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4 **Title: Mediterranean sea cliff plants: Morphological and physiological responses to**
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6
7 **environmental conditions**

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25 **Running title: Sea cliff plants and Mediterranean environment**
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Abstract***Aims***

The plants of Mediterranean sea cliff ecosystems are resistant to several environmental challenges. In the present study, six species typical of the coastal rocky cliffs have been analysed in order to evaluate their diverse morphological and physiological responses to their environment across the seasons, and to examine the strategy of the ecological group to which each species belongs. Since these species are widespread across the Mediterranean region, our aim was also to highlight their ecophysiological features in habitats where the direct influence of the sea is stronger.

Methods

The selected species are characteristic of the sea cliffs of Elba island (Tyrrhenian sea, Italy): the halophyte *Crithmum maritimum*, the semideciduous *Helichrysum italicum* and *Lavandula stoechas*, and the sclerophylls *Myrtus communis*, *Quercus ilex* and *Rhamnus alaternus*. Four morphological traits - canopy height, leaf area, specific leaf area and leaf dry matter content - and two physiological traits - leaf water potential (LWP) and photosynthetic efficiency (PE), measured before the dawn and at midday - were analysed. Water potential was measured by a pressure chamber and photosynthetic efficiency was determined by the analysis of chlorophyll fluorescence. Plant performance was also evaluated by calculating chronic (PI_{chr}) and dynamic photoinhibition (PI_{dyn}).

Important findings

Crithmum maritimum showed high resistance to the recurrent dry periods, because of

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4 the high water storage capacity of its leaves and its PE declined markedly only in July,
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6 under the harshest climatic conditions. Semideciduous taxa utilise primarily an
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8 avoidance strategy, which aims at reducing the overall leaf surface, while sclerophylls
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10 mostly show a tolerance strategy towards the prevailing stressors, as demonstrated by
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12 LWP and PE, that are lower in the sclerophylls than in the semideciduous taxa during
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14 summer, due to osmoregulation and photoinhibition, respectively.
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18 Furthermore, variability of physiological parameters was higher in the sclerophylls than
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20 in the semideciduous taxa, because the former had to withstand wider oscillations of
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22 their LWP and PE. The sclerophyllous taxa underwent a slight loss of PE also in winter,
23
24 likely owing to the combined action of low temperature and high irradiance. In
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26 Mediterranean sea cliff ecosystems, the stressful combination of high irradiance, high
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28 temperatures and low rainfall typical of the summer season may have been intensified
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30 by the shallow soil which displays a poor water storage capacity. On the other hand,
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32 winter stress, caused by high solar radiation and low temperatures, does not seem to
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34 seriously affect the performance of the studied species.
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41 **Keywords:** leaf water potential, phenotypic plasticity, photosynthetic efficiency, plant
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43 functional types
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INTRODUCTION

The Mediterranean region is characterised by recurrent water shortage throughout the year, high values of solar radiation and the occurrence of extreme weather phenomena such as heavy rainstorms and hail. Summer drought is generally considered the primary constraint to plant growth in Mediterranean areas (Mooney *et al.* 1974, Mitrakos 1980, Werner *et al.* 1999). In particular, the synergy of three ecological factors – such as water deficit, high air temperatures and an excess of light - may result in a chronic photoinhibition or down-regulation of photosynthesis. This phenomenon may cause a marked decrease in CO₂ assimilation by plants (Zhou *et al.* 2010). The definition of photoinhibition is based on the concepts of dynamic and chronic photoinhibition, which were firstly introduced by Osmond (1994). Dynamic photoinhibition is a short-term reversible process for the controlled dissipation of excessive light energy and it may be interpreted as a flexible adjustment to actual environmental conditions. On the other hand, chronic photoinhibition is a slowly reversible process that may follow prolonged exposure to excessive photon fluxes and it should reflect the integrated response to long-term environmental stress (Werner *et al.* 2002). A further major attribute of the Mediterranean climate, especially from a biological point of view, is winter cold stress (Mitrakos 1980). Its strength depends on latitude, elevation, and continentality. It is well-known that winter cold stress plays a similarly important role - with respect to summer drought - in the development and distribution of Mediterranean plants. In fact, there is evidence that winter conditions - such as low temperatures, short photoperiods, and clear days with elevated irradiance - ~~canare able to~~ induce photoinhibition in

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4 Mediterranean species (Oliveira and Peñuelas 2000, 2004, Werner *et al.* 2002, Ain-
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6 Lhout *et al.* 2004, Zunzunegui *et al.* 2005). Moreover, for the Mediterranean basin,
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8 changes in global climate are expected to produce a general warming with drier
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10 conditions and an increase of erratic (unpredictable) precipitation (IPCC 2013).
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14 Mediterranean plants have evolved different strategies to adapt to their
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16 environment (Zunzunegui *et al.* 2011). A widely accepted classification identifies two
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18 main strategies within the Mediterranean scrub plants: evergreen sclerophylls and
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20 drought semideciduous species (Mooney and Kummerow 1971). Sclerophylls are
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22 committed to a strategy of tolerance in response to stress, therefore they must withstand
23
24 low water potential and high photoinhibition (Levitt 1980), whereas semideciduous
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26 plants rely on avoidance of cell dehydration by keeping their water potential high,
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28 reducing stomatal conductance and shedding leaves. Among Mediterranean species,
29
30 coastal sea cliff plants are very interesting because they have the ability to cope with a
31
32 series of extreme environmental factors such as shallow and nutrient-poor soils, water
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34 deficit, intense sunlight, salt spray, and strong winds. Mediterranean coastal sea cliff
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36 systems generally consist of distinct plant communities that occur along a coast-to-
37
38 inland ecological gradient. Most of these plant communities correspond to habitat types
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40 included in the Habitat Directive (European Commission 1992), which specifies a list of
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42 habitats and species of conservation interest in Europe (Council Directive 92/43/EEC
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44 Annexes I and II; European Commission 2007). In Italy, the coastal chasmophyllous
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46 series is mainly represented by the following habitats: (i) vegetated sea cliffs of the
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48 Mediterranean coasts with endemic *Limonium* spp. and the halophyte *Crithmum*
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50 *maritimum* (EC 1240), which is the first pioneer plant community living in close
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52 proximity to the water line; (ii) thermo-Mediterranean and pre-desert scrub (EC 5320),
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4 which is openly exposed to marine winds and is composed of small scrubs such as
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7 *Euphorbia* spp. and *Helichrysum* spp.; (iii) arborescent matorral with *Juniperus* spp.
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9 (EC 5210), which is generally mixed with scrub formations of evergreen sclerophyllous
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11 plants.
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14 Recently, renewed interest was raised by the study of morphological and
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16 physiological adaptations of scrub and wood species to the prevailing stress factors
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18 operating under the Mediterranean climate (Mendes *et al.* 2001; Werner *et al.* 1999,
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20 2002; Ain-Lhout *et al.* 2004; Oliveira and Peñuelas 2000, 2004; Zunzunegui *et al.* 2005,
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22 2011; Gratani *et al.* 2006, 2013). However, as far as we know, this topic has never been
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24 studied in Mediterranean sea cliff plants, therefore our data may represent a novel
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26 contribution which may improve our knowledge ~~of~~ the ecophysiological features of
27
28 these species.
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33 In the present paper, we studied six vascular plant species – *Crithmum maritimum*
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35 L., *Helichrysum italicum* (Roth) G. Don, *Lavandula stoechas* L., *Myrtus communis* L.,
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37 *Quercus ilex* L., and *Rhamnus alaternus* L. living on coastal cliffs of Elba island (Italy)
38
39 in Tuscan Archipelago National Park. *Crithmum maritimum* is a halophytic and
40
41 succulent plant growing ~~on~~ rocky shores and rarely on sand or shingle beaches along
42
43 the Mediterranean and Black Sea coasts, as well as along the Atlantic coast of Europe,
44
45 north-wards to Scotland (Tutin 1968a). *Helichrysum italicum* is a small shrub endemic
46
47 to the Mediterranean basin able to colonise open habitats of coastal garrigues and rocky
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49 environments, from ~~the~~ sea level to 800 m a.s.l. *Lavandula stoechas* is a small shrub
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51 growing typically in low maquis and in acidophilus garrigues (frequently associated
52
53 with *Cistus* spp.), occurring in Mediterranean regions ~~from~~ the sea level to 600 m a.s.l.
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59 (Pignatti 1982). *Myrtus communis*, *Q. ilex* and *R. alaternus* are evergreen species
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4 typical of the Mediterranean maquis and forests. *Myrtus communis* is a tall shrub,
5 usually calcifuge, distributed in the Mediterranean region and in SW Europe (Campbell
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7 1968); it grows from the sea level to 500 m a.s.l. (Pignatti 1982). *Quercus ilex* is a tree
8
9 or a tall shrub whose distribution covers a large area extending longitudinally from
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11 Portugal to Syria, and latitudinally from Morocco to France (Valladares *et al.* 2000).
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13 This plant prefers acid soils but it is also able to colonise calcareous soils, growing
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15 from the sea level to 1100 m a.s.l. (Pignatti 1982). *Rhamnus alaternus* is a tall shrub
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17 distributed in the Mediterranean region and extending to Portugal (Tutin 1968b), it
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19 grows from the sea level to 700 m a.s.l. (Pignatti 1982).
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26 The main objective of the research was to evaluate the different morphological
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28 and physiological responses to the Mediterranean climate of these species,
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30 representative of the chasmophylous series, across seasons. With this aim, four
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32 morphological traits - canopy height, leaf area, specific leaf area, and leaf dry matter
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34 content - and two physiological traits - leaf water potential and photosynthetic
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36 efficiency, measured before the dawn and at midday – were analysed. We also evaluated
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38 the seasonal physiological variability of each species through the calculation of
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40 phenotypic plasticity indices, according to Valladares *et al.* (2000, 2006).
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44 The principal aim of the present research was to gain more insight into the
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46 ecophysiological characteristics of these species, which also occur in other
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48 Mediterranean areas where the influence of the sea on the environmental conditions is
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50 less strong. In particular, we wanted to search for possible further adaptations of these
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52 taxa to harder stress conditions such as those occurring on coastal cliffs.
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MATERIALS AND METHODS

