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ORIGINAL PAPER

Resistance of *Posidonia oceanica* seagrass meadows to the spread of the introduced green alga *Caulerpa cylindracea*: assessment of the role of light

Lázaro Marín-Guirao · Jaime Bernardeau-Esteller · Juan Manuel Ruiz · José Miguel Sandoval-Gil

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Abstract *Posidonia oceanica* seagrass meadows are one of most resistant Mediterranean habitats to invasion by the green alga *Caulerpa cylindracea*. We evaluated the hypothesis that light reduction caused by the seagrass canopy can limit algal photosynthesis and growth and hence potentially explain this resistance. To this end, we analysed light regimes and *C. cylindracea* biomass and photoacclimative variables measured outside and within *P. oceanica* meadows at different sites and during contrasting times. The success of photoacclimatory responses was assessed using an ecophysiological, carbon balance approach. *C. cylindracea* abundance significantly varied depending on the sampling site and time, but its biomass was always 10- to 50-fold higher outside the meadow. Outside the canopy, *C. cylindracea* showed characteristic morphological and photosynthetic plasticity closely related to the spatio-temporal variation in light regimes, which varied as expected with depth and season. Under these conditions, the alga was able to perform successful photoacclimation, although some degree of light limitation was observed at the deepest sites and in winter conditions, as indicated by near-zero carbon balance and lower algal abundances. Within the *P. oceanica* canopy, light was reduced by 60–89 % relative to that outside and was at its lowest levels recorded (1–7 % of the sub-surface irradiance), close to the minimum light requirements for growth. Light limitation was evident inside the canopy in the winter sampling, when the photosynthetic plasticity of the alga appears to be exceeded and when carbon balances were clearly negative. Therefore, light appears to play a key role in the apparent incapacity of *C. cylindracea* to penetrate within *P. oceanica* meadow edges.

Keywords Invasive algae · Seagrass · Biotic resistance · Photoacclimation · Species interaction

Introduction

Invasive species are those that are not native to the ecosystem under consideration and that cause, or are likely to cause, economic or environmental harm or harm to human, animal, or plant health (NISC 2006). The green alga *Caulerpa cylindracea* (Sonder) [formerly *Caulerpa racemosa* (Forsskål) J. Agardh var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque; (hereinafter, *C. cylindracea*)], has been described as one

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L. Marín-Guirao · J. Bernardeau-Esteller ·
J. M. Ruiz (✉) · J. M. Sandoval-Gil
Seagrass Ecology Group, Centro Oceanográfico de
Murcia, Instituto Español de Oceanografía, C/Varadero
s/n, 30740 San Pedro del Pinatar, Murcia, Spain
e-mail: juanm.ruiz@mu.ieo.es

J. Bernardeau-Esteller · J. M. Sandoval-Gil
Departamento de Ciencias del Mar y Biología Aplicada,
University of Alicante, P.O. Box 99, 03080 Alicante,
Spain

of the most successful invaders of the Mediterranean Sea (Streftaris and Zenetos 2006). It was first noted in the Western Mediterranean Sea along the coast of Libya in 1990 (Nizamuddin 1991), and has since spread rapidly, gradually invading the entire Mediterranean Sea (Piazzi et al. 2005, Klein and Verlaque 2008). The alga has successfully colonized a wide variety of soft and hard substrata, including dead *Posidonia oceanica* rhizomes or ''matte'' (i.e. the compact biogenic structure resulting from growth of rhizomes intertwined with roots and autochthonous and allochthonous detritus; Boudouresque and Meisnez 1982). It is found at depths between 0 and 60 m, being most abundant between 0 and 30 m (Klein and Verlaque 2008). Overgrowth by *C. cylindracea* on Mediterranean benthic communities can alter biodiversity (Argyrou et al. 1999; Piazzi et al. 2001; Balata et al. 2004; Piazzi and Balata 2008; Vázquez-Luis et al. 2008; Klein and Verlaque 2009), but the degree and extent of this impact depends on many factors, including the type of assemblage (Londsale 1999; Arenas et al. 2006).

At present, little is known about the variation in the resistance of natural Mediterranean communities to *C. cylindracea* invasion, but it has been proposed that benthic assemblages dominated by canopy-forming species are more resistant to invasion since the canopy might limit resources, especially light and space (Piazzi et al. 2001; Ceccherelli and Campo 2002; Klein and Verlaque 2008; Bulleri and Benedetti-Cecchi 2008; Bulleri et al. 2010). However, the mechanisms underlying the resistance to invasion of benthic assemblages have rarely been investigated and are poorly understood (Londsale 1999; Arenas et al. 2006; Britton-Simmons 2006). Meadows of *P. oceanica* are one of the Mediterranean infralittoral biocenoses that is more resistant to invasion by *C. cylindracea* (Klein and Verlaque 2008). The invasive alga is not usually found within *P. oceanica* meadows, whereas it has often been found at the edges of meadows or in very sparse or patchy meadows (Occhipinti-Ambrogi and Savini 2003; Piazzi et al. 1997a, b; Piazzi and Cinelli 1999; Ceccherelli et al. 2000; Montefalcone et al. 2007; Katsanevakis et al. 2010; Infantes et al. 2011; Ruiz et al. 2011). This resistance to the invasion of the alga has been related to *P. oceanica* shoot density, suggesting that some factors correlated with the canopy structure must be involved in the reduced capacity of *C. cylindracea* to penetrate the meadows, such as space limitation, water motion, nutrient supply or canopy shading (Ceccherelli et al. 2000). In the present study we examine the role that light may play in determining the resilience of *P. oceanica* to this highly invasive alga.

P. oceanica is an ecosystem engineer (Koch 2001) that forms conspicuous and extensive meadows from near the surface, to depths of 30–40 m and its ecological importance is widely recognised (e.g. Pergent et al. 2012). *P. oceanica* is a clonal plant consisting of a basal rooted rhizome, with shoots of vertical and horizontal growth, bearing 5–10 blade-like leaves, 12 mm broad and more than 1 m long. This large shoot size, together with the high shoot densities of *P. oceanica* meadows (400–1,000 shoots m^{-2}; Balestri et al. 2003; Procaccini et al. 2003), creates a highly complex canopy structure. In fact, the mean leaf area index (LAI) of *P. oceanica* meadows can reach values as high as 13 $m^2\ m^{-2}$ (e.g. Romero 1989; Balestri et al. 2003), which is comparable with the maximum values measured in terrestrial forest canopies (Scurlock et al. 2001). These LAI values are also very high when compared with those obtained for other seagrass species of similar architecture (e.g. *Thalassia testudinum*, LAI = 0.65–4.34 $m^2\ m^{-2}$; Enríquez and Pantoja-Reyes 2005). These seagrass meadows thus strongly modify the environmental conditions within their leaf canopies, particularly the light climate (Enriquez et al. 1992; Dalla Via et al. 1998; Zimmerman 2006). As previously shown in other canopy-forming plant communities, such as terrestrial (Canhan et al. 1990) and kelp (Clark et al. 2004) forests, modification of the light environment has been shown to be involved in the determination of the structure of the understorey. Accordingly, most algal species that inhabit the basal part of *P. oceanica* meadows are sciaphilic, and a large number of them are undeveloped and do not grow beyond juvenile stages (Templado et al. 2004).

Previous studies have demonstrated the high photosynthetic plasticity of Mediterranean populations of *C. cylindracea*, which could allow the alga to acclimate to reduced light conditions (Bernardeau-Esteller et al. 2011; Raniello et al. 2004, 2006). *C. cylindracea* has been shown to be able to colonise and photoacclimate the basal substratum of *C. nodosa* meadows, another common Mediterranean seagrass, the canopy of which is less complex than that of *P. oceanica* reducing any shading effect (Raniello et al. 2004). However, the capacity of the alga to photoacclimate to the more severe light reductions created by *P.*

oceanica leaf canopies has not yet been investigated. Furthermore, the extent to which the photoacclimatory responses elicited by the alga effectively compensate for imbalances of the metabolic carbon budget, which ultimately determines the availability of resources for survival and growth under reduced-light conditions (Matta and Chapman 1991), has also been neglected.

