DROUGHT STRESS AFFECTS TEMPERATURE RESPONSE OF LEAF DARK RESPIRATION IN MEDITERRANEAN COASTAL MAQUIS SPECIES.

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ABSTRACT – Leaf dark respiration (R_D) plays a key role in plant carbon balance and the knowledge about how environmental stresses affect the response of R_D to temperature under field conditions, might improve the reliability of carbon budget models. This study analyzed the influence of air temperature and drought on the short-term temperature response of R_D of three woody evergreen Mediterranean species. The field activities were carried out in two different coastal sites of Italy and for each site, two different areas located near the shoreline and inland were considered. The respiration–temperature response curves were carried out seasonally, together with leaf water potential and sclerophyll degree measurements. The effect of the average air temperature of the previous two days (T_{2m}) and predawn water potentials on basal respiration measured at 18°C (R_{18}) and temperature sensitivity (Q_{10}), were tested with linear regression analysis. Both R_{18} and Q_{10} were weakly correlated with T_{2m}, while a multifactor model including predawn water potentials yielded a better relation. These findings suggest that, besides air temperature, water availability and in general, plant energy balance, must be taken into account to predict acclimation of leaf dark respiration.

KEYWORDS: ACCLIMATION, EVERGREEN SPECIES, Q_{10}, LEAF RESPIRATION, MULTI-STRESS EXPOSURE.

INTRODUCTION

Drought stress is an important driver of plant carbon balance in Mediterranean ecosystems, affecting both assimilation and respiration (Chaves et al., 2009). Leaf dark respiration (R_D) plays a key role in the net ecosystem exchange of carbon, as well as on plant metabolism, maintenance, and transport of molecules such as ions and nutrients to the growing sinks (Kruse et al., 2011). Previous studies have shown that environmental stresses (i.e abiotic) can significantly affect R_D, although the response is largely variable, depending on plant species, growth stage, stress intensity and duration (Flexas et al., 2006; Galmes et al., 2007a; Loreto & Centritto, 2008; Atkin & Macherel, 2009 Ayub et al., 2011; Fusaro et al., 2015). In particular, under water stress, R_D was generally observed to decrease (Atkin & Macherel, 2009), with herbaceous species

showing a high decrease and faster recovery after drought relative to evergreen trees, reflecting a different metabolic requirement due to different life-span and growth carbon costs (Galmes et al., 2007b). However, in drought tolerant tree species, a complex behaviour was observed, as R_D increased under mild water stress, while it decreased under severe water stress (Gulías et al., 2002; Sanhueza et al., 2013). To date further studies are necessary to have a comprehensive understanding of how drought, and in general interaction among environmental variables, affect leaf dark respiration rate and its acclimation to air temperature (T_{air}) (Griffin & Prager, 2017). During short-term rises in T_{air} (minutes to hours) an exponential increase of R_D is observed (Atkin et al., 2005). However, the responsiveness of R_D to seasonal

temperature rise is reduced because of a thermal acclimation mechanism (Atkin et al., 2005) leading to the adjustment of R_D to compensate for a change in temperature (Lambers et al., 1998). Thermal acclimation of R_D is associated with: i) changes in temperature sensitivity coefficient, Q_{10} (the proportional change in respiration with a 10 °C temperature change) (Type I acclimation, Atkin & Tjoelker, 2003), and ii) changes in the basal respiration rates at standard temperature (R_{Tref}) (i.e. the horizontal asymptote of the short-term temperature response function of R_D, Type II acclimation, Atkin & Tjoelker, 2003). Q_{10} is believed to reflect changes in maximum enzyme activity, while it has been proposed that changes of R_{Tref} are influenced by metabolic or anatomic changes (e.g. amounts of respiratory enzymes, changes in the size and numbers of epidermal and mesophyll cells; Tjoelker et al., 2009). Thus, the seasonal adjustments in R_D depend on changes in Q_{10} and R_{Tref} (Tjoelker et al., 2001), that can vary not only because of seasonal variation of air temperature, but also in response to a complex set of environmental stressors that co-vary with temperature (Smith & Dukes, 2013). In Mediterranean ecosystems, only a few studies have dealt with the seasonal variation of Q_{10} and R_{Tref} (Zaragoza-Castells et al., 2008, Rodríguez-Calcerrada et al., 2011a), even if understanding their seasonal trend is crucial to correctly predict respiration at the daily average temperature, R_{amb}. Indeed Zaragoza-Castells et al., (2008) highlighted the great importance that thermal acclimation has on the maintenance of positive carbon balances in low Net Primary Production (NPP) forest ecosystems, such as those present in coastal Mediterranean regions. Despite their low contribution to the global vegetated surface, coastal ecosystems play a pivotal role in regulating the physical and biological interactions between marine and inland terrestrial ecosystems (Brenner et al., 2010). Coastal ecosystems may represent a good model to study how drought could influence R_D response to temperature since the water shortage is usually present owing to poor water retention capacity of sandy soil (Rawls et al., 1991) or to soil salinity level (Munns and Tester, 2008; Fusaro et al., 2014). Moreover, multiple stress agents as wind, marine spray, soil salinity, and low nutrient availability (Fares et al., 2009) shape the vegetation, often resulting into a sparse canopy with low radiative energy balance, and low penetration of air in the canopy (Spano et al., 2000).

This field study aimed at analyzing the temporal variations of R_D temperature-response curves in three evergreen woody species *Arbutus unedo* L., *Quercus ilex* L., and *Phyllirea latifolia* L. Two sites characterized by different thermal history were compared to investigate if acclimation process was influenced by changes in basal respiration rate measured at 18°C (R_{Tref} at 18°, R_{18}) or Q_{10}, and whether drought conditions alter these parameters. The tested hypothesis is that, because of drought, the temporal pattern of R_{18} and Q_{10} is not consistent with an acclimation response associated with changes in prevailing ambient air temperatures.

