



## CO<sub>2</sub> exchange and biomass development of the herbaceous vegetation in the Portuguese montado ecosystem during spring

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### ABSTRACT

Montado are spatially heterogeneous ecosystems that are economically important for the production of cork and herbaceous biomass that provide fodder for animals. Understanding of how trees and the herbaceous layer interact to determine pasture yield and the overall CO<sub>2</sub> exchange of the herbaceous layer is crucial. Portable chambers were used to study CO<sub>2</sub> exchange by the herbaceous layer component of the montado ecosystem in southern Portugal. Biomass, Net herbaceous layer CO<sub>2</sub> exchange (NEE) and respiration ( $R_{\text{eco}}$ ) were measured in the open and understory locations between March and May, during the active growing period. Parameter fits on the NEE data were performed using empirical hyperbolic light response model, while ecosystem respiration ( $R_{\text{eco}}$ ) data were fitted with a two-parameter exponential model. Annual green biomass productions were  $405.8 \pm 9.0$  and  $250.6 \pm 6.3 \text{ g m}^{-2}$  in the open and the understory, respectively. The respective maximum NEE during the day were  $-24.0 \pm 2.9$  and  $-9.6 \pm 2.2 \mu\text{mol m}^{-2} \text{ s}^{-1}$  while maximum  $R_{\text{eco}}$  were  $20.6 \pm 2.2$  and  $10.0 \pm 1.6 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , occurring in April. Photosynthetic photon flux density (PPFD) explained more than 70% of variations in daytime NEE while soil temperature at 10 cm depth ( $T_{\text{soil}}$ ) explained >50% of the variations in  $R_{\text{eco}}$  under non-limiting soil moisture conditions. Both the herbaceous layer communities shared similar plant functional types and no significant difference in nutrient nitrogen (N) occurred between them. The two herbaceous layer components shared similar physiological characteristics and differences that arose in their CO<sub>2</sub> uptake capacities and green biomass production were the result of microclimatic differences created by tree shading.

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### 1. Introduction

The landscapes of southern Portugal are characterised by a savanna-like, pastoral agro-forestry ecosystem called montado. It consists of scattered trees (85–120 trees ha<sup>-1</sup>) dominated by evergreen oaks, mainly *Quercus suber* (cork oak), and *Q. rotundifolia* (holm oak) and an extensive and species-rich herbaceous layer (Joffre and Lacaze, 1993; Tomé et al., 2007; Bugalho et al., 2008). Montado is economically important for cork production as well as acorns and herbaceous biomass that provide fodder for animals. It covers approximately 2.7 million hectares of the Iberian Peninsula (Tomé et al., 2007). The distribution of tree cover does not follow a uniform pattern as it usually results from natural regeneration. The

varying tree density suggests that despite long-term human intervention in the montado, nature still plays a significant role in shaping the ecosystem and the system has adjusted to local climate (Joffre et al., 1999) and hydrology (Otieno et al., 2006; David et al., 2004).

The nature of tree distribution creates a spatial heterogeneity in the availability of soil-based resources as well as microclimate that could have profound influence on ecosystem composition, functioning and management (Huber-Sannwald and Jackson, 2001). At small spatial scales, the tree distribution pattern promotes a variety of plant responses including the proliferation of roots into resource-rich patches, which modify the competitive ability, survival of individuals and productivity of assemblages. Thus, the spatial tree distribution leads to the creation of two distinct ecological niches: one located outside the tree canopy (open) that is composed of herbaceous plants alone and a second located directly beneath the tree canopy, corresponding to the oak

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tree with its associated herbaceous stratum (understory). Long-term adjustments resulting from differences in the microclimatic and edaphic conditions in these two ecological components are likely to result into distinct differences in their physiological functioning, with significant influence on bioproductivity and ecosystem carbon budget. Trees on the one hand enhance soil nutrient availability, improve soil water holding capacity and ameliorate environmental stress immediately below their canopy (Abrams and Mostoller, 1995; Gallardo et al., 2000) and may stimulate primary production in the herbaceous layer under the canopies.

Gross Primary Production (GPP) increases linearly with photosynthetically active radiation absorbed by the canopies. Plant production is therefore related to plant characteristics that affect the slope of the relationship between Net Primary Production (NPP) and photosynthetic photon flux density (PPFD), light quality (Ehleringer and Björkman, 1977) and the duration of its absorption (Gilmanov et al., 2005). Although vegetation under the trees is subjected to alternate sun and shade periods, which could reduce the amount of CO<sub>2</sub> fixed, increased efficiency in utilizing diffused light and the extended favourable edaphic and microclimatic conditions that prolong the productive period (Percy, 1990) may result into increased production in the tree understory.

The herbaceous layer plays an important role in the annual carbon balance in the montado due to its large leaf area index (LAI) during the spring growth period compared to the trees. Few studies however, have characterised CO<sub>2</sub> exchange and productivity of this layer that is only active for a short period. We report results from an intensive field study conducted during the active vegetative period from February through May in the two distinct herbaceous layer components. We examined how spatial diversity resulting from tree distribution patterns influences the microclimate and resource distribution between the two distinct plant communities and how these differences relate to CO<sub>2</sub> assimilation, biomass production and community composition.

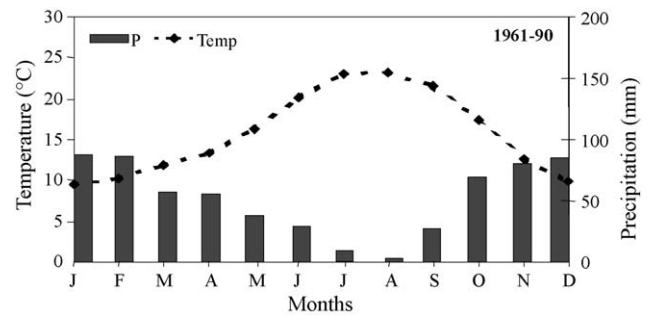


Fig. 1. Long-term averages (30 years) of the monthly temperatures and rainfall at the study site in Mitra, Evora, Portugal.

## 2. Materials and methods

### 2.1. Description of experimental site

Measurements were conducted in the farming (pasture/experimental) plots of the University of Evora's Mitra Campus (38°32'N, 8°01'W, 243 m a.s.l), in the rural district of Evora, 12 km from Evora town, located approximately 150 km south east of Lisbon, Portugal. The site is part of the CarboEurope-IP project (Pereira et al., 2007). The topography is slightly rolling and the soil is shallow (30 cm depth) Cambisol (FAO, 1988) overlying a fractured gneiss rock. The soil texture is sandy loam (>70% sand) and is well drained with a bulk density of 1.56 g cm<sup>-3</sup>. The climate is a typical Mediterranean, with hot and dry summers and cool and wet winters. More than 80% of annual precipitation occurs between October and April. Mean monthly air temperatures and precipitation between 1961 and 1990 are shown in Fig. 1. The understory consisted of grazed pasture dominated by species-rich herbaceous layer composed of native forbs, legumes and grasses (Table 1).

