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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 CO₂ 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 CO₂ 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*

* Correspondence: loretta.gratani@uniroma1.it
Department of Environmental Biology, Sapienza University of Rome, P.le A. Moro, 5 00185, Rome, Italy

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; Way and Sage 2008; Shen et al. 2009). Photosynthesis often decreases sharply at temperatures above its optimum (Sage and Kubien 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 (Llusiá 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 $5.3 \pm 0.2^\circ\text{C}$, the mean maximum air temperature (T_{\max}) of the hottest months (July and August) was $30.9 \pm 0.2^\circ\text{C}$, and the yearly mean air temperature (T_m) was $16.8 \pm 6.5^\circ\text{C}$. 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 $3.8 \pm 3.1^\circ\text{C}$, T_{\max} of the hottest month (July) $34.0 \pm 2.2^\circ\text{C}$, 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: $(\pi \times \text{length} \times \text{width})/4$, 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, cm^2) (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 , $\mu\text{mol (CO}_2\text{) m}^{-2} \text{ s}^{-1}$], photosynthetically active radiation [PAR, $\mu\text{mol (photons) m}^{-2} \text{ s}^{-1}$], stomatal conductance [g_s , $\text{mol (H}_2\text{O) m}^{-2} \text{ s}^{-1}$], leaf temperature (T_l , $^\circ\text{C}$), and leaf chamber air temperature (T_{ch} , $^\circ\text{C}$) 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 (VPD_{leaf} , kPa) was calculated according to Grantz (1990) as: $VPD = e_s - e_a$, where e_s was saturated vapour pressure at leaf temperature and e_a the air vapour pressure. The intrinsic water use efficiency [IWUE, $\mu\text{mol}(\text{CO}_2) \text{mol}(\text{H}_2\text{O})^{-1}$] 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 ($PAR \geq 1200 \mu\text{mol m}^{-2} \text{s}^{-1}$), 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 , $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$] measurements were carried out contemporary 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 CO_2 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_{ch} 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 $311 \pm 10 \mu\text{m}$ (*M. communis*) to

$419 \pm 25 \mu\text{m}$ (*P. latifolia*). SD varied significantly ($p < 0.05$): *M. communis* had the highest SD ($508 \pm 82 \text{ stomata mm}^{-2}$), followed by *Q. ilex* and *P. lentiscus* ($419 \pm 18 \text{ stomata mm}^{-2}$, mean value), then by *P. latifolia* ($238 \pm 21 \text{ stomata mm}^{-2}$). *P. latifolia* had the highest SPL and SPA ($19.5 \pm 2.6 \mu\text{m}$, $170.3 \pm 31.3 \mu\text{m}^2$, respectively), and *Q. ilex* the lowest ones ($9.1 \pm 1.9 \mu\text{m}$, $42.1 \pm 12.4 \mu\text{m}^2$, respectively). LMA varied from $9 \pm 1 \text{ mg cm}^{-2}$ (*M. communis*) to $20 \pm 1 \text{ mg cm}^{-2}$ (*P. latifolia*) (Table 2). *Q. ilex* had the highest LTD ($613 \pm 40 \text{ mg cm}^{-3}$) and *M. communis* the lowest one ($304 \pm 51 \text{ mg cm}^{-3}$).

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 $23.8 \pm 3.0^\circ\text{C}$, 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 [$15.4 \pm 0.2 \mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$, mean value measured in May] than *M. communis* [$10.7 \pm 0.7 \mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$].

g_s of *M. communis* [$0.11 \pm 0.04 \text{ mol}(\text{H}_2\text{O}) \text{m}^{-2} \text{s}^{-1}$, 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 $68 \pm 6 \mu\text{mol}(\text{CO}_2) \text{mol}(\text{H}_2\text{O})^{-1}$, respectively] while *Q. ilex* and *M. communis* in April [81 ± 9 and $98 \pm 7 \mu\text{mol}(\text{CO}_2) \text{mol}(\text{H}_2\text{O})^{-1}$, respectively].