The general aim of the present study was to contribute to the understanding of the mechanisms underlying the resistance by native *P. oceanica* meadows to the spread of *C. cylindracea*. We specifically examined the hypothesis that the light regime within *P. oceanica* leaf canopies might limit *C. cylindracea* growth and survival under canopies of this seagrass species. To this end, we performed a comparative analysis of light regimes and *C. cylindracea* variables related to its abundance (total biomass) and photoacclimative capacities (frond length, pigments and photosynthetic parameters), characterised outside and within *P. oceanica* meadows of different highly-invaded sites of the southeastern coast of Spain (Ruiz et al. 2011). We used an ecophysiological, carbon balance approach by integrating daily light curves and photosynthesis–irradiance (P–E) models to obtain the average daily net productivity of the alga (Bernardeau-Esteller et al. 2011).

Materials and methods

Experimental design

The present study was conducted in 2009 on the Mediterranean coast of Murcia (SE Spain), where the invasive alga *C. cylindracea* was observed for the first time in 2005 (Ruiz et al. 2011). Canopy properties, light regimes and algal abundance were sampled within and outside *P. oceanica* leaf canopies of highly-invaded areas identified in this region (Ruiz et al. 2011; Bernardeau-Esteller et al. 2011). Within this area, sampling was done at different sites and times to assess a variety of environmental situations and encompass as much as possible the spatio-temporal variability of this habitat. The three sites, Isla Grosa (IG; 37°43′N, 00°42′E), Cabo Tiñoso (CT; 37°32′N, 00°44′E) and Calblanque (CB; 37°32′N, 01°07′E), had contrasting depths (11, 18 and 26 m, respectively), with a range that encompassed most of the vertical distribution of *P. oceanica* and *C. cylindracea* in this region. The three sites were in an area with similar climate and oceanography (Vargas-Yáñez et al. 2010) and substratum type (i.e. detritic soft bottoms mixed with dead *P. oceanica* “matte”; Calvín-Calvo et al. 1998, Ruiz et al. 2011). At all sites, the substratum outside the *P. oceanica* meadow was almost totally covered by dense *C. cylindracea* stands, from which some stolons penetrated inside the seagrass leaf canopy, although only up to the first 25–40 cm from the meadow edge. As for the outside, within the *P. oceanica* canopy, the stolons of the alga colonised both sediments and basal seagrass rhizomes (dead and alive). There was no apparent discontinuity in the nature of the substratum or any other environmental feature that could be related to the position of the transition between the meadow and the algal stand. To assess temporal variation, sampling of all three sites was done in both January and July, times that represented the two extremes of the seasonal environmental variation: winter (T1) and summer (T2). In order to avoid temporal resampling of the sites, the sampling of the selected variables at each time and site was done in a randomly-selected meadow area 50 m in length and 10 m in width (i.e. a sampling area of 500 m^2), with the long axis of the rectangle centered on the seagrass meadow edge.

For each sampling site and time, algal variables and light regimes were determined at two positions relative to the edge of the seagrass meadow: (i) an outer position (OUT), on the adjacent densely-invaded detritic sediments within 1–2 m from the meadow edge and (ii) at an inner position (IN), 25–40 cm from the meadow edge. In addition, general descriptive data of the *P. oceanica* meadow structure were also collected.

Caulerpa cylindracea biomass, frond height and pigments

Samples of *C. cylindracea* were gathered in five randomly-selected sampling locations separated by 10 m at each site, time and position. In each sampling location, fronds, stolons and rhizoids of *C. cylindracea* were carefully collected by hand within three 400-cm^2 square frames randomly distributed in the area (Ruitton et al. 2005). Samples were transported to the laboratory in plastic bags together with seawater, in chilled containers. After removing the sediment, debris and other algal species, total *C. cylindracea* biomass (g DW m^{-2}) was determined by drying the samples at 70 °C to constant weight. The three biomass values determined in each sampling location were then averaged to obtain five replicates ($n = 5$) for each site, time and position combination. The frond height (cm) was determined by measuring the height of 10 algal fronds randomly selected from each sample. Measurements were averaged per sample and then per sampling location to constitute one of the five replicates ($n = 5$) of each site, time and position combination.

The pigment content was analysed in 2 randomly-selected healthy and non-epiphytised *C. cylindracea* fronds of approximately 3 cm in height from each *C. cylindracea* sample. Results from the analyses were averaged per sample and sampling location so the final number of replicates was five ($n = 5$) for each site, time and position. The analysis was conducted spectrophotometrically after manual extraction of a homogenised suspension using 90 % acetone (Dennison 1990), with $MgCO_3$ added as a chlorophyll stabiliser. The acetone extracts (10 ml) were stored at 4 °C in the dark for 24 h and centrifuged. The chlorophyll *a* and *b* content was computed using the equations of Lichtenthaler and Wellburn (1983).

Posidonia oceanica meadow and leaf canopy structure

To characterise the structure of the three selected meadows, the shoot density and the percentage of meadow cover were measured at all sampling sites and times, following standard methods adopted for this seagrass species (Ruiz et al. 2010a, b). Shoot density (shoots m^{-2}) was estimated in six randomly-selected locations in each meadow by counting the number of shoots within two 400-cm^2 quadrats randomly placed within each location. The average of each pair of measurements was the individual, independent replicate ($n = 6$). The percentage of meadow cover was visually estimated as the percentage of the bottom covered by seagrass patches within 1,600-cm^2 square frames subdivided into four 20 × 20 cm squares. Visual estimations were performed every meter along three ($n = 3$), 10-m linear transects randomly selected within the meadow at each visit.

The leaf area index (LAI) and the canopy height were used to characterise the leaf canopy. In each site and time, five shoots were collected in four randomly-selected locations. The total leaf surface area (based on one side) was calculated for each shoot by measuring the length and width of all leaves per shoot and averaged for each sampling location. LAI (m^2 m^{-2}), as a descriptor of the degree of leaf packing within the canopy (Enríquez and Pantoja-Reyes 2005), was then calculated by multiplying the total leaf area of each location by the averaged shoot density of each meadow, so the total number of replicates was four ($n = 4$) in each site and time. The canopy height (cm) was estimated in situ by divers, using a ruler and taking two measurements at six different locations along the meadow edges. The average value of the two measurements in each location constituted each one of the replicates per site and time ($n = 6$).

Irradiance measurements

The light field at each site, time and position was characterised 5 cm above the bottom using spherical quantum sensors (Alec MDS MK5). Sensors were programmed to record irradiance values every 10 min and recorded data for at least 2 weeks in each season. Maximum instantaneous irradiance at noon (E_{max}, μmol quanta m^{-2} s^{-1}) and total daily irradiance values (E_{total}, mol quanta m^{-2} d^{-1}) were obtained from the diurnal irradiance cycles.

Light attenuation coefficients were determined for both the water column (water-K_d, m^{-1}) and the meadow canopy (canopy-K_d, m^{-1}) under standard conditions (i.e. between 12:00 h and 14:00 h on standard sunny days with minimal water movement and sediment resuspension; Enríquez and Pantoja-Reyes 2005). Water column down-welling irradiance was measured using a cosine-corrected quantum sensor (LI-190SA; LI-COR). Irradiance data were recorded for each m from the sea subsurface (E_0) to the sea bottom (E_z), integrating the values obtained over 10 s on three different days in both summer and winter. Irradiance within the seagrass canopy was measured using spherical quantum sensors (Alec MDS MK5) at every 5 cm from the base of the meadow to the top of the canopy using a marked vertical bar for reference. Data were averaged for a 10-s period (one measurement s^{-1}) at each height. Light attenuation coefficients (water-K_d and canopy-K_d) were estimated using the Beer-Lambert equation: $E_z = E_0 e^{-Kdz}$; where E_z and E_0 are the irradiance values at a given depth (z in m) and at the sea subsurface/top canopy, respectively; and K_d is the light attenuation coefficient (Kirk 1994).