Table 1

List of plant species of the herbaceous layer vegetation growing in the open and understory locations during spring 2006.

Understory			Open		
Species	Functional type	% Cover	Species	Functional type	% Cover
<i>Stellaria media</i> <sup>a</sup>	Forb	25	<i>Geranium molle</i> <sup>a</sup>	Forb	35
<i>Geranium molle</i> <sup>a</sup>	Forb	20	<i>Rumex bucephalophorus</i> <sup>a</sup>	Forb	15
<i>Coleostephus myconis</i> <sup>b</sup>	Forb	5	<i>Leontodon taraxacoides</i> <sup>a</sup>	Forb	8
<i>Galactites tomentosa</i> <sup>b</sup>	Forb	5	<i>Aphanes microcarpa</i> <sup>a</sup>	Forb	5
<i>Centranthus calcitrapae</i> <sup>b</sup>	Forb	5	<i>Erodium romanum</i> <sup>b</sup>	Forb	5
<i>Anagallis monelli</i> <sup>a</sup>	Forb	5	<i>Spergula arvensis</i> <sup>a</sup>	Forb	5
<i>Leontodon taraxacoides</i> <sup>a</sup>	Forb	5	<i>Coleostephus myconis</i> <sup>b</sup>	Forb	3
<i>Echium plantagineum</i> <sup>c</sup>	Forb	2	<i>Anthemis fuscata</i> <sup>a</sup>	Forb	2
<i>Tuberaria guttata</i> <sup>a</sup>	Forb	2	<i>Crassula tillaea</i> <sup>a</sup>	Forb	2
<i>Sonchus oleraceus</i> <sup>c</sup>	Forb	1	<i>Echium plantagineum</i> <sup>b</sup>	Forb	2
<i>Rumex acetosella</i> <sup>a</sup>	Forb	1	<i>Stachy arvensis</i> <sup>a</sup>	Forb	2
<i>Arum italicum</i> <sup>b</sup>	Forb	1	<i>Cerastium glomeratum</i> <sup>a</sup>	Forb	1
<i>Lamium amplexicaule</i> <sup>a</sup>	Forb	<1	<i>Senecio vulgaris</i> <sup>a</sup>	Forb	1
<i>Aristolochia longa</i> <sup>b</sup>	Forb	<1	<i>Anagallis monelli</i> <sup>a</sup>	Forb	<1
<i>Fumaria adrarica</i> <sup>a</sup>	Forb	<1	<i>Bellardia trixago</i> <sup>b</sup>	Forb	<2
<i>Geranium pupureum</i> <sup>a</sup>	Forb	<1	<i>Calendula arvensis</i> <sup>a</sup>	Forb	<3
<i>Stachys arvensis</i> <sup>a</sup>	Forb	<1	<i>Plnatago coronopus</i> <sup>b</sup>	Forb	<5
<i>Bromus rubens</i> <sup>a</sup>	Grass	12	<i>Raphanus raphanistrum</i> <sup>b</sup>	Forb	<6
<i>Ornithopus sativus</i> <sup>a</sup>	Legume	6	<i>Rumex acetosella</i> <sup>b</sup>	Forb	<7
<i>Medicago sp</i> <sup>a</sup>	Legume	5	<i>Veronica arvensis</i> <sup>a</sup>	Forb	<8
			<i>Poa annua</i> <sup>a</sup>	Grass	2
			<i>Iris sisyryhynchium</i> <sup>b</sup>	Grass	<4
			<i>Trifolium dubium</i> <sup>a</sup>	Legume	8
			<i>Ornithopus sativus</i> <sup>a</sup>	Legume	4

<sup>a</sup> Annual.

<sup>b</sup> Perennial.

<sup>c</sup> Biennial.

## 2.2. Microclimate

Air temperature ( $T_{\text{air}}$ ) and humidity (VAISALA HMP45A, Helsinki, Finland), PPFD (LI-190 Quantum sensor, LI-COR, USA) and precipitation (ARG100 rain gauge, EM Ltd., Sunderland, UK) above the canopy were continuously measured using sensors installed on top of a 30 m high flux tower erected within the study site. Measurements were taken every 5 min, averaged and logged half-hourly. In addition, discontinuous recording of microclimate within and outside the chambers during gas exchange measurements were conducted. Recorded information included PPFD (LI-190, LI-COR, USA) within the transparent chambers, just above the herbaceous vegetation (approx. 50 cm above ground surface),  $T_{\text{air}}$  at 20 cm height inside and outside the chambers (Digital thermometer, Conrad, Hirschau, Germany) and soil temperature ( $T_{\text{soil}}$ ) at 10 cm soil depth (Einstichthermometer, Conrad, Hirschau, Germany) inside the plots/chambers. Data were recorded every 15 s alongside  $\text{CO}_2$  fluxes. This allowed closer monitoring of the microclimate within plots (for understory and open locations) and to relate the fluxes to actual conditions within the chambers during measurements.

## 2.3. Experimental design

The site was divided into two main plots the first within the tree canopy (at least 2 m from the tree trunk) and the second outside the tree canopy, in the open (at least 30 m from the closest tree). The plots measured 10 m by 10 m each and were replicated three times. At any measurement campaign, three soil frames were established on each plot and measurements conducted in rotation between understory and open locations.

## 2.4. $\text{CO}_2$ flux measurements

Portable closed chambers described by Droesler (2005), measuring 40 cm  $\times$  40 cm and 54 cm high, were used to estimate ecosystem  $\text{CO}_2$  fluxes in the herbaceous layer vegetation. The measurement procedure comprised two kinds of chambers of the same size, one transparent (from here on referred to as light chamber) to measure net herbaceous layer  $\text{CO}_2$  exchange (NEE) and the other one opaque (from here on referred to as dark chamber) to measure respiration ( $R_{\text{eco}}$ ). The light chamber was constructed from a 3 mm thick Plexiglas XT type 20070, with >95% light transmittance. Dark chamber was constructed of opaque PVC, and covered with an opaque insulation layer and with reflective aluminum foil.

