



Carbon sink strength of a Mediterranean cork oak understorey: how do semi-deciduous and evergreen shrubs face summer drought?

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Abstract

Questions: How do semi-deciduous and evergreen shrubs exploit environmental resources during summer drought? What is the contribution of the understorey shrubby layer to ecosystem carbon assimilation? To what extent are carbon balance and transpiration impacted by a rain pulse?

Location: Cork oak open woodland in the Mediterranean region.

Methods: We used closed dynamic light and dark chambers to measure gas exchange (CO₂ and H₂O) in the dominant shrub understorey species *Cistus salvifolius*, *Cistus crispus* (semi-deciduous) and *Ulex airensis* (evergreen), together with plant physiological and morphological measurements during summer drought and autumn recovery. A hyperbolic light response model constrained by vapour pressure deficits was fitted for up-scaling shrub photosynthesis to the ecosystem level. The data were compared, on a daily and daytime basis, with gross primary productivity estimates from ecosystem eddy-covariance flux measurements.

Results: The onset of summer drought led to a significant leaf area reduction in semi-deciduous species. A general decrease in photosynthesis in all species was observed, while evapotranspiration and above-ground respiration fluxes contrasted among species during summer progression and autumn recovery. The shallow-rooted *C. salvifolius* was able to use light more efficiently than the other two species, although with poor stomatal control over water loss and consistently higher above-ground respiration rates, leading to lower water and carbon use efficiencies when compared with *C. crispus*. The deep-rooted shrub *U. airensis* maintained higher leaf water potentials and very low photosynthetic rates while decreasing transpiration rates throughout the summer drought. A summer rain pulse showed that shallow-rooted shrubs use water in an opportunistic way, with immediate leaf rehydration and concomitant photosynthesis increments. Conversely, deep-rooted shrubs (*U. airensis*) were unresponsive, only recovering photosynthesis with high soil water content. An opportunistic growth response may be disadvantageous to shallow-rooted shrubs in a future climate with extended dry summers and higher probability of rain pulse events. The prominent increase in transpiration rates and plant respiration costs observed during the dry conditions that followed the rain pulse, led to a reduced plant ability to recover after autumn rains.

Conclusions: The shrubs that naturally colonized this montado understorey showed contrasting strategies to overcome summer drought, suggesting an efficient mosaic exploitation of seasonal environmental resources. The contribution of these shrubs to total ecosystem CO₂ uptake during summer and autumn recovery was 17%. This high contribution implies that shrub density management decisions should consider a carbon balance perspective.

Introduction

As a transition area between subtropical and mid-latitudes climates, the Mediterranean Basin constitutes both a refuge area and one that encourages floral exchange and active plant speciation (Medail & Quezel 1999). It is one of the world's major centres for plant diversity but also a climate change 'hotspot' within the next century (Lionello et al. 2012).

The territory, which was once covered by evergreen oak forests, deciduous and conifer forests, has undergone profound changes in the past centuries as a result of deforestation, intensive grazing and fires. This has led to major changes in plant community structure and the extension of woodlands dominated by competitive species (Blondel & Aronson 1995). The high species richness recorded in Mediterranean Basin ecosystems is a consequence of certain levels of land perturbation without which biodiversity maintenance would be ecologically unsustainable (Scarascia-Mugnozza et al. 2000). The Portuguese *montado* agroforestry system is such an example. This unique landscape has remarkably high biodiversity, including many rare and endangered species, resulting from both the climatic influence and centuries of human presence (Bugalho et al. 2011). Generalized cork oak morbidity and mortality has been occurring in the last decades throughout southern European countries, attributed in part to recent climatic changes (Aronson et al. 2009). Land abandonment in the traditional cork oak–grassland mosaic has led to progressive shrub encroachment. Highly competitive and opportunistic shrubs are becoming dominant in the understorey of abandoned *montados*. Without adequate silvicultural control, these areas are prone to severe and recurrent wildfires (Acacio et al. 2009). However, there is evidence that allowing a certain level of shrub recruitment is not necessarily detrimental and can in fact enhance biodiversity in Mediterranean forests (Torrás et al. 2009). In very depleted sites it can enhance the quantity and distribution of soil and plant carbon and nitrogen pools and increase net ecosystem productivity (McKinley & Blair 2008; Maestre et al. 2009).

The understorey of this cork oak woodland was naturally colonized by highly competitive and successful Mediterranean sclerophyll shrubs, which evolved to either escape or tolerate summer drought stress. Evergreen shrubs (e.g. *Ulex* sp.) are considered tolerant as they maintain a decreasing but positive carbon balance throughout the drought period. On the other hand, semi-deciduous shrubs (e.g. *Cistus* sp.) are drought avoiders, dropping some of their leaves during summer and showing higher productivities only when environmental conditions are favourable (Harley et al. 1987; Werner et al. 1999). The different strategies used to exploit environmental resources may

contribute significantly to net ecosystem carbon productivity. How much they really contribute is still a research gap for the Mediterranean region.

Evidence of such a high understorey contribution to ecosystem carbon balance is presented in Misson et al. (2007), in a study dealing with the partitioning of forest carbon fluxes between the overstorey and understorey in ten FLUXNET sites. In general, these studies have shown that the contribution of understorey gross primary productivity (GPP) in summer can reach 39% of total daytime ecosystem GPP. This study used eddy-covariance flux measurements for determining both understorey and overstorey carbon fluxes. At present, this is the most consensual method for disentangling temporal changes in ecosystem carbon fluxes (Baldocchi 2003). Nonetheless, such partitioning studies are sporadic, especially in Mediterranean ecosystems, and there is still much debate regarding methodological issues with the use of the eddy-covariance technique under forest canopies. An example of such problems is the build up of a strong inversion layer in the understorey of open forests, leading to inconsistent nighttime fluxes. In closed forests, deflection of canopy wind direction leads to a discrepancy in overstorey and understorey flux footprints (Misson et al. 2007). This means that complementary measurements in patches of relatively small plants or uniform areas of the understorey are needed. This is especially important in open forest sites, because both understorey leaf area index and light penetration through the canopy are higher, leading to potentially increased understorey biodiversity and GPP. A more detailed description of the understorey also has the advantage of allowing the comparison of instantaneous fluxes from plants and soil with environmental factors (Kolari et al. 2006) and with replicates in space and time. This information can be incorporated in up-scaling modelling exercises and then compared with overstorey eddy-covariance flux measurements.

Without complementary measurements in the understorey, eddy-covariance flux towers do not provide *per se* a detailed understanding of certain processes occurring in the heterogeneous forest understorey to disentangle responses triggered by soils or vegetation. This is the case when summer rain pulses occur. This is a rewetting event in previously dry soils that causes a rapid and transient increase in soil carbon efflux (Tang et al. 2005; Cheng et al. 2006; Jarvis et al. 2007; Unger et al. 2012). However, the living above-ground biomass component also plays an important role, with species responding differently (Cheng et al. 2006; Chen et al. 2009; Patrick et al. 2009). This means that changes in the timing and magnitude of precipitation may have consequences not also for soil dynamics but for the

plant carbon balance, with the potential to alter community composition.