The percentage of subsurface irradiance that reached the sea bottom was calculated from E_0 (subsurface irradiance) measured at noon on calm sunny days using the LI-COR quantum sensor and the corresponding sea floor values within and outside the meadows measured using the spherical quantum sensors. Both sensor types were intercalibrated in the laboratory, showing a strong linear relationship with a constant factor of 1.02.

C. cylindracea photosynthetic variables

Prior to photosynthetic measurements, *C. cylindracea* samples were kept overnight in the dark under controlled temperature in natural seawater taken from the collection site. Photosynthesis and dark respiration rates were measured using a polarographic oxygen electrode and a magnetic stirrer (DW3, Hansatech Instruments Ltd) under controlled temperature (Bernardeau-Esteller et al. 2011). Incubation was carried out at the same temperature as that measured in the field during sampling: 13 °C in winter for all three sites; and 24 °C at IG and 22 °C at CT and CB in summer. Apical segments of non-epiphytised *C. cylindracea* fronds of approximately 2 cm in length were used for the measurements. Two replicated algal segments from three of the five sampling locations (see above) were randomly selected and employed for the photosynthetic measurements ($n = 3$). Dark respiration rates were measured by maintaining the fronds in the dark for 15 min. Net oxygen production was then determined at 13 different light intensities (from 14 to 2,271 μmol quanta m^{-2} s^{-1}) using a high-intensity light source (LS2, Hansatech Instruments Ltd). Net photosynthetic rates were plotted against the light intensities (P–E curves), and the photosynthetic parameters were calculated as follows: the maximum rate of net photosynthesis (net-P_{max}, μmol O_2 g^{-1} FW h^{-1}) was determined by averaging the maximum values above the saturating irradiance (E_k). The photosynthetic efficiency (α, μmol O_2 g^{-1} FW h^{-1}/μmol quanta m^{-2} s^{-1}) was calculated as the slope of the regression line fitted to the initial linear part of the P–E curve, and the compensation irradiance (E_c) as the intercept on the *X*-axis. E_k was calculated as the ratio P_{max}/α. Mean daily compensation (H_c) and saturation (H_k) periods were calculated from each daily light curve as the number of hours per day that irradiance values exceeded E_c and E_k mean values, respectively.

Daily metabolic carbon balances

Daily carbon balance, as a predictor of plant light limitation (Dennison and Alberte 1985), was calculated according to the Michaelis–Menten function $[P = [\text{gross}-P_{max}\ E/(E + E_k)] + R$ (Baly 1935)] previously applied to *C. cylindracea* (Gatusso and Jaubert 1985; Bernardeau-Esteller et al. 2011), where P is net photosynthesis, gross-P_{max} is the maximum gross photosynthetic rate, E is the irradiance measured in the field, E_k is the saturation irradiance, and R is the respiration rate. Photosynthetic parameters obtained from P–E curves and continuous recordings of field irradiance measurements were entered into the function to generate estimates of net production, which were integrated across 24 h periods to yield daily net production values ($n = 3$). If the photosynthetic quotient is assumed to equal unity, and the ratio g C: g $O_2 = 0.3$ (Matta and Chapman 1991), then the net productivity in oxygen units can be multiplied by 0.012 to obtain the equivalent carbon units (mg C g FW^{-1}). This calculation presumes constant dark respiration throughout the day and does not consider other carbon losses (exudation, grazing) or gains (light-independent carbon fixation).

Data analyses

The spatio-temporal variation of *P. oceanica* meadow structure descriptors was evaluated using a two-way ANOVA with sampling sites (three levels: IG, CT and CB) and times (two levels: T1, in winter, and T2, in summer) as fixed factors. For the analysis of *C. cylindracea* variables a randomized-block design was applied for each sampling time separately, defining sites as blocks and position (two levels: IN vs OUT) as main factor. For both designs, prior to carrying out the ANOVA, the data were tested for heterogeneity of variance using Cochran's *C* test and transformed when necessary. Where variance remained heterogeneous, untransformed data were analysed, as ANOVA is a robust statistical test and is relatively unaffected by the heterogeneity of variances, particularly in balanced designs (Underwood 1997). The Student–Newman–

Keuls (SNK) test was used for a posteriori pairwise comparisons of means. A probability level of 0.05 was regarded as significant except when data transformation was not possible. In such cases, the level of significance was reduced to $P < 0.01$ to minimize type I errors. Randomized block analysis also assumes that there are no interactions between blocks and the main factor. To test this assumption plots of dependent variables versus blocks were examined (Quinn and Keough 2002). Regression analysis was used in order to describe the relationship between irradiance and plant variables with the depth of the site. Univariate statistical analysis was performed using the statistical package STATISTICA (StatSoft Inc. 2001, version 6.0).

We also employed a multivariate approach to explore photoacclimative response patterns between sites, times and positions, but based on the integration of the multiple univariate responses obtained in each case. Principal Components Analysis (PCA) was carried out on the correlation matrix of photoacclimation variables, following fourth square transformation of the data. This analysis provides a measure of association between each original variable and the resulting principal components. Multivariate analysis was conducted using the software package CANOCO version 4.5 for Windows (Ter Braack and Šmilauer 2002).

Results

Light regimes

At both sampling times, mean noon subsurface irradiance (E_0) was similar between sites, but with higher values in the summer sampling (T2) than in the winter sampling (T1) (Table 1). The water column attenuation coefficients (water-K_d) were also similar between sites and times (two-way ANOVA, $P > 0.05$), with mean values ranging from 0.082 to 0.124 m^{-1}.

At T1, the mean total daily irradiance (E_{total}) and the noon maximum irradiance (E_{max}) obtained at the bottom in the OUT-position varied by an order of magnitude between sites, with mean E_{max} values representing 4–27 % of E_0. Irradiance showed a high and negative exponential relation with the depth (z) of the sampling site (e.g. $E_{total} = 1149.5 \cdot e^{-0.13z}$, $R^2 = -0.994$), with highest mean values recorded at the shallowest site IG, and the lowest at the deepest, CB. At T2, these irradiance mean values were 13–41 % of E_o and 2–5 fold higher than at T1, and also showed a similar, but linear negative relation with site depth ($E_{total} = 939-27.9 \cdot z$; $R^2 = -0.992$).

At both sampling times, E_{total} and E_{max} mean values obtained inside the seagrass canopy (IN-position) at the three sites were reduced (82–88 % at T1 and 60–89 % at T2), relative to those determined in the OUT-position. At T1, between-site variation of irradiance mean values in the IN-position reflected that of external light availability (i.e. OUT-position mean values), but not in T2 where mean values determined at the IN-position showed very small variation between sites (1.59–2.30 mol quanta m^{-2} s^{-1}). The sharp light extinction associated with the seagrass canopy shelf-shading corresponded to canopy-K_d mean values that ranged between 5.8 and 8.5 m^{-1}. Sites showed significant differences between mean canopy-K_d values (two-way ANOVA, $P < 0.001$), with the deepest meadow CB always showing the lowest values (Table 1).

Seagrass meadow structure

Meadow structure descriptors showed significant variation between sampling sites and times, except in the case of meadow cover, for which differences were only significant among sites (Fig. 1; Table 2). Spatial variation was the major source of variation of shoot density (54.7 %) and meadow cover (97.6 %), and temporal variation in the case of LAI (70.0 %) and canopy height (87.2 %). For shoot density, LAI and canopy height, the pattern of variation among sites differed between sampling times, as indicated by the significant effect of the ANOVA interaction term (Table 2). At both sampling times, spatial variation showed a high significant negative correlation with the depth of the sampling sites in the case of the shoot density ($R^2 = 0.58$–0.72, $\beta = -0.76$ to -0.85, $P < 0.001$, N = 18), meadow cover ($R^2 = 0.78$–0.88, $\beta = -0.88$ to -0.94, $P < 0.01$, N = 9) and LAI ($R^2 = 0.$ 53–0.93, $\beta = -0.73$ to -0.96, $P < 0.01$, N = 12). In all these cases, maximum values of the variable were found at the shallowest site and minimum values at the deeper one.