To ensure closed-air circulation, collars with base area of 39.5 cm  $\times$  39.5 cm and 10 cm height, externally fitted with a 3 cm wide platform (3 cm from the top) were inserted approximately 4 cm into the soil, at least 3 days before measurements. The chambers were fitted with a 0.5 cm diameter rubber gasket at the base that rests on the platform during the course of measurements, ensuring airtight conditions. To enhance closed circulation further, elastic cords were used to press the chamber gasket onto the collar. Tests indicated that leakage does not occur (see Droesler, 2005 for details) however, this could not be examined regularly in the case of systematic field measurements.

Sudden rise in pressure within the chamber was avoided by having a 12 mm diameter vent at the top of the chambers, which was opened during the placement of the chamber and replaced soon after the chamber was secured on the collars before the onset of  $\text{CO}_2$  flux measurement. Air temperatures inside and outside the chambers were continuously monitored in order to match inside and ambient conditions during the flux measurements and varying number of dry ice packs were lined vertically on the inside wall of the chamber for controlling air temperature inside the chambers.

Fans yielding a wind speed of 1.5 m  $\text{s}^{-1}$  provided a circulation of air within the chambers. Flexible 3 m long and 0.32 cm diameter inflow and outflow tubes (Bev-A-Line, LI-COR, USA) were used for closed-air circulation system at a constant flow rate of 1 l  $\text{min}^{-1}$  and for connecting the chambers to an Infra Red Gas Analyser (LI-820, LI-COR, Nebraska, USA). A battery-driven pump maintained constant flow of air stream through the system.

Light and dark chamber measurements were conducted cyclically on hourly intervals from sunrise to sunset during measurement days, with three observations per treatment. Once a steady state was attained, data was recorded every 15 s for a period of 3 min before shifting to the next collar. 8–11 measurement cycles were accomplished on individual days and a database compiled, together with the microclimate. Measurements were conducted between March and mid-May, in order to develop a picture on  $\text{CO}_2$  exchange occurring during the spring period. Limitation in manpower to carry out the labour intensive chamber measurements prevented continuation of the observations with chambers during nighttime periods. Records of green biomass from each of the collars on any measurement day were then used to normalise the  $\text{CO}_2$  fluxes, in order to compare plots.

## 2.5. Estimation of model parameters

Empirical description of the measured NEE fluxes was accomplished via a non-linear least squares fit of the data to a hyperbolic light response model, also known as the Michaelis–Menten or rectangular hyperbola model (cf. Owen et al., 2007; Gilmanov et al., 2003; Xu and Baldocchi, 2004; Flanagan et al., 2002; Risch and Frank, 2006):

$$\text{NEE} = -\left(\frac{\alpha\beta Q}{\alpha Q + \beta}\right) + \gamma \quad (1)$$

where NEE is net herbaceous layer  $\text{CO}_2$  exchange ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),  $\alpha$  is the initial slope of the light response curve and an approximation of the canopy light utilization efficiency ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ),  $\beta$  is the maximum  $\text{CO}_2$  uptake rate of canopy ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ),  $Q$  is the photosynthetic photon flux density, PPFD ( $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ) measured inside of the gas exchange chamber,  $\gamma$  is an estimate of the average respiration of the herbaceous layer ( $R_{\text{eco}}$ ,  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) occurring during the observation period,  $\alpha/\beta$  is the radiation required for half maximal uptake rate, and  $\beta + \gamma$  is the theoretical maximum uptake capacity. The rectangular hyperbola may sometimes saturate very slowly in terms of light and hence  $\alpha\beta Q/\alpha Q + \beta$  was evaluated at a reasonable level of high light ( $Q = 2000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  used in this study), this is sometimes referred to as GPP approximation and is also regarded as average maximum canopy uptake capacity, described as  $(\beta\gamma)_{2000}$ . Using Sigma Plot 8.0, parameters  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $(\beta + \gamma)_{2000}$  were estimated for both open and understory vegetation using NEE data for each measurement day.

## 2.6. Above- and belowground biomass measurements

At the end of each day's gas exchange measurements, above-ground biomass within every collar was harvested and separated into green biomass and dead biomass. The green biomass was separated into grasses, legumes and forbs before oven drying at 80 °C for 48 h and determining their respective dry weights. Root biomass was sampled from the centre of the collars using a 3 cm diameter core sampler. Due to its labour demands, root sampling was conducted at a lower frequency (larger time intervals) compared to aboveground biomass. The sampled soil cores were separated into three different layers (0–7, 7–12 and 12–20 cm).

Roots from each of the layers were carefully removed from the soil and washed under running tap water using micro-pore soil sieves. The sieved samples were then oven-dried at 80 °C for 48 h before determining dry weights.

### 2.7. Plant and soil nutrient determination

Plant samples (shoots and roots) were oven-dried at 80 °C and re-dried in a desiccator. A known fraction of the dried samples (4–5 g) was then analysed to determine foliar N concentrations (%) by means of elementary analysis (Markert, 1996). N uptake was determined from the above- and belowground biomass. The root samples of different layers were pooled for nitrogen determination. Similar procedure was followed to determine the total nitrogen content in soil. Plant N was expressed either as a percentage of the biomass or per ground area.

### 2.8. Soil water content

From the same plots of CO<sub>2</sub> measurements, a second soil sampling was done with a 3 cm diameter core sampler and the soil cores divided into three layers from 0 to 5, 5 to 10 and 10 to 15 cm. Each sample (layer) was immediately weighed to determine its fresh weight before oven drying at 105 °C for 48 h and dry weight determined. Gravimetric soil moisture content was determined as the relative change in weight between fresh and dry soil samples.

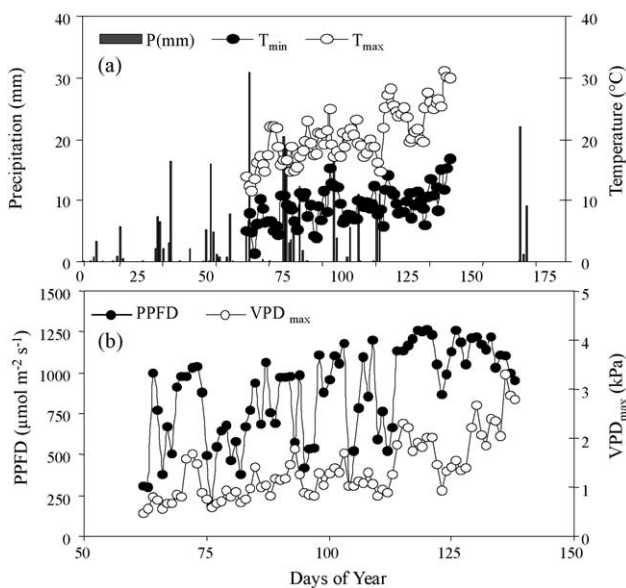
### 2.9. Data analysis

Significant differences in responses between the locations were tested using paired *t*-test analysis with the SPSS statistical software. Best fits for light and temperature response curves for open and understory locations were performed using Sigma Plot version 8.0.