In this study, we hypothesize that shrub recruitment can contribute significantly to the cork oak ecosystem carbon sink. Our investigation focused on the contribution of the shrubby understorey GPP to the ecosystem carbon balance of a Mediterranean cork oak woodland. To evaluate carbon flux partitioning between the understorey and the overstorey, we used net ecosystem productivity data from an eddy-covariance flux tower installed above the tree canopies and closed dynamic chambers to measure carbon fluxes in the dominant species of the understorey, as well as efflux from the soil. Our objectives were to investigate: (1) carbon and water exchange dynamics in the main shrub species and their relative sensitivity to increasing summer drought and recovery after the first autumn rains; (2) the contribution of the understorey to ecosystem carbon assimilation; and (3) shrub species responses to a rain pulse that occurred in the middle of the dry season.

Methods

Site description

The experimental site Herdade da Machoqueira is located in central Portugal (39°08'20.9" N, 9°19'57.7" W, 165 m a.s.l.) and is integrated in the EUI3 IMECC network. The climate is mediterranean, with wet and mild winters and dry and hot summers. Average annual precipitation is 608 mm, and mean annual temperature is 15.9 °C (period 1971–2000 from Évora meteorological station). The site is a 50-yr-old evergreen cork oak open woodland (*Quercus suber*) with an understorey covered with 2-yr-old shrubs (see Table 1 for other characteristics). The soil is a cambisol (FAO), with 81% sand, 5% clay and 14% silt, with roots mainly in the upper horizons (ca. 0–40-cm depth) and some sinker roots taking water from deeper soil horizons and subsoil.

Tree biomass per hectare was estimated by measuring all tree diameters and heights in a representative plot of 40-m radius. Tree above-ground biomass components (leaves, trunk and branch) were estimated subsequently using species-specific allometric equations (Simioni et al. 2008). Tree leaf area index (LAI) was calculated using leaf biomass estimated from allometric equations and species-specific leaf area (SLA) factors (Vaz et al. 2011).

Soil samples were taken randomly (three points) in the understorey from 0 to 10-cm depth, together with undisturbed soil samples for soil bulk density calculations. Soil organic carbon concentration was determined by the dry combustion method according to the International Organization for Standardization 10694, using a CNS elemental analyser (Leco CNS-2000, MI, US). Nitrogen concentration was determined by Kjeldahl digestion analysis (Digestion

Table 1. Site vegetation and soil characteristics. Methods related to biomass sampling are reported later in the text.

Characteristic		Units
Trees		
Tree density	177	Trees·ha ⁻¹
Height	7.9	m
DBH	24.7	cm
LAI	1.6	m ² ·m ⁻²
Total C stock	33.7	tC·ha ⁻¹
Shrubs		
Maximum height	80	cm
Total C stocks		
<i>Ulex airensis</i>	0.156	tC·ha ⁻¹
<i>Cistus crispus</i>	0.093	tC·ha ⁻¹
<i>Cistus salviifolius</i>	0.548	tC·ha ⁻¹
LAI (maximum)	0.38	m ² ·m ⁻²
Grasses		
Above-ground C stock	0.32	tC·ha ⁻¹
% functional groups	43% forbs 45% gram 12% leg	
Soil		
Litterfall input	1.06	tC·ha ⁻¹ ·yr ⁻¹
C stock (up to 60 cm)	62.2	tC·ha ⁻¹
C/N ratio	21.2	

LAI, leaf area index; DBH, diameter at breast height.

System 40; Kjeltex Auto 1030 Analyser, DEcator, SE). Soil organic carbon content was determined using the methodology referred in IPCC (2003).

Ecosystem flux measurements

Standard meteorological data on rainfall (ARG100; Environmental Measurements Ltd., Gateshead, UK), solar radiation (BF2; Delta-T Devices Ltd., Cambridge, UK), air humidity and temperature (CS215; Campbell Scientific, Inc., Logan, UT, US) were collected continuously in 30-min time steps (CR10X; Campbell Scientific).

The fluxes of CO₂, water vapour and energy were continuously measured using an eddy-covariance system installed at the top of a metallic tower (22 m). The system consisted of a 3-D sonic anemometer (R3; Gill Instruments Ltd., Lymington, UK) and a closed-path infrared gas analyser (LI-7000; LI-COR Inc., Lincoln, NE, US) measuring, respectively, the three components of wind velocity and temperature, and the concentration of water vapour and CO₂. Data were continuously acquired on a field laptop with EddyMeas (Meteotools, Jena, DE; Kolle & Rebmann 2007).

Eddy flux data were treated using the eddy-covariance data acquisition and processing software package EddySoft (Meteotools; Kolle & Rebmann 2007). Fluxes were determined on a 30-min basis by block-averaging the 20-Hz

data. Time lags between CO₂ or H₂O signals and vertical wind velocity were determined via cross correlation analysis following Aubinet et al. (2000). The sectorial planar fit method was used for the coordinate rotation of wind vectors (Wilczak et al. 2001). The storage term of CO₂ was calculated according to Greco & Baldocchi (1996) and added to the turbulent CO₂ flux. Final steps of the flux processing procedure included data gap-filling and partitioning of the net CO₂ fluxes into GPP and ecosystem respiration according to Reichstein et al. (2005). Further details on flux data-processing, computation and data quality control are described in Pereira et al. (2007).

Understorey measurements

Soil volumetric water content was measured up to 40-cm depth (2, 10, 20, 30, 40 cm) with dielectric soil moisture sensors in four different places (EC5; Decagon Devices, Inc. Pullman, WA, US). Photosynthetic photon flux density was measured at 0.5 m above the ground with 30 quantum sensors (LI-190SA; LI-COR) randomly placed in the understorey. These measurements were automatically collected with 30-min averages. Litterfall input was estimated with 16 L baskets of 1 m² placed in two transects across the site and sampled every 15 d throughout 2011, with separation of leaves, branches, inflorescences and acorns.

Shrubs and soil gas fluxes

Shrub gas fluxes were measured for the three dominant species of the understorey: *Ulex aircensis*, *Cistus salvifolius* L. and *Cistus crispus* L. *U. aircensis* is an endemic species of the Portuguese flora. It is a perennial evergreen shrub with the shoots and leaves modified into green spines. At maturity, *U. aircensis* retains a high proportion of dead biomass. *Cistus salvifolius* and *Cistus crispus* are both in the Cistaceae family. They are perennial shrubs with xerophytic characteristics, surviving the Mediterranean summer drought by losing part of their leaves in order to reduce the transpiring area (semi-deciduous habit).

We randomly selected four plants per species for flux chamber measurements. In addition, two plots were selected with bare soil and two with an herbaceous layer. Closed portable chambers of 40 cm × 40 cm and 54-cm high were used to measure CO₂ and H₂O fluxes in each selected plot. With one transparent chamber (light chamber) we measured net CO₂ exchange (F_{nee}) and transpiration (F_{et}), and with an opaque chamber (dark chamber) we measured plant and soil respiration (F_{reco}) in the plot. Both chambers were constructed in our laboratory facilities.