Table 1 Characteristics of light regimes determined at sampling sites, times and positions from irradiance measurements performed across vertical profiles of the water column and the *Posidonia oceanica* seagrass canopy and from continuous light measurements performed on the seabed (see methods)

Variable	Time	Site		
		IG (11 m)	CT (18 m)	CB (26 m)
Water column				
Noon subsurface irradiance (E_0, μmol quanta m^{-2} s^{-1})	T1	1059 ± 113	1017 ± 34	1084 ± 17
	T2	1496 ± 63	1305 ± 124	1506 ± 138
Water column K_d (m^{-1})	T1	0.122 ± 0.008	0.082 ± 0.010	0.106 ± 0.012
	T2	0.124 ± 0.020	0.082 ± 0.010	0.095 ± 0.010
Seabed OUT-position				
Max. noon bottom irradiance–OUT (E_{max}, μmol quanta m^{-2} s^{-1})	T1	286 ± 17 (27.0 %E_0)	100 ± 13 (9.8 %E_0)	40 ± 6 (3.7 %E_0)
	T2	622 ± 51 (41.5 %E_0)	459 ± 13 (35.2 %E_0)	204 ± 15 (13.5 %E_0)
Total daily bottom irradiance–OUT (E_{total}, mol quanta m^{-2} d^{-1})	T1	5.06 ± 0.31	1.61 ± 0.23	0.63 ± 0.10
	T2	15.74 ± 0.92	10.50 ± 0.30	4.75 ± 0.34
Seabed IN-position				
Canopy K_d (m^{-1})	T1	8.43 ± 0.56	8.52 ± 0.71	5.79 ± 0.54
	T2	6.69 ± 0.51	7.96 ± 0.83	6.37 ± 0.70
Max. noon bottom irradiance–IN (E_{max}, μmol quanta m^{-2} s^{-1})	T1	51 ± 4 (4.8 %E_0)	17 ± 3 (1.7 %E_0)	7 ± 1 (0.7 %E_0)
	T2	101 ± 9 (6.7 %E_0)	90 ± 16 (6.9 %E_0)	82 ± 7 (5.4 %E_0)
Total daily bottom irradiance–IN (E_{total}, mol quanta m^{-2} d^{-1})	T1	0.71 ± 0.06	0.20 ± 0.04	0.11 ± 0.02
	T2	1.78 ± 0.13	2.30 ± 0.26	1.59 ± 0.15

Data are presented as mean ± standard error

T1 = winter sampling time, T2 = summer sampling time, IN = inside canopy position, OUT = outside canopy position, K_d = light attenuation coefficient, %E_0 = proportion of the subsurface irradiance reaching the seabed

Total algal biomass

The position of the alga relative to the meadow edge showed a significant effect on the total alga biomass at both times (Fig. 2; Table 3). The stands of *C. cylindracea* growing outside the seagrass meadow showed up to a 50-fold higher biomass than that of stands growing beneath the leaf canopy, except at T1 at the shallower IG site where this variable was similar in OUT and IN-positions. Algal biomass was also significantly affected by sites at both times (Table 3) and showed a strong negative correlation with the depth of the sampling site ($R^2 = 0.64$, $\beta = -0.80$, $P < 0.001$, $N = 15$), but only at T2 at the OUT-position.

Caulerpa cylindracea frond variables

In relation to the height of the fronds, the position of *C. cylindracea* stands was the factor that explained the major component of its total variance in both sampling times (59–66 %), and on average, fronds growing within the meadows (i.e. IN-position) were almost twice as tall as those growing in the OUT-position. The blocking factor 'site' had also a significant effect on this variable at both sampling times (Fig. 3; Table 3), which showed a significant positive correlation with the depth of the sampling sites outside the leaf canopy at T2 ($R^2 = 0.0.90$, $\beta = 0.95$, $P < 0.001$, $N = 15$).

Position also had significant effects on the pigment content (Chl *a*, Chl *b* and the molar Chl *b*/*a* ratio) of algal fronds in both sampling times, with higher percentages of total explained variance in T2 (Fig. 3; Table 3). In general, those fronds growing within the meadows (i.e. IN-position) had significantly higher content in chlorophyll *a* and *b*, as well as higher Chl *b*/*a* molar ratios, than those growing in the OUT-position. The chlorophyll content also significantly differed among sites at both sampling times and showed a negative correlation with the depth of the

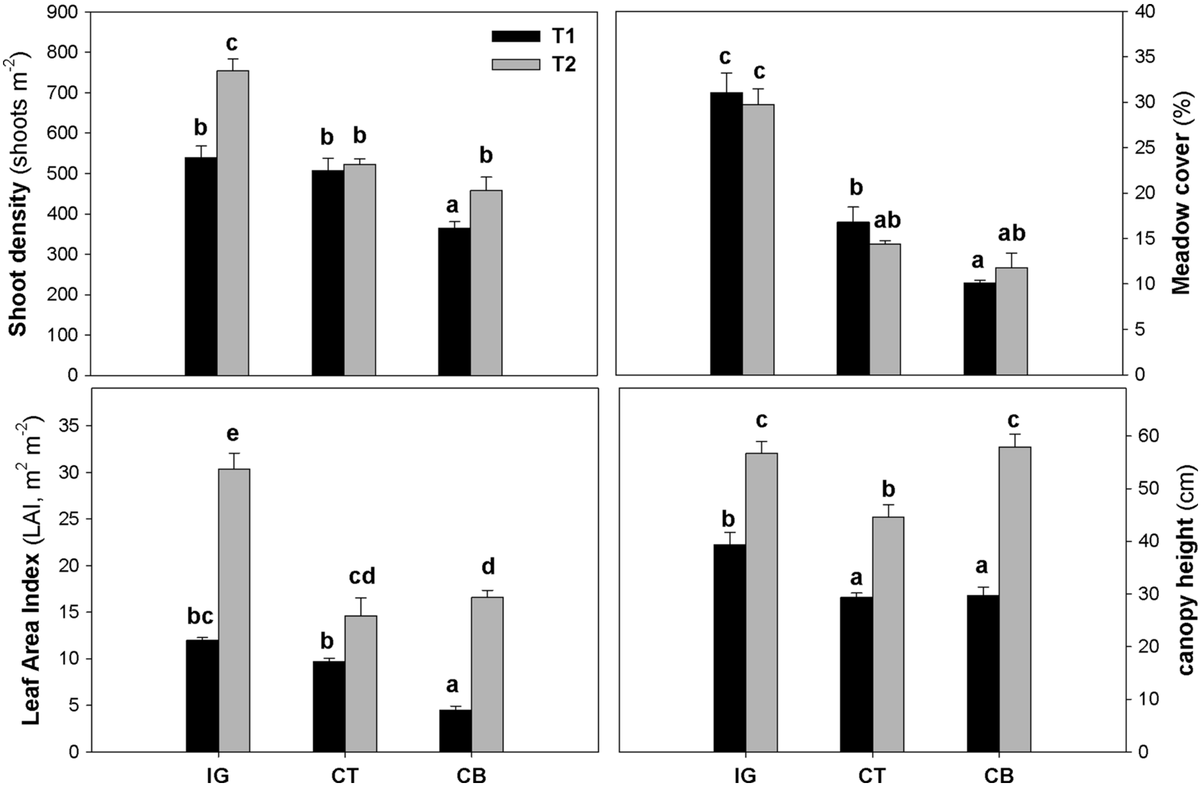

Fig. 1 Mean and standard error of *Posidonia oceanica* meadow structure variables obtained for each combination of sampling site (IG, CT and CB) and time (T1 and T2). *Different letters* indicate groups of homogeneous means obtained in the post hoc SNK test ($P < 0.05$)

sampling site for the Chl *a* and *b* content at T1 at the OUT-position, and for Chl *b* and Chl *a*/*b* ratio at T2 at the IN-position due to the significantly higher mean values observed for these variables at the shallower sites relative to the deeper ones.

