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Biogeosciences Discussions



# *Interactive comment on* "A whole plant approach to evaluate the water use of mediterranean maquis species in a coastal dune ecosystem" *by* S. Mereu et al.

**S. Mereu et al.**

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The Authors wish to thank the Anonymous Referee 4 and Dr. R. Tognetti for their detailed and useful manuscript review.

**Response to Referee 4**

**General comments**

*The results are largely descriptive and confirmatory of previously published studies. The main interest of the paper is the extreme character of the study system itself and the fact that many studies have already been conducted at the same site, providing an opportunity for integrating different aspects of the ecophysiology of the studied species.* As observed by Referee 4, previous studies have focussed on the water relation of the



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three considered species. However, while this topic has been discussed for many years now, specific studies for Mediterranean vegetation in coastal environments are still rare. Moreover, to the best of our knowledge, this is the first time that an integrated approach, based on the integration between measurements at different scales in the soil-water-atmosphere continuum, has been used to study the water use of Mediterranean maquis species in a coastal dune ecosystem. Therefore, we think that our results, even if partially confirmatory of previous studies, may be of interest for a better and more complete understanding of the ecophysiological behaviour of the three studied species, and of their species-specific behaviour in different environments.

*In my view, however, this opportunity has not been fully realized. I suggest the authors restructure the manuscript and focus it around one or two well defined hypotheses. In that respect, the previous paper by Alessio et al. (2004) on the water sources of the studied species at the same site, together with the previous ecophysiological studies on those species, provide a very good opportunity for hypothesizing specific responses for each species. Also, the paper would benefit greatly from a review by a native English speaker and by a careful revision by the authors to correct any remaining mistakes.*
The article already takes into account the previous paper of Alessio et al. 2004, which used isotopes to study the water use and the water use efficiencies of the studied species in the same site. Their work was particularly valuable to us, allowing to make considerations on the relationship between the root structure and the water use dynamics of the species (lines 25-29 pag 1727, line 1-7 pag 1728). We agree with Referee 4 that this previous paper may be used more valuably and hence the introduction has been enriched with findings of previous papers (see also response to comment 5). The manuscript language has been revised.

**Specific comments**
1) *p.1714, l.7; and thereafter: The manuscript contains many ambiguous statements that are not well supported with either data or arguments. For instance, what is meant here by 8220;complexity of the response8221; and by 8220;complexity of the sys-*



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*tem8221;?*

We meant that plant response was specific to the particular environmental conditions of the study site. However, we agree with Referee 4 that this sentence was ambiguous; therefore, the paragraph has been rewritten.

2) *p.1715, l.19-20 and thereafter: It is unclear to me why do you think that 8220;these characteristics of Mediterranean dune ecosystems may prevent the possibility to determine the water use strategy of a species8221; and what is added by your study in that regard. The connection with climate change should be either developed further or deleted.*

The reason for which the site characteristics may prevent the possibility to determine the water use strategy is further explained few lines below the statement. We argue that if some roots, but not all, reach the water table we may assist to a decline in transpiration and stomatal conductance together with a fairly constant predawn and midday water potential. In this situation the strategy of the species would result as an isohydric, but this behaviour is not necessarily the outcome of a physiological control and may be explained in terms of changes in hydraulic properties (in this case changes in absorbing surface). Additionally, plants may differ in root distribution in the soil and root depth. As we argue in the article, the changes in transpiration and midday water potential reflect both the physiology and the root distribution of the species. A species with a higher proportion of roots in the upper soil layer and few roots that tap the water table may keep the midday water potential constant as the SWC declines, but transpiration may decline. In fact a reduced root surface implies a lower hydraulic conductivity and hence a reduced flow at equal difference in water potential between the two extremes. Without the article by Alessio et al. (2004) it would have not been possible to understand the differences in water use between the three species. The paragraph in the manuscript has been rewritten in order to clarify this point.

