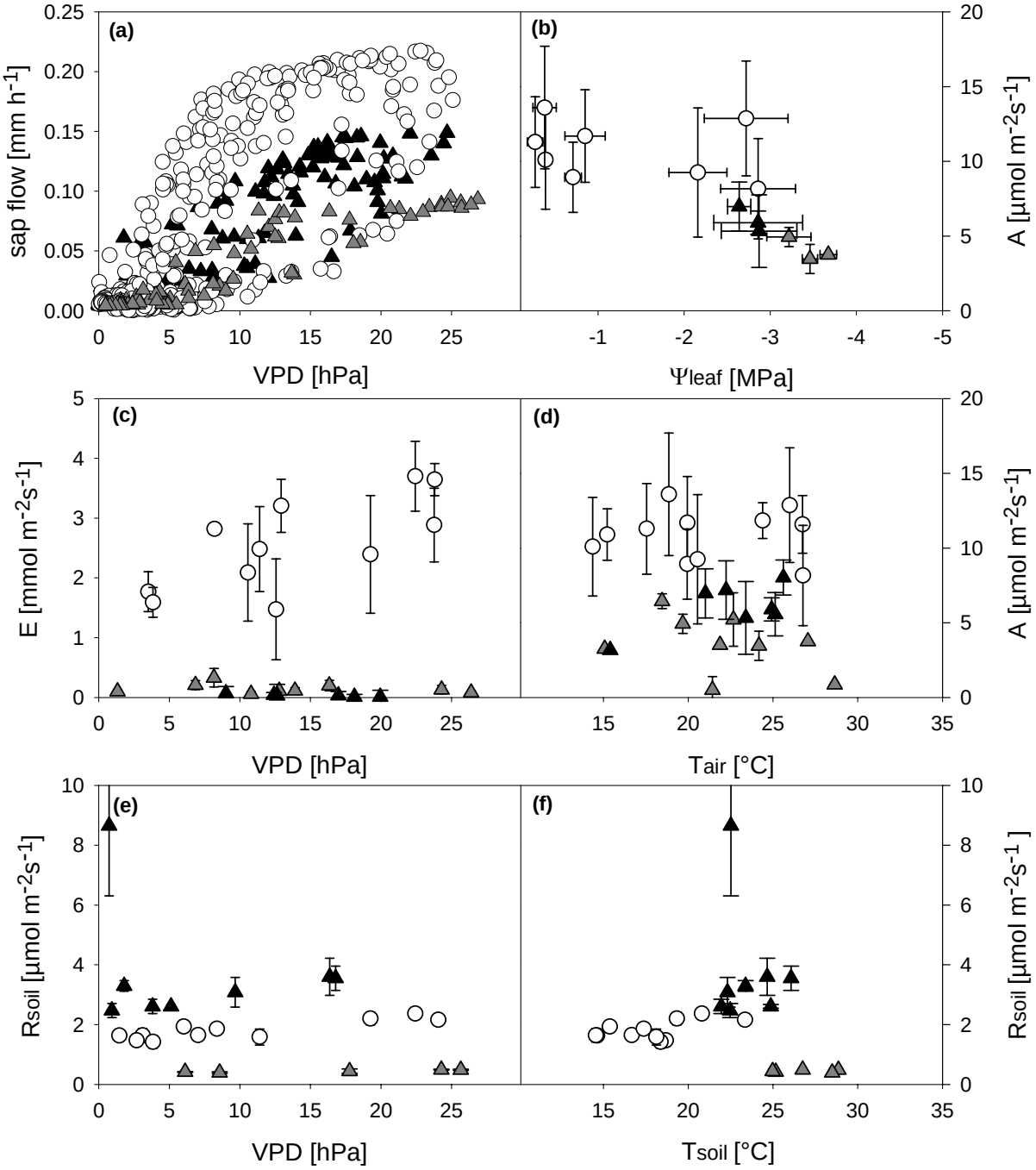

Fig. 3.4 Changes in ecophysiological parameter vs. environmental conditions during the intense sampling periods in May (○) and September before (▲) and after the rain event (▲). Relationship between VPD and **(a)** sap flow, (**c**) stomatal conductance (E) of sunlit leaves and (**e**) soil respiration rates (R_{soil}); leaf net assimilation rates (A) versus (**b**) leaf water potential and (**d**) air temperature (T_{air}), as well as (**f**) soil respiration versus soil temperature (T_{soil}). Outliers in **(e)** and **(f)** are soil respiration rates immediately after the rain pulse. **V**alues plotted from diurnal courses during daylight hours at the beginning and end of each period, for details see material and methods; n = 3-15 ± SD, from Werner *et al*. (2006)

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

During both periods in May and September 2004, 24h-high frequency sampling was conducted to evaluate short-term dynamics in $\delta^{13}C_R$ (**Fig. 3.5**).

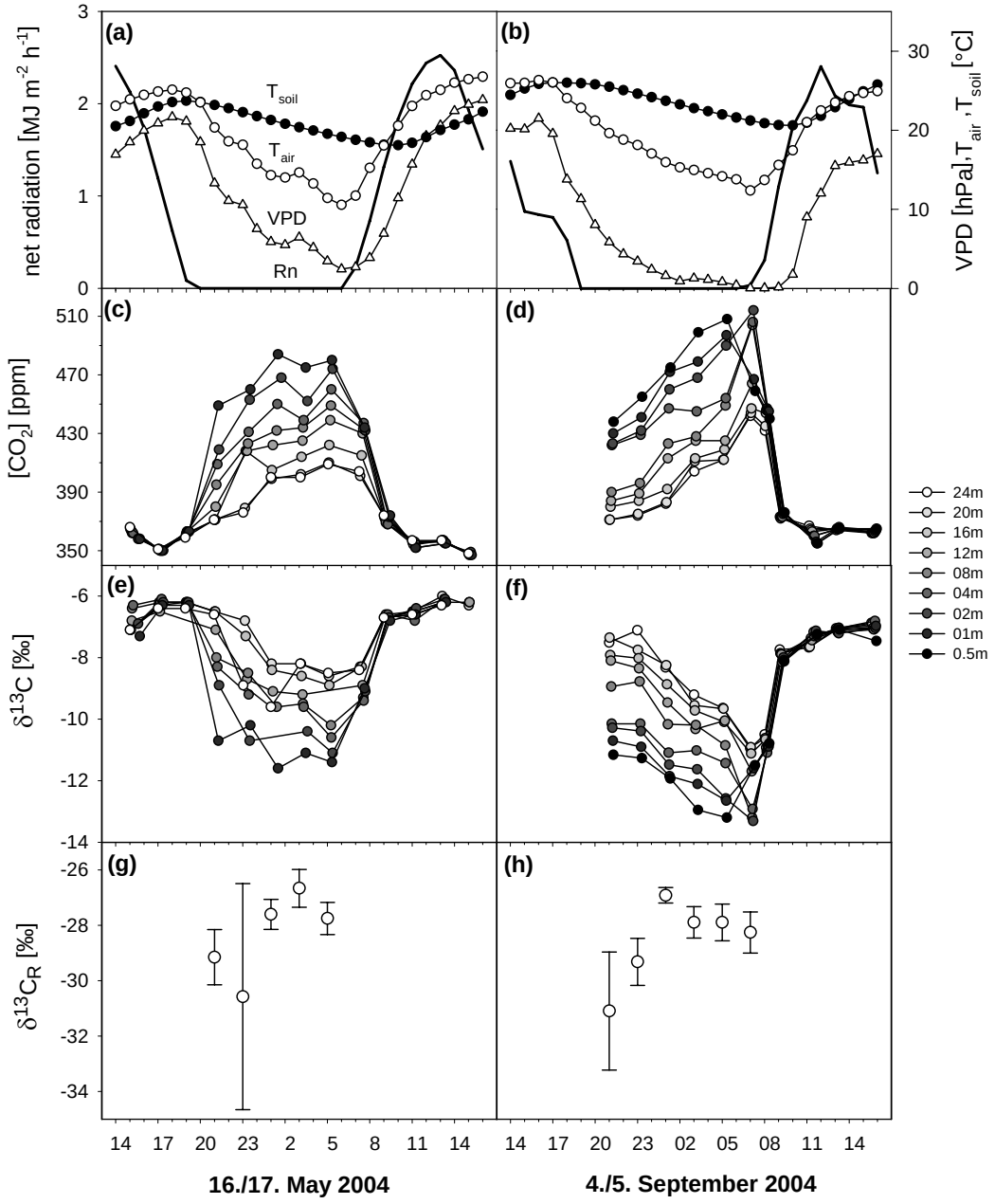

Fig. 3.5 Diurnal variation of climatic variables and $\delta^{13}C_R$ during 24-h cycles in May and September 2004. Hourly means of **a**,**b)** air temperature (T_{air}, open circles), soil temperature (T_{soil}, closed circles), vapor pressure deficit (VPD, open triangles) and net radiation (Rn, closed line) and 2-hourly measurements of CO_2-concentrations **c,d)** and carbon isotope ratios **e**,**f**) of atmospheric air at different heights (24m to 0.5m represented by different shaded circles from open to black, respectively) and **g,h**) nocturnal variation of $\delta^{13}C_R$. Error bars represent standard errors, from Werner *et al*. (2006)

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. 3.5c,d**). Climatic conditions were very similar, with maximum T_{air} reaching ca. 26.5°C on both days. VPD was slightly higher in May compared to September (23.8 and 21.5 hPa, respectively), whereas soil temperature remained on average 2.8°C higher in September (**Fig. 3.5a, b**). During both nights a large nocturnal shift in $\delta^{13}C_R$ was observed (> 4‰, **Fig. 3.5g, h**). On both occasions enrichment at the beginning of the night was followed by stable range 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. **3.2e, f**) and reached more than half of the total annual variation observed at this site (> 7‰, **Fig. 3.1c**).

3.5 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, I will discuss the dynamics in $\delta^{13}C_R$ found at different time scales, 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 two year measuring period, which is similar or slightly lower than 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 (e.g. Fessenden & Ehleringer 2003; Knohl *et al.*, 2005). In spite of the pronounced seasonality of the Mediterranean climate, no annual pattern with enriched $\delta^{13}C_R$-signature during drought (see e.g. Pataki *et al.*, 2003a; 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. Due 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 off-sets in the correlation between environmental variables and $\delta^{13}C_R$ (**Fig. 3.3**) in summer compared to spring (as will be discussed below in detail). This 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 ecosystem respiration.

Similar, $\delta^{13}C$ of organic material of different ecosystem components was a poor proxy for the isotopic content of respiratory fluxes (as was shown by 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 ($p<0.05$), similarly to findings by Badeck *et al.* (2005). They concluded that significant post-photosynthetic 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 (e.g. 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 into 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 release from drought, respectively.

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 sapflow 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 between carbon fixation, assimilate transport to roots, and subsequent release through root respiration and exudates (e.g. Ekblad & Högberg, 2001; Bowling *et al.*, 2002). However, this is only a reasonable assumption if all the 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 for belowground variables and tree water relations in September the correlation coefficients were weak (r^2= 0.33-0.52). These data confirm that ecosystem respiration is closely linked to recent assimilates, as the relationship of $\delta^{13}C_R$ with VPD, T_{air}, Rn, and canopy conductance explained 86-95% of the variation. Time-lags of 4-5 days with VPD found in May are in accordance with what has been found by Bowling *et al.* (2002), Ekblad *et al.* (2005) and Knohl *et al.* (2005).

However, I also found a strong relationship of $\delta^{13}C_R$ with belowground 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 belowground 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. I have considered soil water potential and moisture as factors affecting belowground 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 sapflow 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 understory has vanished.

Time-lags were shorter (1-2 days) for below- than aboveground 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 more slow-turnover carbon pools which 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 summer

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 and 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 to ecosystem was also found by McDowell *et al.* (2004b). The lag periods reported by these authors were 1.1 and 4.9 days 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 belowground 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 (see also 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 the summer 2004 resulted in a pulse-like increase in soil respiration (**Fig. 3.4**), and time-lagged responses were 1-2 days shorter in September than in May. Belowground parameters responded immediately (one-day 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 to May (**Fig. 3.4f**) and ecosystem respiration reached highest annual rates after the summer rains (Pereira *et al.*, 2007). 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

re-wetting there is a sudden 'burst' mineralization and CO_2-release – the *Birch* effect (Jarvis *et al*., 2007; Rey *et al*.; 2005), which is visible in **Fig. 3.4f**. The amount of carbon returned to the atmosphere in this way can reduce significantly the annual net carbon gain by the forests (Jarvis *et al*., 2006; Pereira *et al*., 2004). End of summer 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). At that time there are no herbaceous plants to utilise the nutrients released and thus, leading to the loss of carbon and nitrogen from the soil pools (Jarvis *et al*., 2006). In such cases summer 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 soil water content, which was reflected by the slow stomatal response after the rainfall (e.g. **Fig. 3.4b, c**). It is further unlikely that carbon isotope discrimination changed during the days before the rain event, and sapflow progressively increased during the following three days (data not shown). Given the 3-day time-lag, photosynthetic activity would only be expected to influence $\delta^{13}C_R$ four days after the rain pulse (interestingly, these data points are offset compared to the preceding days, **Fig. 3.2**). 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 I was 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 yield 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. 3.3b**), in spite of different absolute temperatures. This may be explained by large seasonal temperature acclimation of maximum photosynthetic capacity during Mediterranean summer reported for these species (e.g. Tenhunen *et al.*, 1990; Larcher *et al.*, 2000). A similar offset (hence, non significant slopes, but significantly different intercepts) were found for T_{soil}, VPD, soil water potential, and sapflow. Solely 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 in $\delta^{13}C_R$. To my knowledge this is the first

report, which 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 24-h cycle revealed large nocturnal shifts in $\delta^{13}C_R$ (> 4‰), both in May and September (**Fig. 3.5**). Until recently, $\delta^{13}C_R$ was considered to remain constant during nighttime (Pataki *et al.*, 2003a). To my 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 over 6 ‰ shift within a single night and they pointed out that this variation of magnitude was nearly as large as the seasonal range reported over a variety of environmental conditions from several years sampling. Large nocturnal variations in $\delta^{13}C_R$ have been confirmed in three other 24-h time-series measured during the following year ($\delta^{13}C_R$ ranging from 1.8 to 4.2‰, data not shown). Furthermore, nocturnal changes occurred also in soil respired CO_2 (measured with 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 a) through changes in respiratory substrate; b) if diurnal changes in photosynthetic discrimination will translate into nocturnal variation of $\delta^{13}C$ of respired CO_2; c) 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 response to nocturnal changes in climatic variables); d) through fractionation in the respiratory pathways, as will be discussed below:

a) 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 my knowledge there is no information on these processes.

b) 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, e.g. during

midday stomatal closure (e.g. Tenhunen *et al.*, 1984). Furthermore, estimated isotopic composition of the photosynthetic flux has shown high variation over the daylight period (e.g. 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 (e.g. 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. I 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 and T_{air}, and sapflow during the daylight period (for example highest correlation was found 3 d + 12-14h 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 might drive nocturnal changes in $\delta^{13}C_R$.

c) It is well established that respiration rates are a function of temperature, which 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 (e.g. **Fig. 3.5**). 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 (e.g. 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 be simply 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.

d) 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 the here studied oak species (Werner *et al.*, 2007b). 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; compare **section 1.3**). 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) due to the non-statistical 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 (see e.g. Ghashghaie *et al.*, 2003) and its impact on both temporal (e.g. **Fig. 3.5**) and spatial (see e.g. Steinmann *et al.*, 2004) variation in ecosystem respiration.

3.6 CONCLUSIONS

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 found during these periods of rapid environmental changes. Different correlations of $\delta^{13}C_R$ and microclimatic factors in May and September indicate different responsiveness of the ecosystem (e.g. through temperature acclimation) during different seasons. Shorter time-lags (1-2 days) for below- than aboveground 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 time-scales of hours. Given our current knowledge on the strong linkage of $\delta^{13}C_R$ with environmental drivers and their well known circadian cycles, I 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, similarly to what has been found for $\delta^{13}C_R$ on coarser time-scales (e.g. 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 in CO_2-concentrations required for a reliable *keeling plot* is difficult to capture in a short time period due to 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 hours, see Pataki *et al.*, 2003a). 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*.

3.7 AUTHORS CONTRIBUTIONS

The author of this thesis accomplished the entire experimental work and important writing contribution to this chapter. Further contributions were given by C. Werner, who took part in the writing and project coordination, C. Máguas who provided the stable isotope lab, J.S. Pereira who provided *eddy-flux* data, T.S. David and J.S. David who provided sapflow and climate data as well as C. Kurz-Besson and D. Otieno, who provided soil climatic data. Further, I acknowledge contributions of R. Maia and B. Teichner for technical support with isotope analyses, P. Oliveira (*Mezão*) for technical help with equipment and J. Banza for *eddy-flux* data treatment.

CHAPTER 4

THE INFLUENCE OF RAIN PULSE EVENTS ON SOIL AND ECOSYSTEM RESPIRED CO_2 – ASSESSING THE *BIRCH* EFFECT USING STABLE CARBON ISOTOPES

4.1 Abstract

Within recent years the *Birch* effect, a phenomenon of rapidly increasing mineralization and soil respiration after rewetting of previously dried soil, has become a new focus of scientific attention. *Eddy covariance* results from a range of arid ecosystems showed the *Birch* effect to be responsible for the sudden pulse-like release of CO_2 in response to rainfalls during and at the end of summer drought, largely affecting ecosystem carbon balance. Understanding the processes involved in the *Birch* effect is still a developing area of research. In this field study straight-forward stable isotope methodology was used to gather new explanations regarding the occurrences after rewetting of dried out soil, focusing on the evaluation of current hypotheses for the *Birch* CO_2-pulse. Two irrigation experiments in a Portuguese *montado* ecosystem during May and August 2005 were conducted, constantly monitoring soil carbon fluxes along with their isotopic composition. Second, first natural rain events after a long summer drought in October 2005 were observed, measuring changes in magnitude and isotopic composition of both ecosystem and soil respiration over a period of two weeks.

The *Birch* pulse largely determined ecosystem respiration during periods of drying-rewetting. As revealed by $\delta^{13}C$ analyses of respired CO_2 the *Birch* pulse is caused by a hypo-osmotic stress response of soil microorganisms, rather than an increase in labile SOM-C. The magnitude of this hypo-osmotic stress probably plays a key role for the microbial answer to the rewetting of soils and thus, for ecosystem carbon sequestration. Very large sudden rainfalls interrupting long drought periods might enhance carbon sequestration, since they induce large stresses to the soil microbial community, while smaller, extended or frequent rainfalls on the other hand, might cause the contrary effect. Studying the impact of soil rewetting under natural conditions will be a major aim for future research, since knowledge on future impacts of increasing drought and rain fall variability in Mediterranean ecosystems is of high importance.

4.2 Introduction

Annual carbon balance of terrestrial ecosystems is controlled by two major processes, respiratory carbon loss and photosynthetic carbon gain. Seasonal changes in climatic conditions can have large influence on both processes, reflecting on the source/sink behavior of the system. Drought is the main limiting growth factor in arid ecosystems, subjected to an irregular seasonal distribution of rain falls, as in the Mediterranean. Here, onset and length of summer drought, largely influence annual net carbon gain and determine, whether the system will be a source or a sink for carbon and thus, decrease or enhance atmospheric CO_2-concentrations.

Within recent years *eddy covariance* measurements in these drought adapted evergreen forests revealed that a phenomenon, first characterized by Birch (1964) and henceforth called the *Birch* effect, can have a significant influence on the sink capacity of the system (Pereira *et al.*, 2004; Jarvis *et al*., 2007). Birch conducted innovative experiments showing that cycles of drying and wetting of soils stimulate mineralization of soil organic matter, leading to the release of mineral nitrogen and carbon dioxide. The *Birch* effect, comprising the nutrient pulse and by implication the carbon pulse in response to rewetting, was observed in several studies at the ecosystem (e.g. Austin *et al*., 2004; Xu *et al*., 2004; Tang *et al*., 2005; Jarvis *et al.*, 2007) and soil scale (e.g. Birch, 1964; Bottner, 1985; Denef *et al.*, 2001; Fierer & Schimel, 2002, 2003). Ecosystem-scale results for the studied *montado* field site showed the *Birch* effect to be responsible for the sudden pulse-like release of CO_2 in response to rainfalls during and at the end of summer drought (Jarvis *et al.*, 2007). These sudden CO_2-pulses amounted to up to 5 g carbon m^{-2} s^{-1} in response to single rainfalls, and decline to values around and lasted several days (Jarvis *et al.*, 2007). Irregular rainfalls during summer drought are of no use for the vegetation (Nahal, 1981) and thus, not increasing carbon gain of the ecosystems. The herbaceous understory vanishes in the beginning of June and deep rooted trees do not have immediate access to the precipitation water and nutrients released. Therefore, the loss of carbon and nitrogen from the soil pools caused by the *Birch* effect can even have a negative effect on plant productivity (Pereira *et al.*, 2004). The cycles of drying and rewetting of soils, combine inhibition of carbon uptake and at the same time stimulation of carbon loss by the *Birch* effect, which has a large impact on annual net carbon gain. Length of summer drought and frequency of irregular summer rainfalls might become more influential, since climate models for Mediterranean ecosystems predict an increase in both, temperature and irregularity of precipitation (Miranda *et al.*, 2002).

Until today four main hypotheses to explain the *Birch* pulse evolved. Birch himself proposed a spontaneous increase in fungal and microbial biomass in response to water availability (Griffiths and Birch, 1961; Jager & Bruins, 1974; Orchard & Cook, 1983; Scheu & Parkinson, 1994). Some authors indicated that drying and rewetting of soils shatters soil aggregates and exposes previously unavailable organic substrates for decomposition (e.g. Denef *et al.,* 2001). Others favor the theory that drying causes an instant increase in dead microbial biomass, which can be rapidly decomposed by new microorganisms and fungi after rewetting (e.g. Bottner, 1985). Finally Kieft *et al.* (1987), Fierer and Schimel (2002, 2003) and Jarvis *et al.* (2007) found evidence that the nutrient and carbon pulses are the response of a microbial hypo-osmotic stress reaction to the sudden changes in soil water status. However, understanding the processes involved in the *Birch* effect is still a developing area of research (Jarvis *et al.*, 2007).

Stable isotopes might help to address these hypotheses from a different view point. Through the imprint of several photosynthetic and post-photosynthetic fractionation processes on assimilated atmospheric carbon, the isotopic signature of respired CO_2 can potentially yield information about origin, source and age of carbon released from soil pools. Thus, monitoring isotopic composition of carbon pulses released after natural and artificially induced precipitation pulses promises to reveal new insights in the origin of the *Birch* effect.

To achieve this aim I first, conducted two irrigation experiments in a Portuguese *montado* ecosystem during May and August 2005, constantly monitoring soil carbon fluxes along with their isotopic composition. Second, I followed the first natural rain events after a long summer drought in October 2005, measuring changes in magnitude and isotopic composition of both ecosystem and soil respiration over a period of two weeks.

In the following, current theories on processes driving the *Birch* effect will be combined with new evidence gained through straight-forward stable isotope techniques to synthesize the most reasonable explanation for this phenomenon. Further, the impact of this effect on short-term carbon dynamics at the soil and the ecosystem scale in Mediterranean ecosystems will be conferred.

4.3 Materials & Methods

Artificial rain pulses

During late May and the middle of August 2005 two irrigation experiments were conducted in *Mitra* (see **section 2.1**). Continuous records of climate variables (see **section 2.2**) were taken.

On May 23, 10a.m. a defined area of grassland (1.5x6.5m), with nine soil patches (each 0.5x0.5m; three patches with intact understory and soil (*plant*), three patches with bare soil (*soil*) and three patches with trenched, solarized soil (*trenching*); see **section 2.5**) was evenly watered with altogether 20 L m^{-2} from a tank with hose and disperser. In August all understory had vanished. The irrigation treatment was performed equally to May (20 L m^{-2}) on August 28, 8a.m. but measurements were only conducted on intact soil. Besides permanent measurements of climate and net ecosystem exchange (see **section 2.2**) diurnal cycles of soil carbon fluxes and their isotopic composition were monitored on the days before, during and after the irrigation treatment at 11, 15, 21, 23, 1 o'clock and 9, 12, 16, 21, 23, 1, 3, 5 o'clock for May and August, respectively. Soil carbon fluxes of *soil* and *trenching* plots were monitored with a closed system soil respirometer (PP-System EGM2 soil respiration system with SRC-1 chamber; PP-Systems, Amesbury, MA, USA) at three + three plots with three replicates per plot. Soil carbon fluxes of three chosen *plant* plots were monitored using ventilated described Plexiglas chamber (see **section 2.5**), connected in an open system with a Minicuvette system (CMS 400, Walz, Effeltrich, Germany), two pumps and an extra Binos 100 for absolute [CO_2] measurements at the exit of reference and measuring gas (for a thorough description see **section 2.5**). Soil temperature (T_{soil}) and soil water content (SWC) were recorded in 5 to 10 cm depth alongside with soil respiration using the temperature sensor of the soil respiration system and a moisture probe (Theta Meter HH1, Delta-T Devices, Cambridge, UK), respectively.

In May gas sampling for chamber *keeling plots*, as described in **section 2.5** was conducted on three chosen irrigated patches (*plant*, *soil* and *trenching*, respectively) with one replicate per patch, while in August gas sampling was conducted only on *soil* plots, both irrigated and dry control patches.

Ecosystem-scale *keeling plots* at the Eddyflux-tower were still sampled in May every day at midnight (see **section 2.4** and **2.5**), but almost all of them had to be rejected for non-significant regressions due to low [CO_2] gradients.

Natural rain pulses

On October 9, 2005 after a long summer drought a succession of days with natural, partially strong precipitation pulses, began. Apart from continuous measurements of NEE and micro climatic parameters (see **section 2.2**), soil respiration was monitored almost every day during a period of 15 days in diurnal cycles (every 2 to 4 hours), at least in the morning, at midday and at midnight (PP-System EGM2 soil respiration system with SRC-1 chamber; PP-Systems,

Amesbury, MA, USA) on five plots with three replicates. Soil temperature (T_{soil}) and soil water content (SWC) were recorded along with soil respiration as in **4.3.1**. At the same times chamber *keeling plots* were conducted as described in **section 2.2**. During nighttime (approximately at the same time as soil *keeling plots*) *keeling plot* sampling at the tower was done accordingly to **section 2.4** and **2.5**. Additionally, leaf gas exchange (LI-6400 open-flow gas exchange system, LI-COR, Lincoln, NE, USA) and water potentials (Scholander-type pressure bomb, Manofrigido, Portugal) were recorded during daytime every 2-4 hours on marked branches of three *Q. ilex* trees (five leaves per tree). Standard deviations were calculated through error propagation procedures.

Statistics

Analyses of correlation matrices between key climatic variables and ecosystem and soil scale carbon fluxes as well as their respective isotopic compositions were performed using *STATISTICA* 6.0 software (StatSoft, Tulsa, USA). R^2-coefficients of Pearson product-moment correlations and significances ($p<0.05$) were computed.

4.4 Results

4.4.1 Artificial rain pulses

Climate

Irrigation experiments were conducted in late May 2005, when summer drought started but understory plants still dominated the site and during the middle of August 2005, when drought was severe and all understory vegetation cover had vanished. Days in both periods were almost cloudless sunny with high irradiance levels (~1000 W m^{-2}; **Fig. 4.1a**, **b**). Air and soil temperatures (**Fig. 4.1e**, **f**) during May were considerably lower (10 to 15°C) than in August and soil temperature differences between day and night were not as large, due to a buffering effect of the vegetation cover. Soil moistures (in the upper 10cm) were already low in May (~0.12 m^3 m^{-3}) but decreased to almost zero in August (**Fig. 4.1c**, **d**).

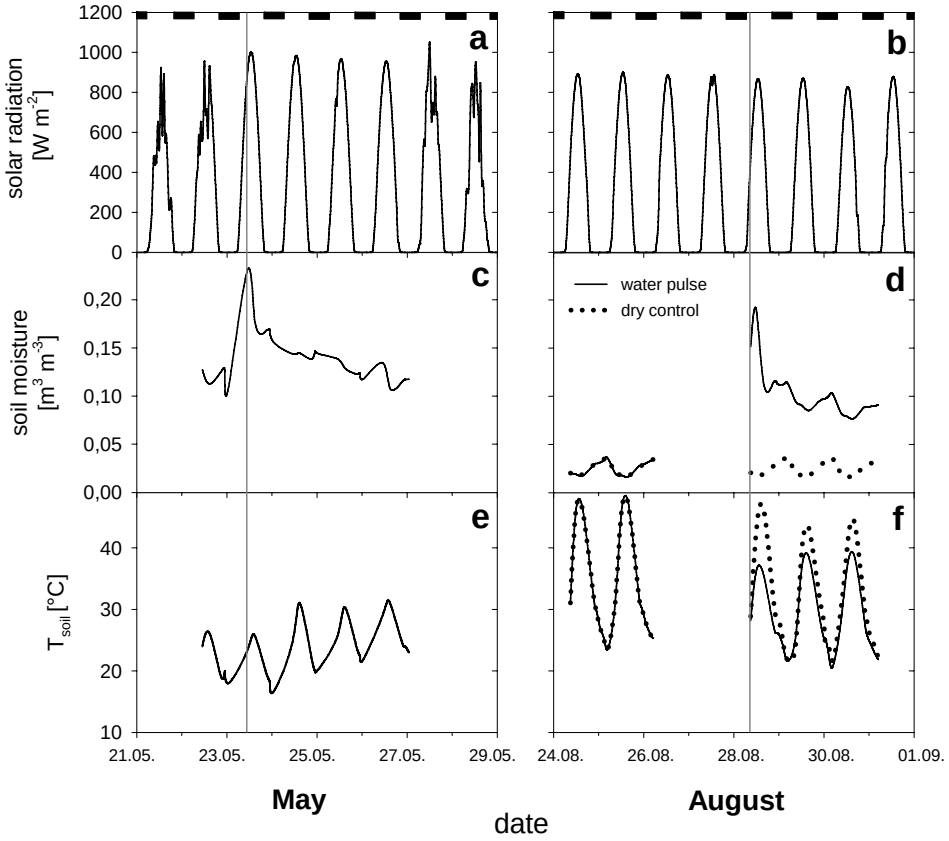

Fig. 4.1 Climatic conditions during late spring and under progressed summer drought in 2005 before and after irrigation (grey vertical bars), (**a**,**b**) solar radiation; (**c**,**d**) soil moisture in 10cm depth; (**e**,**f**) soil temperature at the surface; water pulse treatment (black line); dry control (dottet); black horizontal bars indicate nighttime.

Immediately after the irrigation treatment (20mm) soil moistures in both months rose to ≥0.2 m^3 m^{-3} and than declined rapidly within three days to 0.12 and 0.08 m^3 m^{-3} for May and August, respectively. As expected, soil temperatures responded to watering with a slight decrease in May and a rapid decrease in August, and afterwards increased relative to the decrease in soil moisture.

Carbon fluxes

Before the irrigation treatment net carbon flux of *plant plots* (including soil respiration) showed a typical diurnal cycle (**Fig. 4.2a**), exhibiting carbon uptake rates (-4 µmol m^{-2} s^{-1}) during daytime and CO_2-release during nighttime (4 µmol m^{-2} s^{-1}). After irrigation however, net carbon exchange of these plots became increasingly dominated by respiration, which resulted in positive net fluxes during daytime especially on May 24, the day immediately after

the watering (4 µmol m^{-2} s^{-1} during daytime). Carbon flux of *soil* and *trenching plots* was very similar in range and course (~1.5 to 2 µmol m^{-2} s^{-1}; **Fig. 4.2b**,**c**). Respiration of intact soil was on average only 0.75 µmol m^{-2} s^{-1} higher than respiration of *trenching plots*, suggesting low autotrophic contribution to soil respiration. During daytime differences between *soil* and *trenching plots* were generally higher than during nighttime (up to 1.4 µmol m^{-2} s^{-1}). Immediately after irrigation both *soil* and *trenching plots* responded with a rapid increase in respiration rate (up to 6 µmol m^{-2} s^{-1}), which exceeded two thirds of the pretreatment values. The increase was abrupt and pulse-like and respiration rates decreased by 40% already four hours after the water pulse. Within the following days, respiration rates of *soil* and *trenching plots* decreased slowly about 1 µmol m^{-2} s^{-1} but did not reach the values previous to irrigation.

In August 2005 soil respiration rates were initially five times lower compared to May (~0.5 µmol m^{-2} s^{-1}; **Fig. 4.2d**) and the CO_2-pulse in response to irrigation was substantially higher (12 µmol m^{-2} s^{-1}), exhibiting a 24-fold increase. Within 12 hours respiration rates decreased rapidly to 4 µmol m^{-2} s^{-1} and further within the following days, reflecting the desiccation pattern of the soil.