The light chamber was constructed from 3-mm thick plexiglas, with more than 95% light transmittance

(Hussain et al. 2009). The dark chamber was constructed of opaque PVC, and covered with reflective aluminium foil. To ensure the chambers were completely airtight during the measurements, they were placed over a collar platform with a basal area of 39.5 cm × 39.5 cm and 10-cm high, which was previously buried in the soil leaving a border of 4 cm above the ground. Both the chambers and the collars were fitted with rubber gaskets at the base to ensure airtight conditions. Air temperatures inside and outside the chambers were continuously monitored in order to match ambient conditions during the flux measurements. Ice packs were used in order to keep temperature within 1 °C of ambient. Fans placed inside the chambers provided constant air circulation. Vapour pressure deficit (VPD) changes in the chambers were limited to 1 hPa during the readings when the chambers were placed on the vegetation. We assumed that such small VPD changes should not affect CO₂ exchange via stomatal effects (Otieno et al. 2009). Light levels above the vegetation were monitored using a PAR quantum light sensor (model QSO-S; Decagon Devices) at the beginning and at the end of each measurement. Measurements were performed with an infrared gas analyser (LI-840A; LI-COR) connected to the chamber. Additional information on the methodology is available in Kolari et al. (2006), Li et al. (2008), Otieno et al. (2009) and Langensiepen et al. (2012).

Measurements were conducted from June to November on a fortnightly time step. Each plot was measured in the early morning, midday and in the afternoon, using the light chamber, immediately followed by the dark chamber. To avoid feedback effects on stomata due to an increase in relative humidity inside the chamber, each measurement was reduced to 3 min, with values being recorded each second. During the measurements, no condensation was observed inside the chambers. The time series collected in each measurement were validated for CO₂ concentration linearity with time. Only time series with correlations above 90% were chosen for the calculation of CO₂ and H₂O flux rates, using the equation and methods described in Wohlfahrt et al. (2005) and Li et al. (2008).

To estimate net plant assimilation rates (F_a), we used the equation: $F_a = (F_{nee} + F_{reco})/LA$, where F_{nee} is the net CO₂ exchange flux measured in the light chamber, F_{reco} is the soil and plant respiration efflux measured in the dark chamber, and LA is plant leaf area measured in each plot. The units are in μmol CO₂·m⁻² leaf area·s⁻¹. Plant respiration (F_r) was calculated with the equation: $F_r = (F_{reco} - F_{soil})/ABG$, where F_{soil} is the CO₂ efflux measured in the bare soil plot and ABG is the above-ground biomass measured in each plot. The units are in μmol CO₂·g⁻¹ above-ground biomass·s⁻¹. Water flux measured with the light chamber was used to determine plant

transpiration (F_{et}) and expressed on a leaf area basis; he units are mmol H₂O·m⁻² leaf area·s⁻¹. Soil evaporation corresponds to the flux measured with the light chamber in the bare soil plots and is expressed in mmol H₂O·m⁻² soil·s⁻¹.

Shrub morphological and physiological measurements

Shrub morphological parameters were measured from plant harvesting at the end of the experiment. The four plants used in the plots for gas flux measurements were harvested and the root system completely extracted from a soil volume of 40 × 40 × 40 cm. These plants were used to determine morphological parameters (height, biomass partition, leaf area and root surface and length). SLA was calculated as the ratio between leaf area and leaf dry mass. Leaves and roots were scanned and leaf area and root parameters (length, diameter, area) were calculated using the WinRhizo software (Regent Instruments Inc., Quebec, Canada). All dry mass values were obtained after 48 h at 65 °C.

For shrub biomass extrapolation to the stand level, we sampled four plots in a total of 120 m² in the tower footprint area. All shrubs inside the plots were harvested and separated per species for biomass determination. Leaf to total above-ground biomass and SLA were used for leaf area index (LAI) calculations for each species at the stand level.

Leaf xylem water potential was measured at predawn (Ψ_{pd}) with a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, US) in four plants per species in the summer drought stress period (DOY 215). Shrub leaf samples were taken periodically during the experiment for chloroplast pigment analysis (chlorophyll *a*, *b* and total) describe in detail in Lichtenthaler (1987).

Water, light and carbon use efficiencies

To quantify the trade-off between the amount of carbon assimilated and the amount of water lost by transpiration and evaporation (ET) we used water use efficiency (WUE), which is the ratio between Fa and Fet. Light use efficiency (LUE) is defined as the efficiency with which plants harvest available light to fix carbon via photosynthesis. It was calculated as the ratio of Fa on a leaf area basis and incident PAR. Carbon use efficiency (CUE) was calculated as the ratio between Fa and Fr, which corresponds to the carbon gain via assimilation per each carbon lost via respiration. Two representative days from early summer (June, DOY 173 and 187), summer end (September/October, DOY 270 and 288) and mid-autumn (November, DOY 321 and 333) were selected for the calculations.

Up-scaling to the stand level

In order to model shrub assimilation rates (Fa), we tested the significance of a series of climatic candidate variables (Tair, Tsoil, H%, PAR, SWC and VPD) measured on a 30-min basis in predicting Fa. PAR and VPD were always highly significant at $P < 0.001$. Fa estimates were obtained by a non-linear least square fit of the data to a rectangular hyperbolic light response curve:

$$Fa = \frac{\alpha\beta Q}{\alpha Q + \beta} \quad (1)$$

where Fa is CO₂ assimilation rate (μmol CO₂·m⁻²·s⁻¹), α is the initial slope of the light response curve, which is an approximation of the LUE, β is the maximum Fa rate (μmol CO₂·m⁻²·s⁻¹), and Q is the photosynthetic photon flux density (μmol photon·m⁻²·s⁻¹). To account for the influence of dry air on canopy conductance, we included an algorithm that takes into account the effect of VPD on Fa, where the β coefficient varies according to VPD (Lasslop et al. 2010):

$$\beta = \begin{cases} \beta = \beta_0, & \text{VPD} < \text{VPD}_0 \\ \beta_0 \exp(-k(\text{VPD} - \text{VPD}_0)), & \text{VPD} \geq \text{VPD}_0 \end{cases} \quad (2)$$

where VPD is vapour pressure deficit measured in the atmosphere (in hPa) and VPD₀ is set up as 10 hPa according to Lasslop et al. (2010). Models were fitted using a free and open-source software package for the development of nonlinear statistical models (AD Model Builder; Fournier et al. 2012). For up-scaling to the stand level, 30-min values of Fa estimates were obtained using the VPD measured in the under-canopy (VPD_{uc}) and the photosynthetically active radiation under-canopy (PAR_{uc}) for the days of shrub gas exchange measurements and then integrated over a daylight basis. We used biomass distribution described above for extrapolation to the footprint area and compared the estimated shrub assimilation rates with the daylight GPP estimated from the partitioning of net ecosystem productivity from the eddy-covariance flux tower installed above the tree canopies. Only the days with high-quality data (quality flags and gap-filling) were used for this comparison.

Fluxes footprints were estimated for the period between DOY 172 and 329 using the ART footprint tool (Neftel et al. 2008), in which the input values are measurements made by the eddy-covariance system (wind direction and speed, SD of lateral wind speed, Obukhov length, friction velocity and measurement height). The diurnal fluxes during this period are predominantly from cork oak woodland with shrubs (average 44.2%). To perform comparisons between the eddy-covariance and

the up-scaled flux chamber measurements, we chose flux tower days that were closest to the measurement dates and that had over 60% of the footprint within the measurement area.

