Morphological, anatomical and physiological leaf traits of *Q. ilex*, *P. latifolia*, *P. lentiscus*, and *M. communis* and their response to Mediterranean climate stress factors

Loretta Gratani*, Rosangela Catoni and Laura Varone

Abstract

**Background:** Limitations to plant growth imposed by the Mediterranean climate are mainly due to carbon balance in response to stress factors. In particular, water stress associated to high air temperature and irradiance in summer causes a marked decrease in CO$_2$ assimilation. Air temperature sensitivity of photosynthesis ($P_N$) differs from that of leaf respiration ($R_D$). $P_N$ often decreases sharply at temperature above its optimum while $R_D$ increases exponentially over short term rises in temperature. Nevertheless, the impact of water deficit on $R_D$ is still far from clear with reports in literature including decreases, maintenance or increases in its rates. The ratio $R_D/P_N$ can be considered a simple approach to leaf carbon balance because it indicates the percentage of photosynthates that is respired.

**Results:** The results underline different morphological, anatomical and physiological traits of the evergreen species co-occurring in the Mediterranean maquis which are indicative of their adaptive capability to Mediterranean stress factors. The ratio $R_D/P_N$ varies from 0.15 ± 0.04 in autumn, 0.24 ± 0.05 in spring through 0.29 ± 0.15 in winter to 0.46 ± 0.11 in summer. The lower $R_D/P_N$ in autumn and spring underlines the highest $P_N$ rates during the favorable periods when resources are not limited and leaves take in roughly three to five times more CO$_2$ than they lose by respiration. On the contrary, the highest $R_D/P_N$ ratio in summer underlines the lowest sensitivity of respiration to drought. Among the considered species, *Quercus ilex* and *Pistacia lentiscus* have the largest tolerance to low winter temperatures while *Phillyrea latifolia* and *Myrtus communis* to drought, and *Phillyrea latifolia* the highest recovery capability after the first rainfall following drought.

**Conclusions:** The Mediterranean evergreen specie shows a different tolerance to Mediterranean climate stress factors. The predicted global warming might differently affect carbon balance of the considered species, with a possible change in Mediterranean shrublands composition in the long-term. Understanding the carbon balance of plants in water limited environments is crucial in order to make informed land management decisions. Moreover, our results underline the importance of including seasonal variations of photosynthesis and respiration in carbon balance models.

**Keywords:** Air temperature; Drought; Leaf respiration; LMA; *Myrtus communis*; *Phillyrea latifolia*; Photosynthesis; *Pistacia lentiscus*; *Quercus ilex*
Background

The Mediterranean Basin has long been recognized as a model region for studying global change effects on terrestrial ecosystems (Lavorel et al. 1998). Climatic models indicate that rainfall patterns are changing in the Mediterranean Basin as a consequence of the climate change, with a marked decrease up to 15-20% occurring mainly during summer, associated to an increase in the mean maximum air temperature of about 5.1°C by the end of the 21st century (IPCC 2007). These changes will result in extended periods of soil moisture deficit (Hlavinka et al. 2009). Limitations to plant growth imposed by the Mediterranean climate are mainly due to plant carbon balance in response to stress factors (Galmés et al. 2007). In particular, water stress associated to high air temperatures and an excess of light during summer, may result in a chronic photo-inhibition or down-regulation of photosynthesis causing a marked decrease in CO₂ assimilation (Zhou et al. 2010). Carbon balance depends on the ratio between photosynthesis and respiration (Lambers et al. 1998), and both these factors change in response to climatic conditions (Baldocchi and Amthor 2001). Nevertheless, they do not necessarily respond identically to changes in these conditions (De Boeck et al. 2007). Air temperature sensitivity of photosynthesis differs from that of respiration (Morison and Morecroft 2006; W’ay and Sage 2008; Shen et al. 2009). Photosynthesis often decreases sharply at temperatures above its optimum (Sage and Kuben 2007; Hüve et al. 2011), with most temperate species exhibiting a broad temperature optimum in the range of 15–30°C (Atwell et al. 1999; Larcher 2004), while leaf respiration increases exponentially over short term rises in temperature (Rodríguez-Calcerrada et al. 2011). In particular, the temperature sensitivity of leaf respiration is quantified using Q₁₀ i.e. the proportional increase in respiration for every 10°C rise in temperature (Armstrong et al. 2006). Photosynthesis provides soluble sugar as substrates for leaf respiration (Atkin et al. 2007) and availability of respiratory substrates determines the effect of temperature on respiratory enzymes and consequently on respiration temperature sensitivity (Atkin et al. 2002; Rodríguez-Calcerrada et al. 2011). Respiration decrease depends partially on the photosynthesis decrease in response to water deficit (Gimeno et al. 2010). Under water stress a lower photosynthetic activity limits the soluble sugar availability (Pinheiro and Chaves 2011). A lower soluble sugar level may reduce the temperature sensitivity of respiration and then to cause a respiration decrease (Rodríguez-Calcerrada et al. 2011). Nevertheless, the impact of water deficits on leaf respiration is still far from clear, with reports in literature including decreases, maintenance, or increases in the rates of this process (Gimeno et al. 2010).

Since the magnitude of photosynthetic and respiratory acclimation varies among species, these processes are still poorly understood, especially under field conditions (Shen et al. 2009). In dry-land forests of the Mediterranean region, the rates of carbon loss by plant respiration often equal or exceed the rate of carbon uptake by photosynthesis during the year, except in spring and autumn, when air temperatures and water availability are favorable (Zaragoza-Castells et al. 2008; Gratani et al. 2008).