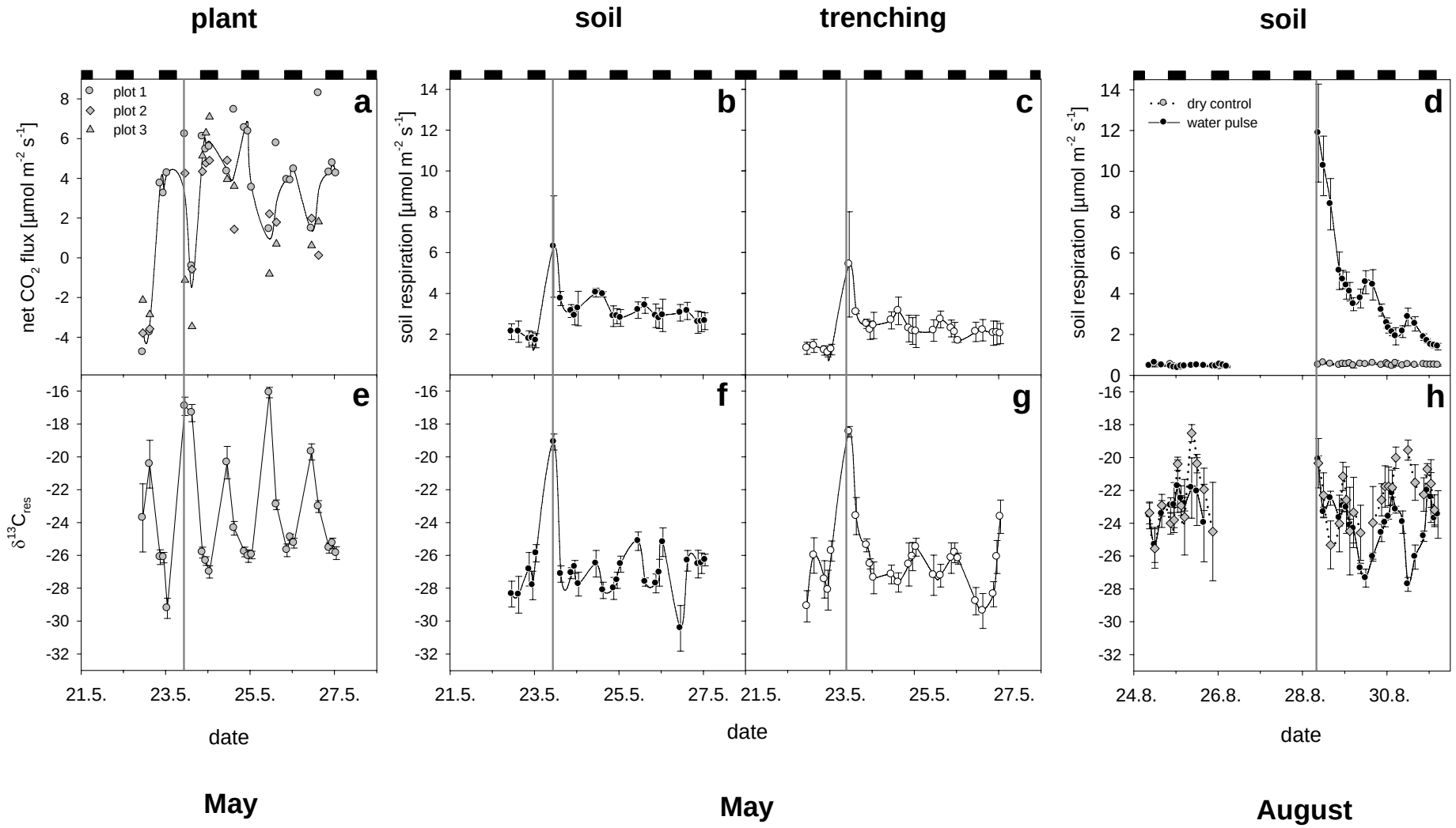

Fig. 4.2 Carbon fluxes (**a-d**) and respective isotopic compositions (**e-h**) before and after irrigation treatment (grey vertical bars) of differently treated soil patches: (**a**,**e**) intact understory vegetation, grey circles (grey triangles and diamonds indicate replicate plots); (**b**,**f**) intact soil without vegetation (black circles); (**c**,**g**) root-free soil without vegetation (white circles) during May 2005; (**d**,**h**) intact soil without vegetation (black circles, lined – water pulse treatment; grey circles, dotted – dry control) during August 2005. Black horizontal bars indicate nighttime; n = 3 ± SD. Error bars of $\delta^{13}C_{res}$ represent standard errors of intercept of OLS regressions.

Isotopic composition of carbon fluxes

$\delta^{13}C$ from chamber *keeling plots* of *plant plots* exhibited a typical pattern of strongly enriched values (-20‰ to -16‰) at midday and in the afternoon due to photosynthetic enrichment. At night intercepts shifted to more depleted values (-29‰ to -25‰) influenced only by respiration (**Fig. 4.2e**). Both *soil* and *trenching plots* respired $\delta^{13}CO_2$ exhibited depleted values ranging between -29‰ and -26‰ (**Fig. 4.2f,g**). Immediately after the water pulse on May 23 $\delta^{13}C_{res}$ of *soil* and *trenching plots* exhibited strongly enriched values (-19‰). Plant plots responded similarly with even higher enrichment (-17‰) caused by the additional effect of photosynthetic discrimination. As with the respiration pulse (see above) $\delta^{13}C_{res}$ of all plots only peaked for a short period and returned to the original range within a few hours. During August however, the range of $\delta^{13}C_{res}$ (**Fig. 4.2h**) previous to the irrigation treatment was considerably more enriched than during May (-24‰ to -19‰, compare **Fig. 4.2f**). Thus, the increase in $\delta^{13}C_{res}$ (to -20‰) in response to watering was not a noticeable pulse-like one. An interesting aspect is that at the day of watering continuous depletion in $\delta^{13}C_{res}$ occurred throughout the day (Fig. **4.3 a**,**b**). Contrastingly, during the days after the irrigation treatment in both months, a diurnal cycle in $\delta^{13}C_{res}$ of soil respiration seemed to appear, with depleted values during daytime and enrichment at night (Fig. **4.3 a**,**b**). In August this effect was even more pronounced than in May and ranged over 5‰. Here, the water pulse treatment exhibited much more depleted values during daytime than the dry control, although these plots displayed nonetheless distinct dynamics, not visible in May.

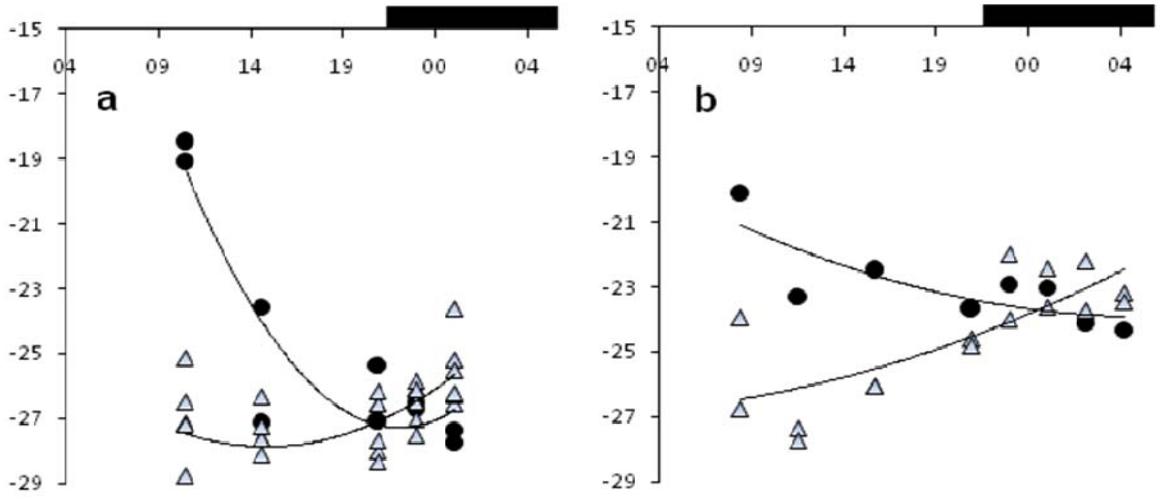

Fig. 4.3 Diurnal courses in $\delta^{13}C_{res}$ of (**a**) soil and trenching plots in May 2005 and (**b**) soil plots in August 2005 for the day of irrigation (black circles) and the following days (white triangles), black bars indicate nighttime.

4.4.2 Natural autumn rain pulses

Climate

On October 9, first natural rain pulse events after an extended summer drought occurred, followed by a series of precipitation pulses (most significant on October 10, 34 mm; **Fig. 4.4c**), all accompanied by strongly reduced net radiation (**Fig. 4.4a**). VPD and T_{air} (**Fig. 4.4b**) dropped instantly after first rainfalls and did not increase again even during the small periods without precipitation (e.g. October 13-15).

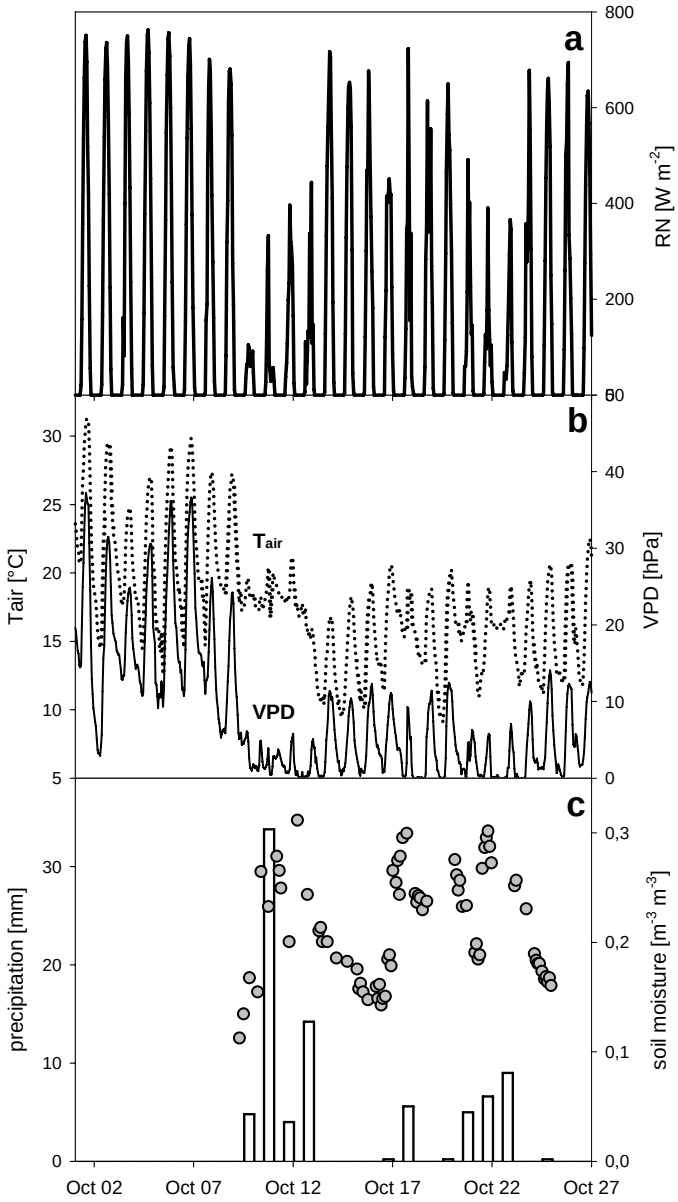

Fig. 4.4 Variation in (**a**) net radiation, (**b**) air temperature (dotted), VPD (lined), (**c**) precipitation (bars) and soil moisture (grey circles) during October 2005.

Comparison of ecosystem and soil scale results

Both nighttime and daytime NEE increased immediately after the first precipitation pulses to 5 and 4 µmol m^{-2} s^{-1}, respectively (**Fig. 4.5a**). The main respiration pulse occurred on October 11, one day after the largest rainfall. Afterwards R_{eco} (nighttime NEE) dropped to ~2 µmol m^{-2} s^{-1} and increased slowly towards the end of October with short pulse-like increases to 4 µmol m^{-2} s^{-1} in response to rainfalls on October 17, 19 and 21. Daytime NEE on the other hand became increasingly smaller after the first heavy rains and reached low net uptake rates again on October 23. $\delta^{13}C_R$ followed smoothly the precipitation pattern with rapid enrichment after days with heavy rainfalls and subsequent depletion during the dry days (**Fig. 4.5b**).

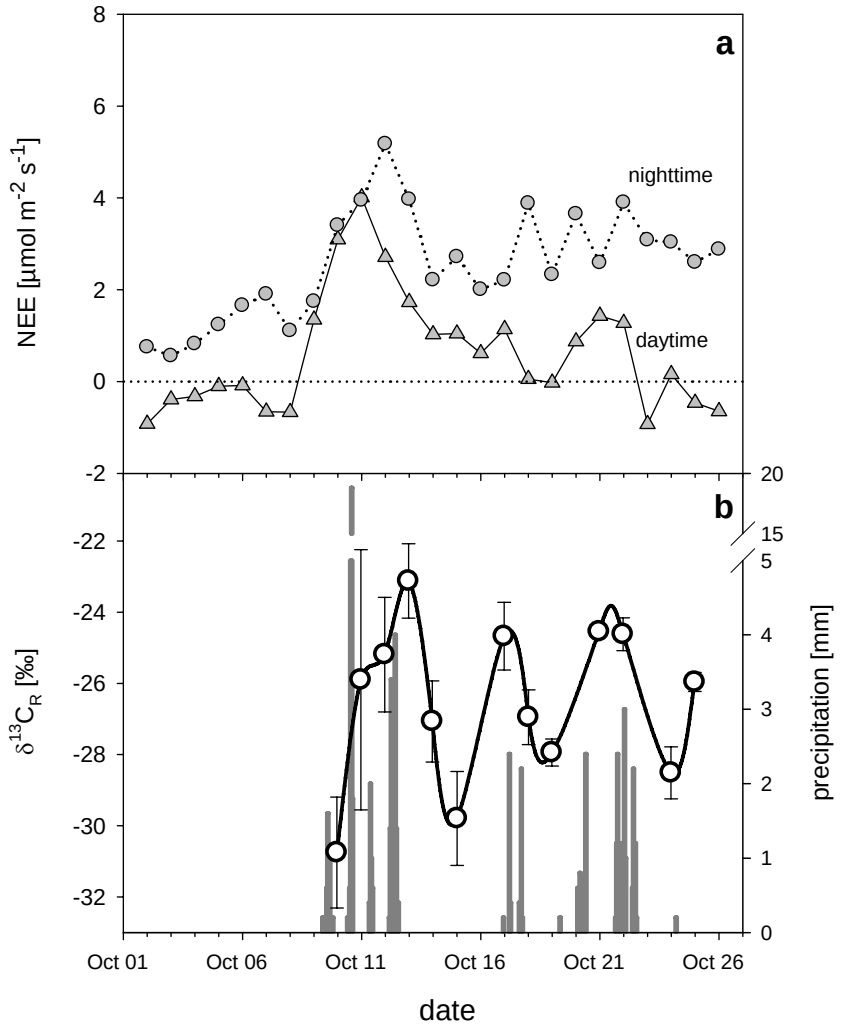

Fig. 4.5 (**a**) averages of nocturnal (circles) and diurnal (triangles) net ecosystem exchange (NEE); (**b**) precipitation (bars) and nighttime $\delta^{13}C_R$ during October 2005. Error bars represent standard errors of intercept for OLS regressions.

Similar results were obtained at the soil scale (**Fig. 4.6**).

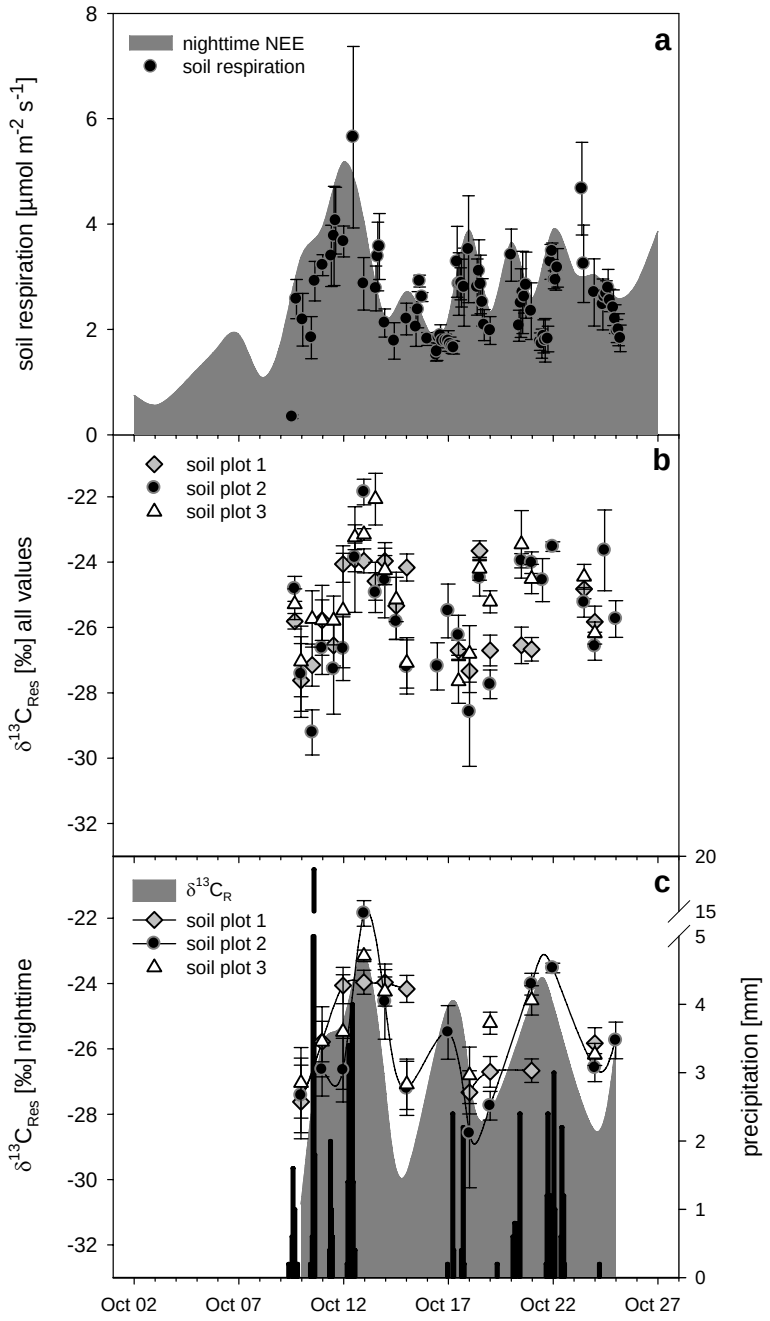

Fig. 4.6 (**a**) soil respiration (daytime and nighttime; black circles) and nighttime NEE (shaded area); (**b**) daytime and nighttime values and (**c**) exclusively nighttime values of $\delta^{13}C_{res}$ of soil from soil plot 1 (grey diamonds); 2 (black circles) and 3 (white triangles), precipitation (bars) and nighttime $\delta^{13}C_R$ (shaded area) during October 2005. Error bars of $\delta^{13}C_{res}$ represent standard errors of intercept for OLS regressions. Error bars of soil respiration represents standard deviations for n=5.

As with R_{eco}, soil respiration increased pulse-like in response to the first water pulses. Moreover, the pattern of R_{eco} and R_{soil} were almost congruent to each other (**Fig. 4.6a**) and

revealed a highly significant relationship (R^2=0.91, **Fig. 4.7b**), which suggests that soil processes were the main cause for the variation in ecosystem respiration. This is reinforced by the good correlations between $\delta^{13}C_R$ and $\delta^{13}C_{res}$ of soil respiration from soil plot 2 (R^2=0.71) and 3 (R^2=0.79), whereas there was no significant relationship with soil plot 3. Overall $\delta^{13}C_R$ and $\delta^{13}C_{res}$ were found to correlate relatively well (R^2=0.61; **Fig. 4.7a**). This gives significant evidence that ecosystem respiration during this time consisted for the most part of soil respiration.

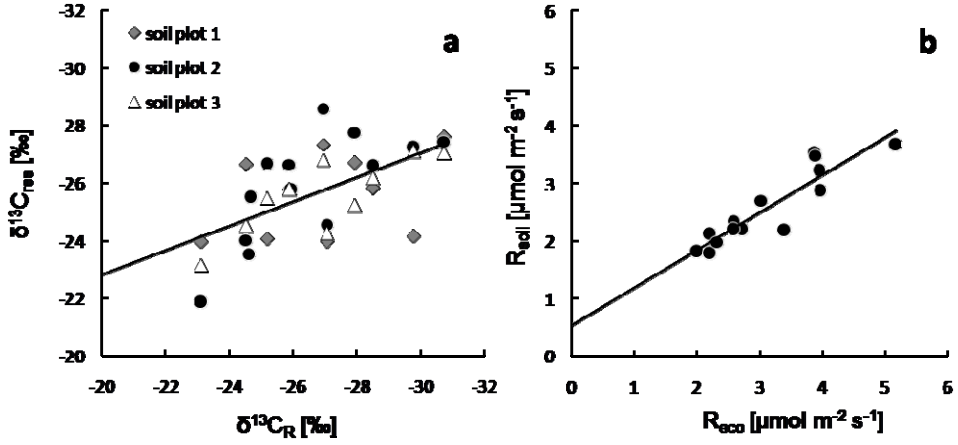

Fig. 4.7 Regressions between (**a**) $\delta^{13}C_R$ and $\delta^{13}C_{res}$ of soil respiration from soil plot 1 (grey diamonds); 2 (black circles) and 3 (white triangles), regression line covers all plots (n=33; R^2=0.37) and (**b**) between soil respiration and ecosystem respiration at nighttime (n=14; R^2=0.86).

Regression analysis (fortnight-scale)

Tab. 4.1 displays correlation coefficients between key climatic variables, $\delta^{13}C_R$, $\delta^{13}C_{res}$, R_{soil} and R_{eco}. Best fits were achieved for regressions between R_{soil} and R_{eco} with soil climatic variables, where R_{soil} correlated best with soil moisture (R^2=0.69), while regressions of R_{eco} and soil temperature revealed highest R^2 (0.74). From all other climatic variables VPD and predawn water potentials of *Q. ilex* correlated best with soil and ecosystem fluxes, though correlations were rather weak and not significant (R^2=0.37-0.49).

$\delta^{13}C_R$ and $\delta^{13}C_{res}$ of soil on the other hand did not correlate well with any of the compared parameters. Here, relationships between one day time-lagged fluxes and soil parameters were best (for a thorough explanation of time-lags compare **chapter 3**). Time-lagged R_{soil} and R_{eco} revealed significant relationships with $\delta^{13}C_R$ and $\delta^{13}C_{res}$ (R^2=0.61-0.87). $\delta^{13}C_R$ was weakly correlated with time-lagged predawn water potentials of *Q. ilex* and soil temperature. $\delta^{13}C_{res}$

of soil on the other hand was partially significantly correlated with time-lagged soil moisture, soil temperature and water potentials. However, correlations were not consistent in all measured plots, thus, variability in soil $\delta^{13}C_{res}$ is likely to be influenced by a combination of these parameters

Tab. 4.1 Pearson correlation coefficients for regressions between midnight values of soil and ecosystem fluxes (R_{soil}, R_{eco}) and their isotopic compositions ($\delta^{13}C_R$ and $\delta^{13}C_{res}$ - plot 1,2,3) with each other and climatic variables for the period between October 9 and October 24 in 2005 are given. RN (daytime sum); T_{air}, VPD (daytime means); WP_{PD} (n=5); SM, T_{soil} at midnight (n=5). Below part of the table shows one day time-lagged regressions between $\delta^{13}C_R$ and $\delta^{13}C_{S1,2,3}$ with the above mentioned parameters. Significant relationships ($p<0.05$) are in bold, n = 10-16.

	RN	T_{air}	VPD	WP_{PD}	T_{soil}	SM	R_{eco}	R_{soil}	$\delta^{13}C_R$	$\delta^{13}C_{res1}$	$\delta^{13}C_{res2}$	$\delta^{13}C_{res3}$
R_{eco}	-0.34	-0.10	-0.49	0.40	**0.74**	0.45	-	**0.91**	0.17	0.25	0.07	0.17
R_{soil}	-0.23	-0.11	-0.37	0.45	**0.54**	**0.69**	**0.91**	-	0.25	0.25	0.13	0.29
$\delta^{13}C_R$	0.00	-0.36	-0.06	-0.29	-0.04	0.24	0.17	0.25	-	0.35	**0.71**	**0.79**
$\delta^{13}C_{res1}$	0.58	-0.58	0.42	0.06	-0.18	-0.03	0.25	0.25	0.35	-	0.51	0.46
$\delta^{13}C_{res2}$	0.19	-0.50	0.14	-0.45	-0.31	0.29	0.07	0.13	**0.71**	0.51	-	**0.88**
$\delta^{13}C_{res3}$	0.36	-0.57	0.25	-0.28	-0.22	0.53	0.17	0.29	**0.79**	0.46	**0.88**	-
$\delta^{13}C_R$ + 1d	-0.13	-0.03	-0.28	0.40	0.41	0.15	**0.68**	**0.61**	-	-	-	-
$\delta^{13}C_{res1}$ + 1d	0.43	-0.49	0.05	0.28	0.32	**0.84**	**0.68**	**0.79**	-	-	-	-
$\delta^{13}C_{res2}$ + 1d	-0.11	-0.46	-0.29	**0.75**	0.26	0.52	**0.73**	**0.72**	-	-	-	-
$\delta^{13}C_{res3}$ + 1d	-0.02	-0.36	-0.36	0.48	**0.64**	0.47	**0.87**	**0.75**	-	-	-	-

Diurnal courses

Fig. 4.8 shows an example of three diurnal courses of soil and ecosystem respiration as well as their isotopic compositions during the studied period in October 2005. On October 16, without rain during the previous four days, soil respiration was constant during the entire diurnal cycle (**Fig. 4.8a**). Two small precipitation pulses occurred after midnight, which led to an increase in R_{eco} but were not reflected by soil respiration measurements. This might be due to the fact, that soil respiration was measured instantaneously when rainfall started and sampling time was too short to reflect the impact of the rain pulse, whereas R_{eco} is an integrated value. Stronger rain pulses, however, on October 21 caused nocturnal increase in

both ecosystem and soil respiration (**Fig. 4.8b**). On the dry day of October 24 (**Fig. 4.8c**) there was no nocturnal variation in R_{eco} nor R_{soil}, as was to be expected.

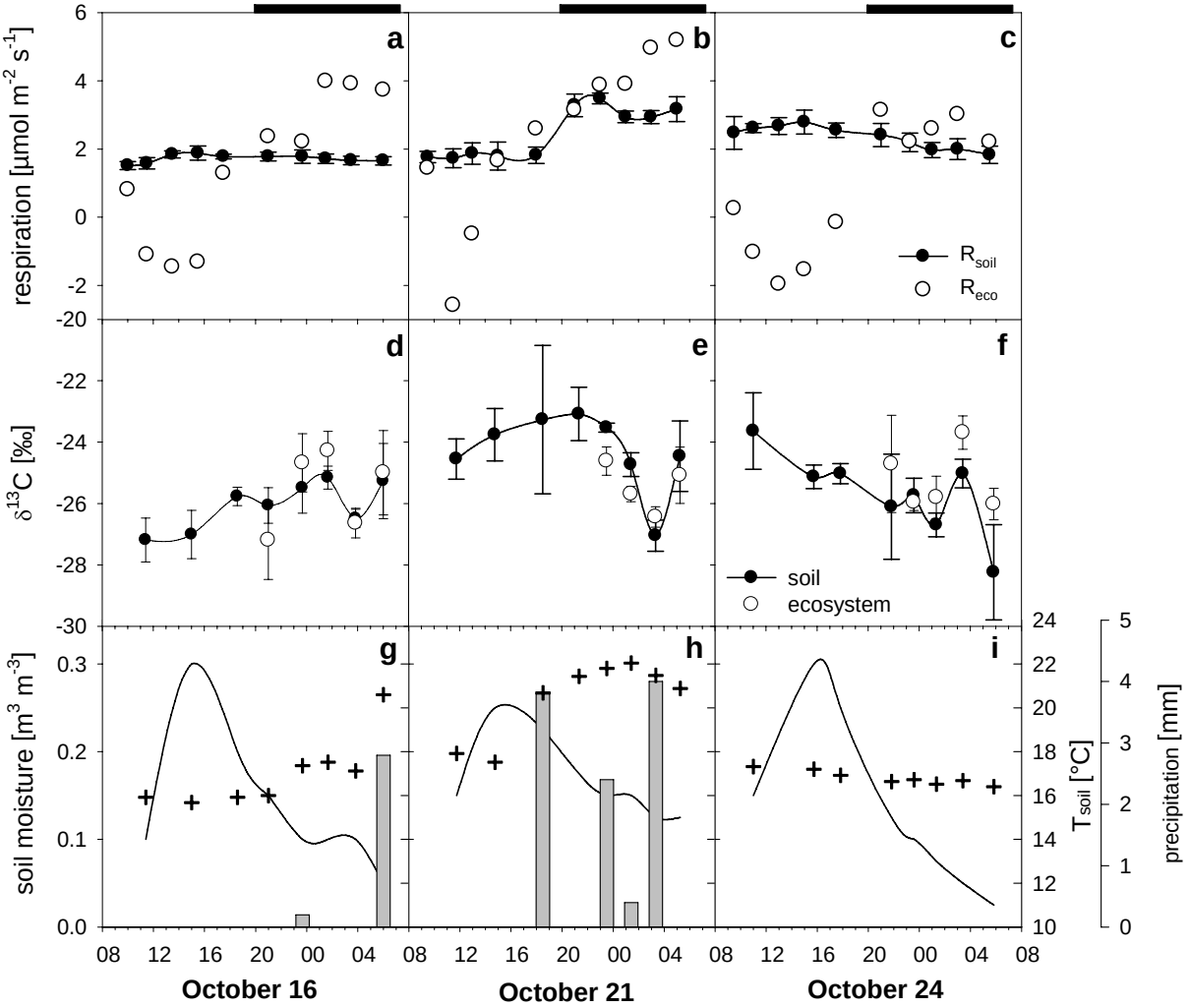

Fig. 4.8 Diurnal courses of (**a,b,c**) soil respiration (black circles, lined) and NEE (white circles), (**d,e,f**) $\delta^{13}C_R$ (white circles) and $\delta^{13}C_{res}$ of soil respiration (black circles, lined), and (**g,h,i**) soil moisture (black crosses), soil temperature (black line) and precipitation (grey bars) for October 16 (**a,d,g**) 21 (**b,e,h**) and 24 (**c,f,i**) 2005. Error bars of *keeling plots* represent standard errors of intercept for OLS regressions. Error bars of soil respiration represents standard deviations for n=5.

During all three nights both $\delta^{13}C_R$ and $\delta^{13}C_{res}$ of soil exhibited substantial variations of 3 to 4‰. Although the course of these variations can be regarded as rather irregular (no clear pattern of nocturnal enrichment, as observed in other campaigns), it strikes that $\delta^{13}C_R$ and $\delta^{13}C_{res}$ of soil followed generally the same course, as already observed at the fortnight-scale (see **Fig. 4.7a**). In correlation analyses, $\delta^{13}C_{res}$ of soil explained 89, 96 and 69% of the nocturnal variation in $\delta^{13}C_R$ for October 16, 21 and 24, respectively.

Regression analysis (diurnal scale)

Tab. 4.2 Pearson correlation coefficients for regressions between diurnal courses (10, 12, 15, 18, 21, 23, 01, 03 and 05:00) of soil and ecosystem fluxes (R_{soil}, NEE) and their isotopic compositions ($\delta^{13}C_R$ (only nighttime) and $\delta^{13}C_{res}$) with each other and climatic variables for October 16, 21 and 24 in 2005 are given. RN, T_{air}, VPD (hourly averages); SM, T_{soil} (n=5). Shaded area contains regression parameters exclusively for nighttime. Significant relationships ($p<0.05$) are in bold, n = 5-9.