Study area and plant species

Elba island belongs to [the](#) Tuscan Archipelago National Park in Northern Italy, in the Tyrrhenian Sea. The study area (Fig. 1; 42° 44' N, 10° 14' E) was located in the southern part of the island close to the village of Marina di Campo, and it included a pocket beach, without any sand dunes, surrounded by a well-developed coastal cliff system. The site was exposed to full sunlight, had a gentle slope toward east and was about 8,000 m² wide. Vegetation of the study area appeared to have undergone very low disturbance: no fire had been recorded throughout several decades prior to our investigation and the closest buildings were a sparse group of small houses about one hundred meters [away](#) ~~apart~~. Within this area we delimited one plot of about 200 m², which [lay](#) ~~ied~~ above the pocket beach, where all the investigated species were represented by a substantial number of individuals. The plot was quite homogeneous for both climate and soil features.

The analyses were carried out on six species that were selected for being representative of the plant communities found in the study area: the halophyte *Crithmum maritimum* (C.marit) typical of the pioneer vegetation of sea cliffs (EC 1240); *Helichrysum italicum* (H.italic) and *Lavandula stoechas* (L.stoech), two semideciduous plants that dominate thermo-Mediterranean and pre-desert scrub (EC 5320), and garrigue shrubland, respectively; *Myrtus communis* (M.comm), *Quercus ilex* (Q.ilex), and *Rhamnus alaternus* (R.alat), three sclerophylls characteristic of Mediterranean evergreen oak woodland (EC 9340). Although *C. maritimum* was very close to the water line (1-2 m a.s.l.), the other species were located along the sea cliffs [at](#) a few metres from the water (2-5 m a.s.l.), all openly exposed to marine winds. All the

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4 individuals of each species analysed in the present study were tagged for subsequent
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7 measurements across seasons.
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10 11 **Climate and soil characteristics**

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13 Elba island is characterised by a Mediterranean semi-arid climate, with mild winters and
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15 hot summers. Temperatures and rainfall for the period 1955-2004 were recorded by the
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17 meteorological station of San Piero in Campo (42° 45' N, 10° 12' E; 226 m a.s.l.). Solar
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19 irradiance and global radiation were measured by a station located in the Isle of Pianosa
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21 (27 m a.s.l.), 20 km SW from the coast of Elba. Given the absence of natural barriers
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23 and the short distance, we assumed that the solar data recorded in Pianosa were
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28 representative of the area under investigation.
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31 This site was characterised by an average annual temperature of 16°C with
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33 January as the coldest month (8.7°C monthly average temperature), and August as the
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35 hottest one (24.7°C monthly average temperature). In the period 1955-2004, the mean
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37 annual rainfall was 672 mm. October and November were the most humid months
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39 (precipitations of 97.5 and 92.1 mm, respectively), while July was the driest one, with
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41 10.9 mm; 37% of the rain fell in autumn, 30% in winter, 23% in spring and only 10% in
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43 summer. Mean annual absolute solar irradiance was 2607 hours, with 110 hours in
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45 December and 343 hours in July. Mean annual global radiation was 149 W m⁻², with 52
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47 W m⁻² in December and 244 W m⁻² in July. For a better interpretation of our results, we
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49 analysed temperature and rainfall data for the period 2005-2006, in which we carried
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52 out our study. Monthly average temperatures (°C) and monthly total rainfalls (mm)
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54 were reported graphically in Fig. 2. In this case, climatic data were available from
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56 Tuscany Region (Sviluppo Rurale Settore "Rete dati agrometeo-climatici",
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60 www.consiglio.regione.toscana.it/articolazioni/Strutture.aspx?cmu=04172) and they

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4 were relative to the meteorological station of Portoferraio (42° 49' N, 10° 19' E; 4 m
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6 a.s.l.), 10 km NE from the study site.
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9 The study plot was partially sheltered from the prevailing winds. These were
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11 mainly breezes, from light (1.6 - 3.3 m s⁻¹) to moderate (5.5 - 7.9 m s⁻¹) and their
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13 frequency was 70.6% (nearly 257 d y⁻¹). The prevailing winds blewblowed from North-
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15 West (mainly in summer) and South-East (throughout the year, with lower frequency in
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17 winter), with frequencies of 15.8% and 15.5%, respectively. The frequency of winds
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19 blowing from North and North-East was about 23%; these could reach the highest speed
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21 (> 14 m s⁻¹), although rarely (frequency: 0.4%). The frequencies of fresh/strong breezes
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23 (8 - 14 m s⁻¹) were 5% from South-East and 2% from North-East; they were fairly
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25 distributed throughout the year. Owing to this wind regime, with its low occurrence of
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27 strong events and to the partial shielding from the prevailing air streams, the salt spray
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29 deposition was not really abundant in the site under study. Data of winds were taken
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31 from the thematic atlas MedAtlas (www.mediterranean-marinedata.eu), which reports
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33 the values relative to the point of coordinates 10.5°E - 42.5°N, located about 15 nautical
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35 miles off the coast, south of the study site.
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42 Moreover, the selected plants grew on a shallow soil, characterised by a coarse-
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44 texture made of quaternary deposits, while only bare limestone was found closer to the
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46 sea.
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51 **Morphological traits**

52 For each species we collected data on four morphological traits: canopy height, leaf
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54 area, specific leaf area, and leaf dry matter content. Each trait was quantified by
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56 measuring at least ten replicate samples from ten different individuals, randomly
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4 selected at the time of the first sampling. For leaves, sample storing and processing
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6 followed the standardised methodologies detailed by Pérez-Harguindeguy *et al.* (2013).
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8 Canopy height (CH) is the shortest distance between the upper boundary of the main
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10 photosynthetic tissues (excluding inflorescences) of a plant and the ground level,
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12 expressed in metres. This trait is generally associated with growth form, competitive
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14 vigour, and potential lifespan. Leaf area (LA) is the one-sided projected surface area of
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16 a fresh leaf, expressed in mm^2 . Leaf area is not only related to climatic variability,
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18 geology, altitude and latitude, but also to allometric factors and ecological strategy with
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20 respect to environmental nutrient stress and disturbances. Specific leaf area (SLA) is LA
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22 divided by its oven-dry mass, expressed in $\text{mm}^2 \text{mg}^{-1}$. Specific leaf area tends to scale
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24 positively with mass-based light-saturated photosynthetic rate and with leaf nitrogen
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26 (N) concentration, and negatively with leaf longevity and C investment in quantitatively
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28 important secondary compounds such as tannins or lignin. Leaf dry matter content
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30 (LDMC) is the oven-dry mass of a leaf divided by its water-saturated fresh mass,
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32 expressed in mg g^{-1} . Leaf dry matter content correlates negatively with potential RGR
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34 (Relative Growth Rate) and positively with leaf lifespan. The different leaf traits tend to
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36 be inter-correlated and are major indicators of leaf lifespan, but their outcomes do not
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38 always overlap. Leaf projected area was acquired with a CanoScan LiDE 90 (Canon)
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40 and determined by CompuEye, Leaf & Symptom Area software (available at
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42 www.ehabsoft.com/CompuEye/LeafSArea/). For LA, SLA, and LDMC current-year
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44 leaves were sampled. All the morphological measurements were conducted in June-July
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46 2005.
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58 **Physiological traits**

59 *Water potential*

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4 Water potential (Ψ) was measured monthly on young leaves or terminal shoots by a
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6 Scholander-type pressure chamber (PMS Instruments, Albany, OR, USA).