In the T2 sampling time all photosynthetic variables derived from P–E curves were significantly affected by the position of *C. cylindracea* stands, except for maximum photosynthetic rate (P_{max}), which in addition was the only photosynthetic variable that significantly differed among positions in T1 (Fig. 4; Table 3). In the summer sampling (T2), the significant lower respiratory demands (65 % in average) exhibited by *C. cylindracea* at the IN-positions significantly increased their photosynthetic efficiency (i.e. α) and reduced their E_c and E_k values with respect to fronds growing at the OUT-positions. The factor ‘site’ also significantly affected the photosynthetic parameters P_{max}, R_d, and α at the winter T1 sampling and only α at the summer T2 (Fig. 4; Table 3). For P_{max} and R_d this spatial variability showed a close, negative and significant correlation with the depth of the sampling site at T1 in both positions ($R^2 = 0.54$–0.82, $\beta = -0.69$ to -0.82, $P < 0.05$, $N = 9$). Whereas, between-site differences in photosynthetic efficiency (α) inside the leaf canopy had a negative and significant correlation with the depth of the sampling site at both sampling times ($R^2 = 0.50$–0.61, $\beta = -0.71$ to -0.78, $P < 0.05$, $N = 9$).

The mean daily period of photosynthetic compensation (H_c) and the mean daily period of photosynthetic saturation (H_k) were both significantly affected by the factors ‘position’ and ‘site’ in both sampling times; the former factor explaining in general the higher percentages of the total variance (Fig. 5; Table 3). Under full illumination conditions (i.e. at the outside positions) H_c ranged between 7 and 13 h and H_k varied between 1 and 10 h; in contrast, these daily periods were significantly and consistently shortened by 43 and 72 %, respectively, at the inside positions (Fig. 5). Minimum mean values (1–7 h for H_c and 0–1.1 h for H_k) were usually found in *C.*

Table 2 Summary of the two-way ANOVA test performed to assess the effect of sampling sites and times on *Posidonia oceanica* meadow structure variables

	df	MS	%Var.	F	P
Shoot density					
Site (S)	2	167066	54.7	40.9	***
Time (T)	1	104275	34.1	25.5	***
SxT	2	30087	9.8	7.4	**
Residual	30	4085	1.3		
LAI					
Site (S)	2	261.9	21.9	52.2	***
Time (T)	1	835.1	70.0	166.6	***
SxT	2	91.4	7.7	18.2	***
Residual	18	5.0	0.4		
Meadow cover					
Site (S)	2	621.2	97.6	93.8	***
Time (T)	1	2.2	0.3	0.3	ns
SxT	2	6.7	1.1	1.0	ns
Residual	12	6.6	1.0		
Canopy height					
Site (S)	2	372.6	8.8	14.3	***
Time (T)	1	3700.7	87.2	141.6	***
SxT	2	146.3	3.4	5.6	**
Residual	30	26.1	0.6		

df degrees of freedom, *MS* mean squares, *%Var.* percentage of explained variance, *F* F-statistics, *P* P value, *ns* not significant

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

cylindracea fronds of the inside position at the winter T1 sampling, but also at T2 at the deepest site CB (Fig. 5). Regarding the variation among sites, H_c and H_k periods were in general shorter at deeper sites than at shallower ones (Fig. 5).

According to these results, daily metabolic carbon balances showed significant differences among positions only for the winter T1 sampling, when carbon balances within the meadow canopy (i.e. IN-position) were consistently negative in all sites (−0.14 to −0.20 mg C g^{-1} FW d^{-1}) but positive (site IG: 0.52 mg C g^{-1} FW d^{-1}) or close to zero (sites CT and CB: −0.01 mg C g^{-1} FW d^{-1}) at the OUT-position (Fig. 5; Table 3). *C. cylindracea* carbon balances were also significantly affected by the factor 'site', with the shallowest site IG showing significantly higher carbon balances than the deepest ones CT and CB.

Multivariate analysis

The PCA performed using the selected *C. cylindracea* photoacclimative variables (frond height, pigment content and photosynthetic parameters) yielded eigenvalues of 0.759 and 0.132 for the PCI and PC2 axes, respectively (Table 4). The first PCA axis (PC1) explained 75.9 % of the variance in the original data set. The ordination of the objects along this axis (Fig. 6a) appears to relate to the reported differences in light regime, since those cases that were exposed to the highest light levels (i.e. at T2, in the outside position) are positioned on the right extreme of the axis (the most positive values), whereas those exposed to the lowest irradiances (i.e. T1, inside position) are at the opposite position (the most negative values). Moreover, the position of the objects on the PC1 axis showed a high and positive significant correlation ($r = 0.82$, $P < 0.001$) with the mean total daily irradiance (E_{total}, Table 1; Fig. 6b); the lineal regression model

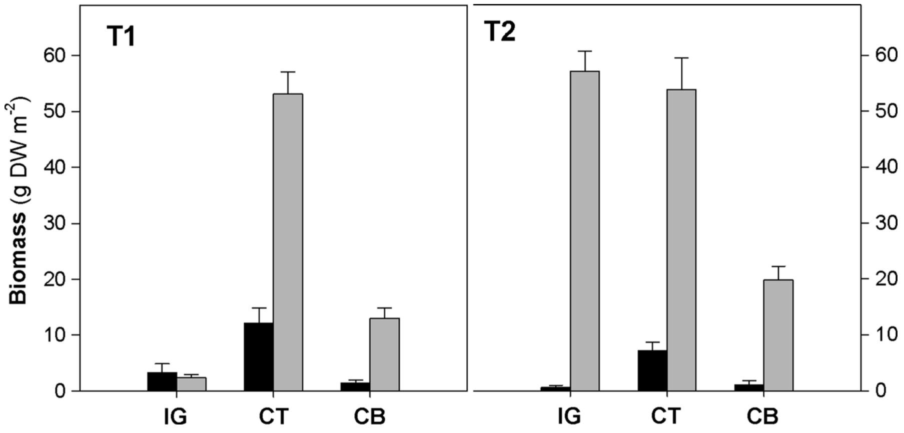

Fig. 2 Mean and standard error of *Caulerpa cylindracea* total biomass determined inside (IN-position, *black bars*) and outside (OUT-position, *grey bars*) *Posidonia oceanica* seagrass meadows for each combination of sampling site (IG, CT and CB) and time (T1 and T2)

Table 3 Summary ANOVA test performed to assess the effect of the position (P) and sampling site (S) on all *Caulerpa cylindracea* variables at the two studied sampling times (T1 & T2)

Effect	df	T1 (Winter)					T2 (Summer)				
		MS	%Var	F	*p*	*SNK-test*	MS	%Var	F	*p*	*SNK-test*
Biomass (g FW m^{-2})											
Position	1	2193.2	22.3	22.35	<0.001	$IN < OUT$	12401.6	68.0	96.30	<0.001	$IN < OUT$
Site	2	2553.9	51.8	26.02	<0.001	$IG = CB < CT$	1241.1	13.6	9.64	<0.001	$IG = CT > CB$
Error	26	98.1	25.9				128.8	18.4			
Frond height (cm)											
Position	1	19.162	58.6	177.7	<0.001	$IN > OUT$	56.255	66.1	100.58	<0.001	$IN > OUT$
Site	2	5.373	32.9	49.8	<0.001	$IG < CT < CB$	7.159	16.8	12.80	<0.001	$IG = CT < CB$
Error	26	0.108	8.6				0.559	17.1			
Chlorophyll *a* (mg g^{-1} FW)											
Position	1	3183.5	10.5	4.795	<0.05	$IN > OUT$	13670.2	71.8	106.6	<0.001	$IN > OUT$
Site	2	4994.1	32.8	7.521	<0.01	$IG > CT = CB$	1021.9	10.7	8.0	<0.01	$IG = CT > CB$
Error	26	664.0	56.7				128.3	17.5			
Chlorophyll *b* (mg g^{-1} FW)											
Position	1	1996.1	22.9	10.809	<0.01	$IN > OUT$	9910.501	81.2	259.8	<0.001	$IN > OUT$
Site	2	950.4	21.9	5.147	<0.05	$IG > CT = CB$	649.282	10.6	17.0	<0.001	$IG > CT > B$
Error	26	184.7	55.2				38.153	8.13			
Chlorophyll *b/a* (molar ratio)											
Position	1	0.009	56.7	40.228	<0.001	$IN > OUT$	0.058	69.9	490.5	<0.001	$IN > OUT$
Site	2	0.001	6.6	2.342	n.s.	–	0.011	26.4	92.4	<0.001	$IG > CT = CB$
Error	26	0.000	36.7				0.000	3.7			
Pmax (mmol O_2 g^{-1} FW h^{-1})											
Position	1	42.25	15.3	7.34	<0.05	$IN < OUT$	31.2	13.9	2.95	n.s.	–
Site	2	76.62	55.5	13.32	<0.001	$IG > CT = CB$	22.9	20.4	2.17	n.s.	–
Error	14	5.75	29.2				10.6	65.8			
Rd (mmol O_2 g^{-1} FW h^{-1})											
Position	1	0.65	5.9	3.28	n.s.	–	30.54	58.7	19.96	<0.001	$IN < OUT$
Site	2	3.81	69.1	19.29	<0.001	$IG > CT = CB$	0.02	0.1	0.014	n.s.	–
Error	14	0.20	25.1				1.53	41.2			
α (mmol O_2 g^{-1} FW h^{-1}/mmol quanta m^{-2} s^{-1})											
Position	1	0.002	2.0	0.816	n.s.	–	0.020	32.4	11.80	<0.01	$IN > OUT$

