Night-time ozone uptake by Mediterranean species

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Abstract

Due to the evident tropospheric ozone impact on plant productivity, an accurate ozone risk assessment for the vegetation has become an issue. There is a growing evidence that ozone stomatal uptake may also take place at night and that the night-time uptake may be more damaging than diurnal uptake. Estimation of night-time uptake in the field is complicated because of instrumental difficulties. Eddy covariance technology is not always reliable because of the low turbulence at night. Leaf level porometry is defective at relative humidity above 70% which often takes place at night. Improved sap flow technology allows to estimate also slow flows that usually take place at night and hence may be, at present, the most trustworthy technology to measure night-time transpiration and hence to derive canopy stomatal conductance and ozone uptake at night. Based on micrometeorological data and the sap flow of three Mediterranean woody species, the night-time ozone uptake of these species was evaluated during a summer season as drought increased. Night-time ozone uptake was 10% of the total when plants were exposed to a weak drought, but increased up to 24% as the drought became more pronounced. The percentage increase is due to a stronger reduction of diurnal stomatal conductance than night-time stomatal conductance.

1 Introduction

Ozone is recognized to be a cause of significant losses of plant Primary Productivity (PP) and, as discussed elsewhere (Musselmann et al., 2006; Karlsson et al., 2007), what is effectively relevant for plants is the ozone stomatal uptake rather than the exposure (Uddling et al., 2004). This implies that ozone risk assessment requires a precise knowledge of canopy stomatal conductance ($G_s$ m s$^{-1}$).

The eddy covariance technique (EC) is recognized to be the most direct method to measure to measure ecosystem-scale fluxes of $H_2O$, $CO_2$, and other gases (Swinbank, 1951; Hicks and Matt, 1988; Grunhage et al., 2000). EC, by the aid of some
assumptions, allows to evaluate canopy conductance \((G_c)\) from the EC dataset by inverting the Penman-Monteith equation (Monteith, 1981) or by using a simple electric analogy (Gerosa et al., 2007). Although the EC method is a powerful tool, eventual gas exchange occurring at night remain “unseen” mainly because of the low atmospheric turbulence (Fisher et al., 2007). Because of this, sap flow technology may be a valid alternative to EC for night-time evaluation of gas exchange (Fisher et al., 2007; Grulke et al., 2004).

Night-time transpiration \((E_n)\) contradicts the predominant view of biology and plant physiology textbooks that stomata are closed at night, however, different authors have detected either \(E_n\) or night-time stomatal conductance \((g_{s,n})\) (Muchow et al., 1980; Furukawa et al., 1990; Wieser and Havranek, 1995; Anderson, 1982; Rawson and Clarke, 1988; Bakker, 1991; Daley and Phillips, 2006; Green et al., 1989). For an accurate review refer to Caird et al. (2007). Dawson et al. (2007) demonstrate that \(E_n\) takes place over a great variety of plant species using datasets from 10 study sites representing six biomes on three continents, plus the Hawaiian Islands. Their work was possible thanks to the advancements in sap flow measurements which allow to measure also very low sap flow rates. They report values of \(g_s\) as high as 20% of the daily maximum, while Caird et al. (2007) report values as high as 50% of the daily maximum. Even if the occurrence of \(E_n\) is a recognized process, a physiological explanation of \(E_n\) is still lacking and to date it is not possible to determine whether \(E_n\) represents a passive process, possibly due to stomatal leaking, or it has an adaptive meaning. The difficulty in explaining the physiological significance arises from the apparently contradictory differences in the response of \(E_n\) and \(g_{s,n}\) to environmental observed between species. Howard and Donovan (2007), for example, demonstrate that the reason for \(E_n\) is not in a higher nutrient uptake and it does not depend on leaf age in nine Helianthus species, but Scholz et al. (2007) and also Ludwig et al. (2006) find an inverse correlation between nutrient availability and \(E_n\). The correlation between \(G_{s,n}\) and Vapour Pressure Deficit (VPD; kPa) is also not clear as, Dawson et al. (2007) found a positive correlation between them, while Cavender-Bares et al. (2007) found a negative correlation. Worth
of notice is the different behaviour of shrub understorey species and of dominant trees reported by Fisher et al. (2007) in the same Mediterranean type ecosystem. In that article it is reported that $E_n$ of the dominant species increases in summer, higher VPD and lower Soil Water Content (SWC; v/v %), while that of the understorey increases in winter, higher SWC and lower VPD. Even if the authors did not estimate $G_{s,n}$, it can be inferred from their data that the $G_{s,n}$ of the shrub species was higher in winter, in fact for transpiration to be greater at a lower VPD a higher $G_{s,n}$ is necessary. The surprising increase in $G_{s,n}$ with VPD is in strong contradiction with a water saving strategy because it means that stomata allow the plant to spend more water when the potential transpiration is higher. This response is also in contrast with the known day-time physiology, where $G_s$ declines with VPD. These species-specific differences suggest a genetic control and hence the possibility for $E_n$ to be an adaptive strategy (Rosenthal et al., 2002; Brouillette et al., 2006), that must still be understood.