October 16	RN	T_{air}	VPD	T_{soil}	SM	NEE	R_{soil}	$\delta^{13}C_R$	$\delta^{13}C_{res}$
NEE	**-0.86**	-0.43	-0.59	-0.32	0.56	-	-0.03	-	**0.94**
R_{soil}	0.13	**0.75**	0.59	**0.89**	-0.23	-0.03	-	-	0.05
$\delta^{13}C_{res}$	**-0.82**	-0.51	-0.60	-0.47	0.64	**0.94**	0.05	-	-
NEE	-	-0.15	-0.21	-0.34	0.42	-	-0.14	0.69	**0.86**
R_{soil}	-	**0.75**	**0.81**	**0.74**	-0.68	-0.14	-	-0.09	0.02
$\delta^{13}C_R$	-	-0.42	-0.45	-0.61	0.51	0.69	-0.09	-	**0.89**
$\delta^{13}C_{res}$	-	-0.34	-0.35	-0.50	0.56	**0.86**	0.02	**0.89**	-
October 21									
NEE	**-0.75**	-0.52	**-0.70**	-0.06	**0.72**	-	**0.72**	-	0.19
R_{soil}	-0.66	-0.49	**-0.70**	-0.44	**0.85**	**0.72**	-	-	-0.10
$\delta^{13}C_{res}$	0.08	0.10	0.11	0.58	-0.13	0.19	-0.10	-	-
NEE	-	-0.23	-0.30	-0.02	0.50	-	**0.71**	**0.89**	0.64
R_{soil}	-	-0.58	-0.13	0.44	-0.07	**0.71**	-	**0.90**	**0.77**
$\delta^{13}C_R$	-	-0.64	0.06	0.45	-0.07	**0.89**	**0.90**	-	**0.96**
$\delta^{13}C_{res}$	-	-0.41	0.27	**0.75**	0.04	0.64	**0.77**	**0.96**	-
October 24									
NEE	**-0.78**	**-0.77**	-0.69	**-0.76**	**0.80**	-	**-0.89**	-	-0.54
R_{soil}	**0.83**	**0.93**	**0.89**	**0.92**	**0.90**	**-0.89**	-	-	**0.71**
$\delta^{13}C_{res}$	0.59	0.57	0.49	0.53	**0.86**	-0.54	**0.71**	-	-
NEE	-	**-0.93**	**-0.87**	**-0.82**	-0.29	-	**-0.87**	0.15	-0.03
R_{soil}	-	**0.99**	**0.98**	**0.95**	0.67	**-0.87**	-	0.24	0.50
$\delta^{13}C_R$	-	0.14	0.31	0.06	0.49	0.15	0.24	-	0.69
δ13Cres	-	0.36	0.41	0.46	**0.94**	-0.03	0.50	0.69	-

As described above, climatic conditions during the three observed diurnal cycles were substantially different. Therefore, it can be expected, that fluxes and $\delta^{13}C_R$ and $\delta^{13}C_{res}$ on the different days were driven by different climatic variables. Consistent, though not in all cases significant were correlations of NEE with RN, VPD and soil moisture (R^2=0.56-0.86), that were found during all three diurnal cycles, which might be simple autocorrelation due to the fact that NEE was negative during daytime and positive during nighttime. At nighttime however, relationships between NEE and climatic variables were not consistent for all three days. On the rainy days October 16 and 21, weak correlations to soil moisture were found (R^2=0.42-0.45), but not to other climatic parameters, while on the day without rain October 24, relationships to T_{air}, T_{soil} and VPD were very strong (R^2=0.95-0.99).

At the soil scale, the stronger dependence of carbon fluxes on water during rainy days and temperature during sunny days was even clearer. On October 24, all climatic factors, but especially T_{air}, VPD and T_{soil} revealed strong relationships with soil respiration. On October 16, a rather sunny day with rainfalls only at late nighttime, these relationships were also the most dominant, while on October 21, with almost constant rainfalls, VPD and soil moisture explained most of the variability in R_{soil} (R^2=0.68-0.72). Interestingly on October 21, these relationships do not hold at nighttime, when soil respiration increased pulse-like with the first rain event and afterwards remained stable.

The observed relationships between $\delta^{13}C_R$ and $\delta^{13}C_{res}$ with the climate variables during the diurnal cycle are more complex to interpret and appear rather random. There were no consistent relationships for all three days. Unexpectedly, October 21 showed a significant correlation of $\delta^{13}C_{res}$ with soil temperature (R^2=0.75), while on the days with less rain $\delta^{13}C_R$ and $\delta^{13}C_{res}$ exhibited partially weak soil moisture relationships (R^2=0.49-0.94). However, nocturnal $\delta^{13}C_R$ and $\delta^{13}C_{res}$ correlated very well with ecosystem respiration rates during the rainy nights of October 16 and 21 (on that particular day I observed also a strong relationship to soil respiration; R^2=0.77-0.90), whereas there was no correlation on October 24.

4.5 Discussion

In this field study the origin of the *Birch* effect following artificial and natural rain pulse events during Mediterranean summer drought was analyzed with straight-forward stable isotope methods.

In the following, current hypotheses on the *Birch* effect will be evaluated under the novel viewpoint of $^{13}CO_2$ expelled in response to rewetting of dried soils. Additionally, the implications of the *Birch* effect for short-term carbon dynamics at both soil and ecosystem scale will be discussed.

4.5.1 Origin of the *Birch* effect

This study confirmed that the carbon pulse after rewetting most likely originates from soil microorganisms, since the pulse-like increase in soil respiration rates was similarly strong in all plots (*plant*, *soil*, *trenching*). However, the most important result of both artificial and natural irrigation studies was the immediate enrichment in $\delta^{13}C$ of soil and ecosystem released CO_2 in response to rewetting.

There are currently a number of hypotheses to explain the *Birch* effect, all combining the assumption that it originates from instantaneously increased mineralization by fungal and microbial biomass in response to water availability (Griffiths and Birch, 1961; Jager & Bruins, 1974; Orchard & Cook, 1983; Scheu & Parkinson, 1994).

The first major hypothesis attributes the CO_2-increase after rewetting to a spontaneous increase in mineralization of soil organic matter carbon (SOM-C), which is explained by rain-pulse induced disruption of aggregate structures and consequent availability of organic matter without physical protection from soil microbes (e.g. Adu & Oades, 1978; Appel, 1998; Denef *et al* 2001). However, the presented isotope results indicate that a shattering of SOM-C from aggregates was not the reason for the *Birch* pulse, as the physical release of bound SOM-C would not change the isotopic composition of the carbon pulse significantly, since soluble SOM-C is the general substrate for mineralization. Contrastingly, the immediate pulse-like increase in soil respiration exhibited a marked enrichment in May 2005.

The second major hypothesis regards microbial carbon as the source of the CO_2-pulse. Bottner *et al.* (1998) proposed this carbon to originate from drought-based dead microbial biomass. Others postulated the rewetting event to cause hypo-osmotic stress in soil microbes, releasing osmoregulatory compounds to dilute with the soil water (Kieft *et al.*, 1987; Fierer & Schimel, 2002, 2003; Jarvis *et al.,* 2007). In a ^{14}C labeling laboratory experiment Fierer & Schimel (2003) brought up experimental evidence that the microbial biomass (and not SOM-C) is respired during the *Birch* pulse. Drought induces soil microorganisms to accumulate low weight organic substances, mainly polyols, amino acids and amines, to lower intracellular

water potentials and maintain cell turgor (e.g. Potts, 1994). To avoid cell lysis after rewetting, the soil microbes must immediately eliminate these osmotic solutes by dilution with the soil water. Within as little as ten minutes after reestablishing cell water potentials the cells can reincorporate and mineralize these substances again (e.g. Wood *et al.,* 2001; Hohmann, 2002).

My results do fit this theory, given that osmotic solutes incorporated in the cytoplasm of soil microorganisms for drought protection were substantially ^{13}C enriched compared to SOM-C, that would be mineralized by the increasing microbial community after establishment of a more accessible environment. So far little is known on possible carbon fractionation processes in microorganisms metabolism, thus, it must be assumed that the input of enriched carbon sources prior to drought will explain enrichment in osmoregulatory substances. Photosynthetic fractionation leaves a time-lagged imprint on soil respiration (e.g. Högberg *et al.*, 2001). In response to drought photosynthetic fractionation of plants decreases (Farquhar *et al.*, 1989) and produced sugars contain progressively more enriched carbon. A major part of the produced photosynthates are translocated within the plant. In the phloem recently fixed carbon is exported from the leaves to the roots, where it might be released as root exudates to the soil and become available substrate for soil mircoorganisms. Therefore, a likely scenario for the observed pulse-like enrichment in May might be a drought-induced increase in assimilation of heavy carbon by soil microorganisms, driven by post-photosynthetic release of enriched root exudates from plants.

Another possible mechanism to explain the incorporation of enriched substrates and subsequent transformation to osmotic substances with drought stress is a positive taxis of soil microorganisms to water, leading to increasing microbial activity in deeper, moister soil layers with enhanced drought. Through a decreased input of photosynthetic assimilates, the carbon cycle of deeper soil layers is increasingly sustained by soil microbial biomass alone (Tu & Dawson, 2005). Soil microbes are known for their ability to assimilate carbon by a process called *anaplerotic* CO_2-fixation, where PEP-carboxylase assimilates CO_2 from the soil air (e.g. Wingler *et al.*, 1996). Through mixing of atmospheric and respired carbon in the soil air spaces, this CO_2 is substantially enriched compared to carbon fixed by C3 plants and microorganisms communities without access to plant carbon become increasingly enriched (Tu & Dawson, 2005). Therefore, substrate for mineralization (SOM-C) is known to become increasingly enriched with soil depths (e.g. Nadelhoffer & Fry 1988; Ehleringer *et al.*, 2000).

In August 2005 initial $\delta^{13}C_{res}$ was already markedly enriched and the $\delta^{13}C$-response after rewetting was small compared to May. Indeed, the variation in $\delta^{13}C_{res}$ in response to the water

pulse in August exhibited about the same magnitude as natural variations indicated by control plots that where not subjected to rewetting. From these results it can be assumed that with enhanced seasonal drought, respired soil carbon originated increasingly from microorganisms of deeper soil layers without access to plant assimilated carbon from the litter. Thus, these results reinforce the above drawn hypothesis that drought induces soil microorganisms to move to deeper soil layers.

During August, summer drought was much more severe than in the end of May. Soil moistures were much lower and the CO_2-pulse after rewetting was substantially higher (~200%). This proportionality in the magnitude of the *Birch* effect and the time of drought has been found in many other studies. Arid ecosystems with high moisture variability generally have a smaller response to rewetting than ecosystems with rare fluctuations in precipitation (e.g. Kieft *et al.*, 1987; Franzluebbers *et al.*, 2000; Fierer & Schimel, 2003) and number of consecutive drying and rewetting cycles decreases the CO_2-pulse (e.g. Bottner, 1985; Denef *et al.*, 2001; Fierer & Schimel, 2002). This has been explained by the hypothesis that recurrent drying-rewetting events should deplete the quantity of labile soil carbon held within aggregates and that soils rarely exposed to precipitation should release more labile SOM-C to be mineralized (e.g. Degens & Sparling, 1995). Alternatively, Fierer & Schimel (2003) supposed that under frequent drying and rewetting soil microbial communities could economically adapt their hypo-osmotic response to a rapid change in soil water potentials. They underlined their theory with the finding that the increase in labile SOM-C after the water pulse, did not significantly contribute to the carbon pulse. The current results suggest that the *hypo-osmotic stress* hypothesis gives the best explanation for the observed differences between May and August, since the hypo-osmotic stress and subsequent release of osmotic substances can be expected to be proportionate to the magnitude in change of soil water potential.

A third explanation for the carbon pulse released by the *Birch* effect might be a very different one. CO_2 is heavier than air and will accumulate by gravitation within the air spaces of the soil. Replacement of this gaseous carbon by dilution will not occur without water and, unstirred by turbulent mixing, accumulation of [CO_2] within the soil will increase. A sudden heavy rainfall might simply replace the captured CO_2 by water and release it back into the air (pers. com. J. S. Pereira). Indeed, some of the results support this idea. Heavy carbon will be retained inside the soil spaces better than light one. Replacement of soil air by precipitation thus, should cause an enriched CO_2-pulse, as observed immediately after irrigation in May

2005. Further, the difference in magnitude of the carbon pulse between May and August might be simply caused by the fact that time span between irrigation and last natural rain event was larger in August. Thus, accumulation of [CO_2] inside the air spaces of the soil increased with time. However, this theory might be rejected, since in that case the carbon pulse in August should have been substantially more enriched than in May, which it was not. Further, in October 2005 several precipitation pulses occurred in succession over a period of two weeks. After each small dry period a new rain pulse caused an increase in enriched soil and ecosystem respired CO_2 of almost the same magnitude as after the first rain fall, which would not be expected if the *replacement* theory was correct.

4.5.2 Implications for short-term carbon dynamics

The *Birch* effect increased soil respiration 3-, 24- and 12-fold in May, August and October, respectively and return to near initial values took between two and three days. There was a general difference between *Birch* pulses observed after irrigation experiments and such caused by natural precipitation events. Irrigation experiments revealed an immediate increase in soil respiration in response to the water pulse (20 mm), while in October soil respiration did not increase pulse-like but peaked a day after the first large rain falls, although water pulses were even larger than in May and August (< 30 mm). Later precipitation pulses on the other hand, led to immediate increases in soil respiration. One reason might be that irrigation causes a more rapid change in soil water potential than a precipitation event over several hours. Furthermore, the natural precipitation pulse is distributed more evenly in space and the capacity to absorb and dispense water to deeper soil layers might be more efficient. Thus, soil moisture increased more slowly in October than in May and August. Therefore, it is reasonable to assume that the *Birch* effect in response to natural precipitation was less drastic, since the hypo-osmotic shock that soil microorganisms experienced was smaller. Nevertheless, the *Birch* effect had large implications for ecosystem carbon balance during October, and ecosystem respiration increased five-fold. Further, R_{eco} was found to be highly dependent on soil respiration and its main climatic drivers, soil moisture and soil temperature. After the first precipitation pulses soil respiration began to largely dominate ecosystem respiration. This was apparent at both seasonal and diurnal time scale and was also reflected in the strong similarity between $\delta^{13}C_R$ and $\delta^{13}C_{res}$ patterns.

In previous field campaigns regularly increasing nocturnal enrichment in $\delta^{13}C_R$ was discovered (for a thorough explanation see **chapter 3**).

Here, during a succession of rain pulses the nocturnal course of neither $\delta^{13}C_R$ nor $\delta^{13}C_{res}$ could be well explained by correlations with key climatic variables and appeared rather randomly. This might be due to the direct influence of the precipitation pulses on the soil microorganisms, thus, the *Birch* effect masked the normal nocturnal pattern. This hypothesis is reinforced by the fact that during May and especially in August clear diurnal cycles of nocturnal enrichment in soil respiration were found on days after the irrigation, whereas during the day of irrigation constant depletion of soil respired CO_2 was observed throughout the entire night. Hence, on the day of irrigation when enriched osmoregulatory compounds are released, soil respiration is mainly fed by the pool of these enriched substrates and continues to become closer to the original depleted value of SOM-C, as the pool decreases.

In contrast to the irrigation experiments, in October the enrichment of both $\delta^{13}C_{res}$ of soil and $\delta^{13}C_R$ was one day time-lagged compared to the CO_2-pulses following the precipitation events. This is another indication for the theory that in October the hypo-osmotic shock on the micro fauna was buffered. Recently, Saetre & Stark (2005) found that bacterial and fungal growth peaked exactly two days after rewetting in different soil types from arid ecosystems in Utah along with depletion in labile soil carbon and than rapidly perished. Soil respiration rates however, peaked immediately after rewetting. This time-lag between the peak in soil respiration and the peak of soil microorganism biomass might explain the time-lag between $\delta^{13}C_R$, $\delta^{13}C_{res}$ and the respective carbon fluxes observed in October, 2005. As the microbial community peaks, the labile soil carbon and soil respiration rates decrease and microbial biomass will increasingly turn to anaplerotic carbon fixation, enriching the substrates for respiration.

This study suggests a combination of both hypo-osmotic stress and increase in microbial biomass, depending on timing and quality of the rain pulse event. In case of strong hypo-osmotic stress, as might be assumed for rapid changes in soil water potentials (e.g. in May and August 2005), the *Birch* effect should be immediate and entirely due to the expulsion of osmoregulatory substances. In response to the stress, the microbial community will respire most of the released carbon immediately after reestablishment of cell water potentials.

In case of moderate or low hypo-osmotic stress in response to slower rewetting, as assumed for the October field campaign, the *Birch* effect is not immediate and the released labile carbon is accumulated in new microbial biomass, prepared to attack SOM-C.

4.6 CONCLUSIONS

This work combines recent theories on the *Birch* effect with new results regarding stable isotope composition of carbon dioxide respired by soils and ecosystems.

The *Birch* pulse largely determined ecosystem respiration during periods of drying-rewetting. As revealed by $\delta^{13}C$ analyses of respired CO_2 the *Birch* pulse is caused by a hypo-osmotic stress response of soil microorganisms, rather than an increase in labile SOM-C.

Amount and timing of the rain pulse event and thus, the magnitude of the hypo-osmotic stress probably played a key role for the microbial answer to the rewetting of soils. When changes in soil water potentials occurred not rapidly but over a longer time-period and hypo-osmotic stress was potentially low or moderate, soil microbes reacted not with a rapid stress response but with immediate production of microbial biomass.

Many studies are conducted by irrigating soils *in vitro*, disregarding that the cause for the *Birch* effect might be substantially different than under natural conditions. Studying the impact of soil rewetting under natural conditions will be a major aim for future research, since knowledge on future impacts of increasing drought and rain fall variability in Mediterranean ecosystems is of high importance. Very large sudden rainfalls interrupting long drought periods might even enhance carbon sequestration, since they induce large stresses to the soil microbial community. Smaller extended or frequent rainfalls on the other hand, might cause the contrary effect.

4.7 AUTHORS CONTRIBUTIONS

The author of this thesis accomplished the entire experimental work and the writing of this chapter. Further contributions were given by C. Werner who coordinated the project, C. Máguas who provided the stable isotope lab, J.S. Pereira who provided the field site and T.S. David who provided climate data. Further, I acknowledge contributions of R. Maia for technical support with isotope analyses and P. Oliveira (*Mezão*) for technical help with equipment.

CHAPTER 5

PARTITIONING CARBON FLUXES IN A MEDITERRANEAN OAK FOREST TO DISENTANGLE CHANGES IN ECOSYSTEM SINK STRENGTH DURING DROUGHT

5.1 ABSTRACT

Partitioning of net carbon fluxes into assimilatory and respiratory fluxes of different ecosystem components provides an important tool to understand different sources and sinks of carbon, their interactions and their responses to abiotic drivers. Particularly in periods of rapidly changing climatic conditions, flux partitioning may disentangle the complex responses of different ecosystem components to these changes. In this study I analyzed the changes in ecosystem assimilation and respiration fluxes in a Mediterranean oak forest during a spring-summer transition period and attribute them to variation in component fluxes (trees, understory, soil microorganisms and roots). Within 15 days a two thirds decrease in both ecosystem carbon assimilation and respiration (R_{eco}) in response to drought was observed. The impact of decreasing R_{eco} on the ecosystem carbon balance was smaller than the impact of decreasing primary productivity. Flux partitioning showed that declining ecosystem sink strength was due to a large drought and temperature induced decrease in understory carbon uptake (from 56 to 21 %). Hence, these shallow-rooted annuals have a surprisingly large impact on the source/sink behavior of the ecosystem during spring to summer transition and the timing of the onset of drought will have a large effect on the annual carbon budget. During nighttime, R_{eco} was increasingly dominated by respiration of heterotrophic soil microorganisms, while the root flux was found to be of minor importance. Soil respiration flux decreased with drought but its contribution to total diurnal CO_2-exchange increased by 11.5%. This partitioning approach disentangles changes in respiratory and photosynthetic ecosystem fluxes that are not apparent from the *eddy covariance* or the soil respiration data alone. Combining measurements of component fluxes (soil, roots understory) with *eddy covariance* data yielded an improved understanding of factors controlling carbon sequestration in Mediterranean ecosystems.

5.2 INTRODUCTION

The carbon balance of ecosystems is controlled by inputs via photosynthetic fixation, storage in various pools (e.g. plant biomass, soil carbon) and loss of carbon by autotrophic and heterotrophic respiration as well as non respiratory processes such as leaching, physical demineralization of inorganic soil carbon, erosion and forest fires (e.g. Schulze *et al.*, 2000; Law *et al.*, 2002; Trumbore, 2006). Carbon cycling in forest ecosystems involves complex interactions between numerous C-pools, which differ both spatially and temporally and may also be differentially affected by environmental variables (e.g. Schimel *et al.*, 2001; Law *et al.*, 2002; Valentini *et al.*, 2003). The desire to understand large scale effects of global warming on ecosystem productivity indicates the necessity for a process based understanding of ecosystem carbon sequestration, which has now become a major objective of ecological research. Increasing frequency and severity of drought as well as changes in the precipitation pattern can cause large reductions in ecosystem carbon exchange of terrestrial ecosystems (Ciais *et al.*, 2005; Granier *et al.*, 2007), and particularly in seasonally dry climates such as the Mediterranean basin (Pereira *et al.*, 2006, 2007).

Net carbon fluxes are currently measured in a wide range of terrestrial ecosystems across the globe using *eddy covariance* techniques (e.g. Janssens *et al.*, 2001), a micrometeorological method particularly suitable to account for net ecosystem carbon exchange (NEE) from local to regional scales (Baldocchi *et al.*, 2001). Assessment of carbon fluxes by *eddy covariance* integrates complex processes (Valentini *et al.*, 2003) and yields high time resolved data sets for empirical modelling (e.g. Papale & Valentini, 2003; Reichstein *et al.*, 2003).

Partitioning net carbon fluxes into assimilatory and respiratory components provides an important tool for analysing abiotic and biotic processes driving carbon cycling. Various methods for measuring and modelling the opposing fluxes have emerged in recent years. One common method is to use *eddy covariance* data in empirical models which apply temperature corrections to the nighttime fluxes to calculate ecosystem respiration (R_{eco}), which subsequently is used to partition daytime fluxes (e.g. Aubinet *et al.*, 2000; Reichstein *et al.*, 2002b; Reichstein *et al.*, 2005). Other approaches combine stable isotopes with *eddy covariance* measurements (e.g. Bowling *et al.*, 2001; Ogée *et al.*, 2003; Knohl & Buchmann, 2005), taking advantage of the isotopic disequilibrium between ecosystem respiration and canopy assimilation.

These partitioning approaches enable the identification of underlying processes controlling changes in the sink strength of ecosystems in response to global climate change. Although many terrestrial ecosystems (e.g. forests) are net sinks for carbon, changes in climate and phenology can result in transformation of ecosystems to net carbon sources (Lindroth *et al.* 1998; Valentini *et al.*, 2003). It has been shown, that ecosystem respiration (R_{eco}) is a main determinant of carbon balance in most ecosystems (Valentini *et al.*, 2000; Rambal *et al.*, 2004). Temporal variation in the ecosystem respiration flux was observed to be substantial in various studies across different biomes (e.g. Baldocchi, 1997; Valentini *et al.*, 2000; Xu *et al.*, 2004; Reichstein *et al.*, 2005). Possible reasons for this variability are concomitant temporal changes in driving climatic factors such as temperature and soil moisture (e.g. Huxman *et al.*, 2003; Xu *et al.*, 2004; Davidson *et al.*, 2006b) as well as differential responses of respiratory sources to these drivers. Autotrophic respiration of leaves and roots including micorrhizal respiration depends mainly on photosynthetic assimilation whereas heterotrophic respiration of soil microorganisms and fungi is a function of the amount of labile soil carbon (Scott-Denton *et al.*, 2006; Subke *et al.*, 2006). Consequently, different respiration sources have different temporal dynamics. Autotrophic soil respiration follows periods of growth- and carbon assimilation cycles throughout the year whereas heterotrophic soil respiration depends on temporal events of carbon input, such as litter fall, priming and other processes (e.g. Kuzyakov & Cheng, 2001).

However, the complex interplay between various assimilatory and respiratory sources and their responsiveness to abiotic changes such as drought and temperature remain poorly understood. Hence, there is a need to disentangle component fluxes for a better understanding of changes in source/sink behavior of ecosystems in response to climate change. Apart from a range of long-term studies regarding annual carbon budgets (e.g. Goulden *et al.*, 1996; Lavigne *et al.*, 1997; Law *et al.*, 1999; Law *et al.*, 2001a; Davidson *et al.*, 2006a) partitioning between all major respiratory fluxes (foliage, roots and soil microorganisms) on the ecosystem scale has seldom been achieved.

In this field study of a Mediterranean oak-savannah ecosystem I took advantage of the rapidly changing environmental conditions during the transition period from spring to summer, which is of special relevance because timing and length of this event can strongly determine the period when the system turns from a net carbon sink into a net source (Werner *et al.*, 2006; Pereira *et al.*, 2007). I partitioned ecosystem fluxes into assimilatory and respiratory components during a spring to summer transition period with increasing drought and

temperatures. To my knowledge this is the first short-term attempt at disentangling all major assimilation and respiration fluxes of an ecosystem and their respective biotic and abiotic drivers. I aimed at identifying i) changes in sink strength in response to drought; ii) the component fluxes responsible for these changes and iii) their dependencies on environmental factors. I hypothesized that certain ecosystem components would be more sensitive to water deficits and rising temperature than others and thus, could significantly influence ecosystem carbon budgets under future climate scenarios.

5.3 MATERIALS AND METHODS

An intensive 14-days field campaign was conducted during May and June 2006 at the *Mitra* site (see **section 2.1**).

Continuous records of micrometeorological measurements of CO_2- and H_2O-fluxes as well as climate variables (see **section 2.2**) were taken.

Diurnal and nocturnal courses of leaf gas exchange (LI-6400 open-flow gas exchange system, LI-COR, Lincoln, NE, USA) and soil respiration (R_{soil}, PP-System EGM2 Soil Respiration System with SRC-1 chamber; PP-Systems, Amesbury, MA, USA) were recorded in 24-h cycles every 2-4 hours on marked branches or plots for leaf and soil measurements, respectively. Soil temperature (T_{soil}) and soil water content (SWC) were recorded in 5 to 10cm depth alongside with soil respiration using the temperature sensor of the soil respiration system and a moisture probe (Theta Meter HH1, Delta-T Devices, Cambridge, UK), respectively.

Leaf gas exchange was measured on three *Q. ilex* trees in at least three sun-exposed leaves per tree and in 10 sun exposed leaves of different *T. guttata* plants. During daytime a black plastic shield was used to cover the cuvette after each measurement in light to obtain dark respiration rates for each leaf. Predawn and midday leaf water potentials (pressure chamber, Manofrigido, Portugal) were also obtained for the same species.

Soil respiration (R_{soil}) was measured with three replicates on i): three plots of bare soil without further treatment and ii) three plots with root exclusion, which was achieved by solarization with black foils and trenching by metal rings buried in the soil three weeks before measurements were started (see **section 2.5**). Standard deviations were calculated through error propagation procedures.

Carbon flux partitioning was achieved by the *ecoflux* mass balance approach (see **section 2.7**).

For flux partitioning between understory and tree canopy I used data from a small *eddy covariance* system at a height of 2,5m (3D sonic anemometer, 1210R3, Gill Instruments Ltd., Lymington, UK; IRGA. LI-7500, LI-COR, Lincoln, NE, USA) installed at a nearby field site with similar conditions.

Analyses of correlation matrices between the modeled component fluxes and climate parameters were performed using *STATISTICA* 6.0 software (StatSoft, Tulsa, USA). R^2-coefficients of Pearson product-moment correlations and significances ($p<0.05$) were computed.

5.4 RESULTS

Variation in climate parameters and CO_2-fluxes

Meteorological conditions during the spring of 2006 were characteristic for Mediterranean regions with highest precipitation in March (104 mm) and April (57 mm) and increasing air temperature, radiation and VPD. Last rainfall event was on April 22, hence, when measurements were conducted during the end of May, the ecosystem already showed signs of drought stress (**Fig. 5.1a-h**).

During the study midday values of air temperatures and VPD increased from 24 to 31°C and from 19 to 36 hPa, respectively (**Fig. 5.1a-d**). Most days were sunny, except May 22 with rather cloudy conditions. On May 30 a particular combination of very high temperatures and comparatively low VPD occurred due to a very small nighttime rainfall event, not detected by precipitation sensors (**Fig. 5.1c**).

The soil (at 10 cm depth) was already very dry on May 20 (ca. 0.1 m^3 H_2O m^{-3}) and soil moisture did not exhibit a further decrease during the study (**Fig. 5.1e-h**).

Daytime ecosystem net CO_2-uptake decreased from -11 to -3 µmol m^{-2} s^{-1} with highest uptake rates in the morning and midday hours (**Fig. 5.1i-l**). Ecosystem respiration (R_{eco}) decreased from 3.5 to 1.5 µmol m^{-2} s^{-1} with a clear diurnal cycle of increasing respiration rates during daytime and a subsequent decrease at night (**Fig. 5.1q-t**). Soil efflux showed a similar pattern as R_{eco} though exhibiting lower rates (2.5 to 1 µmol m^{-2} s^{-1}).

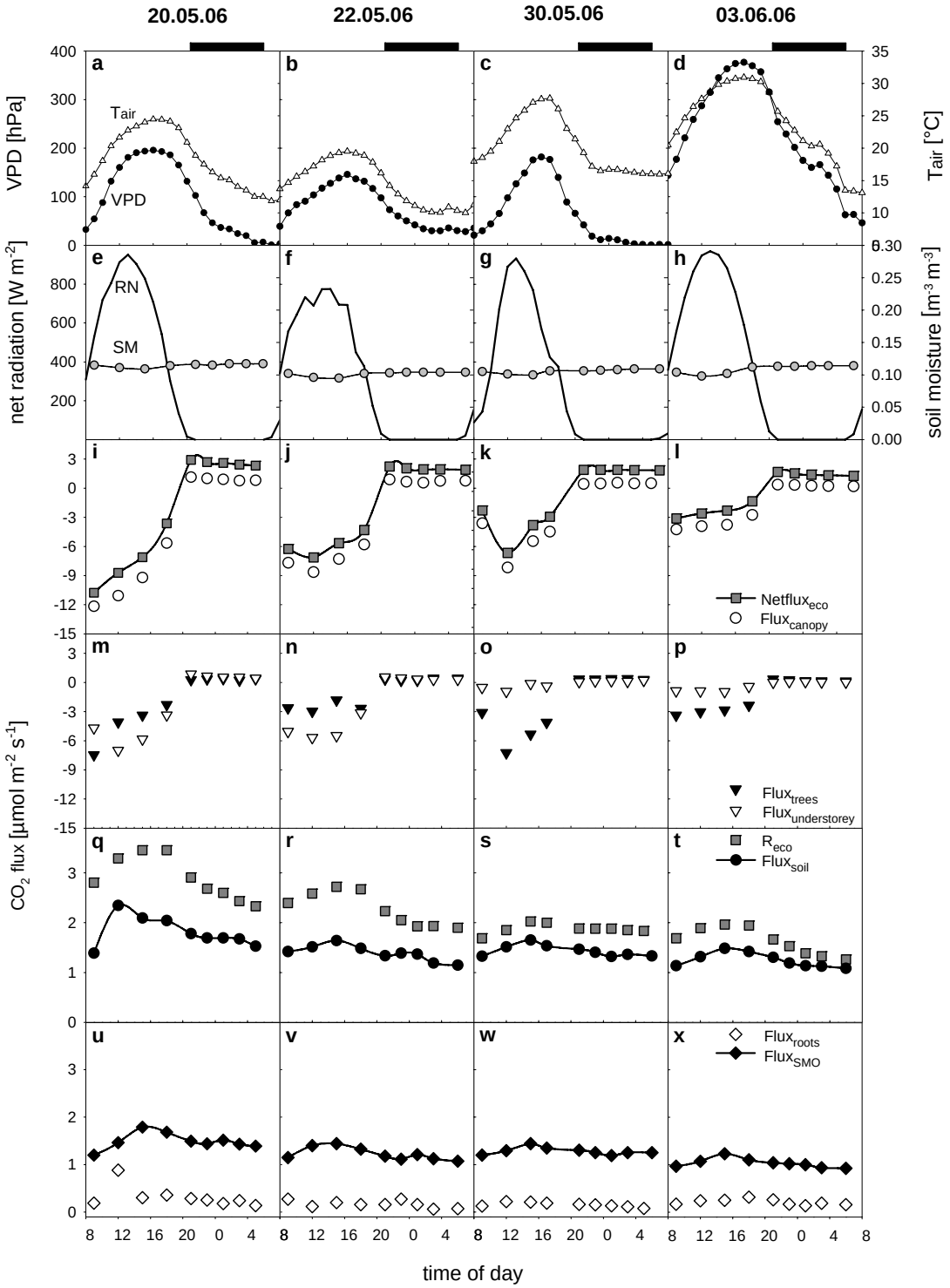

Fig. 5.1 Temporal variation in **a-d**: air temperature (T_{air}, white triangles) and VPD (black circles), **e-h**: net radiation (RN, lines) and soil moisture (grey circles), **i-l**: ecosystem CO_2-netflux (grey squares, lined) and foliage-derived flux (white circles), **m-p**: tree- (black reversed triangles) and understory-derived (white triangles) fluxes, **q-t**: R_{eco} (grey squares) and soil CO_2-efflux (black circles, lined), **u-x**: soil microorganisms- (black diamonds, lined) and root-derived (white diamonds) respiration for four diurnal cycles with increasing summer drought (May – June 2006). Please note that foliage flux values are referred to m^2 ground and not leaf area, all measured values are lined. Carbon uptake is denoted as negative flux, whereas respiration is denoted as positive flux. Black bars indicate nighttime.

Autotrophic and heterotrophic soil respiration was calculated from soil respiration measurements on intact soil and root-free trenching plots (**Fig. 5.1u-x**). Heterotrophic respiration of soil microorganisms (SMO) showed slightly smaller rates and a similar pattern as total soil efflux (2 to 1 µmol m^{-2} s^{-1}) whereas autotrophic respiration exhibited little variation over the observed period with very low rates of about 0.1 to 0.2 µmol m^{-2} s^{-1}.