Table 3 continued

Effect	df	T1 (Winter)					T2 (Summer)				
		MS	%Var	F	*p*	*SNK-test*	MS	%Var	F	*p*	*SNK-test*
Site	2	0.024	63.2	12.73	<0.001	*IG > CT = CB*	0.009	29.3	5.34	<0.05	*IG = CT > CB*
Error	14	0.002	34.8				0.002	38.4			
Ek (mmol quanta m^{-2} s^{-1})											
Position	1	1118.6	29.6	8.15	n.s. (0.012)	–	12068.8	49.4	16.3	<0.01	*IN < OUT*
Site	2	369.6	19.6	2.69	n.s.	–	986.1	8.1	1.3	n.s.	–
Error	14	137.2	50.8				741.6	42.5			
Ec (mmol quanta m^{-2} s^{-1})											
Position	1	21.4	11.4	2.53	n.s.	–	2345.0	74.7	44.5	<0.001	*IN < OUT*
Site	2	24.2	25.7	2.87	n.s.	–	28.7	1.8	0.5	n.s.	–
Error	14	8.4	62.9				52.7	23.5			
Hc (h)											
Position	1	77.3	55.5	141.9	<0.001	*IN < OUT*	78.2	29.4	13.6	<0.01	*IN < OUT*
Site	2	27.2	39.1	50.0	<0.001	*IG > CT = CB*	53.4	40.2	9.3	<0.01	*IG = CT > CB*
Error	14	0.5	5.47				5.8	30.4			
Hk (h)											
Position	1	44.7	42.6	30.3	<0.001	*IN < OUT*	112.7	61.1	72.6	<0.001	*IN < OUT*
Site	2	19.7	37.7	13.4	<0.001	*IG > CT = CB*	25.1	27.2	16.1	<0.001	*IG = CT > CB*
Error	14	1.5	19.7				1.6	11.8			
Carbon balance (mg C g^{-1} FW d^{-1})											
Position	1	0.496	43.6	20.7	<0.001	*IN < OUT*	0.383	10.8	3.07	n.s.	–
Site	2	0.154	27.1	6.4	<0.05	*IG > CT = CB*	0.702	39.7	5.61	<0.05	*IG = CT > CB*
Error	14	0.024	29.4				0.125	49.5			

df degrees of freedom, *MS* mean squares, *%Var.* percentage of explained variance, *F* F-statistics, *P* P value, *SNK-test* SNK post hoc analysis, *ns* not significant

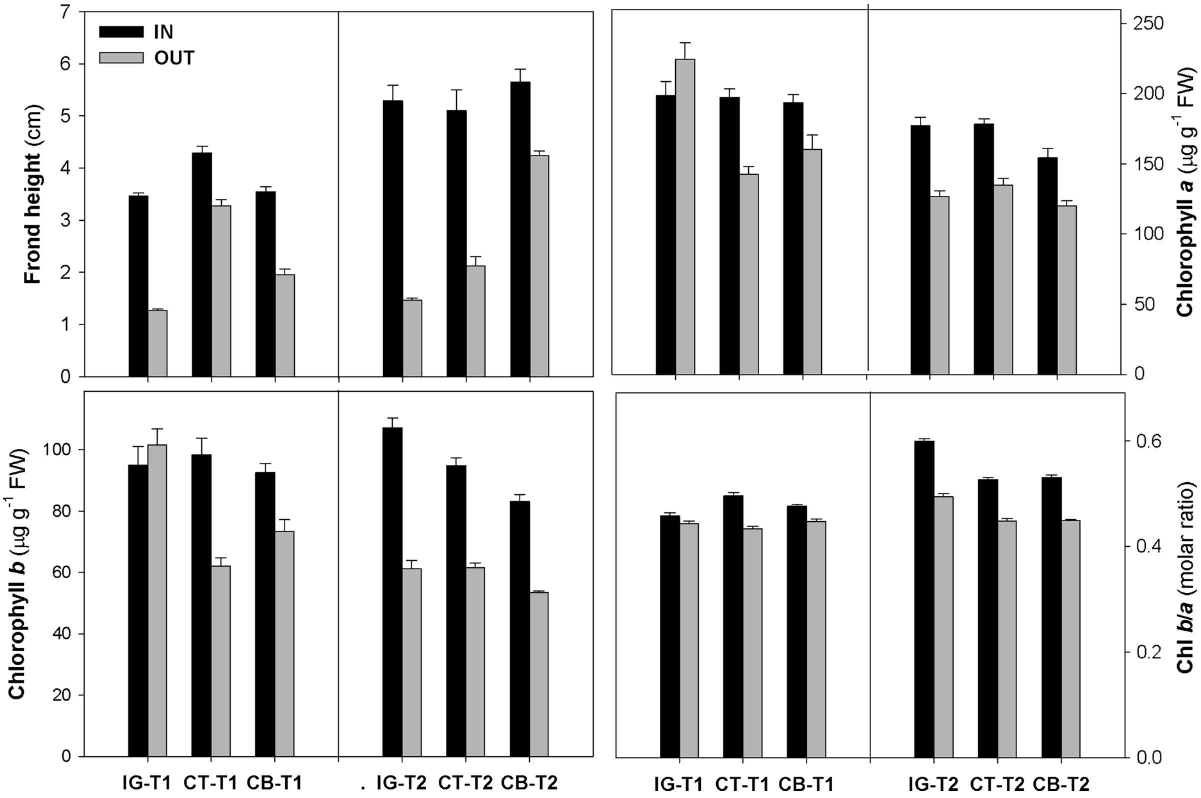

Fig. 3 Mean and standard error the height and pigment content (chlorophyll *a*, *b* and *b*/*a*) of *Caulerpa cylindracea* fronds determined inside (IN-position, *black bars*) and outside (OUT-position, *grey bars*) *Posidonia oceanica* seagrass meadows for each combination of sampling site (IG, CT and CB) and time (T1 and T2)

fitted to these data revealed that this factor explained 68 % of the total variation along the PC1 axis (Fig. 6b).

The vectors depicted in the plot (Fig. 6a), with the arrow pointing to the higher values of the variable, indicate that this first axis had strong positive correlations (scores > 0.8) with E_k, E_c, and R_d, and negative correlations (scores < -0.8) with the concentration of chlorophyll *a* and *b* (Table 4). These strong correlations identified the importance of this set of responses in the photoacclimative response of *C. cylindracea* to cope with the shady conditions found within the meadows.