Although the night-time physiology of $g_s$ is still not understood, it has a relevant implication on the water balance of ecosystems. Howard et al. (2007), Dawson et al. (2007) and Bucci et al. (2005) quantify $E_n$ to be between 5% and 20% of daily maximum transpiration.

The occurrence of night-time stomatal opening could also be relevant for pollutant uptake by plants (Grulke et al., 2004; Matyssek et al., 1995). Stomatal represent in fact the passage way for ozone to enter the leaf lamina. In Mediterranean ecosystems ozone damage may be partially avoided because high concentrations of O$_3$ during day light co-occur with drought, which brings to stomatal closure (Gerosa et al., 2008; Manes et al., 2005). Since night-time $g_s$ may be significantly different from 0, it is plausible that ozone stomatal uptake takes place at night. Ozone night-time may represent a significant fraction of the total ozone uptake since night-time ozone concentrations in Italy vary between 10 and 45 ppb across both rural and suburban areas (Paoletti, 2006).

However, the estimation of night-time stomatal uptake must face both physiological (Barbour and Bucley, 2007) and technical difficulties (Aubinet, 2008). The physiolog-
cal problem is mostly due the poor knowledge of the $G_{s,n}$ response to environmental parameters, which differs substantially among species and hence is hardly predictable. The technical problem, instead, consists in the difficulties to measure fluxes and in estimating $G_{s,n}$ in an environmental situation typically characterized by low turbulence and high humidity. These difficulties imply that, even if ozone night-time uptake may be relevant for the Mediterranean ecosystems and forests in general, the estimation of the dose requires both knowledge of the species specific response to environmental parameters and of an appropriate instrumental approach.

In this work, we first analyse the species night-time physiology of three Mediterranean species (Arbutus unedo L., Quercus ilex L. and Erica arborea L.). Finally, we estimate night-time ozone fluxes, evaluate their significance for the ozone risk assessment and discuss the opportunity to couple EC with sap flow technology.

2 Material and methods

2.1 Study site

The study site is a Mediterranean maquis dominated by Q. ilex. The climate is typically Mediterranean with a pronounced summer drought and rain events concentrated in autumn and spring, with a mean annual total precipitation of 780 mm per year. Mean monthly temperatures range between a minimum of 6°C and a maximum of 24°C while the relative humidity, RH, is rarely below 50%. For a more detailed description refer to Fares et al. (2009).

2.2 Environmental monitoring

Three thermo-hygrometer probes (50Y, Campbell, USA) were placed at 0.1, 1 and 3.5 m height. Two leaf wetness sensor (237, Campbell, USA) were placed at 1.5 m height. Three thermocouples (PT100, Delta T, UK) were used to measure leaf temperature, $T_a$, of three Q. ilex leaves in three different positions of the canopy. The three
PT100 were intercalibrated with the thermo-hygrometers before and after the measuring period. All sensors were connected to a CR10x data logger (Campbell, USA) which acquired data from the sensors every 15 s and stored them as 30 min averages.

2.3 Sap flow measurements

Sap flow measurements were performed on *Q. ilex* and on *A. unedo* and are the same described in Mereu et al. (2009), for this study, an additional species (*Erica arborea* L.; *n*=3) was monitored. The sap flow sensors are based on the Heat Field Deformation system (HFD) (Nadezhdina et al., 2004). The method is part of a family of sensors based on the heat transfer methods, in this case two pair of thermocouples measure changes in the temperature difference, \( \Delta T \), caused by the deformation of the heat field around the heater due to the moving of sap. The so-called symmetric thermocouple measures the difference in temperature between two equidistant points above and below the heater, while the asymmetric thermocouple measures the temperature between the lower point and another point set at 0.5 cm abreast the heater. This system has been shown to be adequate for both high and low sap flow rates (Nadezhdina et al., 2008). The sensors were insulated from the surrounding environment by coating them with silicon and wrapping them in aluminum cover.

