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Photosynthetic performance of lichen transplants as early indicator of climatic stress along an altitudinal gradient in the arid Mediterranean area

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Abstract A climatic change was simulated transplanting samples of the lichens *Evernia prunastri* (L.) Ach. and *Pseudevernia furfuracea* (L.) Zopf v. furfuracea along a 1,400 m altitudinal gradient in the northern side of the island of Crete (Greece). The working hypothesis was that the photosynthetic performance (i.e. pigment content, chlorophyll degradation and photosynthetic efficiency) of transplanted lichens varies along the altitudinal gradient. The overall effect observed was a general depression of the photosynthetic performance along the gradient. Concentrations of chlorophyll *a*, chlorophyll *b* and carotenoids decreased with decreasing elevation and along with the hottest and driest months of the year, with chlorophyll *b* being the most sensitive parameter to dry conditions. Chlorophyll degradation decreased with increasing elevation. The exposure period was the main factor affecting photosynthetic efficiency, with lower values during summer months. We argued that the water content of lichen thalli is the most important factor determining differences in photosynthesis under the experimental conditions. This allowed to suggest that the lichen photosynthetic performance deserves further investigation as early biological indicator of atmospheric stress induced by dry conditions and, to a greater extent, for the assessment of the desertification risk in the arid Mediterranean environment.

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1 Introduction

Global changes, and particularly climate change, are currently influencing ecosystems at different scales (Walther et al. 2002). In the Mediterranean basin, climate change is expected to have diverse and far-reaching consequences. An immediate concern is the potential to exacerbate existing problems of desertification, water resources and food production, but ultimately, the impacts will be much wider as the effects cascade through the social and economic system.

Disturbance of global climate by anthropogenic activity is predicted to have a major impact on ecosystem functioning (Melillo et al. 1990) exacerbating the anthropogenic stress already existing on the environment (Oppenheimer 1989; Kappelle et al. 1999). Regional changes are more relevant in the context of ecological responses to climate change (Melillo et al. 1990) and in the arid Mediterranean area this process is enhanced by non-sustainable human activities such as intensive agriculture, overgrazing, air pollution, deforestation, changes in local population, in combination with adverse physical environmental conditions (Brandt et al. 2003), leading to overall desertification of southern European areas (Grove et al. 1991).

In the Mediterranean basin, environmental consequences of climate change are affecting photosynthesis and plant productivity through higher temperatures, lower water availability, higher vapour pressure deficit, increased UV-B radiation and salinity (Kakani et al. 2003; Chartzoulakis and Psarras 2005). These parameters are very important in arid and semi-arid environments, where water is the main limiting factor for land use performance and biomass production of ecosystems (Kosmas et al. 1999) and drought is the major factor shaping vegetation and controlling plant functions in water-limited ecosystems (Rambal et al. 2003). All the above factors influence sensitive organisms such as lichens, either directly or indirectly through modifications in their habitats, so that ecophysiology, growth, biomass, community structure and distribution can all change in space and time (Insarov and Insarova 1996; Insarov and Schroeter 2002). Lichens, as symbiotic organisms able to regain active metabolism any time the amount of hydration is high enough (Lange 2003), base their survival on the absorption of water and nutrients from the air, differing in that from higher plants, which depend mainly on soil for their nutrition. Therefore, they provide a peculiar and specific monitoring view of the atmospheric environment.

Lichens are responding to global warming (Parmesan and Yohe 2003) and some long-term monitoring programmes proved their sensitivity, mainly through a community approach (van Herk et al. 2002), while only few studies investigated ecophysiological responses to a climatic change (Bjerke et al. 2003).

Changes in photosynthetic processes are sensitive indicators of lichen responses to stress, depending on environmental conditions as well as internal characteristics of lichen thalli (Kershaw 1985). The environment determines (1) intensity, quality and period of light radiation reaching lichen thalli during the day as well as the year; (2) temperature, affecting lichen optimal metabolism; (3) water availability in the form of precipitation, humidity, fog and dew, influencing the degree of thallus hydration (Kershaw 1985).

High light irradiance is known to damage sensitive species, resulting in photoinhibition (Gauslaa and Solhaug 2000), decrease in chlorophyll concentration and change in chlorophyll reciprocal ratios (Vráblíková et al. 2006). On the other side, high light irradiation can also enhance photoprotective mechanisms (Barták et al. 2004). High light influx influences temperature and evaporation, and a combination of these factors is what lichens really face in the environment (Barkman 1958). Photoinhibition depends on actual irradiance, time of exposure, water status, temperature, previous conditions and capacity of recovery from injury (Osmond and Chow 1988). Lichens are generally tolerant to high-temperature stress (Lange 1953), mainly in their dry state (Kappen 1973), nevertheless high temperatures can degrade photosynthetic pigments and damage cell membranes (Pisani et al. 2007). Moreover, tolerance to high summer temperature is species-specific, since prolonged exposure to environmental temperatures over 35°C can influence sensitive lichens even in their dry state, resulting in decline of net photosynthesis (MacFarlane and Kershaw 1978). Furthermore, photosynthesis and respiration are largely dependent on thallus water content, being negatively influenced by both low and high hydration levels (Kershaw 1985; Green et al. 1994).

Lichens, as poikilohydric organisms, are strictly dependent on external water availability and therefore their photosynthetic activity and occurrence in desert areas as well as in extremely cold environments, reflect the presence of a regular water supply (Lange et al. 2001). The morphology of lichen thalli, as well as some algal and mycobiont adaptations, represent internal specific aspects which play an important role in facing and buffering the external conditions, thus determining the different susceptibility and responses of these organisms to the environment. Such responses are potential early indicators of stress (air pollution, climatic changes, eutrophication, etc.) on the ecosystems. In this scenario, the present research aimed at investigating the biological responses of sensitive organisms exposed along a regional climatic gradient in the arid Mediterranean area, providing issues for the development of a biological observing/monitoring system of climate-induced stress in arid environments, based on early warnings in sensitive organisms. Since programmes aimed at assessing biological effects of climate change should be undertaken where climatic gradients are steep and changes are foreseen to be faster, e.g. along biome boundaries, polar regions, coastal zones especially in small islands, and mountain areas (Insarov et al. 1999; Insarov and Schroeter 2002), a change of climatic conditions was simulated by transplanting lichen samples along an altitudinal gradient in the northern side of the island of Crete (Greece). Our hypothesis was that the photosynthetic performance (i.e. assimilation pigment content, chlorophyll degradation and photosynthetic efficiency) of transplanted lichens varies along the gradient.

2 Experimental

2.1 Study area

Depicting a landscape consisting of limestone mountain areas, the island of Crete represents the prosecution of the chains of continental Greece in the SE part of the Mediterranean Sea. The study was carried out in the northern side of the island, along an altitudinal gradient ranging from the hilly massif 10 km (370 m asl) SW of Heraklion, through the village of Anogeia (the highest of Crete, ca. 750 m asl), up to the Skinakas astronomical observatory in the Psiloritis Mountains (1,750 m asl, ca 25 km from the sea; Fig. 1). Climatic data along the altitudinal gradient are available from the meteorological stations of Heraklion (nearby the sea) and Anogeia (in

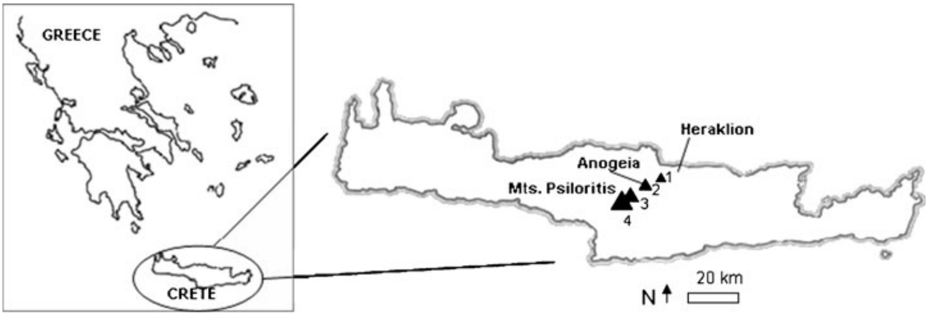
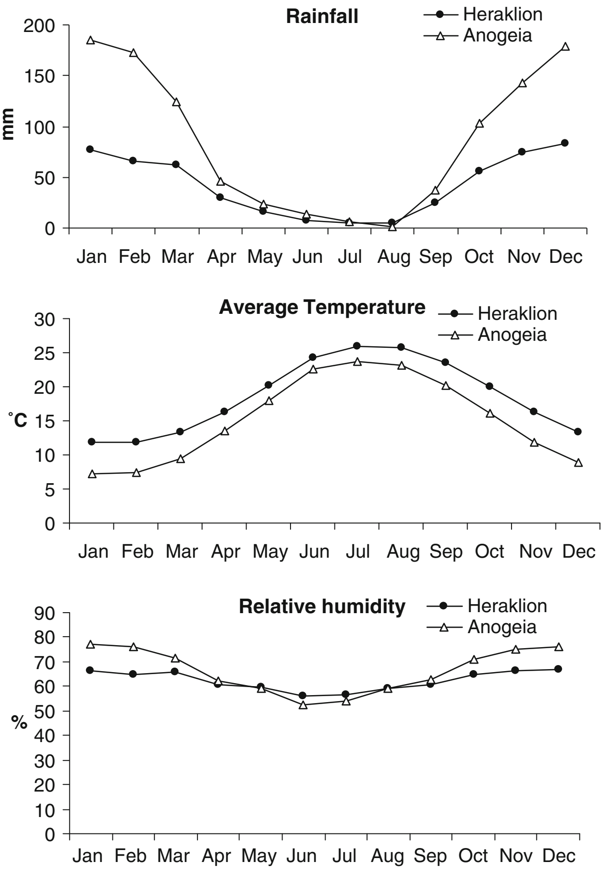