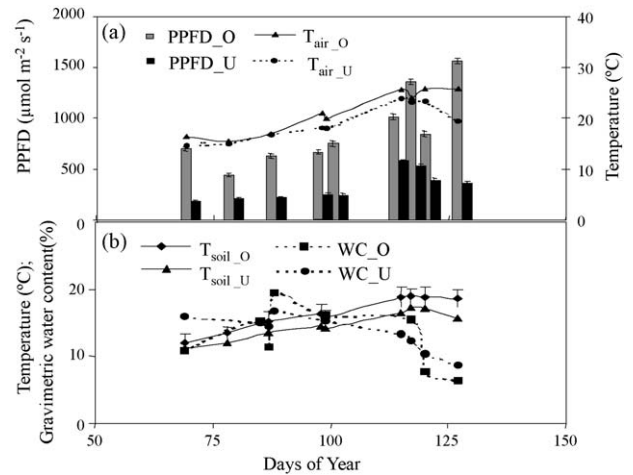
## 3. Results

### 3.1. Climate

Long-term rainfall and temperature trends at the Mitra study site are shown in Fig. 1, while the prevailing weather conditions



**Fig. 2.** (a) Daily amounts of precipitation during the early part of the year, minimum and maximum temperatures and (b) light intensity and VPD records above the canopy at the study site in Mitra during spring when field measurements were carried out.



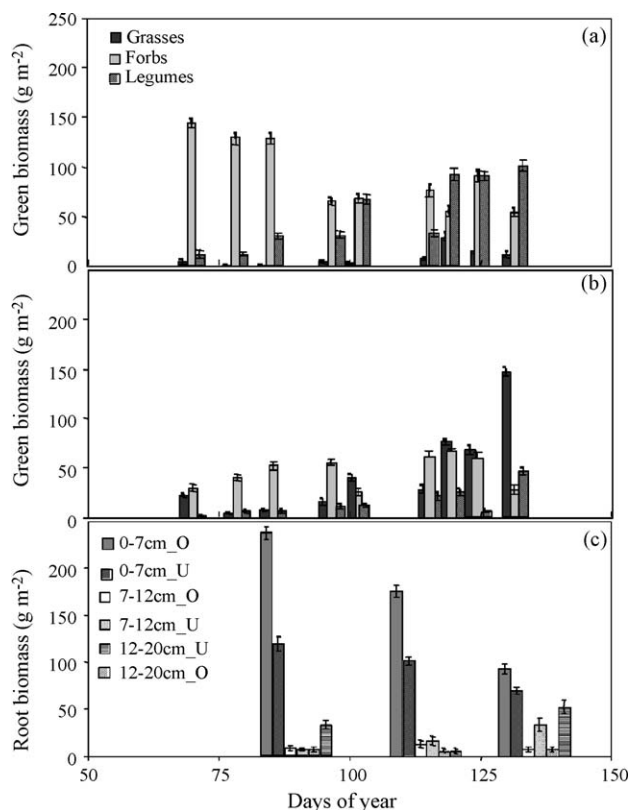
**Fig. 3.** (a) Comparison of daily averages of photosynthetic photon flux densities (PPFD), mean daily air temperatures ( $T_{air}$ ). (b) Soil temperature at  $-10$  cm depth ( $T_{soil}$ ) and soil moisture content (WC) within 0–15 cm depth in the open (O) and understory (U) locations during chamber measurements. Bars are standard errors.

during the experimental period are shown in Fig. 2. Based on precipitation amounts and distribution in 2006, the study year was considered a normal hydrological year for this Mediterranean region (Pereira et al., 2007). Precipitation declined while radiation, air temperature and VPD increased during spring. Fig. 3 shows the prevailing microclimatic and soil moisture conditions in the open and understory locations during the study period. Higher light intensities and temperatures occurred in the open locations compared to the understory (Fig. 3a). On a daily basis, the open locations experienced longer periods with high light intensities, averaging  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , while average light intensity in the understory was  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Due to limited instrumentation, VPD under the tree canopy was not monitored. Previous studies in the same site however, show that VPD is about 3–5 hPa lower under the tree canopy (Otieno et al., 2006). Soil moisture content rapidly dropped at the end of the rainy season (Fig. 3b), a time when VPD and radiation were increasing. Although soil moisture dropped both in the open and understory locations, the relative drop was higher in the open compared to understory, reflecting the microclimatic differences between them.

### 3.2. Species composition and biomass development

Table 1 shows the composition of plant species of the herbaceous layer recorded during the early spring and categorised into three functional types of forbs, legumes and grass. Forbs were dominating during early spring and were major contributors (<50%) to the total biomass, both in the open and understory (Fig. 4a and b). *Geranium molle* constituted 20–35% of the total vegetation cover in both locations. Later in spring, legumes showed a strong increase in biomass in the open locations, while grass biomass increased in the understory. The annual green biomass accumulated were  $405.8 \pm 9.0$  and  $250.6 \pm 6.3 \text{ g m}^{-2}$  in the open and understory, respectively. No further increase in biomass was observed after April.

Root biomass declined between March and May (Fig. 4c). Highest root biomass recorded were  $236.7 \pm 7.6$  and  $118.2 \pm 7.8 \text{ g m}^{-2}$  for open and understory, respectively and occurred in early April. These results include both dead and live roots and it is likely that some of the root biomass considered here originated from the previous year. More than 80% of the roots were concentrated in the top 0–12 cm soil layer and the pattern of root distribution was similar in both locations.



**Fig. 4.** Contribution of different plant functional types to the total green biomass development ( $\text{g m}^{-2}$ ) in the (a) open, (b) understory and (c) root biomass ( $\text{g m}^{-2}$ ) in different soil layers in the open (O) and understory (U) vegetation. Bars are standard errors of the mean.

### 3.3. Soil and plant nitrogen content

Percentage (%) and total N contents in the aboveground biomass are shown in Table 2a. N increased by about 43% and 19% between March and April in the aboveground biomass in the open and understory vegetation, respectively. We did not observe any differences in N content in the aboveground green biomass between

**Table 2**

(a) Percentage (%) nitrogen and total nitrogen content ( $\text{g m}^{-2}$ ) in the aboveground green biomass from the open and understory vegetation during the active growing period in spring. (b) Nitrogen distribution in the roots of open and understory vegetation.