A different R_D trend was observed in spring: *P. lentiscus* and *Q. ilex* R_D peaked in May [$3.7 \pm 0.3 \mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$, mean value], while *P. latifolia* in March [$R_D = 4.3 \pm 0.6 \mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$]. *M. communis* R_D was not significantly different from March to May [$2.7 \pm 0.1 \mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$, 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).

Table 1 Anatomical leaf traits at full leaf expansion of the considered species

Species	L (μm)	SPL (μm)	SPW (μm)	SPA (μm^2)	SD (stomata mm^{-2})
<i>P. lentiscus</i>	378 ± 54 a	11.7 ± 1.3 a	7.8 ± 1.3 a	71.8 ± 12.5 a	406 ± 20 a
<i>P. latifolia</i>	419 ± 25 a	19.5 ± 2.6 b	11.1 ± 1.3 b	170.3 ± 31.3 b	238 ± 21 b
<i>M. communis</i>	311 ± 10 b	10.5 ± 2.0 ac	5.4 ± 1.0 c	45.5 ± 15.2 c	508 ± 82 c
<i>Q. ilex</i>	314 ± 59 b	9.1 ± 1.9 c	5.9 ± 0.6 c	42.1 ± 12.4 c	432 ± 34 a

L, leaf thickness; SPL, stomatal pore length; SPW, stomatal pore width; SPA, stomatal pore area; SD, stomatal density. Mean values (\pm SE) are shown ($n = 20$). Mean values with the same letters are not significantly different (Tukey Test, $p \geq 0.05$).

Table 2 Morphological leaf traits at full leaf expansion of the considered species

Species	DM (mg)	LA (cm ²)	LMA (mg cm ⁻²)	LTD (mg cm ⁻³)
<i>P. lentiscus</i>	229.7 ± 53.4 a	11.7 ± 2.3 a	19 ± 2 a	521 ± 57 a
<i>P. latifolia</i>	105.4 ± 11.3 b	5.2 ± 0.4 b	20 ± 1 a	472 ± 24 a
<i>M. communis</i>	31.5 ± 7.8 c	3.5 ± 0.4 c	9 ± 1 b	304 ± 51 b
<i>Q. ilex</i>	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).