Fig. 1 Study area with location of experimental sites, datum WGS84. Site 1 (370 m): 35°17′N - 24°59′E; site 2 (740 m): 35°16′N – 24°54′E; site 3 (1200 m): 35°14′N - 24°53′E; site 4 (1750 m): 35°12′N – 24°53′E

the middle of the altitudinal gradient, facing the Psiloritis Mountains) (Fig. 2). The climate is characterised by long hot and dry summers and relatively humid and cold winters. Yearly rainfall ranges from ca. 500 mm in the coastal area of Heraklion to ca. 1,000 mm in the Psiloritis and is concentrated mainly in autumn. The dry period

Fig. 2 Comparison of the climatic data from the meteorological stations of Heraklion (39 m a.s.l., nearby the sea) and Anogeia (740 m a.s.l., in the middle point of the altitudinal gradient): rainfall (mm), temperature (°C) and relative humidity (%) (historical data from the National Meteorological Hellenic Service, 1975–1997)



extends from May to October, with summer rainfall almost absent; moreover, about 65% of total precipitation is rapidly lost by evapo-transpiration. In the coastal area of Heraklion, average monthly temperature usually ranges from 12°C in January to 26°C in July–August. In the mountain area of Anogeia, average monthly temperature ranges from 7°C in January to 23°C in July–August. Average temperature decreases 6°C every 1,000 m of elevation (Grove et al. 1991). Winds from the N are dominant, exacerbating the dry conditions during summer.

The combination of over-exploitation of resources (soil, water and vegetation) and bioclimatic stress, place the study area within areas subject to desertification-risk (Greek National Action Plan for combating desertification 2001). Estimates of regional trends in temperature, precipitation, relative humidity, etc. in the region of Crete are available through the database EMERIC of the Region of Crete (http://www.ims.forth.gr/joint_projects/emeric/emeric-gr.html).

2.2 Experimental design

Four sites, all located far from any local pollution source, were selected at increasing distance from the sea (10, 15, 20, 25 km) and elevation (370, 740, 1,200, 1,750 m asl), featuring quite differing climatic conditions. All experimental sites are located in remote areas of Crete, where overall, scarce information is available. Therefore, meteorological variables at the experimental sites, exactly where lichens were transplanted, have been recorded weekly at noon with a LI-COR data-logger (average values are shown in Table 1).

The transplant experiment was carried out in the period of the year when the amount of solar irradiance was maximum (June–September 2005), with a PAR photon flux measured at noon ranging from ca. 1,500 μmol m^{-2} s^{-1} at the lowest site to ca. 1,800 μmol m^{-2} s^{-1} at the highest elevation, and with a corresponding 4–7°C temperature decrease (36–39°C at the bottom and 32°C at the top of the gradient during July and August). Rainfall was almost absent in the period June–August (2005), with sporadic raining events only during May and September. Relative

Table 1 Meteorological variables recorded weekly during the transplant period

		Site 1 (370 m)	Site 2 (740 m)	Site 3 (1200 m)	Site 4 (1750 m)
June	PAR photon flux (μmol m^{-2} s^{-1})	1615	1742	1766	1780
	Temperature (°C)	33	31	27	25
	Relative humidity (%)	52	58	64	65
July	PAR photon flux (μmol m^{-2} s^{-1})	1631	1756	1769	1810
	Temperature (°C)	35	34	31	29
	Relative humidity (%)	58	56	59	61
August	PAR photon flux (μmol m^{-2} s^{-1})	1568	1724	1745	1788
	Temperature (°C)	36	34	32	31
	Relative humidity (%)	58	57	61	62
Sept.	PAR photon flux (μmol m^{-2} s^{-1})	1564	1747	1740	1763
	Temperature (°C)	34	32	30	28
	Relative humidity (%)	54	57	60	63

Average values in correspondence of exposed lichens; all parameters were recorded during sunny days between 12 A.M. and 2 P.M humidity (RH) increased just after the precipitation events and rapidly dropped because of evapo-transpiration, which normally occurred within 1 day. Climatic data from the meteorological station of Heraklion (nearby the sea, 10 km from site 1) indicated a mean RH in the range 68% in May and September to 62–64% in June–August in the morning; RH typically decreased to 56–60% in the afternoon, with lower values occurring in July–August. Along with increasing altitude, climatic data from the station of Anogeia, in the middle of the altitudinal gradient (close to site 2), indicate about 2°C decrease in mean temperature, similar RH and higher precipitation; however during summer rainfall is almost absent (Fig. 2).

To warrant uniform experimental conditions and avoid any possible influence of environmental factors other than climatic parameters, only the top of poorly vegetated rocky peaks (hills or mountains) were selected along the gradient. Twigs carrying at least five thalli of the lichens *Evernia prunastri* (L.) Ach. and *Pseudevernia furfuracea* (L.) Zopf v. furfuracea were harvested in May 2005 from pine trees (*Pinus nigra*) in a remote mountain forest of northern Greece and immediately transferred to Crete. These species were selected being commonly used in biomonitoring surveys. They have a fruticose growth form and a different ecology: *P. furfuracea* is a xerophytic to mesophytic species and *E. prunastri* a hygrophytic to mesophytic one, both living in habitats with a low level of eutrophication, especially *P. furfuracea*. In fact, along the altitudinal gradient, *P. furfuracea* can be found at higher elevations, preferring protected microhabitats within the canopy of evergreen shrubs (*Quercus* or *Juniperus*), far away from extensively grazed areas and sheltered from wind flows. *E. prunastri* is common in the southern area of Psiloritis at the bottom of humid and afforested valleys, while in the upper part of the gradient it is sporadically present; both species tolerate direct solar irradiation in open space.

Biotic and abiotic habitat conditions for lichen thalli at their native area were roughly similar to those in the selected mountains in Crete, with the sole exception of wind intensity. The average wind intensity at the native site was estimated between 7–9 km/h, while along the altitudinal gradient in Crete samples were exposed to stronger winds; intensity rises with elevation overtaking 12–14 km/h as an average value at Anogeia (data from the National Observatory of Athens).

Therefore, to prevent loss of material by strong winds, lichens were exposed together with their substrate inside fishing nets (mesh size 2.5 cm) containing twigs of both species. At each site, the lichen-nets were carefully transplanted on the upper surface (50–150 cm from ground) of five evergreen shrubs (chiefly *Quercus* and *Juniperus*), simulating natural conditions, so that lichens resulted fully sun-exposed. At each site, five lichen-nets were retrieved monthly, after 1–4 months of exposure (June–September 2005). The experiment was carried out during summer to account for a higher environmental stress during the hottest and driest period of the year in the arid Mediterranean environment. In addition, July and August 2005 were particularly hot in Greece, since the average of maximal temperatures exceeded up to 2°C the average values of the period 1961–1990 (data from the National Observatory of Athens).

2.3 Assimilation pigments

After retrieval, lichen samples were air dried and stored at −20°C, which is the temperature recommended for conservation and subsequent use in lichen physiological studies (Honegger 2003). Samples of 20–25 mg were used for the analysis of pigment content and chlorophyll integrity, selecting only the outermost 2 cm of the lobes. Samples were first rinsed six times for 5 min each in pure acetone buffered with $CaCO_3$ to remove lichen acids (Barnes et al. 1992) and subsequently pigments were extracted in PVPi-buffered DMSO for 24 h at room temperature (23 ± 1°C) in the dark. All steps were carried out in semi-dark conditions, using a dim green light to avoid chlorophyll degradation.

Absorbance of the extracts was measured using a UV-visible spectrophotometer (Agilent 8453). Turbidity was checked at 750 nm and values were always lower than 0.010 absorbance units. Other wavelengths measured were 665, 649, 480, 435, 415 nm and values obtained were used to calculate concentrations (expressed in μg/mg dry weight) of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total carotenoids (Car) using the equations proposed by Wellburn (1994). Chlorophyll degradation was expressed by the ratio between absorbance at 435 and 415 nm ($OD_{435/415}$), as suggested by Ronen and Galun (1984).

