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ORIGINAL PAPER

Comparative study of diurnal and nocturnal sap flow of *Quercus ilex* and *Phillyrea latifolia* in a Mediterranean holm oak forest in Prades (Catalonia, NE Spain)

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Abstract Droughts are a cyclical disturbance in Mediterranean ecosystems and climate change models forecast an increase of their frequency and severity. Some experimental and observational studies have shown that co-occurring species may cope with drier conditions with different strategies and present different responses. Here, we investigate sap flow response to drought in order to explain the observed differential growth and mortality of *Quercus ilex* and *Phillyrea latifolia* at Prades Holm oak forest (NE Spain). We measured sap flow of these species and compared their diurnal, nocturnal and seasonal patterns and their relationship with environmental variables. Both species described qualitatively similar daily patterns, either during daylight or night. Sap flow rates were significantly higher in *P. latifolia* except in autumn and spring. *P. latifolia* was more sensitive to soil moisture. Nocturnal sap flow was detected in both species with no significant differences and hourly rates suffered a progressive increase from 3 a.m. to dawn in most sampled nights. Our results indicate a better adaptation of *P. latifolia* to this site as it can take better advantage of wet periods while maintaining higher sap flow rates during dry periods. Along with previous ecophysiological studies at the same location it may be inferred that at its drier distributional limit *Q. ilex* would be at disadvantage with respect to other species like *P. latifolia*, as the latter would cope better with increasing xeric conditions already occurring and further predicted for Mediterranean ecosystems. Our results also show nocturnal sap flow to be relevant in individual water losses in these two species as they can be up to 35–40 % of daily sap flow. Further research on the underlying mechanisms of this nocturnal sap flow is required since it may also enhance early morning CO_2 fixation or nutrient supply to leaves.

Keywords *Phillyrea latifolia* · *Quercus ilex* · Sap flow · Nocturnal sap flow · Drought

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Introduction

Water scarcity represents one of the major constraints for plant communities and its importance is likely to increase in the next decades as projected by global circulation models (IPCC 2007). Main consequences of droughts include growth decline, reduced primary production, widespread defoliation, tree mortality and changes of species distribution ranges (Allen et al. 2010; Carnicer et al. 2011; Mueller et al. 2005; Penuelas and Boada 2003; Penuelas et al. 2007). This is especially crucial for Mediterranean ecosystems where rising temperatures and the projected decrease in rainfall will magnify drought risk, as pointed out by many studies (David et al. 2007; Lindner et al. 2010; Lloret et al. 2009; Ogaya and Penuelas 2007b). Although most of Mediterranean species are relatively well adapted to drought, they do not cope equally with the longer dry periods predicted by models. Specific water use strategies are thus of significance to understand possible changes on stand composition and species' distribution range.

Holm oak (*Quercus ilex*) seems to effectively tolerate drought effects by reducing its leaf area and by stomatal

closure control (Limousin et al. 2009; Ogaya and Penuelas 2006; Villar-Salvador et al. 1997) and have a high plasticity in its hydraulic features, as yearly vessel diameter are well coupled with annual rainfall (Corcuera et al. 2004). Photosynthetic and transpiration rates during drought of *Q. ilex* are not always lower than those from co-occurring species with different water use strategies (Quero et al. 2011). However, it has been reported that nearby its drier distributional limit *Q. ilex* would be at disadvantage with respect to *Phillyrea latifolia*, a tall shrub more resistant to drought, which could eventually lead to a shift in community composition (Martinez-Vilalta et al. 2002a, 2003; Ogaya and Penuelas 2003a, 2006, 2007a, b; Ogaya et al. 2003, 2011; Penuelas et al. 1998, 2000). Sap flow measurements can provide further insights on the water use strategies of these two species and on possible future changes on stand composition for these areas, where differential responses of the leaf water potential, hydraulic conductivity and net photosynthetic rates of these two species to drought have been already demonstrated (Martinez-Vilalta et al. 2003; Ogaya and Penuelas 2003a).