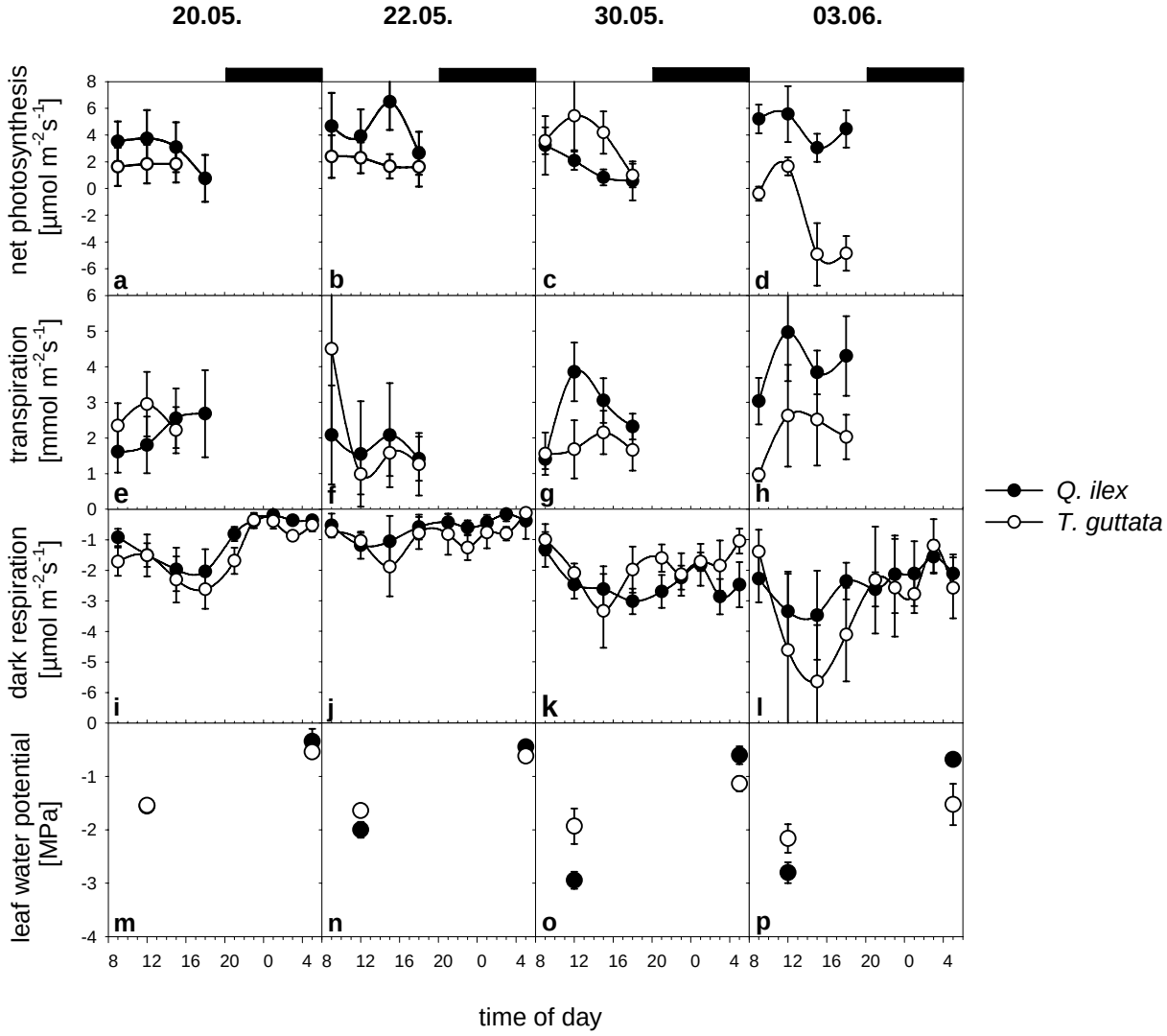

Fig. 5.2 Diurnal cycles of net carbon assimilation (**a-d**), transpiration (**e-h**), dark respiration (**i-l**) and leaf water potentials (**m-p**) from single leaf measurements of *Q. ilex* (tree, black circles) and *T. guttata* (understory species, white circles) during May and June 2006. Please note that net photosynthesis (positive rates) and respiration (negative rates) are referred to m^2 leaf area. Black bars indicate nighttime, n = 3-10 ± SD.

Using **equ. (1)** I calculated the canopy flux (comprising trees and understory) as the difference between net ecosystem flux and soil efflux (**Fig. 5.1i-l**). Given the small

contribution of soil efflux to daytime net ecosystem exchange, variation in calculated canopy flux was similar to variation in net ecosystem flux.

Single leaf photosynthesis and respiration measurements (**Fig. 5.2**) on trees (*Quercus ilex*) and understory plants (e.g. *Tuberaria guttata*) reflected different responses to increasing water deficit and temperature. Even with decreasing water availability and increasing temperature stress *Q. ilex* was able to maintain predawn leaf water potentials around -0.5 MPa (**Fig. 5.2m-p**) and maximum daytime photosynthetic carbon uptake did not vary much (4 to 6 µmol m^{-2} s^{-1}, **Fig. 5.2a-d**). Daytime transpiration (**Fig. 5.2e-h**) increased with temperature from 1 to 3.5 mmol m^{-2} s^{-1}, indicating sufficient water supply for cooling through transpiration. In contrast, understory plants like *T. guttata* were severely affected by the changing climatic conditions and died off during the study period. Despite the low predawn leaf water potentials -1.5 MPa (**Fig. 5.2m-p**) transpiration rates (**Fig. 5.2e-h**) of *T. guttata* were persistently in a similar range as *Q. ilex* between 1.5 and 3 mmol m^{-2} s^{-1}. The low net photosynthetic uptake on May 20 still being about 2.5 µmol m^{-2} s^{-1} decreased on June 3 below 0, since carbon loss by respiration especially in the afternoon (up to -6 µmol m^{-2} s^{-1}) was higher than carbon gain by photosynthesis (**Fig. 5.2a-d**; **i-l**). Leaf respiration in *Q. ilex* also increased throughout the study, but with a lower magnitude (-3 µmol m^{-2} s^{-1}; **Fig. 5.2 i-l**).

Daytime net canopy flux of the understory, as calculated by subtracting soil respiration from the understory flux, showed a constant decrease in daytime CO_2-uptake (-7 to -0.5 µmol m^{-2} s^{-1}) and in nighttime respiration (0.5 to 0.1 µmol m^{-2} s^{-1}) reflecting the effects of water deficit on understory plants (**Fig. 5.1m-p**). Net canopy flux of the trees was calculated as difference of canopy flux and net canopy flux of the understory. Daytime net canopy flux of *Q. ilex* varied between -3 and -7 µmol m^{-2} s^{-1} exhibiting lowest values in the morning and midday hours of May 20 (**Fig. 5.1m**) and 30 (**Fig. 5.1o**). May 22 being rather cloudy with lower light intensities (see **Fig. 5.1f**) and June 3 with exceptional high VPD (**Fig. 5.1d**) revealed a higher daytime canopy flux for *Q. ilex* (**Fig. 5.1n, p**). Nighttime respiration decreased only slightly from 0.3 to 0.2 µmol m^{-2} s^{-1}.

These results show that daytime net ecosystem exchange rate was dominated by CO_2-uptake through the canopy (up to 86%), whereas nighttime ecosystem exchange was mainly due to soil respiration (up to 82%).

Response of component fluxes to drought and relative contribution to ecosystem flux

Decreases in both ecosystem respiration and carbon uptake were mainly caused by a strong decrease in understory activity with progressive water deficit (**Fig. 5.3a, b**). Canopy respiration decreased strongly (1.3 to 0.3 µmol m^{-2} s^{-1}), whereas soil respiration decreased only slightly (1.9 to 1.3 µmol m^{-2} s^{-1}), to a large part due to a loss in root respiration of about 0.2 µmol m^{-2} s^{-1}. Nighttime respiration was always smaller than daytime respiration (**Fig. 5.3a**).

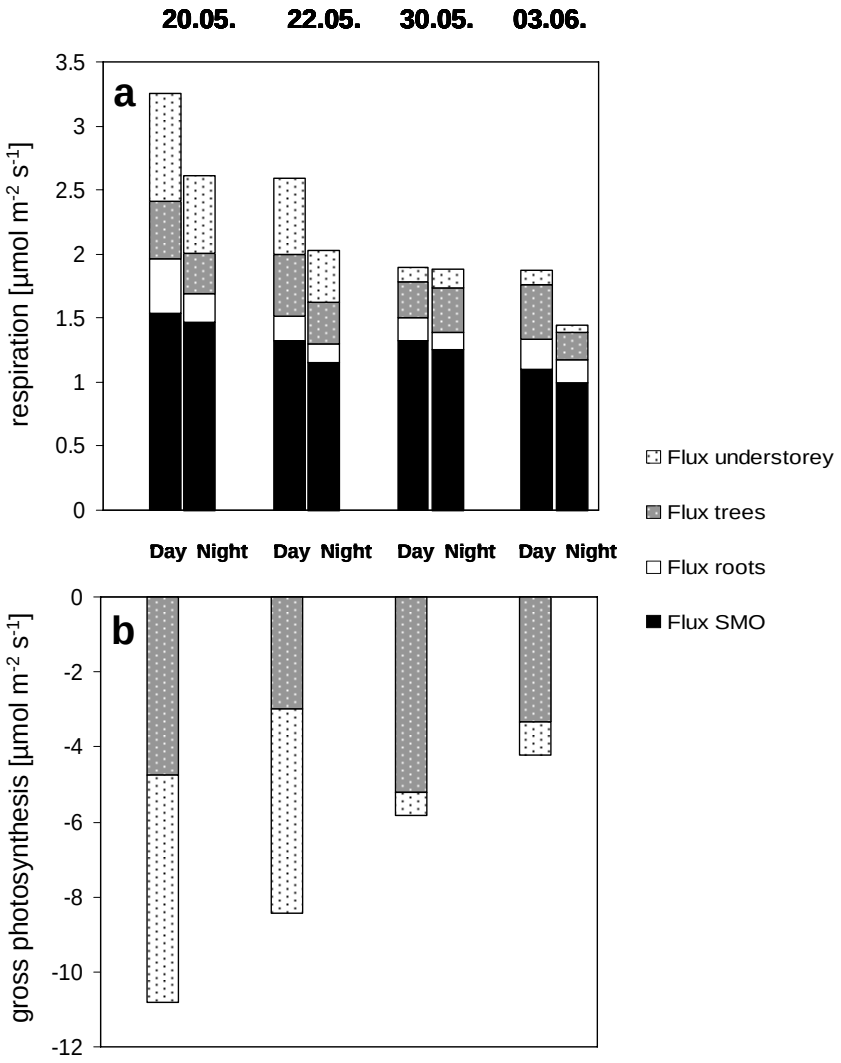

Fig. 5.3 Mean diurnal and nocturnal respiration fluxes (**a**) and gross carbon assimilation (**b**) from understory (dotted), trees (grey), roots (white) and soil microorganisms (black) during May and June 2006. Please note that foliage flux values are referred to m^2 ground and not leaf area. Carbon uptake is denoted as negative flux, whereas respiration is denoted as positive flux.

Daytime respiration and photosynthesis of the canopy was calculated by subtracting soil efflux during daytime from R_{eco} and R_{eco} from net flux, respectively. I assumed that proportionate contributions of trees and understory to canopy respiration remain constant between day and night and thus, used nighttime canopy respiration to partition daytime net flux of trees and understory into respiration and gross photosynthesis. Gross CO_2-uptake (**Fig. 5.3b**) remained more or less constant in trees (-3 to -5 µmol m^{-2} s^{-1}) but decreased strongly in the understory (-6 to -0.8 µmol m^{-2} s^{-1}) with increasing temperature and VPD.

Fig. 5.4 shows the relative contributions of component fluxes to the total ecosystem flux. During the study contribution of tree respiration to daytime and nighttime ecosystem respiration increased about 3 and 9%, respectively. In contrast, the influence of understory respiration decreased dramatically from 33.2 to 3.5% and from 25.7 to 5.7% for night and day, respectively (**Fig. 5.4a**). Heterotrophic soil respiration was the main respiration source in the ecosystem and exhibited an increase of ~12%, whereas the generally small contribution of autotrophic soil respiration only showed small changes (+4.3% and -0.2% for night and daytime, respectively; **Fig. 5.4a**). Canopy respiration varied more between day and night (30-50%) than soil respiration (10%).

Gross ecosystem CO_2-uptake on May 20 was still dominated by the understory (56%), with a marked reduction to only 21% at the beginning of June (**Fig. 5.4b**).

While understory plants were still the main contributors to total diurnal CO_2-exchange (45%) on May 20, on June 3 trees (52.8%) and soil microorganisms (27.6%) became most significant contributors to the sink strength of the ecosystem. Thus, although the system remained a net sink for CO_2, in early June it was on the verge of becoming a net source (**Fig. 5.4c**; see also **Fig. 5.3**).

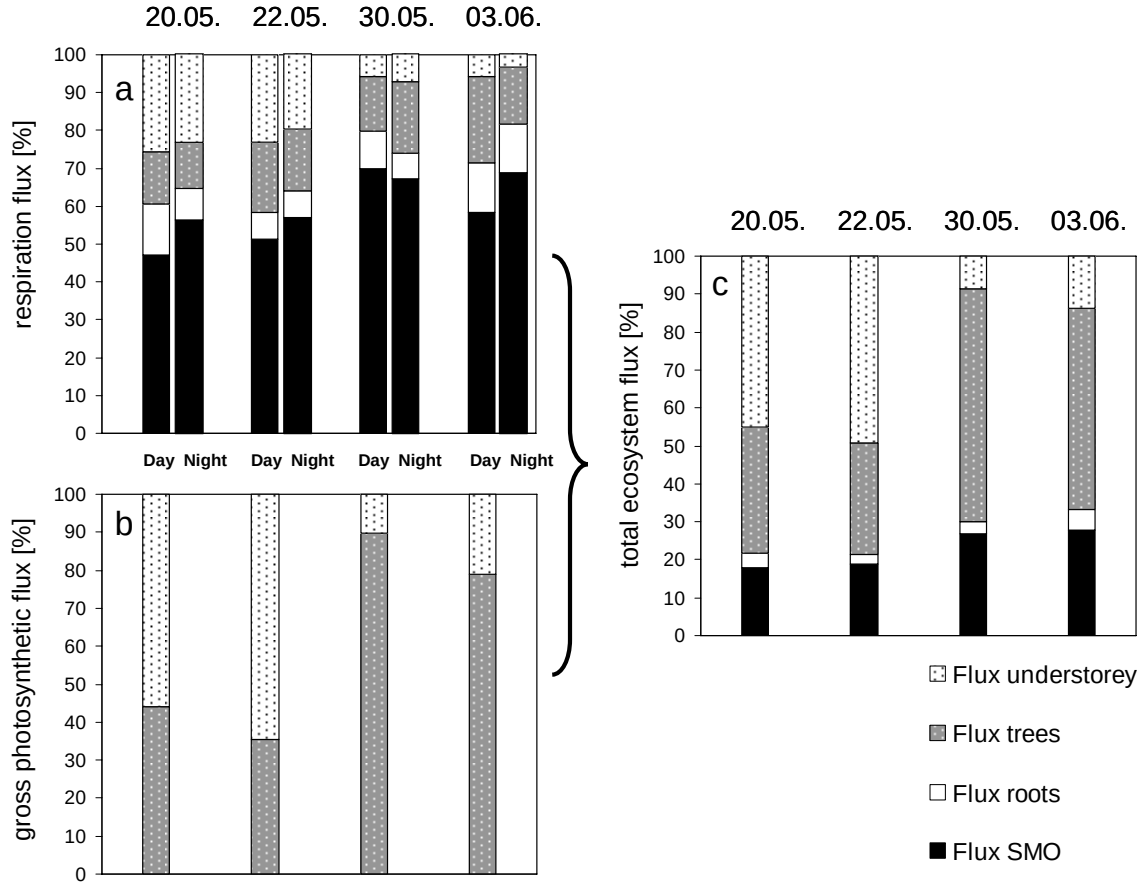

Fig. 5.4 Relative contributions of component fluxes (%) to (**a**) total ecosystem respiration, (**b**) total ecosystem carbon assimilation and (**c**) to total diurnal CO_2-exchange during day and nighttime; calculated from data shown in Fig. 5.3; understory (dotted), trees (grey), roots (white) and soil microorganisms (black).

5.5 DISCUSSION

In this field study I partitioned assimilatory and respiratory contributions of the main ecosystem components from the respective overall carbon fluxes in an evergreen oak-savannah ecosystem in response to increasing water deficit at the onset of increasing drought in the Mediterranean. To my knowledge, this is the first approach to disentangle all major assimilation and respiration fluxes of an ecosystem and their respective biotic and abiotic drivers in response to short-term changes in environmental conditions.

In the following, the differential responses of ecosystem flux components (trees, understory, soil microorganisms and roots) to changes in the main abiotic drivers of drought (particularly air temperature and soil moisture) and their impact on the sink strength of the ecosystem will

be discussed. Further, the effects of changes in carbon input via photosynthesis on the respiratory fluxes of these ecosystem components in response to drought will be assessed.

Contributions of ecosystem components to overall ecosystem fluxes

Ecosystem respiration (R_{eco}) was mainly dominated by soil CO_2-efflux, which contributed on average ~70% during daytime and ~65% during nighttime. This is in the range of soil efflux accounting for 60 to 80% of R_{eco}, as observed in other studies (e.g. Goulden *et al.*, 1996; Lavigne *et al.*, 1997; Law *et al.*, 2001a; Davidson *et al.*, 2006a). The major component of soil respiration was heterotrophic, i.e. soil microorganisms, which comprised up to 69% of R_{eco}. Autotrophic soil respiration rates were very small and did not show significant changes with decreasing water availability. The autotrophic component of soil respiration (ca. 20%) and R_{eco} (ca. 10%) was rather small, but in the range of other studies, which found a strong variability in contribution of root respiration to soil efflux ranging from >10 to <90% over a wide range of ecosystems and climate types (Hanson *et al.*, 2005; Subke *et al.*, 2006).

During the day NEE was controlled to 86% by canopy activity. Therefore, diurnal courses of canopy flux and NEE had similar patterns. Contributions of canopy respiration to R_{eco}, including both foliage and trunk respiration, ranged between 19 and 35% during the night and 28 to 40% during the day. These proportions were slightly lower than contributions observed by Lavigne *et al.* (1997) in a boreal forest (foliage 25-43%, wood 5-15%), but in the range of studies from Goulden *et al.* (1996, foliage 27%, wood 5%) and Law *et al.* (1999, foliage 18%, wood 6%) for a temperate deciduous and a dry climate ponderosa pine forest, respectively. The contribution of tree respiration to total ecosystem respiration flux increased with drought by about 6%, whereas influence of understory respiration decreased dramatically (from ~30% to ~5%).

While understory plants on May 20 were still the main contributors to total diurnal CO_2-exchange (assimilation and respiration), trees and heterotrophic soil respiration became increasingly significant for ecosystem carbon balance with increasing water deficit, turning the system slowly towards becoming a net source for carbon. Data from previous years for this system showed that net fluxes turn positive between the middle of June and the beginning of July (DOY 170 – 200), depending on the last significant rain falls (data not shown).

Responses of component fluxes to changes in abiotic drivers

With ongoing transition from wet and mild spring period to dry and hot summer conditions I observed a constant decrease of more than 50% in both NEE and R_{eco} within a single fortnight (see **Fig. 5.1**). Decreasing NEE towards the summer is generally due to constrained carbon assimilation by the vegetation with decreasing water availability and increasing temperature (e.g. Beyschlag *et al.*, 1987; Pereira *et al.* 2007) and is a characteristic of Mediterranean type ecosystems (Reichstein *et al.*, 2002b; Rambal *et al.*, 2003). Similarly, strong declines in GPP (and also in NEE) in response to drought have been reported in various studies (e.g. Pereira *et al.*, 1995; 2007; Baldocchi, 1997; Reichstein *et al.*, 2002b; Jarvis *et al.*, 2007) and were also observed in previous years at the same site, depending on the timing of the onset of seasonal drought (Jarvis *et al.*, 2007; Pereira *et al.*, 2007).

R_{eco} exhibited similar values as observed in studies by Rambal *et al.* (2004) and Reichstein *et al.* (2002a,b) for late spring in other Mediterranean *Q. ilex* dominated sites. A wide range of studies have shown that R_{eco}, as with all respiration processes, is positively correlated with temperature. Moreover it is correlated with soil moisture, which under drought conditions can have a substantial influence and mask the temperature response (e.g. Davidson *et al.*, 2000; Reichstein *et al.*, 2002a,b; Xu *et al.*, 2004; Gaumont-Guay *et al.*, 2006; Jarvis *et al.*, 2007). Several studies have observed that temperature dependence (Q10) of R_{eco} increases with soil moisture (Reichstein *et al.*, 2002a,b; Knohl, 2003; Flanagan & Johnson, 2005). In systems without water limitation R_{eco} tends to be mainly determined by temperature (e.g. Huxman *et al.*, 2003; Griffis *et al.*, 2004). However, in this study soil moisture was limiting, but did not vary significantly during the observed period and thus, does not explain the decrease in R_{eco} (see **Fig. 5.1e-h**). Furthermore, the increasing air temperatures should have caused an increase in R_{eco}. Hence, a more likely cause of the decrease in R_{eco} is dwindling contribution of understory biomass (mainly herbs), which died off during the observed period (observation at field site). However, soil moisture may have played a more important role than I was able to detect by only monitoring changes in the upper 10cm of soil. I might have missed significant changes in deeper soil layers, which could have affected overall soil respiration (Buchmann, 2000). Predawn water potentials of shallow-rooted understory species (*T. guttata*, see **Fig. 5.2m-p**) were still high (-0.5 MPa) at the beginning of the experiment, indicating that soil moisture measurements in the upper soil layer did not accurately reflect the general soil water status. For precise interpretation of temporal variation in R_{eco} that cannot be attributed to

temperature, Reichstein *et al.* (2005) highlights the importance of chamber methods in order to measure component fluxes in addition to *eddy covariance*.

Soil CO_2-efflux followed a similar trend as R_{eco} with smaller absolute rates, comparable to other studies in Mediterranean and semiarid sites (e.g. Irvine *et al.,* 2005; Tang & Baldocchi, 2005). Towards June R_{eco} and R_{soil} became increasingly similar, as found by Davidson *et al.* (2006a), where summer drought effects were visible in an initial increase in R_{soil}/R_{eco}, followed by stabilization with severe drought. This observed increase in R_{soil}/R_{eco} was due to the strong drought effect on canopy respiration.

Heterotrophic and autotrophic portions of soil respiration responded differently to increasing water deficit. The decrease in heterotrophic soil respiration was stronger than in autotrophic soil respiration, whose contribution was small but stable. This is in agreement with other studies (Borken *et al.*, 2006; Scott-Denton *et al.*, 2006), who found that root respiration was less susceptible to drought than heterotrophic soil respiration.

The decrease in heterotrophic soil respiration was small (about 20%), compared to the strong decrease observed in R_{eco} and cannot be explained by measured soil water content or temperature, as discussed above. A reason for this decrease could be temperature acclimatization of heterotrophic soil respiration (e.g. Zhang *et al.*, 2005), which can be explained by simple substrate depletion of labile C-pools with increasing temperature, as found by Eliasson *et al.* (2005). In spite of this decrease with increasing water deficit, the relative contribution of heterotrophic soil respiration to R_{eco} increased by 12%. Since changes in relative contributions of tree- and root fluxes to the ecosystem flux are negligible, this increase can be attributed to the decrease in relative contribution of the understory flux. This indicates clearly that total ecosystem response to changing environmental conditions might be different from the response of single component fluxes, because it depends on the interplay of all sources.

Respiration at nighttime was always smaller than daytime respiration, which can be explained by the nocturnal decrease in air temperature. Soil as well as ecosystem respiration at a short time scale (a diurnal cycle) were still positively correlated with temperature (data not shown). On a larger time scale (a fortnight) the correlation turned negative. Tang & Baldocchi (2005) showed similar results for another Mediterranean oak-grass savannah, concluding that the seasonal pattern of soil respiration was driven mainly by soil moisture but that the diurnal

pattern was controlled by temperature and in root rich patches (under tree crowns) also by photosynthesis.

The magnitudes of canopy respiration and canopy scale carbon uptake both decreased by two thirds with increasing water deficit and temperature. Neither assimilation nor respiration of trees (*Q. ilex*) responded markedly to warming and drought, indicating an apparent ability to extract sufficient water from deeper soil layers, as was also observed in others studies at this site (e.g. Kurz-Besson *et al.*, 2006). Assimilation rates were mainly controlled by radiation and VPD, rather than temperature and soil moisture. Further, respiration rates did not change significantly, although most studies show positive relationships between foliage respiration and increases in temperature and soil moisture (e.g. Damesin, 2003; Atkin *et al.*, 2005; Lee *et al.*, 2005). Hartley *et al.* (2006) suggests that canopy photosynthesis regulates leaf respiration to a much larger extent than below-ground respiration and that temperature effects might play a more significant role in the latter, thus, explaining stable respiration rates in trees. Single leaf measurements on *Q. ilex* confirmed the canopy scale results. Single leaf assimilation patterns were very similar to patterns observed at the canopy scale and predawn water potentials of *Q. ilex* (see **Fig. 5.2**) remained stable even in June, as observed by David *et al.* (2007).

Understory plants, on the other hand, were severely affected by seasonal drought, decreasing their assimilation and respiration rates as well as their contribution to overall CO_2-uptake (56 to 21%) and canopy respiration (23.2 to 3.5%). Accordingly, predawn water potentials and net assimilation rates of *T. guttata* (main understory species) showed a strong decrease. Tissue repair will increase foliage respiration (Law *et al.*, 1999), but reduced assimilation decreases respiration (Amthor, 1994). In spite of the canopy scale decrease, dark respiration of single leaves of the understory species *T. guttata* increased with temperature to extraordinarily high rates of up to 6 μmol m^{-2} s^{-1}, which can be attributed to accelerated metabolism in dying tissues (see **Fig. 5.2i-l**). The decrease in understory respiration at the canopy scale was therefore, entirely due to a decrease in leaf area index (LAI) by senescence with increasing water deficit.

Tab. 5.1 Pearson coefficients for linear regressions of temporal changes in component fluxes from soil microorganisms, roots, understory and tree foliage with daytime averages of climate parameters and predawn water potentials of *T. guttata*. Significant relationships ($p<0.05$) are in bold.

		RN	T_{air}	T_{soil}	VPD	SWC	WP_{PD}
trees	*Nighttime CO_2-release*	**-0.86**	**-0.70**	-0.68	**-0.99**	-0.06	0.67
	Daytime CO_2-release	0.60	-0.41	-0.44	0.14	0.05	0.44
	Daytime CO_2-uptake	0.42	0.15	0.16	-0.38	0.55	0.1
understory	*Nighttime CO_2-release*	0.04	**-0.72**	**-0.74**	-0.52	0.51	**0.94**
	Daytime CO_2-release	0.15	**-0.72**	**-0.74**	-0.43	0.46	**0.92**
	Daytime CO_2-uptake	0.12	**-0.79**	**-0.82**	-0.44	0.29	**0.92**
SMO	*Nighttime CO_2-release*	-0.24	-0.42	-0.42	-0.61	**0.70**	**0.73**
	Daytime CO_2-release	-0.26	-0.64	-0.64	**-0.71**	0.57	**0.88**
	Daytime CO_2-uptake	-	-	-	-	-	-
roots	*Nighttime CO_2-release*	**0.81**	0.16	0.13	0.42	**0.84**	0.22
	Daytime CO_2-release	**0.73**	0.00	-0.03	0.10	**0.95**	0.43
	Daytime CO_2-uptake	-	-	-	-	-	-

I correlated daytime averages of T_{air}, VPD, soil water content and T_{soil} as well as cumulative net radiation and predawn water potentials of *T. guttata* with temporal changes in component fluxes (**Tab. 5.1**). Most significant correlations were obtained for understory fluxes with temperature and predawn water potentials as a measure for plant accessible soil water. As expected, I found a strong negative relationship for air and soil temperatures and a positive relationship for predawn water potentials with understory respiration and assimilation rates. The strong relationships with predawn water potentials confirm the above discussed importance of soil moisture for the carbon balance of the system. Relationships of understory

fluxes with predawn water potentials were even stronger (R^2 0.92 - 0.94) than temperature correlations (R^2 max. -0.82). Soil water content, on the other hand, was not significantly correlated with the understory flux, revealing the insufficiency of soil moisture data collection as restricted to the upper soil layer.

Temporal dynamics of respiration from soil microorganisms showed a positive relationship with predawn water potentials of understory foliage (*T. guttata*) but no significant correlation with temperature. This suggests that, as discussed above, the effects of soil moisture on respiration processes under seasonal drought overlaid the temperature effects. I found a relationship for both night- and daytime CO_2-release of roots with soil water content. Since neither soil water content, nor root respiration changed to a substantial extent, these relationships are regarded as random and non-causal. Similarly, the relationships between nighttime respiration in trees and RN and VPD are probably coincidental. As described above, contribution of the understory to overall ecosystem respiration decreased, whereas proportion of heterotrophic soil flux increased with increasing water deficit (see **Fig. 5.4a**). Similarly, trees became most significant contributors to ecosystem carbon uptake (**Fig. 5.4c**). Regarding that neither trees nor soil microorganisms increased their actual flux rates in response to drought, it might therefore, be concluded that their increasing contribution to overall ecosystem flux was entirely due to the disappearance of the understory, which was mainly triggered by decreasing water availability and increasing temperatures.

Link between R_{eco} and carbon assimilation

A strong relationship between aboveground carbon assimilation and belowground allocation and therefore, respiration has been observed in several studies (e.g. Ekblad & Högberg, 2001; Bowling *et al.*, 2002; Mortazavi *et al.*, 2005; Werner *et al.*, 2006) and nowadays is widely accepted. Assimilation is linked to below ground respiration with a time-lag, ranging from hours to days, which is generally attributed to the time of carbon transport to the roots and subsequent release via root exudation and/or rhizospheric respiration (e.g. Ekblad & Högberg, 2001). Hence, diminishing substrate supply by photosynthesis to autotrophic and heterotrophic soil respiration (compare Knohl, 2003; Verburg *et al.*, 2004; Davidson *et al.*, 2006b) might have been an important factor affecting temporal changes in R_{eco} with increasing water deficit. Plant activity was found to be temporally (e.g. Boone *et al.*, 1998; Kuzyakov & Cheng, 2001; Ekblad *et al.*, 2005; Irvine *et al.*, 2005) and spatially (e.g. Law *et*

al., 2001b; Søe & Buchmann, 2005; Tang & Baldocchi, 2005) associated with soil respiration. Some authors conclude that photosynthesis could trigger soil respiration by accelerating the autotrophic respiration (e.g. Boone *et al.*, 1998; Tang *et al.*, 2005) due to higher substrate production while others have found an acceleration of heterotrophic respiration via root exudates, which can cause priming (e.g. Kuzyakov & Cheng, 2001; Heath *et al.*, 2005). However, these effects of photosynthesis on soil respiration are difficult to separate. Subke *et al.* (2006) showed that roots contributed to a larger extent in systems with high soil CO_2-effluxes. They discussed this pattern with smaller carbon allocation to the roots and subsequent smaller autotrophic respiration flux in ecosystems with low productivity. The results for the low-productive Mediterranean oak-savannah fit this theory. Low soil respiration rates coincided with low root contribution (15 - 28%). Root respiration rates were small and remained stable even with senescence of aboveground biomass, thus, a large effect of photosynthesis during the studied period is not very likely. Since the system was already subjected to prolonged drought when measurements started, it may be argued that understory root metabolism was already inhibited and therefore, did not play a significant role for ecosystem carbon balance. Respiration from deep tree roots could not be accounted for, since trenching only affected the upper soil layer (20 cm). Nevertheless the largest fraction of root biomass is found in the upper soil layers (Kurz-Besson *et al.*, 2006; Otieno *et al.*, 2006). Soil respiration plots were fairly distant from trees, thus, effects of tree root respiration are expected to be negligible. Furthermore, soil respiration was not decoupled from diurnal temperature correlation as in Tang *et al.* (2005). Hence, root respiration data in this study may only reflect severe drought effects on understory plants, unaffected by canopy gas exchange of trees. For this partitioning study I therefore, expect tree root as well as trunk respiration to be included in the tree foliage flux.

As discussed above I found that autotrophic soil respiration remained stable, whereas heterotrophic soil respiration decreased with increasing water deficit. Carbohydrates stored in roots may buffer effects of reduced assimilate supply and sustain root metabolism for several days or even weeks after removal of aboveground biomass (Pregitzer *et al.*, 2000; Högberg *et al.*, 2001). This was confirmed by another study, where clipping did not affect root respiration for several days but soil microbial respiration responded strongly to short-term changes in assimilate supply (Bahn *et al.*, 2006).

Foliage derived respiration is even more closely linked to assimilate supply from photosynthesis. Many studies find relationships between foliage respiration and soluble

sugars, specific leaf area, leaf nitrogen and light habitat (e.g. Lusk & Reich, 2000; Atkin & Tjoelker, 2003; Damesin, 2003; Lee *et al.*, 2005). Rambal *et al.* (2004) partitioned respiration of *Q. ilex* attributed to growth from whole ecosystem respiration and found highest respiration rates in May of about 0.8 to 1 µmol m^{-2} s^{-1} declining rapidly towards the end of June. In the studied system, leaf growth and development of *Q. ilex* was already completed by the end of May, thus, trees exhibited lower respiration rates of about 0.3 to 0.4 µmol m^{-2} s^{-1}.