Mediterranean plant species are distributed along different gradients of water availability, according to their capacity to withstand drought (Medrano et al. 2009). Nevertheless, if dry season lasts too long, plant water deficit may negatively affect plant species capacity for carbon assimilation, as a result of the lowest photosynthetic rates and leaf surface area produced (Pereira et al. 2007). Carbon assimilation is also related to stomatal conductance with a strong impact on plant water use efficiency (i.e. the amount of water used per carbon gain) that links plant performance with water availability (Craven et al. 2013). The strength and direction of the relationship between water use efficiency and plant performance can illustrate interspecific differences in drought tolerance strategies (Craven et al. 2013).

Considering global change, variations in water supply will induce important changes in Mediterranean plant species that suffer of water scarcity, especially during drought (Llusíá et al. 2011). Different species can respond to global change by developing different mechanisms both at physiological and morphological levels. Nevertheless if the length or strength of the dry season increases, the distribution area of the species could shrink (Díaz-Barradas et al. 2010) and affect the composition of vegetation in the long-term (Gebrekirstos et al. 2011). Assessment of vegetation level vulnerability and climate change resilience require understanding of the diversity among plant species in the current vegetation, and of their growth strategies in response to fluctuating water availability (Dawson et al. 2009). A rapid adaptation to an increased aridity will be crucial for the future of many species in the Mediterranean region (Sánchez-Gómez et al. 2011). To predict how climate change might affect future Mediterranean species presence and distribution and, as a consequence, community structure and ecosystem functioning, it is essential to have a broad knowledge of which climatic factors are constraining plant species physiological traits, and how these constraints are manifested temporally (Llorens et al. 2003). Morphological adaptations as small, thick, layered leaves with high stomatal density of small size in many Mediterranean evergreen species could favor carbon gain profits over transpiration losses (Rotondi et al. 2003; Gratani and Varone 2004, 2006), Quercus ilex L., Phillyrea latifolia L., Pistacia lentiscus L., and Myrtus communis.
L. are evergreen shrub species largely distributed in the vegetation of the Mediterranean Basin. *Q. ilex* extends longitudinally from Portugal to Syria and latitudinally from Morocco to France (Valladares et al. 2000); it occurs in the Mediterranean maquis and forests, growing in different soil conditions and over a broad range of elevations, from the sea level to 1100 m a.s.l. (Khatouri 1992; Terradas and Savé 1992; Gratani et al. 2003). *P. latifolia* is a drought- and salt-stress-tolerant evergreen shrub species growing in the Mediterranean maquis, forests (Gratani and Bombelli 2000; Ogaya and Peñuelas 2003) and on seashore dunes, where excess soil salinity and salt spray are additional stress agents (Ogaya and Peñuelas 2003). *P. lentiscus* occurs in a wide variety of habitats, from open communities in garigue to closed ones in more mesic sites (Correia and Diaz Barradas 2000). *M. communis* is the only species of the *Myrtaceae* in the actual flora of the Mediterranean Basin (González-Varo 2010). It grows on fertile soils of warm habitats in the Mediterranean region (González-Varo et al. 2009) and in the maquis (Pignatti 1982).

The main objective of this research was to investigate morphological, anatomical and physiological leaf traits of *Q. ilex, P. latifolia, P. lentiscus* and *M. communis* and their involvement in carbon acquisition. Moreover, the ratio respiration to photosynthesis which is indicative of the capacity of plants to produce new biomass for growing and reproductive structures (Galmés et al. 2007; Millar et al. 2011) was analyzed over the year. Improving knowledge on carbon acquisition capability of the Mediterranean species will allow us to hypothesize their presence into the distribution area over the long-term, also in consideration of global change.

### Methods

#### Study site and plant material

Experiments were carried out in the period from December 2009 to October 2010, on *Q. ilex, P. latifolia, P. lentiscus,* and *M. communis* shrubs (5 shrubs per species) growing in the open, under the same environmental conditions, at the Botanical Garden of Rome (41°53’53”N, 12°28’46”E; 53 m a.s.l.). The selected shrubs had comparable size (height = 1.36 ± 0.19 m, mean value of the considered shrubs). During the study period the selected shrubs were not watered and they received only natural rain.

#### Climate

The climate of the study area was of the Mediterranean type: the mean minimum air temperature (*T* min) of the coldest months (January and February) was 5.3 ± 0.2°C, the mean maximum air temperature (*T* max) of the hottest months (July and August) was 30.9 ± 0.2°C, and the yearly mean air temperature (*T* m) was 16.8 ± 6.5°C. Dry period was from the beginning of June to the end of August (65.5 mm total rainfall of the period). Total annual rainfall was 708 mm, most of it occurring in autumn and in winter (Data from UCEA for the years 1995 to 2010). During the study period *T* min of the coldest month (January) was 3.8 ± 3.1°C, *T* max of the hottest month (July) 34.0 ± 2.2°C, and total rainfall was 709 mm, most of it occurring in winter.

### Anatomical leaf traits

Leaf thickness (L, μm) was measured by leaf sections from fresh, fully expanded sun leaves (20 per species), collected at the end of September 2010 from the selected shrubs, and measured by light microscope. Stomatal density (SD, stomata mm−2) was measured from nail varnish impressions (n = 20 per species) of the inferior lamina, according to Sack et al. (2003), each of them 0.5 × 1.0 cm, obtained by a Zeiss Axiocam MRc 5 digital camera (Carl Zeiss), with Axiovision AC software (Release 4.5). Stomatal pore length (SPL, μm) and width (SPW, μm) were measured on the same recorded digital images. Dimension of the stomata was used to calculate the equivalent area of the ellipsoid representing the stomatal pore area (SPA) by the following formula: (π × length × width)/4, according to Minnocci et al. (1995) and Bartolini et al. (1997).

### Morphological leaf traits

Measurements of leaf morphological traits were carried out on fully expanded sun leaves (n = 20 per species), collected at the end of September 2010. The following parameters were measured: projected fresh leaf surface area (LA, cm2) (excluding petioles), obtained by the Image Analysis System (Delta-T Devices, UK), and leaf dry mass (DM, mg), determined drying leaves at 80°C to constant mass. Leaf mass per unit leaf area (LMA, mg cm−2) was calculated by the ratio of DM and LA (Reich et al. 1992).