All sensors were connected to a DL2 data logger (Delta-T devices, UK) which acquired data from the sensors every minute and stored them as 30 min averages. During three subset of data acquisition of 24 h, data were recorded as 1 min averages to estimate the time lag between environmental drivers (radiation, PPFD, and VPD) and sap flow.

2.4 Stomatal conductance

Leaf stomatal conductance measurements taken with steady state or dynamic diffusion porometers are complicated to perform at relative humidity (RH) values above 70% (McDermitt, 1990). In our case leaf stomatal conductance measurements at night
were not reliable because RH was often above 80%.

Sap flow density \( (Q_l; \text{Kg m}^{-2} \text{s}^{-1}) \), measured per unit area of sapwood (SA) can also be expressed per unit of Leaf Area (LA) after multiplication by the sapwood/leaf area ratio (LA/SA). For such a derivation, we used the LA/SA ratio of the species collected in Mereu et al. (2009). Using the transpiration per unit leaf area, \( G_s \) can be derived from sap flow measurements, based on a simplification of the Penman-Monteith equation (Whitehead and Jarvis, 1981; Pataki et al., 1998; Martinez-Vilalta et al., 2003; Kurpius and Goldstein, 2003):

\[
G_s = \frac{\gamma \times \lambda \times Q_l}{\rho \times cp \times VPD}
\]  

where \( \gamma \) is the psychrometric constant (kPa K\(^{-1}\)), \( \lambda \) is the latent heat of vaporization of water (J kg\(^{-1}\)), \( \rho \) is the density of air (kg m\(^{-3}\)), \( cp \) is the specific heat of air at constant pressure (J kg\(^{-1}\) K\(^{-1}\)) and VPD is the vapour pressure deficit (kPa) of the air. The simplification can be considered valid if \( G_s \) is predominant over the leaf boundary layer conductance, \( g_b \) (Whitehead and Jarvis, 1981) i.e. when the canopy is strongly coupled with the atmosphere. The Mediterranean vegetation is usually assumed to be well coupled with the atmosphere (Martinez-Vilalta et al., 2003; Infante et al., 1997) given its general small leaf size (Gratani and Varone, 2004, Gratani and Bombelli, 2000). This assumption is not necessarily valid at night when RH may be high and wind intensity is usually low, two conditions that are known to cause the decoupling of the vegetation (Monteith and Unsworth, 1990). To test the degree of coupling with the atmosphere, the value of \( g_b \) was calculated for all three species and compared with \( G_s \) using the equation proposed by Jones (1992):

\[
g_b = 6.62 \times \left( \frac{u}{d} \right)^{0.5}
\]  

where \( d \) is the average leaf size and \( u \) is the wind speed. The samples that were not well coupled were determined by means of an iterative procedure where samples
with increasing \( g_b \) values were excluded at each step until the correlation coefficient between \( E_n \) and VPD did not increase of more than one hundredth.

2.5 Eddy covariance flux measurements

The micrometeorological instrumentation was mounted over a scaffold at 3.8 m from the ground. The three components of the wind vector and the temperature were measured at high sampling frequency (20 Hz) by means of a ultrasonic anemometer (mod. USA-1, Metek, Germany). Air moisture was recorded with a fast-response sensor (mod. 7500, LiCor, USA). Half hourly ozone concentration means were measured with an automatic analyzer (S-5014, SIR, E) based on UV absorption.

Starting from the fast anemometer data, the friction velocity \( u^* \), a good descriptor of the atmospheric turbulence, was calculated as:

\[
 u^* = \sqrt{\left(w'v'ight)^2 + \left(w'u'ight)^2} \tag{3}
\]

where \( u' \) and \( v' \) are the wind speed fluctuations along the x and y axis respectively and \( w' \) is the vertical wind speed.