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9 Measurements were made on five leaves/shoots per plant, on five individuals per
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11 species, at two times during the day: before the dawn (between 3 and 6 a.m., Ψ_{pd}) and at
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13 midday (between 12 and 1 p.m., Ψ_{md}). Values were recorded with greater frequency in
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15 spring and summer, i.e. concomitantly with the resumption of growth and, afterwards,
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17 with the harshest environmental conditions (drought, high temperature and irradiance).
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20 21 22 *Photosynthetic efficiency*

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24 It was evaluated by analysing chlorophyll fluorescence, which was measured monthly
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26 on fully expanded, sun-exposed leaves, by a portable fluorometer (MINI-PAM Walz,
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28 Effeltrich, Germany). Four to five records per plant were taken from five individuals per
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30 species, at two times during the day: $(Fv/Fm)_{pd}$ before dawn (between 3 and 6 a.m.) and
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32 $(Fv/Fm)_{md}$ at midday (between 12 and 1 p.m.). The former parameter represents the
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34 maximum Photosystem II (PSII) quantum yield of dark-adapted samples, while
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36 $(Fv/Fm)_{md}$, which was measured on the leaves previously shaded for 30 minutes with
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38 dedicated clips, gives an estimation of the potential photosynthetic efficiency of PSII,
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40 during the most stressful period of the day. These measurements allowed us to calculate
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42 both chronic- (PI_{chr}) and dynamic photoinhibition (PI_{dyn}); PI_{chr} represents the loss of
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44 photosynthetic efficiency with reference to the maximum value of the year, while PI_{dyn}
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46 is the additional decrease of Fv/Fm occurring in leaves at daytime, which may be
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48 recovered during the night at nighttime (Werner et al., 2002). These indices were
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50 calculated by the following equations:
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$$57 \quad PI_{chr} = [(Fv/Fm)_{max} - (Fv/Fm)_{pd}] / (Fv/Fm)_{max}$$

$$58 \quad PI_{dyn} = [(Fv/Fm)_{pd} - (Fv/Fm)_{md}] / (Fv/Fm)_{max}$$

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4 where $(Fv/Fm)_{\max}$ is the highest value recorded for each species throughout the year.

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7 Physiological analyses were carried out in June, July, August and October 2005;

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10 January, March, April, and May 2006.

11 12 13 **Phenotypic plasticity index (PPI)**

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15 According to Valladares et al. (2000), an index of phenotypic plasticity (PPI), ranging
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17 from 0 to 1, was calculated for each species and each physiological parameter analysed,
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19 i.e. $(Fv/Fm)_{pd}$, $(Fv/Fm)_{md}$, Ψ_{pd} and Ψ_{md} , based on annual maximum and minimum
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21 means, following the formula: (maximum value – minimum value)/maximum value.
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25 Mean phenotypic plasticity was calculated for each species by averaging the indices of
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27 plasticity for each of the four traits.
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30 31 32 **Statistical analyses**

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34 Canopy height, LA, SLA and LDMC were compared by the non-parametric test of
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36 Kruskal-Wallis with the Bonferroni correction for multiple comparisons to verify if
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38 there were significant differences. Morphological and physiological traits were arranged
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40 in a matrix of 6 species x 12 functional parameters - such as CH, LA, SLA, LDMC, PS-
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42 PDA (annual pre-dawn Fv/Fm), PS-PDS (Fv/Fm pre-dawn in Jun-Oct), PS-MDA
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44 (annual midday Fv/Fm), PS-MDS (midday Fv/Fm in Jun-Oct), WP-PDA (annual pre-
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46 dawn Ψ), WP-PDS (pre-dawn Ψ in Jun-Oct), WP-MDA (annual midday Ψ), WP-MDS
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48 (midday Ψ in Jun-Oct) - and were analysed using two multivariate techniques, Cluster
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50 analysis and Non-metric Multidimensional scaling (NMDS). The matrix was prior
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52 standardised and square-root transformed, then was subjected to the Cluster analysis
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54 using average-linkage clustering and Euclidean distance as the dissimilarity index.
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60 Cluster analysis is a classification technique that [makes it possible](#) to disclose

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4 the natural groupings among the samples that are characterised by the dataset (Podani
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6
7 2007). The same resemblance matrix was used to perform NMDS, which is an
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9 ordination method that [allows](#) a two-dimensional visualisation of the position of
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11 the samples in relation to each other (Podani 2007). The Pearson product-moment
12
13 correlation coefficient was also calculated in order to point out which functional trait
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15 was more correlated to the NMDS axes. All statistical tests were performed using R
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17 2.14.1 software (vegan package, Oksanen *et al.* 2011, <http://www.r-project.org/>).
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25 RESULTS

26 Morphological traits

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28 Canopy height (CH) was significantly greater in sclerophyllous taxa (*M. communis*, *Q.*
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30 *ilex* and *R. alaternus*, ranging from 2 m to about 4 m) than in semideciduous plants (*H.*
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32 *italicum* and *L. stoechas*), and in *C. maritimum*, which were below 1 m (Fig. 3;
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34 Supplementary Table S1). Apparently, [this](#) trait was not affected by winds and salt
35
36 spray, because we did not find dwarf or twisted plants in the investigated area: the
37
38 reason for this might be the limited exposure of the site to strong winds. Leaf area (LA)
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40 was significantly greater (more than ten times) in sclerophyllous taxa than in
41
42 semideciduous ones. Specific leaf area (SLA) did not vary significantly between
43
44 sclerophyllous and semideciduous plants, oscillating between 5.81 and 18.03 mm² mg⁻¹
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46 (Fig. 3; Supplementary Table S1). Leaf dry matter content (LDMC) differed
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48 significantly among all species, ranging from 117.25 mg g⁻¹ in *C. maritimum* to 532.21
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50 in *Q. ilex* (Fig. 3; Supplementary Table S1). *Crithmum maritimum* exhibited the
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52 significantly highest mean LA and SLA (1987.95 mm² and 5944.67 mm² mg⁻¹,
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4 respectively), and the significantly lowest LDMC, while *Q. ilex* showed the
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6 significantly greatest mean CH and LDMC (Fig. 3; Supplementary Table S1).
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10 11 **Physiological traits**

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13 Leaf water potential (Ψ) showed the lowest values, for all species, at midday in
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15 summer, particularly in July and August, when the weather was mostly dry and hot
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17 (Fig. 2, 4). For some species (*M. communis*, *H. italicum*, *R. alaternus*), the difference
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19 between Ψ_{pd} and Ψ_{md} was minimum in this period. The lowest Ψ was recorded in July
20
21 for *R. alaternus* (-5.2 MPa). In August 2005, following some weak rainfalls (Fig. 2b),
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23 two species (*R. alaternus* and *L. stoechas*) promptly exhibited a recovery (i.e. their Ψ
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25 became less negative), while in others (*M. communis*, *Q. ilex* and *H. italicum*) Ψ
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27 changed negligibly or only slightly in comparison with July. The Ψ of *C. maritimum*
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29 was markedly higher than in the other species: during summer, Ψ_{md} varied between -
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31 0.24 and -0.37 MPa. Overall, the sclerophyllous taxa (*M. communis*, *Q. ilex*, *R.*
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33 *alaternus*) showed more negative Ψ values than the semideciduous ones (*H. italicum*, *L.*
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35 *stoechas*) during the critical summer months. Water potential was high and steady for
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37 all species in winter, while in spring there was a general decline, less pronounced in the
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39 semideciduous species, in coincidence with a dry period between March and May 2006
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41 (Fig. 2b) and with the temperature rise (Fig. 2a).
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50 Regarding the photosynthetic efficiency of PSII, which was evaluated by
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52 measuring chlorophyll fluorescence, the semideciduous species showed quite steady
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54 high F_v/F_m values throughout the year (around 0.8), with minor or no differences
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56 between pre-dawn and midday data (Fig. 5). Photosynthetic efficiency of the
57
58 sclerophyllous taxa decreased markedly in summer (July-August), while in *C.*
59
60 *maritimum* the decline was limited to July, in coincidence with the most intense drought