MATERIALS AND METHODS

Study sites

The experimental activity was conducted on three woody evergreen Mediterranean species: *A. unedo* (Au), *Q. ilex* (Qi) and *P. latifolia* (Pl), sampled on two coastal areas of Italy: the Presidential Estate of Castelporziano (CP), in the Latium region near the Metropolitan area of Rome (41° 41'54.56'' N, 12° 21'0 9.50'' E), and Piscinas (PSN) on the South West coast of the Sardinian Island (39° 34'9.37'' N, 8° 27'41.48'' E). In each site, an area was selected near the shoreline (hereafter referred to as Shl), and a second area was selected in the inland (hereafter referred to as Inl). In CP site, *A. unedo* was present only in the Shl area, and in PSN *Q. ilex* was present only in the Inl area. In both sites, all sampled individuals were not dominated by neighbours and were characterized by multiple stems and of comparable size within the same species. The sites characteristics, and the distances between Shl and Inl areas in the two sites are summarized below, and in Table 1.

Castelporziano

Castelporziano (CP) belongs to a Thermo-Mediterranean/ Meso-Mediterranean sub-region, and ombrotypes between the upper dry and the lower sub-humid region, with low precipitationsduring the summer period, and cold winters (Blasi 1993). In the shoreline area (Shl), the water table is shallow in winter, and remains a source of freshwater even in summertime, when – however – it drops to a ca. 2 m depth (Mereu et al., 2009). This area is subjected to soil salinity, because of seawater intrusion in the water table (Banzato et al., 2013). The vegetation is composed by patches of "garigue", with an average height of 0.96 m. In the CP inland area (Inl), the water table is deeper than Shl and its level is subjected to strong seasonal fluctuations (Mereu et al., 2009). In the Inl area the vegetation is composed of a high maquis, with an average height of 2.9 m, with a dense and uniform cover (Table 1). Daily meteorological data in CP were collected from a monitoring station located 3 Km south of the experimental areas.

Piscinas

Piscinas (PSN) belongs to a Mediterranean pluviseasonal bio-climate (Rivas-Martínez', 2002), with thermotypes ranging from the upper thermo- to the lower meso-mediterranean, and ombrotypes from the upper dry to the lower sub-humid region (Bacchetta et al., 2007). In PSN, severe drought periods are frequent during summer months (Table 1) and vegetation is exposed to strong prevailing sea

Table 1. Summary of the sites characteristics. Shoreline and Inland represent nearest and farthest areas from the shore respectively. Mean annual temperature (MAT), annual rainfall and drought length are reported based on 30-year climatic series (from 1982 to 2012). The distance from the shore and the altitude above sea level (ASL) are reported. Measured species are marked in bold.

Site	Castelporziano		Piscinas	
Sampling Month	2010 April, May, June, July, August 2011 February, April, July		2010 April, August 2011 February, April, July	
Annual rainfall (mm)	740		460	
MAT (°C)	15.9		18.5	
Drought length (months)	2		3	
	Shoreline	**Inland**	**Shoreline**	**Inland**
Distance from shore (m)	250	450	75	1200
AMSL (m)	15	3	6	60
Soil type	Sandy	Sandy	Sandy	Sandy
Bedrock	Ancient coastal dune	Ancient coastal dune	Scists	Scists
Mean canopy height (cm)	96	290	60	150
Canopy Cover (%)	90%	100%	65%	98%
Main woody species	*A. unedo* *Q. ilex* *P. latifolia* C. salvifolius E. Arborea J. oxycedrus J. phoeniceae R. officinalis H. litoreum	*Q. ilex* *P. latifolia* E. arborea J. phoeniceae P. lentiscus	*A. unedo* *P. latifolia* C. monspeliensis J. phoeniceae P. lentiscus H. litoreum C. spinosa	*A. unedo* *Q. ilex* *P. latifolia* C. salvifolius J. oxycedrus J. phoeniceae M. comunis P. angustifolia P. lentiscus Q. suber R. officinalis C. spinosa

wind causing mechanical stresses and sea spray deposition. Vegetation structure and community composition are similar to those observed in CP: the Shl area is composed by a garigue, where low and prostrate shrubs, strongly shaped by the wind (plants height of 1.5 m), are prevailing, whereas the Inl area is composed by a high maquis (plants height of 2.5 m). Daily meteorological data in PSN were collected from the Capo Frasca meteorological station (39° 40'42.85'' N, 8° 26'49.48'' E) located 22 Km north of the experimental areas.

Field measurement campaigns

The experimental campaigns were carried out in 2010 (April and August) and 2011 (February, April and July). In May, June and July 2010, further measurements were carried out only in the CP site (Table 1).

Morphological and physiological traits
Leaf Mass Area

On June 2010, twenty fully expanded leaves of each species (*A. unedo*, *Q. ilex*, *P. latifolia*) and experimental areas in each

site, were collected to determine the Leaf Mass Area (LMA, g/cm^2). Leaf area was measured immediately after excising the petiole with a scanner and the images were elaborated with Image Lab free software (http://en.freedownloadmanager. org/Windows-PC/Image-Lab.html). Leaf dry mass (DM) was obtained by drying leaves at 80 °C to constant mass.

Predawn leaf water potential

Predawn leaf water potential (Ψ_{PD}, MPa) was measured on three leaves, for three to five representative adult plants of each species in both considered areas (Shl and Inl) of the two experimental sites, with a pressure chamber (Scholander, PMS Instruments, Oregon, USA).