Latent heat was measured by using the micrometeorological approach, assuming that fluxes are independent from height in the lowest air layers (Stull, 1988). Hence, a flux measured at several meters above ground level is assumed to be equal to the flux at the air–vegetation interface. The flux was determined by the eddy covariance method, considered the most reliable one (Hicks and Matt, 1988). Fluxes are proportional to the covariance between turbulent fluctuations of the vertical wind vector component and of the scalar entity of interest: water vapour for latent heat fluxes, and air temperature for sensible heat fluxes. Calculation of the fluctuations requires averaging over successive periods of time, in order to eliminate non-turbulent, long-term variations. The averaging time was chosen such as to include all turbulent fluctuations (at least 10 min) occurring in the atmospheric surface layer, but it had to be short enough to avoid the synoptic-scale fluctuations (less than 1 h, Van der Hoven, 1957). A 30-min
averaging time was taken as a compromise. Ozone concentrations at the height of interest (1.5 m, the average height of the vegetation) were not available, hence they were estimated by the gradient method taking the “turbulent diffusion” coefficient for heat, $K_H$, directly derived from the EC measurements:

$$C_h = C_{Z_m} + \frac{F}{K_H} \cdot (z_m - h)$$  \hspace{1cm} (4)

with

$$K_H = k \cdot u^* \cdot (z - d) / \Theta_H(\zeta)$$ \hspace{1cm} (5)

and the similarity function $\Theta_H(\zeta)$ accounting for the atmospheric stability (Dyer, 1974) being

$$\Theta_H(\zeta) = \begin{cases} 
[1 - 16 \cdot (\zeta)]^{-1/2} & \text{for } \zeta < 0 \text{ unstable conditions} \\
[1 - 5 \cdot (\zeta)]^{-1} & \text{for } \zeta \geq 0 \text{ stable conditions}
\end{cases}$$

with $\zeta=(z-d)/L$. $L$ is the Monin-Obukhov length, obtained from $u^*$ and the sensible heat flux measurements (Stull, 1988), $C_h$ is the ozone concentration at 1.5 m, $C_{Z_m}$ is the ozone concentration at the measurement height $z_m=3.8$ m, $h$ is the canopy height equal to 1.5 m and $F$ is the ozone flux (ppb m s$^{-1}$).

2.6 Ozone fluxes

Ozone fluxes were calculated using:

$$F_{O_3, \text{stom}}(i) = [O_3(i)] \times G_S(i) \times 0.613$$ \hspace{1cm} (6)

where $F_{O_3, \text{stom}}$ is the ozone stomatal flux (nmol m$^{-2}$ s$^{-1}$), $[O_3]$ is ozone concentration (nmol m$^{-3}$), $G_S$ is stomatal conductance (mm s$^{-1}$) and 0.613 is the ratio between the diffusion coefficient of O$_3$ and that of water vapour (Massman, 1998). Ozone stomatal fluxes were calculated only when the leaves could be assumed coupled with the atmosphere using the procedure described above.
3 Results and discussion

3.1 Environmental monitoring

During the measuring period only two precipitation events occurred in the first half of the campaign, totalling 14 mm of rain. VPD at 3.8 m height varied between 0.1 and 2.9 kPa and air temperatures ranged between 10 and 32°C, both parameters had higher values as the season progressed. SWC at 100 cm depth declined constantly from 11% to 4% during the campaign, however the response of the studied species was also determined by the presence of a water table (Mereu et al., 2009).

Mean $T_a$ and mean VPD during the night (21:30–06:00) at 1m height were 14.46°C and 0.2 kPa. Typically, leaf wetness increased from 19:00 and reached complete wetness around midnight, while leaves dried from 6:30 to 9:30 a.m. (Fig. 1a). The slope of the vertical gradients of VPD and $T_a$ were negative during the day and positive at night, typically inverting after sunset (19 p.m.) and after dawn (7 a.m.), while the vertical gradient of RH showed the opposite change in gradients, from positive in the day to negative at night (Fig. 1a, b, and c).

Ozone concentrations at 3.8 m height were comprised between 0 and 108 ppb with an average of 35 ppb (Gerosa et al., 2009), while ozone estimated at 1.5 m height was between 0 and 87 ppb. At night, ozone concentrations were on average between 30 and 11 ppb, with the higher concentrations occurring in the first hours after sunset (Fig. 2a). The mean night-time ozone concentration at 1.5 m was 23 ppb, but higher concentrations occurred when the wind blew from the sea (Fig. 2b).