Leaf tissue density (LTD, mg cm−3) was calculated by the ratio of LMA and leaf thickness (Wright and Westoby 2002).

### Gas exchange measurements

Measurements of gas exchange were carried out using an infrared gas analyser (ADC LCA4, UK), equipped with a leaf chamber (PLC, Parkinson Leaf Chamber). Measurements were made on fully expanded sun leaves (10 leaves per species per each sampling occasion) during the study period.

Net photosynthetic rate [P*N*, μmol (CO2) m−2 s−1], photosynthetically active radiation [PAR, μmol (photons) m−2 s−1], stomatal conductance [gs, mol (H2O) m−2 s−1], leaf temperature (*T* p, °C), and leaf chamber air temperature (*T* ch, °C) were measured.

The *P*N, *g*s, and *E* rates shown were the mean of the maximum rates for the four days measurement per
month, carried out in comparable weather conditions. During gas exchange measurements, the leaf to air vapour pressure deficit (VPDleaf, kPa) was calculated according to Grantz (1990) as: VPD = es – ea, where es was saturated vapour pressure at leaf temperature and ea the air vapour pressure. The intrinsic water use efficiency [IWUE, μmol (CO2) mol (H2O)\(^{-1}\)] was calculated as \( P_{N}/g_{s} \) ratio, according to Medrano et al. (2009).

Measurements were carried out under natural conditions, on cloud – free days (PAR ≥ 1200 μmol m\(^{-2}\) s\(^{-1}\)), in the morning, from 8.00 a.m. to 12.00 p.m., according to Reich et al. (1995).

On each sampling occasion, leaf respiration rate \( R_{D} \) (μmol (CO2) m\(^{-2}\) s\(^{-1}\)) measurements were carried out contemporarily to \( P_{N} \) ones (on the same leaves) by darkening the leaf chamber with a black paper, according to Cai et al. (2005), for 30 min prior to each measurement to avoid transient post-illumination bursts of CO2 releasing (Atkin et al. 1998a, 1998b). The \( R_{D} \) rates shown were the mean of the maximum rates for the four days measured per month, carried out in comparable weather conditions. The ratio between \( R_{D} \) and \( P_{N} \) was also calculated. \( Q_{10} \) was calculated according to Rodríguez-Calcerrada et al. (2012) as: \( Q_{10} = e^{10k} \) where \( k \) is the slope of the linear regression between \( T_{ds} \) and the natural logarithm of \( R_{D} \) (Atkin et al. 2005).

**Statistics**

Differences in the considered variables were determined by the analysis of variance (ANOVA), and Tukey test for multiple comparisons, performed using a statistical software package (Statistica, Statsoft, USA). The regression analysis was carried out to evaluate correlations among the considered variables. The principal component analysis (PCA) was carried out in order to summarise the considered anatomical (L, SD, SPL, SPA), morphological (LMA, LTD) and physiological (\( P_{N}, R_{D}, g_{s}, E, \) IWUE) leaf traits into major components which explained their variation in the considered species.

**Results**

**Anatomical and morphological leaf traits**

Anatomical leaf traits of the considered species are shown in Table 1. L ranged from 311 ± 10 μm (M. communis) to 419 ± 25 μm (P. latifolia). SD varied significantly (p < 0.05): M. communis had the highest SD (508 ± 82 stomata mm\(^{-2}\)), followed by Q. ilex and P. lentiscus (419 ± 18 stomata mm\(^{-2}\), mean value), then by P. latifolia (238 ± 21 stomata mm\(^{-2}\)). P. latifolia had the highest SPL and SPA (19.5 ± 2.6 μm, 170.3 ± 31.3 μm\(^{2}\), respectively), and Q. ilex the lowest ones (9.1 ± 1.9 μm, 42.1 ± 12.4 μm\(^{2}\), respectively). LMA varied from 9 ± 1 mg cm\(^{-2}\) (M. communis) to 20 ± 1 mg cm\(^{-2}\) (P. latifolia) (Table 2). Q. ilex had the highest LTD (613 ± 40 mg cm\(^{-3}\)) and M. communis the lowest one (304 ± 51 mg cm\(^{-3}\)).

**Seasonal gas exchange and leaf respiration variations**

Gas exchange and leaf respiration data of the considered species during the study period are shown in Figures 1 and 2.

**Spring measurements**

During the study period all the considered species had the highest \( P_{N} \) and \( g_{s} \) in spring (March, April and May), peaking in May, when \( T_{max} \) was 23.8 ± 3.0°C, and water availability 113.8 mm (total rainfall of May). Q. ilex, P. lentiscus and P. latifolia had the significantly (p < 0.05) highest \( P_{N} \) [15.4 ± 0.2 μmol (CO2) m\(^{-2}\) s\(^{-1}\)], mean value measured in May] than M. communis [10.7 ± 0.7 μmol (CO2) m\(^{-2}\) s\(^{-1}\)].

\( g_{s} \) of M. communis [0.11 ± 0.04 mol (H2O) m\(^{-2}\) s\(^{-1}\)], in May] was 52%, 39%, and 35% lower than P. latifolia, P. lentiscus and Q. ilex, respectively. P. lentiscus and P. latifolia had the lowest IWUE in May [84 ± 10 and 68 ± 6 μmol (CO2) mol (H2O)\(^{-1}\), respectively] while Q. ilex and M. communis in April [81 ± 9 and 98 ± 7 μmol (CO2) mol (H2O)\(^{-1}\), respectively].