3) *p.1716, l.11: I don8217;t think this equation is required. In any case, it is unclear what 8220;g8221; stands for in Eq.1, and the same symbol is repeated with a different*



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*meaning in Eq.3. Also, you should make clear at which level you are focusing the discussion: is it at the leaf level? at the whole-plant level? This is very relevant.*
We agree with Referee8217;s suggestion, therefore the equation 1 has been deleted. The text has been modified in order to be clear that we focused on the integration of studies at different levels: leaf level, whole plant level and structural parameters. Also the title has been revised according to this suggestion: the term 8220;whole plant approach8221; has been corrected with 8220;integrated approach8221;.

4) *The sentence 8220;The implication of our findings for the quantification of the interactions between Mediterranean vegetation and the atmosphere will be finally discussed, in the frame of the ACCENT-VOCBAS campaign8221;; at the end of the Introduction is never substantiated.*
Following Referee8217;s suggestion, this sentence has been removed from the introduction and added to the revised Abstract.

5) *Overall, the Introduction is too general, and should be streamlined focusing on the specific hypotheses that the authors want to address in their study.*
An experimental hypothesis has been made explicit after the findings of Alessio et al. (2004), and the introduction has been revised accordingly, following Referee8217;s suggestion.

6) *p.1719, l.24-26: why were predawn leaf water potentials not measured? (see below) Please also specify whether the four leaves per species were sampled from different individuals*
Yes, the four leaves were sampled from different individuals.

7) *A general methodological question is why measurements were not continued after the end of July, as conditions would have been presumably (even) drier and might have highlighted different responses to those observed. It would also be useful to know how the meteorological conditions of 2007 compared to those of an 8220;average8221; year.*



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The ACCENT-VOCBAS field campaign ended at the beginning of June. Our group and that of Gerosa made an effort to prolong the campaign. The meteorological conditions of year 2007 have been already described in detail in the introductory article of the ACCENT-VOCBAS campaign (by Fares et al.), where the Bagnouls-Gaussen and Mitrakos diagrams for the years 2005, 2006 and 2007 are reported; therefore we did not think it was important to repeat these information. However we have modified the discussion, taking into account the difference between our year and the average year.

8) *p.1720, l.3-5 and thereafter: The fact that sap flow was not measured for P. latifolia remains an important limitation of the study. Why did you not use another technique, such as the heat balance method, allowing the measurement of small stems? Also, four stems per species is a low sample size provided the variability of sap flow. Finally, it is unclear how sapwood depth was estimated, and how the radial integration of sap flow was achieved.*
We are aware that the lack of sap flow measurements on P. latifolia is a limitation, however we did not own sensors based on the heat balance method. Four plants per species is in the range of replicates often used in field campaigns for the comparison of species water relations. The method used to calculate sapwood depth and to integrate sap flow is described in the paper cited as reference in the text (Cermak et al. 2004) Additional details have been added to materials and methods.

9) *p.1722, l.19-24: the soil water contents (SWCs) reported in the study are extremely low. Were the TDR probes calibrated using soil from the study site? This is critical in this case as SWCs are used to estimate predawn leaf water potentials (see below) and whole-tree hydraulic conductance.*
Soil water content (SWC) values were used to estimate the soil water potential and not the predawn water potential. This approach was preferred because it allows to calculate the plant hydraulic conductance without errors due to unreached hydraulic equilibrium between the plant and the soil at night (Donovan et al., 2001), or changes in root-to-soil adherence. In this sense the plant hydraulic conductance reflects the