3.2 Water fluxes

The experimental period was characterized by two periods (15 May–12 June and 13 June–31 July) with a different water availability that caused a reduction in the Latent Heat (LE) of the ecosystem (Fig. 3a, b) (Gerosa et al., 2009). In both periods sap flow rised during the day (Fig. 3a, b) following the trend of the net radiation and VPD.
(Fig. 1c), the two driving forces of LE (Monteith, 1965). The effect of the different water availability was reflected mostly by a diurnal decrease of sap flow, with *A. unedo* being the species that underwent the higher reduction (Fig. 3a, b). Night-time sap flow instead did not vary greatly between the two periods.

Night-time values of VPD, the only potential driver of transpiration in the absence of radiation, was greater than zero at all heights (Fig. 1a), hence allowing for a potential transpiration.

A positive sap flow (from roots to leaves) was recorded by the sensors also at nights when EC measurements could not be considered reliable because of the low turbulence and canopy wetness (Gerosa et al., 2009). Sap flow represents an indirect measurement of transpiration and its reliability must be judged with attention. The reliability of the measurement was strengthen by the constantly lower temperature of the lower end of the symmetrical pair of thermocouples (cfr. Sect. 2.3), meaning that heat was transferred upwards from the heater to the upper end of the thermocouple. The occurrence of transpiration at night is also confirmed by the leaf temperature $T_l$ of *Q. ilex*: $T_l$ was higher than $T_a$ at 1 m height during the day because of the incoming solar radiation, but lower at night, typically inverting at 7:30 and 19:30 (Fig. 1b). Between 23:30 and 5:00, $T_l$ was almost constantly 1.26°C lower than $T_a$. This difference can be sustained only if heat is continuously extracted from the leaf, i.e. transpiration occurs at the expenses of the internal heat of the leaves and not at the expenses of the surrounding air. A further evidence is that when wind intensity rises, the difference between $T_l$ and $T_a$ ($\Delta T$) tends to 0 with a logarithmic relationship (Fig. 4a). High wind intensities, in fact, lead to a thinning of the leaves boundary layer (increased $g_b$, refer to Eq. 2) to which plants respond with a reduction of $G_{s,n}$ (Fig. 4b). The reduction of $g_b$ also favours an exchange of heat from the warmer air to the colder leaves that together with the reduction of $G_{s,n}$, favours the thermal re-equilibration of the canopy with the surrounding air.

Caution was also given in differentiating night-time refilling from actual water loss. Refilling occurs during the night in order for the plant to re-equilibrate the leaf water
potential with that of the soil, hence for a determined period of time the leaf water potential ($\Psi_l$) tends to equal the soil water potential ($\Psi_s$). The time necessary for this process largely depends on the amount of water that must be replenished in the plant tissues at the end of the day and on the capacitance of the plant, which in turn depends on plant volume, wood elasticity and wood density. In this study, plants were rather small in size (Fares et al., 2009) and had a high wood density as most Mediterranean woody species (Cherubini et al., 2003), in accordance with their adaptation with a dry environment (Hacke et al., 2001). The influence of refilling on the sensors also depends on the distance that separates the sensor from the transpiring surfaces and an additional reduction of the influence of refilling on transpiration was achieved by placing the sensors at less than one meter from the leaves.

A time lag between sap flow at stem level and sap flow at branch level or between sap flow and environmental variables is often considered as a proxy of the capacitance of the plant tissues (Phillips et al., 2008), where capacitance is the change in tissue water content per unit change in water potential. However, Burgess and Dawson (2008) observed that the time lag is not always clearly proportional to the capacitance and that this variability may be explained by other water movement inside the tissues or instrumental artefacts. In fact, sap flow based on continuous heating, such as in this study, may bring to a thermal inertia where the increase or decrease in sap flow is not immediately sufficient to determine changes in the temperature gradients around the heater. The fast recording sub-sampling periods allowed to determine a time lag of about eight minutes between radiation or VPD (data not shown) hence suggesting a reasonable accuracy of the HFD sensors and confirming a limited capacitance of the three species.