Dark respiration

The response of dark respiration (R_D, μmol CO_2 m^{-2} s^{-1}) to changes in leaf temperature was measured in the afternoon from 17:00 (GMT+1), using the CIRAS 2 (PP Systems, Hitchin, UK) infrared gas analyser. Measurements were performed in the field, on three sun-exposed, fully

developed leaves belonging to the current-year, for three to five representative adult plants for each species (N = 9-15). Ambient and leaf temperature (T_a, T_l, °C), and relative air humidity (RH, %) were also recorded by the instrument. The leaf temperature was varied in six steps starting from 18 °C (the reference temperature, R_{Tref}), to 36 °C, using the Peltier system of the CIRAS cuvette. Considering our experimental conditions in terms of air temperature trend over the seasons in the two sites, the reference temperature was set at 18° C in order to compare R_{Tref} over all species and sites. Moreover, this temperature allows keeping the air humidity in the CIRAS flow lower than 70% to avoid instrumental errors. Indeed the measurements were performed in the field in the late afternoon.

A supporting external battery was used to ensure a better leaf temperature control. Leaves were dark adapted with aluminium foil for 45 min, prior to performing R_D measurements, to avoid transient post-irradiation bursts of CO_2 release (Atkin et al., 1998). For each temperature step, the measurements were taken when R_D was stable (approximately after 10 min from the change of temperature). The values of Q_{10} were determined following Gunderson et al., (2000):

$$Q_{10} = 10^{(10*\alpha)} \qquad \text{Eq. 1}$$

where a is the slope of the regression line between $\log_{10}$ of R_D measured at each step of the response curve and leaf temperature.

The linear correlation between R_D and a daily average of the air temperature of two days prior to the measurements (T_{2m}, °C), was assessed to test the acclimation of R_D (Tjoelker et al., 2009). T_{2m} was calculated using data from the meteorological stations.

Respiration at the daily average temperature of the sampling day (R_{amb}) was extrapolated for each replicate using:

$$R_{amb} = R_{18} * Q_{10}^{[\frac{T-Tref}{10}]} \qquad \text{Eq. 2}$$

where T is the mean air temperature of the sampling day, and T_{ref} is the leaf reference temperature (18°C).

Statistical analysis

As for LMA the difference between Shl and Inl was assessed for PSN and CP. One-way ANOVA, followed by Tukey's post hoc, was applied for evaluating the differences between R_{18} and Q_{10} between species. For each species, Factorial ANOVA was applied for testing the effect of distance from the shoreline (D), seasonality (Time) and their interaction (D*T) on physiological traits (Ψ_{PD}, Q_{10}, R_{18}, R_{amb}). Distance from the shoreline (Shl and Inl) and sampling date (Time) were used as random factors for *A. unedo* and *Q. ilex*. For *P. latifolia* also the site (PSN and CP) was considered

as a random factor. When differences were significant, ANOVA was followed by the Newman-Keuls test at p<0.05. We tested single correlations between seasonal values of R_{18} or Q_{10} and T_{2m} or Ψ_{PD} by linear regressions. P values and R^2 were used to test statistical significance and strength of the regression, respectively (Warton et al., 2006)and there has been much debate on this issue in the literature. In this review, we describe for the practitioner the essential features of line-fitting methods for estimating the relationship between two variables: what methods are commonly used, which method should be used when, and how to make inferences from these lines to answer common research questions. A particularly important point for line-fitting in allometry is that usually, two sources of error are present (which we call measurement and equation error. The combined effect of T_{2m} and Ψ_{PD} on R_{18} or Q_{10} was tested using multiple linear regressions. Linear Model was run pooling all data together for each species, for sites separately, and for each position along the coast (i.e Shl and Inl). The best model was evaluated using the Akaike Information Criterion (AIC) (Akaike, 1974). This test measures the relative quality of statistical models for a given set of data, thus providing a tool for highlighting the most performing model in explaining the variability of the dependent variable. All statistical analyses were made using the Statistica 7 software package (StatSoft, Inc. – Tulsa, OK – USA).

