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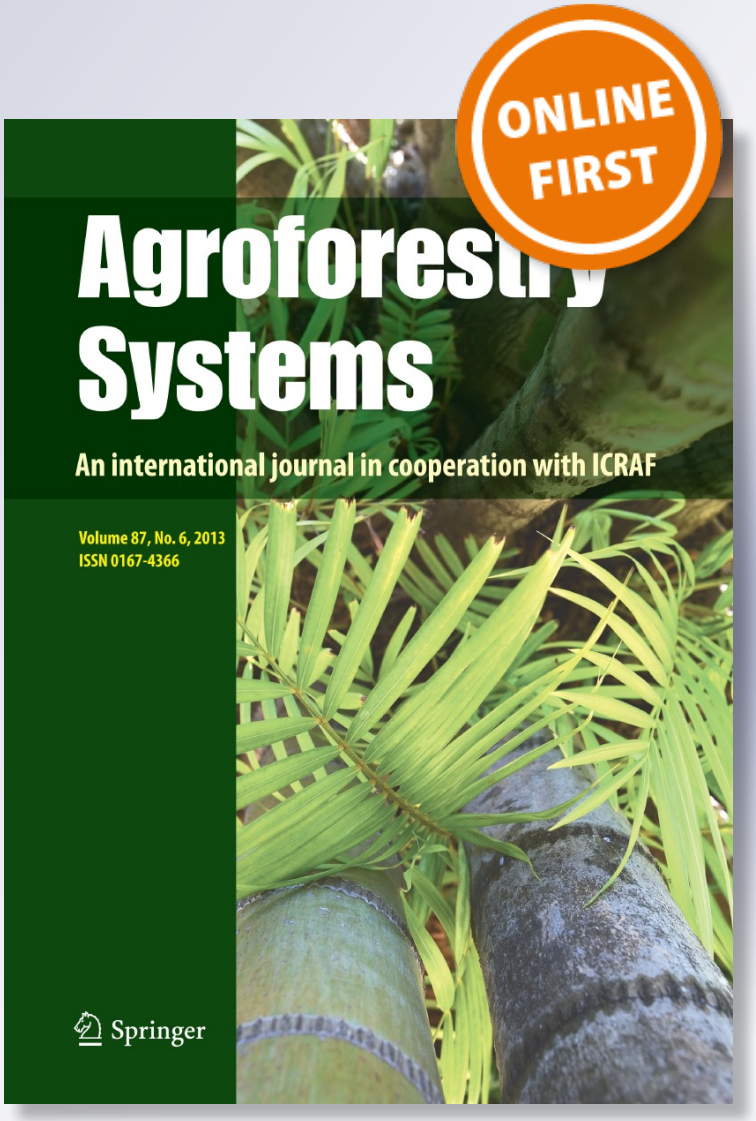
Agroforestry Systems
An International Journal incorporating Agroforestry Forum

ISSN 0167-4366

Agroforest Syst
DOI 10.1007/s10457-014-9672-y



Springer

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Received: 28 May 2013 / Accepted: 15 January 2014

Abstract The accurate assessment of respiration by woody vegetation, still a challenge in plant productivity models, is generally a problem of correctly scaling-up the process from organs to the whole plant. We used a large (41.6 m^3), canopy chamber to enclose mature olive trees and to measure aboveground respiration (R_{ag}) under natural environmental conditions in an irrigated olive orchard in Córdoba (Spain). The 3-year study assessed nocturnal and seasonal R_{ag} patterns in terms of temperature (T), plant dry matter composition, and phenology. The relative contributions of maintenance and growth respiration to R_{ag} were determined empirically via an independent experiment. Although short-term variations in R_{ag} rates were explained mainly by T variations, over seasonal time-scales this relationship was modulated by the vegetative composition of the olive trees and the contribution of growth respiration to R_{ag} when the plants, in different seasons, allocated most of the new assimilates to actively growing shoots, flowers or fruits. Leaf mass and fruit load were the main determinants of R_{ag}, which was weakly affected by differences in woody biomass since woody tissue respiration accounted for just 15 % of R_{ag}. Respiration in olive trees during fruit setting periods is composed of approximately 30 % growth and 70 % maintenance. This study provides an independent evaluation of how, and to what degree, seasonally varying plant organ composition determines total respiration. Improved modelling of ecosystem respiration can be achieved by accounting for plant biological patterns characterising energy-requiring growth and maintenance processes, since biochemical kinetics alone cannot explain the observed seasonal variability.

Keywords CO_2 efflux · Canopy chamber · Aboveground respiration · Maintenance respiration · *Olea europaea*

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Introduction

Olive groves (*Olea europaea* L.) are among the most significant agroforestry systems in the Mediterranean

Published online: 23 January 2014

area, and could play an important role in biogeochemical cycling as a possible sink of greenhouse gases (GHGs) as well as providing goods and services (Dixon 1995). Plant biomass production depends on the balance between photosynthesis and respiration. Compared to photosynthesis, plant respiration processes have been poorly represented in productivity models and little information is available about the carbon exchange capacity of olive orchards (Testi et al. 2008; Villalobos et al. 2012).