2.4 Photosynthetic efficiency

A physiological recovery of the lichens was achieved prior to measurements of photosynthetic efficiency. To avoid any osmotic stress by air humidity after the freezing, samples were left 15 min in dry ambient conditions. They were subsequently sprayed with water until completely moisten and the excess water was removed by hand-shaking. Samples were then stored at 4°C in the dark for 24 h. The outermost 2 cm of the thalli were then selected for measurements. Measurements were carried out with a Plant Efficiency Analyser (PEA, Hansatech Ltd, UK). After samples were dark-adapted for 10 min, they were lightened for 1 sec with a saturating excitation pulse (1,800 $\mu mol\ s^{-1}\ m^{-2}$) of red light (650 nm) from a LED into the fluorometer sensor and fluorescence emission recorded. Photosynthetic efficiency was expressed by the F_V/F_M ratio, reflecting the potential quantum yield of PSII.

2.5 Statistical analysis

Significance of difference between unexposed and transplanted samples was checked by one-way analysis of variance (ANOVA). A one-way ANOVA, using the Tukey test for post-hoc comparisons, was run separately for each species to check for differences in photosynthetic parameters at different climates and exposure lengths. A three-way ANOVA was run to investigate the effects of climate, length of exposure and species, as well as the interactions among them, on each investigated

Table 2 Photosynthetic parameters and thallus water content (mean±SD, N = 5) measured before samples were transplanted

	E. prunastri	*P. furfuracea*
Chlorophyll *a*	1.73 ± 0.20	1.35 ± 0.13
Chlorophyll *b*	0.49 ± 0.06	0.37 ± 0.04
Chlorophyll *a* + *b*	2.22 ± 0.27	1.72 ± 0.17
Carotenoids	0.47 ± 0.03	0.42 ± 0.03
$OD_{435/415}$	1.29 ± 0.08	1.34 ± 0.03
F_V/F_M	0.58 ± 0.06	0.63 ± 0.04
Thallus water content (%)	12.5	9.0

Pigment concentrations in $\mu g{\cdot}mg^{-1}$ (d.w.)

parameter. Prior to analysis, data not matching a normal distribution (Shapiro–Wilk *W* test at the 95% confidence interval) were transformed using the Box–Cox method (Legendre and Legendre 1998). Statistical analysis was run using the software Statistica 6.0 (StatSoft).

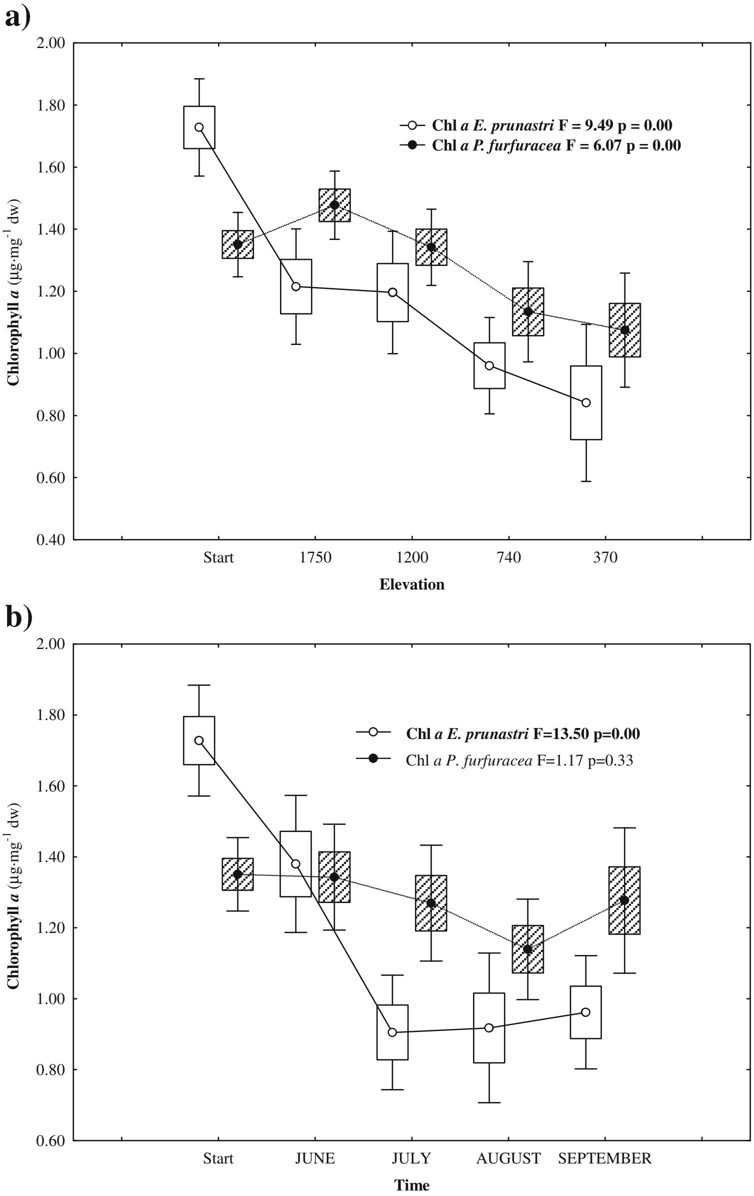


Fig. 3 Comprehensive comparison (ANOVA) of the chlorophyll *a* content for **a** elevation and **b** time. Mean ± SE (*box*) and confidence interval (95%, *whisker*)

3 Results

The transplantation in the arid Mediterranean area affected lichens along the altitudinal gradient and according to the length of exposure.

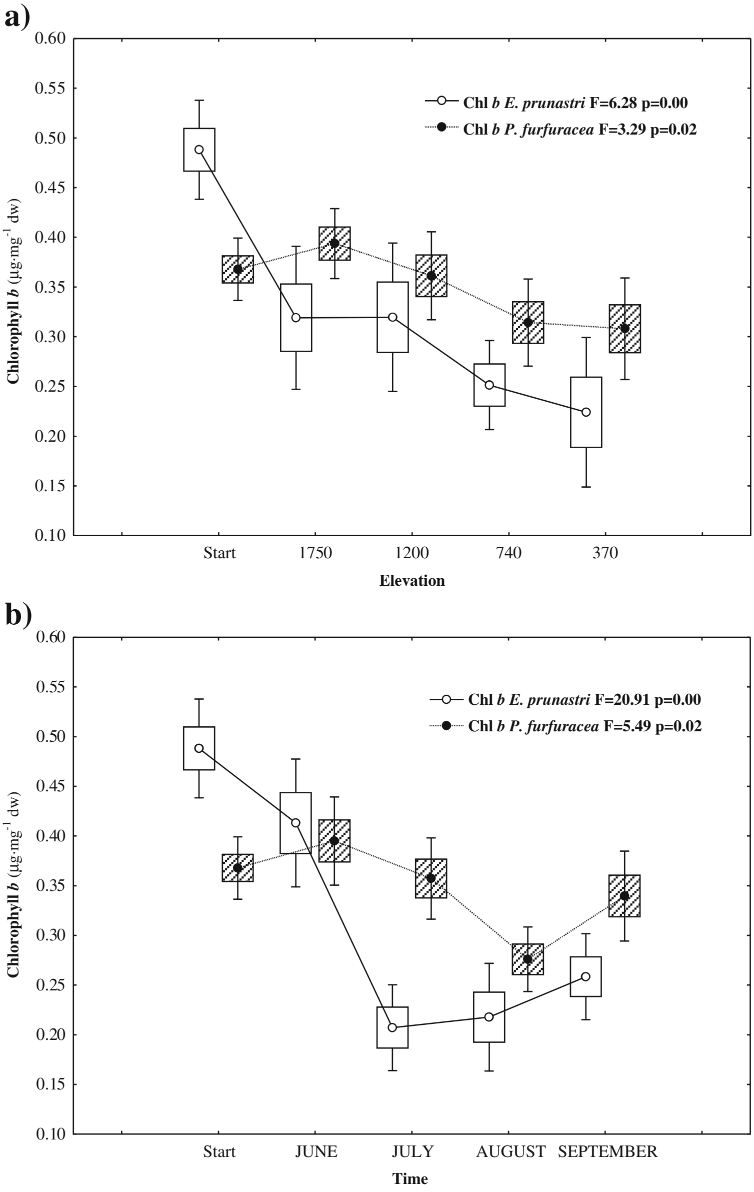


Fig. 4 Comprehensive comparison (ANOVA) of the chlorophyll *b* content for **a** elevation and **b** time. Mean ± SE (*box*) and confidence interval (95%, *whisker*)

Table 2 summarises the status of the samples at the beginning of the experiment. An overall view of the main effects of elevation, length of exposure and species is reported graphically in Figs. 3, 4, 5, 6, 7, 8 and 9. All investigated parameters (Chl a, Chl b, Chl $a + b$, Car, Chl a/b, $\mathrm{OD}_{435/415}$ and $\mathrm{F_V/F_M}$) along with their statistical descriptors (mean, standard deviation, F- and P-values of ANOVA) are shown in