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4 of summer (Fig. 2). Midday Fv/Fm records were below 0.5 in the sclerophylls (in July),
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6 while in *C. maritimum* they were between 0.5 and 0.6. During summer, *Q. ilex* and *R.*
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8 *alaternus* behaved differently compared to *M. communis*: the former two species,
9
10 particularly *R. alaternus*, displayed an appreciable recovery of photosynthetic efficiency
11
12 at night. Instead, in July and August, in *M. communis* (Fv/Fm)_{pd} exceeded (Fv/Fm)_{md}
13
14 only slightly; moreover, the pre-dawn values were lower than in the two aforementioned
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16 species. The recovery of PSII efficiency, likely attributable to light rainfall between July
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18 and August (Fig. 2b), proceeded at a lower rate in *Q. ilex* than in *R. alaternus* and the
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20 process was even slower in *M. communis* (see the data of August in Fig. 5); in the latter
21
22 species the resumption of a high photosynthetic efficiency occurred only in autumn,
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24 following more abundant precipitations (Fig. 2b). The sclerophylls seem to suffer from
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26 a loss of photosynthetic efficiency also in winter, although to a lesser extent than in
27
28 summer. This slight winter decline may be related to the concomitant occurrence of low
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30 temperatures (the average daily values of the period were below 10°C, see Fig. 2a) and
31
32 scarce rainfall (especially in January-February 2006, see Fig. 2b). Such transient crisis
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34 was followed, in the subsequent months, by a prompt recovery, which led to (Fv/Fm)_{pd}
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36 values around 0.8 as early as in March-April, while (Fv/Fm)_{md} was only slightly lower.
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38 The recovery of *M. communis* did not proceed at the same rate.
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47 Seasonal changes of PI_{chr} and PI_{dyn}, calculated from the pre-dawn and midday
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49 Fv/Fm values, showed different patterns among species (Fig. 6). Total photoinhibition
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51 (PI_{tot}), i.e. the sum of PI_{chr} + PI_{dyn}, was approximately 5 – 10% in the semideciduous
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53 taxa, with a slight increase during summer. The sclerophyllous species and *C.*
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55 *maritimum* showed wide oscillations of PI_{tot} all over the year. The highest values
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57 coincided with the periods characterised by low rainfalls and high or low temperatures:
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4 summer, with more than 40% loss of photosynthetic efficiency in the sclerophylls and
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6 more than 30% in *C. maritimum* (although for the latter species the crisis was
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8 particularly severe only in July), and winter, when PI_{tot} was around 15% in the
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10 sclerophylls, with a peak of 20% for *M. communis*. As to the semideciduous taxa, *H.*
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12 *italicum* exhibited a modest PI_{chr} throughout the year, while in *L. stoechas* PI_{chr} was of
13
14 some relevance, albeit limited, only in early summer. During both critical periods
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16 (summer and winter) the sclerophyllous species showed different patterns of
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18 photoinhibition: in *R. alaternus* PI_{tot} was dominated by PI_{dyn} , in *Q. ilex* the contributions
19
20 of PI_{dyn} and PI_{chr} were nearly equivalent, and in *M. communis* PI_{chr} was dominant. In *C.*
21
22 *maritimum*, PI_{tot} in the most critical month (July) was attributable almost entirely (over
23
24 90%) to PI_{chr} . Photoinhibition was moderate in the sclerophylls between summer and
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26 winter and it was due almost completely to PI_{dyn} .
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35 **Physiological plasticity indices (PPI)**

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37 Regarding the single physiological trait, the greatest PPI value (Table 1) was shown by
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39 Ψ_{pd} of *R. alaternus* (0.96), while $(Fv/Fm)_{pd}$ of *H. italicum* displayed the lowest one
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41 (0.05). According to our data, leaf Ψ displayed greater PPIs than photosynthetic
42
43 efficiency, because the averages of the indices calculated on Ψ were higher than those
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45 calculated on (Fv/Fm) . Comparison of the mean values showed that the species with the
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47 lowest PPI was *H. italicum*, while *M. communis* exhibited the highest one. More
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49 generally, the averages of the PPIs of the semideciduous taxa were lower than those of
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51 the sclerophylls and of *C. maritimum* (Table 2). Apparently, this difference may be
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53 attributed primarily to the variability of (Fv/Fm) , which was notably lower in *H.*
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55 *italicum* and in *L. stoechas*.
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Multivariate analysis

Both Cluster analysis and NMDS (Fig. 7 and Fig. 8) highlighted three groups of species: one cluster formed by only one species, the halophyte *C. maritimum*; a second group with the semideciduous taxa (*H. italicum* and *L. stoechas*); a third cluster made by the sclerophylls (*M. communis*, *Q. ilex*, *R. alaternus*). Leaf Area, SLA and WP-MDS (midday ψ in Jun-Oct) showed a high correlation with the first two dimensions of the NMDS ordination (Pearson's coefficient > 0.98): LA and SLA were positively correlated to *C. maritimum*, while WP-MDS showed a positive correlation with *Q. ilex* and *R. alaternus* (Fig. 8).

DISCUSSION

In the present study, we have investigated some species representative of a Mediterranean sea cliff environment. The association of morphological and physiological traits by multivariate analysis has been applied to distinguish three functional groups: the halophyte *Crithmum maritimum*, the semideciduous *Helichrysum italicum* and *Lavandula stoechas*, the sclerophylls *Myrtus communis*, *Quercus ilex*, and *Rhamnus alaternus*.

The tolerance to salt and drought of *C. maritimum*, which grows all around the Mediterranean area, depends on the ability to accumulate high levels of ions in the tissues (i.e. the water parenchyma in the leaves) for osmotic adjustment (Ben Amor *et al.* 2005). In the present study, the high values of Ψ (close to 0) found in plants of *C. maritimum* highlighted a strong resistance to the recurrent dry periods. This feature is confirmed by the high values of LA and SLA and the low LDMC of this species. The