Q₁₀

Q. ilex and *P. latifolia* showed the highest Q₁₀ 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).

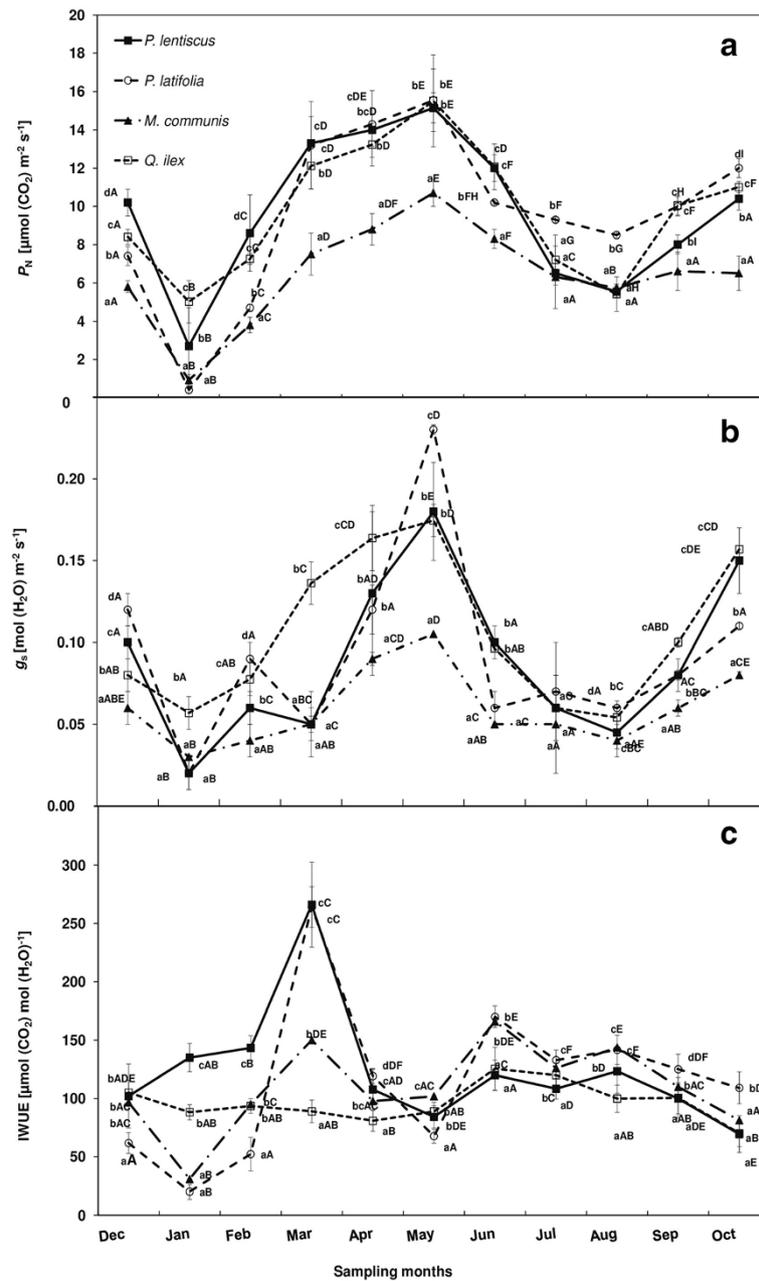


Figure 1 Trend of a) net photosynthetic rate (P_N), b) stomatal conductance (g_s), c) intrinsic water use efficiency (IWUE) of *P. lentiscus* (close squares), *P. latifolia* (open circles), *M. communis* (close triangles), and *Q. ilex* (open squares) during the study period. The mean values for each month (\pm 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.

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

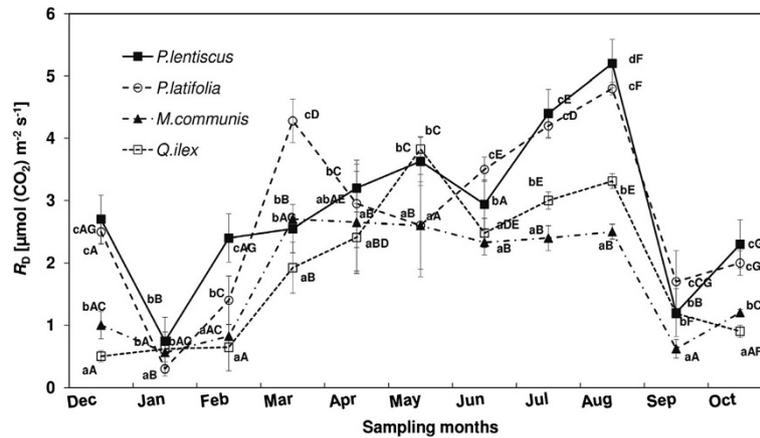


Figure 2 Leaf respiration (R_D) trend of *P. lentiscus* (close squares), *P. latifolia* (open circles), *M. communis* (close triangles) and *Q. ilex* (open squares) during the study period. The mean values for each month (\pm SE) are shown ($n = 40$ leaves). Mean values with the same letters are not 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.

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, *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). *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. *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, *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