Canopy respiration varied more between day and night (30-50%) than soil respiration (10%). This may be explained by the positive correlation between temperature and leaf respiration.

Q10 in foliage respiration is assumed to be higher than in root respiration or soil respiration (e.g. Loveys *et al.*, 2003). Furthermore, light affects foliage respiration positively through enhanced substrate supply by photosynthesis as well as by enhanced maintenance respiration (e.g. Lambers *et al.*, 1983; Lusk & Reich, 2000). In contrast, soil respiration reacts more slowly to photosynthetic activity and is merely a function of temperature.

Root respiration was positively correlated to incident solar radiation (**Tab. 5.1**), which is a well known driver of photosynthesis. Increasing root respiration rates with higher light levels are therefore, not surprising. One could argue that in this case understory foliage should also be related to RN, which it is not. An explanation might be that proportions in root respiration to total ecosystem respiration did not decrease but increased with drought, while understory foliage decreased strongly due to senescence (see **Fig. 5.4a**). While, as discussed above, roots may well sustain on stored carbohydrates over a certain period after foliage death (e.g. Bahn *et al.*, 2006), they are inside the soil and thus, protected from heat and water loss. Therefore, they die off more slowly as compared to leaves. The fact that RN correlates with root respiration fluxes but not with foliage respiration leads also to the assumption that remaining assimilated carbon in senescent plant foliage is transferred to the root system in order to conserve energy for a possible resprout when conditions change.

5.6 CONCLUSIONS

This is the first report to disentangle abiotic and biotic drivers of all important component fluxes influencing the overall sink strength of a Mediterranean ecosystem during a spring to summer transition period. The drought sensitive understory plant species were found to play the most important role, determining the rapid decrease in CO_2-uptake of the open Q. ilex forest with increasing water deficit, since their contribution to total diurnal CO_2-exchange

exhibited the most significant decrease (from 45 to 14%). This surprisingly high importance of the understory is crucial for the source/sink behavior of the whole ecosystem and the timing of the onset of drought may largely determine annual carbon budget.

Further, the partitioning approach revealed that total ecosystem respiration was increasingly dominated by heterotrophic soil respiration with decreasing water availability, even though the associated rates of soil microorganisms decreased. Similarly, the relative contribution of tree carbon assimilation to diurnal ecosystem CO_2-uptake increased, although absolute rates did not change. Hence, *eddy covariance* measurements without simultaneous chamber measurements of component fluxes (soil, roots, understory) are not sufficient for accurate interpretations of changes in ecosystems sink strength and their underlying processes. I therefore, recommend using component flux measurements and subsequent partitioning along with *eddy covariance* measurements in more ecosystems to better explain changing ecosystem sink strength with global climate change.

5.7 AUTHORS CONTRIBUTIONS

The author of this thesis accomplished the entire experimental work and the writing of this chapter. Further contributions were given by C. Werner who coordinated the project, C. Máguas who provided the stable isotope lab, J.S. Pereira who provided the field site and *eddy-flux* data, L. Aires who provided *eddy-flux* data of the understory and T.S. David who provided climate data. Further, I acknowledge contributions of P. Oliveira (*Mezão*) for technical help with equipment and V. Andrade for *eddy-flux* data treatment.

CHAPTER 6

STABLE ISOTOPE MASS BALANCES - A NEW TOOL TO IDENTIFY VARIATION IN ISOTOPIC COMPOSITION OF ECOSYSTEM AND SOIL RESPIRATION

6.1 ABSTRACT

A thorough understanding of processes driving respiration and its isotopic composition at the ecosystem level has become of major interest. Partitioning ecosystem respiration (R_{eco}) into its component fluxes (e.g. R_{soil}, R_{root}, R_{leaf}) is an important tool to disentangle these processes. Her, I partitioned R_{eco} into all major component fluxes during a spring to summer transition period with increasing water deficit and temperature with both respiration flux partitioning (*ecoflux*) and stable isotope partitioning (*eco-isoflux*) approaches. I was able to validate the isotope model at the soil scale, while at the ecosystem scale uncertainties in R_{eco} due to small CO_2-gradients for *keeling plot* calculation were responsible for partial deviations between *ecoflux* and *eco-isoflux* modeled data. Decreasing respiration from drought sensitive understory plants was found to be the major reason for the decrease in R_{eco} with seasonal drought. Furthermore, I found large variation in isotopic composition of CO_2 respired ($\delta^{13}C_{res}$) from foliage and roots in response to seasonal drought, at both diurnal and fortnight time scales. While foliage respiration exhibited enrichment during day (up to 6‰) and a subsequent depletion during night, $\delta^{13}C_{res}$ from roots exhibited an opposite pattern, showing increasing enrichment at nighttime (up to 5.5‰). This effect became more pronounced with increasing drought and temperatures. These findings are in accordance with recent theories regarding post-photosynthetic fractionation in the dark respiratory pathways and during phloem loading. The results shown here contribute to a process-based understanding of isotopic variation in ecosystem respired CO_2, which is crucial to model carbon exchange between ecosystems and atmosphere and possible future climate scenarios.

6.2 INTRODUCTION

Ecosystem respiration (R_{eco}) is one of the major determinants of the carbon balance in most terrestrial ecosystems (Valentini *et al*., 2000; Rambal *et al*., 2004). Depending on key climatic variables such as temperature and moisture (e.g. Huxman *et al*., 2003; Xu *et al*., 2004;

Davidson *et al*., 2006b), changes in ecosystem respiration with increasing global warming might be substantial. Thus, having a process-based understanding of respiration at the ecosystem level has become increasingly desirable within recent years. However, understanding the complex interplay of processes underlying the response of ecosystem carbon sequestration to climate change is not simple, since different carbon pools as well as respiratory sources (e.g. autotrophic and heterotrophic respiration) are expected to respond differentially to changes in the abiotic environment.

Partitioning the ecosystem respiration flux (R_{eco}) into its component fluxes (soil, roots, foliage respiration) is an important tool to estimate their contribution to the ecosystem carbon budget as well as to identify their responses to abiotic drivers. Many approaches comprising mass balance calculations at the ecosystem (e.g. Law *et al*., 2001; Davidson *et al*., 2006a) and soil scale (see Subke *et al*., 2006) emerged within recent years. However, apart from several long-term studies regarding annual carbon budgets (Goulden *et al*., 1996; Lavigne *et al*., 1997; Law *et al*., 1999; Law *et al*., 2001; Davidson *et al*., 2006a; Misson *et al*., 2007; Zha *et al*., 2007) partitioning between all major respiratory fluxes (foliage, roots and soil microorganisms) on the ecosystem scale has seldom been achieved.

Stable carbon isotopes can help to disentangle the drivers of respiration as well as the complex exchange processes between carbon pools on a wide range of temporal and spatial scales (Dawson *et al*., 2002). Still, a general understanding of isotope effects during carbon sequestration has not yet been achieved and the interplay of processes driving ecosystem scale variation in $\delta^{13}C$ of respiration remains largely unknown. Isotopic composition of CO_2 respired by ecosystem components ($\delta^{13}C_{res}$) such as soil, roots and leaves can be measured with chamber and incubation methods and serves to acquire information about processes driving carbon fluxes at the ecosystem scale. Substantial seasonal as well as $\delta^{13}C_{res}$ from leaves (e.g. Hymus *et al*., 2005; Mortazavi *et al*., 2005; Werner *et al*., 2007b), roots (e.g. Klumpp *et al*., 2005), soils (e.g. McDowell *et al*., 2004b; Ekblad *et al*., 2005) and ecosystems (e.g. Bowling *et al*., 2003; Knohl *et al*., 2005; Werner *et al*., 2006, 2007a) have been observed. Variability in photosynthetic discrimination is thought to largely determine isotopic composition of leaf, root, soil and even ecosystem respiration. Several studies showed the close linkage between carbon assimilation and respiration, which exhibits a time-lag ranging from hours to days (e.g. Ekblad & Högberg, 2001; Bowling *et al*., 2002; Mortazavi *et al*., 2005; Werner *et al*., 2006). Another reason for variation in $\delta^{13}C_{res}$ and $\delta^{13}C_R$ might be temporal changes in supply and utilization of different respiratory substrates (e.g. old vs. fresh

carbon). Additionally, there is now significant evidence for substantial metabolic fractionation in the dark respiratory pathways of leaves (Tcherkez *et al*., 2003; for a review see Ghashghaie *et al*., 2003 and Werner *et al*., 2007a; compare **section 1.3**) and during carbon transport and allocation to stem and roots (Tcherkez *et al*., 2004; compare **section 1.3**).

Variability in $\delta^{13}C_{res}$ of different ecosystem components will translate into changes of isotopic signature of respiration at the ecosystem level ($\delta^{13}C_R$). $\delta^{13}C_R$, as derived by *keeling plots* (a two-source mixing model describing mixing between atmospheric and ecosystem derived CO_2 (Keeling, 1958)) can potentially be applied to isotopic mass balance approaches for partitioning studies of the respiration flux at the ecosystem scale. $\delta^{13}C_R$ is known to vary seasonally with changes in climatic conditions, driving photosynthetic discrimination and respiratory activity (e.g. Bowling *et al*., 2001, 2002; Ponton *et al*., 2006; Werner *et al*., 2006, 2007a). Recent studies showed small (Ogée *et al*., 2003; Still *et al*., 2003; Schnyder *et al*., 2004) to large (up to 6‰, e.g. Bowling *et al*., 2003; Werner *et al*., 2006) variation in $\delta^{13}C_R$ even within a single night**.**

However, there are various problems associated with the determination of $\delta^{13}C_R$, since the *keeling plot* method assumes a stable background signal during the sampling period as well as a sufficiently high CO_2-gradient (~30 to 50ppm) for reliable *keeling plot* calculations (Pataki *et al*., 2003a). In ecosystems with low respiratory activity (e.g. during Mediterranean summer drought) it can be difficult to capture these CO_2-gradients within a time frame of 30 min, where presumably no variation in the background signal occurs.

Measuring all major respiratory component fluxes and their respective isotopic signals does enable $\delta^{13}C_R$-estimates independently from the *keeling plot* approach, which could be particularly useful in low productive ecosystems in seasonally dry climates such as the Mediterranean basin. In this field study I partitioned ecosystem respiration (R_{eco}) into its component fluxes (R_{res}) from trees, understory plants, autotrophic (roots and micorrhizal associations) and heterotrophic soil components (bacteria and non-micorrhizal fungi), henceforth referred to as root and soil microorganisms respiration, during a spring to summer transition period with increasing water deficit and temperature. I aim to i) identify short-term changes in respiratory fluxes of both ecosystem (R_{eco}) and ecosystem components (roots, soil microorganisms, understory and trees) in response to seasonal drought; ii) determine the variation in isotopic composition of respired CO_2 from these sources and iii) compare two different model approaches (flux and isoflux-based mass balances) to model respiration fluxes on the ecosystem and soil level.

6.3 Materials and Methods

An intensive 14-days field campaign was conducted during May and June 2006 at the *Mitra* site (see **section 2.1**). Continuous records of micrometeorological measurements of CO_2- and H_2O-fluxes as well as climate variables (see **section 2.2**) were taken.

Diurnal and nocturnal courses of leaf gas exchange (LI-6400 open-flow gas exchange system, LI-COR, Lincoln, NE, USA) and soil respiration (R_{soil}, PP-System EGM2 Soil Respiration System with SRC-1 chamber; PP-Systems, Amesbury, MA, USA) were recorded in 24-h cycles every 2-4 hours on marked branches or plots for leaf and soil measurements, respectively. Soil temperature (T_{soil}) and soil water content (SWC) were recorded in 5 to 10cm depth alongside with soil respiration using the temperature sensor of the soil respiration system and a moisture probe (Theta Meter HH1, Delta-T Devices, Cambridge, UK), respectively.

Leaf gas exchange was measured on three *Q. ilex* trees in at least three sun-exposed leaves per tree and in 10 sun exposed leaves of different *T. guttata* plants. During daytime a black plastic shield was used to cover the cuvette after each measurement in light to obtain dark respiration rates for each leaf. Predawn and midday leaf water potentials (pressure chamber, Manofrigido, Portugal) were also obtained for the same species.

Soil respiration (R_{soil}) was measured with three replicates on i): three plots of bare soil without further treatment and ii) three plots with root exclusion, which was achieved by solarization with black foils and trenching by metal rings buried in the soil three weeks before measurements were started (see **section 2.5**). Standard deviations were calculated through error propagation procedures.

Samples for *keeling plot* analyses (see **section 2.4, 2.5**) were collected during four 24 h cycles with one measurement series every 2 to 3 hours. Daytime *keeling plots* could often not 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.

CO_2-sampling for chamber *keeling plots* of soil respiration was done with a properly built, well ventilated and gas tight Plexiglas chamber (17L) in a closed system (see **section 2.5**).

For collection and analysis of leaf and root respired CO_2 the *Intube-incubation* method was applied as described in **section 2.5**.

Carbon flux partitioning was achieved by two different mass balance approaches (see **section 2.7**) at the ecosystem scale (*ecoflux*, *eco-isoflux*) and at the soil scale (*soil-isoflux*). Root respiration was calculated independently of trees and understory from soil respiration of intact and trenched soil plots. Therefore, the *ecoflux* approach could be applied autonomously at the soil scale (*soilflux*).
For flux partitioning between understory and tree canopy I used data from a small *eddy covariance* system at a height of 2,5m (3D sonic anemometer, 1210R3, Gill Instruments Ltd., Lymington, UK; IRGA. LI-7500, LI-COR, Lincoln, NE, USA) installed at a nearby field site with similar conditions.

To quantify the degree of congruency between isotopic and non-isotopic mass balances, I linearly correlated data from the different approaches and calculated an average offset (AO; %) between them. Correlation coefficients (R^2) give information on similar trends in both models, while the average offset (AO) indicates differences in the data range. Highest congruency is expected for correlations with high R^2 and small AO between data points.

Analyses of correlation matrices between modeled fluxes were performed using *STATISTICA* 6.0 software (StatSoft, Tulsa, USA). R^2-coefficients of Pearson product-moment correlations and significances ($p<0.05$) were computed.

6.4 Results

Variation in climatic parameters and respiration fluxes

Meteorological conditions during spring 2006 were typical for Mediterranean climate with highest precipitation in March (104 mm) and April (57 mm) and continuously increasing air temperature (T_{air}), net radiation (NR) and vapor pressure deficit (VPD; data not shown). Last rainfall was on April 22, hence, when measurements were conducted by the end of May, the ecosystem already showed signs of extended drought. The upper soil layer (at 10 cm depth) was already very dry on May 20 (ca. 0.1 m^3 m^{-3}).
Most days were sunny (around 950 W m^{-2}), except May 22 with rather cloudy conditions (**Fig. 6.1a**). Midday values for air temperatures and VPD increased to 31°C and to 36 hPa, respectively (**Fig. 6.1b, d**). Soil temperatures in 5-10 cm depth followed changes in T_{air} but revealed slightly higher values (**Fig. 6.1c**).

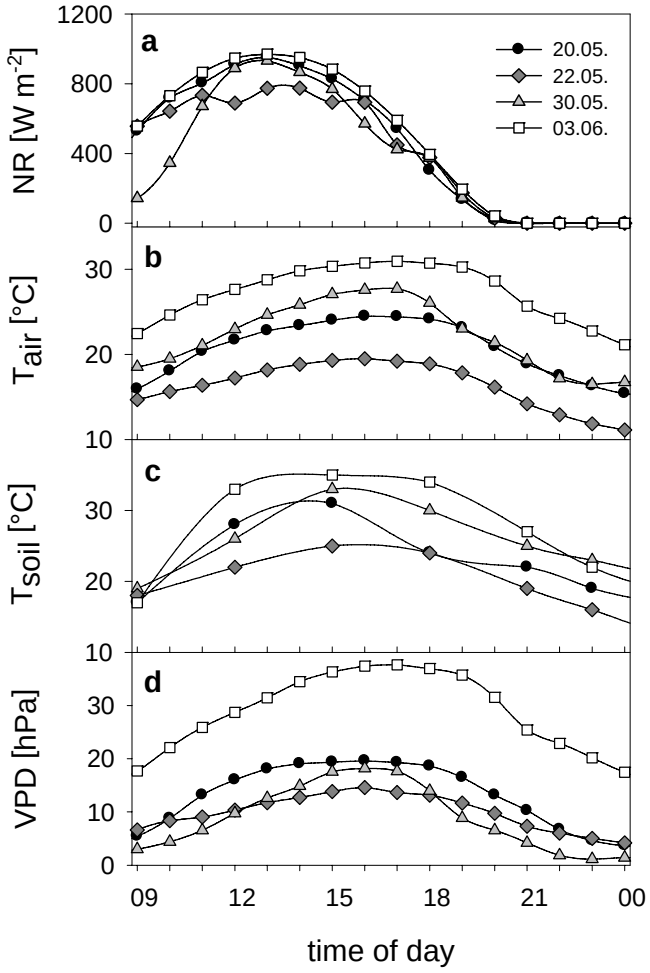

Fig. 6.1 Diurnal variation in a: net radiation (NR), **b**: air temperature (T_{air}), **c**: soil temperature (T_{soil}) and **d**: vapor pressure deficit (VPD) for May 20 (black circles), 22 (dark grey diamonds), 30 (light grey triangles) and June 3 (white squares) 2006.

Single leaf gas exchange and water potential measurements on trees (*Quercus ilex*) and understory plants (e.g. *Tuberaria guttata*) reflected a differential response to increasing water deficit and temperature (**Tab. 6.1**, **Fig. 6.2a-h**). While *Q. ilex* was able to preserve predawn leaf water potentials around -0.5 MPa and maintained net photosynthesis rates of ~3 to 4 μmol m^{-2} s^{-1} (**Tab. 6.1**), understory plants like *T. guttata* were severely affected by seasonal drought and died off during the study period. Predawn leaf water potentials became increasingly similar to midday values (-1.5 MPa) and generally low net photosynthesis turned into net respiration towards June 3 (**Tab. 6.1**). Dark respiration rates of single leaves revealed a clear diurnal cycle and increased strongly throughout the study in both species (**Fig. 6.2a-h**), but especially in *T. guttata* (up to 6 μmol m^{-2} s^{-1}, **Fig. 6.2h**).

Tab. 6.1 Net photosynthesis rates, predawn and midday leaf water potentials of trees (*Q. ilex*) and understory plants (*T. guttata*) during late spring 2006. n = 3-10 ± SD

		May 20	May 22	May 30	June 3
Q. ilex	**WP**$_{predawn}$ (MPa)	-0.3 ± 0.2	-0.5 ± 0.1	-0.6 ± 0.2	-0.7 ± 0.1
	WP$_{midday}$ (MPa)	-1.6 ± 0.1	-2.0 ± 0.2	-2.9 ± 0.2	-2.8 ± 0.2
	NP (µmol m^{-2} s^{-1})	2.8 ± 0.9	4.4 ± 1.0	3.5 ± 0.8	4.6 ± 0.7
T. guttata	**WP**$_{predawn}$ (MPa)	-0.5 ± 0.1	-0.6 ± 0.1	-1.1 ± 0.1	-1.5 ± 0.4
	WP$_{midday}$ (MPa)	-1.5 ± 0.1	-1.6 ± 0.1	-1.9 ± 0.3	-2.2 ± 0.3
	NP (µmol m^{-2} s^{-1})	1.8 ± 0.7	2.0 ± 0.7	1.7 ± 0.7	-2.1 ± 0.7

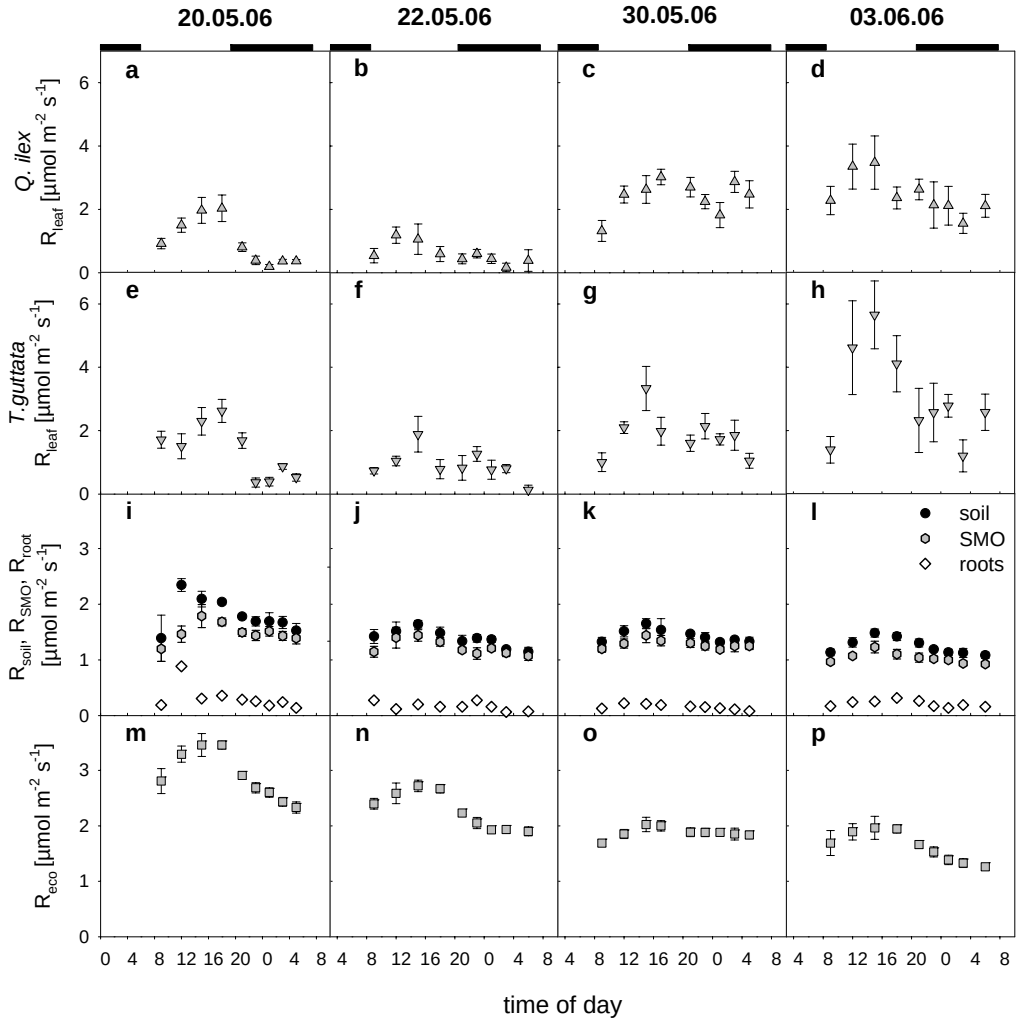

Fig. 6.2 Temporal variation in **a-d**: single leaf respiration of *Q. ilex* (grey upward triangles), **e-h**: single leaf respiration of *T. guttata* (grey downward triangles), **i-l**: respiration fluxes from soil (black circles), soil microorganisms (SMO, grey hexagons), roots (white diamonds) and **m-p**: ecosystem respiration (R_{eco}, grey squares) for four diurnal cycles with increasing summer drought (May – June 2006). Please note that foliage flux values (**a-h**) are referred to m^2 leaf area. Black bars indicate nighttime. n = 3-5 ± SE.

Soil respiration (R_{soil}) showed a slight decrease (~1 µmol m^{-2} s^{-1}) in response to seasonal drought and exhibited higher rates during daytime than during night (**Fig. 6.2i-l**). Heterotrophic respiration of soil microorganisms (R_{SMO}) was measured on root-free trenching plots, while root respiration was calculated as difference between R_{soil} and R_{SMO} (**equ. 2**). Soil microorganism's respiration showed slightly smaller rates and a similar pattern as total soil efflux, whereas root respiration exhibited little variation over the observed period with very low rates of about 0.2 µmol m^{-2} s^{-1} (**Fig. 6.2i-l**).

Ecosystem respiration (R_{eco}) decreased similarly to soil respiration from 3.5 to 1.5 µmol m^{-2} s^{-1} and showed similar diurnal dynamics (**Fig. 6.2m-p**).

Variation in isotopic composition of respired CO_2

Respired CO_2 of plant material was substantially more enriched than soil respiration. $\delta^{13}C_{res}$ of *Q. ilex* leaves on May 20 varied around -19.8‰ and exhibited a continuous decrease to -22.1‰ with increasing drought (**Fig. 6.3a-d**). The same tendency was observed in leaves of *T. guttata* but the depletion in $\delta^{13}C_{res}$ was even stronger from -21.3‰ to -25.9‰, while roots of *T. guttata* did not exhibit such strong changes over time with only a small decrease from -21.8 to -22.9‰ (**Fig. 6.3e-h**). At the diurnal scale leaf respired $\delta^{13}C_{res}$ exhibited enrichment during the day and subsequent depletion during night in both species. Maximum differences between day and nighttime values were up to 3.6‰ and 5.3‰ in *Q. ilex* and *T. guttata,* respectively.

Roots of *T. guttata* exhibited an opposite pattern to leaves, showing progressive enrichment during night (**Fig. 6.3e-h**). Daytime values were similar to leaves, but after dark the difference between $\delta^{13}C_{res}$ of leaves and roots increased continuously. This effect became more pronounced with increasing drought and temperatures and biggest differences of up to 7‰ were found in the night of June 3 (**Fig. 6.3h**).

$\delta^{13}C_{res}$ of soil respiration did not change over time and was on average -26.8‰ (**Fig. 6.3i-l**) with no obvious diurnal pattern.

$\delta^{13}C_R$ showed, apart from $\delta^{13}C_{res}$ of soil, the most depleted values (on average -25.9‰, **Fig. 6.3m-p**) but also exhibited a strong nocturnal enrichment between 2 and 4‰ (7.2‰ on May 30, **Fig. 6.3o**). With increasing drought mean values became slightly enriched and amplitude in the nocturnal cycle decreased.

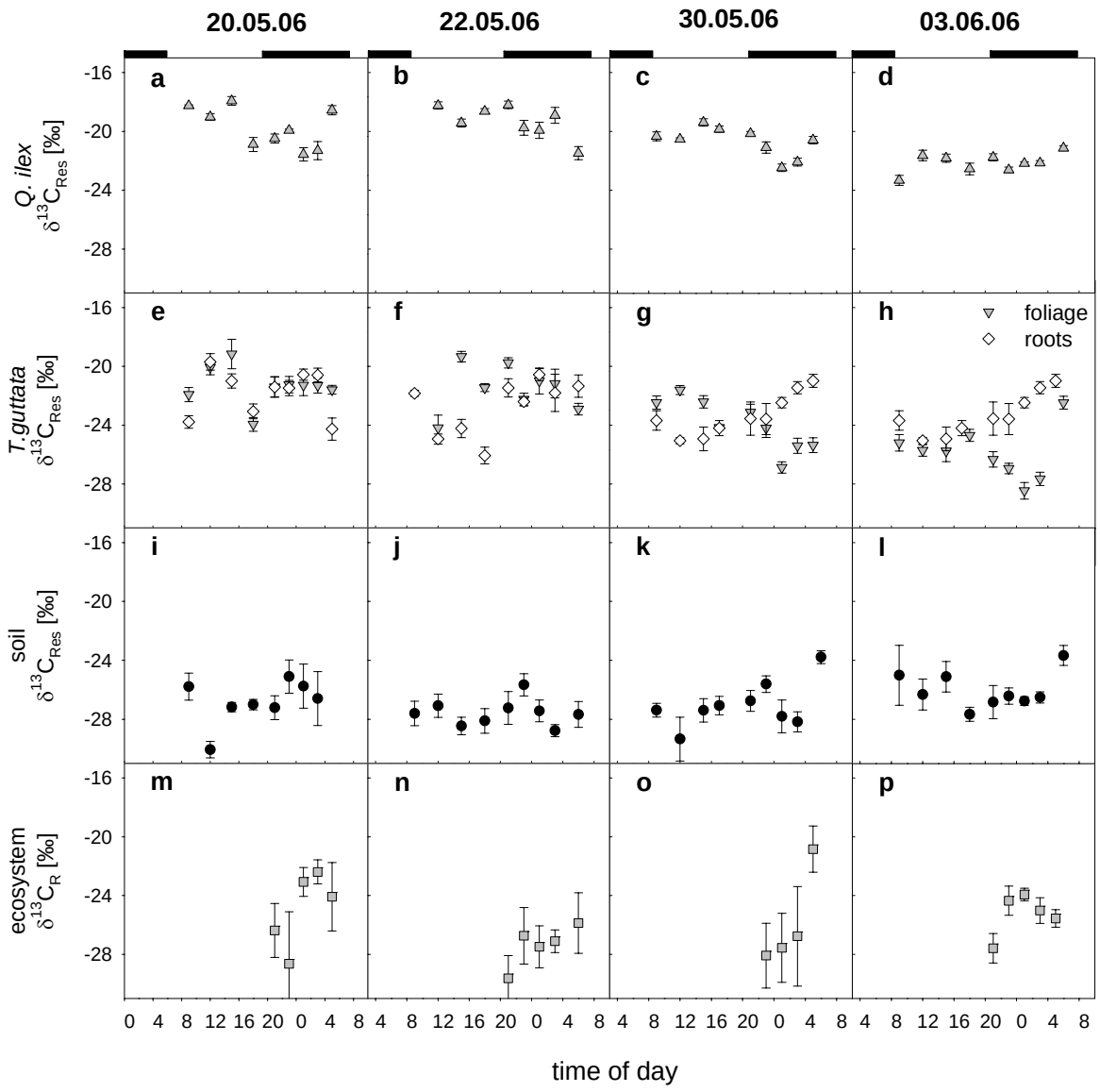

Fig. 6.3 Diurnal cycles of carbon isotopic composition ($\delta^{13}C$) of respired CO_2 from **a-d**: *Q. ilex* leaves (grey upward triangles), **e-h**: *T. guttata* leaves (grey downward triangles) and roots (white diamonds), **i-l**: soil (black circles) and **m-p**: ecosystem respiration ($\delta^{13}C_R$) during May and June 2006. Black bars indicate nighttime, n = 10 ± SE.

Partitioning of isotopic fluxes and comparison of different partitioning approaches

To evaluate changes in contribution of component fluxes to total ecosystem respiration with increasing water deficit and temperature, I used two different partitioning approaches. Since almost all component fluxes were measured, I was able to partition the missing tree respiration flux from R_{eco} (*ecoflux* approach, **equ. 2**). Additionally, stable isotope measurements were used to calculate isotopic fluxes for an *eco-isoflux* partitioning (**equ. 3**).

Fig. 6.4 shows ecosystem and component isofluxes calculated as products of respiration fluxes and their respective isotopic compositions. The isofluxes illustrate clearly the drought-induced changes in both respiration fluxes and their isotopic composition, as well as the magnitude in contribution of component fluxes to R_{eco}.

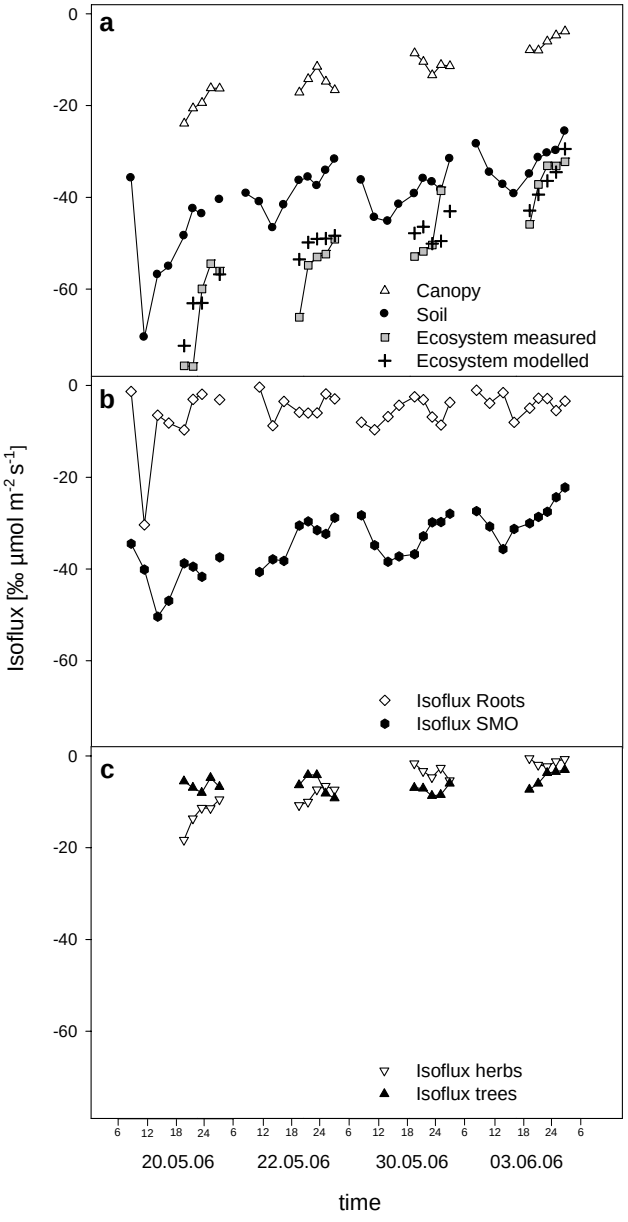

Fig. 6.4 Temporal variation in isofluxes as calculated from **a**: ecosystem (grey squares), soil (black circles), canopy (white upward triangles) and modeled ecosystem respiration (crosses), **b**: roots (white diamonds) and soil microorganisms (SMO, black hexagons), **c**: herbs (*T. guttata*, white downward triangles) and trees (*Q. ilex*, black upward triangles) during May and June 2006. Please note that apart from soil respiration all respiration flux partitioning was generally during nighttime and that foliage flux values are referred to m^2 ground and not leaf area.