Results

Meteorological conditions

The 30 years time series of meteorological data highlighted that the values of maximum and minimum temperature were lower in CP relative to PSN. Mean annual temperature (MAT) in CP was 2.6 °C lower than at the PSN site (Table 1). The differences in air temperature between the sites were evident even when the values were averaged over the entire experimental period 2010-2011: MAT in CP was 3.1 °C lower relative to PSN. The difference in T_{air} between sites was more pronounced during the coldest and the warmest months, reaching a delta of 6.2 and 1.5 °C in February and in July respectively (Fig. 1 a, b). In CP, the temperature dropped below the freezing point 27 times, while in PSN freezing temperatures never occurred. Based on the time series of meteorological data, the annual precipitation was higher in CP than in PSN, where the length of the drought period is longer (Table
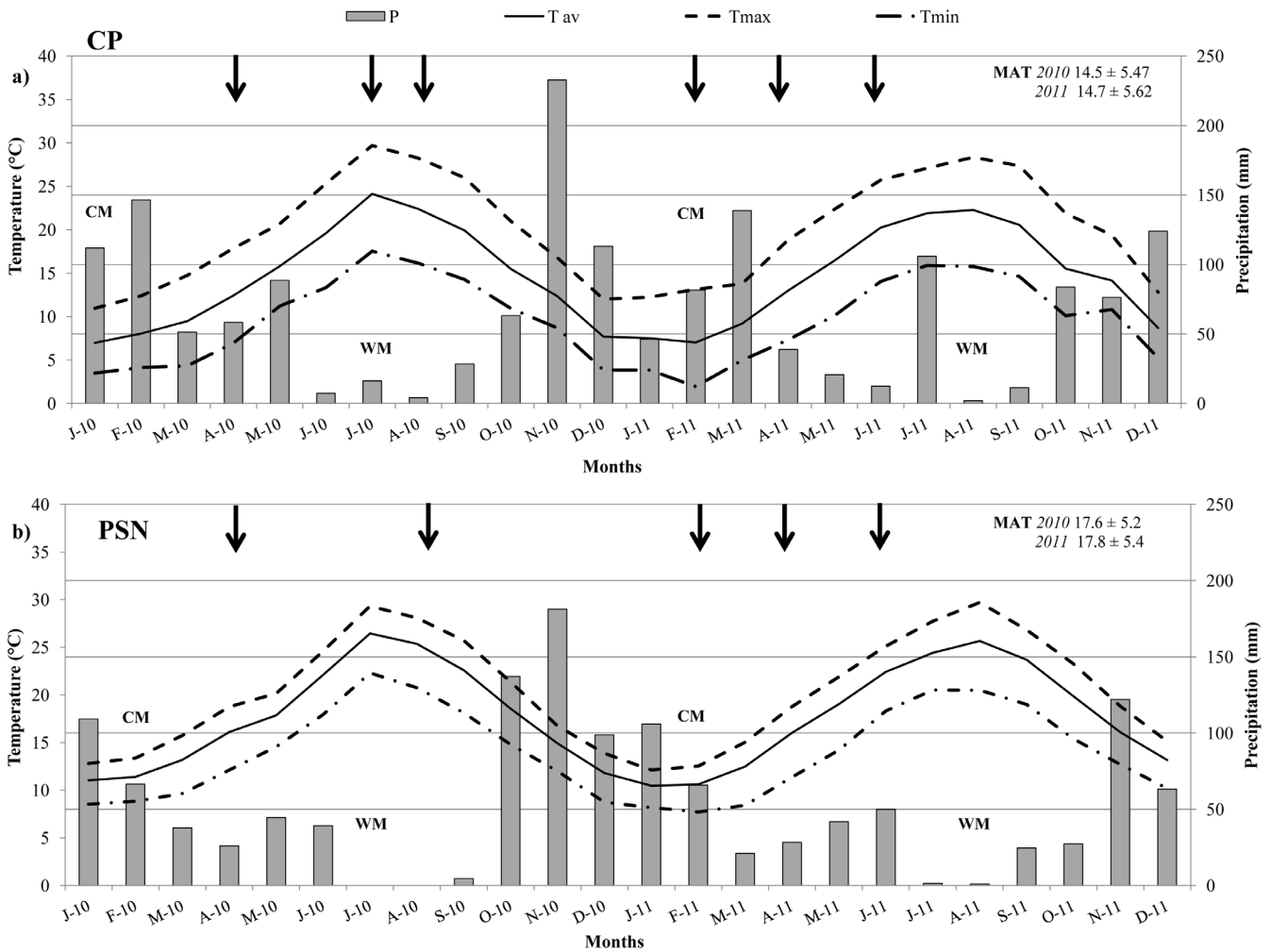
Figure 1. Monthly trend of air temperatures (Tav=Average; Tmax=Average of the maximum; Tmin= Average of the minimum) and rainfall from January 2010 to December 2011 in the two studied sites: a) Castelporziano (CP) and b) Piscinas (PSN). CM and WM indicate the coldest and warmest month respectively of the two years. The text in the inset shows the Mean Annual Temperature (MAT). The arrows indicated the months in which the field measurement carried out in both sites.

1). Precipitation over the years 2010–2011 (Fig. 1 a, b) was higher in CP (832.1 mm) than in PSN (648 mm), and in both sites year 2011 had the lowest rainfall (742 mm in CP, and 552 mm in PSN). In PSN, the potential evapotraspiration was higher due to higher temperatures, Vapour Pressure Deficit (VPD) and wind speed (data not shown). Because of the short distance between Shl and Inl sites, meteorological conditions were assumed equal between the two sites.

Physiological traits
Leaf Mass Area and plants water status

The LMA value of *A.unedo* was higher in PSN than in CP, whereas that of *Q. ilex* was comparable between sites, and

that of *P. latifolia* was higher in CP than in PSN (Table 2 and Table 3). In both site, CP and PSN, the LMA of *A.unedo* and *P. latifolia*, were slightly higher in Shl study area than in the Inl area. The water availability declined in summer and was higher in the Shl than in the Inl sites, both at CP and PSN (p<0.001, Table 3). In CP, the Ψ_{PD} measured in the Shl never fell below -1.1 MPa (Fig. 2a).

Dark respiration

R_{18} did not differ between species, being on average 0.83, 0.84 and 0.75 μmol CO_2 m^{-2} s^{-1} for *A. unedo*, *P. latifolia*, and *Q. ilex*, respectively (Fig. 3a). Similar values were observed when referring R_{18} on the leaf mass basis (data not shown). On the other hand, the mean value of Q_{10} was lower in *Q.*

Table 2. Leaf Mass Area (LMA, g/cm²) of the three species for Castelporziano (CP) and Piscinas (PSN) at different distance from the coastline, nearest shore (Shoreline) and farthest shore (Inland) respectively. n.a. indicates data not available for the analysis. The data are mean value ± standard error. For each species and distance from the coast, different letters indicate significant differences between Castelporziano and Piscinas.

	Species	Castelporziano	Piscinas
Shoreline	*A. unedo*	0.015 ± 0.002a	0.020 ± 0.003b
	Q. ilex	0.019 ± 0.004	n.a
	P. latifolia	0.029 ± 0.003b	0.021 ± 0.004a
Inland	*A. unedo*	n.a	0.018 ± 0.04
	Q. ilex	0.019 ± 0.002	0.019 ± 0.001
	P. latifolia	0.021 ± 0.005ab	0.018 ± 0.01a

Table 3. Analysis of variance for the Distance from shoreline (D) and seasonal trend (Time), on the three studied species. Only for *P. latifolia* the Site effect has been tested. LMA, Leaf Mass area (g/cm²); Ψ_{PD}, predawn leaf water potential (MPa); Q_{10}, temperature sensitivity; R_{18}, dark respiration measured at given temperature, 18 °C (μmolm²s⁻¹); R_{amb}, dark respiration (μmolm²s⁻¹) extrapolated using Eq 2 (see Materials and Methods). Significant factors (p < 0.05) are marked in bold. n.a. indicates data not available for the analysis.