A different \( R_{D} \) trend was observed in spring: P. lentiscus and Q. ilex \( R_{D} \) peaked in May [3.7 ± 0.3 μmol (CO2) m\(^{-2}\) s\(^{-1}\), mean value], while P. latifolia in March \( [R_{D} = 4.3 ± 0.6 \mu{\text{mol (CO}}_2{\text{) m}}^{-2}{\text{ s}}^{-1}] \). M. communis \( R_{D} \) was not significantly different from March to May [2.7 ± 0.1 μmol (CO2) m\(^{-2}\) s\(^{-1}\), mean value of the three months]. M. communis had the highest \( R_{D}/P_{N} \) ratio (0.30 ± 0.06, mean of March, April and May), followed by P. latifolia (0.23 ± 0.08), P. lentiscus (0.22 ± 0.03) and Q. ilex (0.20 ± 0.05).

| Species    | L (μm)       | SPL (μm)  | SPW (μm)  | SPA (μm\(^{2}\)) | SD (stomata mm\(^{-2}\)) |
|------------|--------------|-----------|-----------|------------------|-------------------------|
| P. lentiscus| 378 ± 54 a   | 11.7 ± 1.3 a | 7.8 ± 1.3 a | 11.8 ± 1.5 a     | 406 ± 20 a              |
| P. latifolia| 419 ± 25 a   | 19.5 ± 2.6 b | 11.1 ± 1.3 b | 170.3 ± 31.3 b   | 238 ± 21 b              |
| M. communis| 311 ± 10 b   | 10.5 ± 2.0 ac | 5.4 ± 1.0 c  | 45.5 ± 15.2 c    | 508 ± 82 c              |
| Q. ilex    | 314 ± 59 b   | 9.1 ± 1.9 c  | 5.9 ± 0.6 c  | 42.1 ± 12.4 c    | 432 ± 34 a              |
Table 2 Morphological leaf traits at full leaf expansion of the considered species

| Species    | DM (mg)     | LA (cm²)   | LMA (mg cm⁻²) | LTD (mg cm⁻³) |
|------------|-------------|------------|---------------|---------------|
| P. lentiscus | 229 ± 53.4 a | 11.7 ± 2.3 a | 19 ± 2 a      | 521 ± 57 a    |
| P. latifolia | 105.4 ± 11.3 b | 5.2 ± 0.4 b | 20 ± 1 a      | 472 ± 24 a    |
| M. communis | 31.5 ± 7.8 c  | 3.5 ± 0.4 c  | 9 ± 1 b       | 304 ± 51 b    |
| Q. ilex     | 195.2 ± 35.4 d | 10.1 ± 1.6 d | 19 ± 2 a      | 613 ± 40 c    |

DM, leaf dry mass; LA, projected leaf surface area; LMA, leaf mass per unit leaf area; LTD, leaf tissue density. Mean values (±SE) are shown (n = 20). Mean values with the same letters are not significantly different (Tukey Test, p ≤ 0.05).

Winter measurements

During winter (December, January and February) $P_N$, $g_s$ and $R_D$ decreased in respect to the spring maximum, reaching the lowest rates in January ($T_{min}$ 3.8 ± 3.1°C; 118.2 mm = total rainfall of the month). In particular, Q. ilex had the lowest $P_N$ decrease (68% compared to the spring maximum) followed by P. lentiscus (82%), M. communis (92%) and P. latifolia (97%). In the same month, Q. ilex had the significantly ($p < 0.05$) highest $g_s$ [0.06 ± 0.01 mol (H₂O) m⁻² s⁻¹], followed by M. communis [0.03 ± 0.01 mol (H₂O) m⁻² s⁻¹], P. lentiscus, and P. latifolia [0.02 ± 0.01 mol (H₂O) m⁻² s⁻¹, mean value].

Among the species the highest IWUE was measured in P. lentiscus [127 ± 22 μmol (CO₂) mol (H₂O)⁻¹, mean of December, January and February] and the lowest one in P. latifolia [45 ± 22 μmol (CO₂) mol (H₂O)⁻¹]. $R_D$ in January was, on an average, 84% lower than the spring maximum. M. communis and P. latifolia had the highest $R_D/P_N$ ratio (0.61 ± 0.02 and 0.75 ± 0.03, respectively) and Q. ilex and P. lentiscus had the lowest one (0.12 ± 0.02 and 0.27 ± 0.01, respectively).

Summer measurements

In summer (June, July, August), $P_N$ significantly decreased, reaching the lowest rates in August, when $T_{max}$ was 32.3 ± 2.0°C, and the total rainfall of the month 4.4 mm. In particular, $P_N$ decreased, on an average, by 64% in P. lentiscus and Q. ilex, and 46% in P. latifolia and M. communis. IWUE was lower in P. lentiscus (24%) and P. latifolia (1%), and higher in M. communis and Q. ilex, (24% and 33%, respectively), compared to the spring values. M. communis had the lowest $g_s$ decrease (64%) compared to the spring maximum, followed by Q. ilex (70%), P. lentiscus (75%), and P. latifolia (74%).

$R_D$ was 43% and 12% higher than the spring maximum in P. lentiscus and P. latifolia, respectively, while $R_D$ was 13% lower than the spring maximum in Q. ilex. There were no significant $R_D$ differences in M. communis between spring and summer measurements. P. lentiscus and Q. ilex had the highest $R_D/P_N$ rate (0.94 ± 0.05 and 0.61 ± 0.06, respectively), followed by P. latifolia (0.56 ± 0.04), and M. communis (0.43 ± 0.02).

Autumn measurements

$P_N$ recovered 64% of the spring maximum in Q. ilex, P. latifolia and M. communis (mean value), and 53% in P. lentiscus at the end of September ($T_{max}$ 28.2 ± 3.1°C after the first rainfall (22.9 mm from the middle to the end of September) following drought. $g_s$ recovered 59% of the spring maximum in Q. ilex, 54% in M. communis, 44% in P. lentiscus, and 35% in P. latifolia.