All isofluxes increased throughout the study (**Fig. 6.4a**), mainly caused by decreasing respiration rates and, for R_{eco} and R_{soil}, also to a small part by isotopic enrichment (see **Fig. 6.3**). Isoflux of soil respiration followed the diurnal cycle of the soil flux (see **Fig. 6.2 i-l**) and became increasingly similar to the isoflux of R_{eco} throughout the study (**Fig. 6.4a**). This variation was generally due to heterotrophic soil respiration, which was in the range of the soil efflux, while roots only exhibited very small respiration rates, resulting in isofluxes close to 0 without significant changes during the study (**Fig. 6.4b**). Similarity between the isofluxes of R_{eco} and R_{SMO} indicates that the main component of R_{eco} was heterotrophic soil respiration.

Isoflux of canopy respiration (both understory and trees) was high, as compared to the isofluxes of R_{eco} and soil and showed the smallest increase (**Fig. 6.4a**). Isofluxes of trees were small but stable and increasing isofluxes from understory respiration (**Fig. 6.4c**) were the main cause for the increase in canopy isoflux (**Fig. 6.4a**).

To test the *eco-isoflux* approach for consistency, I modeled all fluxes and isotopic compositions of ecosystem and component fluxes independently (**Fig. 6.5**). I found that not all measured fluxes and corresponding isotope ratios led to a sensible solution of the mass balance. The most reasonable mass balance was achieved using isofluxes of $\delta^{13}C_{res}$ from soil, herbs and tree respiration and the respective measured fluxes in combination with modeled $\delta^{13}C_R$. This approach led to high similarity between modeled and measured isofluxes of $\delta^{13}C_R$ (**Fig. 6.4a**).

However, using measured $\delta^{13}C_R$ for the *eco-isoflux* approach I detected a significant deviation from *ecoflux* modeled component fluxes. I found that this deviation (R^2 and average offset) was larger, when the calculated component flux was small (**Fig. 6.5**; **Tab. 6.2**). Thus, highest congruency between the isotope model and measured fluxes was achieved for R_{eco} (**Fig. 6.5a**; Tab.2) and largest deviations were found for the smallest component fluxes (root and tree respiration, **Fig. 6.5c, d**; **Tab. 6.2**). Lowest uniformity between *ecoflux* and *eco-isoflux* model was observed for R_{soil}, where non congruencies of summed model data from R_{root} and R_{SMO} magnified the difference between the models (**Fig. 6.5b**, **Tab. 6.2**).

Since respiration of soil, roots and SMO was measured independently to the ecosystem mass balance I was able to compare the different partitioning approaches exclusively at the soil scale (*soil-isoflux* approach, **Fig. 6.5b, d, f**). In contrast to the *eco-isoflux* results, root respiration calculated by *soil-isoflux* partitioning was in the same range and followed nicely the pattern of the *ecoflux* model (**Fig. 6.5d**; **Tab. 6.2**). Outliers were only observed for the

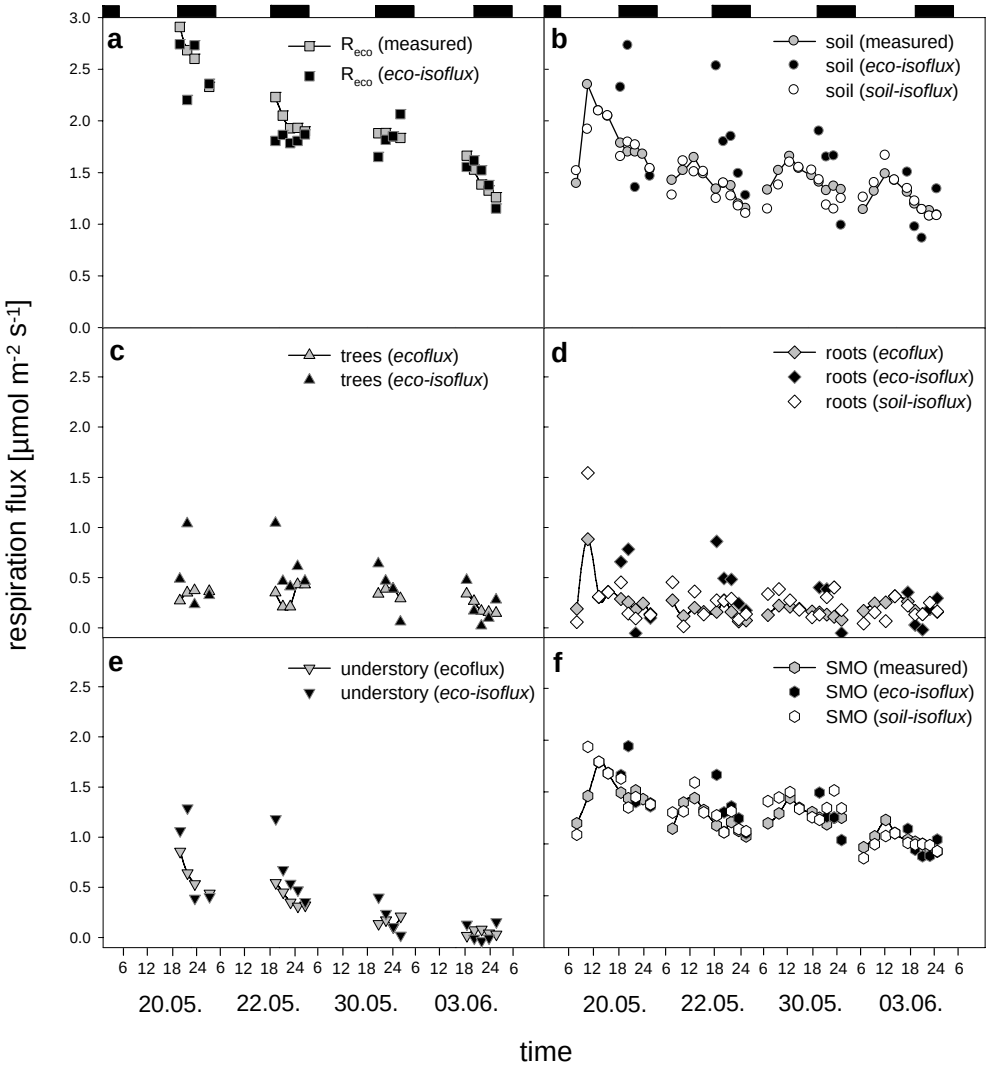

Fig. 6.5 Temporal changes in measured and modeled fluxes from **a**: ecosystem respiration (squares), **b**: soil respiration (circles), **c**: tree respiration (upward triangles), **d**: root respiration (diamonds), **e**: understory respiration (downward triangles) and **f**: soil microorganisms respiration (hexagons); measured and *ecoflux* modeled values - lined grey; *eco-isoflux* modeled values – black, *soil-isoflux* modeled values – white. Please note that apart from soil respiration all respiration flux partitioning was generally during nighttime and that foliage flux values are referred to m^2 ground and not leaf area. Black bars indicate nighttime.

Midday value of May 20, where isoflux partitioning overestimated the root flux. Given that the root respiration flux was very small (<0.5 µmol m^{-2} s^{-1}) these outliers are expected and negligible. At the soil scale patterns in $\delta^{13}C_{res}$ still reflected the flux patterns very well, since modeled and measured values of soil and SMO respiration were at all times congruent in range and course (**Fig. 6.5b, f**; **Tab. 6.2**).

Tab. 6.2 Correlation coefficients (R^2) and average offset (%) between correlated data points (AO) for correlations of measured and *ecoflux* modeled respiration fluxes from ecosystem and ecosystem components (trees, understory, soil, soil microorganisms (SMO), roots) with the respective isotopic flux models (*eco-isoflux* and *soil-isoflux*), n = 20.

vs.	R_{eco} (measured)		R_{trees} (*ecoflux*)		$R_{understory}$ (*ecoflux*)		R_{soil} (measured)		R_{SMO} (measured)		R_{root} (*ecoflux*)	
	R^2	AO	R^2	AO	R^2	AO	R^2	AO	R^2	AO	R^2	AO
(*eco-isoflux*)	0.72	8	0.02	75	0.66	59	0.21	31	0.3	14	0.33	187
(*soil-isoflux*)	-	-	-	-	-	-	0.81	6	0.69	8	0.77	64

I might summarize that isotopic flux partitioning did reveal very high congruency to the *ecoflux* model at the soil scale, while at the ecosystem scale best uniformity between the models was achieved when modeling $\delta^{13}C_R$ by summing up the respective component isofluxes.

6.5 Discussion

Stable isotopes have been used to partition net carbon fluxes into gross photosynthetic and respiratory fluxes (Bowling *et al*., 2001; Ogee *et al*., 2003; Knohl & Buchmann, 2005), but seldom for the partitioning of all major ecosystem components from R_{eco}.

i) Apart from McDowell *et al*. (2004b), who used an isotopic mass balance to explain temporal variation in $\delta^{13}C_R$, this is the first study to include stable isotopes in a partitioning approach of ecosystem respiration into all major component respiratory fluxes. In the following I will compare the traditional *eddy-flux* partitioning approach with an isotopic partitioning approach at the soil (*soilflux* vs. *soil-isoflux*) and ecosystem scale (*ecoflux* vs. *eco-isoflux*) and test the validity of both approaches.

ii) In contrast to McDowell *et al*. (2004b) my results show substantial short-term variation in the isotopic composition of the main component fluxes contributing to $\delta^{13}C_R$ within a fortnight and even within a nocturnal cycle. I will discuss this variability with isotopic disequilibria in substrate usage, respiratory metabolism, carbon allocation and transport and at the ecosystem scale also with differential responses of different ecosystem component fluxes to seasonal drought.

6.5.1 Validity of isotopic partitioning approaches

All measured and *ecoflux* modeled values were in agreement. Soil was the main respiratory component, comprising ~70% of ecosystem respiration, as observed in other studies (Goulden *et al*., 1996; Lavigne *et al*., 1997; Law *et al*., 2001; Davidson *et al*., 2006a; Zha *et al*., 2007). Contributions of canopy respiration to R_{eco}, including both foliage and trunk respiration, ranged between 19 and 40%. These proportions were slightly lower than contributions observed by Lavigne *et al*. (1997) in a boreal forest (foliage 25-43%, wood 5-15%), but in the range of studies from Goulden *et al*. (1996), Law *et al*. (1999) and Zha *et al*. (2007).

However, including stable isotopes into the mass balance at the ecosystem scale (*eco-isoflux*) to model either single fluxes or their isotopic composition did not always result in sensible solution of the mass balance. Especially fluxes with small relative contributions (roots, trees) were found to deviate between the *ecoflux* and the *eco-isoflux* approach (see **Fig. 6.5**; **Tab. 6.2**). Highest congruency between isotopic and non-isotopic approach was obtained for R_{eco} by summing up isofluxes of its components. Modeling the component fluxes with measured R_{eco} and $\delta^{13}C_R$ however, revealed partially large uncertainties in the modeled fluxes. These congruency tests led to the assumption that $\delta^{13}C_R$ was the main cause of error in the *eco-isoflux* approach. McDowell *et al*. (2004b) similarly, found measured $\delta^{13}C_R$ always depleted to modeled $\delta^{13}C_R$, even using various theoretical combinations of relative contributions from soil and foliage respiration. It is known that $\delta^{13}C_R$ as obtained from *keeling plots* depends strongly on the CO_2-gradient used for calculation of the regression (e.g. Pataki *et al*., 2003a). Thus, small gradients (below 30ppm) might still yield a significant *keeling plot* regression but might cause errors in the intercept. Windy conditions and small respiratory activity (e.g. during Mediterranean summer drought) can cause difficulties in capturing a sufficiently high gradient (Hemming *et al*., 2005; Werner *et al*., 2006). Indeed, the CO_2-gradients obtained for *keeling plots* were on average only 36 ppm and uncertainties (SE of OLS, model I) were partially large. CO_2-gradients of chamber retrieved soil *keeling plots* on the other hand were generally large (~70 ppm) and *soil-isoflux* partitioning revealed a good congruency with the *soilflux* model. Apart from $\delta^{13}C_R$ another cause of error in the *eco-isoflux* model might be a missing flux. Although all main ecosystem components and their respective $\delta^{13}C_{res}$ have been measured, tests revealed that already a very small additional flux with extreme isotopic signature might explain differences in measured and modeled $\delta^{13}C_R$. This hypothesis is reinforced by finding, that for soil (a comparatively smaller scale, with less possible contributors) the *soil-isoflux* approach worked very well.

Nevertheless, the isotope approach to partition respiratory fluxes was also found to be valid at the ecosystem scale. Moreover, measuring all component fluxes of R_{eco} and their respective carbon isotopic signatures with chamber and incubation methods might be a new and powerful tool to estimate $\delta^{13}C_R$ in times of low activity, when *keeling plot* measurements are restricted by low nocturnal CO_2-gradients.

6.5.2 Influence of seasonal drought on respiration and its isotopic composition

Ecosystem scale

During a spring to summer transition which constituted a period of substantial short-term change in respiratory fluxes for the studied ecosystem, R_{eco} decreased by ~50% within a single fortnight (see **Fig. 6.2**). The main cause for this decline was the large decrease in ecosystem scale canopy respiration, which decreased by two thirds as drought progressed (data not shown). This was mainly due to the strong wilting in understory foliage during the study (observation at the field site) and understory respiration flux decreased by ~90% (see **Fig. 6.5e**). In contrast respiration of trees (*Q. ilex*) at the ecosystem scale did not respond markedly to warming and drought (**Fig. 6.5c**), indicating the ability to extract sufficient water from deeper soil layers (e.g. Kurz-Besson *et al*., 2006).

Along with this decrease in R_{eco} carbon isotopic composition of ecosystem respiration ($\delta^{13}C_R$) showed a variability of up to 8.8‰. In spite of earlier assumptions that $\delta^{13}C_R$ remains relatively constant on a monthly to seasonal basis (e.g. Flanagan *et al*., 1996; Buchmann *et al*., 1998), several recent studies showed substantial short-term variation in $\delta^{13}C_R$. Seasonal differences of up to 8‰ were observed (e.g. Fessenden & Ehleringer 2003; McDowell *et al*., 2004a; Lai *et al*., 2005; Ponton *et al*., 2006; Werner *et al*., 2006, 2007a) and thus, confirm my results. Some authors reported large shifts of up to 6‰ within even a single night (Bowling *et al*., 2003; Werner *et al*., 2006, 2007a). Indeed, similarly largest nocturnal variability in $\delta^{13}C_R$ was 7.2‰, almost as large as the maximum variability within the entire study period. Meteorological events have been linked to seasonal short-term variation in $\delta^{13}C_R$, assuming that ecosystem respiration is partially controlled by carbon assimilation and its environmental drivers. Nowadays it is widely accepted that after a transport time ranging from hours to days, recently assimilated carbon is respired by roots and their micorrhizal associations, and after

root exudation also by soil heterotrophic organisms (Ekblad & Högberg, 2001; Bowling *et al*., 2002; McDowell *et al*., 2004a; Knohl *et al*., 2005; Mortazavi *et al*., 2005; Werner *et al*., 2006). Changes in photosynthetic discrimination might therefore, be one of the main causes of short-term variation in $\delta^{13}C_R$. Decreasing canopy discrimination in response to seasonal drought (T_{air}↑, VPD↑, soil moisture↓) should result in increasing enrichment in $\delta^{13}C_R$ (e.g. Ekblad & Högberg, 2001; Ponton *et al*., 2006; Werner *et al*., 2006, 2007a). Although VPD and T_{air} increased markedly especially in the beginning of June the results do not show a very pronounced enrichment. Mean nocturnal $\delta^{13}C_R$ was most depleted on May 22 and increased only about 2‰ towards June 3.

Net photosynthesis of trees (*Q. ilex*) remained stable and given that increasing difference between predawn and midday leaf water potentials coincided with increasing VPD, mean canopy conductance of trees probably remained stable as drought progressed. Therefore, I can conclude that photosynthetic discrimination of trees, which were able to access ground water, should not have changed much throughout the study. In understory plants (*T. guttata*) on the other hand net photosynthesis decreased dramatically and predawn and midday leaf water potentials became increasingly similar with drought. The effect on photosynthetic discrimination in understory species is thus, expected to be small. Further, as outlined above, understory species were severely affected by drought and vanished during the study. Thus, the influence of understory assimilates on isotopic composition of ecosystem respiration probably only played a minor role. Given this information, it is possible that small effects of drought on photosynthetic discrimination of trees and understory translated into a minor response of $\delta^{13}C_R$. On the other hand, it is reasonable to assume that the changes in $\delta^{13}C_R$ occurred upon changes of respiratory activity of different ecosystem components. The largest influence on $\delta^{13}C_R$ had probably soil respiration (particularly soil microorganisms), as it contributed to a major part to R_{eco} (~70%). Accordingly, it was the most depleted source compared to the other components and in the range of $\delta^{13}C_R$. Isotopic values of foliage and soil respiration became increasingly similar on June 3, which coincided with lowest nocturnal variability in $\delta^{13}C_R$ on that date. This might indicate that changes in relative contributions from plant and soil microorganisms respiration to R_{eco} were the main reason for nocturnal isotopic shifts in ecosystem respired CO_2.

Soil scale

Absolute soil respiration rates were equal to other studies in Mediterranean and semiarid sites (e.g. Irvine *et al*., 2005; Tang & Baldocchi, 2005). Towards June R_{eco} and R_{soil} became increasingly similar, which was caused by a strong drought-induced decrease in canopy respiration. A similar decrease in R_{eco}/R_{soil} ratio with drought was observed by Davidson *et al*. (2006a). Values became slightly more enriched on June 3. As discussed above for $\delta^{13}C_R$, $\delta^{13}C_{res}$ of soil was found to undergo annual and seasonal variations, mainly attributed to the influence of differences in photosynthetic discrimination and substrate supply to root respiration (e.g. Ekblad & Högberg 2001; Bhupinderpal-Singh *et al*., 2003; McDowell *et al*., 2004b; Ekblad *et al*., 2005; Mortazavi *et al*., 2005). Indeed, many studies using carbon tracers or girdling methods found that soil respiration was to a large part controlled by photosynthesis (Bhupinderpal-Singh *et al*., 2003; Søe *et al*., 2004; Steinmann *et al*., 2004; Göttlicher *et al*., 2006). Most of theses studies, however, were conducted in dense forest ecosystems with very high relative root contribution to soil CO_2-efflux. Contrarily, in the here studied system soil respiration was not controlled by rhizospheric activity but mainly by heterotrophic organisms (up to 85%, see **Figs. 6.4**; **6.5**), while the autotrophic portion of soil respiration comprised a very small flux, which did not change in response to seasonal drought (see **Fig. 6.2**). Autotrophic soil respiration is largely variable and contributes to total soil efflux from >10 to <90% depending on ecosystem and climate type (Hanson *et al*., 2005; Subke *et al*., 2006). Small autotrophic contributions in the studied low productive Mediterranean ecosystem are therefore, expected.

During the study period heterotrophic soil microorganisms obviously did not use recent carbon as main respiratory substrate, as $\delta^{13}C_{res}$ was substantially more depleted than foliage and root respiration (see **Fig. 6.3**). Furthermore, R_{SMO} probably did not change respiratory carbon source in response to the seasonal drought. While heterotrophic soil respiration was inhibited by drought on June 3, root contribution remained stable (see **Fig. 6.5**) thus, the ratio R_{SMO}/R_{root} decreased. Increasing relative contribution of roots to total soil respiration, therefore, might explain the slight enrichment in soil $\delta^{13}C_{res}$ during the study. In contrast to $\delta^{13}C_R$ there was no marked nocturnal variation in $\delta^{13}C_{res}$ of soil, pointing to a stable equilibrium of respiratory carbon pools used by the soil organisms.

Foliage scale

Single leaf respiration increased during the study in both *Q. ilex* and *T. guttata* (see **Fig. 6.2**). This increase was accompanied by a gradual depletion in $\delta^{13}C_{res}$ of about 4‰ and 8‰ in *Q. ilex* and *T. guttata*, respectively (see **Fig. 6.3**). Contrastingly, several studies observed increasing enrichment in substrates (e.g. phloem sap, soluble sugars, starch) with enhanced drought (e.g. Pate & Arthur 1998; Ghashghaie *et al*., 2001). Duranceau *et al*. (1999) found that CO_2 respired from well watered *Phaseolus* plants was substantially more depleted than from drought stressed plants. These results were confirmed in another study for *Nicotiana*, but not for *Helianthus* (Ghashghaie *et al*., 2001). Those studies suggest that the drought induced decrease in photosynthetic discrimination results in enriched substrates and thus, increasing $\delta^{13}C_{res}$. However, during extreme drought and high respiratory demand photosynthetic discrimination might not always well reflect carbon isotopic composition of foliage respired CO_2. Within recent years arose increasing evidence that substantial post-photosynthetic fractionation in dark respiration of foliage during PDH-reaction and Krebs cycle metabolism occurs through heterogeneous distribution of heavy carbon (Tcherkez *et al*., 2003; for a review see Ghashghaie *et al*., 2003 and Werner *et al*., 2007a; compare **section 1.3**), which might be linked to changes in metabolic pathways during growth and enhanced secondary metabolism (P. Priault & C. Werner, unpublished results). Tcherkez *et al*. (2003) showed that under high respiratory demand (temperature increase) respired CO_2 was more depleted than under reduced respiration in the cold. They explained this with increased Krebs cycle activity and decreased biosynthetic deviation of pyruvate. This finding confirms clearly my results for drought and temperature induced depletion in foliage respiration coinciding with increasing foliage respiration rates. Additionally the increasing demand on substrate to respire coupled with a decreasing carbon fixation might have caused enhanced respiration of older, more depleted carbon sources (e.g. fatty acids). Hence, under conditions of very small or even negative net carbon balance, these effects seem to mask the effect of photosynthetic discrimination.

The marked diurnal dynamics in isotopic composition of foliage respiration (especially in *T. guttata*) with increasing enrichment during the light period and subsequent depletion after dark was observed in some previous studies (Hymus *et al*., 2005; Mortazavi *et al*., 2006; Prater *et al*., 2006, Barbour *et al*., 2007; Werner *et al*., 2007b) and was linked to cumulative photosynthesis. Tcherkez *et al*. (2003) showed that, as outlined above for seasonal patterns, diurnal variability can also be explained by post-photosynthetic discrimination due to

decreasing biosynthetic deviation of pyruvate during nighttime. In contrast to Werner *et al*. (2007b) diurnal variability was larger in *T. guttata* than in *Q. ilex* and increased with enhanced seasonal drought. A similar pattern was observed by Duranceau *et al*. (1999), who found a nocturnal depletion in respired CO_2 of drought subjected *Phaseolus* but none in well watered ones. I figure that, as outlined above, the small amount of sugars produced under extreme drought conditions do not fully sustain respiratory demand at nighttime and stored carbon from more depleted carbon sources has to be respired. Indeed, Nogués *et al*. (2004; 2006) showed with labeling experiments that nighttime foliage respiration consisted to more than half of old carbon even in unstressed plants.

Root scale

Isotopic composition of root and foliage respired CO_2 from *T. guttata* ranged between the values of *Q. ilex* foliage and soil respiration (**Fig. 6.3**). Interestingly there was a diurnal pattern in $\delta^{13}C_{res}$ of roots, which increased during the study. It showed the inverse pattern of foliage $\delta^{13}C_{res}$: a nocturnal enrichment on May 30 and June 3, while during daytime root respiration was more depleted or equal to foliage respiration. Largest nocturnal enrichment was to be found in root respiration of severely drought stressed individuals (May 30, June 3).
These results are in contrast to Klumpp *et al*. (2005) and Badeck *et al*. (2005), who observed that root respiration was always more depleted than shoot respiration in different herbal species and explained this pattern mainly with the the enzyme PEPc contained in roots of C3 plants. PEPc assimilates HCO^{3-} that is comparatively enriched to the putative substrates and thus, might be responsible for depleted respiration.
However, other studies are in favor of my results and found root- (Gessler *et al*., 2007) as well as trunk respired CO_2 (Damesin & Lelarge 2003; Brandes *et al*., 2006) to be ^{13}C-enriched as compared to the potential respired substrate. Gessler *et al*. (2007) found even a marked diurnal cycle in $\delta^{13}C$ of phloem sap from *Eucalyptus* with nocturnal enrichment, explaining this with post-photosynthetic fractionation during starch remobilization at night (Fructose from starch breakdown being enriched as compared to primary assimilates - described by Tcherkez *et al*. (2004). This fractionation and possible further fractionation processes associated with phloem transport seem to play a major role, as many studies found leaf organic matter to be depleted as compared to roots (see Badeck *et al*., 2005).
I propose here that post-photosynthetic fractionation during dark respiration explains diurnal enrichment in leaf $\delta^{13}C_{res}$, while nocturnal enrichment in root respiration was probably due to

fractionation with by drought enhanced starch break down and export to the roots. The fact that net radiation was well correlated with root respiration fluxes but not with foliage respiration (data not shown) support the idea that in wilting herbal plants carbon transfer to the roots is accelerated in order to conserve energy for a possible resprout when conditions change.

6.6 Conclusions

This study is the first to include carbon isotopic compositions of respired CO_2 in a complete ecosystem scale mass balance approach to partition R_{eco} into all major component fluxes. I was able to validate the model at the soil scale, while at the ecosystem scale uncertainties in R_{eco} due to small CO_2-gradients for *keeling plot* calculation were responsible for partial deviations between *ecoflux* and *eco-isoflux* modeled data. Nevertheless, modeling $\delta^{13}C_R$ from the component fluxes revealed very high congruencies between isotopic and non-isotopic approach. Therefore, I propose the *eco-isoflux* approach as an alternative bottom-up model of $\delta^{13}C_R$ during times when sufficient CO_2-gradients for *keeling plots* are difficult to capture.

Partitioning revealed that the large decrease in R_{eco} in response to seasonal drought was mainly triggered by disappearance of drought sensitive understory species, which in the beginning of the study contributed significantly to R_{eco} (> 20%).

Moreover, I discovered substantial diurnal and seasonal variation in $\delta^{13}C_R$ that were mainly induced by the interplay of changing $\delta^{13}C_{res}$ from foliage and soil. The marked seasonal and diurnal variation in $\delta^{13}C_{res}$ from foliage was in accordance with recent findings of post-photosynthetic fractionation in the dark respiratory pathways. Nocturnal enrichment in $\delta^{13}C_{res}$ of roots from drought stressed individuals agreed with current theories on fractionation during phloem loading. These results will contribute to a process-based understanding of isotopic variation in ecosystem respired CO_2, which may improve models of carbon exchange between ecosystems and atmosphere that are crucial to predict the impact of future climate scenarios on ecosystem functioning.

6.7 AUTHORS CONTRIBUTIONS

The author of this thesis accomplished the entire experimental work and the writing of this chapter. Further contributions were given by C. Werner who coordinated the project, C.

Máguas who provided the stable isotope lab, J.S. Pereira who provided the field site and *eddy-flux* data, L. Aires who provided *eddy-flux* data of the understory and T.S. David who provided climate data. Further, I acknowledge contributions of R. Maia for technical support with isotope analyses, P. Oliveira (*Mezão*) for technical help with equipment and V. Andrade for *eddy-flux* data treatment.

CHAPTER 7

CONCLUDING REMARKS

A variety of isotope effects driven by the environmental conditions leave an imprint on $\delta^{13}C$ of photosynthetic products and respired CO_2. This work took advantage of stable isotopic composition of carbon fluxes to gain new insights in ecosystem carbon dynamics and their dependence on climatic variables.

One major aim of this study was to determine the environmental drivers of ecosystem and soil scale carbon fluxes and their isotopic compositions. To achieve this goal, temporal dynamics in carbon fluxes and $\delta^{13}CO_2$ were studied during climatic transition periods from spring to summer drought (**chapter 3, 5, 6**) and vice versa (**chapter 3, 4**).

The second major aim was the quantification of component fluxes contributing to net ecosystem exchange and to gain new insights from the combination of stable isotope methodology with flux measurements. This study is the first to disentangle all biotic drivers of drought induced changes in ecosystem source/sink behavior from net ecosystem exchange (*ecoflux*, **chapter 5, 6**) and to include carbon isotopic compositions of respired CO_2 in a complete ecosystem scale mass balance approach to partition R_{eco} into all major component fluxes (*eco-isoflux*, **chapter 6**).

7.1 INFLUENCE OF ENVIRONMENTAL DRIVERS ON CARBON FLUXES AND THEIR ISOTOPIC COMPOSITION

As a multitude of studies have shown, ecosystem scale carbon fluxes (both assimilation and respiration) depend on a variety of environmental drivers, such as radiation, temperature, VPD and soil moisture. Besides temperature and water availability, ecosystem carbon uptake is mainly limited by radiation and leaf area index, while ecosystem respiration (R_{eco}) depends largely on the size of labile carbon pools, as well as on temperature and moisture. In Mediterranean ecosystems, the increase in air temperature along with a substantial decrease in soil water potentials during summer drought is the major limiting factor for carbon sequestration, largely determining annual carbon balance. Further, precipitation pulses during

drought periods were shown to release significant amounts of carbon from the soil pools (*Birch* effect), with relevant effect on annual carbon budgets.

Earlier works considered isotopic composition of ecosystem respiration ($\delta^{13}C_R$) to remain relatively constant at short-term scales and only to vary slightly in response to substrate changes in soil respiration during the annual cycle.
This is one of the first studies showing contrary results. $\delta^{13}C_R$ did not remain constant on a monthly to seasonal time-scale and varied even within a time-scale of only some hours by almost the same magnitude as observed during the entire annual cycle (**chapter 3,4,6**).

Long-term dynamics (seasonal) $\delta^{13}C_R$ were linked to variations in $\delta^{13}C_{res}$ of the ecosystem component fluxes from foliage, roots and soil (**chapter 4, 6**) and to changes in climatic drivers of photosynthetic discrimination (**chapter 3**) and edaphic conditions in the soil (**chapter 3, 4**). Additionally, the responsiveness of ecosystem respiration to key climatic factors was shown to vary substantially within the annual cycle (e.g. by temperature acclimatization; **chapter 3**).

The rapid nocturnal variations in $\delta^{13}C_R$ pointed towards even faster dynamics in respiratory pathways on the time-scale of hours (**chapter 3, 4, 6**). To explain these dynamics variation in respired $^{13}CO_2$ of ecosystem components were studied. Nocturnal variation in $\delta^{13}C_{res}$ from foliage and roots was found to increase with enhanced respiratory demand and decreased photosynthetic capacity and could be attributed to substrate changes as well as post-photosynthetic fractionation steps in the respiratory pathways and during phloem loading (**chapter 6**). Nocturnal variation in $\delta^{13}C_{res}$ of soil microorganisms was generally low, except for times of rapid changes from dry to wet conditions (**chapter 4**). These new findings led to the conclusion that short-term variation in $\delta^{13}C_R$ are caused by three main processes, (***i***) through changes in respiratory substrate, (***ii***) 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 response to nocturnal changes in climatic variables) and (***iv***) through post-photosynthetic fractionation in the respiratory pathways.

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 in CO_2 concentrations required for a reliable *keeling plot* regression is difficult to capture in a short time period due to 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 hours, see Pataki *et al.*, 2003a). As was shown, these nocturnal shifts are frequent phenomena. Therefore, we might need to verify one of the basic assumptions for sampling nocturnal *keeling plots*.

Furthermore, the results shown in this work confirm that both ecosystem respiration and GPP experienced a large decrease in response to drought. Evidence was given that herbaceous understory species played the major role for the rapid decline in ecosystem carbon sequestration after spring (**chapter 5**), while afterwards heterotrophic respiration of soil microorganisms gained strongest influence on ecosystem carbon balance. Thus, a combination of these two processes is responsible for the rapid turn of ecosystem carbon balance towards a CO_2-source in summer and timing and onset of drought might have a larger effect on annual carbon budget than its length.

It was shown, that during summer drought, soil moisture is the major limiting factor for soil and ecosystem respiration at the seasonal scale, whereas temperature regulates both fluxes at the diurnal time scale (**chapter 5**). The heterotrophic portion of soil respiration generally made up the largest part of ecosystem respiration, while contribution of autotrophic soil respiration from roots was only marginal.