Even if in this study the effect of capacitance on sap flow measurements is minimized, a refilling process is assumed to be taking place after sunset and for a period of time the amount of water entering the plant is higher than the amount transpired. It has often been observed that sap flow declines after sunset and tails off to a quasi plateau in a period of time dependent on the species. Such a behaviour is recogniz-
able in the mean days presented in Fig. 3 and in the sample days for *Q. ilex* and *E. arborea* (Fig. 5) and persist for a period of about three hours after sunset. The period between sunset and the achievement of the quasi plateau can be considered a period where transpiration occurs together with refilling (Fisher et al., 2007) and where it is complicated to estimate the relative proportion of the two processes. The following period, instead, can be considered in a steady state equilibrium where the amount of water entering the plant equals the amount transpired. Difficulties in differentiating refilling from transpiration can cause inaccuracies in the estimation of transpiration at night which can be quantitatively relevant especially for big trees. In order to avoid a potential overestimation during this period, transpiration in the first three hours after sunset was assumed to be equal to the average night transpiration. However, this is likely to underestimate the night-time contribution to transpiration as VPD in the first three hours after sunset was generally higher than in the rest of the night.

Even if the occurrence of transpiration at night has been reported for several species in a wide range of environmental conditions (Caird et al., 2007) either with sap flow gauges, leaf level measurements of gas exchange or EC, night-time transpiration remains a controversial topic as it is a complicated measurement and because of its implication for plant physiology and ecosystem gas exchange measurements. In fact, in the presence of high humidity and low turbulence, as in this study at night, LE measurements with EC are largely impaired and, as expected, EC was not able to deliver reliable LE measurements during the night, in fact only 156 cases out of 1593 satisfied the imposed stationarity and dry canopy conditions (Gerosa et al., 2009) between 21:00 p.m. and 6:00 a.m.(h). Even when the conditions were satisfied, caution should be used in considering the measured LE as true evapo-transpiration. In fact, in presence of a stable boundary layer, intermittent turbulence produces strongly non stationary events during which the validity of turbulent transport is uncertain (Aubinet, 2008). In these conditions, that characterized our site at night, the measured LE peaks may be the result of the release of water accumulated in the precedent hours in the lower atmospheric strata. This is supported by the fact that the few EC measured water fluxes
were, at least, one order of magnitude greater than the transpiration measured with the sap flows.

In agreement with Fisher et al. (2007), sap flow measurements appear to be more reliable than EC in these conditions and at night in general. However, besides the technological complications discussed above, low humidity and low turbulence, represent a complication also for the estimation of transpiration from sap flow technology. In fact, in these conditions it is necessary to establish the degree of coupling of the vegetation with the atmosphere.

$E_n$ occurred throughout the season in all three species (Fig. 3) and had a positive correlation with VPD (Fig. 6) in agreement with other authors (Dawson et al., 2007; Howard and Donovan, 2007; Fisher et al., 2007). A significant improvement of the correlation was achieved by discarding data for low values of $g_b$ with the procedure described in section 2.4. Species differed in the $g_b$ value below which the vegetation was considered partially decoupled from the atmosphere: a value of 17 and 21 mm s$^{-1}$ was found for A. unedo and Q. ilex while a value of 32 mm s$^{-1}$ was found for E. arborea. While the values for the first two species correspond to the same wind intensity (0.41 m s$^{-1}$), the value found for E. arborea corresponds to a lower wind speed of 0.33 m s$^{-1}$. The percentage of data that was discarded varied between species both because of the different coupling and because of the different number of measurements available for each species. More precisely, 112 measurements out of 1058 for A. unedo, 104 out of 942 for Q. ilex, and 58 out of 1158 for E. arborea were discarded from the further data processing. The procedure allowed for an appreciable increase in the $R^2$ of the best curve fit: from 0.49 to 0.64 for A. unedo, from 0.39 to 0.46 for Q. ilex, and from 0.40 to 0.46 for E. arborea. The $R^2$ of these correlations is slightly lower than that reported by other authors, but it must be considered that in our experimental period VPD varied only between 0.2 and 1 kPa while Fisher et al. (2007) report mean night VPD values in a range from 0 to 2.5 kPa, a range that allows for a better correlation to be achieved. Also differently from Fisher et al. (2007), our best fit consisted in a logarithmic and not in a linear relationship for A. unedo and Q. ilex (the $R^2$ of the linear
fit was 0.56 and 0.42 for *A. unedo* and *Q. ilex*, respectively). Given the improvement achieved the $R^2$ of these relationships, we strongly recommend such or similar tests before further processing sap flow datasets.