DOY	% N in aboveground green biomass		N ( $\text{g m}^{-2}$ ) in aboveground green biomass	
	Open	Understory	Open	Understory
68	1.77 ± 0.54	2.08 ± 0.43	1.67 ± 0.81	1.30 ± 0.78
70	1.76 ± 0.38	2.33 ± 0.34	2.80 ± 0.39	1.29 ± 0.60
99	2.03 ± 0.41	2.47 ± 0.56	4.76 ± 1.02	2.11 ± 0.40
100	2.13 ± 0.66	2.26 ± 0.26	4.57 ± 0.94	1.83 ± 0.86
114	2.10 ± 0.40	2.14 ± 0.29	4.85 ± 1.64	3.79 ± 1.0
135	1.63 ± 0.73	1.57 ± 0.56	5.0 ± 1.22	4.01 ± 0.92

DOY	% N in root biomass		N ( $\text{g m}^{-2}$ ) in root biomass	
	Open	Understory	Open	Understory
95	1.02 ± 0.52	1.00 ± 0.29	0.52 ± 0.74	0.40 ± 0.38
114	0.98 ± 0.60	1.12 ± 0.51	0.46 ± 0.42	0.28 ± 0.49
135	0.93 ± 0.41	0.9 ± 0.30	0.26 ± 0.20	0.36 ± 0.37

±Standard error.

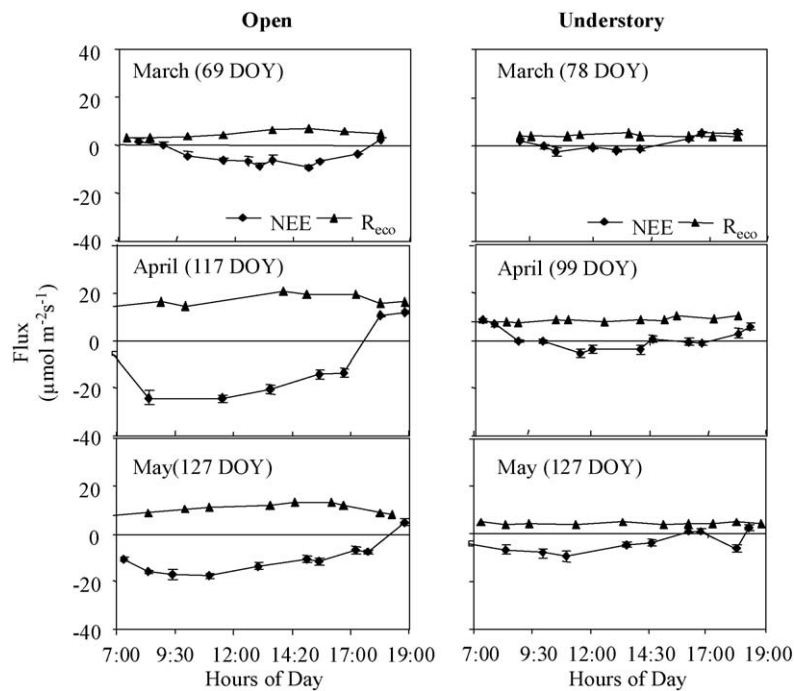
the two locations during the active growth period. Root N content remained relatively constant in both locations with no significant differences between them (Table 2b). Soil N content was analysed in May and no differences were observed between the open ( $0.06 \pm 0.15 \mu\text{mol g dry wt}^{-1}$ ) and understory ( $0.09 \pm 0.2 \mu\text{mol g dry wt}^{-1}$ ).

### 3.4. Ecosystem $\text{CO}_2$ exchange

Fig. 5 shows diurnal courses of NEE and  $R_{\text{eco}}$  during representative days, while prevailing weather conditions for the corresponding days of gas exchange measurements are shown in Fig. 6. On clear sky days, net ecosystem  $\text{CO}_2$  uptake increased from sunrise and reached maximum around midday. Similar patterns of NEE were observed for both open and understory, but with different peak values and higher NEE at any particular time during spring were recorded in the open locations.  $R_{\text{eco}}$  remained relatively constant throughout the day but was higher in the open. Maximum NEE during March (low light and temperatures and high soil moisture conditions) were  $-9.7 \pm 1.1$  and  $-2.7 \pm 1.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ , while concurrent maximum  $R_{\text{eco}}$  were  $6.7 \pm 1.2$  and  $4.8 \pm 1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  in open and understory, respectively. Respective maximum NEE rates in April (high light, temperatures and soil moisture) averaged between  $-24.4 \pm 1.5$  and  $-5.5 \pm 1.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ . This was associated with mean maximum light intensities of 1800 and  $600 \mu\text{mol m}^{-2} \text{s}^{-1}$  in open and understory locations, respectively. During this period, the respective soil temperatures ranged between 16.9 and  $20.2^\circ\text{C}$  and 13.5 and  $15.0^\circ\text{C}$ . In March, the maximum flux rates were attained in the morning, at around 11:00 h and remained stable till 16:00 h. Peak flux rates in April however, occurred earlier (9:00 h) and declined soon after midday. This was the time, when VPD and air temperatures were rapidly rising. Corresponding maximum  $R_{\text{eco}}$  during April were  $20.6 \pm 2.2$  and  $10.0 \pm 1.6 \mu\text{mol m}^{-2} \text{s}^{-1}$  for open and understory, respectively. During May (high light and temperatures and low soil moisture), NEE rose to a maximum early in the day but started to drop soon after 11:00 h. Maximum NEE recorded varied between  $-10.7 \pm 1.2$  and  $-15.7 \pm 1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $R_{\text{eco}}$  ranged  $17.4 \pm 1.2 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the open locations while maximum NEE and  $R_{\text{eco}}$  were  $-9.6 \pm 2.2$  and  $5.2 \pm 1.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively in the understory. Light intensity during May remained above  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$  while air temperature was above  $25^\circ\text{C}$  for most part of the day. May was also characterised by low soil moisture and high VPD. A summary of the maximum NEE and  $R_{\text{eco}}$  for the representative days during the entire active growing period in spring are presented in Fig. 7, demonstrating the response patterns of the open and the understory vegetation.