Mechanicistic representation of respiration (R) in whole plant or crop models remains a challenge (Atkin 2011). Mechanisms underlying R have been represented semi-empirically using the "growth and maintenance respiration concept" (McCree 1970), in which R is partitioned into the energy-requiring processes for constructing new tissues (growth respiration, R_g) and those for maintaining existing cells (maintenance respiration, R_m). Although there is no rigorous division between growth and maintenance energy-requiring processes (Amthor 2000; Thornley and Cannell 2000; Cannell and Thornley 2000), the paradigm of considering total respiration as a sum of R_g and R_m is generally accepted as a basis for modelling carbon fluxes (Gifford 2003). Time series analyses have been performed on measurements of CO_2 exchange at the leaf or ecosystem level to study seasonal changes in R_g and R_m throughout vegetation development (Wullschleger et al. 1992; Adu-Bredu et al. 1997; Van Iersel and Seymour 2000; Rambal et al. 2004; Van Iersel 2006). Other approaches have been proposed to independently estimate R_m or R_g. These include assessing respiration in continual darkness (the "starvation method"; McCree 1986) or in mature tissue (Wilson and Jones 1982) to estimate R_m, and either the chemical composition (Penning de Vries FWT 1975) or combustion heat (Williams et al. 1987) approaches to isolate R_g. However, in comparison with R_g, the physiological bases of R_m are largely unknown and difficult to predict (Gifford 2003).

Maintenance respiration represents a large portion of the carbon budget of a plant (Amthor 1984) and can be empirically described with the following equation (McCree 1970):

$$R_m = R_s(T) \times M, \quad (1)$$

where R_s is the mass-specific respiration rate (μmol CO_2 kg^{-1} plant dry matter s^{-1}) that varies with temperature (T, °C), and M is the plant dry mass (kg). Respiration rates typically increase with T and can be easily modelled with T response functions (e.g. Q_{10}, exponential functions; Richardson et al. 2006). However, energy-requiring maintenance processes vary noticeably among aboveground parts of the woody vegetation (namely, foliage and wood; Edwards and Hanson 1996; Ryan et al. 1996; Lavigne and Ryan 1997; Ryan et al. 1997), as does the T, and thus there is no general value of R_s that can be assumed for a whole plant. Therefore, it is clear that the relative distribution of plant dry matter among wood and foliage determines R_m, and thus environmental variables alone may not explain the seasonal variability of aboveground respiration rates, especially in deciduous trees. As a consequence, models that use this approach require reliable experimental relationships to express R_m in terms of the T and biomass of individual tissues.

Chamber or cuvette systems have been used widely to provide high-resolution respiration measurements for a given specific tissue of the plant (Ryan et al. 1994; Yokota and Hagihara 1995; Edwards and Hanson 1996; Ryan et al. 1996; Lavigne and Ryan 1997; Stockfors and Linder 1998). However, small errors in plant-organ estimates (surface or dry mass of foliage and woody tissues, leaf N content) may lead to large errors in upscaled values (Damesin et al. 2002; Liu et al. 2010) and thus estimates based on simple or even complex allometric relationships require validation with whole-plant measurements. A motivation of this study was to use a transitorily closed canopy chamber, large enough to enclose mature olive trees (Pérez-Priego et al. 2010), with the overall objective of studying respiratory CO_2 fluxes from the aboveground parts of an olive orchard. We present three years of monitoring aboveground respiration (R_{ag}) to assess the nocturnal and seasonal dynamics of R_{ag} as a function of temperature, plant dry matter composition and phenology. Additionally, we conducted two experiments;

(1) in Experiment I, two tests were carried out to determine the contributions of woody and non-woody respiration to R_{ag} and to define the best scalar factor to estimate R_{ag}; and
(2) in Experiment II, young olive trees were exposed to prolonged darkness, and independent

evaluations determined the relative importance of maintenance (R_m) and growth (R_g) respiration. Fits for respiration per unit of dry mass and its Q_{10} temperature response were obtained for fruit, leaves and wood using the integration method.

Finally, we used the results from Experiment II to estimate the relative importance of R_m to R_{ag} measured in adult trees. The growth component (R_g) was estimated during different phenological periods by subtraction.

Materials and methods

Experimental site

The orchard is located near Córdoba, in southern Spain (37.85°N, 4.8°W, altitude 110 m). An automated weather station within 300 m of the experimental plot provided meteorological data during the study, which spanned the period from August 2006 to November 2008 for the different experiments. The climate in the area is Mediterranean, with an average annual rainfall of around 600 mm concentrated from autumn to spring, and reference evapotranspiration (ET_0) of 1390 mm. The orchard was drip irrigated to replace loss by evapotranspiration (ET) using 7 emitters per tree (with discharge rates of 4 L h^{-1} each). The orchard irrigation water requirements were estimated following Orgaz et al. (2007) and the soil water balance was recalculated monthly to avoid water stress. The well-watered status of the trees was confirmed by periodically measuring leaf water potential with a Scholander bomb; more details can be found in Villalobos et al. (2012).

Trees in olive groves are very homogenous, with negligible differences in structural variables and yield (Leaf area index, number of fruits per unit ground or leaf area; Iniesta et al. 2009), and furthermore present low inter-tree variability in physiological functioning (e.g. photosynthesis, transpiration, stomatal conductance, chlorophyll fluorescence, temperature; Pérez-Priego et al. 2005; Pérez-Priego et al. 2010). This similarity among trees, as well as the difficulty in managing a 41-m^3 chamber at night, justifies the simplified sampling scheme of working with three mature trees (seasonal monitoring and Experiment I below), as well as four potted, young trees (Experiment II below).