Doubts may rise on the reliability of $G_{s,n}$ through Eq. (1), since the equation does not include an energy balance as the original Penman-Monteith equation, nor it includes leaf temperature as in the Evaporation-Resistance approach proposed by Thom (1975). $G_{s,n}$ was indeed sensible to VPD values, but it must be considered that if the leaf temperature is lower than that of air, as it is at night, the resultant difference in vapour pressure between leaf and air ($VPD_l$) is lower than the atmospheric VPD. Hence Eq. (1) is likely to underestimate $G_s$ at night when leaf temperature is lower than $T_a$ ($VPD_l < VPD$) and overestimate it during the day when $T_l$ is higher than $T_a$ ($VPD_l > VPD$).

$G_{s,n}$ had a negative correlation with VPD for all three species (Fig. 7) and did not change relevantly between the first period (higher water availability) and the drier second period (Fig. 8), bringing to a weak correlation between soil water content and $G_{s,n}$ (data not shown).

In this study, $G_{s,n}$ of all three species responds to wind (Fig. 4b) and VPD (Fig. 7) indicating a water saving strategy in accordance with their daytime physiology and with their adaptation to the dry Mediterranean climate. However, given the high variability of responses to environmental parameters reported in the literature, such a conclusion cannot be extrapolated to other Mediterranean species or to the same species in different growing conditions. This response also suggests that $E_n$ is not a passive process as considered by Fisher et al. (2007), which would imply a linear relationship between $E_n$ and VPD and a constant $G_{s,n}$. The physiological control of $G_{s,n}$ is also testified by the variation of $G_{s,n}$ during in the hours precedent to dawn (Bucci et al., 2004; Dodd et al., 2005), a variation that is also evident in Fig. 8, where $G_{s,n}$ diminishes before day light.
3.3 Ozone fluxes

Besides the importance to the water budget of the species, significant values of $G_{s,n}$ may imply important ozone stomatal uptake during the night. Despite the generally low ozone concentrations of the site, but notice that in some nights they were above 30 ppb, a significant fraction of the total (day and night) ozone uptake occurred at night (Table 1).

On average, night-time ozone fluxes remained similar in the first and in the second period (Fig. 8) and were on average below 1 nmol m$^{-2}$ s$^{-1}$, except for A. unedo in the second period where values of 1.5 nmol m$^{-2}$ s$^{-1}$ were reached around midnight. The small difference between the two periods is a consequence of similar night-time ozone concentrations and similar $G_{s,n}$ values across the experimental period. Using the concentrations at the two different levels, determines a substantial difference in ozone uptake only at night with an overestimation for the concentrations at 3.8 m. The $G_{s,n}$ response to the increasing VPD and aridity during the experimental period is weaker than that of diurnal $G_s$ (Mereu at al., 2009), and this determines a higher fraction of the total ozone uptake to occur at night (Table 1).

The instantaneous individual flux threshold of 1.6 nmol m$^{-2}$ s$^{-1}$ proposed by Karlsson et al. (2004) to account for ozone detoxification at cellular level, was almost never exceeded at night, but it should be taken into account that at night the detoxification apparatus is likely to be less efficient (Musselman and Minnick, 2000). The experiments of Winner et al. (1989), for example, demonstrate a higher level of injury and reduced growth of Brassica rapa L. exposed to ozone at night with respect to plants exposed during the day, even though nighttime uptake was lower than daytime because $G_{s,n}$ was 75–80% lower than daytime $G_s$. Similarly, Matyssek et al. (1995) showed that also woody plants, Betula pendula L., were more effected by night-time ozone resulting in higher reduction in growth than those exposed to ozone in daylight, even if leaf injury was similar for both treatments.

In our case it is possible that a similar effect is taking place also in maquis species
but it is also possible that in these species – already adapted to oxidative stress drought and light driven – the effect may be less evident. Unfortunately knowledge on night-time physiology and ozone effects on Mediterranean maquis species is still lacking and should be addressed. However, our night-time ozone uptake represents a significant portion of the dose (Fig. 8; Table 1) and shouldn’t be neglected in ozone risk assessment. It is also worth noticing that this “additional” dose partially counterbalances the dose “avoided” by Mediterranean plants as a consequence of stomatal closure during the typical Mediterranean summer drought.