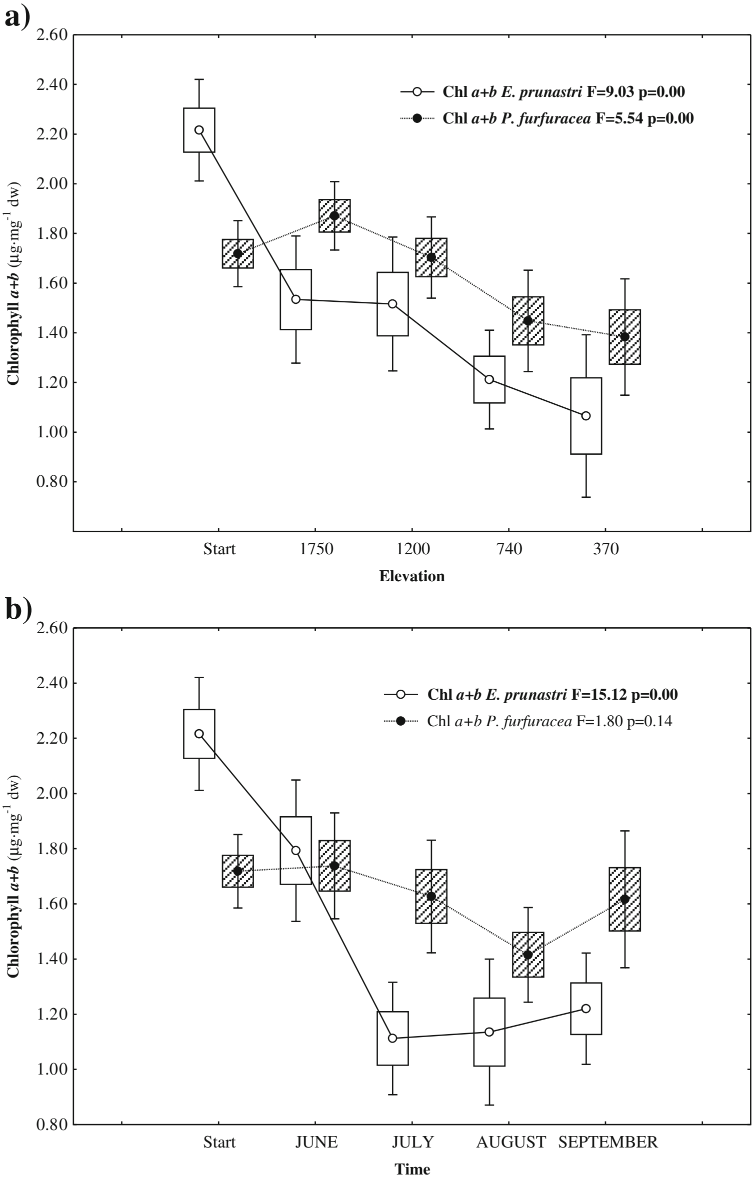


Fig. 5 Comprehensive comparison (ANOVA) of the chlorophyll $a + b$ content for **a** elevation and **b** time. Mean ± SE (*box*) and confidence interval (95%, *whisker*)

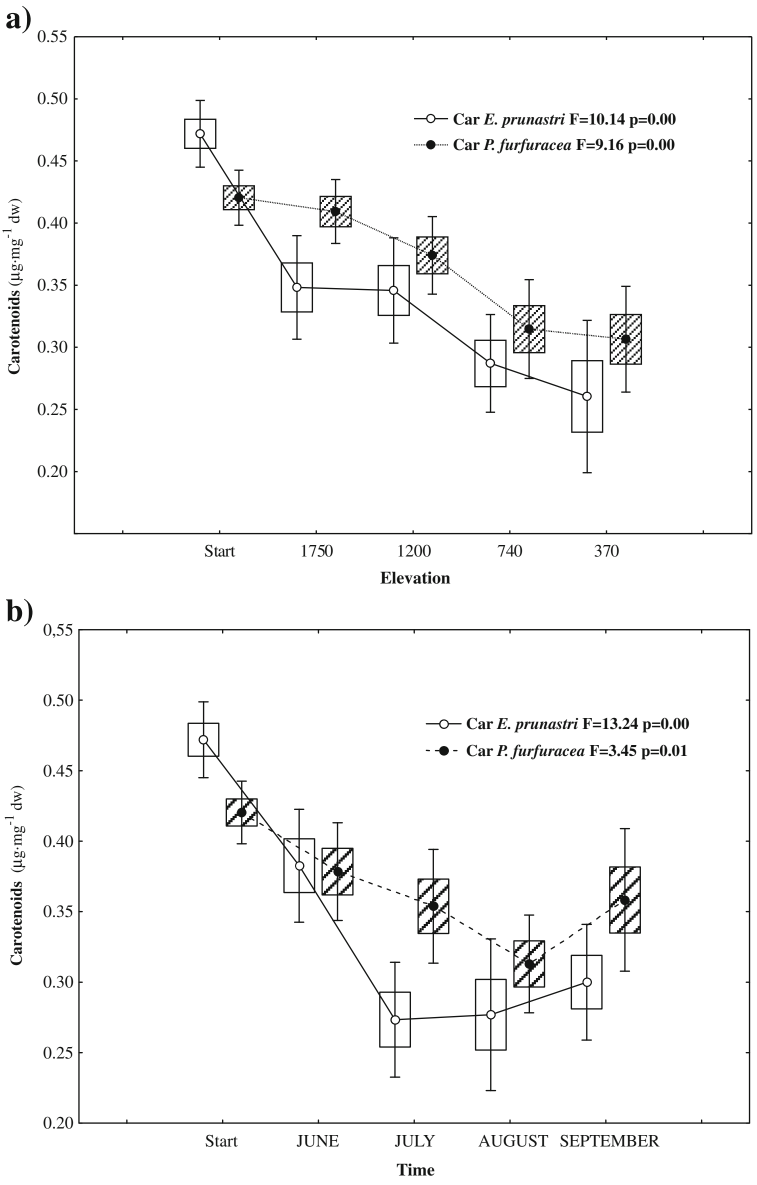


Fig. 6 Comprehensive comparison (ANOVA) of the carotenoids content for **a** elevation and **b** time. Mean ± SE (*box*) and confidence interval (95%, *whisker*)

detail in Tables 3, 4, 5, 6, 7, 8 and 9. Results of three-way ANOVA are shown in Table 10. In general, all parameters were influenced along the altitudinal gradient and, with the exception of the F_V/F_M ratio, had a species-specific performance. The period of retrieval mainly influenced the F_V/F_M ratio and the content of Chl *b*, but had almost no effect on the $OD_{435/415}$ ratio. Only Chl *a*/*b* ratio was sensitive to the

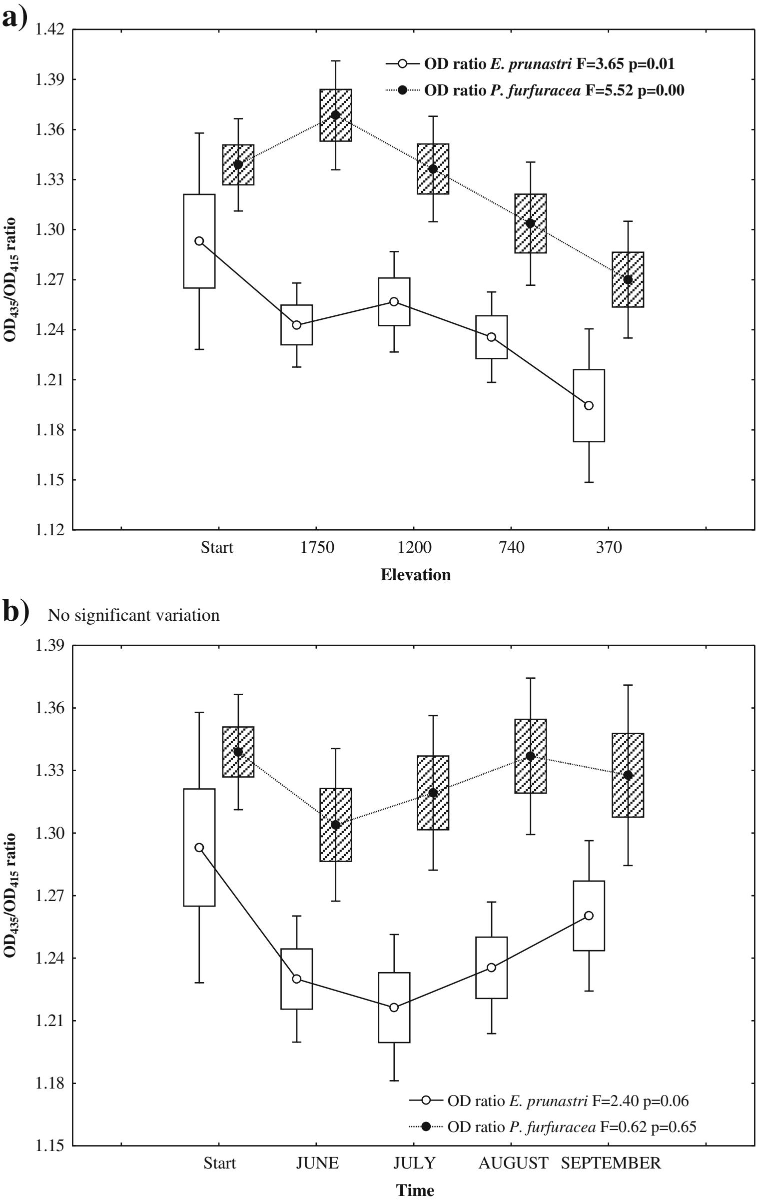


Fig. 7 Comprehensive comparison (ANOVA) of the chlorophyll *a* integrity by OD_{435}/OD_{415} ratio for **a** elevation and **b** time. Mean ± SE (*box*) and confidence interval (95%, *whisker*)