All around the Mediterranean basin, several studies have already focused on sap flow of these species either together or separately. *Q. ilex* diurnal sap flow has been well studied at several different sites. It usually reaches a similar hourly maxima at any time of the year except at the end of the dry season (Infante et al. 2003) and decreases with summer drought (Infante et al. 2001, 2003; Limousin et al. 2009; Mereu et al. 2009; Tognetti et al. 1998; Van der Zande et al. 2009). After a midsummer minimum and after the rainfalls in late summer or autumn, sap flow peaks recover. Summer reduction in transpiration follows the reduction in soil water content, and is driven by stomata closure control (Martinez-Vilalta et al. 2003) in parallel to an increase of hydraulic resistance in the soil–plant-atmosphere continuum (Tognetti et al. 1998). Sometimes this is accompanied by a decline in leaf area (Limousin et al. 2009; Ogaya and Penuelas 2006; Villar-Salvador et al. 1997) although others did not observe it (Mereu et al. 2009). By contrast, there are not many studies of *P. latifolia*'s sap flow dynamics and results disagree even when conducted on climatically similar locations. While some authors observed that sap flow remained relatively stable over the course of the year (Gucci et al. 1999), others found declines in peaks of sap flow as summer drought progressed (Schiller et al. 2002), similarly than *Q. ilex*. Specific differences in sap flow quantities and seasonal variability are partly explained by differences in hydraulic architecture that provide *P. latifolia* with a vulnerability to xylem embolism lower than in *Q. ilex* (Martinez-Vilalta et al. 2002b). The same study found *Q. ilex* to have wider hydraulic conduits and higher hydraulic conductivity of branches and leaves. Consequently, the pressure causing 50 % loss of hydraulic conductivity is lower in *Q. ilex* than in *P. latifolia*. In another study, Martínez-Vilalta et al. (2003) monitored sap flow of both species in these mountains and reported a greater sensitivity of *Q. ilex* to soil moisture along with a more important decrease in whole-plant hydraulic conductivity coupled with drought. That specific water use behavior may partly explain the different physiological and demographical responses that were found in a nearby site submitted to the same experimental drought for 5 years (Ogaya and Penuelas 2007b). In the latter study, *Q. ilex* suffered a reduction in mean annual stem diameter increment at drought plots, while no significant differences were detected in *P. latifolia*. *P. latifolia* experienced also the lowest mortality. Furthermore, in another comparative study in the same experimental system, *P. latifolia* was the only species to increase water use efficiency by reducing transpiration losses during midday and the net photosynthetic rate showed no response to water availability above the threshold of 17 % of volumetric soil water content (Ogaya and Penuelas 2003a). In the frame of current and future climate change, it is now warranted to elucidate whether *Q. ilex* and *P. latifolia* sap flow have similar patterns or not in environmentally more extreme sites for *Q. ilex*.

Nocturnal water losses have been documented in a wide range of genera and life forms (Caird et al. 2007; Dawson et al. 2007) and can represent a significant fraction of the total daily water used (maximum between 20 and 50 %) when VPD and soil water content are high (Caird et al. 2007; Daley and Phillips 2006; Dawson et al. 2007; Phillips et al. 2010; Zeppel et al. 2010). It may constitute an important factor for water relations in Mediterranean ecosystems, where water availability represents a major environmental constraint (Penuelas et al. 1998). Hence, the investigation of the magnitude of nocturnal sap flow is warranted as it could imply significant differences on the water use strategy of *Q. ilex* and *P. latifolia*. Adaptive meanings of nocturnal sap flow include facilitation of early morning carbon fixation, nutrient supply to distal parts of the crown and delivery of dissolved O_2 via the parenchyma to woody tissue sinks, all of them mediated by a partial stomata aperture (Caird et al. 2007; Daley and Phillips 2006; Scholz et al. 2007; Snyder et al. 2003). Although there are no studies focused on nocturnal sap flow of *P. latifolia* or *Q. ilex*, Fisher et al. (2007) reported relative percentages of nighttime transpiration up to 20 % of the whole-day flow in *Q. douglasii* at the Californian oak-savannah. Furthermore, drought seemed to reduce absolute nighttime water losses in saplings of live oaks (*Q. virginiana* and *Q. oleoides*) (Cavender-Bares et al. 2007). In the same study, water losses appeared as a strict function of VPD and thus it was likely an unavoidable and non-adaptive process. Zeppel et al. (2010) found water losses to

be a greater contribution to nocturnal sap flow than stem refilling in two evergreen species (*Angophora bakeri* and *Eucalyptus parramattensis*) and mainly through stomata rather than the cuticle as Howard and Donovan (2007) observed in *Helianthus* species.

The aim of this study was to characterize sap flow of these two co-occurring species by comparing diurnal, nocturnal and seasonal sap flow patterns and relate them to environmental variables, mainly soil water availability and vapor pressure deficit (atmospheric evaporative demand), in order to gain knowledge on the mechanisms that drive to the drought effects on growth and mortality described on these populations by Ogaya and Peñuelas (2003a, b, 2007b). We aimed to conduct such characterization in a site where *Q. ilex* is suffering a clear decline caused by periodical droughts, which is exacerbated by shallow soils and high stem density. Our initial hypothesis was that the more drought tolerant *P. latifolia* would maintain higher relative sap flow quantities during dry periods, but would not increase them significantly during wet periods. On the other hand, we expected that *Q. ilex* would suffer a more drastic reduction in sap flow during summer drought while being more water consuming in wet periods thanks to their wider xylem conduits (Martinez-Vilalta et al. 2002b). Regarding nocturnal sap flow, we expected to observe higher rates in nights with high VPD and high soil moisture and in nights following days with high sap flow rates, since the diurnal depletion of water stores would require its subsequent refilling at night.