IWUE ranged from 100 ± 13 μmol (CO₂) mol (H₂O)⁻¹ in P. lentiscus to 125 ± 13 μmol (CO₂) mol (H₂O)⁻¹ in P. latifolia. P. latifolia had the highest $R_D$ [1.7 ± 0.5 μmol (CO₂) m⁻² s⁻¹], followed by P. lentiscus and Q. ilex [1.2 ± 0.3 μmol (CO₂) m⁻² s⁻¹, mean value], and M. communis [0.6 ± 0.1 μmol (CO₂) m⁻² s⁻¹]. In October, $P_N$, $g_s$ increased compared to the rates monitored in September in all the considered species while $R_D$ increased in P. latifolia, P. lentiscus and M. communis and decreased in Q. ilex.

In September, P. latifolia had the highest $R_D/P_N$ ratio (0.17 ± 0.02) followed by P. lentiscus (0.15 ± 0.04), Q. ilex (0.12 ± 0.01) and M. communis (0.09 ± 0.02).

$Q_{10}$

Q. ilex and P. latifolia showed the highest $Q_{10}$ value (1.78 ± 0.01, mean value) followed by P. lentiscus (1.47 ± 0.03) and M. communis (1.44 ± 0.02).

Leaf to air vapour pressure deficit

The seasonal VPD trend of the considered species is shown in Figure 3. The considered species had similar VPD trend with the lowest values in January ranging from 0.21 ± 0.06 kPa (in P. latifolia) to 0.33 ± 0.05 kPa (in M. communis). VPD increased from February (0.62 ± 0.06 kPa, mean value) to August (1.42 ± 0.21 kPa, mean value) when Q. ilex had the highest VPD (1.70 ± 0.12 kPa) and P. lentiscus the lowest one (1.20 ± 0.09). In September and October VPD, on an average, decreased by 7% and 45% respectively, compared to August.

Statistical analysis

The results of the regression analysis showed a significant relationship between $R_D$ and $T_{ch}$ and between $g_s$ and VPD (Figures 4 and 5).
The PCA analysis extracted two factors accounting for 76% of the total variance among the considered species (48% and 28% for the 1st and the 2nd factor, respectively). The 1st factor was related to physiological traits ($P_N$ in summer, $g_s$ in spring, IWUE in spring and summer, $R_D$ in summer and spring) and anatomical leaf traits ($L$, $SPL$, $SPA$ and $SD$). The 2nd factor was mainly related to morphological leaf traits ($LMA$ and $LTD$), and to $P_N$ in winter and spring, and IWUE in winter. According to these results, the considered species were divided into three groups (Figure 6): the 1st group included $P. lentiscus$ and $Q. ilex$, the 2nd group $P. latifolia$, and the 3rd group $M. communis$.

**Discussion**
Knowledge of plant species response to limited soil moisture is important for providing insights into potential...
ecological impacts on wild populations (Wu et al. 2010) also in consideration of climate change scenarios which hypothesizes an increasing aridity in many regions worldwide (Canadell et al. 2007). Our results on the whole underline different morphological, anatomical and physiological leaf traits of the considered shrub species which are indicative of their adaptive capability to Mediterranean climate stress factors. Among the considered species, \textit{Q. ilex} has the highest \( P_N \) in spring associated to the highest \( R \) which may be related to the concomitance of vegetative activity (spring shoots production) and flowering (Gratani et al. 1996). \textit{Q. ilex} has the largest tolerance to low winter air temperatures evidenced by the lowest \( P_N \) decrease (68\% of the maximum) and low \( R_D \) rates (87\% of the maximum). Under drought conditions, plants optimize carbon assimilation and minimize water loss by decreasing \( g_s \) (Medrano et al. 2002), and IWUE may be considered a good indicator of carbon assimilation optimization. \textit{Q. ilex} has a high responsiveness to drought showing a high \( g_s \) decrease at the beginning of June (41\% lower compared to the maximum) associated with a 22\% \( P_N \) decrease determining a 40\% IWUE increase compared to the maximum. The responsive stomatal behaviour is also underlined by the significant relationship between \( g_s \) and VPD (\( R^2 = 0.46 \)). As drought stress progresses in July, \textit{Q. ilex} IWUE does not significantly increase because of \( P_N \) and \( g_s \) change to the same extent. At the highest drought intensity (August) IWUE decreases by 10\% compared to June, due to a higher \( P_N \) decrease than \( g_s \). Despite the high \( P_N \) decrease in August

**Figure 2** Leaf respiration (\( R_D \)) trend of \textit{P. lentiscus} (close squares), \textit{P. latifolia} (open circles), \textit{M. communis} (close triangles) and \textit{Q. ilex} (open squares) during the study period. The mean values for each month (± SE) are shown (\( n = 40 \) leaves). Mean values with the same letters are not significantly different (\( p \geq 0.05 \)). Lowercase letters indicate the differences among the species for each month, capital letters indicate the intra-specific differences during the study period.