CO_2 exchange measurements

The canopy chamber used in this study (Pérez-Priego et al. 2010) operates as a transitorily closed system and consists of a 4-m high hexagonal prism with base of 10.4 m^2. Windows and tops are made of Llumar® "NRS90 clear" polyester film of 75 μm thickness, stretched and fixed to aluminium frames. The bottom is sealed around the tree trunk through a thick polyethylene panel to exclude fluxes from the soil. Revolving sides and top windows allow quick opening and closure. During closure periods (lasting 1–3 min) a vacuum pump circulates chamber air through the sampling circuit and a sample is diverted to a CO_2/H_2O infrared gas analyser (IRGA; model LI-840, LI-COR, Lincoln, NE, USA) which measures variations in the CO_2 molar fraction at 1 Hz; the output is recorded by a datalogger (model CR23X, Campbell Scientific, Logan, UT, USA). Dilution errors due to humidification were corrected (Webb et al. 1980). After each measurement the chamber reverts to the open position to minimise perturbation of the tree environment.

Aboveground respiration in an olive tree orchard

Aboveground respiration rates (R_{ag}) were measured over time against the phenological cycle of the plant, distribution of dry matter among organ classes (dry matter fraction of wood, fruits and foliage) and temperature. The canopy chamber was installed in a 4-ha olive (*Olea europaea* L. 'Arbequina') orchard planted in 1997 with a 3.5 m × 7 m spacing to measure night-time R_{ag} at different times over a range of phenological stages. Measurements were made on one tree on 10 October 2006 (final stage of fruit set), 15 December 2006 (winter dormancy) and 31 October 2007 (final stage of fruit set), and on two other trees, one on 12 May 2008 (initial flowering) and the other on 17 July 2008 (initial stage of fruit set, Rapoport et al. 2012). Leaf area of the enclosed trees was determined non-destructively by measuring canopy transmissivity using a plant canopy analyser (model LAI-2000, LI-COR). In the case of 12 May 2008 and 17 July 2008, leaf area and organ dry mass were measured destructively; leaf area with an electronic

area meter (model LI-3100, LI-COR) and mass of the removed organ class (leaves, fruit and wood) after drying at 70 °C for 48 h. The leaf mass per unit leaf area (LMA; g m^{-2}) was calculated as the ratio between the dry mass of leaves and their fresh area.

Experiment I: mass-specific woody and non woody respiration

The significant within-canopy variation in both leaf respiration and its temperature response related to leaf characteristic (Griffin et al. 2002) must be taken into account, as should the fact that wood respiration may be a large fraction of the ecosystem carbon budget (Ceschia et al. 2002). Other specific objectives of this experiment were: (1) to determine how to scale-up the CO_2 efflux from organs to the whole plant (especially the partitioning of the aboveground CO_2 efflux into woody and non-woody organs); and (2) to quantify the temperature dependence of respiration by these organs.

Two tests were carried out: In test (A) during initial flower development (May 2008), the canopy chamber measured R_{ag} of a single 11-year-old tree during 3 nights at constant temperature, with defoliations at branch level performed each day to progressively increase the fraction of wood biomass. Wounds were covered with a latex pruning seal to avoid bias from CO_2 diffusion from the xylem into the chamber (Teskey and McGuire 2005). Sap flow decreased in parallel with the reductions in leaf area (López-Bernal et al. 2010). Studies using removal of some organ of the plant as a tool to determine the individual contributions suggest that respiration measurements should be conducted within few hours after excision (Liu et al. 2006) before non-structural carbohydrate mobilisation (Chesney and Vasquez 2007). Accordingly, variations of aboveground respiration (R_{ag}) were related with total aboveground dry mass (plant mass-specific respiration, R_{spm}, μmol kg^{-1} plant dry matter s^{-1}), leaf area (leaf area-specific plant respiration, R_{spla}, μmol m^{-2} leaf surface s^{-1}) and leaf dry mass (leaf mass-specific plant respiration, R_{splm}, μmol kg^{-1} leaf dry weight s^{-1}) after cuts performed over the 3 days of measurements. Similarly, in test (B) during the initial fruit set period (July 2008), measurements of R_{ag} were performed during two nights over a wide range of temperatures. The second night, following complete defoliation of the tree, wood respiration was measured and non-woody respiration was calculated as the reduction in aboveground CO_2 efflux versus that measured on the previous night.