Methods

Study site and species

The present study was carried out in Prades holm oak forest in Southern Catalonia (NE Spain) (41°21′N, 1°2′E), at 950 m asl and on a south-facing slope (25 % slope). The climate is Mediterranean, with a mean annual rainfall of 610 mm and a mean temperature of 12.2 °C during the period of experimentation (1999–2009). Seasonal distribution of rainfall is irregular, with spring and autumn being the more rainy seasons and with a strong and usually long water shortage during summer months, which are also the hottest. Interannual variability is also high and ranges from 376 to 925 mm. The soil is a Dystric Cambisol over Paleozoic schist, and its depth ranges from 35 to 90 cm. The forest studied has a very dense multistem crown (20,700 stems ha^{-1}) and is dominated by *P. latifolia* (11,700 stems ha^{-1}) and *Q. ilex* (7,700 stems ha^{-1}), with limited presence of other evergreen species well adapted to dry conditions (*Arbutus unedo* L., *Erica arborea* L., *Juniperus oxycedrus* L., *Cistus albidus* L.). This forest has not suffered any significant disturbance since 60 years ago, and the maximum height of the dominant species is about 3–4 m tall (Ogaya et al. 2011).

Sap flow and meteorological measurements

Four sap flow sensors (Sap Flow Meter P4.2, Jiri Kucera-Environmental Measuring Systems, Brno, Czech Republic) were initially installed on two trees of each species studied, *Q. ilex* and *P. latifolia* (Table 1). These sap flow sensors are based on the tissue heat balance model (THB) with internal heating and sensing. They measure and log the sap flow in kg hr^{-1}. The heat balance of xylem through which the sap flow passes can be described by the general equation:

$$Q = \frac{P}{c_w \times d \times dT} - \frac{z}{c_w}$$

where Q is the sap flow rate (kg s^{-1}), P is the heat input power (W), dT is the temperature difference in the measuring point, c_w is the specific heat of water (J kg^{-1}) and z is the coefficient of heat losses from the measuring point (W degree^{-1}). Q value is obtained per cm of stem circumference, from the triangle-shaped heated space among the four sensors (d). In order to obtain the whole tree sap flow rate in terms of kg hr^{-1}, the initial values were extrapolated using the following formula:

$$Q_{tree} = Q \times (A - 6.28 \times B)$$

where A is the stem circumference (with bark) (cm) and B the bark plus phloem thickness (cm). Q_{tree} was later corrected by the proportion of sapwood area of each individual which is the effective conducting section. Finally, the values obtained (kg h^{-1}) were normalized dividing them by the individual basal area.

Sap flow sensors were powered by a battery, and when they did not receive enough energy to measure properly,

Table 1 Characteristics of the monitored trees

	Diameter at 50 cm height (cm)	Leaves biomass (kg)	Aboveground biomass (kg)	Sapwood area (cm^2)	Sapwood width (cm)
Phillyrea latifolia 1	9.33	0.94	17.16	33.11	1.5
Phillyrea latifolia 2	10.60	1.29	23.52	36.06	1.4
Quercus ilex 1	11.20	1.95	32.92	30.18	1.2
Quercus ilex 2	12.03	2.20	38.72	40.60	1.4

Aboveground and leaves biomass were obtained with the allometric relationships calculated by Ogaya et al. (2003) at the same location

they stopped recording automatically in order to avoid unreal fluctuations caused by low power. Thus, we only monitored sap flow from representative days per each season. An automatic meteorological station installed in the study site monitored temperature, photosynthetic active radiation, air humidity, precipitation and wind speed and direction each half hour. Soil moisture at 25 cm depth was measured continuously with ECH2O probes (Decagon Devices Inc., Pullman, WA, USA) (Fig. 1).