**Figure 3** Leaf to air vapor pressure deficit (\( \text{VPD}_{\text{leaf}} \)) trend of \textit{P. lentiscus} (close squares), \textit{P. latifolia} (open circles), \textit{M. communis} (close triangles) and \textit{Q. ilex} (open squares) during the study period. The mean values for each month (± SE) are shown (\( n = 40 \) leaves). Mean values with the same letters are not significantly different (\( p \geq 0.05 \)). Lowercase letters indicate the differences among the species for each month, capital letters indicate the intra-specific differences during the study period.
(by 65% compared to the maximum), *Q. ilex* is able to recover 65% of the spring rates in September. Gratani and Varone (2003) underline the sufficiently high leaf water potential and relative water content during drought in *Q. ilex*. Moreover, the results underline that *Q. ilex* does not seem to suffer significant metabolic damage that could make a demand on respiratory products as drought stress progresses, according to the results of Rodríguez-Calcerrada et al. (2011). This is also pointed out by a 13% $R_D$ decrease in August compared to the spring rates. Due to the high $P_N$ decrease, *Q. ilex* shows a relatively high $R_D/P_N$ ratio (0.61 ± 0.06) in August. The most important factor determining how negative the plant carbon balance becomes under water stress is the absolute and proportional change in $P_N$ rates since drought has typically a greater proportional inhibitory effect on photosynthesis than on respiration thus, resulting in a higher $R_D/P_N$ ratio (Galmés et al. 2007). As regards leaf anatomy and morphology, *Q. ilex* high SD and low SPL and SPA, associated to a high LMA and LTD, contribute to an...
efficient control of gas exchange. Niinemets (2001) under-
lines that the adaptive significance of leaves characterised
by thick cell walls and low fractions of intercellular air
spaces (i.e. high LMA and LTD) lies in their large elastic
module which upholds water flow from drying soils.

P. lentiscus strategy to stress factors is similar to that of
Q. ilex (i.e. high $P_N$ in spring and a relatively high $P_N$
in winter associated to a high $R_D$). In August $g_s$ and $P_N$
decrease by 72% and 63%, respectively, and $R_D$ increases by
43% resulting in a high $R_D/P_N$ ratio (0.94 ± 0.05).

The similar strategy of P. lentiscus and Q. ilex is also
underlined by their similar IWUE values during the
study period and their $P_N$ recovery capability in September.
Moreover, P. lentiscus shows a higher relationship be-
tween $g_s$ and VPD ($R^2 = 0.33$) as well as Q. ilex. A higher
$P_N$ recovery capability might be related to the capacity
of this species to have low leaf water potential and rela-
tive water content variations during the year (Gratani
and Varone 2004). At morphological and anatomical
levels, P. lentiscus is characterised by a high LMA and
LTD. In particular, the larger SPL, SPW and SPA in P.
lentiscus with respect to Q. ilex may be related to its
origin from the semi-arid steppes of central Asia with
an exceptionally hot summer and an exceptionally cold
and dry winter (Blondel and Aronson 1999). Billing et al.
(1971) and Cunningham and Read (2003) hypothesize that
plant species which have originated in climates with more
fluctuating temperatures may have a higher gas-exchange
acclimation to air temperature than those originated in
more constant climate. Thus, Q. ilex and P. lentiscus
capability to maintain sufficiently high photosynthetic
rates both in cold and drought stress periods seem to
be related to their origin under a climate characterized
by a pronounced seasonality.

Compared to the considered species, P. latifolia has the
lowest $P_N$ decrease in drought (45% compared to the
spring maximum) associated to a 74% $g_s$ decrease resulting
in a high IWUE. The lower $R_D/P_N$ ratio (0.56 ± 0.04) in
P. latifolia compared to P. lentiscus and Q. ilex, is due to
the lowest $P_N$ decrease in drought. The high P. latifolia
photosynthetic recovery capacity in September (64% of
the maximum) after the first rainfall following drought
attests to its greater drought tolerance through the
maintenance of a high $P_N$ rate even at low leaf water
potential (Bombelli and Gratani 2003). Moreover, the
high P. latifolia LMA, due to the presence of thick cell
walls and sclereids (Gratani and Bombelli 1999) and
the high LTD (i.e. a densely packed mesophyll cells
with few air spaces, Gratani and Bombelli 2000) con-
tribute to improve drought resistance by improving water
use efficiency (Niinemets 2001) and limiting photochem-
ical damage to the photosynthetic apparatus through the
reduction of the incident irradiance (Jordan et al. 2005).
On the contrary, the lower P. latifolia $P_N$ in winter
compared to the maximum underlines its lower tolerance to cold temperatures, according to the results of Ogaya and Peñuelas (2003), and Ogaya et al. (2011), also pointed out by the highest $R_D/P_N$ (0.75 ± 0.03). The lowest $R_D$ rates in January underline the limitation of the enzyme activity of the respiratory apparatus (i.e. glycolysis, the TCA cycle and mitochondrial electron transport chain) (Atkin and Tjoelker 2003).

$M. \text{ communis}$ has a physiological response to drought similar to that of $P. \text{ latifolia}$, which may be related to their common origin in the dry tropics of the continental Africa and adjacent regions (Blondel and Aronson 1999). $M. \text{ communis}$ has a low $P_N$ decrease (by 46%) during drought associated to stable $R_D$ rates which determine a lower $R_D/P_N$ ratio (0.43 ± 0.02). In winter a 92% $P_N$ decrease associated to a 79% $R_D$ decrease results in a higher $R_D/P_N$ ratio (0.61 ± 0.02). Hernández et al. (2010) underline that $M. \text{ communis}$ has a low capacity to transport water from roots to leaves also under water availability. Gratani et al. (1980) show its low biomass production capability respect to other Mediterranean shrubs which are pointed out by the significant lowest $M. \text{ communis}$ $P_N$ rates during the study period compared to the other considered species. Moreover, the low stomatal control of $M. \text{ communis}$ is pointed out by a lower relationship between $g_s$ and VPD ($R^2 = 0.29$). Despite the highest SD, $M. \text{ communis}$ has a very small SPL and SPW which could explain the low $g_s$. The lower LMA and LTD $M. \text{ communis}$ with respect to $P. \text{ latifolia}$ underline a lower leaf consistency. The above considerations are confirmed by the PCA showing a higher similarity between $Q. \text{ ilex}$ and $P. \text{ lentiscus}$ compared to $P. \text{ latifolia}$ and $M. \text{ communis}$.