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4 photosynthetic efficiency of *C. maritimum* declined markedly only in July, when
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6 temperatures and irradiance were high and water availability was low, while it was at
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8 optimal levels throughout the rest of the year. Summer drought seems to be the main
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10 constraint for this species.
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14 The semideciduous *H. italicum* and *L. stoechas* showed low values of LA and
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16 LDMC, while the sclerophylls *M. communis*, *Q. ilex* and *R. alaternus* - with high values
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18 of CH, LA and LDMC - have tough and long-lived leaves, which are assumed to be
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20 more resistant to physical hazards (Pérez-Harguindeguy *et al.* 2013). These results are
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22 in accordance ~~with~~ Levitt's classification (1980). The author stated that
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24 semideciduous taxa utilise primarily an avoidance strategy, which relies on the
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26 reduction of the overall leaf surface, achieved through leaf abscission or desiccation,
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28 while the sclerophylls mostly show a tolerance strategy towards the stress agents
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30 prevailing in the coastal environment. These morphological features of semideciduous
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32 taxa and sclerophylls are in agreement with the analysis of Ψ and photosynthetic
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34 efficiency, particularly when considering the data recorded under the most adverse
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36 climatic conditions. In fact, in July and August Ψ was lower in sclerophyllous taxa than
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38 in semideciduous plants, suggesting the involvement of tolerance processes (i.e.,
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40 osmoregulation), particularly in the former group. The response pattern of the studied
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42 sclerophylls emerges also from the NMDS analysis, that emphasises the positive
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44 correlation of *Q. ilex* and *R. alaternus* with Ψ_{pd} values recorded from June to October
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46 (WP-MDS). The divergent behaviour of the semideciduous and sclerophyll taxa is also
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48 highlighted by their photosynthetic efficiency, which was higher and steady in the
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50 former group. Low photoinhibition in semideciduous species is ~~probably~~ likely
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52 attributable to their leaf morphology (restricted area, high reflectance, vertical
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4 orientation) and physiology (reduction of chlorophyll content), that may effectively
5 contribute to limit the amount of intercepted radiation, light harvesting and electron
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7 flow capacity, as was demonstrated for some species of this group (Kyparissis *et al.*
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9 1995; Nuñez-Olivera *et al.* 1996; Zunzunegui *et al.* 1999). Hence, we may highlight
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11 that summer drought represents the most critical environmental limitation for both
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13 semideciduous and sclerophyll species, although to a different degree. In fact, the
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15 physiological responses (particularly Fv/Fm and PI) suggest that, at least under the
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17 environmental conditions that occurred in the sea cliffs throughout the present research,
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19 the semideciduous taxa performed better than the sclerophyll ones. Within the latter
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21 group, during summer *Q. ilex* and *R. alaternus* appeared to behave differently from *M.*
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23 *communis*: the former species, particularly *R. alaternus*, showed a satisfying recovery of
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25 PSII efficiency during the night, which prevented a sustained loss of photosynthetic
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27 efficiency (moderate PI_{chr}). *Myrtus communis* appeared to be the least resistant species.
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29 Summer drought considerably lowered its Ψ : although this could be dependent on the
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31 operation of osmotic adjustment mechanisms (Mendes *et al.* 2001), the low leaf Ψ and
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33 the limited degree of rehydration at night over a prolonged time might have negatively
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35 affected the physiological performance of the plant. In *M. communis*, a marked decline
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37 of photosynthetic efficiency occurred in summer and a smaller one in winter (in the
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39 latter case the decline was ~~likely owed~~ probably due to the combined action of low
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41 temperature and high irradiance), that were attributable to PI_{chr} . The recovery was
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43 slower, in comparison to the other sclerophylls studied. These plants underwent a slight
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45 decrease of photosynthetic efficiency in winter, but to a lower degree than in *M.*
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47 *communis*. *Myrtus communis* appears particularly vulnerable and its spread is
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49 potentially jeopardized by winter chill and, mostly, summer drought, whose occurrence
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4 might become more frequent, according to the foreseen climate changes. This makes *M.*
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7 *communis* a good candidate as a bioindicator for tracking the changes in Mediterranean
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10 ecosystems.

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12 The comparison of the behaviour of the species living in the coastal cliff
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14 ecosystem with respect to the behaviour of the same species growing in different
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16 habitats of the Mediterranean basin has been carried out only for sclerophyll taxa,
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18 because of the scarcity of published data. In South Portugal, under a slightly more
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20 humid climate than that of Elba Island, less negative values of both Ψ_{pd} and Ψ_{md} were
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22 recorded for *M. communis* (Mendes *et al.* 2001). In South-West Spain, where summer
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24 drought stress was mitigated by a sufficient water availability in the soil, *M. communis*
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26 showed a higher efficiency of PSII (Fv/Fm) in summer than in winter (Ain-Lhout *et al.*
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28 2004), while in our study site the opposite behaviour was observed. In the present study,
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30 during the period of the investigation (2005-2006), *M. communis* plants underwent the
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32 most critical conditions during summer, when the stress induced by the climate may
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34 have been exacerbated by the low water storage capacity of the shallow soil. This
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36 feature might also be a major cause for the differences between the data recorded on *Q.*
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38 *ilex* in the present research and the others collected in different Mediterranean localities.
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40 In Central (peninsular) Italy, under a climate slightly hotter than that of Elba but with a
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42 higher soil water availability, in summer *Q. ilex* showed higher Ψ_{pd} and Ψ_{md} (Gratani *et*
43
44 *al.* 2000) than at our site. Potential photochemical efficiency of PSII was higher in
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46 summer and slightly lower in winter than that of Elba plants. The same result, with even
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48 greater differences, was obtained from a comparison between our data and those
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50 collected from a colder and more humid site in Northern Italy (Gratani *et al.* 2000).
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52 Therefore, in the coastal cliff environment that we have studied also *Q. ilex* underwent
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the most critical conditions in summer.

According to Mitrakos (1980), summer drought and winter chilling temperatures may be considered as the two main constraints for plant growth and distribution in Mediterranean areas, that are characterised by a polarisation between these two stresses, i.e. the higher the summer drought the lower the winter temperatures in a given area, and vice versa. Our data suggest that, at least during the period of the study, drought and not chilling has been the major seasonal limitation for the selected plants in the coastal cliff area of Elba.

In conclusion, our data suggest that in Mediterranean sea cliff ecosystems the stressful action of the combined high irradiance, high temperatures and low rainfall might have been enhanced by the fact that the plants grow on a shallow soil, which has a poor water storage capacity. Conversely, winter stress (caused by high irradiance along with low temperatures) does not seem to seriously depress the physiological performance of the studied species, probably ~~likely~~ because the temperatures are mild during this season and the plants are not exposed to strong winds.

The present work may add to our knowledge of ~~on~~ the responses of Mediterranean plants to the environmental challenges and it highlights the need for further research on this topic.

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Conflict of interest statement. None declared.

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For Peer Review

Legends to figures

Figure 1: (a) Satellite image of the site under study indicated by the white arrow (modified after Google Earth 5.2.1.1588), (b) a picture of Italy with Tuscany. The black arrow shows the location of Elba island.

Figure 2: (a) Monthly average temperatures ($^{\circ}\text{C}$) for 2005 (full line) and 2006 (dashed line), (b) monthly total rainfalls (mm) for 2005 (full line) and 2006 (dashed line). Data were obtained from the meteorological station at the site of Portoferraio (LI).

Figure 3: Box and whisker graph of (a) CH (canopy height), (b) LA (leaf area), (c) SLA (specific leaf area), (d) SLA in detail, (e) LDMC (leaf dry matter content) values for the species analyzed. Squares are medians; boxes are 25% and 75% percentiles; whiskers are maximum and minimum values. Specie abbreviations: *Crithmum maritimum* = C.marit; *Helichrysum italicum* = H.italic; *Lavandula stoechas* = L.stoech; *Myrtus communis* = M.comm; *Quercus ilex* = Q.ilex; *Rhamnus alaternus* = R.alat.

Figure 4: Seasonal changes of water potential (MPa) measured before dawn, Ψ_{pd} (filled circles) and at midday, Ψ_{md} (empty circles) from June 2005 to May 2006. Data are the mean \pm SE. 1 = January; 3 = March; 4 = April; 5 = May; 6 = June; 7 = July; 8 = August; 10 = October.

Figure 5: Seasonal changes of photosynthetic efficiency of PSII (Fv/Fm) measured before dawn (filled symbols) and at midday (open symbols), in sun leaves from June 2005 to May 2006. Midday values were recorded on dark adapted leaves. Data are the mean \pm SE. 1 = January; 3 = March; 4 = April; 5 = May; 6 = June; 7 = July; 8 = August; 10 = October.

Figure 6: Seasonal changes of chronic and dynamic photoinhibition (PI_{chr} , filled bars and PI_{dyn} , empty bars, respectively), in sun leaves from June 2005 to May 2006.

Photoinhibition is expressed as % reduction of Fv/Fm compared to its maximum annual value. Total photoinhibition (PI_{tot}) is the sum of $PI_{chr} + PI_{dyn}$. 1 = January; 3 = March; 4 = April; 5 = May; 6 = June; 7 = July; 8 = August; 10 = October.