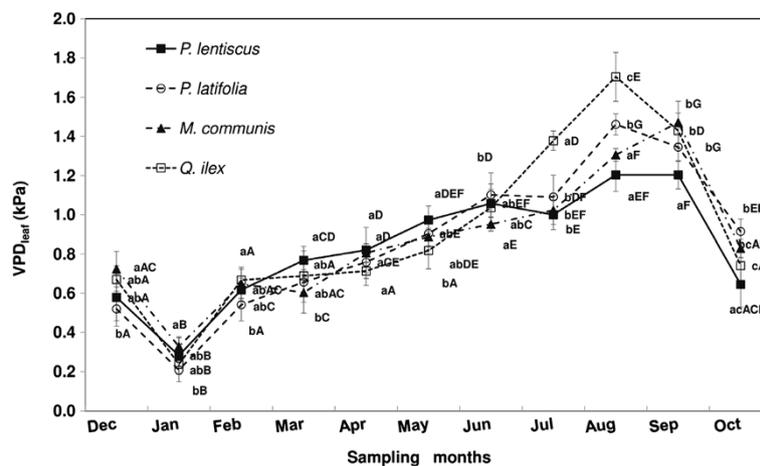


Figure 3 Leaf to air vapor pressure deficit (VPD_{leaf}) trend of *P. lentiscus* (close squares), *P. latifolia* (open circles), *M. communis* (close triangles) and *Q. ilex* (open squares) during the study period. The mean values for each month (\pm SE) are shown ($n = 40$ leaves). Mean values with 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.

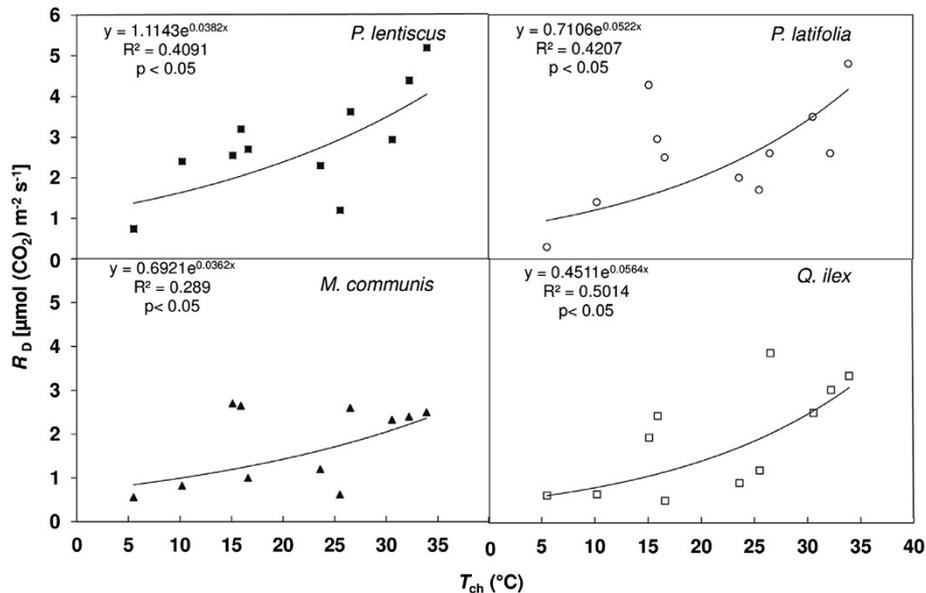


Figure 4 Regression analysis between leaf respiration (R_D) and leaf chamber air temperature (T_{ch}) for the considered species. Regression equation, determination's coefficient (R^2) and P-level are shown.