Chu et al. (2011) suggest that $R_D/P_N$ ratio can be considered as a simple approach to leaf carbon balance because it indicates the percentage of photosynthates that is respired. Our results show that $R_D/P_N$ ratio of the considered species, calculated over the study period, varies from 0.15 ± 0.04 in autumn, 0.24 ± 0.05 in spring, through 0.29 ± 0.15 in winter to 0.46 ± 0.11 in summer, and it is indicative of the different sensitivity of both $R_D$ and $P_N$ to water availability and air temperature changes, according to results of Zaragoza-Castells et al. (2008). The low $R_D/P_N$ ratio in autumn and spring of the considered Mediterranean evergreen species (i.e. during vegetative activity) underlines the highest $P_N$ rates during the favorable periods, when resources are not limited, and leaves take in roughly three to five times more CO$_2$ than they lose by dissimilatory processes during the same period of time (Larcher 2003). On the contrary, the highest $R_D/P_N$ ratio in summer underlines the lower sensitivity of respiration to drought (Atkin and Macherel 2009) that is indicative of a higher proportion of fixed carbon which is respired at elevated temperature (Gratani et al. 2011; Riikonen et al. 2012). Thus, summer drought can reduce the carbon assimilation because of $R_D$ rates increasing more than $P_N$ rates.

It is known that over short-term rises in temperature, $R_D$ increases exponentially but the seasonal temperature sensitivity of $R_D$ is often lower than that observed over hours, a phenomenon known as thermal acclimation (Rodrííguez-Calcerrada et al. 2012). This phenomenon involves adjustments in $R_D$ rates to compensate for changes in air temperature (Atkin et al. 2000). In particular, acclimation of $R_D$ to high temperatures can result in a lower slope (i.e. lower $Q_{10}$) for the temperature-response curve of acclimated tissue (Atkin et al. 2000). Among the considered species, $M. \text{ communis}$ has the highest acclimation to high temperatures compared to the other species pointed out by the lower $Q_{10}$ value (1.44 ± 0.02) and by more stable $R_D$ rates during the year. There is growing evidence that acclimation of $R_D$ to heat and drought reflects the metabolic down-regulation that reduces carbon depletion and helps plants to grow and survive in Mediterranean-type environments (Rodríguez-Calcerrada et al. 2010, 2011). Understanding the function of plant species in water limited environments is crucial in order to make informed land management decisions (Maseyk et al. 2008). Moreover, under a Mediterranean type of climate, our results underline the importance of including seasonal variations of photosynthesis and respiration in carbon balance models.

**Conclusions**

Limitations to plant growth imposed by the Mediterranean climate are mainly due to carbon balance in response to stress factors. In particular, water stress associated to high air temperature and irradiance in summer causes a marked decrease in CO$_2$ assimilation. The results underline the response of the evergreen species co-occurring in the Mediterranean maquis to Mediterranean stress factors. In particular, the lower $R_D/P_N$ in autumn and spring underlines the highest $P_N$ rates during the favorable periods while the highest $R_D/P_N$ ratio in summer shows the lower sensitivity of respiration to drought. Among the considered species, $Q. \text{ ilex}$ and $P. \text{ lentiscus}$ have the largest tolerance to low winter temperatures while $P. \text{ latifolia}$ and $M. \text{ communis}$ to drought. Among the considered species, $M. \text{ communis}$ has the higher acclimation to high temperatures compared to the other species and this is underlined by the lower $Q_{10}$ value and the more stable $R_D$ rates during the year. The predicted global warming might differently affect carbon balance of the considered species, with a possible change in Mediterranean shrublands composition in the long term.

**Abbreviations**

DM: Leaf dry mass; $g_s$: Stomatal conductance; L: Leaf thickness; LA: Leaf surface area; LMA: Leaf mass area; LTD: Leaf tissue density; SD: Stomatal
density; SPA: Stomatal pore area; SPL: Stomatal pore length; SPW: Stomatal pore width; Pn: Net photosynthetic rate; Rr: Respiration rate; Ti: Leaf chamber air temperature; Lf: Leaf temperature; IWUE: Intrinsic water use efficiency.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
LG wrote the manuscript. RC carried out the experimental research and contributed to statistical analysis. LV carried out the experimental research and statistical analysis. All authors read and approved the final manuscript.

Acknowledgements
This paper was supported by the grants from Ministry of Agricultural, alimentary and Forestry politicians (MIPAF) for the years 2007–2010.