combination of elevation and time of exposure. The water content of *E. prunastri* samples was 7–11% d.w. and that of *P. furfuracea* 7–9% d.w. Both species, retrieved during sunny days (12 A.M.–2 P.M.), had a lower water content during June and August and higher in September. Although the water content tended to be higher in samples from mountain sites, only few variations were evident along the gradient.

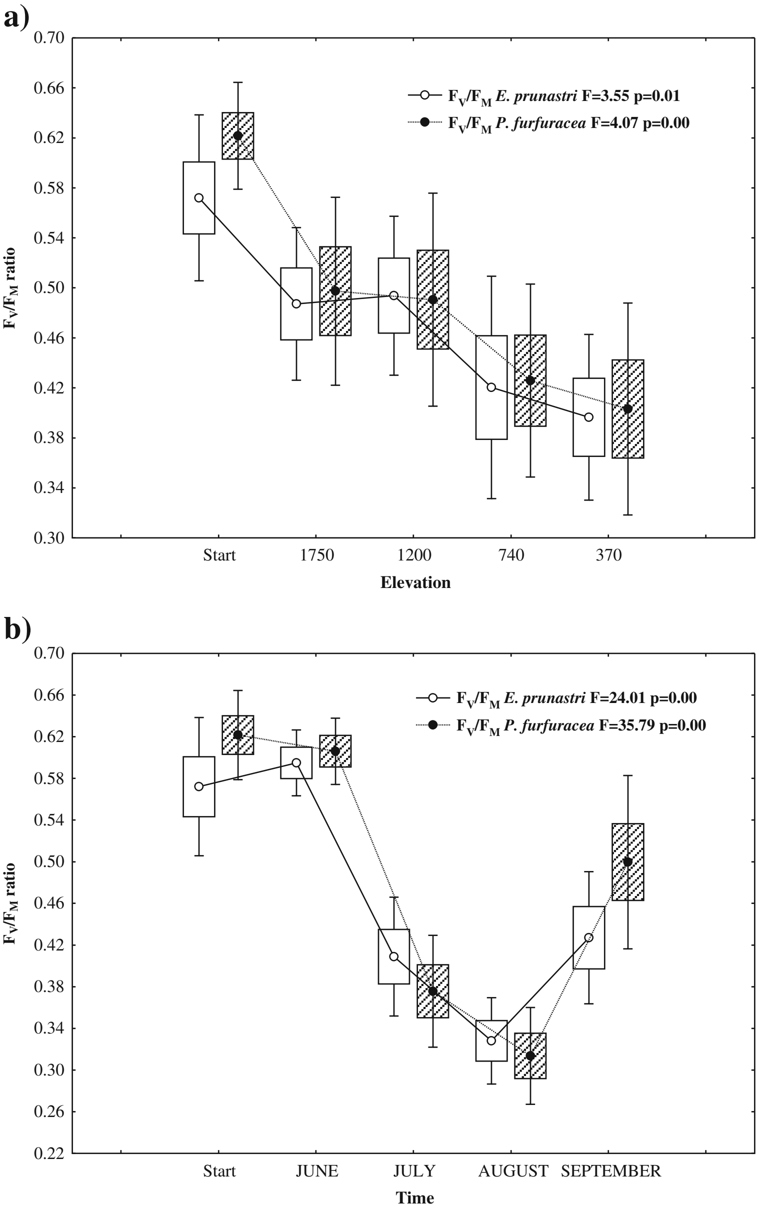


Fig. 8 Comprehensive comparison (ANOVA) of the potential quantum yield of PSII by F_V/F_M ratio for **a** elevation and **b** time. Mean ± SE (*box*) and confidence interval (95%, *whisker*)

For both species, morphological damage, consisting in bleaching of thallial surface occurred after 3 and 4 months of exposure (in August and September), mainly at lower elevations.

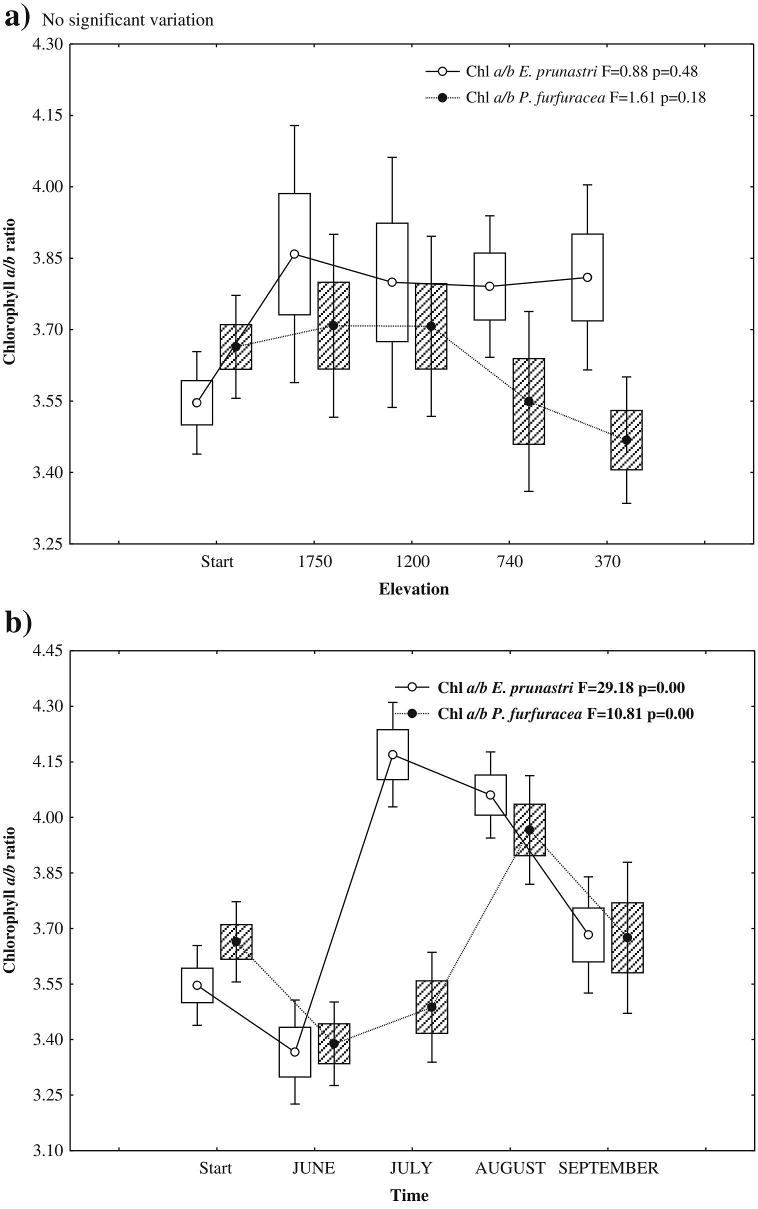


Fig. 9 Comprehensive comparison (ANOVA) of the chlorophyll *a*/*b* ratio for **a** elevation and **b** time. Mean ± SE (*box*) and confidence interval (95%, *whisker*)

3.1 Assimilation pigments

Unexposed samples of *E. prunastri* had a higher content of photosynthetic pigments than *P. furfuracea*, while at the end of the transplantation higher concentrations were

Table 3 Mean concentrations ± SD ($\mu g \cdot mg^{-1}$ dw, N = 5) of chlorophyll *a* in *E. prunastri* [a] and *P. furfuracea* [b], after transplanting to different elevations and harvesting in different months