A. unedo	Distance from the shore	Time	D*T	D*T*Site
LMA	0.054	n.a	n.a	n.a
Ψ_{PD}	**0.046**	**<0.001**	**0.002**	n.a
Q_{10}	0.247	**0.001**	**0.029**	n.a
R_{18}	0.121	**<0.001**	**<0.001**	n.a
R_{amb}	**0.030**	**<0.001**	**<0.001**	n.a
Q. ilex				
LMA	0.22	n.a	n.a	n.a
Ψ_{PD}	**0.038**	**<0.001**	**<0.001**	n.a
Q_{10}	**0.004**	**0.001**	0.062	n.a
R_{18}	**<0.001**	**<0.001**	**<0.001**	n.a
R_{amb}	0.118	**<0.001**	**<0.001**	n.a
P. latifolia				
LMA	**0.031**	n.a	n.a	n.a
Ψ_{PD}	**<0.001**	**<0.001**	**<0.001**	**0.032**
Q_{10}	**<0.001**	**0.009**	**<0.001**	**<0.001**
R_{18}	**<0.001**	**<0.001**	**<0.001**	**0.001**
R_{amb}	**<0.001**	**<0.001**	**<0.001**	**<0.001**

ilex (1.92 ± 0.32), relative to the value calculated for *A. unedo* (2.10 ± 0.31) and *P. latifolia* (2.13 ± 0.33).
The outputs of the linear models are reported in Table 4. The analysis was carried out pooling all data together (section I), analysing the sites separately (section II: CP and PSN), and considering Shl and Inl separately for each site (sections III: CP Shl, CP Inl; PSN Shl, PSN Inl).
Pooling all data together (Table 4, section I) the correlation between R_{18} and T_{2m} for each species was low, although significant ($R^2 = 0.16$) for *Q. ilex* and *P. latifolia*, whereas

the correlation between R_{18} and Ψ_{PD} reached higher R^2 for the three species. A regression model using both T_{2m} and Ψ_{PD} as independent variables slightly improved the determination coefficient.
In both sites (Table 4, sections II), for each species, R_{18} and Ψ_{PD} were negatively correlated, with higher determination coefficient relative to that obtained with T_{2m}. Regression model using both T_{2m} and Ψ_{PD} as independent variables, improved the determination coefficient only for *A. unedo* at PSN. When the regression model was applied considering

Shl and Inl separately (Table 4, sections III) both in CP and PSN, for all considered species, no correlation between T_{2m} and R_{18} was found in the Shl, whereas taking into account data from Inl areas, the correlation between T_{2m} and R_{18} resulted in *Q. ilex* (CP) and in *P. latifolia* (PSN). On the other hand, the relation between R_{18} and Ψ_{PD} showed significant R^2 for all the species. The model that considers both T_{2m} and Ψ_{PD} as independent variables slightly increased the capacity of predicting R_{18}. On the other hands, Q_{10} presented a weaker correlation with T_{2m} and Ψ_{PD}. A significant correlation was present between Q_{10} and T_{2m} only in CP Inl (*Q. ilex*) and PSN Shl (*P. latifolia* and *A. unedo*). Correlation between Q_{10} and Ψ_{PD} was present in CP site for *Q. ilex* and *P. latifolia* (section II) and for the same species in CP belonging to Shl area. Including both T_{2m} and Ψ_{PD} as explanatory variables, slightly improve the predictability of Q_{10} for Shl areas in CP and PSN. In the three species seasonal changes of R_{amb} showed similar trends in CP for both sampled areas, Shl and Inl (Fig. 4; Table 3), reaching the highest values in the Shl during summer, when T_a was the highest. In PSN the highest values of R_{amb} were reached in Jul-11 when higher VPD was measured (data not show).

DISCUSSION

In this study, the acclimation process of R_D was investigated in three Mediterranean species (*A. unedo, Q. ilex, P. latifolia*) growing in coastal environments, analysing the regression model between R_{18}, Q_{10} and air temperature. No differences in R_{18} between species are present, suggesting that they have convergence in metabolic pathways that control the basal respiration changes (Lawlor and Cornic, 2002). On the other hand, Q_{10} varied among the studied species, being lower in *Q. ilex* compared to the other two species, resulting in a general lower daily respiration rate, R_{amb}. This difference suggests that among the three studied species, *Q. ilex* has lower responsiveness to changes in temperature as a part of a conservative resource-use strategy (Valladares et al., 2000). The change in Q_{10} was not the predominant factor underpinning respiratory temperature acclimation, since a minor temporal pattern was detected (i.e low correlation with T_{2m}).