### 3.5. Abiotic influence on ecosystem $\text{CO}_2$ exchange

Fig. 8 shows NEE responses to changing light conditions in the open and understory locations during early spring when soil moisture was favourable and available to the vegetation. Similar correlation was performed with data from May period (not shown) when soil moisture was limiting. Cloudy days are considered for this evaluation in order to minimise the influence of variable air temperatures on assimilation, since relatively uniform and similar air temperatures existed both in the open and understory under such conditions. A strong correlation ( $R^2 > 0.65$ ) was observed between PPFD and NEE for both locations when soil moisture was high. Correlation was however, weaker ( $R^2 \sim 0.5$ ) in dry soils. Model derived physiological parameters (Table 3) showed no significant differences in light use efficiency ( $\alpha$ ) between the open and understory vegetation. Light use efficiency increased between DOY 69 and DOY 99 depicting increases in NEE and biomass. Maximum canopy carboxylation rates ( $\beta$ ) and average ecosystem



**Fig. 5.** Net ecosystem exchange (NEE) and ecosystem respiration ( $R_{eco}$ ) during the day in the open (left panel) and understory (right panel) vegetation at different times during the active growing period in spring. The time periods indicated are those with different microclimate and soil moisture conditions. Negative NEE indicate carbon uptake by the ecosystem. Bars are standard errors.

respiration ( $\gamma$ ) were higher for the open locations compared to the understory. The results also showed higher maximum  $CO_2$  uptake capacity ( $\beta + \gamma$ )<sub>2000</sub> in open locations compared to the understory.

The influence of soil temperature at 10 cm soil depth on the herbaceous layer respiration was examined with a two-parameter exponential fit described by Rogiers et al. (2005) and Schmid et al. (2000) shown in Eq. (2):

$$R_{eco} = a \exp(bT_{soil}) \quad (2)$$

where  $a$  and  $b$  are the fitting parameters. Single data sets from each of the chambers are presented in Fig. 9 (left panel). There was positive correlation ( $R^2 = 0.58$  and  $0.73$  for open and understory, respectively) between  $R_{eco}$  and soil temperature at temperature ranges below  $15^\circ C$ . Soil temperatures ( $T_{soil}$ ) above  $15^\circ C$  coincided with the period when soil moisture was low, leading to a shift in the relationship between  $R_{eco}$  and  $T_{soil}$  at higher temperatures (data not shown).

Responses of  $R_{eco}$  to changes in soil moisture conditions, both in the open and understory are shown in Fig. 9 (right panel). Peak  $R_{eco}$  occurred at soil moisture content of around 12.0–14.0% in both the open and understory, with a substantial decrease in  $R_{eco}$  on both sides of the optimum soil moisture level. A strong relationship was established between soil moisture and ecosystem respiration, however both soil temperature and soil moisture changed simultaneously and it was difficult to discern the interactive effect of soil temperature and soil moisture on ecosystem respiration in the constantly changing natural conditions.

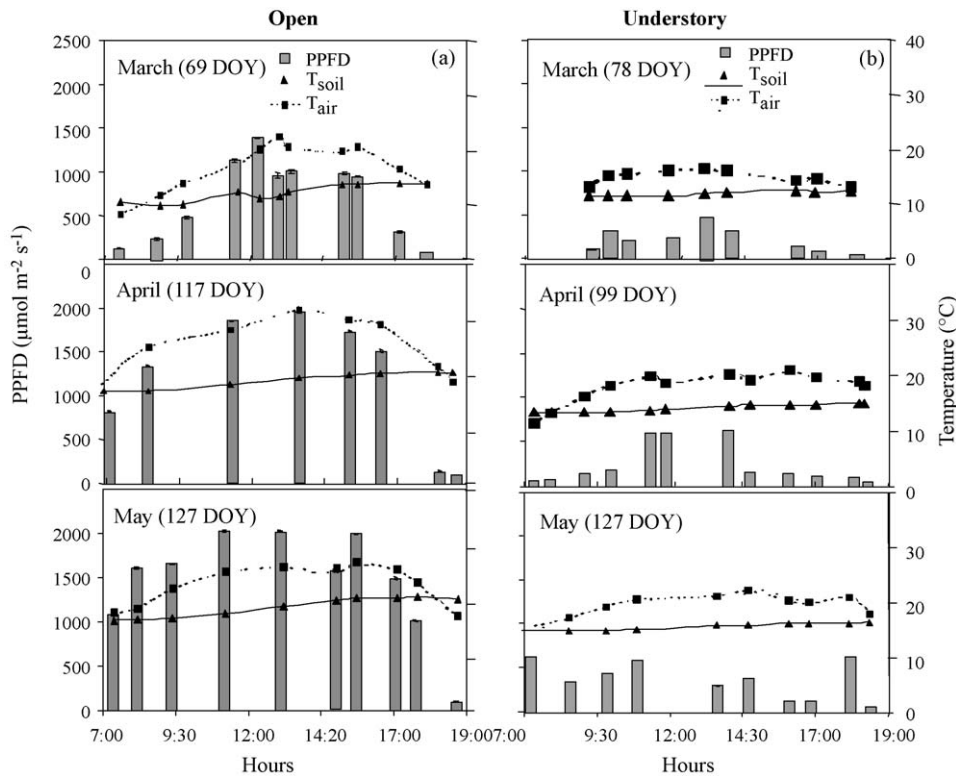
#### 4. Discussion

The period between March and April was characterised by significant increases in green biomass both in the open and understory. Biomass development is determined by the availability of soil moisture, soil nutrients and the prevailing microclimatic conditions (Xu and Baldocchi, 2004; Ma et al., 2007).

Increase in biomass of the herbaceous layer during this period was attributed to high soil moisture and suitable microclimate. Low soil moisture, plant maturity and senescence may account for the lack of growth observed towards the end of our experimentation (Tenhunen et al., 1990; Aires et al., 2008; Pereira et al., 2007).

Trees had a negative effect on the herbaceous layer production, with significantly ( $p < 0.05$ ) lower biomass production in the understory compared to the open locations. Our results contradict those reported for similar ecosystems in Spain (Moreno, 2008) and savanna (Scholes and Archer, 1997; Cech et al., 2008), but are in agreement with those reported for Mediterranean ecosystems (Silva-Pando et al., 2002; Moreno et al., 2007a) and other grazing ecosystems in NW Spain (González-Hernández et al., 1998). Differences in biomass production in the open and shade locations in the Mediterranean grasslands have been attributed to differences in species composition (Caldeira et al., 2005). In our case, both the open and the understory shared similar plant functional types during most part of the active production (vegetative) stage, and were expected to function similarly (Diaz Barradas et al., 1999). Thus species composition was not the reason for differences in production in the two locations at our study site. Higher nutrient-N content was the reason for higher biomass production in the understory compared to the open locations in the Spanish Mediterranean ecosystem (Moreno et al., 2007b; Moreno, 2008). Increased foliar N content leads to higher photosynthetic rates and high production (Hodge, 2004; Ries and Shugart, 2008). We did not observe any significant differences ( $p > 0.05$ ) in leaf N content between the open and understory during the period of our study and similar N concentrations were found in soils at both locations, indicating that differences in N availability could not explain the observed differences.