(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

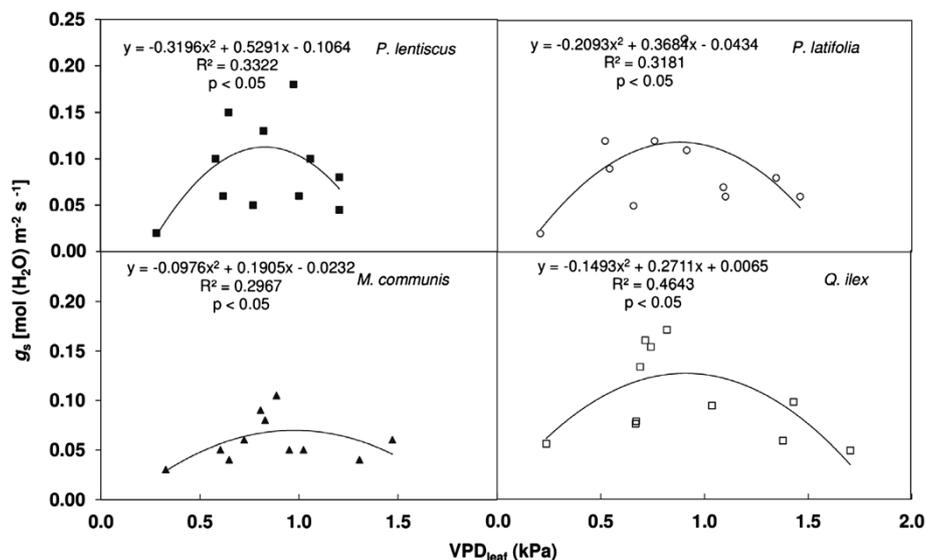


Figure 5 Regression analysis between stomatal conductance (g_s) and leaf to air vapour pressure deficit (VPD_{leaf}) for the considered species. Regression equation, determination's coefficient (R^2) and P-level are shown.

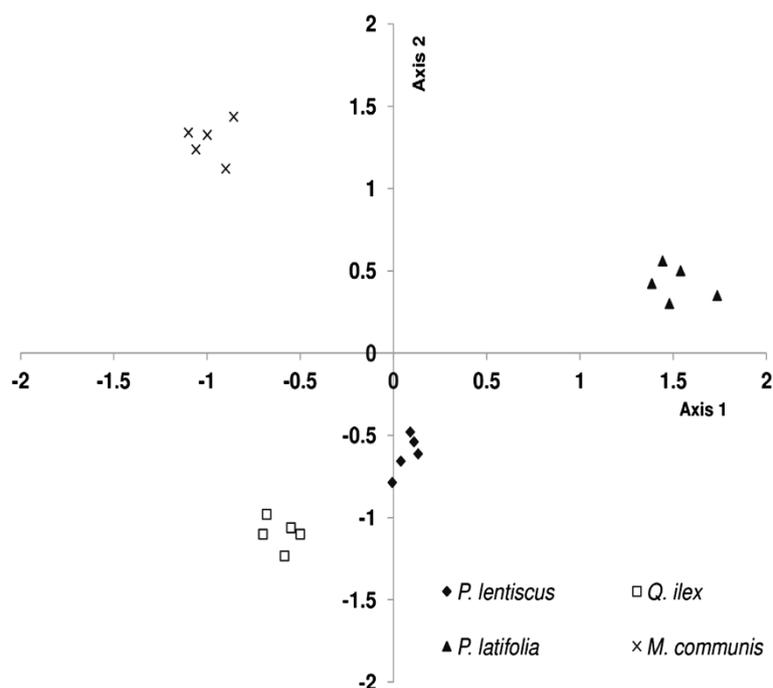


Figure 6 Principal component analysis (PCA) carried out using physiological traits in winter, spring and summer (photosynthetic rates stomatal conductance transpiration rates intrinsic water use efficiency and leaf respiration) and morphological and anatomical leaf traits (LMA, LTD, leaf thickness, stomatal density, stomatal pore length and stomatal pore area) for the considered species.

efficient control of gas exchange. Niinemets (2001) underlines 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 between 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 relative 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) contribute to improve drought resistance by improving water use efficiency (Niinemets 2001) and limiting photochemical 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. communis has a physiological response to drought similar to that of *P. 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. 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. 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. communis* P_N rates during the study period compared to the other considered species. Moreover, the low stomatal control of *M. communis* is pointed out by a lower relationship between g_s and VPD ($R^2 = 0.29$). Despite the highest SD, *M. communis* has a very small SPL and SPW which could explain the low g_s . The lower LMA and LTD *M. communis* with respect to *P. latifolia* underline a lower leaf consistency. The above considerations are confirmed by the PCA showing a higher similarity between *Q. ilex* and *P. lentiscus* compared to *P. latifolia* and *M. 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 (Rodríguez-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. communis* has the higher 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. ilex* and *P. lentiscus* have the largest tolerance to low winter temperatures while *P. latifolia* and *M. communis* to drought. Among the considered species, *M. 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; P_N : Net photosynthetic rate; R_D : Respiration rate; T_{ch} : Leaf chamber air temperature; T_l : 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.

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