These results can contribute to the debate on the assumption of many carbon balance models that use Q_{10} values at or near 2.0, and a fixed value for basal respiration as a proportion of
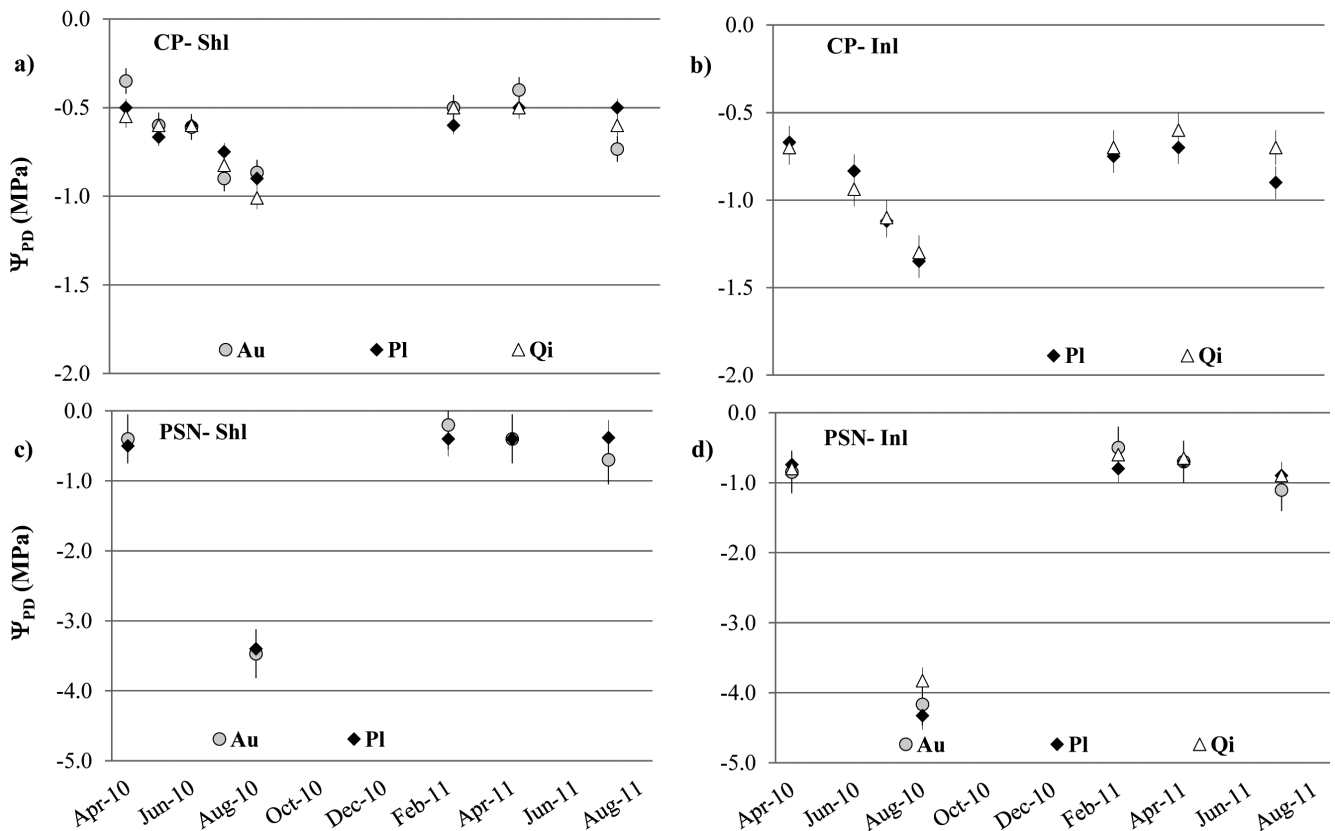
Figure 2. Pre-dawn leaf water potential (Ψ_{PD}, MPa), measured in Castelporziano (a, b) and Piscinas (c, d) at different distance from shoreline (Shl, nearest sites; Inl, farthest sites), on *A. unedo* (Au), *Q. ilex* (Qi) and *P. latifolia* (Pl). Value are showed as mean ± standard error, N = 3-5.
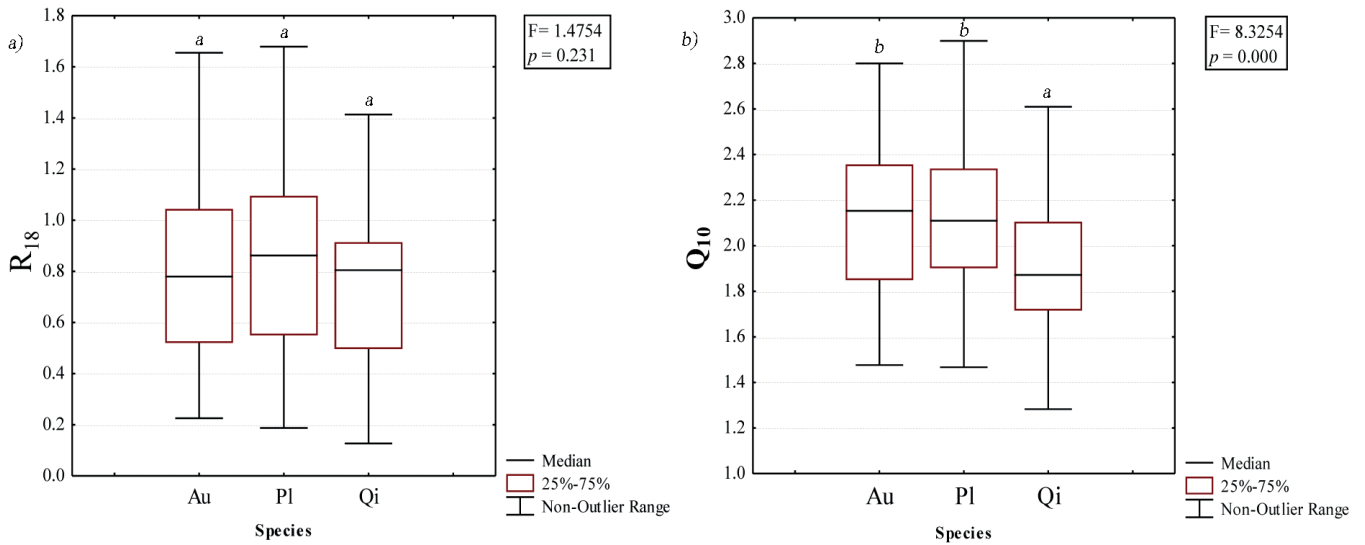
Figure 3. a) Basal respiration (R_{18}) and b) Q_{10} in the three studied species. The box plots indicate the median, 25th and 75th percentile for each species. Box plots not accompanied by the same letter are significantly different at P < 0.05.
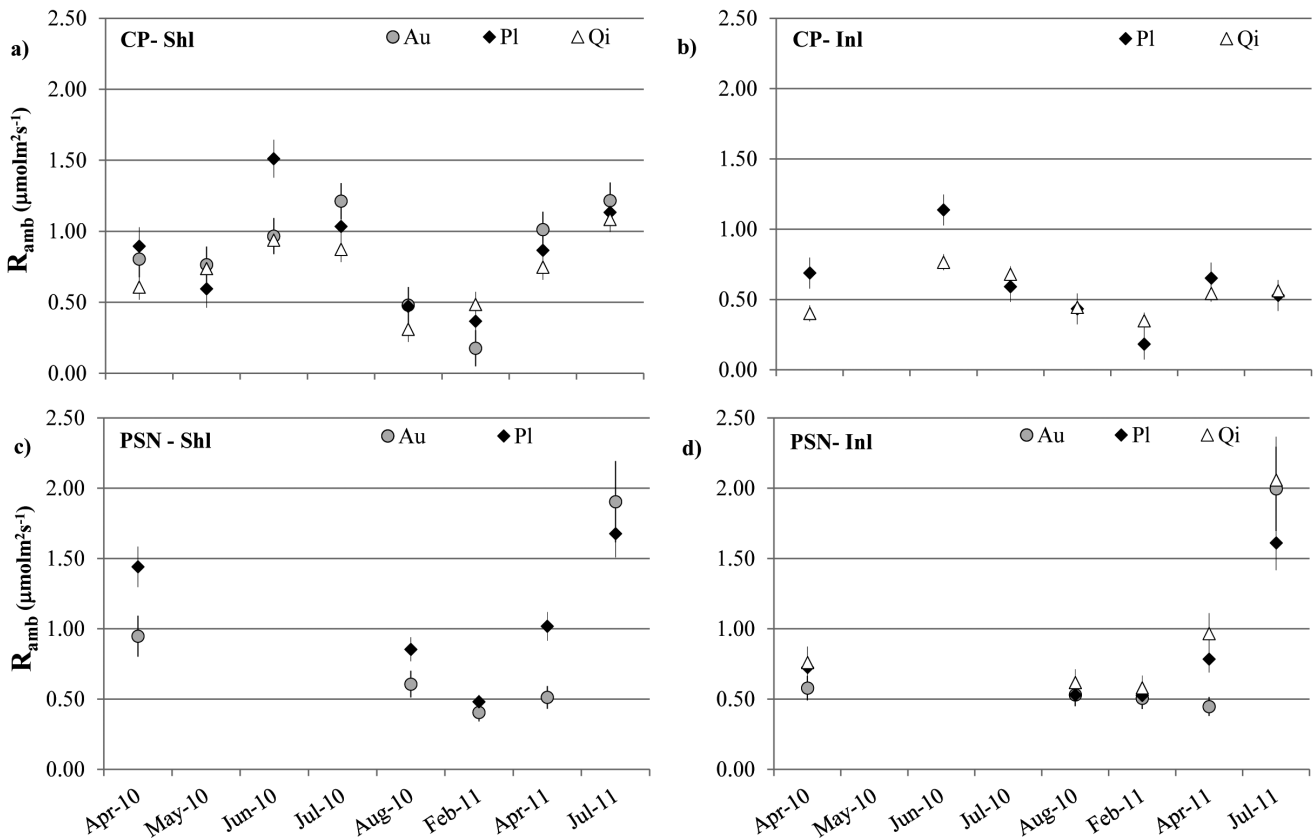
Figure 4. Seasonal changes in leaf dark respiration rate calculated at the daily ambient temperature (R_{amb}) of the sampling day, for Castelporziano (CP) and Piscinas (PSN), on sites at different distance from the shoreline (Shl, nearest sites; Inl, farthest sites). R_{amb} was extrapolated for each replicate using Eq 2 (see Materials and Methods). The data are presented as the mean ± standard error with N includes between three and five.