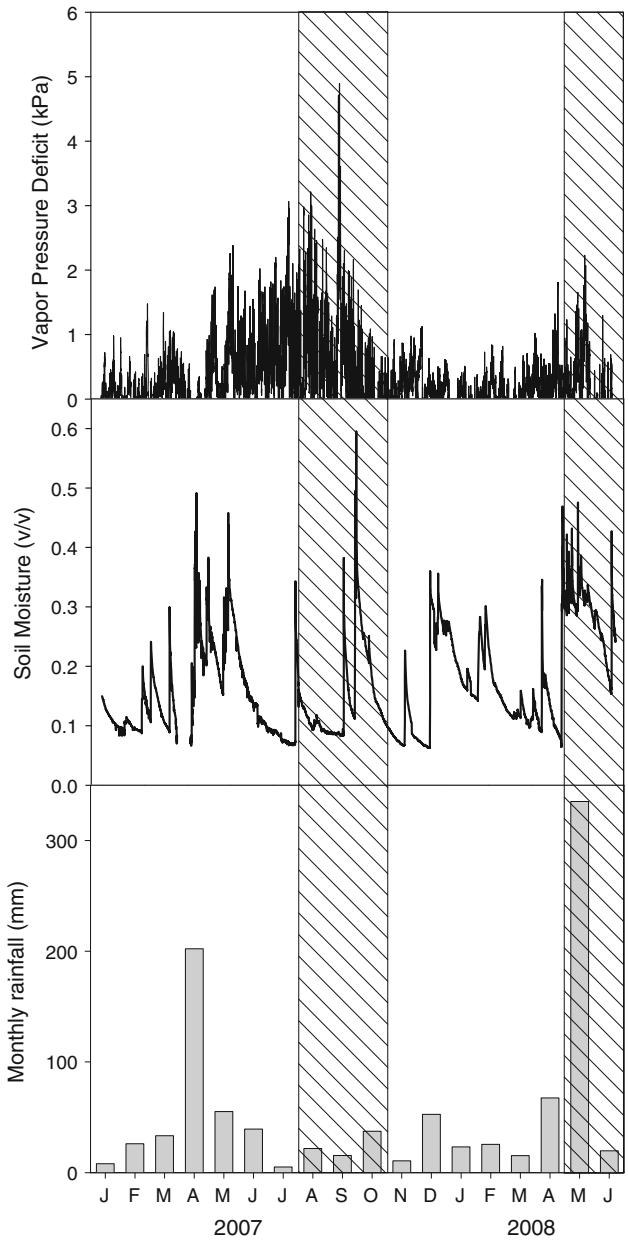

Fig. 1 Environmental conditions (vapor pressure deficit, soil moisture and monthly rainfall) from January 2007 to June 2008. Sampling periods are *highlighted*

Statistical analyzes

We performed a repeated measures ANOVA comparing species sap flow daily quantities. From the regression equation of sap flow hourly quantities as a function of vapor pressure deficit (VPD), we extracted the coefficient β for each day and registered its corresponding soil moisture. After that, we conducted a regression analysis of coefficient β (Sap flow vs. VPD) and soil moisture. We assessed species-specific differences in the response of β to soil moisture by using a separate slopes model after ensuring that the interaction between species and soil moisture was significant. Moreover, we conducted a multiple regression model for each species including sap flow hourly quantities as a dependent variable and continuous values of VPD and soil moisture as predictor variables. We used sap flow measured in the night (when PAR was zero) as our nocturnal sap flow variable. All statistical tests were conducted with the software Statistica (version 6.0, StatSoft, Tulsa, OK, USA).

Results

Both species followed similar daily sap flow patterns. Diurnal sap flow began before dawn and increased rapidly during the first 2 h of light. Then, it presented a sustained rise along midday and usually reached a peak just before nightfall (Fig. 2). Also nocturnal sap flow was noticeable and only in very few occasions sap flow was totally stopped. In both *Q. ilex* and *P. latifolia*, sap flow rates were low and stable until 3 a.m., where a progressive rise until dawn occurred. Such increase of sap flow rates was observed at different magnitudes in all seasons but spring (Fig. 3). As during daytime, there were no remarkable differences between species.

The mean of daily sap flow of both species varied among seasons spring > autumn > midsummer > late summer for *P. latifolia* and spring > midsummer > autumn > late summer for *Q. ilex*) although differences were not significant (Fig. 4). Nonetheless, some seasons presented high fluctuations among days. The maximum daily sap flow rates per cm^2 of basal area were found in midsummer (*Q. ilex*) and autumn (*P. latifolia*). However, in both species the seasonal mean of daily sap flow was higher in spring, matching up with the rainy seasons in Prades which at the same time had days with practically no flow due to a low vapor pressure deficit, the driving force of sap flow (Fig. 2). Late summer was the least active period for both species and also the maximum daily values were lower than in the other seasons (Figs. 2, 4). Sap flow series described different patterns depending on the season. The curve of sap flow was wider in midsummer than in autumn in response to the longer photoperiod (Fig. 2).

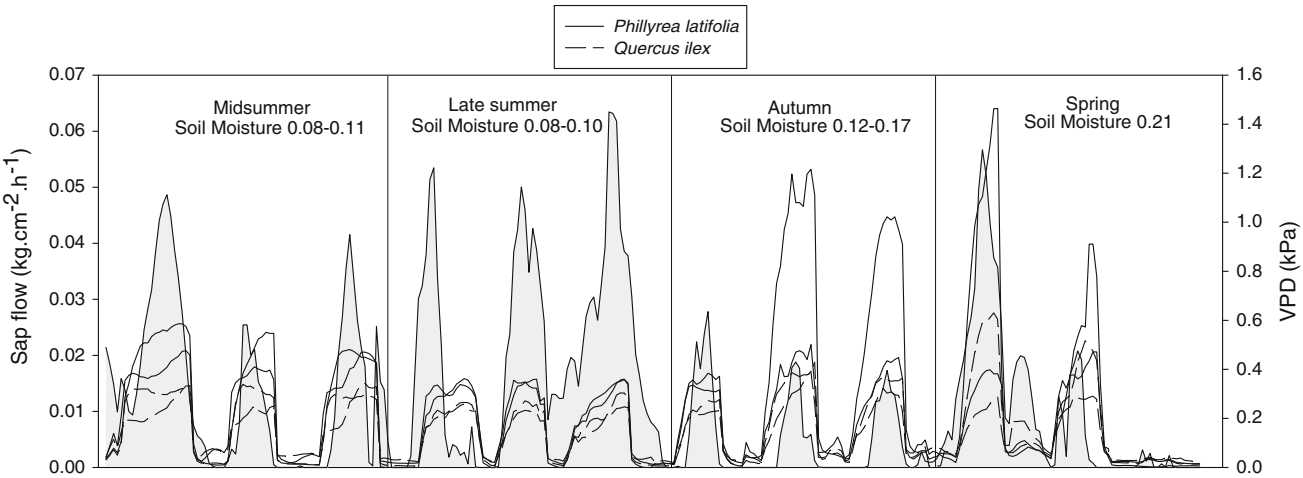

Fig. 2 Daily cycles of sap flow of two individuals of each species (*solid line P. latifolia* and *Q. ilex dashed line*). Three representative days per season are shown. *Filled area* corresponds to VPD (*right axis*) and soil moisture ranges for each 3-day period are depicted under season's name