Statistical analysis and model performance

To examine differences among species we used one-way ANOVA. We used the paired *t*-test to examine variable changes in each species on a temporal scale. When statistically significant differences were found, differences between group means were identified by *post-hoc* Tukey HSD tests. When ANOVA assumptions were not met, namely normal distribution of the data and homogeneity of variances, non-parametric tests were carried out, performing a comparison on ranks, and Dunn's test was used for *post-hoc* pair-wise comparisons. The Pearson product moment correlation coefficient was used to display the strength of the association between pairs of variables. All statistical relationships were considered significant at $P < 0.05$. All statistical analyses were carried out using SigmaStat (SigmaStat for Windows v. 3.5; Dundas Software, Germany).

Statistical analysis of the models used in this study followed the approach proposed in Miehle et al. (2006) and Simioni et al. (2008). We examined the accuracy and the bias of predictions and observations on an average basis through calculation of the relative mean error of prediction ($\bar{e}\%$):

$$\bar{e}\% = 100 \frac{\sum_{i=1}^n (O_i - P_i)}{n\bar{O}} \quad (3)$$

where O_i and P_i are the observed and predicted values, \bar{O} is the average of observations and n is the number of measurements. To account for the significant of prediction errors, we used the difference between the relative mean square root error of prediction (RMSE%) and the mean absolute relative error of prediction (MAE%):

$$\text{MAE}\% = 100 \frac{\sum_{i=1}^n |P_i - O_i|}{n\bar{O}} \quad (4)$$

$$\text{RMSE}\% = \frac{100}{\bar{O}} \sqrt{\frac{\sum_{i=1}^n (P_i - O_i)^2}{n}} \quad (5)$$

We used a modification of the Nash–Sutcliffe index to obtain a quantitative measure of agreement between the predictions and the observations indicating the level of precision. This modification was suggested by Simioni et al. (2008) and provides a more apparent method to

assess general model accuracy than the original formulation:

$$\text{ME} = 1 - \frac{\sum_{i=1}^n |O_i - P_i|}{\sum_{i=1}^n |O_i - \bar{O}|} \quad (6)$$

Results

Climate

The meteorological conditions during 2011 (Fig. 1) were abnormal compared with the long-term averages. April and May recorded a total of 218 mm rain, double the average of 108 mm observed in the same period for 1971–2000. On the other hand, summer was relatively dry, with one large rain pulse of 22 mm (around DOY 240) at the beginning of September (Fig. 1a) and lasted longer than normal. Maximum VPD values in the first 2 wk of October 2011 (Fig. 1c) were three times higher (36 hPa) than values observed for the same period in 2010 (12 hPa). The drought period continued from summer throughout early autumn, and the first consecutive autumn rains were only recorded in November (Figs 1a, 2a for the days of chamber measurements).

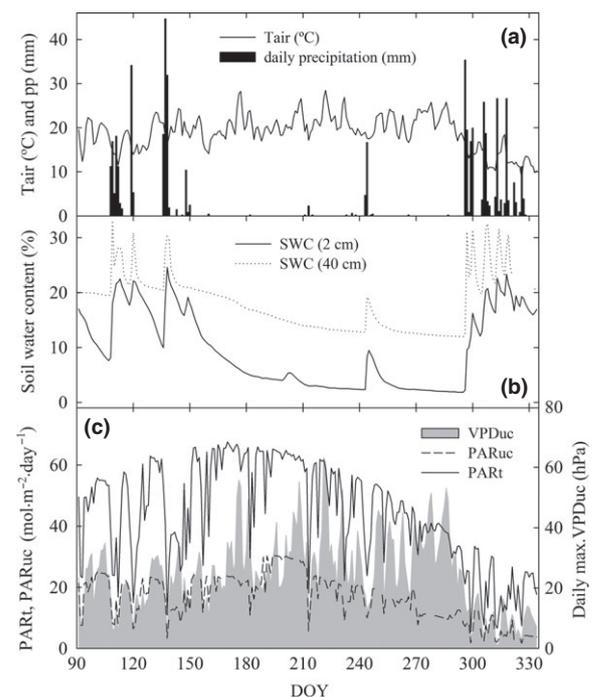


Fig. 1. Daily meteorological conditions during 2011. (a) Daily precipitation (pp, mm) and daily average air temperatures (Tair, °C). (b) Soil water content (SWC, %) at 2- and 40-cm depth. (c) Total photosynthetically active radiation (PART, mol·m⁻²·d⁻¹), under-canopy PAR (PARuc, mol·m⁻²·d⁻¹) and maximum daily VPD measured in the under-canopy (VPDuc, hPa).

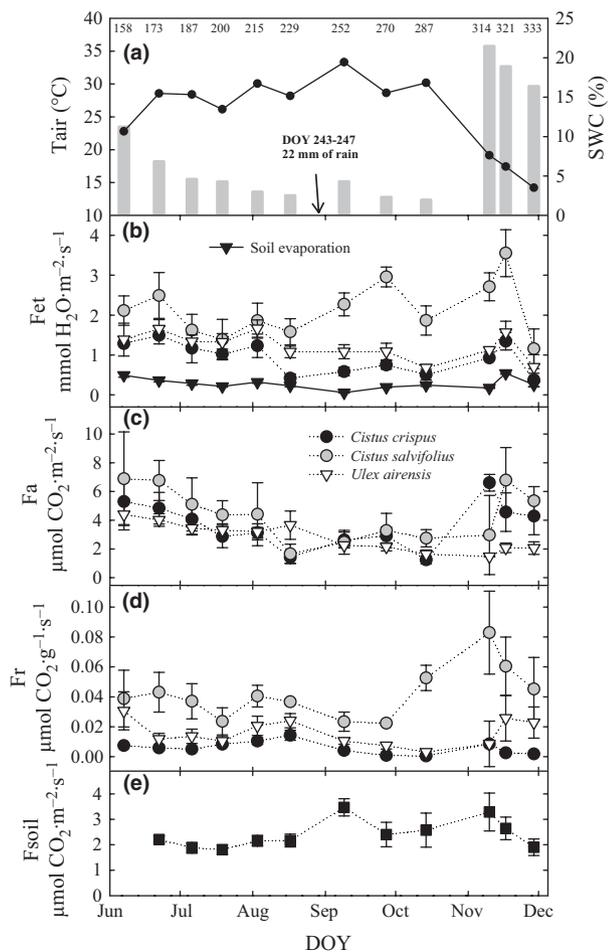


Fig. 2. General meteorological understorey conditions and gas exchange fluxes for the three shrub species measured from June until November 2011. (a) Soil water content at 2-cm depth (SWC, %, grey bars), air temperature (T_{air} , °C, continuous line). (b) Transpiration per shrub species on a leaf area basis (F_{et} , $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and soil evaporation on an area basis ($\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). (c) Net assimilation rates per shrub species (F_a , $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) on a leaf area basis. (d) Above-ground biomass respiration (F_r , $\mu\text{mol CO}_2\cdot\text{g}^{-1}\cdot\text{s}^{-1}$) on a plant dry mass basis. (e) Soil respiration (F_{soil} , $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Julian days are shown in the top x-axis. Error bars represent SE, with $n = 2-4$.