Microclimatic conditions, including light, temperature and soil moisture however differed between the open and understory locations. The understory was about 3–4 °C cooler, with higher soil moisture in May compared to the open locations while light intensities were more than twice as high in the open compared to



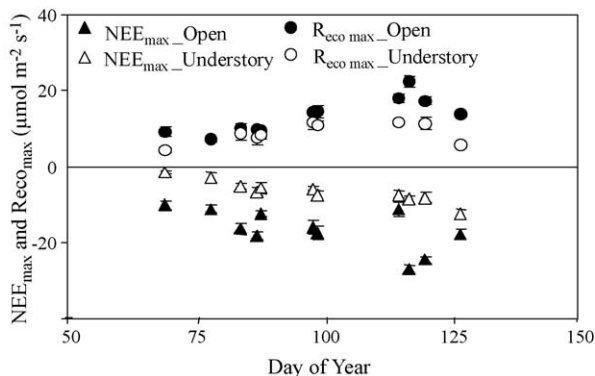
**Fig. 6.** Prevailing microclimatic conditions (PPFD,  $T_{air}$  and  $T_{soil}$ ) in the (left panel) open and (right panel) understory locations during the corresponding times when gas exchange measurements (Fig. 5) were carried out.

the understory. Biomass production is the result of long-term fixation of carbon through photosynthesis (Ma et al., 2007). Variations in light intensity explained more than 70% of the NEE fluctuations and also accounted for differences in NEE observed between the open and understory when soil moisture content was high. Past studies in the Mediterranean (Xu and Baldocchi, 2004; Aires et al., 2008) and in temperate (Metting et al., 2001; Rogiers et al., 2005) grassland ecosystems have emphasised the significant role of light in determining NEE during the day. Model derived physiological parameters showed higher maximum CO<sub>2</sub> uptake rates ( $\beta$ ) in the open compared to the understory vegetation. Normalised CO<sub>2</sub> fluxes also showed higher NEE fluxes per unit biomass in the open locations. Similar light use efficiencies ( $\alpha = 0.04$ ) were however, observed between the open and understory vegetation under conditions of ample soil water availability, suggesting that both the open and understory

vegetation were physiologically similar (Ehleringer and Björkman, 1977). Thus differences in NEE and biomass production between the open and understory vegetation were likely the result of differences in incident light levels, leading to higher assimilation rates and rapid growth (higher biomass) in the open locations. Under conditions of ample soil water availability, therefore, light intensity and green biomass interact in a feed-forward process that determines the overall herbaceous layer production (Ma et al., 2007; Flanagan et al., 2002; Xu and Baldocchi, 2004; Li et al., 2005).

The situation however, changed in late spring. Compared to early spring (March), significantly low NEE and lack of increase in biomass were recorded both in the open and shade locations during this time (May). We attributed the low/lack of biomass increase to low assimilation rates, probably due to low soil moisture availability (Aires et al., 2008; Pereira et al., 2007), increase in temperatures and VPD and ageing plant population (Aires et al., 2008). Compared to daily NEE, correlation between NEE and PPFD during May were weaker ( $R^2 = 0.47$  and  $0.50$  for open and understory, respectively), suggesting that factors other than light were involved. This was the period when soil moisture was low and temperatures and VPD were high. Soil moisture has an overriding effect on carbon assimilation in the Mediterranean region (Tenhunen et al., 1990; García-Barrios and Ong, 2004; Pereira et al., 2007; Aires et al., 2008; Moreno, 2008) and low soil moisture leads to a decline in NEE. We could not discern the direct effect of soil moisture on NEE and plant growth, since many factors changed simultaneously when soil moisture was declining.

Ecosystem respiration changed considerably during spring and highest  $R_{eco}$  occurred in April, but subsequently declined in May. Previous studies have shown a strong dependence of respiration on both temperature and soil moisture (Davidson et al., 1998; Ma et al., 2007). Due to large variations in soil moisture at our study



**Fig. 7.** Seasonal trends of peak NEE ( $NEE_{max}$ ) and peak  $R_{eco}$  ( $R_{eco,max}$ ) in the open and understory vegetation during the spring period. Bars are standard error.

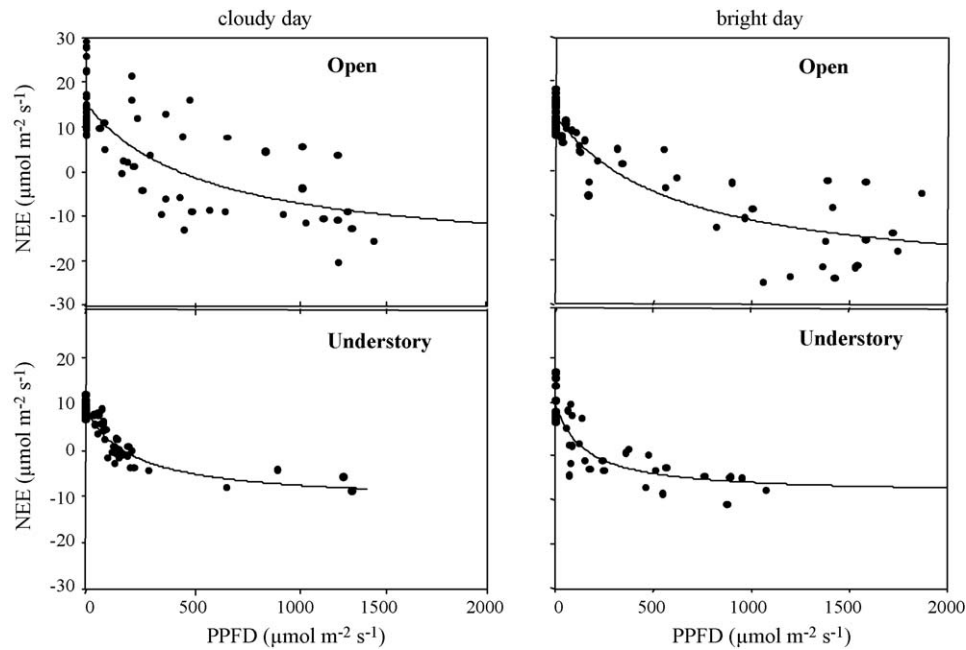


Fig. 8. Light response curves derived from rectangular hyperbola fitting to the NEE–PPFD data for open and understory vegetation under bright and cloudy day conditions.

site, we analysed separately, the temperature dependence of  $R_{\text{eco}}$  only for the period when soil moisture was favourable and also for the entire range of soil moisture during the study period. It became apparent that as long as soil moisture was high,  $R_{\text{eco}}$  increased with increasing soil temperature at 10 cm soil depth, both in the open and the understory and that the responses were similar in both locations. Our findings agree with previous results reported for the Mediterranean ecosystem (Aires et al., 2008; Pereira et al., 2007) and elsewhere (Dilustro et al., 2002; Flanagan et al., 2002; Xu and Baldocchi, 2004; Falk et al., 2005).