Experiment II: maintenance respiration

This experiment was carried out in the Instituto de Agricultura Sostenible (IAS) of Córdoba from 1 to 7 August 2008, when annual shoot growth is already reduced. Its objectives were (A) to quantify the contribution of maintenance respiration (R_m) to total respiration (R_{ag}) in young olive trees and assess its temperature response, and (B) to evaluate mass-specific respiration coefficients for different organ classes (respiration per unit of dry mass of fruit, leaves and fine branches). Aboveground respiration (R_{ag}) was partitioned into maintenance (R_m) and growth (R_g) components using the "dark method" (McCree and Kresovich 1978) or "starvation method" (Gary et al. 2003; Gifford 2003). This experimental approach relies on the theoretical assumption that, after an extended period of darkness (48 h), plants use up their carbohydrate reserves and respiration associated with the growth of new structures ceases. At that time, the respiration rate represents energy only for functions such as protein turnover and maintenance of ionic gradients (Gary et al. 2003). According to this, the difference between R_{ag} taken after the photoperiod and the steady-state respiration rate obtained after 48 h of darkness (taken as R_m) is assumed to be R_g (Amthor 1984). Afterwards, once plants had achieved a steady-state flux (R_m) in the dark, we applied the component integration method to estimate the relative contribution of each organ class to the total flux. This method measures the respiration rates of spatially separable contributors to total CO_2 fluxes (similarly, autotrophic respiration in soils is estimated from the rate of CO_2 production by roots excised from soil; Hanson et al. 2000). Accordingly, differences in R_m before and after excision of different organ classes (leaves, fruit) determine the relative contributions of each organ class to the total flux.

Four three-year-old olive trees (*Olea europaea* L. 'Arbequina'), growing in a soil-peat mixture in 50 L pots, were placed in a smaller (8 m^3) version of the transitorily closed chamber system described in Pérez-Priego et al. (2010) during 5 days. The entire

chamber was covered with aluminium foil to maintain the trees in total darkness. The chamber, kept outside to generate a wide range of temperatures, was maintained at ambient temperature by a ventilation system when not measuring. Two respiration data sets were collected with trees in the chamber, the first after 6–8 h and the second after 48 h of continuous darkness, theoretically following growth cessation (McCree and Kresovich 1978; Amthor 1984; Gary et al. 2003; Gifford 2003) and representing the steady-state respiration rate R_m. Afterwards, measurements of CO_2 exchange were made over 3 days between 9:00 and 13:00 GMT (daytime). At the end of each measurement day a class of plant organs was removed; first fruits and second leaves such that finally only wood maintenance respiration was measured. The temperature response of R_m for fruit was obtained from the difference of the best fits between whole plant respiration and that obtained with the fruit removed. Similarly, the temperature response of R_m for leaves was obtained from the difference of the best fits between whole plant respiration with fruits removed and wood respiration. The mass of the removed organs was measured after drying at 70 °C for 24 h. The area of the leaf samples was measured with an electronic area meter (model LI-3100, LI-COR). The leaf mass per unit leaf area (LMA; g m^{-2}) was calculated as the ratio between the dry mass of leaves and their fresh area.

Experimental summary

Table 1 summarises conditions for aboveground respiration measurements in the above mentioned experiments.

Temperature sensitivity

The relationship between mass-specific respiration rates and air temperature was fitted to the following equation;

$$R_s = R_{ref} e^{\beta T} \quad (2)$$

where R_s is the aboveground CO_2 efflux per unit of plant or organ dry mass (μmol CO_2 kg^{-1} s^{-1}, mass-specific respiration rates), T is air temperature (°C), and R_{ref} (the reference respiration rate at $T_{ref} = 0$ °C) and β are fitted parameters. The Q_{10} (factor by which R_s increases for a 10 °C rise in the T) was calculated as follows:

$$Q_{10} = e^{10\beta} \quad (3)$$

Parameters R_{ref} and β were fitted by non-linear regression analysis using "OriginPro 8" software (OriginLab, Northampton, MA, USA); the procedure used the simplex algorithm followed by Levenberg–Marquardt minimisation.

Results

Nocturnal and seasonal variations of aboveground respiration

The aboveground CO_2 efflux (R_{ag}; expressed per unit of ground surface) showed a decreasing pattern over the course of the night. As expected, temperature (T) fell during the night and so R_{ag} and T were positively correlated. On the October 2006 night shown (Fig. 1), R_{ag} values decreased from 3.5 μmol CO_2 m^{-2} s^{-1} a couple of hours after sunset to 1.9 μmol CO_2 m^{-2} s^{-1} just before sunrise, during overnight cooling from 24 to 15 °C.

Table 1 Overview of experiments affecting aboveground respiration (R_{ag})

Experiment	Test	Dates	Temperature	Perturbation	Notes
I	A	May 2008	ca. 17 °C	Progressive defoliation	Increasing fraction of woody biomass
	B	July 2008	Variable	Complete defoliation	Whole-tree versus wood respiration
II	A	August 2008	Variable	48 h of darkness	Whole plant maintenance respiration
	B			Component excision following prolonged darkness	Whole plant (less fruit) maintenance respiration
					Woody tissue maintenance respiration (fruits and leaves removed)

Two different mature trees were used in Experiment I, and four potted, young trees in Experiment II

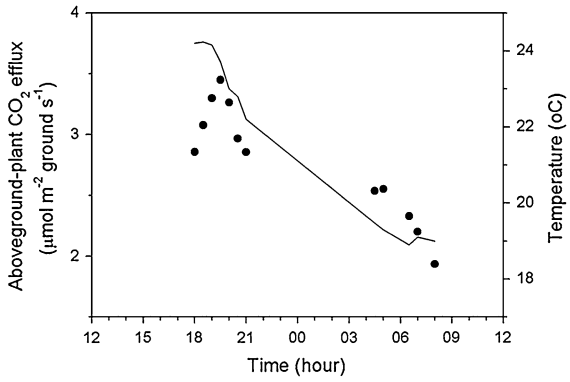

Fig. 1 Air temperature (*line*) and night-time aboveground CO_2 efflux (R_{ag}; *Black symbols*) of an adult tree measured with a canopy chamber on the night of 10–11 October 2006 in the field