Table 4. Correlation coefficients of basal respiration measured at given temperature (R_{18}) and Q_{10} (proportional increase in R_D per 10°C temperature increase),with T_{2m} (mean value of the air temperature of two days prior to the measurements),Ψ_{PD} (pre-dawn leaf water potential) and T_{2m} together with Ψ_{PD}. Significant correlations are marked in bold. * is for p<0.05, *** is for p<0.001. Values highlighted in grey indicate the best model based on the Akaike Information Criterion. n.a., data not available.

			R_{18}			Q_{10}		
		Species	T_{2m}	Ψ_{PD}	$T_{2m} + \Psi_{PD}$	T_{2m}	Ψ_{PD}	$T_{2m} + \Psi_{PD}$
Section I	All Data	*A. unedo*	0.04	**0.20***	**0.21***	0.10	0.00	**0.16***
		Q. ilex	**0.16***	**0.18***	**0.22***	0.04	0.01	0.04
		P. latifolia	**0.16***	**0.21***	**0.23***	0.01	0.01	0.013
Section II	CP	*A. unedo*	n.a	n.a	n.a	n.a	n.a	n.a
		Q. ilex	**0.39***	**0.50***	**0.51***	0.05	**0.15***	**0.16***
		P. latifolia	**0.23***	**0.56***	**0.56***	0.01	**0.13***	**0.18***
	PSN	*A. unedo*	0.00	**0.26***	**0.50***	0.08	0.00	**025***
		Q. ilex	n.a	n.a	n.a	n.a	n.a	n.a
		P. latifolia	0.14	**0.47***	**0.50***	0.07	0.05	0.08
Section III	CP Shl	*A. unedo*	0.11	**0.37***	**0.43***	0.10	0.00	**0.23***
		Q. ilex	0.21	**0.34***	**0.34***	0.01	**0.13***	**0.20***
		P. latifolia	0.11	**0.48***	**0.50***	0.00	**0.28***	**0.56***
	CP Inl	*A. unedo*	n.a	n.a	n.a	n.a	n.a	n.a
		Q. ilex	**0.68***	**0.66***	**0.59***	**0.16***	**0.15***	0.04
		P. latifolia	**0.40***	**0.59***	**0.74***	0.04	0.04	0.17
	PSN Shl	*A. unedo*	0.01	**0.37***	**0.68***	**0.36***	0.10	**0.40***
		Q. ilex	na	na	na	n.a	n.a	n.a
		P. latifolia	0.16	**0.45***	**0.45***	**0.75***	**0.21***	**0.78***
	PSN Inl	*A. unedo*	0.00	**0.19***	**0.39***	0.00	**0.13***	**0.33***
		Q. ilex	0.00	**0.41***	**0.82***	0.06	0.04	0.06
		P. latifolia	**0.25***	**0.72***	**0.83***	0.06	0.00	0.19