Daily sap flow rates were 11.44 ± 3.65 kg d^{-1} for *Q. ilex* and 14.29 ± 6.12 kg d^{-1} for *P. latifolia* in the sampled days. *P. latifolia* had significantly higher daily sap flow rates per cm^2 of basal area than *Q. ilex* throughout the sampling season (0.23 ± 0.08 vs 0.15 ± 0.04 kg cm^{-2} d^{-1}, repeated measures ANOVA (RMA), Wilks' Lambda (WL) $= 0.82$, $p < 0.01$). Although mean of *P. latifolia* was higher for each season alone, differences were statistically significant in midsummer (0.27 ± 0.05 vs. 0.20 ± 0.02 kg cm^{-2} d^{-1}, RMA, WL $= 0.60$, $p < 0.05$), late summer (0.15 ± 0.02 vs. 0.11 ± 0.02 kg cm^{-2} d^{-1}, RMA, WL $= 0.54$, $p < 0.001$), and autumn (0.29 ± 0.11 vs. 0.16 ± 0.02 kg cm^{-2} d^{-1}, RMA, WL $= 0.64$, $p = 0.056$). Likewise, spring values were 33 % lower for *Q. ilex* than for *P. latifolia* but a large variance prevented to obtain significant differences (0.31 ± 0.10 vs. 0.21 ± 0.04 kg cm^{-2} d^{-1}, RMA, WL $= 0.77$, $p = 0.53$). We used allometric relationships calculated by Ogaya et al. (2003) at the same site to estimate total leaf area and from that to obtain sap flow relative to leaf area. Overall differences increased because *P. latifolia* has a lower leaf area (*P. latifolia* $= 1.97 \pm 0.26$ kg d^{-1} m^{-2} and *Q. ilex* $= 1.14 \pm 0.16$ kg d^{-1} m^{-2}).

Nighttime sap flow was detected in both species. Unlike the overall sap flow rates commented above, there were no differences between the two species in the whole sampling season nor when analyzing each season separately. The amount of nighttime sap flow was considerable since some days it represented up to 38 % (*P. latifolia*) and 39 % (*Q. ilex*) of whole-day flow. The average mean for the sampled days was 18.2 ± 8.9 % (*P. latifolia*) and 22.6 ± 7.5 % (*Q. ilex*). No significant differences were found among seasons although autumn values were typically higher than the others (Fig. 5). VPD alone could not explain the detection of sap flow at night globally nor seasonally, as even when VPD fell to 0 sap flow was appreciable, and in addition, hourly values of VPD and sap flow were not correlated.

Daily sap flow of both species seemed to be mainly determined by the atmospheric evaporative demand (VPD) and by soil moisture. However, the relationship of daily sap flow with these two environmental variables differed between *Q. ilex* and *P. latifolia* (Fig. 6). Since VPD is the driving force of sap flow, we evaluated changes in the relationship between sap flow and VPD at different levels of soil moisture (Fig. 7). Linear regression for *P. latifolia* fitted better than for *Q. ilex* ($R^2 = 0.62$ vs. $R^2 = 0.35$, $p < 0.01$ and $p < 0.05$, respectively) and furthermore, coefficient β was higher for *P. latifolia* (0.16 vs. 0.05, separate slope design with $F = 12.56$ and $p < 0.001$). In an exploratory analysis, we also performed a multiple regression model to assess soil moisture and VPD weight on sap flow hourly quantities. Results showed soil moisture as predominant on *P. latifolia* (soil moisture $\beta = 0.34$, VPD $\beta = 0.23$, $p < 0.001$). On the other hand, in *Q. ilex* there was a similar influence of VPD and soil moisture on sap flow performance (soil moisture $\beta = 0.32$, VPD $\beta = 0.32$, $p < 0.001$). Nevertheless, when soil moisture was below 10 %, sap flow peaks of both species did not follow VPD patterns.

Discussion

Overall sap flow rates of *P. latifolia* were unexpectedly higher than those of *Q. ilex* all along the sampling seasons although only clearly significantly in mid and late summer, the driest periods. Martinez-Vilalta et al. (2003) observed a similar difference between both species at the end of summer at a nearby site. In our study, *P. latifolia* integrated quantities and peaks of sap flow were clearly higher during