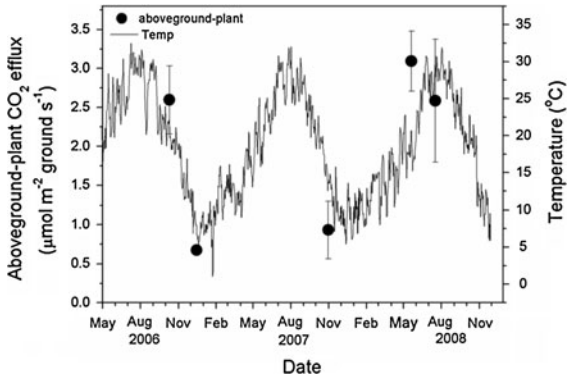

Fig. 2 Seasonal variations in night-time aboveground CO_2 efflux (R_{ag}; *Black symbols*) and daily air temperature (T; *line*) from May 2006 to December 2008. *Bars* on data represent ± SEM

The average night-time R_{ag} measured during different phenological periods followed the seasonal variations in daily average air T (Fig. 2). For example, R_{ag} measured on the same tree at different times in 2006 showed lower values during winter dormancy (0.7 µmol m^{-2} s^{-1} at 8 °C, December 2006) and higher during the final period of fruit setting (2.6 µmol m^{-2} s^{-1} at 22 °C; October 2006). No significant difference in leaf area index was observed for this tree (LAI about 1.6 m^2 of leaf per m^{-2} of ground area). However, R_{ag} measured during the same phenological period (October 2007) for the same tree but with lower LAI (1.22 m^2 m^{-2}) and T of 14 °C was almost as low as in December 2006 (0.9 µmol m^{-2} s^{-1}). From Fig. 2 there seems to be a relationship between average night-time R_{ag} and daily average air T. However, this relationship was not observed in measurements taken during the initial flowering period (May 2008) against those belonging to the initial fruit setting period (July 2008); whereas the T in May was notably lower than in July, R_{ag} was higher in May, with the same LAI but different LMA.

Experiment I: woody and non woody mass-specific respiration

In test I-A, conducted during the flowering period (May 2008), defoliations led to changes in the specific composition of the tree (Table 2), with a decrease in leaves (f_{leaves} fell from 0.22 to 0.17) and corresponding increase in the wood fraction from 0.78 to 0.83. Aboveground CO_2 effluxes expressed per total plant dry mass unit (R_{ag} per unit of whole plant dry mass) measured at nearly constant T (around 17 °C) decreased over the three study days from 0.95 to 0.79 µmol CO_2 kg^{-1} s^{-1}. However, no significant differences were found between the last two days (0.80 and 0.79 µmol CO_2 kg^{-1} of whole plant dry matter s^{-1}) when leaf fraction decreased from 0.19 to 0.17. Rather, the leaf area-specific aboveground CO_2 efflux (R_{ag}; per unit leaf area) increased from 1.03 to 1.28 µmol CO_2 m^{-2} s^{-1}, with no significant difference between the first two days, but clearly higher on the third day. However, no significant differences were found over 3 days when R_{ag} was expressed per unit of leaf mass (4.4 µmol CO_2 kg^{-1} of leaf dry matter s^{-1}).

In test I–B, performed on a different tree two months later during the initial fruit setting period (July 2008), following complete defoliation, aboveground CO_2 effluxes expressed per total plant dry mass unit decreased greatly (by up to 75 %). Over the T range of 17–30 °C, R_{ag} varied from 0.2 to 0.9 µmol CO_2 kg^{-1} s^{-1} (square symbols, Fig. 3). These discrepancies appeared similarly when R_{ag} was expressed per unit of leaf mass, corresponding to a reduction of 70 %.

Mass-specific respiration rates measured in wood (0.04–0.32 µmol CO_2 kg^{-1} s^{-1}) were noticeably lower than those for the whole plant (triangular symbols, Fig. 3). The CO_2 efflux estimated for non-woody organs presented the highest values at a given T (circular symbols, 0.73–2.67 µmol CO_2 kg^{-1} s^{-1}). The relative importance of woody and non-woody CO_2 effluxes indicated that those from wood accounted on average for 15 % of whole-crown respiration over the T range of 17–30 °C.