Another main result of this work regards precipitation pulses in the middle and at the end of summer in Mediterranean ecosystems, which have large implications on ecosystem carbon dynamics through a strong increase in soil respiration (**chapter 4**). Results of both flux measurements and stable isotopes indicated that the influence of heterotrophic soil respiration on ecosystem respiration increases to >90% during such periods, caused by rapid changes in labile substrate pools inside the soil in response to the precipitation pulse (*Birch* effect, **chapter 4**). This study gives new support to the theory that these sudden changes occur through a hypo-osmotic stress response of soil microorganisms caused by the rapid changes in soil water potentials. It was also shown that the extent of the *Birch* effect and thus, its impact on carbon budget might largely depend on the duration and suddenness of soil moisture changes. Hence, an increase in respiration by a growing soil microorganism population after slow rewetting might cause the opposite effect on carbon sequestration than a pulse-like stress reaction in response to rapid changes in soil moisture, potentially damaging microbial

community. This finding might be important for correct interpretations of the influence of precipitation pulses during drought on ecosystem carbon sequestration.

The here presented results contribute significantly to a better understanding of ecosystem carbon exchange and its environmental dependencies. Studying the temporal variability in stable isotope composition of component fluxes added new valuable information to disentangle ecosystem net carbon balance of the studied Mediterranean oak savannah. However, we are only beginning to comprehend the complex processes responsible for isotopic exchange between carbon pools and their responses to changes in key climatic parameters. Thus, carbon cycle studies by stable isotopes will remain of major interest for future research, aiming to generate and improve regional and global carbon flux models that are crucial to predict the impact of future climate scenarios on ecosystem functioning.

7.2 VALUE OF *ECOFLUX* AND *ECO-ISOFLUX* MODEL APPROACH TO PARTITON ECOSYSTEM SCALE CARBON FLUXES

Partitioning net ecosystem carbon exchange (NEE) into photosynthetic and respiratory component fluxes has become an important tool to estimate their contributions to ecosystem carbon balance as well as to identify their responses to environmental drivers. Many approaches comprising mass balance calculations at the ecosystem (e.g. Law *et al*., 2001; Davidson *et al*., 2006a) and soil scale (see Subke *et al*., 2006) emerged within recent years and stable isotopes were used to separate net carbon fluxes into fractions of assimilation and respiration (e.g. Bowling *et al*., 2001).

The straight-forward *ecoflux* model approach allowed a detailed separation of diurnal and nocturnal respiration fluxes of soil microorganisms, trees, understory and roots from R_{eco} as well as a partitioning of assimilation fluxes of trees and understory from ecosystem carbon uptake. The separation of these component fluxes permitted very new insights into ecosystem scale carbon dynamics that were not visible from *eddy covariance* data alone. The data indicate that temporal changes in relative contribution of single component fluxes to the overall ecosystem flux can have a substantially different response to climatic variables than the changes in these fluxes (e.g. heterotrophic soil respiration decreased but its relative contribution to R_{eco} increased in response to drought, **chapter 5**). Thus, for correct interpretations of temporal changes in ecosystem sink strength, I recommend to always

monitor component fluxes (soil, roots, understory) simultaneously to *eddy covariance* measurements.

The *eco-isoflux* model (**chapter 6**) combined isotopic composition of respiration from different ecosystem components with carbon flux measurements to a mass balance of isotopic carbon fluxes (*isofluxes*). In contrast to the *ecoflux* approach it had a general shortcoming, since it could only be used to separate respiratory fluxes. However, the general advantage of this approach is that it accounts for variation in isotopic composition of respiratory components that could not be assessed by measurements (e.g. canopy respiration).

The isotopic mass balance revealed reasonable results at the soil scale, while at the ecosystem scale uncertainties in *eco-isoflux* modeled R_{eco} due to small CO_2 gradients for *keeling plot* calculation were responsible for partial deviations from the *ecoflux* approach. However, modeling $\delta^{13}C_R$ from the component fluxes indicated very high congruencies between *ecoflux* and *eco-isoflux* model.

Small CO_2 gradients owing to low respiratory activity or strong vertical mixing are known to complicate the calculation of significant *keeling plot* regressions and thus, impede $\delta^{13}C_R$ measurements (e.g. Pataki *et al*., 2003a). The results shown here suggest the *eco-isoflux* model as a powerful alternative tool to bottom-up model $\delta^{13}C_R$ during times when sufficient CO_2 gradients for *keeling plots* are difficult to capture.

The presented models have proofed to realize a good partitioning of soil and ecosystem carbon fluxes into their assimilatory and respiratory components. They provide the possibility to directly access the different biotic responses in isotopic carbon fluxes to changes in abiotic drivers and might be a promising and innovative appliance for future carbon cycle research. Therefore, both *ecoflux* and *eco-isoflux* approach can be recommended for the use at larger spatial and temporal scales in order to achieve an accomplished comprehension of ecosystem and soil carbon dynamics.

7.3 POSSIBLE IMPACTS OF FUTURE CLIMATE CHANGE ON THE CARBON CYCLE IN THE MEDITERRANEAN

Anthropogenic green house gas emission (mainly CO_2) has led to substantial climatic changes all over the world (IPCC, 2007). Mediterranean ecosystems are especially endangered by the

consequences of this green house effect, namely an increase in length and severity of seasonal drought (Pereira *et al*., 2007), as well as larger unpredictability of precipitation events.

As mentioned above, the turn of the studied Mediterranean ecosystem from a net carbon sink to a net carbon source is caused by a strong decrease in understory carbon uptake with beginning summer drought (**chapter 5**) and annual carbon balance might largely depend on timing of the onset, rather than on the length of summer drought. Future climate impacts will thus, cause an even shorter growing period for understory species and shift the time when the ecosystem changes from a sink to a source more towards the spring.

Carbon sequestration of the ecosystem however, might not necessarily decrease by such a development, since winter will become warmer and the growing period might start earlier in the year. However, results of Pereira *et al*. (2007) showed a stronger drought effect on GPP than on R_{eco} for the studied oak woodland during the extremely dry hydrological year 2004/2005, resulting in positive annual NEE. Hence, drought shifted the ecosystem more towards a carbon source.

Increasing length and severity of drought will also increase the extent of hypo-osmotic stress responses of soil microorganisms to rapid rain pulse events during summer. The carbon released by these *Birch* pulses is lost to the atmosphere. However, results of this study indicate that this carbon is likely to originate from anaplerotic CO_2 fixation and carbon recycling of soil microbes rather than from photosynthetic products (**chapter 4**). Therefore, an increase in severity of hypo-osmotic stresses might even increase carbon sequestration by a long-term decrease in the microbial community and thus, in mineralization.

Predicting carbon fluxes in Mediterranean ecosystems under future climate scenarios is still an emerging field of research and genuine models incorporating a process-based view of carbon exchange between the major C-pools are required. This work separated short-term responses of ecosystem components from the overall ecosystem response to changes in environmental conditions that might be expected on a larger time scale for future climate scenarios. Thus, the main goal, to contribute to a mechanistic understanding of ecosystem scale carbon cycle processes, that might be important under the impact of future climatic conditions, was achieved.

REFERENCES

Adu J, Oades J (1978) Physical factors influencing decomposition of organic materials in soil aggregates. *Soil Biol. Biochem*., **10**, 109–115.

Amthor JS (1994) Higher plant respiration and its relationship to photosynthesis. In ED Schulze, MM Caldwell, eds, Ecophysiology of Photosynthesis. *Ecological Studies*. Springer-Verlag, Berlin, 71–101.

Appel T (1998) Non-biomass soil organic N: The substrate for N mineralization flushes following soil drying-rewetting and for organic N rendered $CaCl_2$–extractable upon soil drying. *Soil Biol. Biochem*., **30**, 1445–1456.

Aschman H (1973) Distribution and peculiarity of mediterranean ecosystems. In:*Mediterranean type ecosystems. Origin and structure* (eds Di Castri F, Mooney HA), Chapman & Hall, London, 11-19.

Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in plant science*, **8**, 343-351.

Atkin OK, Bruhn D, Hurry VM, Tjoelker MG (2005) The hot and the cold: unravelling the variable response of plant respiration to temperature. *Functional Plant Biology*, **32**, 87-105.

Aubinet M, Grelle A, Ibrom A, Rannik U, Moncrieff J, Foken T, Kowalski AS, Martin PH, Berbigier P, Bernhofer C, Clement R, Elbers J, Granier A, Grunwald T, Morgenstern K, Pilegaard K, Rebmann C, Snijders W, Valentini R, Vesala T (2000) Estimates of the annual net carbon and water exchange of forests: The EUROFLUX methodology. *Adv.Ecol.Res.,* **30**, 113-175.

Austin AT, Yahdjian L, Stark JM, Belnap J, Porporato A , Norton U, Ravetta DA, Schaeffer SM (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia*, **141**, 221–235.

Badeck F, Tcherkez G, Nogués S, Piel C, Ghashghaie J (2005) Post-photosynthetic fractionation of stable carbon isotopes between plant organs- a widespread phenomenon. *Rapid Communications in Mass Spectrometry*, **19**, 1381-1391

Barbour MM, McDowell NG, Tcherkez G, Bickford P, Hanson DT (2007) A new measurement technique reveals rapid post-illumination changes in the carbon isotope composition of leaf-respired CO2. *Plant Cell and Environment in press*, **30**, 1-14.

Bahn M, Knapp M, Garajova Z, Pfahringer N, Cernusca A (2006) Root respiration in temperate mountain grasslands differing in land use. *Global Change Biology*, **12** ,995-1006.

Baldocchi D, Valentini R, Running S, Oechel W, Dahlman R (1996) Strategies for measuring and modelling carbon dioxide and water vapour fluxes over terrestrial ecosystems. *Global Change Biology*, **2**, 159-168.

Baldocchi D (1997) Measuring and modelling carbon dioxide and water vapour exchange over a temperate broad-leaved forest during the 1995 summer drought. *Plant, Cell and Environment*, **20**, 1108-1122.

Baldocchi D, Falge E, Gu LH, Olson R, Hollinger D, Running S, Anthoni P, Bernhofer C, Davis K, Evans R, Fuentes J, Goldstein A, Katul G, Law B, Lee XH, Malhi Y, Meyers T, Munger W, Oechel W, U K, Pilegaard K, Schmid HP, Valentini R, Verma S, Vesala T, Wilson K, Wofsy S (2001) FLUXNET: A new tool to study the temporal and spatial variability of ecosystem-scale carbon dioxide, water vapor, and energy flux densities. *Bulletin of the American Meteorological Society*, **82**, 2415-2434.

Baptista JA, Rodrigues JS (1991) Geografia, *Universitária Editrora*, 223-230

Barbour MM, Hunt JE, Dungan RJ, Turnbull MH, Brailsford GW, Farquhar GD, Whitehead D. (2005) Variation in the degree of coupling between delta ^{13}C of phloem sap and ecosystem respiration in two mature *Nothofagus* forests. *New Phytologist*, **166**, 497-512.

Barbour MM, McDowell NG, Tcherkez G, Bickford P, Hanson DT (2007) A new measurement technique reveals rapid post-illumination changes in the carbon isotope composition of leaf-respired CO2. *Plant Cell and Environment in press*, **30**, 1-14.

Bender MM (1968) Mass spectrometric studies of carbon-13 variations in corn and other grasses. *Radiocarbon*, **10**, 468-472

Bender MM (1971) Variations in the $^{13}C/^{12}C$ ratios of plants in relations to the pathway of photosynthesic carbon dioxide fixation. *Phytochemistry*, **10**, 1239-1244

Beyschlag W, Lange OL, Tenhunen JD (1987) Photosynthese und Wasserhaushalt der immergrünen Hartlaubpflanze *Arbutus unedo* L. am Freilandstandort in Portugal. II. Charakteristische Gaswechselparameter von CO_2-Aufnahme und Transpiration. *Flora*, **179**, 399-420.

Bhupinderpal, S., A. Nordgren, M. Ottosson Löfvenius, M. N. Högberg, P.-E. Mellander and P. Högberg (2003) Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year, *Plant Cell Env.*, **26**, 1287-1296.

Birch HF (1964) Mineralisation of plant nitrogen following alternate wet and dry conditions. *Plant Soil*, **20**, 43–49.

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

Borken W, Savage K, Davidson EA, Trumbore SE (2006) Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change Biology*, **12**, 177-193.

Bottner P (1985) Response of microbial biomass to alternate moist and dry conditions in a soil incubated with 14-C and 15-N labelled plant material. *Soil Biol. Biochem*., **17**, 329–337.

Bottner P, Austrui F, Cortez J, Billes G, Coûteaux MM (1998) Decomposition of ^{14}C- and ^{15}N-labelled plant material, under controlled conditions, in coniferous forest soils from a northsouth climatic sequence in western Europe. *Soil Biol Biochem*., **30**, 597–610

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

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

Bowling DR, McDowell NG, Welker JM, Bond BJ, Law BE, Ehleringer JR. (2003a) Oxygen isotope content of CO_2 in nocturnal ecosystem respiration: 1. Observations in forests along a precipitation transect in Oregon, USA. *Global Biogeochemical Cycles*, **17**, 31-1-31-14.

Bowling DR, McDowell NG, Welker JM, Bond BJ, Law BE, Ehleringer JR. (2003b) Oxygen isotope content of CO_2 in nocturnal ecosystem respiration: 2. Short-term dynamics of foliar and soil component fluxes in an old-growth ponderosa pine forest. *Global Biogeochemical Cycles*, **17**, 34-1-34-12.

Bowling DR, Sargent SD, Tanner BD, Ehleringer JR (2003c) Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem-atmosphere CO2 exchange. *Agricultural and Forest Meteorology*, **118**, 1-19.

Brandes E, Kodama N, Whittaker K, Weston C, Rennenberg H, Keitel C, Adams MA, Gessler A (2006) Short-term variation in the isotopic composition of organic matter allocated from the leaves to the stem of Pinus sylvestris: effects of photosynthetic and postphotosynthetic carbon isotope fractionation. *Global Change Biology*, **12**, 1922-1939.

Brugnoli E, Farquhar GD. (2000) Photosynthetic fractionation of carbon isotopes. In: Leegood RC, Sharkey TD, Caemmerer v.S, eds. *Photosynthesis: Physiology and Metabolism*. The Netherlands: Kluwer Akademic Publisher, 399-434.

Buchmann N, Kao WY, Ehleringer J. (1996) Carbon dioxide concentrations within forest canopies - variation with time, stand structure, and vegetation type. *Global Change Biology*, **2**, 421-432.

Buchmann N, Guehl JM, Barigah TS, Ehleringer JR. (1997) Interseasonal comparison of CO_2 concentrations, isotopic composition, and carbon dynamics in an Amazonian rainforest (French Guiana). *Oecologia*, **110**, 120-131.

Buchmann N, Brooks JR, Flanagan LB, Ehleringer JR (1998) Carbon isotope discrimination of terrestrial ecosystems, in *Stable Isotopes - integration of biochemical, ecological and geochemical processes*, edited by H. Griffiths, BIOS Scientific Publishers Ltd, Oxford, 202-221.

Buchmann N (2000) Biotic and abiotic factors controlling soil respiration rates in Picea abies stands. *Soil Biology and Biochemistry*, **32**, 1625-1635.

Canadell JG, Mooney HA, Baldocchi D, Berry JA, Ehleringer JR, Field CB, Gower ST, Hollinger DY, Hunt JE, Jackson RB, Running SW, Shaver GR, Steffen W, Trumbore SE, Valentini R, Bond BY. (2000) Carbon Metabolism of the Terrestrial Biosphere: a multi-technique approach for improved understanding. *Ecosystems*, **3**, 115-130.

Carreiras JMB, Pereira JMC, Pereira, JS (2006) Estimation of tree canopy cover in evergreen oak woodlands using remote sensing. *Forest Ecol. Managem.*, **223**, 45–53

Ciais P, Reichstein M, Viovy N, Granier A, Ogee J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A, Chevallier F, De Noblet N, Friend AD, Friedlingstein P, Grunwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature*, **437**, 529-533.

Cohen, Y (1994) Thermoelectric methods for measurement of sap flow in plants. *Advances in Bioclimatology*, **3**, 63-89.

Cox PM, Betts RA, Jones CD, Spall SA, Totterdell IJ (2000) Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature*, **408**, 184-187

Damesin C (2003) Respiration and photosynthesis characteristics of current-year stems of *Fagus sylvatica*: from the seasonal pattern to an annual balance. *New Phytologist*, **158**, 465-475.

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

David TS, Ferreira MI, Cohen S, Pereira JS, David JS (2004) Constraints on transpiration from an evergreen oak tree in southern Portugal. *Agricultural and Forest Meteorology* ,**122**, 193-205.

David TS, Gash JHC, Valente F, Pereira JS, Ferreira MI, David JS (2006) Rainfall interception by an isolated evergreen oak tree in a Mediterranean savannah. *Hydrol. Process.* **20**, 2713–2726

David TS, Henriques MO, Kurz-Besson C, Nunes J, Valente F, Vaz M, Pereira JS, Siegwolf R, Chaves MM, Gazarini LC, David JS (2007) Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. *Tree Physiology*, **27**, 793-803.

Davidson EA, Verchot LV, Cattanio JH, Ackerman IL, Carvalho JEM (2000) Effects of soil water content on soil respiration in forests and cattle pastures of eastern Amazonia. *Biogeochemistry*, **48**, 53-69.

Davidson EA, Richardson AD, Savage KE, Hollinger DY (2006a) A distinct seasonal pattern of the ratio of soil respiration to total ecosystem respiration in a spruce-dominated forest. *Global Change Biology*, **12**, 230-239.

Davidson E, Janssens IA, Luo Y (2006b) On the variability of respiration in terrestrial ecosystems: moving beyond Q10. *Global Change Biology*, **12**, 154-164.

Davis FW, Michaelsen J (1995) Sensitivity of fire regime in chaparral ecosystems to climate change. In: Moreno JM, Oechel WC (eds.) *Global Change and Mediterranean-Type ecosystems*, Ecological studies 117, Springer Verlag, Berlin, 435-456

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable Isotopes in Plant Ecology. *Annu.Rev.Ecol.Syst.* ,**33**, 507-559.

Degens BP, Sparling GP (1995) Repeated wet-dry cycles do not accelerate the mineralization of organic C involved in the macro-aggregation of a sandy loam soil. *Plant Soil*, **175**,197–203.

Denef K, Six J, Bossuyt H, Frey SD, Elliott ET, Merckx R, Paustian K (2001) Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial activity dynamics. *Soil Biol. Biochem*., **33**, 1599–1611.

Di Castri F, Goodall DW, Specht RL (1981) Ecosystems of the world 11 - *Mediterranean-type shrublands*. Elsevier Scientific Publishing Company, Amsterdam, Oxford, New York, 1-52.

Duranceau M, Ghashghaie J, Badeck F, Deléens E, Cornic G (1999) δ13C of leaf carbohydrates in relation to dark respiration in *Phaseolus vulgaris* L.under progressive drought. *Plant, Cell and Environment*, **22**, 515-523.

Ehleringer JR, Buchmann N, Flanagan LB. (2000) Carbon isotope ratios in belowground carbon cycle processes. *Ecological Applications* **10**: 412-422.

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

Ekblad A, Bostrom B, Holm A, Comstedt D (2005) Forest soil respiration rate and delta C-13 is regulated by recent above ground weather conditions. *Oecologia*, **143**, 136-142.

Eliasson PE, McMurtrie RE, Pepper DA, Stromgren M, Linder S, Agren GI (2005) The response of heterotrophic CO_2 flux to soil warming. *Global Change Biology*, **11**, 167-181.

Eugster W, Senn W (1995) A cospectral correction model for measurement of turbulent NO_2 flux. *Bound. Layer Meteorol*., **74**, 321-340

Farquhar GD, O´Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology*, **9**, 121-137.

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503-537.

Fessenden J, Ehleringer JR (2002) Age-related variations in$\delta^{13}C$ ecosystem respiration across a coniferous forest chronosequence in the Pacific Northwest. *Tree Physiology*, **22**, 159-167.

Fessenden JE, Ehleringer JR (2003) Temporal variation in $\delta^{13}C$ of ecosystem respiration in the Pacific Northwest: links to moisture stress. *Oecologia*, **136**, 129-136.

Fierer N, Schimel JP (2002) Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biol. Biochem*., **34**, 777–787.

Fierer N, Schimel JP (2003) A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Sci. Soc. Am. J.*, **67**, 798–805.

Flanagan LB, Varney GT. (1995) Influence of vegetation and soil CO_2 exchange on the atmospheric CO_2 within a *Pinus resinosa* canopy. *Oecologia*, **101**, 37-44.

Flanagan LB, Brooks JR, Varney GT, Berry SC, Ehleringer JR (1996) Carbon isotope discrimination druing photosynthesis and the isotope ratio of respired CO2 in boreal forest ecosystems. *Global Biogeochemical Cycles*, **10**, 629-640.

Flanagan LB, Ehleringer J. (1998) Ecosystem-atmosphere CO_2 exchange: interpreting signals of change using stable isotope ratios. *Trends in Ecology and Evolution*, **13**, 10-14.

Flanagan LB, Johnson BG (2005) Interacting effects of temperature, soil moisture and plant biomass production on ecosystem respiration in a northern temperate grassland. *Agricultural and Forest Meteorology*, **130**, 237-253.

Foken T (2003) Angewandte Meteorologie. *Springer-Verlag*, Berlin, Heidelberg.

Franzluebbers A, Haney R, Honeycutt C, Schomberg H, Sorensen F (1974) Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. *Soil Sci. Soc. Am. J.*, **64**, 613–623.

Fritz P, Fontes JC (1980) Handbook of environmental isotope geochemistry. Vol. I, *The terrestrial environment*, Elsevier, Amsterdam.

Gaumont-Guay D, Black TA, Griffis TJ, Barr AG, Jassal RS, Nesic Z (2006) Interpreting the dependence of soil respiration on soil temperature and water content in a boreal aspen stand. *Agricultural and Forest Meteorology*, **140**, 220-235.

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

Ghashghaie J, Duranceau M, Badeck F, Cornic G, Adeline MT, Deléens E (2001) $\delta^{13}C$ of CO_2 respired in the dark in relation to ^{13}C of leaf metabolites: comparison between *Nicotiana sylvestris* and *Helianthus annuus* under drought. *Plant, Cell and Environment* ,**24**, 145-515.

Ghashghaie J, Badeck F, Lanigan G, Nogúes, S., Tcherkez G, Deléens E, Cornic G, Griffiths H (2003) Carbon isotope fractionation during dark respiration and photorespiration in C3 plants. *Phytochemistry Rewies* ,**2**, 145-161.

Göttlicher SG, Knohl A, Wanek W, Buchmann N, Richter A (2006) Short-term changes in carbon isotope composition of soluble carbohydrates and starch: from canopy leaves to the roots. *Rapid Communications in Mass Spectrometry*, **20**, 653-660.

Goulden ML, Munger JW, Fan SM, Daube BC, Wofsy SC (1996) Measurements of carbon sequestration by long-term eddy covariance: Methods and a critical evaluation of accuracy. *Global Change Biology*, **2**, 169-182.

Granier A (1985) Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres. *Annales des Sciences Forestières*, **42**, 193-200.

Granier A (1987) Mesure du flux de sève brute dans le tronc du Douglas par une nouvelle méthode thermique. *Annales des Sciences Forestières*, **44**, 1-14.

Granier A, Reichstein M, Breda N, Janssens IA, Falge E, Ciais P, Grunwald T, Aubinet M, Berbigier P, Bernhofer C, Buchmann N, Facini O, Grassi G, Heinesch B, Ilvesniemi H, Keronen P, Knohl A, Kostner B, Lagergren F, Lindroth A, Longdoz B, Loustau D, Mateus J, Montagnani L, Nys C, Moors E, Papale D, Peiffer M, Pilegaard K, Pita G, Pumpanen J, Rambal S, Rebmann C, Rodrigues A, Seufert G, Tenhunen J, Vesala I, Wang Q (2007) Evidence for soil water control on carbon and water dynamics in European forests during the extremely dry year: 2003. *Agricultural and Forest Meteorology*, **143**, 123-145.

Griffis TJ, Black TA, Gaumont-Guay D, Drewitt GB, Nesic Z, Barr AG, Morgenstern K, Kljun N (2004) Seasonal variation and partitioning of ecosystem respiration in a southern boreal aspen forest. *Agricultural and Forest Meteorology*, **125**, 207-223.

Griffiths E, Birch HF (1961) Microbiological changes in freshly moistened soil. *Nature*, **189**, 424.

Hanson PJ, Wullschleger SD, Norby RJ, Tschaplinski TJ, Gunderson CA (2005) Importance of changing CO_2, temperature, precipitation, and ozone on carbon and water cycles of an upland-oak forest: incorporating experimental results into model simulations. *Global Change Biology*, **11**, 1402-1423.

Hartley IP, Armstrong AF, Murthyw R, Barron-Gafford G, Ineson P, Atkin OK (2006) The dependence of respiration on photosynthetic substrate supply and temperature: integrating leaf, soil and ecosystem measurements. *Global Change Biology*, **12**, 1954-1968.

Heath J, Ayres E, Possell M, Bardgett RD, Black HIJ, Grant H, Ineson P, Kerstiens G (2005) Rising atmospheric CO_2 reduces sequestration of root-derived soil carbon. *Science*, **309**, 1711-1713.

Hemming D, Yakir D, Ambus P, Aurela M, Besson C, Black K, Buchmann N, Burlett R, Cescatti A, Clement R, Gross P, Granier A, Grunwald T, Havrankova K, Janous D, Janssens IA, Knohl A, Ostner BK, Kowalski A, Laurila T, Mata C, Marcolla B, Matteucci G, Moncrieff J, Moors EJ, Osborne B, Pereira JS, Pihlatie M, Pilegaard K, Ponti F, Rosova Z, Rossi F, Scartazza A, Vesala T (2005) Pan-European delta C-13 values of air and organic matter from forest ecosystems. *Global Change Biology*, **11**, 1065-1093.

Herrera MA, Salamanca CP, Barea JM (1992) Inoculation of woody legumes with selected arbuscular mycorrhizal fungi and rhizobia to recover desertified Mediterranean ecosystems. *Appl. Environ. Microbiol.*, **59**, 129-133

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

Hofrichter R (2002) Das Mittelmeer – Fauna, Flora, Ökologie (Spektrum, Akademischer Verlag, Heidelberg, Berlin.

Hohmann S (2002) Osmotic stress signaling and osmoadaptation in yeasts. *Microbiol. Mol. Biol. Rev.*, **66**, 300–372.

Hollis GE (1992) Implications of climatic changes in the Mediterranean Basin. In: Jeftic L, Milliman JD, Sestini G (eds.), *Climatic Change and the Mediterranean,* United Nations Environment Programme, UK, 602-665

Huxman TE, Turnipseed AA, Sparks JP, Harley PC, Monson RK (2003) Temperature as a control over ecosystem CO_2 fluxes in a high-elevation, subalpine forest. *Oecologia*, **134**, 537-546.

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

IPCC (1996) Houghton J, Filho LM, Callander B, Harris N, Lattenberg A, Maskell K, Climate change 1996: The science of climate change. *Contribution of working group I to the second assessment report of the intergovernmental panel on climate change*, Cambridge University Press, Cambridge, U.K., 1-364.

IPCC (2001) Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA, Climate change 2001: the scientific basis. *Contribution of working group I to the third assessment report of the intergovernmental panel on climate change*., Cambridge University Press, Cambridge, U.K., 881.

IPCC (2007) IPCC WGI Fourth Assessment Report. Summary for Policymakers. Available on-line http://www.ipcc.ch/WG1_SPM_17Apr07.pdf

Irvine J, Law BE (2002) Seasonal soil CO_2 effluxes in young and old ponderosa pine forests. *Global Change Biology*, **8**, 1183-1194.

Irvine J, Law BE, Kurpius MR (2005) Coupling of canopy gas exchange with root and rhizosphere respiration in a semi-arid forest. *Biogeochemistry*, **73**, 271-282.

Jager G & Bruins EH (1974) Effect of repeated drying at different temperatures on soil organic matter decomposition and characteristics, and on the soil microflora. *Soil Biol. Biochem*., **7**, 153–159.

Janssens IA, Kowalski AS, Ceulemans R (2001) Forest floor CO_2 fluxes estimated by eddy covariance and chamber-based model. *Agricultural and Forest Meteorology*, **106**, 61-69.

Jarvis PG, Rey A, Petsikos C, Rayment M, Pereira JS, Banza J, David JS, Miglietta F, Valentini R (2007) Drying and wetting of soils stimulates decomposition and carbon dioxide emission: the "Birch Effect". *Tree Physiology*, **27**, 929-940.

Joffre R, Rambal S, Damesin C (1999) Functional attributes in Mediterranean-type ecosystems. In:*Handbook of functional plant* ecology (eds Pugnaire FI, Valladares F), Marcel Dekker, Inc., New York, Basel, 347-380.

Keeling CD (1958) The concentration, and isotopic abundance of atmospheric carbon dioxide in rural areas, *Geochimica et Cosmochimica Acta*, **13**, 322-334.

Keeling RF (1995) The atmospheric oxygen cycle: the oxygen isotope of atmospheric CO_2 and O_2 and the O_2/N_2 ratio. *Reviews of Geophysics*, supplement, 1253-1262.

Keeling CD, Whorf TP (2002) Atmospheric CO_2 records from sites in the SIO air sampling network. In Trends: *A Compendium of Data on Global Change. Oak Ridge* (TN): Carbon Dioxide Information Analysis Center,Oak Ridge National Laboratory. (29 July 2003; http://cdiac.esd.ornl. gov/trends/co2/sio-keel.htm).

Kieft TL, Soroker E, Firestone MK (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biol. Biochem*., **19**, 119–126.

Klumpp K, Schäufele R, Lötscher M, Lattanzi FA, Feneis W, Schnyder H (2005) C-isotope composition of CO_2 respired by shoots and roots: fractionation during dark respiration. *Plant, Cell and Environment*, **28**, 241-250.

Knohl A (2003) Carbon dioxide exchange and isotopic signature (13 C) of an unmanaged 250 year-old deciduos forest. *PhD-Thesis.*

Knohl A, Buchmann N (2005) Partitioning the net CO_2 fluxes of a deciduous forest into respiration and assimilation using stable carbon isotopes. *Global Biogeochemical Cycles*, **19**, 1-14.

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

Kurz-Besson C, Otieno DO, Lobo do Vale R, Siegwolf R, Schmidt M, Herd A, Nogueira C, David TS, David JS, Tenhunen J, Pereira JS, Chaves M (2006) Hydraulic lift in cork oak trees in a savannah-type Mediterranean ecosystem and its contribution to the local water balance. *Plant and Soil*, **282**, 361-378.

Kuzyakov Y, Cheng W (2001) Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biology & Biochemistry*, **33**, 1915-1925.

Lai CT, Ehleringer JR, Schauer AJ, Tans PP, Hollinger DY, Paw U, Munger JW, Wofsy SC (2005) Canopy-scale delta 13C of photosynthetic and respiratory CO_2 fluxes: observations in forest biomes across the United States. *Global Change Biology*, **11**, 633-643

Lambers H, Szaniawski RK, Devisser R (1983) Respiration for Growth, Maintenance and Ion Uptake - An Evaluation of Concepts, Methods, Values and Their Significance. *Physiologia Plantarum*, **58**, 556-563.

Larcher W (2000) Temperature stress and survival ability of Mediterranean sclerophyllous plants. *Plant Biosystems*, **134**, 279-295.

Lavigne MB, Ryan MG, Anderson DE, Baldocchi DD, Crill PM, Fitzjarrald DR, Goulden ML, Gower ST, Massheder JM, McCaughey JH, Rayment M, Striegl RG (1997) Comparing nocturnal eddy covariance measurements to estimates of ecosystem respiration made by scaling chamber measurements at six coniferous boreal sites. *Journal of Geophysical Research-Atmospheres*, **102**, 28977-28985.

Law BE, Ryan MG, Anthoni PM (1999) Seasonal and annual respiration of a ponderosa pine ecosystem. *Global Change Biology*, **5**, 169-182.

Law BE, Thornton PE, Irvine J, Anthoni PM, Van Tuyl S (2001a) Carbon storage and fluxes in ponderosa pine forests at different developmental stages. *Global Change Biology*, **7**, 755-777.

Law BE, Kelliher FM, Baldocchi DD, Anthoni PM, Irvine J, Moore D, Van Tuyl S (2001b) Spatial and temporal variation in respiration in a young ponderosa pine forests during a summer drought. *Agricultural and Forest Meteorology*, **110**, 27-43.

Law BE, Falge E, Gu L, Baldocchi DD, Bakwin P, Berbigier P, Davis K, Dolman AJ, Falk M, Fuentes JD, Goldstein A, Granier A, Grelle A, Hollinger D, Janssens IA, Jarvis P, Jensen NO, Katul G, Mahli Y, Matteucci G, Meyers T, Monson R, Munger W, Oechel W, Olson R, Pilegaard K, Paw KT, Thorgeirsson H, Valentini R, Verma S, Vesala T, Wilson K, Wofsy S (2002) Environmental controls over carbon dioxide and water vapor exchange of terrestrial vegetation. *Agricultural and Forest Meteorology*, **113**, 97-120.

Lee TD, Reich PB, Bolstad PV (2005) Acclimation of leaf respiration to temperature is rapid and related to specific leaf area, soluble sugars and leaf nitrogen across three temperate deciduous tree species. *Functional Ecology*, **19**, 640-647.

Lindroth A, Grelle A, Morén A-S (1998) Long-term measurements of boreal forests. *Global Change Biology*, **2**, 231-240

Lloyd J, Farquhar GD. (1994) ^{13}C discrimination during CO_2 assimilation by the terrestrial biosphere. *Oecologia*, **99**, 201-215.