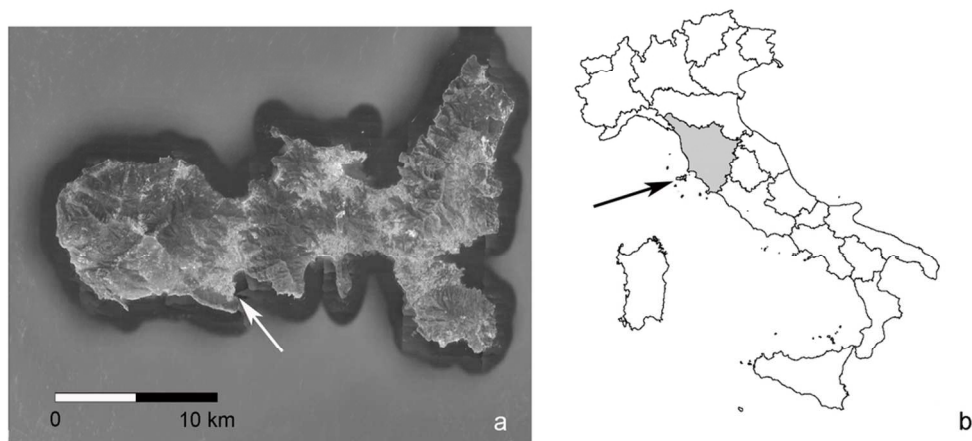
Figure 7: Agglomerative cluster analysis of the matrix formed by 6 species x 12 functional parameters - CH, LA, SLA, LDMC, PS-PDS (pre-dawn Fv/Fm in Jun-Oct), PS-PDA (annual pre-dawn Fv/Fm), PS-MDS (midday Fv/Fm in Jun-Oct), PS-MDA (annual midday Fv/Fm), WP-PDS (pre-dawn water potential in Jun-Oct), WP-PDA (annual pre-dawn water potential), WP-MDS (midday water potential in Jun-Oct), WP-MDA (annual midday water potential). Three groups of species were clustered: *C. maritimum*, semideciduous taxa and sclerophyllous taxa. Species abbreviations: *Crithmum maritimum* = C.marit; *Helichrysum italicum* = H.italic; *Lavandula stoechas* = L.stoech; *Myrtus communis* = M.comm; *Quercus ilex* = Q.ilex; *Rhamnus alaternus* = R.alat.

Figure 8: Non-metric Multidimensional Scaling ordination based on the matrix formed by 6 species x 12 functional parameters - CH, LA, SLA, LDMC, PS-PDS (pre-dawn Fv/Fm in Jun-Oct), PS-PDA (annual pre-dawn Fv/Fm), PS-MDS (midday Fv/Fm in Jun-Oct), PS-MDA (annual midday Fv/Fm), WP-PDS (pre-dawn water potential in Jun-Oct), WP-PDA (annual pre-dawn water potential), WP-MDS (midday water potential in Jun-Oct), WP-MDA (annual midday water potential). Three groups of species were clustered: *C. maritimum*, semideciduous taxa and sclerophyllous taxa. Traits with a Pearson correlation coefficient > 0.9 with the first two dimensions are shown. Species abbreviations: *Crithmum maritimum* = C.marit; *Helichrysum italicum* = H.italic;

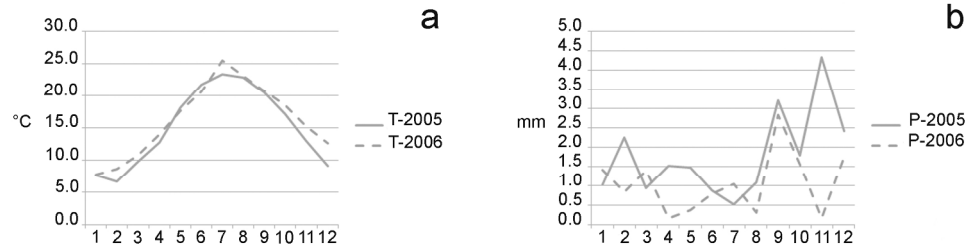
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4 *Lavandula stoechas* = L.stoech; *Myrtus communis* = M.comm; *Quercus ilex* = Q.ilex;
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7 *Rhamnus alaternus* = R.alat.
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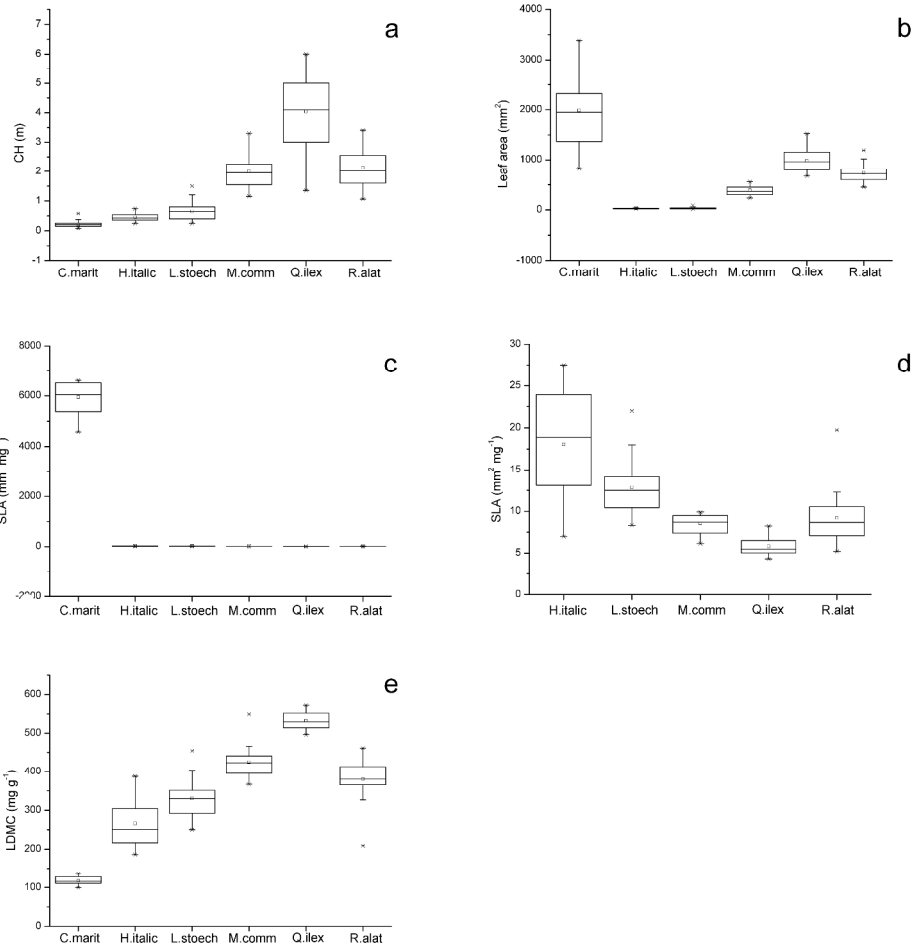
For Peer Review



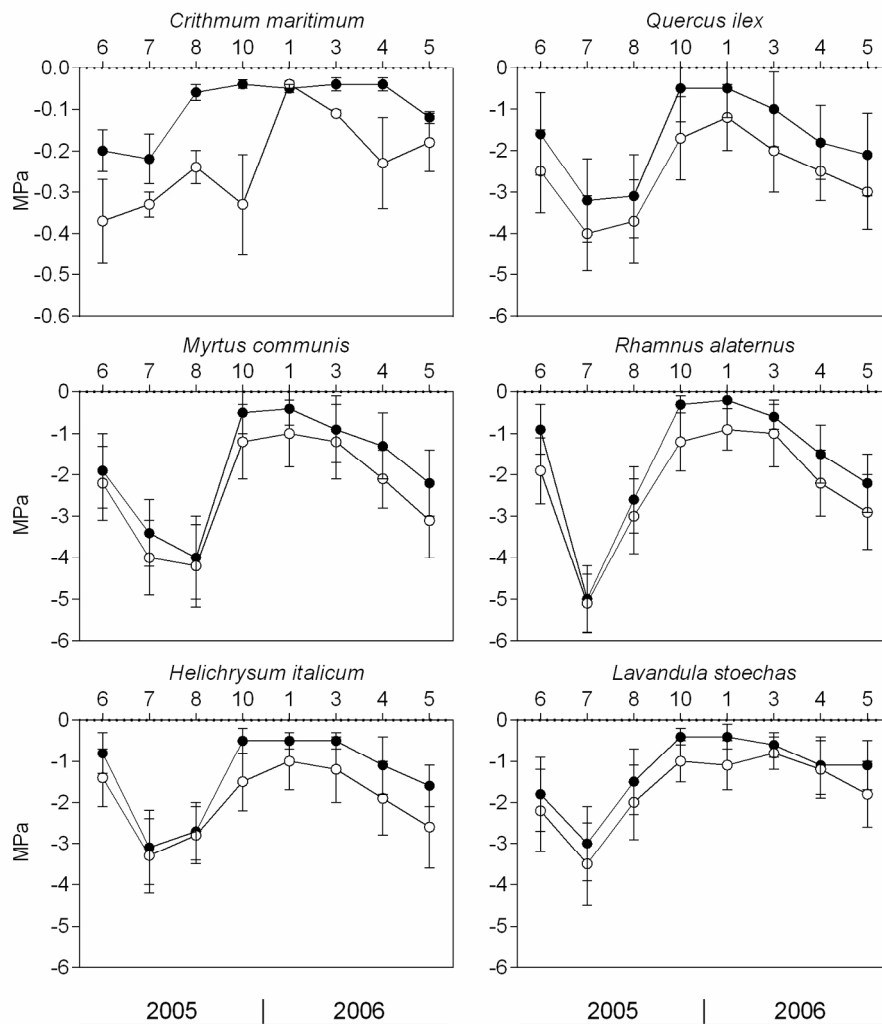
(a) Satellite image of the site under study indicated by the white arrow (modified after Google Earth 5.2.1.1588), (b) a picture of Italy with Tuscany. The black arrow shows the location of Elba island.
94x52mm (300 x 300 DPI)