Discussion

Outside the seagrass leaf canopy, between-site variability of the light regime showed a high negative correlation with the depth of the site that was consistent with the characteristic pattern of light extinction that occurred together with water column vertical profiles (Kirk 1994). Sites had similar mean water-K_d values and were also very similar in many other climatic, geological and oceanographic features (Marín-Guirao et al. pers. obs.; Vargas-Yáñez et al. 2010). Therefore, the possibility that the reported spatial variation in light regimes was caused by other local factors apart from depth, is assumed to be very low. Similarly, the pronounced variation in irradiance between sampling times (about one order of magnitude), matched typical differences in underwater irradiance between winter and summer at similar depths and latitudes (e.g. Enríquez et al. 2004; Raniello et al. 2004; Vargas-Yañez et al. 2010). Therefore, it can be considered that the reported differences in light regime are representative of the typical spatio-temporal variation of this factor associated with depth and seasonality, at least in benthic macroalgal assemblages within the depth range and region considered in this study. The invasive *C.*

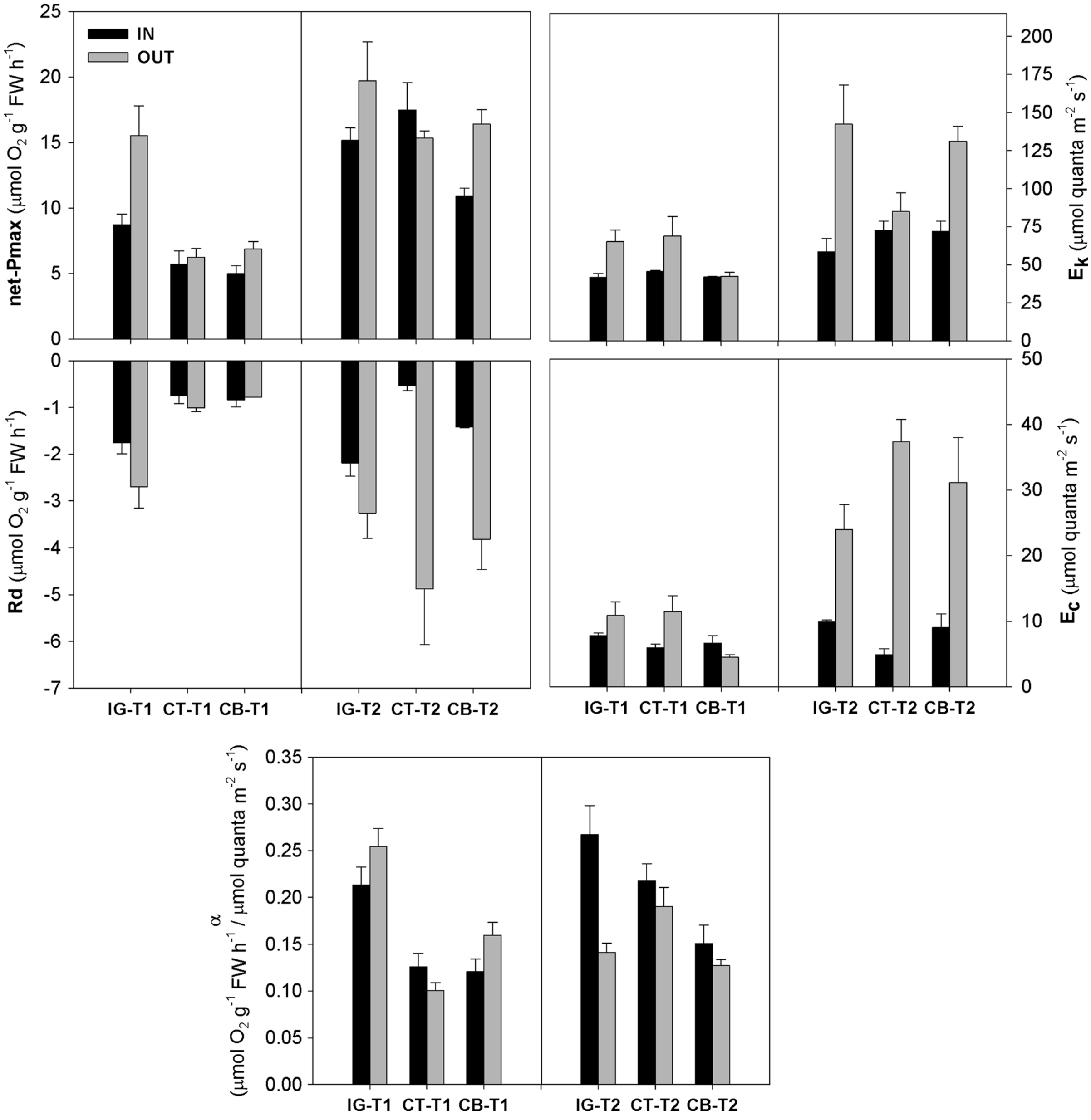

Fig. 4 Mean and standard error of photosynthetic characteristics derived from P–E curves obtained from *Caulerpa. cylindracea* fronds inside (IN-position, *black bars*) and outside (OUT-position, *grey bars*) *Posidonia oceanica* seagrass meadows for each combination of sampling site (IG, CT and CB) and time (T1 and T2)

cylindracea has been shown to be able to photoacclimate and persist across the environmental light gradient associated with depth and season in sublittoral Mediterranean environments (Raniello et al. 2004, 2006; Bernardeau-Esteller et al. 2011), which has been invoked as one of the key mechanisms involved in its colonisation success in native habitats. In support of this, many of the significant effects associated with sampling sites and times observed in the analysed variables of algal stands growing outside the seagrass canopy reflected photoacclimatory responses previously observed in response to variability in the light regime.

During the summer sampling (T2), *C. cylindracea* stands growing outside the canopy showed in general

