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Are carbon and oxygen isotope compositions of bulk leaf material reliable predictors of water use efficiency in slow-growing drought-adapted species?

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Introduction

Regulation of water use efficiency (WUE) is particularly important in Mediterranean ecosystems, where plants are periodically exposed to severe water stress. An important technique to measure WUE is carbon isotope discrimination ($\Delta^{13}C$) in leaf material, which represents an integrative measure over the leaf lifetime. However, in bulk leaf material of slow-growing evergreen species the influence of seasonal environmental constraints may be buffered by leaf longevity. Furthermore, under conditions of limited resources, interpretation of $\Delta^{13}C$ may not be straightforward, since regulation may occur through a coherent change in stomatal restriction of CO_2 diffusion and down-regulation of photosynthetic capacity, which may result in similar WUE. Recently it has been suggested that the oxygen isotope ratio ($\delta^{18}O$) of bulk leaf material may reflect evaporative conditions and may be used to determine whether variations in $\Delta^{13}C$ result from differences in photosynthetic capacity or in stomatal conductance (Farquhar et al. 1998). While ^{13}C is depleted during photosynthesis, leaf water is enriched in ^{18}O during transpiration, since diffusion and enzymatic incorporation generally favours the lighter isotope. This enrichment in ^{18}O of leaf water is passed on to organic molecules due to an isotopic exchange of the water oxygen and the carbonyl group oxygen during biosynthesis. The oxygen isotope composition of organic material is further influenced by the isotopic composition of the source water. The degree of leaf water enrichment depends on the ratio of the vapour pressure differences in the atmosphere and the intercellular spaces (e_a/e_i), whereby low relative humidity causes an increase in leaf water enrichment. In general, bulk leaf water is somewhat less enriched than water at the evaporating surface, due to a gradient within the leaf as a result of the shifting balance between the convective evaporation flux of unfractionated water through the leaf, and the back diffusion of isotopically enriched water away from the evaporating sites (Yakir & Sternberg 2000). This phenomenon, termed the Péclet effect, has been modelled by

Farquhar and Lloyd (1993) predicting an inverse relationship between $\delta^{18}O$ of bulk leaf water and the rate of transpiration.

The combination of $\delta^{18}O$ and $\delta^{13}C$ has the advantage to allow a differentiation between stomatal regulation and carbon assimilation (see Farquhar et al. 1998): When humidity is the underlying source of variation, as humidity increases both $\delta^{13}C$ and $\delta^{18}O$ will decrease. This should also be the case when the source of variation is stomatal conductance. However, when the source of variation is increasing photosynthetic capacity, which will tend to draw-down C_i, then carbon isotope discrimination will diminish. Thus, if stomata do not respond, when $\delta^{13}C$ becomes less negative, $\delta^{18}O$ is unaffected.

Here, we will analyse these effects for 11 species of a Mediterranean plant community, which comprise different functional groups regarding their drought adaptation mechanisms. The usefulness of additional oxygen isotope analysis of the bulk leaf material to distinguish different strategies will be discussed.

Materials and methods

The study was performed in the *Parque Natural da Serra da Arrábida* in SW Portugal, in a well-preserved macchia formation, situated on a costal south-east facing slope (38° 28' 40" N, 8° 59' 34" W, elevation 280 m). The plant community at the study site is dominated by the evergreen sclerophylls *Quercus coccifera* L., *Arbutus unedo* L., *Olea europaea* var. *silvestris* Brot., *Phillyrea latifolia* L., *Phillyrea angustifolia* L., *Pistacia lentiscus* L., *Erica arborea* L. and *Juniperus phoenicea* L., and the drought semi-deciduous shrubs *Cistus albidus* L., *Cistus monspeliensis* L. and *Rosmarinus officinalis* L. 10-20 south-facing sun leaves of 5 marked plants per species were collected once per month. Only fully mature leaves from the latest growth period were used. Leaves were oven dried at 60°C for 48h and milled to fine powder for carbon and oxygen isotopic analysis. The isotopic composition was determined using standard mass spectrometric techniques (at the ICAT Stable Isotope Laboratory, Lisbon). Sample preparation was performed in an elemental analyzer (Carlo Erba, Italy) where the samples are automatically combusted to water and CO_2 for the analysis of C isotopes and through pyrolysis for O isotopes, respectively, and analysed in a SIRA II VG mass spectrometer (Micromass, UK).

Results

Within the macchia ecosystem pronounced differences in $\Delta^{13}C$ of > 4‰ were found among all woody species and a ranking of the species to their WUE is presented in Fig. 1. Drought semi-deciduous species (*Cistus* spp.) showed the lowest WUE. These species have an extended growth period during favourable environmental conditions and a high leaf-turnover, which was reflected in seasonal variations ranging from 18.2‰ to 21.0‰ from autumn to spring (data not shown). Two species, namely *J. phoenicea* and *P. angustifolia*, showed remarkably higher

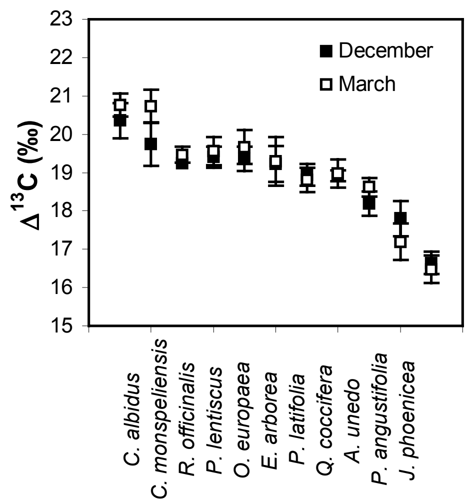

Fig.1. Carbon isotope discrimination ($\Delta^{13}C$) of 11 mediterranean macchia species measured in December and March, n=5 ± SE.