Table 2 Specific respiration rates of a large olive tree measured on the 3 days of Experiment I-A

Dagagay	Temperature (°C)	R_{ag} per unit plant dry matter	R_{ag} per unit leaf area	R_{ag} per unit leaf mass	Leaf mass area (LMA)	Fraction of standing biomass	
		(μmol CO_2 kg^{-1} s^{-1})	(μmol CO_2 m^{-2} s^{-1})	(μmol CO_2 kg^{-1} s^{-1})	(g m^{-2})	(f_{leaves})	(f_{wood})
1	17.5 ± 0.3	0.95 ± 0.02b	1.03 ± 0.02a	4.38 ± 0.08a	234.2	0.22	0.78
2	17.4 ± 0.2	0.80 ± 0.05a	1.02 ± 0.07a	4.27 ± 0.27a	238.5	0.19	0.81
3	16.1 ± 0.2	0.79 ± 0.06a	1.28 ± 0.09b	4.64 ± 0.34a	275.7	0.17	0.83

The variation of woody and leaf dry biomass distribution in the same tree is also presented. Respiration values are averages ± SEM for n = 6 measurements

Values with different letters (a and b) indicate significant differences ($p < 0.05$, Tukey test) among days

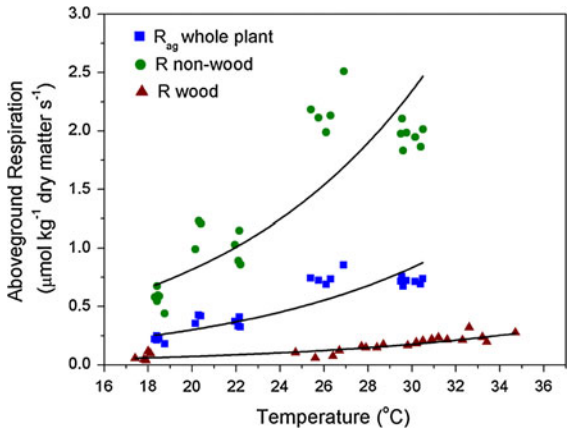

Fig. 3 Temperature responses of respiration rates of aboveground (R_{ag}), wood- and non-wood plant components measured in Experiment I–B. Wood respiration was measured once the tree had been completely defoliated. *Lines* represent the fit to Equation [2]. Optimized parameters were $R_{ref} = 0.0394$ µmol CO_2 kg^{-1} aboveground dry matter s^{-1}, $\beta = 0.1017$ and $Q_{10} = 2.8$ ($r^2 = 0.83$) for aboveground respiration, $R_{ref} = 0.0119$ µmol CO_2 kg^{-1} wood dry matter s^{-1}, $\beta = 0.0899$ and $Q_{10} = 2.5$ ($r^2 = 0.75$) for wood respiration and $R_{ref} = 0.0995$ µmol CO_2 kg^{-1} non-wood dry matter s^{-1}, $\beta = 0.1053$ and $Q_{10} = 2.9$ ($r^2 = 0.76$) for non-wood respiration

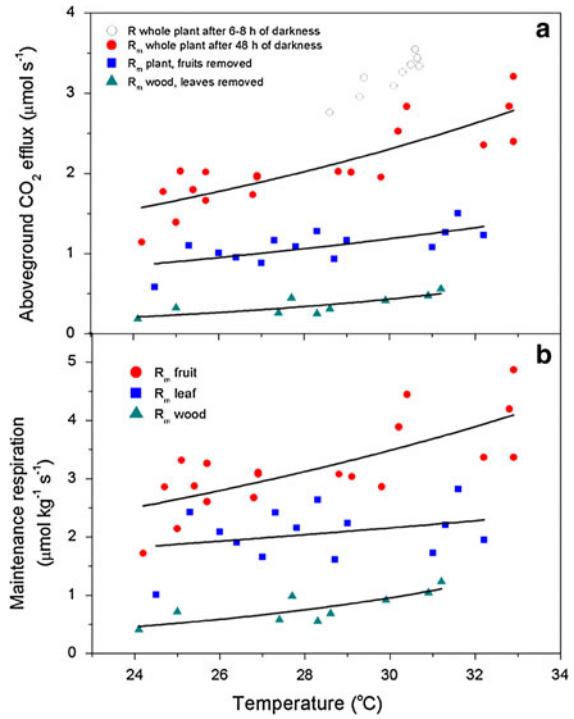

Fig. 4 CO_2 efflux of 4 three-year-old olive trees in August 2008 *vs.* temperature. **a** Total and maintenance respiration rates were measured after 6–8 h and 48 h of darkness, respectively (Experiment II-A). Afterwards, while plants were still kept in continuous darkness, measurements of CO_2 exchange when a class of organs of the plants was removed (fruits and leaves) are represented. **b** Maintenance respiration for fruits, leaf and wood (Experiment II-B)

The T sensitivities (Q_{10}) of wood and non-wood respiration were 2.5 and 2.9, respectively.

Experiment II: maintenance respiration

In test II-A, the CO_2 effluxes of whole plants measured 6–8 h after placing the young trees in the chamber (with T between 28 and 31 °C) showed a mean value of 3.22 µmol CO_2 s^{-1} (white circles, Fig. 4a), equivalent to 2.39 µmol CO_2 kg^{-1} s^{-1} when expressed per whole plant dry matter. The average CO_2 efflux for the same T range after 48 h of continuous darkness was 2.27 µmol s^{-1} (1.69 µmol kg^{-1} s^{-1}), or a reduction of 30 %. The CO_2 efflux of whole plants measured after 48 h in continuous darkness varied from 1.14 to 3.2 µmol CO_2 s^{-1} over the 24–33 °C range of T (red circles, Fig. 4a). Once fruits had been removed, the

Table 3 Maintenance respiration (R_m) coefficients at 25 °C of whole plant and different organ classes in young olive trees, as measured in Experiment II-B