	370 m	740 m	1200 m	1750 m	*F* ratio	*F* probability
[a] *E. prunastri*						
JUNE	1.14 ± 0.44	1.21 ± 0.34	1.61 ± 0.45**A**	1.55 ± 0.30**A**	1.884	0.173
JULY	0.77 ± 0.52	1.01 ± 0.33	0.89 ± 0.23**B**	0.95 ± 0.31**B**	0.403	0.753
AUGUST	0.52 ± 0.12**b**	0.79 ± 0.15**b**	1.19 ± 0.25**a**AB	1.27 ± 0.42**a**AB	**7.884**	**0.004**
SEPTEMBER	0.92 ± 0.76	0.76 ± 0.15	1.06 ± 0.15AB	1.08 ± 0.08AB	1.204	0.358
F ratio	1.483	2.771	**5.257**	**3.842**		
F probability	0.269	0.081	**0.012**	**0.036**		
[b] *P. furfuracea*						
JUNE	1.25 ± 0.43	1.17 ± 0.36	1.49 ± 0.23	1.47 ± 0.11	1.327	0.301
JULY	1.09 ± 0.36	1.21 ± 0.47	1.34 ± 0.25	1.43 ± 0.29	0.893	0.466
AUGUST	0.82 ± 0.17**b**	1.18 ± 0.23**b**	1.12 ± 0.18**b**	1.43 ± 0.13**a**	**7.286**	**0.004**
SEPTEMBER	1.12 ± 0.10ab	0.93 ± 0.25**b**	1.38 ± 0.22ab	1.60 ± 0.33**a**	**4.810**	**0.025**
F ratio	1.226	0.580	2.029	0.470		
F probability	0.343	0.637	0.156	0.708		

Significance of differences (in bold) by one-way ANOVA and Tukey test at $p < 0.05$. Values in each horizontal line followed by different small letter are significantly different along the altitudinal gradient, values in each column followed by different capital letter are significantly different according to the exposure month

overall observed in *P. furfuracea*, suggesting for the two species a different acclimation to the new environment. In *E. prunastri* assimilation pigments significantly decreased at all sites, while in *P. furfuracea* dropped only at lower elevations.

In general, lower Chl *a*, Chl *b* and Car where recorded at lower elevations (Figs. 3a, 4, 5 and 6a). However, only after 3 months of exposure differences along the altitudinal gradient were marked enough and separated mountain (1,200, 1,750 m) from hilly sites (370, 740 m).

Table 4 Mean concentrations ± SD ($\mu g \cdot mg^{-1}$ dw, N = 5) of chlorophyll *b* in *E. prunastri* [a] and *P. furfuracea* [b], after transplanting to different elevations and harvesting in different months

	370 m	740 m	1200 m	1750 m	*F* ratio	*F* probability
[a] *E. prunastri*						
June	0.33 ± 0.14	0.34 ± 0.10**A**	0.51 ± 0.13**A**	0.48 ± 0.10**A**	3.172	0.053
July	0.19 ± 0.13	0.26 ± 0.09AB	0.18 ± 0.05**B**	0.20 ± 0.09**B**	0.610	0.618
August	0.12 ± 0.02**b**	0.18 ± 0.02**bB**	0.28 ± 0.07**aB**	0.31 ± 0.13**a**AB	**6.323**	**0.009**
September	0.26 ± 0.20	0.21 ± 0.05AB	0.29 ± 0.04**B**	0.28 ± 0.04**B**	0.709	0.569
F ratio	2.198	**3.766**	**12.72**	**8.309**		
F probability	0.141	**0.036**	**0.000**	**0.002**		
[b] *P. furfuracea*						
June	0.36 ± 0.11	0.33 ± 0.10	0.44 ± 0.06**A**	0.45 ± 0.05**A**	2.948	0.064
July	0.33 ± 0.10	0.34 ± 0.12	0.38 ± 0.07**A**	0.38 ± 0.07**A**	0.413	0.746
August	0.22 ± 0.03**b**	0.30 ± 0.08**a**	0.34 ± 0.05**aB**	0.32 ± 0.01**aB**	**3.260**	**0.046**
September	0.32 ± 0.00	0.28 ± 0.08	0.34 ± 0.06AB	0.41 ± 0.07**A**	2.357	0.133
F ratio	1.966	0.394	**7.797**	**3.851**		
F probability	0.173	0.759	**0.003**	**0.034**		

Significance of differences (in bold) by one-way ANOVA and Tukey test at $p < 0.05$. Values in each horizontal line followed by different small letter are significantly different along the altitudinal gradient, values in each column followed by different capital letter are significantly different according to the exposure month

Table 5 Mean concentrations ± SD (μg·mg^{-1} dw, N = 5) of total chlorophylls in *E. prunastri* [a] and *P. furfuracea* [b], after transplanting to different elevations and harvesting in different months

	370 m	740 m	1200 m	1750 m	*F* ratio	*F* probability
[a] *E. prunastri*						
June	1.47 ± 0.58	1.55 ± 0.44	2.12 ± 0.58**A**	2.03 ± 0.39**A**	2.163	0.132
July	0.96 ± 0.65	1.26 ± 0.42	1.07 ± 0.28**B**	1.15 ± 0.40**B**	0.402	0.754
August	0.64 ± 0.14**b**	0.96 ± 0.17ab	1.47 ± 0.32**a**AB	1.58 ± 0.55**a**AB	**7.599**	**0.005**
September	1.18 ± 0.96	0.97 ± 0.20	1.35 ± 0.19**B**	1.36 ± 0.12AB	1.093	0.396
F ratio	1.631	2.973	**6.649**	**4.752**		
F probability	0.234	0.068	**0.005**	**0.019**		
[b] *P. furfuracea*						
June	1.60 ± 0.54	1.49 ± 0.46	1.94 ± 0.29	1.92 ± 0.16	1.626	0.223
July	1.42 ± 0.46	1.56 ± 0.59	1.72 ± 0.32	1.81 ± 0.36	0.776	0.524
August	1.04 ± 0.20**b**	1.48 ± 0.31**a**	1.76 ± 0.24**a**	1.75 ± 0.13**a**	**6.397**	**0.007**
September	1.44 ± 0.10ab	1.21 ± 0.33**b**	1.72 ± 0.27ab	2.00 ± 0.40**a**	**4.337**	**0.033**
F ratio	1.365	0.511	2.933	0.618		
F probability	0.300	0.681	0.070	0.615		

Significance of differences (in bold) by one-way ANOVA and Tukey test at $p < 0.05$. Values in each horizontal line followed by different small letter are significantly different along the altitudinal gradient, values in each column followed by different capital letter are significantly different according to the exposure month

The length of exposure also played an important role, with Chl *a*, Chl *b* and Car (Figs. 3b, 4, 5 and 6b) dropping during the dry season and reaching their lowest values in August. An overall recovery of pigment content occurred in September. However, temporal changes were evident only at higher elevations, while no significant change was observed at lower sites. Noteworthy is the fact that all pigments were affected in *E. prunastri* while only Chl *b* in *P. furfuracea*.

Table 6 Mean concentrations ± SD (μg·mg^{-1} dw, N = 5) of total carotenoids in *E. prunastri* [a] and *P. furfuracea* [b], after transplanting to different elevations and harvesting in different months

	370 m	740 m	1200 m	1750 m	*F* ratio	*F* probability
[a] *E. prunastri*						
June	0.33 ± 0.09	0.36 ± 0.08	0.43 ± 0.09**A**	0.41 ± 0.06	1.498	0.253
July	0.25 ± 0.14	0.29 ± 0.09	0.28 ± 0.05**B**	0.28 ± 0.07	0.211	0.887
August	0.18 ± 0.04**b**	0.24 ± 0.04ab	0.34 ± 0.06**a**AB	0.36 ± 0.12**a**	**5.979**	**0.011**
September	0.27 ± 0.18	0.25 ± 0.04	0.32 ± 0.04AB	0.35 ± 0.01	1.905	0.193
F ratio	1.427	2.787	**4.613**	2.839		
F probability	0.283	0.079	**0.019**	0.079		
[b] *P. furfuracea*						
June	0.35 ± 0.09	0.34 ± 0.09	0.41 ± 0.05	0.41 ± 0.02	1.534	0.234
July	0.30 ± 0.08	0.34 ± 0.12	0.37 ± 0.06	0.41 ± 0.06	1.403	0.278
August	0.25 ± 0.04**b**	0.30 ± 0.06ab	0.39 ± 0.05ab	0.39 ± 0.04**a**	**5.349**	**0.013**
September	0.32 ± 0.03ab	0.27 ± 0.05**b**	0.39 ± 0.05ab	0.43 ± 0.09**a**	**5.193**	**0.020**
F ratio	1.391	0.652	2.585	0.433		
F probability	0.293	0.594	0.095	0.733		

Significance of differences (in bold) by one-way ANOVA and Tukey test at $p < 0.05$. Values in each horizontal line followed by different small letter are significantly different along the altitudinal gradient, values in each column followed by different capital letter are significantly different according to the exposure month