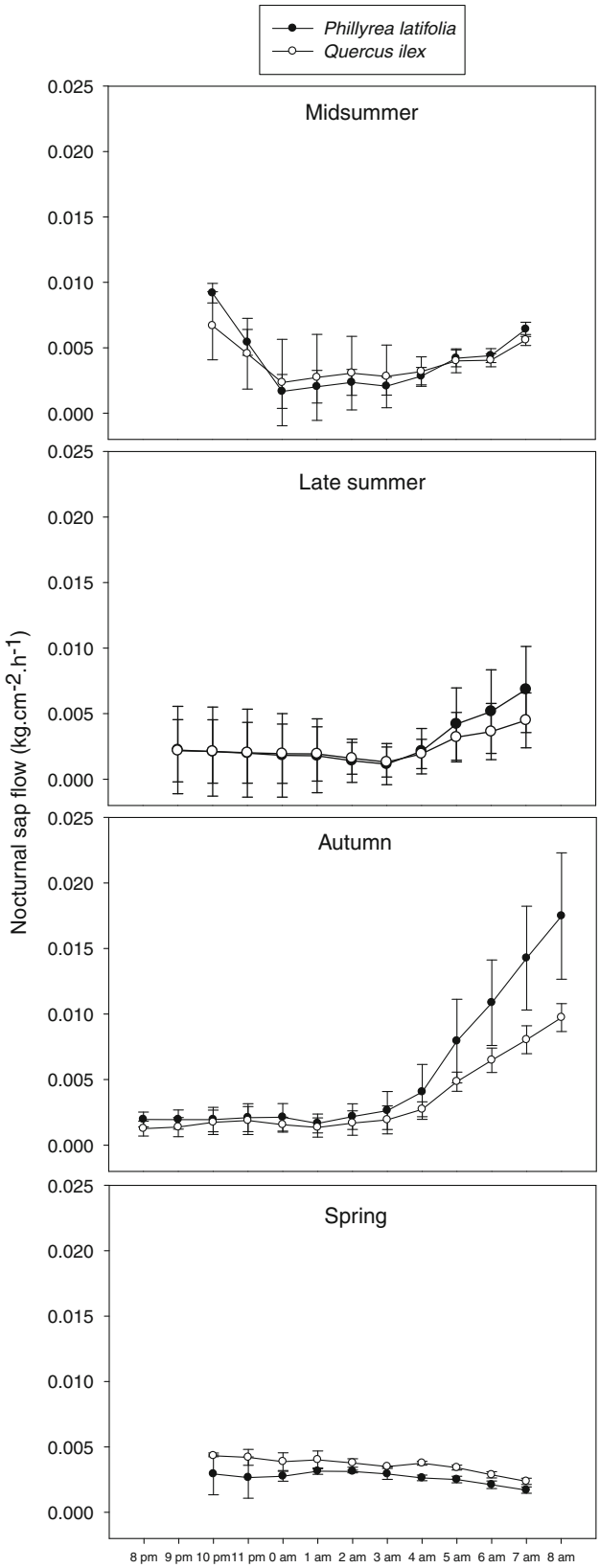

Fig. 3 Hourly sap flow rates at nighttime for the four sampling periods. *Error bars* are standard errors of the mean ($n = 2$)

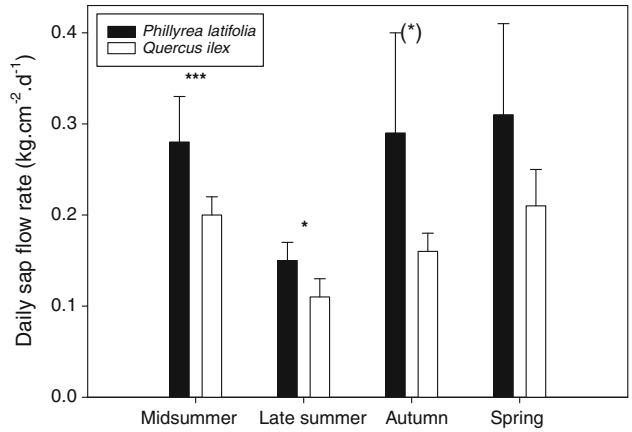

Fig. 4 Seasonal means of daily sap flow rates. The *error bars* are standard error of the mean ($n = 2$). Significant differences are marked with $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $(*)p = 0.056$

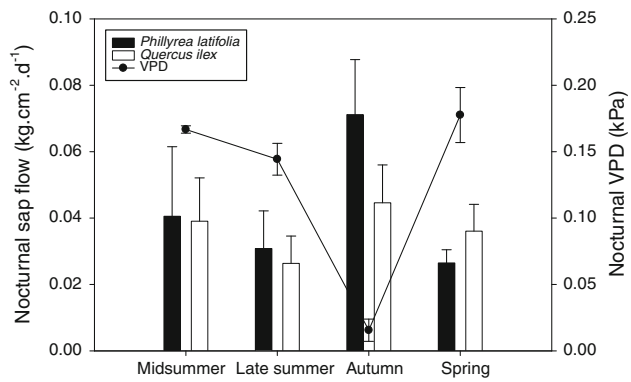

Fig. 5 Mean VPD and mean nocturnal sap flow rates per season and species. *Error bars* are standard errors of the mean ($n = 2$)