	Maintenance respiration rates per unit dry matter (μmol CO_2 kg DM^{-1} s^{-1}) calculated at 25 °C	R_{ref} (μmol CO_2 kg DM^{-1} s^{-1})	β	Q_{10}
Whole plant	1.2	0.2	0.0675	2.0
Fruits	2.6	0.6	0.0577	1.8
Leaves	1.8	0.7	0.0374	1.5
Wood	0.5	0.03	0.1134	3.1

For fruits, leaves and wood, R_m was obtained as the difference between measurements at that temperature on different days

CO_2 efflux varied from 0.58 (at 24.5 °C) to 1.5 μmol CO_2 s^{-1} (at 31.5 °C, square symbols). Finally, with trees defoliated, CO_2 effluxes from wood (triangles) ranged from 0.19 (at 24 °C) to 0.56 (at 31.3 °C) μmol CO_2 s^{-1}.

From test II-B, the T sensitivities of maintenance respiration R_m by plant parts are presented in Fig. 4b. Fruits showed the highest maintenance respiration rates ranging from 1.7 to 4.9 μmol CO_2 kg^{-1} s^{-1} (circles, Fig. 4b). Leaf maintenance respiration rates were somewhat lower over the T range explored (1–2.8 μmol CO_2 kg^{-1} s^{-1}, squares). Wood maintenance respiration presented the lowest range of values (0.4–1.2 μmol CO_2 kg^{-1} s^{-1}, triangles). From the T response functions, R_m of plant parts at 25 °C and expressed as a function of dry mass were: 1.8 μmol CO_2 kg^{-1} s^{-1} for leaves, 2.6 μmol CO_2 kg^{-1} s^{-1} for fruits and 0.5 μmol CO_2 kg^{-1} s^{-1} for wood (Table 3).

Discussion

Short-term variations in aboveground respiration (R_{ag}) rates such as those occurring overnight are explained mainly by variations in air temperature (T). Both aboveground respiration rates and T show decreases throughout the night (Fig. 1), as in all the measurements carried out. Olive canopies, well coupled with the atmosphere (Villalobos et al. 2012), quickly respond adjusting their respiration rates to T. At the seasonal timescale, however, although R_{ag} responded to variations in T, some divergence was associated with leaf mass area (LMA; g of dry leaf matter per unit of leaf surface) and phenological factors (Fig. 2). For example, R_{ag} obtained in May 2008 was much higher than in July 2008 despite lower T. Although differences were observed in LMA between those periods, this behaviour is explained mainly by the variable contribution of the growth component of R_{ag} when plants, in different seasons, allocate most of the new assimilates to different organ classes: actively growing shoots and flowers in May, versus fruits in July. These findings suggest that the seasonal growth patterns and the organ allocation priority of the plant noticeably influence R_{ag}. Therefore, models of ecosystem respiration can be improved by including the phenology of growth and maintenance respiration, whose seasonal variability cannot be described purely in terms of biochemical kinetics.

In general, the respiration of structural wood of the olive tree is relatively small, while foliage and fruits are the main contributors to R_{ag}. The results of experiment I–A over three nights in May 2008 (at nearly constant T) show that R_{ag} expressed per total plant dry mass decreases significantly when the non-woody fraction is reduced (Table 2). Thus, R_{ag} was more sensitive to leaf mass (LMA) than total plant dry mass. The aboveground CO_2 efflux expressed per unit leaf area remained unaffected by partial defoliation (between days 1 and 2) despite the differences found between woody and non-woody fractions, but was clearly higher on the third day when LMA was higher. Results revealed that R_{ag} per total leaf dry mass unit is very stable despite differences in wood fraction, which suggest that dry leaf mass is the best scaling factor to represent plant respiration.

One of the difficulties in comparing results in the literature arises from methodological approaches used to obtain wood respiration: clamp-on chambers (Matyssek and Schulze 1988; Sprugel 1990; Ryan et al. 1996; Damesin et al. 2002) or chemical absorption, gas chromatography, mass spectrometry and

microelectrodes techniques (Cai et al. 2000; McGuire and Teskey 2002; Ubierna et al. 2009). Some authors have reported that woody tissue respiration may represent up to 1/3 of the CO_2 released in temperate forests (Damesin et al. 2002). Contrarily, studies by Lavigne et al. (1997) in six boreal forests indicated that woody tissue respiration contributed only 5–15 % of ecosystem respiration. Our results reveal that woody tissue has much lower respiration rates than non-ligneous parts in olive orchards and accounted for just 15 % of the total R_{ag}. In experiment I–B, measurements of woody-organ respiration rates at 15 °C (0.048 μmol CO_2 kg^{-1} s^{-1}) amount to 27 μmol CO_2 m^{-3} s^{-1} when expressed per volume of wood, within the 18–110 μmol CO_2 m^{-3} s^{-1} range reported by Ryan et al. (1997) at the same T among eight boreal forest stands. Our relatively low value may be due, to some extent, to wood characteristics, since olive wood density is nearly double that of most conifers, and owing to low actively respiring tissue content. Accordingly, live cell volume content and biochemical properties in olive wood need further characterisation.