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whole path from soil to atmosphere. But we are aware that also this method has an intrinsic error, in this case due to the conversion from soil water content, SWC to soil water potential, using the pF curve. However, the presence of a water table does not allow for extreme changes of predawn water potential during the studied period, this was also tested on the same species during the following year (2008 growing season) when predawn water potentials measured at similar soil water contents varied between -1.2 and -1.8 MPa.
The default table for mineral soils of the Delta T sensor library was used to calibrate the TDR probes. Following the referee comment, we have noticed that this calibration yields a minimum value of 4.3% while the soil samples used for the pF curve have a SWC of 5.7% at -4.2 MPa. Therefore, the TDR probes were recalibrated accordingly. The new calibration was obtained moving the curve proportionally between the default curve for mineral soils and that for organic soils, in order for the minimum and maximum measured values measured by the TDR to match the same values obtained in controlled conditions: minimum values found at -4.2 MPa in the pF curve, and maximum SWC value measured gravimetrically after abundant irrigation around the probe. The new calibration, even if more accurate, does not modify the interpretation of the results as the difference is on average of 1.3% at low SWC.
The measured SWC values are so low because the site is characterized by a sandy soil, with low water retention capacity, as described both in section 2.1 of this manuscript and in the introductory paper of this special issue (The ACCENT-VOCBAS field campaign on biosphere-atmosphere interactions in a Mediterranean ecosystem of Castelporziano (Rome): site characteristics, climatic and meteorological conditions, and eco-physiology of vegetation. S. Fares, S. Mereu, G. Scarascia Mugnozza, M. Vitale, F. Manes, M. Frattoni, P. Ciccioli, and F. Loreto). This site characteristic has also been deeply discussed in section 4-Discussion of our manuscript. However, the low values at 10 cm depth should be taken with caution because soil disturbance is likely to have occurred at this depth and also because the organic matter at this depth clearly shrinked as the soil dried, creating open spaces between the soil and the probe. The



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text has been edited in order to clarify this aspect

10) *p.1724, l.23: you should justify why an exponential function is used instead of the more usual logarithmic fit.*
We used the best curve fit (exponential) and not an a-priori determined curve. However, following Referee8217;s suggestion, the exponential fit has been replaced with the logarithmic fit as in Martìnez-Vilalta et al. (2003), Schäfer et al. (2000), Oren and Pataki (2001). This change does not modify our interpretation of the results.

11) *p.1724, l.24-26: it is unclear what changes in the environment on the 20th of June that justifies splitting the data there. Also, from Fig.7 it is not clear that the relative change before and after that date is different for the two species*
The choice to separate the data on the 20th of June is given by the change in slope of the VPD vs Gs curve, more evident for A. unedo. This moment also coincides with the moment when the soil water content at 100 cm depth reaches the values of 7.4%. This happens more or less one week earlier the moment in which the SWC at 100 cm reaches the constant value of 5.8%. We argue that the slope change of June, 20, could mark the beginning of the change in the absorbing root surface as the SWC progressively declines from the superficial layers, and consequently the water uptake of both species shifts from the shallow soil layers to the water table. In agreement with the discussed relationship between Ql,max and SWC, as well as with the change in the radial pattern profiles, this shift is more evident for A. unedo, a species which should have more evenly vertically distributed roots, being able to use both water resources (superficial water, when present, and water table), while Q.ilex partially compensates the reduction of the hydraulic conductance, induced by soil water stress, by enhancing it in the roots that absorb water from a reliable resource (water table), as suggested by Tognetti et al. (1998). The percentage reduction in the slope of the logarithmic fit between late spring and early summer is 55.5% and 37% for A.unedo and Q.ilex, respectively; these values have been reported in the text and captions to render this aspect clearer.



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12) *p.1725, l.7-12: the results on the radial sap flow patterns should be presented earlier. Also, if I am not mistaken in l.12 it should say 8220;the radial pattern DID NOT change*

The Referee is right, the radial patter of Q.ilex DID NOT change during the season. The corresponding sentence in the manuscript has been corrected. As for radial sap flow patterns, authors think that the results of this measurements can be better understood if described after the sap flow measurements, as in the original version of the manuscript.