Table 7 Means±SD (μg·mg^{-1} dw, N = 5) of chlorophyll *a* integrity by OD$_{435}$/OD$_{415}$ in *E. prunastri* [a] and *P. furfuracea* [b], after transplanting to different elevations and harvesting in different months

	370 m	740 m	1200 m	1750 m	*F* ratio	*F* probability
[a] *E. prunastri*						
June	1.17 ± 0.06	1.24 ± 0.08	1.28 ± 0.07	1.25 ± 0.04AB	2.565	0.091
July	1.16 ± 0.16	1.25 ± 0.05	1.22 ± 0.08	1.21 ± 0.05**B**	0.342	0.795
August	1.20 ± 0.06	1.22 ± 0.06	1.26 ± 0.04	1.30 ± 0.05**A**	2.749	0.093
September	1.21 ± 0.20	1.25 ± 0.05	1.30 ± 0.04	1.27 ± 0.01AB	0.467	0.712
F ratio	0.228	0.207	1.177	**3.742**		
F probability	0.875	0.890	0.354	**0.039**		
[b] *P. furfuracea*						
June	1.26 ± 0.07	1.28 ± 0.09	1.34 ± 0.05	1.33 ± 0.06	1.990	0.156
July	1.29 ± 0.09	1.31 ± 0.12	1.31 ± 0.06	1.36 ± 0.03	0.878	0.473
August	1.29 ± 0.06**b**	1.33 ± 0.04ab	1.35 ± 0.04ab	1.39 ± 0.04**a**	**4.621**	**0.021**
September	1.28 ± 0.04**b**	1.28 ± 0.04**b**	1.37 ± 0.03**a**	1.35 ± 0.06**a**	**6.156**	**0.015**
F ratio	0.195	0.796	1.376	1.367		
F probability	0.898	0.515	0.291	0.293		

Significance of differences (in bold) by one-way ANOVA and Tukey test at p < 0.05. Values in each horizontal line followed by different small letter are significantly different along the altitudinal gradient, values in each column followed by different capital letter are significantly different according to the exposure month

3.1.1 Chlorophyll degradation

Chlorophyll degradation was more pronounced in *E. prunastri*. In both species the OD$_{435/415}$ ratio rose with increasing elevation, but the effect becomes significant only accounting jointly all retrievals (Fig. 7a). Within single collections, this trend was marked after 3 months of exposure only in *P. furfuracea*. Within each site, the

Table 8 Means±SD (μg·mg^{-1} dw, N = 5) of potential quantum yield of PSII by F$_V$/F$_M$ ratio in *E. prunastri* [a] and *P. furfuracea* [b], after transplanting to different elevations and harvesting in different months

	370 m	740 m	1200 m	1750 m	*F* ratio	*F* probability
[a] *E. prunastri*						
June	0.57 ± 0.05**A**	0.61 ± 0.06**A**	0.63 ± 0.03**A**	0.63 ± 0.04**A**	1.444	0.267
July	0.49 ± 0.11**A**	0.34 ± 0.19**A**	0.48 ± 0.08**CB**	0.47 ± 0.05**CB**	1.025	0.426
August	0.37 ± 0.09**B**	0.42 ± 0.11AB	0.38 ± 0.08**C**	0.39 ± 0.06**C**	0.598	0.629
September	0.41 ± 0.09**bB**	0.42 ± 0.13**bB**	0.55 ± 0.08**aAB**	0.51 ± 0.11**aAB**	**7.623**	**0.004**
F ratio	**8.041**	**5.977**	**10.79**	**20.83**		
F probability	**0.002**	**0.011**	**0.001**	**0.000**		
[b] *P. furfuracea*						
June	0.59 ± 0.02**A**	0.62 ± 0.04**A**	0.63 ± 0.04**A**	0.63 ± 0.07**A**	0.890	0.468
July	0.49 ± 0.14**B**	0.37 ± 0.11**B**	0.43 ± 0.09**B**	0.47 ± 0.09**B**	0.963	0.439
August	0.32 ± 0.07**B**	0.40 ± 0.07**B**	0.32 ± 0.16**B**	0.33 ± 0.07**C**	1.770	0.210
September	0.42 ± 0.18**B**	0.44 ± 0.23AB	0.40 ± 0.20AB	0.62 ± 0.07**AB**	2.040	0.210
F ratio	**19.69**	**12.89**	**5.491**	**9.559**		
F probability	**0.000**	**0.000**	**0.017**	**0.001**		

Significance of differences (in bold) by one-way ANOVA and Tukey test at $p < 0.05$. Values in each horizontal line followed by different small letter are significantly different along the altitudinal gradient, values in each column followed by different capital letter are significantly different according to the exposure month

Table 9 Means±SD (µg·mg^{-1} dw, N = 5) of chlorophyll *a*/*b* ratio in *E. prunastri* [a] and *P. furfuracea* [b], after transplanting to different elevations and harvesting in different months

	370 m	740 m	1200 m	1750 m	*F* ratio	*F* probability
[a] *E. prunastri*						
June	3.55 ± 0.35**aB**	3.63 ± 0.18**aB**	3.16 ± 0.16**bC**	3.25 ± 0.15**aC**	**5.665**	**0.008**
July	4.23 ± 0.50**bA**	3.96 ± 0.25**b**AB	4.89 ± 0.31**aA**	5.00 ± 0.75**aA**	**6.607**	**0.004**
August	4.23 ± 0.41**A**	4.36 ± 0.56**A**	4.19 ± 0.23**B**	4.27 ± 0.46**AB**	0.780	0.971
September	3.52 ± 0.17**B**	3.60 ± 0.30**B**	3.73 ± 0.24**B**	3.95 ± 0.42**B**	0.923	0.465
F ratio	**4.415**	**4.805**	**47.06**	**18.48**		
F probability	**0.026**	**0.017**	**0.000**	**0.000**		
[b] *P. furfuracea*						
June	3.46 ± 0.21	3.58 ± 0.31	3.34 ± 0.11**B**	3.25 ± 0.18C	2.240	0.123
July	3.32 ± 0.16	3.43 ± 0.40	3.54 ± 0.13AB	3.76 ± 0.14B	2.168	0.132
August	3.71 ± 0.29**b**	3.98 ± 0.30**ab**	4.28 ± 0.30**aA**	4.42 ± 0.44**aA**	**4.769**	**0.019**
September	3.52 ± 0.30	3.48 ± 0.03	4.05 ± 0.46**A**	3.91 ± 0.15**A**	2.739	0.105
F ratio	2.009	2.366	**15.45**	**21.29**		
F probability	0.167	0.112	**0.000**	**0.000**		

Significance of differences (in bold) by one-way ANOVA and Tukey test at $p < 0.05$. Values in each horizontal line followed by different small letter are significantly different along the altitudinal gradient, values in each column followed by different capital letter are significantly different according to the exposure month

OD$_{435/415}$ ratio did not show temporal fluctuations, irrespective of an initial and weak reduction as a consequence of the transplantation (Fig. 7b).

3.2 Photosynthetic efficiency

The potential quantum yield of PSII represented by the F$_V$/F$_M$ ratio was higher in unexposed samples (Fig. 8a) and *P. furfuracea* showed higher values. During the

Table 10 Three way ANOVA for the effects of elevation, time, species and their interactions on photosynthetic parameters by three-way ANOVA

	Elevation	Time	Species	Elevation *Time	Elevation *Species	Time *Species	Elevation *Time *Species
Chlorophyll *a*	**F = 11.42**	**F = 8.11**	**F = 14.45**	F = 1.22	F = 0.26	**F = 3.07**	F = 0.57
	P = 0.000	**P = 0.000**	**P = 0.000**	P = 0.288	P = 0.854	**P = 0.031**	P = 0.823
Chlorophyll *b*	**F = 8.00**	**F = 22.54**	**F = 19.01**	F = 1.77	F = 0.39	**F = 6.43**	F = 0.83
	P = 0.000	**= 0.000**	**P = 0.000**	P = 0.081	P = 0.759	**P = 0.000**	P = 0.593
Chlorophyll *a* + *b*	**F = 10.73**	**F = 10.71**	**F = 15.54**	F = 1.25	F = 0.27	**F = 3.68**	F = 0.61
	P = 0.000	**P = 0.000**	**P = 0.000**	P = 0.270	P = 0.844	**P = 0.014**	P = 0.785
Carotenoids	**F = 12.89**	**F = 8.62**	**F = 10.07**	F = 1.05	F = 0.35	F = 2.30	F = 0.44
	P = 0.000	**P = 0.000**	**P = 0.002**	P = 0.408	P = 0.787	P = 0.082	P = 0.913
Chl *a*/Chl *b*	**F = 3.80**	**F = 44.39**	**F = 19.76**	**F = 5.67**	F = 1.52	**F = 17.43**	F = 1.06
	P = 0.012	**P = 0.000**	**P = 0.000**	**P = 0.000**	P = 0.214	**P = 0.000**	P = 0.397
OD$_{435}$/OD$_{415}$ ratio	**F = 7.61**	F = 1.07	**F = 52.24**	F = 1.32	F = 1.25	F = 0.67	F = 0.29
	P = 0.000	P = 0.363	**P = 0.000**	P = 0.234	P = 0.297	P = 0.574	P = 0.975
F$_V$/F$_M$	**F = 9.51**	**F = 71.13**	F = 0.19	F = 1.58	F = 0.26	F = 0.90	F = 0.18
	P = 0.000	**P = 0.000**	P = 0.662	P = 0.133	P = 0.852	P = 0.442	P = 0.995

P values in bold are statistically significant transplantation both species achieved the same F_V/F_M ratios. A linear increasing of values along the gradient was only evident when all retrievals were combined, since analysing the single data, only *E. prunastri* samples retrieved in September showed significant changes along the gradient. The period of retrieval was the main factor affecting photosynthetic efficiency, since during the second month of exposure (July), F_V/F_M dramatically dropped at all sites along the altitudinal gradient, featuring a recovery only in samples retrieved in September (Fig. 8b).