Relative soil water content (SWC) followed closely precipitation events and decreased sharply after May (around DOY 150) reaching the lowest values at 2-cm depth (ca. 2.5%) in midsummer. At 40-cm depth, SWC showed a lower decrease and maintained values above 12% during summer and until the autumn rains (Figs 1b, 2a for days of chamber measurements).

The understorey received on average 36% of the PAR measured in the top canopy (Fig. 1c).

Shrub and soil gas fluxes

Water fluxes

Plant transpiration (F_{et})

Shrub transpiration (F_{et}) decreased moderately (Fig. 2b) in early summer following soil water availability, and then varied little among species until the 22-mm rain event. This soil rehydration led to a prominent F_{et} increase in *C. salviifolius*, while the other two species only slightly increased F_{et} . In general, summer caused a significant decrease in F_{et} in all species, and *C. crispus* had a statistically significant lower F_{et} ($P < 0.001$) than the other shrubs from August to October.

Carbon fluxes

Photosynthesis (F_a)

The evergreen *U. airensis* had consistently lower assimilation rates during the study period compared with the semi-deciduous *Cistus* spp. Although *C. salviifolius* presented the highest F_a rates, it also showed higher variability. On a leaf area basis, differences among the three species were not statistically significant, except in autumn and between *U. airensis* and *Cistus* spp.

The onset of the drought season led to a significant leaf area reduction in semi-deciduous *Cistus* spp., with a concomitant decrease in assimilation rates (F_a). A drop of 60% in leaf assimilation rates was observed, on average, in *Cistus* spp. from DOY 215 to 229, while *U. airensis* maintained similar values. All species presented the lowest F_a rates in summer, although the 22-mm rain pulse from DOY 243–247 led to a slight photosynthesis increase in *Cistus* spp. while *U. airensis* continuously decreased. After DOY 270, the combination of very low soil moisture and higher VPD (>40 hPa; Figs 1c) led to the lowest assimilation rates in *Cistus* spp. and *U. airensis*.

The onset of the rainy season led to an increase in assimilation rates for *Cistus* spp., while *U. airensis* was practically unresponsive throughout the autumn ($P > 0.05$). *C. crispus* had an earlier response to water availability than *C. salviifolius*, which only on DOY 321 showed a marked F_a increase. *C. salviifolius* showed high variability in the observations, so the fluctuations before and after the rain pulse led to differences lacking statistical significance.

Plant respiration (F_r)

Shrub respiration was significantly higher ($P < 0.05$) in *C. salviifolius* than the other two species (Fig. 2d), which had similar respiration rates during the summer. Respiration rates were relatively constant until mid-August and then decreased until DOY 287, with the exception of *C. salviifolius*, which presented a pronounced increase (Fig. 2d). With the onset of rain *C. salviifolius* respiration increased greatly, while *C. crispus* was practically unresponsive. *U. airensis* respiration increased moderately.

Soil respiration (F_{soil})

Soil respiration remained constant (ca. $2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) throughout the dry season (Fig. 2e) and clearly responded to the rain event in September (DOY 252). This soil rehydration was sufficient to stimulate soil microorganism activity and possibly some root activity, as F_{soil} never dropped below $2.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for the rest of the dry season and until the first rains in November. This rain pulse effect was also noticed in the eddy-covariance measurements as an increase in ecosystem respiration on DOY 252 (Fig. 4).

Shrub morphology and physiology

The three shrub species showed contrasting morphological and physiological traits (Table 2). Among the species, *U. airensis* has the highest leaf area as well as the highest investment in root system development, with a root/

shoot ratio of 0.68 ± 0.04 . This species has a much deeper root system than the other two, exploring the soil beyond 40-cm depth. Conversely, *C. salviifolius* has a lower leaf area and the lowest investment in root development, with a root/shoot ratio of 0.29 ± 0.06 ; noteworthy, this species has a very shallow root system restricted to the first 15-cm soil layer but with a very dense distribution and the highest root surface area per volume of soil. Also, *C. salviifolius* has a higher SLA than *C. crispus*, denoting a better adaptation to high light conditions, and grows taller than *C. crispus*. In general, *C. crispus* presented intermediate values in leaf area and root development.

Under summer drought stress, *U. airensis* had a significantly ($P < 0.001$) higher Ψ_{pd} than both *Cistus* spp. (Table 2), confirming its higher capacity for soil water absorption due to its deeper root system.

Table 2. Morphological and physiological traits per species: total plant biomass, specific leaf area, leaf area, root/shoot, root surface area/volume of soil, root distribution, summer predawn leaf water potential (Ψ_{pd}), total chlorophyll and chlorophyll *a/b*. ES is early summer, SE is summer end and MA is mid-autumn.

Characteristics		<i>Ulex airensis</i>	<i>Cistus crispus</i>	<i>Cistus salviifolius</i>
Total plant biomass (g)		60 ± 24.8	29.1 ± 5.1	13.4 ± 2.3
Specific leaf area ($\text{m}^2\cdot\text{kg}^{-1}$)		7.9 ± 0.47	8.9 ± 0.28	11.5 ± 1.71
Leaf area (m^2)		0.12 ± 0.05	0.06 ± 0.01	0.03 ± 0.01
Root/shoot		0.68 ± 0.04 ^a	0.45 ± 0.10 ^{ab}	0.29 ± 0.06 ^b
Root surf./vol. soil ($\text{m}^2\cdot\text{m}^{-3}$)		1.02 ± 0.3	0.79 ± 0.2	1.16 ± 0.3*
Maximum root depth		>60 cm	40–50 cm	10–20 cm

General plant morphology (example from harvested plants)				
SE Ψ_{pd}	(MPa)	−1.4 ± 0.2	−2.5 ± 0.1	−2.9 ± 0.3
Total chlorophyll ($\text{mg}\cdot\text{g}^{-1}$)	ES	4.11 ± 0.8 ^a	8.1 ± 1.18 ^b	6.42 ± 1.11 ^b
	SE	4.83 ± 0.53 ^a	9.52 ± 0.88 ^b	11.36 ± 0.48 ^b
	MA	2.31 ± 0.15 ^a	5.36 ± 0.55 ^b	4.6 ± 0.25 ^b
Chlorophyll <i>a/b</i>	ES	1.63 ± 0.17	1.02 ± 0.18 ^b	1.51 ± 0.21
	SE	1.73 ± 0.11 ^a	1.04 ± 0.13 ^b	0.82 ± 0.07 ^c
	MA	2.21 ± 0.02 ^a	1.44 ± 0.12 ^b	1.71 ± 0.23 ^b

*Roots found only in the 15-cm top soil.

Different letters represent statistical significance at $P < 0.05$, no letters means no differences (average ± SE).

Regarding chlorophyll content, total chlorophyll increased for all species from early summer to the end of summer, dropping sharply in the moist season. Total chlorophyll was two times higher in the semi-deciduous *Cistus* spp. compared to *U. airensis*. Regarding chlorophyll *a/b*, there was a clear decrease for *C. salviifolius* from early summer to the end of summer, resulting from chlorophyll *a* degradation, but *U. airensis* and *C. crispus* were able to maintain the ratio. A recovery occurred in mid-autumn for all species, but was more pronounced for the *Cistus* spp.