Unless growth and maintenance components are explicitly resolved, regular measurements of R_{ag} are of limited help for linking CO_2 effluxes to specific physiological processes, and thus for modelling plant respiration. Maintenance respiration is theoretically explained mostly by the environmental T and plant organ composition; therefore, of the two components it is the easier to model. Experiment II-A showed that CO_2 effluxes after 6–8 h of darkness were higher than those after 48 h. Applying the theoretical assumption that prolonged darkness arrests energy-requiring growth processes, our results show that respiration in olive during fruit setting periods is composed of roughly 30 % growth and 70 % maintenance.

In both experiments I and II, respiration following organ removal or tissue wounding could have been increased due to the mobilization of assimilates from the reserve pool first, and the re-growth or healing wounded tissue later. However, the energy cost of assimilate mobilization is relatively small (Bouma et al. 1995), compared with that of growth and maintenance sub-processes (Amthor 2000), and the experiments were too short to capture re-growth mechanisms (Maillard et al. 2004), which in any event were not observed. The results of experiment II-B showed that after 48 h of continuous darkness and after removing an important carbon consumer of assimilates (fruit), the CO_2 efflux declined significantly; the same occurred when leaves were removed. In this sense, we considered mobilization effects on respiration to be negligible over the time-scales of our experiments (2–5 days).

Measurements of maintenance respiration (R_m) for the whole plant, as well as for fruit, leaves and wood individually obtained in experiment II-B, show a direct relationship with T. At 25 °C, olive leaf R_m is slightly lower than the 0.8 μmol CO_2 m^{-2} s^{-1} reported for the same T by Díaz-Espejo et al. (2006). In fruits, R_m at 25 °C was higher than in leaves (Table 3). Proietti et al. (1999) reported a similar value at this stage of fruit development ($\approx$ 2 μmol CO_2 kg^{-1} s^{-1}) when the fruit stops growing. However, the low fruit respiration coefficient found in our experiment, versus the high values observed by Proietti et al. (1999), suggests that growth respiration of fruits may be a large component during periods of intense growth.

Finally, we tested the ability of the Equation [1] to represent the energy-requiring maintenance process in each R_{ag} measurement carried out on adult olive trees. We applied the T response functions of mass-specific R_m rates for leaves, fruits and wood obtained from Experiment II-B to the respective amount of plant dry mass of different organs (M) (Fig. 5). The difference between estimated R_m and measured R_{ag} for each period may be attributed to growth respiration, which resulted as 26 % (October 2006), 40 % (December 2006), 27 % (October 2007), 78 % (May 2008) and 31 % (July

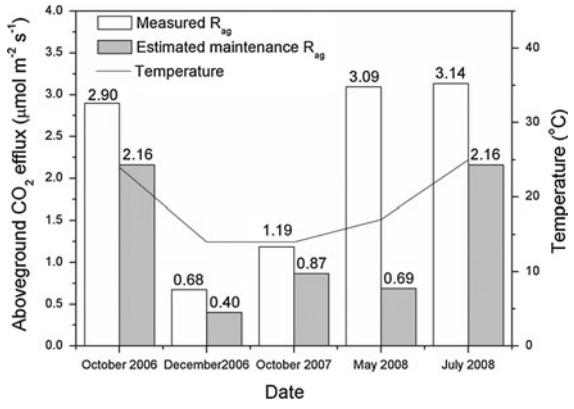

Fig. 5 Aboveground respiration measured in October 2006, December 2006, October 2007, May 2008 and June 2008. Maintenance respiration was estimated using the relationships between maintenance respiration coefficients of leaves, fruit and fine branches and temperature measured in Experiment II-B and for wood in Experiment I–B

2008) of the total R_{ag}. With the exception of May 2008, the percentage of growth-to-total respiration was near that found in experiment II-A during 48 h of prolonged darkness (30 %). The largest discrepancy occurred during flowering (May 2008) when aboveground growth was at its peak. This is consistent with the findings of Rambal et al. (2004), who estimated growth respiration as the residual between measured ecosystem respiration (R_{eco}) and a basal R_{eco} in a *Quercus ilex* Mediterranean forest, finding similarly high growth respiration during flowering (also May in that case). The effect of growth respiration on the seasonal variability of R_{ag} seems quite important, especially during inflorescence formation, floral development or flowering. Although R_m represents a large component of the carbon budget in olive and can be determined in terms of dry matter and T, no carbon balance model for olive orchards would be reliable over the whole season unless growth respiration were explicitly taken into account and modelled independently.

Conclusions

Approximately 30 % of plant respiration was associated to growth and 70 % to maintenance respiration (R_m), whose dependence on temperature was empirically determined for different organs. Maintenance respiration in fruits was close to that found in leaves, while R_m for branches was lowest. The above-ground CO_2 efflux was more closely related to leaf mass than whole plant mass or leaf area. Therefore, leaves were the main contributors to above-ground plant respiration. The low maintenance respiration of structural wood indicates that above-ground plant respiration is weakly affected by differences in woody biomass.

Acknowledgments This work was co-funded by Spanish Science Ministry projects CGL2011-15276-E, AGL-2004-05717 and CONSOLIDER CSD2006-67, and Andalusian regional projects P08-AGR-4202 and RNM-7186, with European Regional Development Fund (ERDF) support. OPP is funded by a postdoctoral fellowship from the European Commission (FP7) through GHG-Europe project (Call FP7-ENV-2009-1.1.3.1; Project Code 244122).

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