Our study shows that the open and understory vegetation of the montado herbaceous layer are physiologically similar, with respect to  $\text{CO}_2$  exchange, and differences in assimilation and biomass production that were observed between them are the result of tree crowns that alter the light environment in the understory. Thus under conditions of ample soil moisture availability, tree density could be a major factor determining the herbaceous layer production in the montado (1996). Cubera and Moreno (2007) arrived at the same conclusions for similar ecosystem (dehasa) in Spain.

**Table 3**  
Model derived plant physiological parameters obtained from the light response curves (NEE vs. PPFD) for single day NEE measurements in the (a) open and (b) understory locations during active growing period in spring.

a									
DOY	$\alpha^a$	$\beta^b$	$\gamma^b$	$(\beta + \gamma)_{2000}^b$	$SE_{\alpha}$	$SE_{\beta}$	$SE_{\gamma}$	$R^2$	$p$
69	-0.03	-19.5	4.7	-14.7	0.01	2.0	0.33	0.92	<0.0001
78	-0.03	-24.7	5.6	-19.8	0.01	2.2	0.30	0.95	<0.0001
87	-0.04	-31.8	7.5	-25.9	0.01	2.0	0.35	0.96	<0.0001
98	-0.06	-34.3	11.0	-26.7	0.01	3.1	0.50	0.93	<0.0001
99	-0.06	-29.5	15.1	-23.7	0.03	5.4	1.34	0.60	<0.0001
115	-0.07	-75.2	11.4	-15.8	0.01	69.0	0.76	0.76	<0.0001
117	-0.06	-53.8	16.5	-37.1	0.01	11.0	0.94	0.90	<0.0001
120	-0.08	-35.2	12.5	-28.9	0.02	3.7	1.03	0.84	<0.0001
127	-0.05	-32.8	10.4	-24.7	0.01	4.9	0.52	0.93	<0.0001
b									
DOY	$\alpha^a$	$\beta^b$	$\gamma^b$	$(\beta + \gamma)_{2000}^b$	$SE_{\alpha}$	$SE_{\beta}$	$SE_{\gamma}$	$R^2$	$p$
69	-0.02	-9.2	3.6	-7.5	0.03	2.5	0.19	0.79	<0.0001
78	-0.02	-11.1	4.3	-8.7	0.01	3.7	0.24	0.69	<0.0001
87	-0.03	-15.8	5.8	-14.2	0.01	1.5	0.30	0.87	<0.0001
98	-0.06	-20.2	8.4	-17.3	0.01	3.4	0.41	0.79	<0.0001
99	-0.08	-19.9	8.7	-17.7	0.01	1.7	0.34	0.86	<0.0001
115	-0.05	-	9.0	-	0.01	-	0.43	0.83	<0.0001
117	-0.02	-26.2	6.6	-15.8	0.01	9.0	0.52	0.78	<0.0001
120	-0.02	-18.6	8.2	-16.9	0.02	2.5	0.67	0.75	<0.0001
127	-0.03	-22.0	4.6	-18.0	0.01	3.2	0.33	0.89	<0.0001

<sup>a</sup> ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )/( $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ ).

<sup>b</sup> ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ).



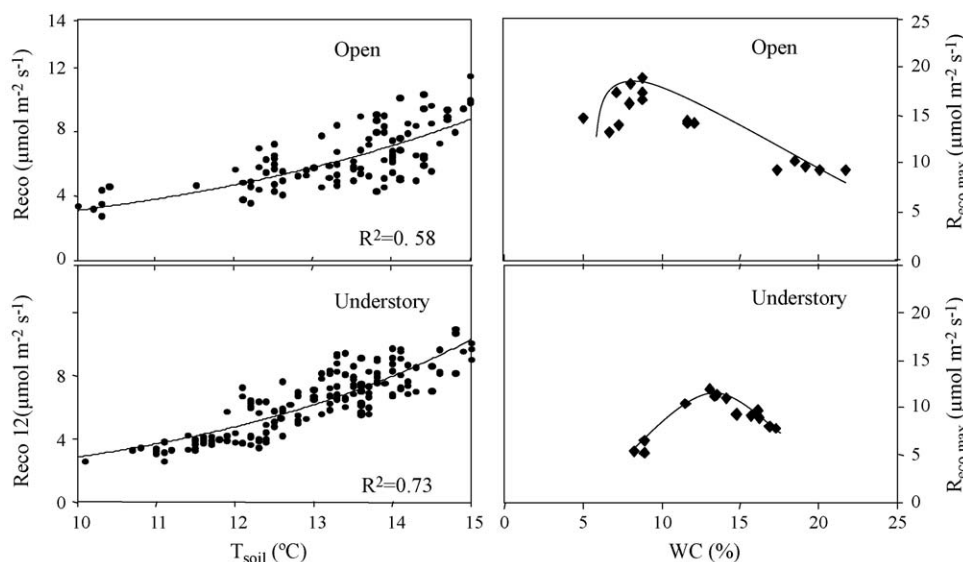


Fig. 9. Influence of soil temperature ( $T_{soil}$ ) (left panel) and soil moisture (right panel) on ecosystem respiration ( $R_{eco}$ ) in the open and understory locations.

## 5. Conclusions

1. The montado herbaceous vegetation demonstrated similar physiological responses, with similar light use efficiencies ( $\alpha$ ) both in the understory and in the open locations. Tree canopy however, reduced the overall capacity of the herbaceous layer component to assimilate carbon by lowering radiation reaching the understory and leading to lower maximum assimilation ( $\beta$ ). Plant production is a linear function of light, depending upon leaf area index (biomass) and canopy nutrition. Thus high light intensities recorded in open location lead to higher NEE and biomass accumulation compared to the understory when soil moisture was high.
2. Ecosystem respiration of the understory during the period when soil moisture was high was driven by soil temperature, with similar responses in both locations. Influence of temperature on respiration, however, became less dominant as the soil dried out. Both the decline in soil moisture after the rain events and increase in soil temperature with the advancing spring period however, were delayed under the tree canopies compared to the open locations.
3. Overall, biomass production and  $CO_2$  exchange of the montado herbaceous layer therefore, are determined by tree density, which influences light distribution, soil temperature and soil moisture conditions.

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