Additionally, in other sites the night-time uptake may be even higher than ours, since the dunes orography determined particularly stable atmospheric conditions for the rear dune ecosystem at night – i.e. air masses were stratified – which brought to low ozone concentrations in the lower atmospheric strata.

4 Conclusions

Sap flow technology allowed to determine the night-time water loss and $G_{s,n}$ response to environmental parameters of three Mediterranean shrub species, in conditions of high humidity and low wind speed where EC resulted to be heavily impaired. The night-time physiological response to environmental parameters of the three studied species was assessed and found to be consistent with the diurnal water saving strategy. The approach also allowed to determine night-time ozone stomatal uptake by these species, an additional dose 10 to 20% higher than the dose estimated by considering stomata to be closed at night. This additional dose could be particularly relevant for vegetation risk assessment (Fuhrer et al., 1997) especially if the higher toxicology of ozone at night will be proven. The night-time fraction may partially compensate the dose usually considered avoided by Mediterranean species as a consequence to the diurnal stomatal response to drought. Given the technological difficulties for the estimation of night-time fluxes, more research needs to be focused on this issue.
Acknowledgements. The authors wish to thank Mr. De Michelis, Head of the Castelporziano Estate, and Mr. Tinelli whose assistance was essential in providing logistical co-ordination. This research was supported grants of the Accademia delle Scienze detta dei XL grants, and of the Ateneo “Sapienza” MIUR.

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Table 1. Average daily ozone dose (nmol m² s⁻¹) for the three species in the first and in the second period and using ozone concentrations at two heights. Night fraction (%) corresponds to the fraction of the total ozone attributable to night fluxes only.

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Fig. 1. Average of the half-hourly measurements of environmental parameters calculated using data from the entire experimental campaign (from 15 May to 31 July 2007). (a) Vapour Pressure Difference (VPD, kPa) at tree different heights (3.8 m; 1 m; 0.3 m), and Leaf Wetness (relative frequencies). (b) Air temperature $T_a$ ($^\circ$C – 3.8 m ;1 m; 0.3 m) and leaf temperature $T_l$ ($^\circ$C). (c) Relative Humidity (RH, % – 3.8 m; 1 m; 0.3 m) and solar radiation (PPFD, $\mu g$ mol m$^{-2}$ s$^{-1}$).
Fig. 2. (a) Average of the half-hourly measurements of ozone concentrations at 1.5 m height. (b) Night-time ozone concentration as function of wind direction. Concentrations are higher when the wind is blowing from the sea.
Fig. 3. Average of the half-hourly measurements of sap flow rates of tree species and total ecosystem LE from Eddy Covariance. Values shown are for the mean day of (a) first period and (b) second period. Modified from Gerosa et al. (2009).
Fig. 4. (a) The $\Delta T$ between air and leaves of *Q. ilex* diminishes logarithmically with wind intensity (m s$^{-1}$) (b) Median of canopy conductance, $G_s$ (mm s$^{-1}$) as function of wind intensity (m s$^{-1}$) for the tree species, showing how stomata tend to limit transpiration. Maximum wind speed in the two panels does not coincide because sap flow measurements were not available (instrumental break down) in the only day when wind speed exceeded 5 m s$^{-1}$.
**Fig. 5.** Daily course of VPD and sap flow of *Q. ilex*, and *E. arborea* from 5 to 9 June 2007.
Fig. 6. Relationship between canopy transpiration at night ($E_n$) and mean vapour pressure deficit (VPD) for each studied species. Best curve fit was: logarithmic for *A. unedo* and *Q. ilex* and linear for *E. arborea*. 
Fig. 7. Relationship between canopy stomatal conductance ($G_s$) and vapour pressure deficit (VPD) for each species using data collected at night (11:00 p.m. to 6:00 a.m.).
Fig. 8. Average of the half-hourly measurements of $G_s$ (red line) and ozone stomatal fluxes of the three species in the first (left panels) and in the second period (right panels). Ozone fluxes were calculated using concentrations at two different heights. Grey lines show trend using ozone concentrations at 3.8 m and black lines at 1.5 m. Night-time flows slightly increase between the two periods for Q. ilex and A. unedo, while the day-time fluxes decrease for all three species.