WUE, however most eve green sclerophylls exhibited similar carbon isotope discrimination, ranging from 18.3 to 19.7‰. Furthermore, only minor seasonal changes in $\Delta^{13}C$ occurred in their long-lived leaves, which generally emerge during a short flush-period in spring (data not shown).

However, regarding oxygen isotopic composition, remarkable differences between species appeared (Fig. 2). The semi-deciduous *Cistus* species show the lowest isotopic composition of both carbon and oxygen, whereas *J. phoenicea*, the species with the highest WUE did exhibit a high $\delta^{18}O$ values. Interestingly, *Q. coccifera*, a very drought tolerant evergreen oak revealed the highest $\delta^{18}O$. In all species a decline in $\delta^{18}O$ was observed from December to March, however the differences were less pronounced in *Q. coccifera* and *O. europaea*. In the semi-deciduous *Cistus* spp. this reduction in $\delta^{18}O$ was accompanied by a coherent reduction in $\delta^{13}C$.

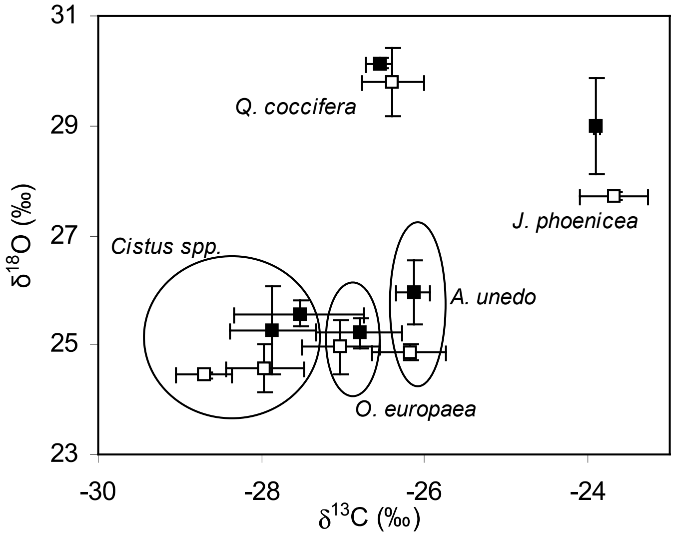

Fig.2. Carbon ($\delta^{13}C$) versus oxygen ($\delta^{18}O$) isotope composition (‰) of bulk leaf material in selected semi-deciduous and sclerophyllous species in December and March (closed and open symbols, respectively), n=3 ± SE.

Discussion

A high variation in $\Delta^{13}C$ was found among all macchia species, revealing clear differences between functional groups: drought semi-deciduous exhibited lower water use efficiencies than evergreen sclerophylls, which is in agreement with their lower stomatal regulations of transpirational water loss (Correia et al. 1987). Highest WUE was found in *J. phoenicea*, followed by *P. angustifolia*, however all other sclerophylls revealed a similar $\Delta^{13}C$. Furthermore, seasonal changes in these evergreen leaves were small (data not shown). Fig. 2 indicates that the simultaneous analysis of carbon and oxygen isotope composition of bulk leaf material allows a more detailed analysis. Scheidegger et al. (2000) developed a conceptual model linking $\delta^{13}C$ and $\delta^{18}O$ to changes in stomatal conductance and photosynthetic capacity. Following the model, the decrease in $\delta^{18}O$ from December to March indicates an increase in relative humidity, in agreement with the very rainy and humid weather (data not shown). In the evergreen species, changes in $\delta^{18}O$ were only accompanied by small or no changes in $\delta^{13}C$, indicating that an increase in stomatal conductance was balanced by a proportional increase in photosynthetic capacity, without alteration in WUE. On the other

hand, in the semi-deciduous *Cistus* species both $\delta^{18}O$ and $\delta^{13}C$ declined, indicating that an increase in stomatal conductance exceeded any increase in photosynthetic capacity, resulting in a lower WUE.

Since all species were growing in the same microenvironment, we do not expect the isotopic composition of the air water vapour or rain water to cause any differences among species. However, these species strongly deviate in their rooting depth. Differential utilization of soil water from different depths or ground water may have accentuated the differences in $\delta^{18}O$ among species. Especially *Q. coccifera* is known to develop a very deep root system and may probably tap into a durable water source which allows the maintenance of much higher water potentials throughout the year as compared to other evergreen sclerophylls on this site (data not shown, see Werner et al. 1999). Semi-deciduous species have a shallow root system which restricts their water uptake to the upper soil layers. Temporary use of rain water during winter could explain the similar $\delta^{18}O$ values for *O. europaea*, *A. unedo* and *Cistus* spp., however further information on the water source utilization and annual changes is necessary for a thorough interpretation.

The marked differences in $\delta^{18}O$ of *Q. coccifera* and other sclerophylls with similar WUE may be at least partially due to the very conservative strategy of this species: it is highly drought tolerant but reveals lower stomatal conductance and low maximum photosynthetic capacity during winter and spring, as compared for example with *A. unedo* (see Beyschlag et al. 1986, Tenhunen et al. 1987). Hence *Q. coccifera* may work on a lower operational point, resulting in the same WUE.

Even though more data are needed, the present work indicates the advantage of combined use of carbon and oxygen isotopes of leaf dry matter for the interpretation of WUE and drought tolerance strategies, especially in long-lived evergreen species.

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