photosynthesis. Our data suggest that for evergreen species the first assumption could be suitable, as the Q_{10} values obtained in this study divert little from that used by models (Wythers et al., 2005; Vanderwel et al., 2015), whereas some concerns arise regarding the second generalization. When water stress occurs, it is likely to influence differently photosynthesis and R_D (Joseph et al., 2014). Our results do not allow to give a mechanistic explanation of the possible causes of R_D change, neither to disentangle the direct effect of water stress on R_D from the indirect one (through photosynthesis) (Flexas et al., 2006). However, they indicate that, at least in the Mediterranean environment, water availability affects R_D by disrupting the acclimation process. Indeed, analysing the acclimation process through the linear correlation between R_{18} and T_{2m}, the seasonal pattern of R_{18} did not exhibit the expected negative linear correlation with air temperature (i.e. R_{18} decreases as T_{air} increases, Tjoelker et al., 2008; Rodrìguez-Calcerrada et

al., 2010). However, it is worth notice that, as hypothesised, acclimation was more evident in the CP site, which presents moderate water stress. Otherwise, in the PSN site, where water stress is more pronounced, T_{2m} was not sufficient to explain the observed seasonal trends of R_{18}. Considering the water availability together with air temperature in acclimation models might help in predicting R_D of species in coastal Mediterranean ecosystems characterized by high seasonal variability in water availability (Mereu et al., 2009; Bussotti et al., 2014). The evidence that R_{18} presents a higher correlation with Ψ_{PD} relative to T_{2m}, might be due to a general decline of both R_{18} and R_D as drought progressed (Fusaro et al., 2015). Air temperatures and Ψ_{PD} partially hold the same information, but Ψ_{PD} is also influenced by atmospheric and physiological conditions that plants have experienced in a given time period namely wind speed, VPD, radiation and the species-specific responses to environmental conditions. T_{2m} could be the best

predictor of R_{18} when water stress is low. Accordingly, it would be expected that the goodness of fit between R_{18} and T_{2m} is higher in the Shl sites that have a higher water availability, compared to the Inl. However, the results show the opposite behaviour. This contradiction could be explained by the fact that in the Shl the studied species are exposed to higher stressful conditions (i.e salty wind, soil salinity, nutrient scarcity), as suggested by the LMA values which are higher in the shoreline stands of *P. latifolia* and *A. unedo*. This result highlights that these species are facing stress factors that influence foliar anatomy (Daas-Ghrib et al., 2011).

Additionally, R_{amb} is also higher in the Shl than in Inl denoting an increase of metabolic needs, possibly to withstand the higher intensity of stresses (Slot et al., 2008) occurred in the proximity of the shoreline (Mereu et al., 2011). Multi-factor models can describe more precisely the variations of basal respiration in the field conditions, since an array of environmental factors co-vary with temperature and could alter the temperature acclimation process of respiration (Smith & Dukes, 2013; Smith, 2015). In accordance with these authors in our case study, Ψ_{PD} improved the linear regression model for R_{18}, confirming that at least water availability should be included in respiration models (Rodríguez-Calcerrada et al., 2011b).

Finally, we speculate that the low correlation between R_{18} and T_{2m} could be due to the fact that, when water stress occurs, R_D, acclimates better to the average leaf temperature rather than to the air temperature of the previous days.

Indeed, a general drought-related decrease of stomatal conductance can reduce the latent heat loss, thereby increasing leaf temperature (Warren et al., 2011), which directly affects R_D. We argue that acclimation models should use the average leaf temperature of the previous days, which can better accounting for water stress effects, or for the effects that multiple stress factors might have on anatomical (leaf mass, size, angle, reflectance properties), physical (incoming radiative energy) and biological (transpiration) leaf traits (Leuzinger et al., 2010) that, in turn, influence leaf respiration. For example, the leaf angle of Mediterranean species was shown to vary during the season (Gratani & Bombelli 2000), thereby modifying leaf energy budget and thus leaf temperature. Additionally, monitoring leaf temperature would allow distinguishing the true acclimation process to temperature from the direct effect of stress factors on leaf respiration.

Conclusions

This study highlighted that seasonal acclimation of leaf dark respiration in all the three studied species was related to adjustments in basal respiration rate, R_{18}, more than to Q_{10} regulation This evidence provides a relevant indication for

improving the reliability of carbon balance models applied to low NPP ecosystems, such as those present in coastal Mediterranean regions.

However, the temporal pattern of R_{18} was not consistent with a clear acclimation response entirely associated with changes in the air temperatures, highlighting that other interacting environmental factors can affect the acclimation process. This latter is better characterized when the leaf pre-dawn water potential was considered as an explanatory factor, together with the average air temperature of the previous days. These findings support the need for developing R_D models based on multiple combined factors, and in particular, for Mediterranean coastal ecosystems, oxidative stressors should be considered. This is consistent with the different acclimation response observed between shoreline and inland, sites exposed to different environmental pressures. Furthermore, we suggest that acclimation models should take into account the average leaf temperature of the previous days rather than the air temperature, since the latter could synthetize better the overall functional condition of vegetation. To determine how the acclimation process of foliar respiration takes place in plants that cope with multi-stresses, such as those growing in coastal areas, experimental work using new technologies (infrared camera; remote sensing) would be beneficial.

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