Received: 4 March 2011 Accepted: 1 April 2013
Published: 17 September 2013

References
Armstrong AF, Logan DC, Atkin OK (2006) On the development dependence of leaf respiration: responses to short- and long-term changes in growth temperature. Amer J Bot 93:1633–1639
Atkin OK, Machereil D (2009) The crucial role of plant mitochondria in orchestrating drought tolerance. Ann Bot 103:581–597
Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. Trends Plant Sci 8:340–351
Atkin OK, Evans JR, Ball MC, Siebel K (1998a) Relationship between the inhibition of leaf respiration by light and enhancement of dark leaf respiration following light treatment. Aust J Plant Physiol 25:437–433
Atkin OK, Evans JR, Ball MC, Siebel K, Pons TL, Lamberts H (1998b) Light inhibition of leaf respiration: the role of irradiance and temperature. In: Möller M, Gardestrom P, Gliminius K, Glaser E (ed) Plant Mitochondria: from Gene to Function. Backhays Publishers, Leiden, pp 567–574
Atkin OK, Edwards EJ, Lavery BR (2000) Response of root respiration to changes in temperature and its relevance to global warming. New Phytol 147:141–154
Atkin OK, Zhang QS, Wilschut JT (2002) Effect of temperature on rates of alternative and cytochrome pathway respiration and their relationship with the redox poise of the quinone pool. Plant Physiol 128:212–222
Atkin OK, Bruhn D, Tjoelker MG (2003) Response of plant respiration to changes in temperature: mechanisms and consequences of variations in Q10 values and acclimation. In: Lambers H, Ribas-Carbo M (ed) Plant Respiration. From Cell to Ecosystem. Springer, Dordrecht, The Netherlands, pp 95–135
Atkin OK, Scheuwer L, Pons TL (2007) Respiration as a percentage of daily photosynthesis in whole plants is homeostatic at moderate, but not high, growth temperatures. New Phytol 174:367–380
Atwell BJ, Kriedemann PE, Turnbull CGN (ed) (1999) Plants in Action. Adaptation to Environment. Macmillan Education Australia Pty Ltd, South Yarra
Baldocchi DD, Amthor JS (2001) Canopy photosynthesis. In: Roy J, Saugier B, Mooney HA (ed) Terrestrial global productivity. Academic Press, San Diego, CA, USA, pp 9–31
Bartoloni S, Minnoci A, Vitagliano C (1997) Influence of temperature on morpho-
Bombelli A, Gratani L (2003) Interspecific differences of leaf gas exchange and whole-plant performance of nine tropical tree species at two sites with contrasting water availability in Panama. Trees 27:639–653
Cunningham SC, Read J (2003) Do temperate rainforest trees have a greater ability to acclimate to changing temperatures than tropical rainforest trees? New Phytol 157:55–64
Dawson IK, Lengkeek A, Weber JC, Jamnadass R (2009) Managing genetic variation in tropical trees: linking knowledge with action in agroforestry ecosystem for improved conservation and enhanced livelihood. Biodivers Conserv 18:969–986
De Boeck HJ, Lemmens CMHM, Vicca S, den Berge AV, Van Dongen S, Lammens IA, Ceulemans R, Njøl H (2007) How do climate warming and species richness affect CO2 fluxes in experimental grasslands? New Phytol 175:512–522
Díaz-Barradas MC, Zunzunegui M, Ain-Lhou F, Járquej H, Boutealeb S, Álvarez-Cansino L, Esquivias MP (2010) Seasonal physiological responses of Argania spinosa tree from Mediterranean to semi-arid climate. Plant Soil 337:217–231
Galmés J, Ribas-Carbo M, Medirando H, Flexas J (2007) Response of leaf respiration to water stress in Mediterranean species with different growth forms. J AEd Environ 68:206–222
Gebrekristos A, van Noordwijk M, Neufeldt H, Mittler R (2011) Relationships of stable carbon isotopes, plant water potential and growth: an approach to assess water use efficiency and growth strategies of dry land agroforestry species. Trees 25:195–202
Gimeno TE, Somerville KE, Valladares F, Atkin OK (2010) Homeostasis of respiration under drought and its important consequences for foliar carbon balance in a drier climate: insights from two contrasting Acacia species. Funct Plant Biol 37:233–233
González-Varo JP (2010) Fragmentation, habitat composition and the dispersal/predation balance in interactions between the Mediterranean myrtle and avian frugivores. Ecography 33:185–197
González-Varo JP, Rafael G, Albareldo RG, Aparicio A (2009) Mating patterns and spatial distribution of conspecific neighbours in the Mediterranean shrub Myrtus communis (Myrtaeae). Plant Ecol 203:207–215
Grant DA (1990) Plant response to atmospheric humidity. Plant Cell Environ 13:667–679
Gratani L, Bombelli A (1999) Leaf anatomy, inclination, and gas exchange relationships in evergreen sclerophyllous and drought semideciduous shrub species. Photosynthetica 37:573–585
Gratani L, Bombelli A (2000) Correlation between leaf age and other leaf traits in three Mediterranean maquis shrub species: Quercus ilex, Phyllaea latifolia and Cistus incanus. Environ Exp Bot 43:141–153
Gratani L, Varone L (2005) Drought-adaptive responses of the Mediterranean shrub species. Atti del Tredicesimo Congreso Nazionale della S.I.T.E. Como (I), 8–10 Settembre. In: Processing of the 13th National Congress of the Italian Ecology Society (S.I.T.E.), Como, Italy, pp 8–10
Gratani L, Varone L (2004) Adaptive photosynthetic strategies of the Mediterranean maquis species according to their origin. Photosynthetica 42:551–558
Gratani L, Varone L (2006) Long-time variations in leaf mass and area of Mediterranean evergreen broad-leaf and narrow-leaf maquis species. Photosynthetica 44:161–168
Gratani L, Amendol A, Verl I, Bruno F, Poni M (1988) Determinazione di un metodo di stima della Biomassa nella macchia di Castelporziano (Lazio). Annali di Botanica XXXI:131–151
Gratani L, Tisi F, Crescentino MF, Pesoli P, Larcher W (2006) Thermal acclimation and the development dependence of leaf respiration: responses to short- and long-term changes in growth temperature. Trends Plant Sci 11:325–331
Gratani L, Meneghini M, Pesoli P, Crescente MF (2003) Structural and functional relationships in evergreen sclerophyllous and drought semideciduous shrub species. Photosynthetica 41:619–625
Cai ZQ, Ston M, Fan ZX (2005) Leaf development and photosynthetic properties of three tropical species with delayed greening. Photosynthetica 43:91–98
Canadell JG, Pataki DE, Peltola LF (ed) (2007) Terrestrial ecosystems in a changing World. Springer, Berlin
Chu Z, Yijun L, Chang J, Wang M, Jiang H, He J, Peng C, Ge Y (2011) Leaf respiration/photosynthesis relationship and variation: an investigation of 39 woody and herbaceous species in east subtropical China. Trees 25:301–310
Correia O, Díaz Barradas MC (2000) Ecophysiological differences between male and female plants of Pistacia lentiscus L. Plant Ecol 149:131–142
Craven D, Hall JS, Ashton MS, Berlyn GP (2013) Water-use efficiency and whole-plant performance of nine tropical tree species at two sites with contrasting water availability in Panama. Trees 27:639–653

http://www.as-botanicalstudies.com/content/54/1/35

Gratani et al. Botanical Studies 2013, 54:35

Page 11 of 12