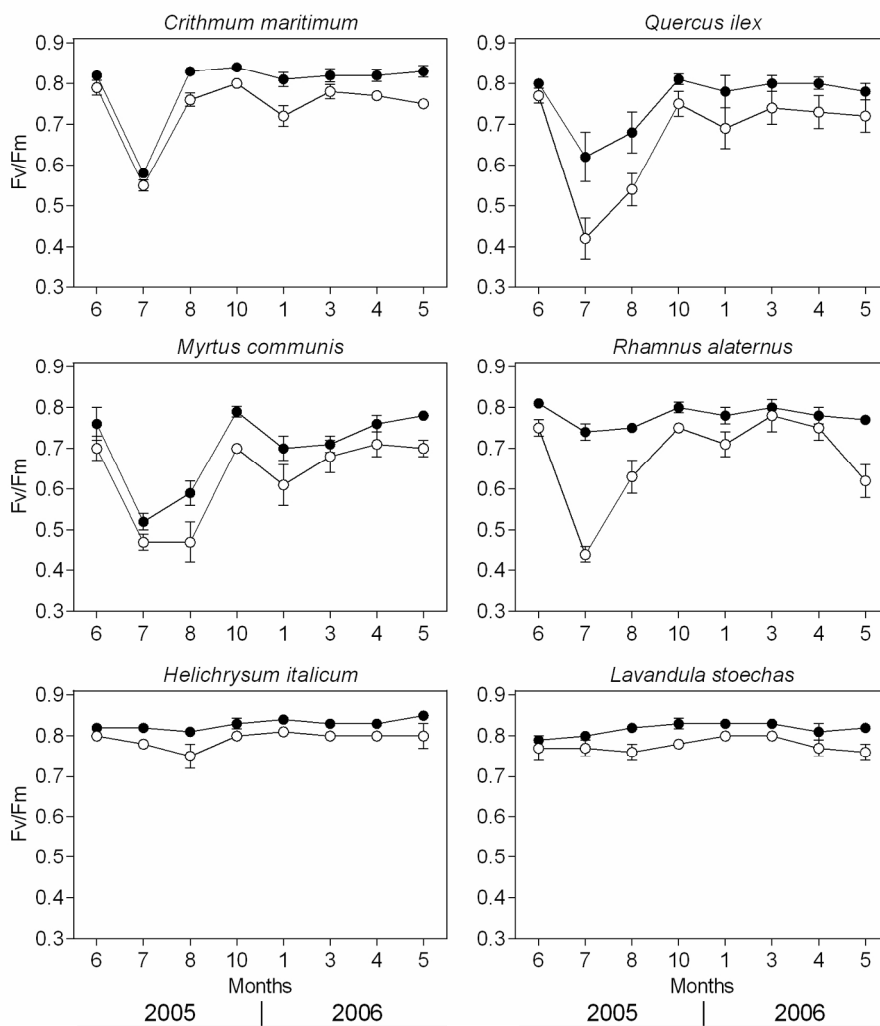
(a) Monthly average temperatures (°C) for 2005 (full line) and 2006 (dashed line), (b) monthly total rainfalls (mm) for 2005 (full line) and 2006 (dashed line). Data were obtained from the meteorological station at the site of Portoferraio (LI).



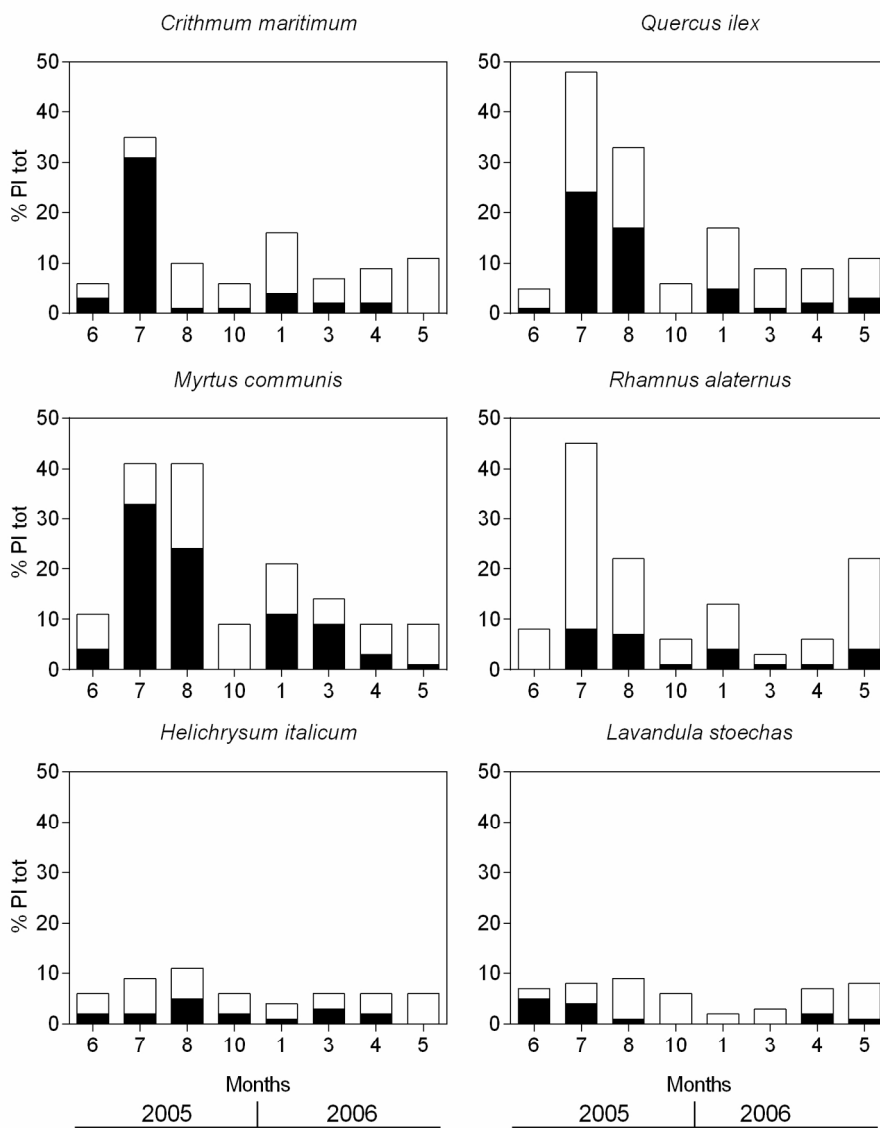
Box and whisker graph of (a) CH (canopy height), (b) LA (leaf area), (c) SLA (specific leaf area), (d) SLA in detail, (e) LDMC (leaf dry matter content) values for the species analyzed. Squares are medians; boxes are 25% and 75% percentiles; whiskers are maximum and minimum values. Specie abbreviations: *Crithmum maritimum* = C.marit; *Helichrysum italicum* = H.italic; *Lavandula stoechas* = L.stoech; *Myrtus communis* = M.comm; *Quercus ilex* = Q.ilex; *Rhamnus alaternus* = R.alat.