13) *p.1725, l.21-27: were your estimates of LA/SA at the branch level as in Martínez-Vilalta et al. (2003)? Otherwise that could explain the observed discrepancy. Please clarify.*

As reported in Section 2.3, the protocol used to derive the LA/SA values is described in detail in the introductive paper of this special issue (8220;The ACCENT-VOCBAS field campaign on biosphere-atmosphere interactions in a Mediterranean ecosystem of Castelporziano (Rome): site characteristics, climatic and meteorological conditions, and eco-physiology of vegetation8221;. S. Fares, S. Mereu, G. Scarascia Mugnozza, M. Vitale, F. Manes, M. Frattoni, P. Ciccioli, and F. Loreto). Briefly, the LA/SA ratio of each species was estimated as the slope of the linear fit of the LA vs. SA regression using thirty branches for each species with diameters ranging between 0.3 and 5 cm. These diameters often corresponded to an entire shoot or to the whole plant. The ratio between LA and SA did not vary greatly excluding or including the stem level measurements. All in all, the method we used to measure LA/SA show values that are about double of those of Martinez-Vilalta et al (2003), a difference which is unlikely to be attributable only to the different methodologies.

14) *p.1726, l.15-16: I do not see how this sentence follows from the previous discussion. Please reword or delete*

The Referee is right; the sentence has been deleted.

15) *p.1726-1728: I found this part of the Discussion confusing and anecdotic. In my view, one of the most intriguing results of this study is the fact that the studied species*



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*managed to keep leaf water potentials relatively high while there was basically no water in the soil (Fig.1), suggesting that they had access to deep water resources. However, a proper understanding of this result would require knowledge on the root distribution of the studies species, as well as detailed measurements of predawn leaf water potentials. In this regard, a figure showing the time patterns of the estimated soil water potential would be useful (alternatively, this information could be added into Fig.2).*
The text has been revised, in order to be more clear, but we think that in the discussion it is clearly stated that these species have access to the water table. It is also suggested that they differ in the amount of roots that reach the deeper soil layers and that the different flows are determined by the different root surfaces that reach this water. As argued in the discussion this would imply similar water potentials but different flows with the same difference in water potential. The additional information regarding the soil water potentials used to calculate the hydraulic conductance have been added to the article. In particular, the soil water potentials extrapolated from the pF curve have been added to figure 2, as suggested by the referee

16) *p.1728: As I have said before, the results of the Alessio et al. (2004) study may provide a good starting point to structure the paper around one or two relevant hypotheses regarding how the study species may respond to drought*
As suggested by Referee, the results of the Alessio et al. (2004) study are now used also in the introduction to hypotize the possible drought response of these species (see also response to general comments and to point 5).

17) *p.1728: the apparent increase in whole-plant hydraulic conductance in P. latifolia is intriguing, but the data is not conclusive enough to reach solid conclusions, and the discussion on that point remains highly speculative. To begin with, the estimation of whole-plant hydraulic conductance in P. latifolia is not based on sap flow (as in the other two species) but on leaf-level gas exchange measurements. The authors do not say how many leaves were sampled and, at any rate, they should show that the estimates of whole-plant hydraulic conductance are similar for the other two species regardless*



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*of whether they are based on sap flow or leaf-level transpiration.*
More information has been added to this point. The number of measurements and leaves used for the estimation of the whole plant hydraulic conductance of P.latifolia has been specified in material and methods (the 95% percentile of 15-20 measurements taken between noon and 15.00 pm). The qualitative trend of K estimated by sap flow or by leaf level transpiration theoretically should be similar. As a counterproof, K estimated from leaf level also for the other two species has been added in Figure 8 in order to strengthen the comparison, following Referee8217;s suggestion.

18) *p.1729, l.7: see comment (11) above*
We have added some of the consideration reported in response to comment (11) in different part of the discussion, and reformulated this sentence in order to better clarify this point.

19) *p.1730, l.13-15: the reference to climate change is far too general to be of interest*
The reference to climate changes has been deleted from the conclusions, introduction and abstract of the manuscript

20) *Fig.1: I am surprised that the VPD values are so low, never reaching values >1.6 kPa. Do they correspond to average daily values or average daytime values?*
The VPD values correspond to average daytime values (from sunrise to sunset). These values are low because, as described in section 2.1, the proximity of the sea determines a high air humidity (rarely below 50%) in our studied site especially in the morning. Maximum values can be extrapolated from figure 7 and were never higher than 3.1 kPa.