4 Discussion

Monitoring changes in photosynthetic parameters of sensitive species along a climatic gradient can provide a rapid tool to investigate productivity and ecological functioning of arid ecosystems. In the Mediterranean area, natural vegetation has to face a strong seasonality, with hot, long and dry summers alternate to cold, wet winters (Rambal et al. 2003). The effects of this severe environment are evident also observing lichen communities growing on bushes: at the bottom of the studied gradient, only few crustose species are present, reflecting the extreme habitat conditions. With increasing elevation, the lichen flora becomes enriched in nitrophytic and xerophytic species, and under humid conditions, also some fruticose lichen appears. On bushes, the latter only grow sheltered within the inner part of the canopy, protected from strong winds, which at the top of relieves model the shape of bushes and limit the performance of fruticose lichens.

Transplanted lichens faced severe dry conditions determined by the combination of low relative humidity, high temperature and high irradiance, which are typical of Mediterranean summer.

4.1 Assimilation pigments

Overall, the arid environment depressed the pigment content, especially at lower elevations and during driest months, with lower values shown by *E. prunastri* (hygrophytic to mesophytic species) and higher values by *P. furfuracea* (xerophytic to mesophytic), reversing their initial condition. Therefore, the auto-ecological requirements of the two transplanted species, rapidly reflected the status of the environment, allowing information on regional trends of biological productivity to be deduced.

In fact, during the study period, water availability to lichens was predominantly dependent on dew and on the presence of low clouds, being hence higher for samples transplanted to the mountain sites, in particular in June and September.

Water availability has been shown to have main effects on the productivity of most terrestrial biomes and especially within Mediterranean-type ones under a global change scenario (Mouillot et al. 2002). Based on these observations, water supply to lichens was more subjected to variations during the exposure time rather than along the altitudinal gradient. Water availability was in fact the most important element differentiating lichen photosynthetic parameters, depending on scarce precipitation, limited air humidity and the ability of the species to maintain a hydrated state. The decrease in Chl *a*, Chl *b* and Car during summer months was paralleled by the rise in the Chl a/b ratio, which was determined at once by the stronger decrease of Chl

b. Also previous studies indicated that Chl b is more sensitive than Chl a to high temperatures (Pisani et al. 2007). Furthermore, the decrease in Chl b occurred earlier in *E. prunastri* (July) and only one month later (August) in *P. furfuracea*, suggesting a larger susceptibility of *E. prunastri*, probably caused by a limited ability of this species to maintain an active hydrated state (Paoli et al. 2010). On the other side, no clear trend of Chl a/b was detected along the altitudinal gradient. Also acclimation of higher plants to warm and sunny conditions is known to involve a rise in Chl a/b ratios, reflecting changes in concentrations of light-harvesting Chl complexes relative to PSII and PSI reaction centres (Pearcy and Sims 1994). Lichen photobionts acclimate seasonally to the environment by changing their chlorophyll content and photosynthetic parameters (Vráblíková et al. 2006) with a seasonal tendency to a rise in the Chl a/b ratio during summer (Czeczuga and Krukowska 2001).

4.2 Photosynthetic efficiency

Photosynthetic efficiency dropped following substantially the lacking rainfall in the period June-August, to slightly recover after some precipitation in September, especially in the mountains. However, the amplitude of F_V/F_M fluctuations is on the whole larger than that normally found in humid habitats (Vráblíková et al. 2006) owing to a strong depression of fluorescence values caused by the dry conditions.

A low water potential was shown to decrease the rate of photosynthesis in *E. prunastri* exposed to atmospheric desiccation (Nash et al. 1990) and, in addition, under these conditions a high irradiance exacerbates the susceptibility of this species to other stress factors, e.g. salt stress. Green-algal lichens have an extraordinary ability to regain photosynthetic activity by taking up water from air humidity (Nash et al. 1990; Lange et al. 2001), allowing a much better acclimation to arid conditions than lichens with cyanobacterial photobionts, which require wetting of thalli by liquid water to gain CO_2 for net photosynthesis (Lange et al. 1989). Nevertheless, acclimation requires active metabolism to occur and involves several mechanisms, e.g. production of sun-screening pigments and secondary metabolites, change of the absorbance of cortical layers, increase of non-photochemical quenching (Demmig-Adams et al. 1990; Valladares et al. 1995; Solhaug and Gauslaa 1996). It is possible to suggest that, irrespective of the common water stress, lichens exposed in the mountains could benefit of a longer hydrated state, probably dependent on air humidity and dew, being thus metabolically active for acclimation and featuring higher pigment concentrations and reduced chlorophyll degradation.

Photoinhibition was shown to occur in *E. prunastri* thalli after 3 days of continuous irradiance at 1000 μmol photons m^{-2} s^{-1} with recovery being possible after rewetting, while only prolonged irradiances are likely to photoinhibit irreversibly this lichen species (Manrique et al. 1993). Irreversible photoinhibition involves inactivation of the electron transport system and chlorophyll bleaching determined by photooxidation (Krause 1988). However, irrespective of the presence of visible discoloration of thalli, the partial recovery of the F_V/F_M ratio in samples retrieved in September indicated that photoinhibition was not irreversible and that this parameter does not allow an estimation of long-term, chronic stress effects, as also suggested by Gauslaa and Solhaug (2000).

Multiple stresses by water-deficit, high irradiance and elevated temperature caused functional consequences, as shown by the reversible fluctuation in the F_V/F_M

ratio, and slowly modified the structure of the photosynthetic apparatus, as indicated by changes in the concentration of photosynthetic pigments. Injury was slowly apparent, as depicted by chlorophyll degradation to phaeophytin and visible symptoms that occurred moving from the high mountains to the lower sites.

4.3 Detecting responses to a climatic stress in arid environments

Detecting the effect of climate change in arid areas is complicated by several environmental factors, including background air pollution and eutrophication from animal grazing (Pirintsos and Loppi 2003; Pirintsos et al. 1998). The experimental design adopted in the present study aimed at detecting responses induced by a regional climatic gradient and at reducing the noise of other disturbances that make difficult attributing an observed change to a specific cause (Insarov and Schroeter 2002). Background air pollution generally increases oxidant levels throughout the lower atmosphere and the hydrosphere. These oxidants, being phytotoxic, contribute both directly and indirectly, along with other stressors, to the acceleration of S, N and C cycles (Oppenheimer 1989). To avoid any direct effect of air pollution and accounting for the prevalence of NW winds, the experimental gradient was selected in the SW direction with respect to the town of Heraklion, which is the main source of urban and industrial pollutants in the central part of Crete. Agricultural activities, especially animal grazing, enhance dust diffusion and atmospheric N-enrichment (Ferm 1998), and therefore, experimental sites were placed at the top of remote peaks, where crop farming was not feasible and the sole possible source of disturbance along the gradient was sheep and goat grazing. However, considering that during the favourable season animals are regularly transferred from pastures at lower elevations to the mountainous areas, the influence of NH_3 emission from animals between 1,200 and 1,750 m was estimated by passive samplers and found in line with background values reported for rural areas (Fowler et al. 1998; Frati et al. 2007).

In the assessment of the desertification-risk in the Mediterranean environment, extensive attention has mainly been devoted to physical, chemical and biological processes related to soil degradation, to finally devise diagnostic techniques and criteria for appraising the status and trends of desertification through a system of indicators (Rubio and Bochet 1998). However, in the framework of a desertification scenario, attention should also be paid to the biological effects on sensitive atmospheric-depending organisms such as lichens, which might be profitably used. In fact, the rapid response induced on the photosynthetic performance of transplanted lichens can provide early warnings on the effects of bioclimatic stress that are specifically referred to the atmospheric environment, integrating and complementing the information provided by other indicators, such as higher plants, for the soil. In this direction, in addition to biodiversity surveys, the present study showed that the photosynthetic performance of lichen transplants deserves to be further investigated as early indicator of the biological consequences of stress induced by the drying up of the habitats.

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