Water, light and carbon use efficiencies

All three species were able to maintain similar WUE from early summer to the end of summer (Fig. 3a). Soil water rehydration in autumn, together with a significant drop in VPD, led to an increase in WUE in all species except *U. airensis*, in which it only moderately increased. This WUE increase for *Cistus* spp. was the result of canopy photosynthesis recovery with a high control of water loss. Nevertheless, *C. salviifolius* presented a modest increase in WUE in mid-autumn (Fig. 3a), resulting from a proportionally higher transpiration compared with recovery in assimilation rates (Fig. 2b,c).

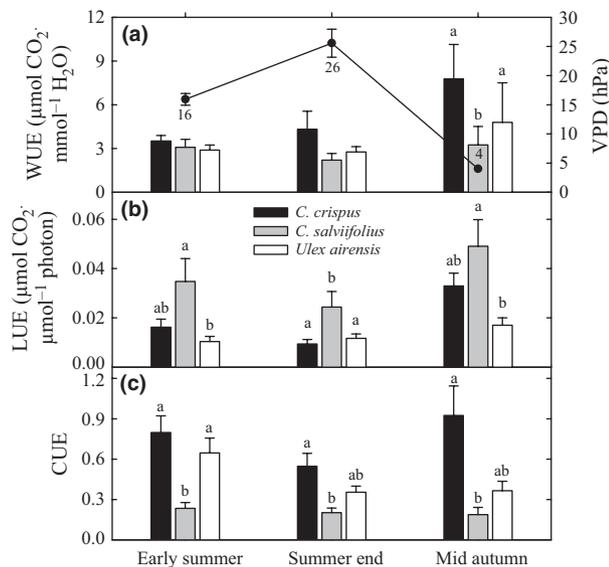


Fig. 3. Temporal variation in water, light and carbon use efficiencies for *Cistus crispus* (black bars), *C. salviifolius* (grey bars) and *Ulex airensis* (white bars). **(a)** Water use efficiency (WUE, $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$) and average max. daily VPD (hPa). **(b)** Light use efficiency (LUE, $\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{ photon}$), **(c)** carbon use efficiency (CUE, dimensionless). Letters represent significant differences ($P < 0.05$) between species for each period.

Throughout the summer, LUE was higher in *C. salviifolius* when compared to the other two species (Fig. 3b). No differences were observed at a seasonal level, although *C. crispus* increased LUE after the first rains mainly due to a recovery in assimilation rate. On the other hand, *C. crispus* always showed a significantly higher CUE than *C. salviifolius* (Fig. 3c), which was mainly due to increased differences in respiration rates during the summer and autumn (Fig. 2d). The drought at the end of summer was accompanied by a decrease in CUE for *C. crispus* and *U. airensis*, followed by a recovery in mid-autumn only for *C. crispus*.

Up-scaling to the stand level

Modelling results

The inclusion of VPD limitation improved the model ability to predict instantaneous F_a for *C. crispus* and *U. airensis* but not for *C. salviifolius*.

The results show that model bias (given by $e\%$) was considerably higher for *C. salviifolius* with the inclusion of VPD, meaning that the predictions were under-estimated by an average of 56%. The difference between RMSE% and MAE%, which reflects the existence of significant prediction errors, also corroborates this result. For *C. crispus* and *U. airensis*, the inclusion of VPD in the models decreased the error bias and increased model efficiency.

Eddy-covariance measurements and shrub up-scaling

Eddy-covariance data showed that the ecosystem was a strong carbon sink during the early summer (Fig. 4). This carbon sequestration decreased gradually with the onset of the dry season, although a positive carbon balance was maintained until the first rains in mid-autumn. The

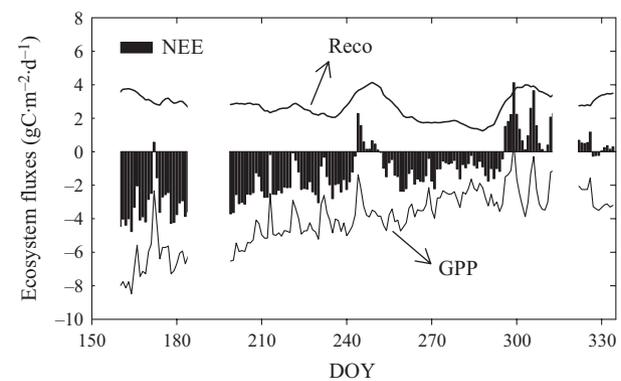


Fig. 4. Net ecosystem exchange (NEE), gross primary productivity (GPP) and ecosystem respiration (Reco) measured using eddy-covariance. Discontinuities in the data are due to rejected data in filtering and/or gap-filling processing.

22-mm rain event in early September caused an ecosystem CO₂ efflux to the atmosphere, mostly due to a soil respiration pulse (Fig. 2e) as a consequence of soil rehydration.

Based on chamber and up-scaling of gas exchange measurements, the shrub contribution to ecosystem CO₂ uptake (GPP) was, on average, 17%. Due to a higher proportion in the understorey (see total C stock estimates in Table 1), *C. salviifolius* made a particular high contribution of 14%, while *C. crispus* and *U. airenensis* only amounted to 2% each. This CO₂ uptake contribution follows an expected seasonal trend in accordance with soil water availability and assimilation rate limitation. Therefore, in the early summer, the average contribution was 7%, increasing to 10% with drought stress at the end of summer. After the first rains in mid-autumn, there was an assimilation rate recovery (Fig. 4), and the contribution to GPP peaked at 35%.

Discussion

The understorey of this cork oak woodland comprises two functional groups (semi-deciduous and evergreen), which have developed different strategies of using light, water and carbon to overcome summer drought and compete successfully under the tree canopies.

Leaf traits, root depth and its relation with summer resource exploitation

Cistus salviifolius

Cistus salviifolius exhibited higher rates of net photosynthesis throughout the study period, which was related to canopy architecture. Leaves are arranged in many layers, with longer and perpendicular branches at the base decreasing in length and angle towards the top, thus optimizing light capture and reducing self-shading (see Table 2; Givnish 1988; Pearcy et al. 2005). Werner et al. (1999) also found that *Cistus* spp. are able to structurally adjust leaf orientation in accordance with water and light availability, which confers some advantage in heterogeneous shade conditions such as in this understorey environment. We also observed that *C. salviifolius* presented summer dimorphic leaves: one group of fully expanded leaves formed in early spring that are mostly lost in summer and another group of small leaves (around 30%) formed in late spring that remain small and attached to the plant during summer. This feature was also observed in other semi-deciduous Mediterranean shrubs (Kyparissis & Manetas 1993; Aroñne & De Micco 2001). The smaller leaves contained five times more chlorophyll per unit dry mass than adjacent old leaves, suggesting plant resources mobilization to the

maintenance of the photosynthetic apparatus. Maintaining these leaves is advantageous to *C. salviifolius* when the first autumn rains start, as they quickly expand and start to photosynthesize prior to other co-occurring species (Harley et al. 1987). So it is likely that these small leaves may be contributing to the higher assimilation rates observed.