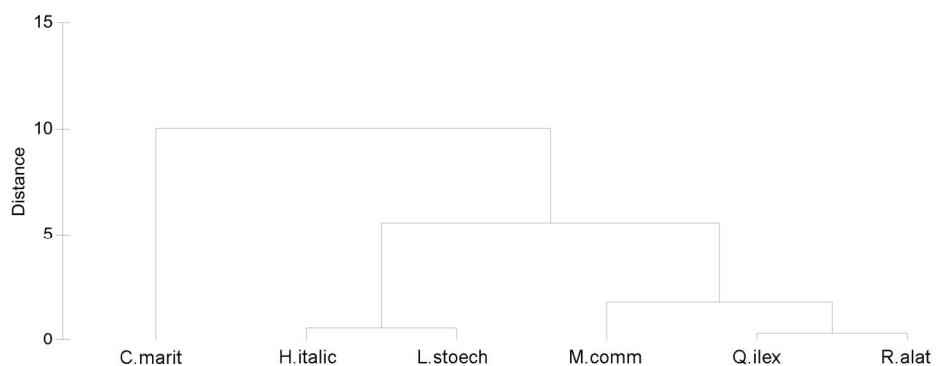
Seasonal changes of water potential (MPa) measured before the dawn, Ψ_{pd} (filled circles) and at midday, Ψ_{md} (empty circles) from June 2005 to May 2006. Data are the mean \pm SE. 1 = January; 3 = March; 4 = April; 5 = May; 6 = June; 7 = July; 8 = August; 10 = October.
160x171mm (300 x 300 DPI)



Seasonal changes of photosynthetic efficiency of PSII (Fv/Fm) measured before the dawn (filled symbols) and at midday (open symbols), in sun leaves from June 2005 to May 2006. Midday values were recorded on dark adapted leaves. Data are the mean \pm SE. 1 = January; 3 = March; 4 = April; 5 = May; 6 = June; 7 = July; 8 = August; 10 = October.
160x171mm (300 x 300 DPI)

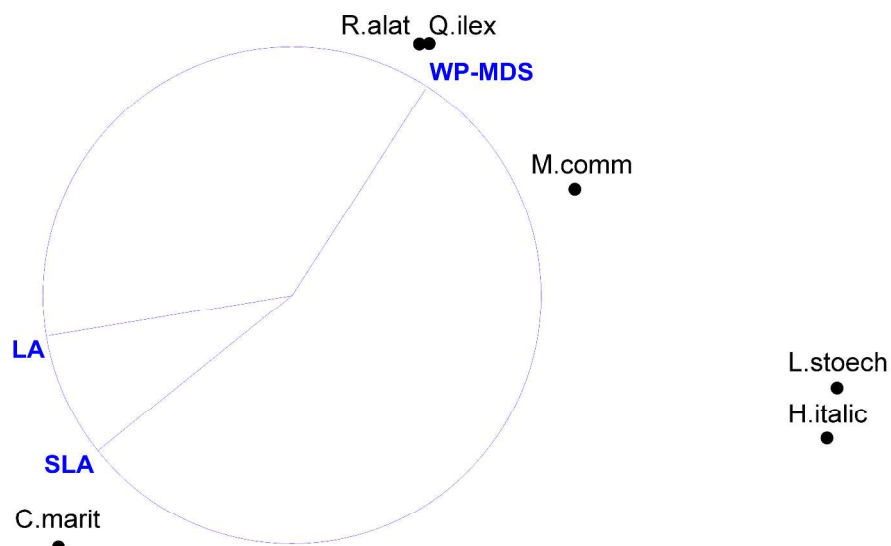


Seasonal changes of chronic and dynamic photoinhibition (PIchr, filled bars and PIdyn, empty bars, respectively), in sun leaves from June 2005 to May 2006. Photoinhibition is expressed as % reduction of Fv/Fm compared to its maximum annual value. Total photoinhibition (PItot) is the sum of PIchr + PIdyn. 1 = January; 3 = March; 4 = April; 5 = May; 6 = June; 7 = July; 8 = August; 10 = October.
160x187mm (300 x 300 DPI)



Agglomerative cluster analysis of the matrix formed by 6 species x 12 functional parameters - CH, LA, SLA, LDMC, PS-PDS (pre-dawn Fv/Fm in Jun-Oct), PS-PDA (annual pre-dawn Fv/Fm), PS-MDS (midday Fv/Fm in Jun-Oct), PS-MDA (annual midday Fv/Fm), WP-PDS (pre-dawn water potential in Jun-Oct), WP-PDA (annual pre-dawn water potential), WP-MDS (midday water potential in Jun-Oct), WP-MDA (annual midday water potential). Three groups of species were clustered: *C. maritimum*, semideciduous taxa and sclerophyllous taxa. Species abbreviations: *Crithmum maritimum* = C.marit; *Helichrysum italicum* = H.italic; *Lavandula stoechas* = L.stoech; *Myrtus communis* = M.comm; *Quercus ilex* = Q.ilex; *Rhamnus alaternus* = R.alat.
170x85mm (300 x 300 DPI)

Review



Non-metric Multidimensional Scaling ordination based on the matrix formed by 6 species x 12 functional parameters - CH, LA, SLA, LDMC, PS-PDS (pre-dawn Fv/Fm in Jun-Oct), PS-PDA (annual pre-dawn Fv/Fm), PS-MDS (midday Fv/Fm in Jun-Oct), PS-MDA (annual midday Fv/Fm), WP-PDS (pre-dawn water potential in Jun-Oct), WP-PDA (annual pre-dawn water potential), WP-MDS (midday water potential in Jun-Oct), WP-MDA (annual midday water potential). Three groups of species were clustered: *C. maritimum*, semideciduous taxa and sclerophyllous taxa. Traits with a Pearson correlation coefficient > 0.9 with the first two dimensions are shown. Species abbreviations: *Crithmum maritimum* = C.marit; *Helichrysum italicum* = H.italic; *Lavandula stoechas* = L.stoech; *Myrtus communis* = M.comm; *Quercus ilex* = Q.ilex; *Rhamnus alaternus* = R.alat.

Table 1: index of phenotypic plasticity for physiological traits

	<i>C.marit</i>	<i>H.ital</i>	<i>L.stoech</i>	<i>M.comm</i>	<i>Q.ilex</i>	<i>R.alat</i>	Mean \pm SE
$(Fv/Fm)_{pd}$	0.31	0.05	0.05	0.34	0.24	0.09	0.18 ± 0.05
$(Fv/Fm)_{md}$	0.31	0.07	0.05	0.34	0.45	0.44	0.28 ± 0.07
Ψ_{pd}	0.82	0.84	0.87	0.90	0.84	0.96	0.87 ± 0.02
Ψ_{md}	0.89	0.67	0.77	0.76	0.70	0.82	0.77 ± 0.03
Mean \pm SE	0.58 ± 0.16	0.41 ± 0.21	0.43 ± 0.22	0.59 ± 0.14	0.56 ± 0.13	0.58 ± 0.20	

Ψ_{pd} and Ψ_{md} , water potential measured before the dawn and at midday, respectively; $(Fv/Fm)_{pd}$ and $(Fv/Fm)_{md}$ photosynthetic efficiency of PSII

measured before the dawn and at midday, respectively. Species abbreviations: *Crithmum maritimum* = *C.marit*; *Helichrysum italicum* = *H.italic*;

Lavandula stoechas = *L.stoech*; *Myrtus communis* = *M.comm*; *Quercus ilex* = *Q.ilex*; *Rhamnus alaternus* = *R.alat*.

Supplementary Table S1: morphological traits for the six species analysed

	CH	LA	SLA	LDMC
	(m)	(mm ²)	(mm ² mg ⁻¹)	(mg g ⁻¹)
C.marit	0.09 ^a ± 0.03	1987.95 ^e ± 756.83	5944.67 ^b ± 663.99	117.25 ^a ± 12.22
H.italic	0.45 ^a ± 0.13	29.05 ^a ± 6.00	18.03 ^a ± 6.53	265.88 ^b ± 62.14
L.stoech	0.65 ^a ± 0.32	36.05 ^a ± 20.32	12.85 ^a ± 3.18	329.58 ^c ± 47.42
M.comm	2.00 ^b ± 0.60	380.85 ^b ± 99.10	8.53 ^a ± 1.14	424.83 ^c ± 41.30
Q.ilex	4.04 ^c ± 1.20	960.15 ^d ± 233.89	5.81 ^a ± 1.08	532.21 ^f ± 22.84
R.alat	2.13 ^b ± 0.63	719.40 ^c ± 179.96	9.20 ^a ± 3.27	379.50 ^d ± 51.77

Data are mean of ten replicates ± SE for each species. Means followed by the same letters are not significantly different at 5% according to the Kruskal-Wallis test with the Bonferroni correction. CH = canopy height; LA = leaf area; SLA = specific leaf area; LDMC = leaf dry matter content.

Species abbreviations: *Crithmum maritimum* = C.marit; *Helichrysum italicum* = H.italic; *Lavandula stoechas* = L.stoech; *Myrtus communis* = M.comm; *Quercus ilex* = Q.ilex; *Rhamnus alaternus* = R.alat.