Lloyd J, Kruijt B, Hollinger DY, Grace J, Francey RJ, Wong SC, Kelliher FM, Miranda AC, Farquhar GD, Gash JHC, Vygodskaya NN, Wright IR, Miranda HS, Schulze E-D (1996) Vegetation effects on the isotopic composition of atmospheric CO_2 at local and regional scales: theoretical aspects ad a comparison between rain forest in Amazonia and a boreal forest in Sibiria. *Australian Journal of Plant Physiology*, **23**, 371-399.

Loveys BR, Atkinson LJ, Sherlock DJ, Roberts RL, Fitter AH, Atkin OK (2003) Thermal acclimation of leaf and root respiration: an investigation comparing inherently fast- and slow-growing plant species. *Global Change Biology*, **9**, 895-910.

Lusk CH, Reich PB (2000) Relationships of leaf dark respiration with light environment and tissue nitrogen content in juveniles of 11 cold-temperate tree species. *Oecologia*, **123**, 318-329.

McDowell NG, Bowling DR, Schauer A, Irvine J, Bond BJ, Law BE, Ehleringer JR (2004a) Associations between carbon isotope ratios of ecosystem respiration, water availability and canopy conductance. *Global Change Biology*, **10**, 1767-1784.

McDowell NG, Bowling DR, Bond BJ, Irvine J, Law BE, Anthoni P, Ehleringer JR (2004b) Response of the carbon isotopic content of ecosystem,leaf, and soil respiration to meteorological driving factors in a *Pinus ponderosa* ecosystem. *Global Biogeochemical Cycles*, **18**, 1013-12.

Miranda P, Coelho FES, Tomé AR, Valente MA (2002) 20th century Portuguese climate and climate scenarios. in: Santos FD, Forbes K, Moita R (eds.) *Climate change in Portugal. Scenarios, Impacts, and Adaptation measures*. Lisbon, Gradiva.

Misson L, Baldocchi DD, Black TA, Blanken PD, Brunet Y, CURIEL YUSTE J, Dorsey JR, Falk M, Granier A, Irvine MR, Jarosz N, Lamaud E, Launiainen S, Law BE, Longdoz B, Loustau D, Mckay M, Paw U, Vesala T, Vickers D, Wilson KB, Goldstein AH (2007) Partitioning forest carbon fluxes with overstory and understory eddy-covariance measurements: A synthesis based on FLUXNET data. *Agricultural and Forest Meteorology*, **144**, 14-31.

Monteith JL, Unsworth MH (1990) Principles of environmental physics. Arnold, London.

Mooney C (1978) Types of climate (II): In middle latitudes. In: Money D.C. (eds.): *Climate, soils and vegetation*. University tutorial press LTD; Slough, 118-139.

Mortazavi B, Chanton JP (2002) Carbon isotopic discrimination and control of nighttime canopy $\delta^{18}O$-CO_2 in a pine forest in the southeastern United States. *Global Biogeochemical Cycles* **16**: 8-1-13.

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

Mortazavi B, Chanton JP, Smith MC (2006) Influence of C-13-enriched foliage respired CO_2 on delta C-13 of ecosystem-respired CO_2. *Global Biogeochemical Cycles*, **20** (3), GB3029, doi:10.1029/2005GB002650

Nadelhoffer KJ, Fry B (1988) Controls on natural 15N and 13C abundances in forest soil organic matter. *Soil Sci Soc Am J*, **52**, 1633-1640

Nadezhdina N, Cermák J, Nadezhdin V (1998) The heat field deformation method for sap flow measurement. In Cérmak J, Nadezhdina N. (eds.). *4th International Workshop on Measuring Sap flow in Intact Plants*. Brno, Czech Republic IUFRO Publications, Publishing house of Mendel University, 72-92.

Nahal I (1981) The mediterranean climate from a biological view point. In:Ecosystems of the world 11; *Mediterranean-type shrublands* (eds Di Castri F, Goodall DW, Specht RL), Elsevier Scientific Publishing, Amsterdam, 63-87.

Naveh Z (1995) Conservation, Restoration, and Research priorities for Mediterranean uplands threatened by Global Change. In: Moreno JM, Oechel WC (eds.) *Global Change and Mediterranean-Type ecosystems*, Ecological studies 117, Springer Verlag, Berlin, 482-507.

Nogués S, Tcherkez G, Cornic G, Ghashghaie J (2004) Respiratory carbon metabolism following illumination in intact french bean leaves using $^{13}C/^{12}C$ isotope labelling. *Plant Physiology*, **136**, 3245-3254

Nogués S, Damesin C, Tcherkez G, Maunoury F, Cornic G, Ghashghaie J (2006) $^{13}C/^{12}C$ isotope labelling to study leaf carbon respiration and allocation in twigs of field grown beech trees. *Rapid Communications in Mass Spectrometry* , **20**, 219-226.

Obukhof AM (1951) Investigation of the micro-structure of the wind in the near-surface layer of the atmosphere. *Izvestia AN SSSR, seria Geofizika*, **3**, 49.

Ogée J, Peylin P, Ciais P, Bariac T, Brunet Y, Berbigier P, Roche C, Richard P, Bardoux G, Bonnefond JM (2003) Partitioning net ecosystem carbon exchange into net assimilation and respiration using $^{13}CO_2$ measurements: A cost-effective sampling strategy. *Global Biogeochemical Cycles*, **17**, 1070.

Ometto JP, Flanagan LB, Martinelli LA, Moreira MZ, Higuchi N, Ehleringer JR (2002) Carbon isotope discrimination in forest and pasture ecosystems of the Amazon Basin, Brazil. *Global Biogeochemical Cycles*, **16/ 4**, 56-1-56-10.

Orchard VA, Cook FJ (1983) Relationship between soil respiration and soil moisture. *Soil Biol. Biochem*., **15**, 447-454.

Oren R, Sperry JS, Ewers BE, Pataki DE, Phillips N, Megonigal JP (2001) Sensitivity of mean canopy stomatal conductance to vapor pressure deficit in a flooded Taxodium distichum L. forest: hydraulic and non-hydraulic effects. *Oecologia*, **126**, 21-29.

Otieno D, Kurz-Besson K, Liu J, Schmidt M, Vale RD, David T, Siegwolf R, Pereira J and Tenhunen J (2006) Seasonal variations in soil and plant water status in a *Quercus suber* L. stand: roots as determinants of tree productivity and survival in the Mediterranean-type Ecosystem. *Plant and Soil*, **283**,119-135.

Papale D, Valentini A (2003) A new assessment of European forests carbon exchanges by eddy fluxes and artificial neural network spatialization. *Global Change Biology*, **9**, 525-535.

Pataki DE, Ehleringer JR, Flanagan LB, Yakir D, Bowling DR, Still CJ, Buchmann N, Kaplan JO, Berry JA (2003a) The application and interpretation of Keeling plots in terrestrial carbon cycle research. *Global Biogeochemical Cycles*, **17**, 1022.

Pataki DE, Bowling DR, Ehleringer JR (2003b) Seasonal cycle of carbon dioxide and its isotopic composition in an urban atmosphere: Anthropogenic and biogenic effects. *Journal of Geophysical Research*, **108**, NO. D23, 4735, doi:10.1029/2003JD003865.

Pate J, Arthur D (1998) delta C-13 analysis of phloem sap carbon: novel means of evaluating seasonal water stress and interpreting carbon isotope signatures of foliage and trunk wood of Eucalyptus globulus. *Oecologia*, **117**, 301-311.

Peñuelas J, Gordon C, Llorens L, Nielsen TR, Tietema A, Beier C, Bruna P, Emmet B, Estiarte M, Gorissen A (2004) Non-intrusive field experiments show different plant responses to warming and drought among sites, seasons and species in a North-South European gradient. *Ecosystems*, **7**, 598-612

Pereira JS, Chaves MM (1995) Plant responses to drought under climate change in Mediterranean-type ecosystems. In:*Global change and Mediterranean-type Ecosystems*. *Ecological Studies*, **117**, (eds Moreno JM, Oechel WC), pp. 140-160. Springer-Verlag, Berlin.

Pereira JS, David JS, David TS, Caldeira MC, Chaves MM (2004) Carbon and Water Fluxes in Mediterranean-Type Ecosystems - Constraints and Adaptations. In Esser K, Lüttge U, Beyschlag W, Murata J. eds *Progress in Botany* Berlin Heidelberg: Springer-Verlag, 467-498.

Pereira JS, Chaves MM, Caldeira MC, Correia AV (2006), Water availability and productivity, in: Plant Growth and climate change, edited by: Morrison JIL and Morecroft D, Blackwell Publishers, London, 118-145

Pereira JS, Mateus JA, Aires LM, Pita G, Pio C, David JS, Andrade V, Banza J, David TS, Paço T and Carbo Europe PT (2007) Net ecosystem carbon exchange in three contrasting Mediterranean ecosystems. The effect of drought. *Biogeosciences Discuss*., **4**, 1-35, in press.

Ponton S, Flanagan LB, Alstad KP, Johnson BG, Morgenstern K, Kljun N, Black TA, Barr AG (2006) Comparison of ecosystem water-use efficiency among Douglas-fir forest, aspen forest and grassland using eddy covariance and carbon isotope techniques. *Global Change Biology*, **12**, 294-310.

Potts M (1994) Desiccation tolerance of prokaryotes. *Microbiol. Rev*., **58**, 755–805.

Prater JL, Mortazavi B, Chanton JP (2006) Diurnal variation of the δ13C of pine needle respired CO2 evolved in darkness. *Plant, Cell and Environment*, **29**, 202-211.

Pregitzer KS, King JA, Burton AJ, Brown SE (2000) Responses of tree fine roots to temperature. *New Phytologist*, **147**, 105-115.

Prentice IC, Farquhar GD, Fasham MJR, Goulden ML, Heiman M, Jaramillo VJ, Kheshgi, HS, Le Quere C, Scholes RJ, Wallace DWR (2001) The carbon cycle and atmospheric carbon dioxide. In: Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA (eds.), *Climate Change 2001: The Scientific Basis, Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press., Cambridge, pp. 183-237.

Quezel P (1977) Forests of the Mediterranean basin. In: *Mediterranean Forests and marquis: ecology conservation and management*. UNESCO ed., Paris, 9-33.

Rambal S (1999) Spatial and temporal variations in productivity of Mediterranean-type ecosystems: a hierarchical perspective. In: Mooney HA, Saugier B, Roy J (eds.): *Terrestrial global productivity: past, present, future*. Elsevier Academic Press, San Diego.

Rambal S, Ourcival JM, Joffre R, Mouillot F, Nouvellon Y, Reichstein M, Rocheteau A (2003) Drought controls over conductance and assimilation of a Mediterranean evergreen ecosystem: scaling from leaf to canopy. *Global Change Biology*, **9**, 1813-1824.

Rambal S, Joffre R, Ourcival JM, Cavender-Bares J, Rocheteau A (2004) The growth respiration component in eddy CO_2 flux from a *Quercus ilex* mediterranean forest. *Global Change Biology*, **10**, 1460-1469.

Reichstein M (2001) Drought effects on carbon and water exchange in three Mediterranean ecosystems. PhD-Thesis, *Bayreuther Forum Ökologie*, BITÖK, 1-150.

Reichstein M, Tenhunen JD, Roupsard O, Ourcival J-M, Rambal S, Dore S, Valentini R (2002a) Ecosystem respiration in two Mediterranean evergreen Holm Oak forests: drought effects and decomposition dynamics. *Functional Ecology*, **16**, 27-39.

Reichstein M, Tenhunen JD, Roupsard O, Ourcival JM, Rambal S, Miglietta F, Peressotti A, Pecchiari M, Tirone G, Valentini R (2002b) Severe drought effects on ecosystem CO_2 and H_2O fluxes at three Mediterranean evergreen sites: revision of current hypotheses? *Global Change Biology*, **8**, 999-1017.

Reichstein M, Tenhunen J, Roupsard O, Ourcival JM, Rambal S, Miglietta F, Peressotti A, Pecchiari M, Tirone G, Valentini R (2003) Inverse modeling of seasonal drought effects on canopy CO_2/H_2O exchange in three Mediterranean ecosystems. *Journal of Geophysical Research-Atmospheres*, **108**, ACL6.1-ACL6.16

Reichstein M, Falge E, Baldocchi D, Papale D, Aubinet M, Berbigier P, Bernhofer C, Buchmann N, Gilmanov T, Granier A, Grunwald T, Havrankova K, Ilvesniemi H, Janous D, Knohl A, Laurila T, Lohila A, Loustau D, Matteucci G, Meyers T, Miglietta F, Ourcival JM, Pumpanen J, Rambal S, Rotenberg E, Sanz M, Tenhunen J, Seufert G, Vaccari F, Vesala T, Yakir D, Valentini R (2005) On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm. *Global Change Biology*, **11**, 1424-1439.

Rey A, Petsikos C, Jarvis PG, Grace J (2005) Effect of temperature and moisture on rates of carbon mineralization in a Mediterranean oak forest soil under controlled and field conditions. *European Journal of Soil Science*, **56**, 589-599.

Rossmann A, Butzenlechner M, Schmidt HL (1991) Evidence for a non-statistical carbon isotope distribution in natural glucose. *Plant Physiology*, **96**, 609-614.

Saetre P, Stark JM (2005) Microbial dynamics and carbon and nitrogen cycling following re-wetting of soils beneath two semi-arid plant species. *Oecologia*, **142**, 247-260

Scartazza A, Mata C, Matteucci G, Yakir D, Moscatello S, Brugnoli E (2004) Comparisons of $\delta\ ^{13}C$ of photosynthetic products and ecosystem respiratory CO_2 and their responses to seasonal climate variability. *Oecologia*, **140**, 340-351.

Scheu S, Parkinson D (1994) Changes in bacterial and fungal biomass C, bacterial and fungal biovolume and ergosterol content after drying, remoistening and incubation of different layers of cool temperate forest soils. *Soil Biol. Biochem*., **26**, 1515–1525.

Schimel DS, House JI, Hibbard KA, Bousquet P, Ciais P, Peylin P, Braswell BH, Apps MJ, Baker D, Bondeau A, Canadell J, Churkina G, Cramer W, Denning AS, Field CB, Friedlingstein P, Goodale C, Heimann M, Houghton RA, Melillo JM, Moore B, Murdiyarso D, Noble I, Pacala SW, Prentice IC, Raupach MR, Rayner PJ, Scholes RJ, Steffen WL, Wirth C (2001) Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. *Nature*, **414**, 169-172.

Schlesinger WH (1997) Biogeochemistry: An analysis of global change. Academic Press, San Diego, 565.

Schmidt H-L, Gleixner G (1998) Carbon isotope effects on key reactions in plant metabolism and ^{13}C-patterns in natural compounds. In:Stable Isotopes - integration of biocemical, ecological and geochemical processes (ed Griffiths H), 13-24. BIOS Scientific Publishers Ltd, Oxford.

Schnyder H, Schaufele R, Wenzel R (2004) Mobile, outdoor continuous-flow isotope-ratio mass spectrometer system for automated high-frequency C-13- and O-18-CO2 analysis for Keeling plot applications. *Rapid Communications in Mass Spectrometry*, **18**, 3068-3074.

Schulze ED, Wirth C, Heimann M (2000) Climate change - managing forests after Kyoto. *Science*, **289**, 2058-2059.

Scott-Denton LE, Rosenstiel TN, Monson RK (2006) Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biology*, **12**, 205-216.

Smith EC, Epstein S (1971) Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiology*, **47**, 380-384.

Søe ARB, Giesemann A, Anderson TH, Weigel HJ, Buchmann N (2004) Soil respiration under elevated CO_2 and its partitioning into recently assimilated and older carbon sources. *Plant and Soil*, **262**, 85-94.

Søe ARB, Buchmann N (2005) Spatial and temporal variations in soil respiration in relation to stand structure and soil parameters in an unmanaged beech forest. *Tree Physiology*, **25**, 1427-1436.

Steinmann KTW, Siegwolf R, Saurer M, Korner C (2004) Carbon fluxes to the soil in a mature temperate forest assessed by C-13 isotope tracing. *Oecologia*, **141**, 489-501.

Still CJ, Berry JA, Ribas-Carbó M, Helliker BR (2003) The contribution of C_3 and C_4 plants to the carbon cycle of tallgrass prairie: an isotopic approach. *Oecologia*, **136**, 347-359.

Subke JA, Inglima I, Cotrufo MF (2006) Trends and methodological impacts in soil CO2 efflux partitioning: A metaanalytical review. *Global Change Biology*, **12**, 921-943.

Swinbank WC (1951) The measurement of vertical transfer of heat and water vapour by eddies in the lower atmosphere. *J. Meteorol.*, **8**, 135-145

Tang JW, Baldocchi DD (2005) Spatial- and temporal variation in soil respiration in an oak-grass savannah ecosystem in California and its partitioning into autotrophic and heterotrophic components. *Biogeochemistry*, **73**, 183-207.

Tang JW, Baldocchi DD, Xu L (2005) Tree photosynthesis modulates soil respiration on a diurnal time scale. *Global Change Biology*, **11**, 1298-1304.

Tcherkez G, Nogués S, Bleton J, Cornic G, Badeck F, Ghashghaie J (2003) Metabolic Origin of Carbon Isotope Composition of Leaf Dark-Respired CO_2 in French Bean. *Plant Physiology*, **131**, 237-244.

Tcherkez G, Farquhar G, Badeck F, Ghashghaie J (2004) Theoretical considerations about carbon isotope distribution in glucose of C3 plants. *Functional Plant Biology*, **31**, 857-877.

Tenhunen JD, Lange OL, Gebel J, Beyschlag W, Weber JA (1984) Changes in the photosynthetic capacity, carboxylation efficiency, and CO2 compensation point associated with midday stomatal closure and midday depression of net CO2 exchange of leaves of Quercus suber. *Planta*, **162**, 193-203.

Tenhunen JD, Sala Serra A, Harley PC, Dougherty RL, Reynolds FJ (1990) Factors influencing carbon fixation and water use by mediterranean sclerophyll shrubs during summer drought. *Oecologia*, **82**, 381-393.

Tirone G (2003) Stima del bilanco del carbonio di due ecosistemi forestali Mediterranei. Confronto tra una lecceta e una pineta. In: PhD Thesis, Department of Forest Science and Resources (DISAFRI), p. 98. University of Tuscia, Viterbo, Italy.

Tomaselli R (1981) Relations with other ecosystems: temperate evergreen forest, Mediterranean coniferous forest, savannahs, stepps and desert shrublands. In:Ecosystems of the world 11; *Mediterranean-type shrublands* (eds Di Castri F, Goodall DW, Specht RL), Elsevier Scientific Publishing, Amsterdam, 123-130.

Trumbore S (2006) Carbon respired by terrestrial ecosystems - recent progress and challenges. *Global Change Biology*, **12**, 141-153.

Tu KP, Dawson TE (2005) Partitioning ecosystem respiration using stable carbon isotope analyses of CO_2. In: Flanagan L, Ehleringer JR, Pataki DE (eds.) *Stable isotopes and biosphere-atmosphere interactions*, Elsevier Academic Press, San Diego, 125-148.

Valentini R, Matteucci G, Dolman AJ, Schulze E-D, Rebmann C, Moors EJ, Granier A, Gross P, Jensen NO, Pilegaard K (2000) Respiration as the main determinant of carbon balance in European forests. *Nature*, **404**, 861-865.

Valentini R, Matteucci G, Dolman AJ, Schulze E-D, Jarvis PG (2003) The Carbon Sink Strength of Forests in Europe: A Synthesis of Results. In: Valentini R (ed.) Fluxes of Carbon, Water and Energy of European Forests, Ecological Studies. Vol **163**. Springer Verlag, Berlin

Verburg PSJ, Arnone JA III, Evans RD et al. (2004) Net ecosystem C exchange in two model grassland ecosystems. *Global Change Biology*, **10**, 498–508.

Walter HW (1990) Vegetation und Klimazonen. 6. Auflage. Ulmer, Stuttgart

Watson RT, Rodhe H, Oeschger H, Siegenthaler U (1990) Greenhouse gases and aerosols. In Climate Change, The IPCC Scientific Assessment (eds. Houghton JT, Jenkins GJ, Ephraums JJ). Cambridge University Press, Cambridge, pp. 1–40.

Watson RT, Zinyowera MC, Moss, RH (1995) Impacts, Adaptations and Mitigation of Climate Change: Scientific-Technical Analyses Contribution of Working Group II to the Second Assessment of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge.

Werner C, Correia O, Beyschlag W (1999) Two different strategies of Mediterranean macchia plants to avoid photoinhibitory damage by excessive radiation levels during summer drought. *Acta Oecologica*, **20**, 15-23.

Werner C, Correia O, Beyschlag W (2002) Characteristic patterns of chronic and dynamic photoinhibition of different functional groups in a Mediterranean ecosystem. *Functional Plant Biology*, **29**, 999-1011.

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

Werner C, Unger S, Pereira JS, Ghashghaie J, Máguas C (2007a) Temporal dynamics in $\delta^{13}C$ of ecosystem respiration in response to environmental changes, in *Isotopes as Tracers of Ecological Change*. Dawson T, Siegwolf R (eds.), Elsevier Academic Press, San Diego, 193-210.

Werner C., N. Hasenbein, R. Maia, W. Beyschlag, and C. Máguas (2007b) Evaluating high time-resolved changes in carbon isotope ratio of respired CO_2 by a rapid in-tube incubation technique, *Rapid Communications in Mass Spectrometry*, **21**, 1352-1360.

Whittaker RH, Likens GE (1975) The Biosphere and man, in *Primary Productivity of the Biosphere.* Lieth H, Whittaker RH (eds.), Springer, New York, 305-328

Wilczak JM, Oncley SP, Stage SA (2001) Sonic anemometer tilt correction algorithms. *Boundary-Layer Meteorology*, **99** (1), 127-150.

Wingler A, Wallenda T, Hampp R (1996) Mycorrhiza formation on Norway spruce (*Picea abies*) roots affects the pathway of anaplerotic CO_2 fixation. *Physiol. Plantarum*, **96**, 699-705

Wood JM, Bremer E, Csonka LN, Kraemer R, Poolman B, van der Heide T, Smith LT (2001)

Osmosensing and osmoregulatory compatible solute accumulation by bacteria. *Comp.Biochem. Physiol*. Part A, **130**A, 437–460.

Xu LK, Baldocchi DD, Tang JW (2004) How soil moisture, rain pulses, and growth alter the response of ecosystem respiration to temperature. *Global Biogeochemical Cycles*, **18**, GB4002.1-GB4002.10.

Yakir D, Wang X-F. (1996) Fluxes of CO_2 and water between terrestrial vegetation and the atmosphere estimated from isotope measurements. *Nature*, **380**, 515-517.

Yakir D, Sternberg L (2000) The use of stable isotopes to study ecosystem gas exchange. *Oecologia*, **123**, 297-311.

Zha T, Xing Z, Wang KY, Kellomaki S, Barr AG (2007) Total and Component Carbon Fluxes of a Scots Pine Ecosystem from Chamber Measurements and Eddy Covariance. *Ann.Bot.*, **99**, 345-353.

Zhang W, Parker KM, Luo Y, Wan S, Wallace LL, Hu S (2005) Soil microbial responses to experimental warming and clipping in a tallgrass prairie. *Global Change Biology*, **11**, 266-277.

APPENDIX

SPECIES LIST

List of understory species occurring between April and May at *Mitra*.

Apiaceae	
Torilis nodosa	annual
Asteraceae	
Anthemis arvensis	annual
Calendula arvensis	annual
Coleostephus myconis	annual
Hypochoeris glabra	annual
Matricaria sp.	annual
Onopordum macracanthum	biannual
Reichardia picroides	perennial
Tolpis barbata	annual
Boraginaceae	
Anchusa sp.	perennial
Echium plantagineum	biannual
Brassicaceae	
Cardamine sp.	annual
Raphanus raphanistrum	annual
Caryophyllaceae	
Cerastium glomeratum	annual
Polycarpon tetraphyllum	annual
Silene gallica	annual
Spergula arvensis	annual
Spergularia rubra	annual
Stellaria sp.	annual
Cistaceae	
Cistus salvifolius	perennial
Tuberaria/ Xolantha guttata	annual
Geraniaceae	
Erodium botrys	annual
Erodium moschatum	annual
Geranium molle	annual
Geranium purpureum	annual
Lamiaceae	
Lamium sp.	annual
Leguminosae	
Ornithopus compressus	annual
Trifolium campestre	annual
Trifolium nigrescens	annual
Vicia lutea	annual
Liliaceae	
Muscari comosum	perennial
Papaveraceae	
Fumaria agraria	annual
Plantaginaceae	
Plantago bellardii	annual
Plantago coronopus ssp. coronopus	perennial
Poaceae	
Bromus rigidus	annual
Avena barbata ssp. lusitanica	annual
Briza maxima	annual
Bromus hordeaceus	annual
Hordeum murinum ssp. leporinum	annual
Vulpia myuros	annual
Polygonaceae	
Rumex acteosella ssp. angiocarpus	perennial
Rumex bucephalophorus	annual
Rumex conglomeratus	perennial
Ranunculaceae	
Ranunculus sp.	annual
Rosaceae	
Rubus sp.	perennial
Rubiaceae	
Sherardia arvensis	annual
Scrophulariaceae	
Linaria spartea	annual
Veronica sp.	annual
Valerianaceae	
Centranthus rigidus	perennial

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PUBLICATIONS

JOURNALS AND BOOK CHAPTERS

Unger S., Pereira J.S., Máguas C. & Werner C. (2007) *Carbon-flux partitioning of ecosystem respiration in a Mediterranean oak forest in response to drought*. In 'MEDECOS IX' Proceedings: 9th International Mediterranean ecosystems conference, (CSIRO Publishing: Perth, Australia).

Werner, C., Unger S., Pereira J.S., Ghashghaie J. & Máguas C. (2007) *Temporal dynamics in $\delta^{13}C_R$ of ecosystem respiration in response to environmental changes.* In: 'Isotopes asTracers of Ecological Change' Eds. T. Dawson & R. Siegwolf. Elsevier Academic Press, 193-210.

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

Unger S., Werner C & Máguas C (2001) *Evaluation of water use efficiency: the contribution of structural vs. functional adaptations to drought stress*. In 'PS2001 Proceedings: 12th International Congress on Photosynthesis.'. (CSIRO Publishing: Melbourne, Australia).

CONGRESSES

Máguas C., Priault P., Unger S., Pereira, J.S., Werner C., (2007) *A rapid In-tube incubation technique reveals high-time resolved dynamics in carbon isotope ratio respired by different ecosystem compartments.,* ESF Final conference, "The role of soils in the terrestrial carbon balance", Pont-à-Mousson, France, Poster

Werner C., Unger S., Máguas C., Pereira, J.S., (2007) *Disentangling components of soil and ecosystem carbon fluxes: a comparison of two different model approaches based on flux and stable isotope measurements.,* ESF Final conference, "The role of soils in the terrestrial carbon balance", Pont-à-Mousson, France, Poster

Unger S., Maia, R., Pereira, J.S., Máguas C., Werner C., (2007) *Carbon-flux partitioning in a Mediterranean oak forest in response to drought.,* Medecos XI conference, Perth, Australia, Poster

Unger S., Maia, R., Pereira, J.S., Máguas C., Werner C., (2007) *Dynamics of $\delta^{13}C$ of soil and ecosystem respired CO_2 in response to rain pulse events after drought. ,*ESF SIBAE workshop, "Disentangling abiotic and biotic effects on soil respiration", Innsbruck, Austria, Poster

Werner, C., Unger S., Maia, R., Pereira, J.S., Máguas C., (2006) *Importance of time scales to evaluate the dynamic in ecosystem respiration (δ13CR) and driving factors in a Mediterranean woodland.,* SIBAE-BASIN Conference, "Isotopes as tracers of ecological change", Tomar, Portugal, Poster

Unger S., Maia, R., Pereira, J.S., Máguas C., Werner C., (2006) *Dynamics of $\delta^{13}C_R$ and $\delta^{13}C_S$ in a Mediterranean type ecosystem following rain pulse events after drought.,* SIBAE-BASIN Conference, "Isotopes as tracers of ecological change", Tomar, Portugal, Poster

Unger S., Maia, R., Pereira, J.S., Máguas C., Werner C., (2005) *Measurements of isotopic fluxes in chamber systems provide information about short term temporal dynamics of $\delta^{13}C_R$ and its drivers.*, IX Congresso Luso-Espanhol de Fisiologia Vegetal, Évora, Portugal, Poster

Werner, C., Unger S., Maia, R., Pereira, J.S., Máguas C., (2004) *Use of $\delta^{18}O$ of atmospheric CO_2 to evaluate photosynthetic activity in a Mediterranean ecosystem.*, SIBAE-BASIN Conference, "Partitioning of fluxes between the biosphere and atmosphere across spatial scales.", Interlaken, Switzerland, Poster

Unger S., Maia, R., Pereira, J.S., Máguas C., Werner C., (2004) *Seasonality of $\delta^{13}C$ and $[CO_2]$ in a Mediterranean ecosystem and the link to ecosystem respiration.* , SIBAE-BASIN Conference, "Partitioning of fluxes between the biosphere and atmosphere across spatial scales.", Interlaken, Switzerland, Poster

Unger S., Werner C., Maia, R., Máguas C., Pereira, J.S. (2003) *First data-set concerning Mediterranean respiratory components by stable isotope analysis: Keeling plots and incubation studies*, "Carbon Balance of Forest Biomes (1) The global and regional perspective", Southampton, UK, Poster

Unger S., Maia, R., Pereira, J.S., Máguas C., Werner C., (2003) *Seasonal changes of carbon fluxes in a Mediterranean woodland and the contribution of ecosystem components to ecosystem respiration.*, ESF SIBAE Workshop, "Stable isotope signals of the terrestrial biosphere: Linking ecosystem C fluxes to isotopic signals of plant components.", Orvieto, Italy, Poster

Unger S., Werner C., Maia, R., Máguas C., Pereira, J.S. (2003) *Contribution assessment of different respiratory components to ecosystem respiration using Keeling plot and incubation methods*, 3. Netcarb Summer-School, "Use of Stable Isotopes in plant Ecology and Ecophysiology", Freising, Germany, Poster

Unger S., Werner C., Maia, R., Máguas C., Pereira, J.S. (2003) *First data-set concerning Mediterranean respiratory components by stable isotope analysis: Keeling plots and incubation studies*, "Carbon Balance of Forest Biomes (1) The global and regional perspective", Southampton, UK, Poster

Unger S., Werner C. (2002) *Effects of mild and severe drought stress on leaf $\Delta^{13}C$ and gas exchange in Mediterranean species*, 2. Netcarb Summer-school "Field-related techniques for the use of stable isotopes in the study of ecosystem functioning", Oeiras, Portugal, Poster

Máguas C, Unger S., Werner C. (2001) *Evaluation of Water Use Efficiency: The contribution of structural vs. functional adaptations to drought stress*, 12th International Congress on Photosynthesis, Melbourne, Australia, Poster

CURRICULUM VITAE

name:	Stephan Unger
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ACADEMIC RECORD

1996-1998	basic course Biology at Ernst-Moritz-Arndt-University Greifswald (Germany)
1998- 2002	main course Biology at University of Bielefeld (Germany)
2001	practical with ERASMUS-scholarship and Data collection for Final Thesis at Universidade de Lisboa, Faculdade de Ciéncias, Seccáo Ecologia, Portugal
2002	master thesis on *Structural and functional adaptations of three Mediterranean species to drought stress - A comparison of water use efficiency as measured by isotope discrimination and leaf gas exchange*
since 2003	PhD-Student at the Department of Experimental and Systems Ecology (University of Bielefeld), experimental part in Portugal working at ICAT (Faculdade Ciências da Universidade de Lisboa) and ISA (Universidade Técnica de Lisboa) in Lisbon, Project: ISOFLUX (German science foundation) DFG, WE 2681/2-1.

PROFESSIONAL APPOINTMENT AND RESEARCH EXPERIENCE

2002-2007	partially contracted as scientific assistant at the University of Bielefeld
2004-2006	contracted in the project: ISOFLUX (German science foundation) DFG, WE 2681/2-1.
2005	coordination of field work for master theses of Nils Hasenbein - *New methods to evaluate rapid dynamics in carbon isotope ratios ($\delta^{13}C$) of dark respired CO_2 of different functional groups* and Alexandra Wiemann - *Ecophysiological examination of dynamics and activity of the understorey in a Mediterranean evergreen forest*
2007	contracted for supervision of *Bachelor* course and practical lessons in *Stress induced processes in plant ecology*, Faculty of Biology, University of Bielefeld

GRANTS AWARDED

2001 ERASMUS-scholarship from University of Bielefeld
2003 PhD-grant by the Deutschen Akademischen Auslandsdienst (DAAD, D/02/04151)

Erklärung

Hiermit versichere ich, daß ich die vorliegende Dissertation selbständig erarbeitet habe und alle Quellen, Hilfsmittel und Beiträge anderer Mitarbeiter angegeben sind.

Weiterhin erkläre ich, daß die vorliegende Dissertation weder vollständig noch teilweise einer anderen Fakultät mit dem Ziel einen akademischen Grad zu erwerben, vorgelegt wurde beziehungsweise wird.

Bielefeld, 19 September 2007