The way in which *C. salviifolius* optimizes light harvesting and uses it to maintain higher photosynthetic rates is in accordance with the higher LUE observed (Fig. 3b). Additionally, light response curves and gas flux data from light chambers are consistent with the finding that *C. salviifolius* always achieves higher photosynthetic rates irrespective of light available and soil drought status. In relation to CUE, *C. salviifolius* presented the lowest values (Fig. 3c) even if it had higher photosynthetic rates. This low efficiency in using the assimilated carbon is mainly due to the very high respiration cost throughout the season and is associated with the maintenance of above-ground components (Fig. 2d). The high success of *C. salviifolius* in the understorey of this cork oak woodland, representing almost 70% of the understorey shrubby carbon stock, may be related, to some extent, to these two features: a higher capacity to exploit the reduced light available under the tree canopy and the ability to exploit water in an opportunistic way due to its shallow roots.

Both semi-deciduous *Cistus* spp. experienced a significant reduction in leaf area, reaching 62% in *C. salviifolius* and 45% in *C. crispus*. Leaf area reduction is a well-known drought stress adaptation in semi-deciduous species that greatly reduces leaf transpiration (Harley et al. 1987; Wedler et al. 1996). On a leaf area basis, this water loss decrease was modest in both *Cistus* spp., probably because spring was wet and moderate levels of soil water were maintained at the beginning of summer. Nonetheless, the two species showed clear and distinct responses after the

Table 3. Parameter values for the hyperbolic light models tested for each species with and without the VPD limitation. Parameters are described in Eqs. (1) and (2). Statistical outputs and model performance tested using the relative mean error of prediction ($\bar{\epsilon}\%$), the difference between the relative mean square-root error of prediction (RMSE%) and the mean absolute relative error of prediction (MAE%) and model efficiency (ME).

With VPD	Parameter estimates			Model performance		
	β_0	α	k	$\bar{\epsilon}\%$	RMSE%–MAE%	ME
<i>C. crispus</i>	8.463	0.030	0.051	1.4	12.3	0.26
<i>C. salviifolius</i>	6.251	0.153	0.042	56.2	23.1	–0.07
<i>U. airenensis</i>	4.184	0.029	0.043	7.1	18.7	0.12
Without VPD	β	α				
<i>C. crispus</i>	4.550	0.039		7.6	14.9	0.16
<i>C. salviifolius</i>	4.949	0.123		10.5	18.1	0.04
<i>U. airenensis</i>	3.844	0.030		7.17	18.8	0.09

22 mm rain pulse in late August (Fig. 2b), as discussed below. Excluding this rain pulse effect from the analysis, *C. salviifolius* was able to maintain the same water use efficiency from early summer to the end of summer, even with an increase of 60% in VPD. Autumn rains led to only a modest recovery of photosynthesis in *C. salviifolius*, which was unexpected for a shallow-rooted shrub which, according to Harley et al. (1987), responds quickly to rainfall.

Ulex airensis

Ulex airensis presented lower photosynthesis per unit leaf area but more homogeneous rates during the studied period (Fig. 2c). Concomitantly, respiration rates remained low, leading to an intermediate CUE when compared to deciduous species, possibly related to the higher cost of leaf construction and maintenance (see Table 3 for SLA values in *Ulex* and *Cistus*; Walters & Reich 1999; Baldocchi et al. 2010). Recovery of photosynthesis and growth only occurs later in spring, when soil moisture and air temperature are not limiting.

During the experiment, *U. airensis* only showed modest signs of senescence at the peak of stress in the lower canopy (average 14% of total above-ground biomass). Lower branch shedding can have several purposes. One is to maximize light penetration in the inner canopy, as *Ulex* tends to produce very dense canopies resulting from the high accumulation of woody tissue (see Table 2 for above-ground biomass; Valladares et al. 2003). This may partly explain the lower LUE found. According to Tyree et al. (1993), this is a mechanism to prevent the whole plant from hydraulic failure by sacrificing some less productive and shaded shoots and thus protecting the stem from cavitation. Finally, lower and old branch shedding can also be a carbon economy strategy in order to optimize the ratio between plant carbon gains and losses.

Increased leaf longevity in an evergreen like *U. airensis* requires a conservative strategy in terms of water and light use in order to avoid irreversible leaf damage (Tenhunen et al. 1990). Leaf transpiration rate was significantly lower in *U. airensis* than in *C. salviifolius* but was similar to *C. crispus*, meaning that *U. airensis* survives summer in a better hydrated condition. This is supported by drought stress peak predawn leaf water potentials, which were lower (more negative) in *C. salviifolius* than in *U. airensis* (Table 2). This latter species seems to have isohydric behaviour, maintaining relatively constant leaf water potential but reducing stomatal conductance as the soil dries out. This is substantiated in *U. airensis* by the root/shoot ratio and access of roots to soil deep water, as the roots easily extend beyond 60 cm soil depth (see Table 2). Nonetheless, the apparent control of transpira-

tion rates in *U. airensis* does not yield higher WUE, meaning that all three species are equally efficient in using water from early summer to the end of summer.

Cistus crispus

This shrub showed a strategy of environmental resource utilization standing between *U. airensis* and *C. salviifolius* and was apparently more efficient. With lower carbon assimilation rates but also the lowest respiration rates (Fig. 2c,d), *C. crispus* presented a significantly higher CUE throughout the experimental period. It also showed high control on water loss leading to a higher (but not statistically significant) WUE. Also, tap roots and shallow feeder roots (Table 2) allow *C. crispus* to access both deep water supplies and surface precipitation, providing a higher ability to explore available water and therefore more control of leaf senescence and abscission during summer. The corrugated pubescent leaves must be contributing also for leaf transpiration inhibition. This ability may also explain the significant increase in WUE and CUE after the first autumn rains. Canopy architecture in *C. crispus* seems to be consistent with the statistically lower LUE found for this species: the plants are stocky, grow in the shade of other plant canopies and the leaves are packed into one layer with a high level of self-shading (Table 2).

Response to a summer rain pulse: when opportunistic strategies do not compensate

A 22-mm rain event occurred at the end of August, followed by a long period of hot and dry conditions (September and October). Species responded differently to this sudden soil water availability. This rain event moistened the soil up to a depth of 40 cm but had returned to pre-rain values after 10 d (Fig. 1b) so it mostly benefited the species with shallow roots. *C. salviifolius* was particularly responsive, showing an increase in assimilation rates related to the expansion of the small summer leaves. Average canopy total chlorophyll per unit dry mass in leaves just before and after the rain event increased 17% in *C. salviifolius* from 9.7 to 11.4 mg·g⁻¹, indicating canopy photosynthetic pigment recovery. Conversely, chlorophyll content only slightly increased in *C. crispus* (10%) and remained stable in *U. airensis*, reinforcing the relative insensitivity of this latter species to summer rain pulses.