wet periods (spring and autumn) than in mid and late summer (Fig. 3). Hence, it may be deduced that at least at this site *P. latifolia* is more water spender regardless of the environmental conditions. However, daily sap flow reduction in the peak of dry respect wet periods was 64.3 % in *P. latifolia* and 47.6 % in *Q. ilex* whilst Martinez-Vilalta et al. (2003) found 57.8 and 87.1 %, respectively. Also Tognetti et al. (1998) found higher sap flow reduction due to summer drought for *Q. ilex* than in our study (~75 %), which is likely due to the lower and stable quantities that we obtained during favorable conditions. Moreover, during our sampling season (Fig. 1), the lowest soil moisture did not coincide with the highest VPD due to a couple of rainfall episodes in early and mid August, so the strongest water stress took place in late summer and was thus probably less severe in comparison to the cited studies. Our results on seasonality of sap flow for *Q. ilex* agree with Infante et al. (2001, 2003) at an oak-savannah, where they described similar patterns with minimum quantities at the end of summer instead of midsummer. Furthermore, *Q. ilex*

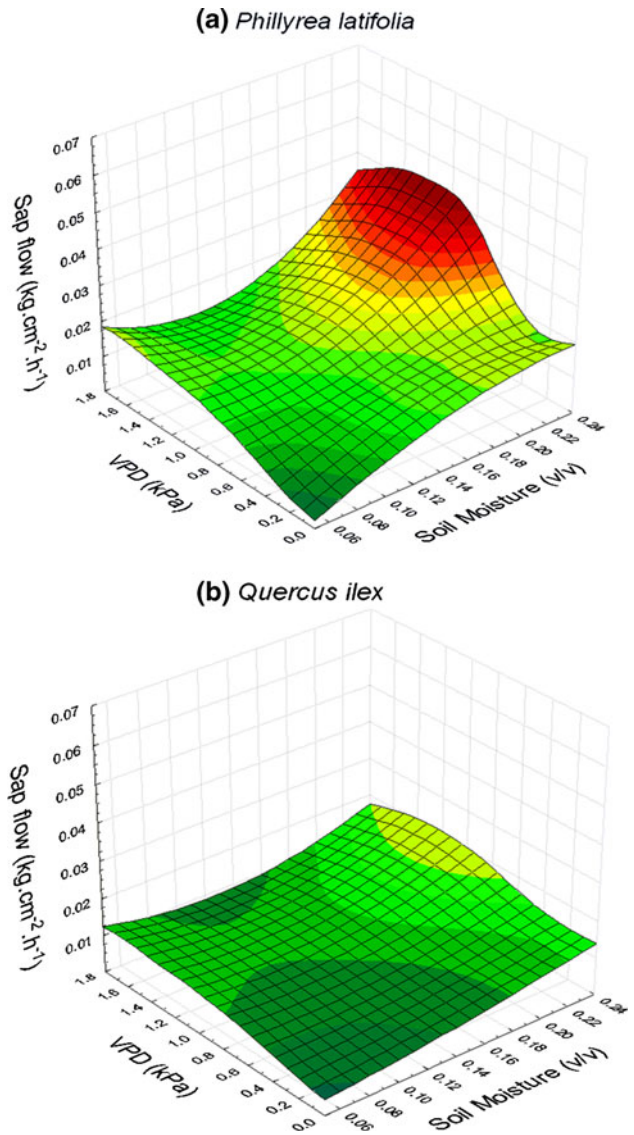

Fig. 6 Sap flow response to instantaneous values of VPD and soil moisture for **a** *Q. ilex* and **b** *P. latifolia*. *Z axis* units are hourly integrated estimations

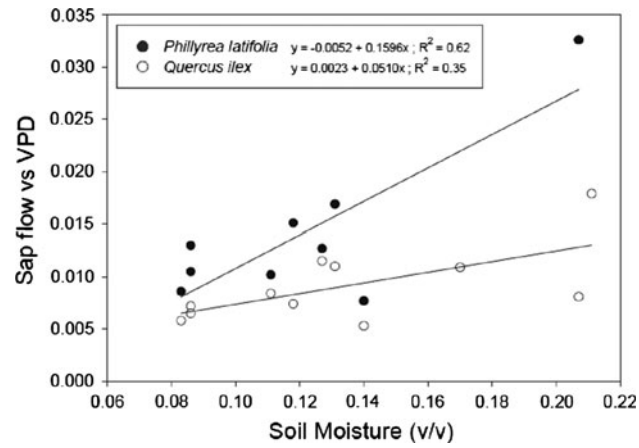

Fig. 7 Daily β coefficients of the sap flow versus VPD regressions at increasing soil moistures. Only β from significant ($p < 0.05$) sap flow versus VPD relationships was used. Soil moisture is the mean for each day

showed the highest sap flow daily mean in spring, coinciding with the maximum radial increment observed in a nearby forest (Gutierrez et al. 2011).