**Technical corrections**

1) Done
2) Done
3) We were not able to understand the comment
4) Done



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5) The site coordinates (41° 408217; 49.38217;8217; N, 12° 238217; 30.68217;8217; E) are reported in the introductive paper of the special issue (The ACCENT-VOCBAS field campaign on biosphere-atmosphere interactions in a Mediterranean ecosystem of Castelporziano (Rome): site characteristics, climatic and meteorological conditions, and eco-physiology of vegetation. S. Fares, S. Mereu, G. Scarascia Mugnozza, M. Vitale, F. Manes, M. Frattoni, P. Ciccioli, and F. Loreto). As explained above, given that our paper is part of the special issue 8220;The ACCENT-VOCBAS field campaign on biosphere-atmosphere interactions in a Mediterranean ecosystem8221;, we have tried to avoid duplications in the site description referring, where possible, to the general introductive paper.
6) Yes, corrected.
7) Done
8) Done
9) Corrected, Gb
10) Done
11) No, we did not use repeated measurements ANOVA. Data were analyzed with a Two-Way ANOVA, with species and date of measurements as factors. The ANOVA test was followed by the Newman-Keuls post-hoc test, at $p<0.05$. The number of replicates was $27<N<45$ for gas exchanges measurements, $12<N<20$ for leaf water potential measurements. The description of Statistical analysis (Paragraph 2.7) was revised, and $p<0.05$ has been deleted from line 7, page 1722, following Referee8217;s suggestions.
12) Done
13) The correct nomenclature is LMA, and it has been corrected also elsewhere in the manuscript.
14) Done
15) Done
16) Done
17) Done



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18) Done
19) Done
20) The sentence has been deleted, as suggested by Referee.
21) Done
22) Done
23) Done
24) Done
25) Done.

**Response to Interactive comment by R. Tognetti**

**General comments**

*A complex set of unspecified mechanisms at whole plant level might be involved in contradictory water use performances of these species, including plant size, water consumption, acclimation processes, species competition, etc., and scaling of results on ecophysiological parameters collected during a short-term experiment to species-specific survival should be done with caution. The high experience in ecophysiological studies of the Authors, induce themselves to extrapolate beyond the domain of the data. In particular, some speculation on root structure and function should be avoided, as well as the relation to plant origin.*

Following the suggestions of both Referees, the references to species-specific survival under climate changes scenarios have been deleted from the manuscript. The comments relating the observed drought response to plant origin have been deleted too; however, the authors think that the discussion about root structure and function is well supported from the experimental data about radial sap flow patterns, from the relationship between Ql,max and SWC, and from previous studies conducted in the same site by Alessio et al. (2004).

**Technical corrections**

1) *mmoli in Fig 8 should be mmol*
Done



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2) *p. 1725, line 12: please revise, it seems there is some contradiction with previous sentence*
Done, it was a typing error.

3) *In the relationship between Gs and VPD there could be the confounding effect of PAR, which varies concomitantly*
Gs was estimated from sap flow measurements (Eq. 3 in the revised ms) at saturating light intensities (PAR>1000); therefore, the effect of PAR on the relationship between Gs and VPD should be negligible.

**Conclusion**
*In conclusion, the manuscript is well written and methodologically sound, though it prevalently corroborates observations and results presented by other studies before. The Authors are well versed in the literature immediately pertaining to their topic. However, discussion of results mainly confirms emerging consensus on resistance/adaptation mechanisms to water stress in Mediterranean plants, without breaking new ground. I think the study would benefit if the discussion also related to in-situ situations and whole-ecosystem responses that have been observed and where similar general patterns were found. I would suggest the editor to accept the manuscript after minor revision.*
Following Referee8217;s suggestions, we have revised the discussion, by adding more references to previous studies

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