A rapid leaf sprouting response to soil moisture is a common feature of semi-deciduous species. This is advantageous once the autumn rains start because nutrient and water uptake occur prior to that of neighbouring species (Harley et al. 1987; Kypris & Manetas 1993; Grammatikopoulos 1999). This allows plants to reach earlier maximum leaf area, thus optimizing carbon assimila-

tion. However, the ecophysiological implications are not the same when a sporadic summer rain event is followed by long and dry conditions. Thus, *C. salviifolius* did not show any sign of stomatal control over water loss, as the soil dried out after the rain pulse, contradicting the findings of Harley et al. (1987), who reported considerable stomatal control of transpirational water loss in *C. salviifolius*. Although some photosynthesis still occurred (Fig. 2c), transpiration rates increased abruptly (Fig. 2b) and then later respiration also increased (Fig. 2d) leading to a plant carbon balance disequilibrium, in some cases irreversible, as field observations (not quantified) revealed plant death. Such a breakdown may be related to the maintenance respiration cost of the small, expanded leaves. In contrast, *U. aërensis* and *C. crispus* did not respond to the rain event, confirming their 'play safe' strategy towards a carbon cost-effective equilibrium (Werner et al. 2001).

Summer rain pulses are known to stimulate soil micro-organism activity due to sudden soil rewetting after a long dry period, where abundant partly decomposed organic matter from the previous spring is available for decomposition. This causes a rapid and pronounced CO₂ efflux to the atmosphere (Fig. 2e). However, there is also plant respiration feedbacks to this rain event. In this study, the over-hasty response of *C. salviifolius* to immediate soil moistening ended in a huge above-ground plant carbon efflux that may have also contributed to ecosystem carbon lost in summer. We may speculate that, under a future climate change scenario of longer drought periods punctuated by extreme rainfall events, survival of *C. salviifolius* may be threatened. However, only a detailed analysis of the various aspects of plant strategy, namely growth patterns and reproduction, would allow elucidation of a more comprehensive scenario for the development of this species.

Shrub contribution to ecosystem CO₂ uptake

Except for *C. salviifolius*, the inclusion of VPD data in models helped to increase their accuracy in estimating Fa (Table 3), with a decrease in carbon assimilation rates when temperature and VPD are higher (Pettigrew et al. 1990; Lasslop et al. 2010). Nevertheless, the approach used failed to accurately describe the high variability in measured carbon assimilation rates, especially for *C. salviifolius*. This variability may be partly explained by the highly dynamic understorey light environment, punctuated by brief and unpredictable periods of direct solar radiation (sun flecks). Shade species are able to efficiently use these sun patches but can also become susceptible to photoinhibition during periods of drought when CO₂ diffusion inside the leaf is limited by low stomatal conductance (Chaves et al. 2004). Diffuse light is known to provide higher assimilation rates, as it is used more efficiently (Rosati &

Dejong 2003), which means that higher Fa does not necessarily coincide with high PAR and lower VPD. An analysis of the sources of error revealed that the models failed to estimate the highest Fa values by default, meaning that our daily estimates are rather conservative. We consider that only a model specifically addressing species-specific photosynthesis optimization under shade and drought would provide a statistically stronger up-scaling exercise.

The average contribution of the understorey GPP to total ecosystem CO₂ uptake was estimated at 17%, very similar to the rates of 20% found by Wedler et al. (1996) in the understorey of a Scots pine plantation, and 25% found by Sakai et al. (2006) in a cool-temperate deciduous broad-leaf forest in central Japan. Furthermore, the values found in this study are within the range of 0–39% reported by Misson et al. (2007) for several forest ecosystems. The 7% contribution of the understorey during early summer results from the proportionally higher contribution of the tree component to ecosystem GPP compared with that of the shrubs. Early summer is the time when cork oaks are physiologically more active: their canopies have new shoots and leaves and their deep roots have access to ground water tables (Chaves et al. 2004; David et al. 2007). We speculate that the slight increase in shrub contribution at the end of summer may result from the more favourable understorey environment for the shrubs at the peak of summer, with lower VPD and lower risk of photoinhibition. The higher shrub contribution occurred in mid-autumn (35%), which may be explained by the rapid shrub response to soil rehydration upon the onset of autumn rains (Fig. 2c), particularly from the two *Cistus* spp.

The 17% contribution of the shrubby understorey to ecosystem carbon sequestration shows that allowing shrub recruitment may increase forest mitigation importance in a climate change context. However, due to the extremely aggressive and competitive feature of Mediterranean shrubs, management of the shrubs growing beneath the trees should be a compromise between benefits and costs, especially in relation to competition with trees for water and nutrients and also the increased fire risk. This is still a research gap in Mediterranean forest management.

Besides the ecological services provided by a shrubby mosaic, namely protection from soil erosion, a biodiversity refuge and an increase in soil fertility (Bugalho et al. 2011), shrub recruitment management can help in ecosystem restoration. For example, the generalized lack of cork oak regeneration is considered one of the main problems of future sustainability of *montados* (Plieninger et al. 2010). Recent studies reveal that in stress-prone ecosystems such as those in the Mediterranean region, the heterogeneity generated by shrubs facilitates the establishment of tree species (Gomez-Aparicio et al. 2005). Tree seedling establishment and survival benefit significantly from modifica-

tion of the abiotic environment promoted by nurse shrubs, where shading appears the crucial facilitating factor (Smit et al. 2008). Although this shrub–tree seedling beneficial interaction seems to be species-specific (Rolo & Moreno 2011), there is a broad general consensus that augmenting populations of nurse shrubs and trees should be considered a promising strategy for restoring woody late-successional communities (Valladares et al. 2003; Prider & Facelli 2004; Gomez-Aparicio et al. 2005; Smit et al. 2008; Plieninger et al. 2010; Rolo & Moreno 2011).

This study allows us to conclude that under a global change perspective, the future of cork oak forests requires a scientifically sound conservation strategy and locally tailored sustainable management that must accommodate various aspects of understorey plant biology and ecosystem functions.

Conclusions

The understorey shrubby vegetation of this *montado* ecosystem contributed 17% to ecosystem net CO₂ uptake, reinforcing our hypothesis of the importance of shrub recruitment in forest mitigation in a climate change context. The mixture of sclerophyllous plants that naturally colonize the understorey, although with contrasting leaf habits and rooting depths, was able to balance carbon gain and water loss during the most critical time in the Mediterranean climate: the summer drought. However, the unexpected summer rain pulse in the middle of the study allowed us to distinguish between species' ability to use water that is immediately available and its impact on whole plant carbon and water balance. We conclude that shallow-rooted, semi-deciduous species exploit environmental resources in an opportunistic way and are more susceptible to longer dry periods following summer rain pulses. This study reinforces that Mediterranean rain pulses are particularly detrimental to ecosystem carbon sequestration, not only due to soil respiration efflux but also due to plant respiration losses. Future studies should focus on understorey plant ecophysiological processes concerning environmental resource exploitation by different functional groups. Due to the difficulty in reproducing water and carbon fluxes, modelling exercises should specifically incorporate realistic photosynthetic responses to the patchy forest understorey light, and also plant water and carbon efficiencies at a seasonal time-scale.

From a sustainable management standpoint, the study of shrub–tree interactions is essential as during drought, Mediterranean shrubs are extremely aggressive, competing directly with trees for water and nutrients. Shrub encroachment can greatly increase fire risk and might jeopardize the potential for long-term carbon sequestration.

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