This study also demonstrates a species-dependent response to environmental conditions. Sap flow needs evaporative demand (VPD) to take place but at varying soil moisture we detected higher slopes of the sap flow versus VPD relation in *P. latifolia* than in *Q. ilex* and (Fig. 7). Such difference indicates a greater sensitivity of *P. latifolia* to increases in soil moisture. This greater sensitivity of *P. latifolia* disagrees with our expectations based on previous studies of hydraulic properties of both species. Martinez-Vilalta et al. (2002a, b) described wider xylem conduits and higher hydraulic conductivity of *Q. ilex* respect to other Mediterranean woody species, among them, *P. latifolia*. Consequently, we expected that under favorable environmental conditions *Q. ilex* would be able to conduct higher amounts of sap flow as a response to the soil moisture increases after autumn and spring rainfalls, but instead, at this site of Prades forest, *P. latifolia* presented a higher plasticity in its sap flow rates, which is also in disagreement with findings of Gucci et al. (1999) who found similar sap flow peaks throughout the seasons except for summer in a meteorologically similar location. This higher fluctuation of *P. latifolia* sap flow rates may be explained by their shallower root system in comparison to *Q. ilex*, as the latter may have access to deeper soil layers with more constant moisture. Moreover, this morphological difference seemed to allow *Q. ilex* to have similar average daily sap flow amounts in midsummer and spring despite a large difference in soil moisture (Fig. 4). As drought progressed, deeper soil layers also dry out and *Q. ilex* would not be able to maintain the same hydraulic conductivity, resulting in lower sap flow rates in late summer (Fig. 4). Ogaya and Penuelas (2003b), reported higher stomatal conductance maximums in autumn and summer in *P. latifolia* than in *Q. ilex*, which would be another species-specific factor enhancing *P. latifolia* capacity of water transport. Therefore, at this site, the trade-off between hydraulic conductivity and resistance to cavitation is more balanced in *P. latifolia*, which has lower branch and leaf hydraulic conductivity (Martinez-Vilalta et al. 2002b) but ends up transpiring greater amounts of water than *Q. ilex*, probably due to a less embolized percentage of xylem conduits at the end of summer. Furthermore, water use efficiency is not different between the studied species (Ogaya and Penuelas 2003b).

The nocturnal sap flow in both species represented a similar percentage of the whole-day flow than reported in

the study of Fisher et al. (2007) at the Mediterranean oak-savannah of deciduous *Q. douglasii* (they found 18 % and we calculated a 22.6 ± 7.5 % for *Q. ilex* and 18.2 ± 8.9 % for *P. latifolia*). Nevertheless, our data lack winter measures while Fisher et al. (2007) monitored almost a whole year. With our data, it is difficult to discern whether nocturnal sap flow responds to water losses or to refilling of water stores or both, since they can occur simultaneously. This is usually tested by coupling nocturnal sap flow with VPD (Cavender-Bares et al. 2007; Fisher et al. 2007). In our study, the hourly rates of nocturnal sap flow shown in Fig. 3 did not follow VPD fluctuations. Therefore, our data does not clarify the mechanism that drives both species to either transpire or refill water stores during the night. It is possible that premature stomata aperture would enhance early morning carbon assimilation or facilitate nutrient supply to distal parts of the crown (Daley and Phillips 2006; Dawson et al. 2007; Scholz et al. 2007). These potential ecological meanings of nocturnal sap flow were further supported by the rise of sap flow since 3 a.m. observed in all sampling seasons but spring (Fig. 3), which has been also detected for stomatal conductance in many species and seems to respond to endogenous circadian rhythms (Caird et al. 2007; Dodd et al. 2005; Donovan et al. 2003; Howard and Donovan 2007). On the other hand, plants could suffer simply unavoidable water leaks through the leaf cuticle or stomata under high VPD as observed by Cavender-Bares et al. (2007) in live oaks under drought and by Zeppel et al. (2010) in two evergreen *Eucalyptus* species. Our results may suggest a pre-dawn activation of sap flow but sampling limitation warrant further research to elucidate the drivers of nocturnal sap flow and the ecological significance for these species, if any. Moreover, if plant transpiration is different to zero, the assumption that plant water potential reaches equilibrium with the soil at night will have to be reconsidered and thus will limit our ability to assess plant water status from pre-dawn water potential measurements (Bucci et al. 2004; Dawson et al. 2007). In any case, further and more extensive monitoring of sap flow at different heights along with nocturnal stomatal conductance is needed to disentangle the function of nocturnal sap flow and its ecophysiological consequences.

In summary, *P. latifolia* seemed to cope better with drought conditions and transpired generally greater amounts of water. This might partly explain and support the more severe effects of an experimental drought (on average 15 % decreased soil moisture) on *Q. ilex* than on *P. latifolia* observed at the same site (Ogaya and Penuelas 2007b). In that experiment, *Q. ilex* presented lower mean annual stem diameter increments and higher mortality rates than *P. latifolia* (Ogaya and Penuelas 2007b). These results provide additional evidence that *P. latifolia* may outcompete *Q. ilex* at the drier edge of its distributional range due to the increase in the intensity and frequency of summer droughts, as stated in several previous studies (Martinez-Vilalta et al. 2002a, 2003; Ogaya and Penuelas 2003a, 2006, 2007a, b; Ogaya et al. 2003, 2011; Penuelas et al. 1998, 2000). The results also showed a significant role of nocturnal sap flow in both species with a frequent pattern of increase from 3 a.m. to dawn that warrants further and in depth research.

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