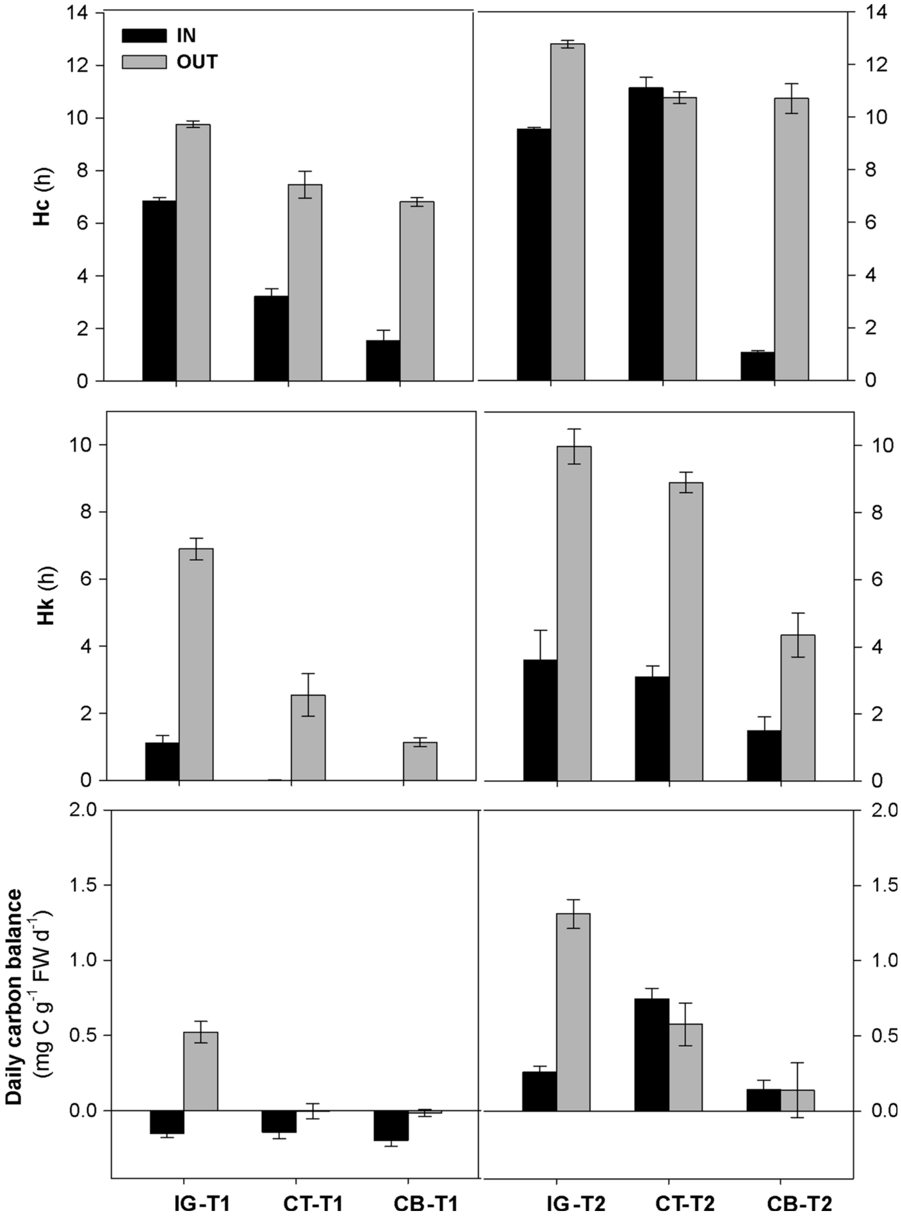

Fig. 5 Mean and standard error of light compensation (H_c) and saturation (H_k) periods and daily carbon balance estimated for *Caulerpa cylindracea* fronds inside (IN-position, *black bars*) and outside (OUT-position, *grey bars*) *Posidonia oceanica* seagrass meadows for each combination of sampling site (IG, CT and CB) and time (T1 and T2)

highest P_{max} and R_d rates, which is consistent with the highest irradiance levels (well above E_c and E_k mean values) and day-length recorded in this time. This high light availability for photosynthesis and growth could explain the lack of significant differences in most of individual photosynthetic characteristics (P–I curves) between sites, as it was also reflected in the integrative multivariate analysis (PCA, Fig. 6b), with cases belonging to T2-OUT occupying a very close position in the PCI axis despite their differences in light climate (E_{total}, Fig. 6b). Accordingly, the alga showed high H_k and carbon balance mean values except in the deepest site (CB) with the lowest light availability and total biomass. This suggest the existence of some degree of light limitation at this site in summer, which is supported by the considerable enhancement of frond height, a typical morphological adaptation of this and other macroalgal species to light-limiting conditions (Calvert 1976; Ohba and Enomoto 1987; Kirk 1994). In addition, these results were also consistent with

Table 4 Eigenvalues of the first two axes of the PCA (PC1 and PC2) and the scores of selected photoacclimative variables

Variable/axis	PC1	PC2
Eigenvalues	0.759	0.132
Ec	0.957	−0.017
Ek	0.940	−0.075
Rd	0.887	0.403
Chl b	−0.731	0.641
Chl a	−0.769	0.570
Pmax	0.759	0.581
Frond height	−0.252	−0.183
Chl b/a	−0.214	0.455
α	−0.006	0.939

those obtained in a previous study performed at the same sampling sites in a similar sampling time (Bernardeau-Esteller et al. 2011), confirming a more limited capacity of colonization by the alga in these deepest areas.

During the winter sampling (T1), physiological variables of *C. cylindracea* growing outside the seagrass canopy reflected a major photoacclimatory effort (relative to T2) according to the more reduced light availability characteristic of this season, particularly in the deepest sites. Thus, photosynthesis and respiration rates, as well as E_c and E_k, were in general lower than in T2 and showed significantly lower mean values in the deepest sites (CT and CB), where frond height was significantly higher than in the shallower site IG. All these are characteristic photoacclimatory responses of marine macrophytes to overcome light limitation (Falkowski and Raven 2007; Lobban and Harrison 1997; Littler et al. 1986; Lüning 1990) that allows the lengthening of H_c and H_k periods and counterbalances the metabolic carbon budget (Dennison and Alberte 1982, 1985; Dunton and Shell 1986; Gómez et al. 1997). Other responses were opposite to those expected under a situation of light limitation, such as the significant reduction in photosynthetic efficiency and pigment content reported in the deepest sites. However, in this case, the adjustments of the photosynthetic metabolism (particularly in respiration) probably avoided further reductions in H_c and H_k and allowed the average carbon balance to remain close to zero at those sites with greater depths. Under such situation the alga can maintain the standing biomass but with a very limited growth. The capacity of *C. cylindracea* to maintain biomass during winter in these deeper areas has been reported at other sites at similar latitudes (Giaccone and Di Martino 1995; Cebrian and Ballesteros 2009), but not in colder areas where a winter decline occurs (e.g. Piazzi et al. 1997a, b; Piazzi and Cinelli 1999; Buia et al. 2001; Capiomont et al. 2005; Ruitton et al. 2005; Lenzi et al. 2007). At the shallowest site (IG), a clear uncoupling between biomass and carbon balance was evident, and we attribute this to the effect of abiotic factors other

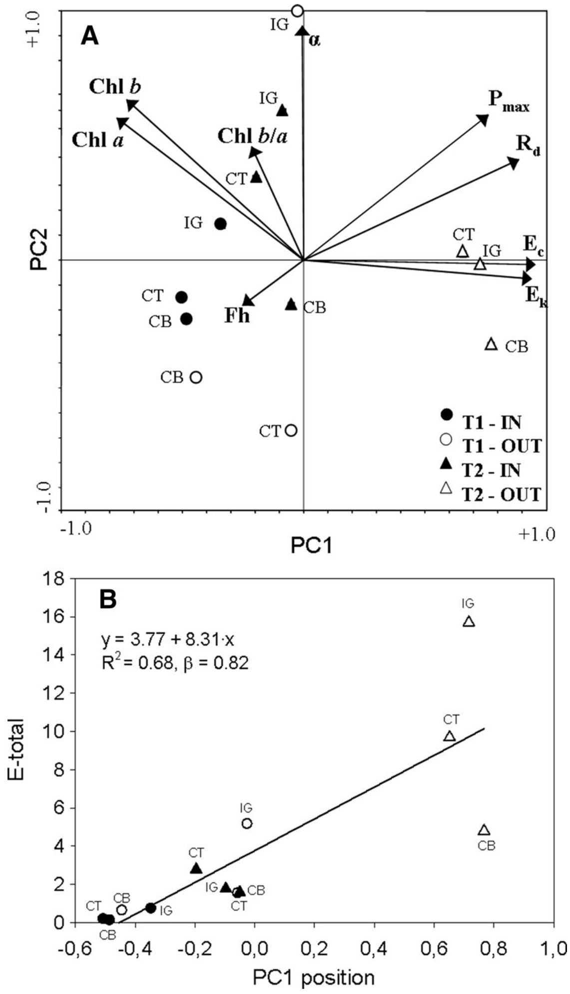

Fig. 6 **a** Ordination diagram of the principal components analysis with all selected photoacclimative variables. **b** Lineal relationship between *X*-axis positions and mean total daily irradiance (E_{total}, mol quanta m^{-2} d^{-1}; Table 1), indicating the lineal regression model, slope (i.e. regression coefficient; $P < 0.05$) and coefficient of determination (r^2). Sampling sites = IG, CT and CB. Fh = frond height

than light that might influence the pattern of vertical distribution of the alga, such as winter storms (e.g. Cebrian and Ballesteros 2009; Marín-Guirao et al. pers. obs.).

Within the *P. oceanica* leaf canopy, light availability was drastically reduced up to levels that were always 3.0–8.8 times lower than those recorded outside. Such low irradiance levels are typically measured inside *P. oceanica* meadows (1–7 % of E_o) and reflect the elevated K_d values associated with the strong self-shading caused by complex canopies formed by this seagrass species (Dalla Via et al. 1998). The complexity of the leaf canopy showed significant spatio-temporal variation characteristic of *P. oceanica* meadows elsewhere (Romero 1989; Buia et al. 1992; Pergent et al. 1995; Dalla Via et al. 1998; Olesen et al. 2002). On one hand, shoot density, meadow cover and LAI decreased with depth, which explains the lowest mean K_d values observed in the deepest site CB; on the other hand, LAI and canopy height were lower in the winter time, accordingly with the seasonal variation of the seagrass production, but K_d values were equal or even higher than those in the summer time, contributing to the explanation of the considerably low light levels within the canopy at that time. In fact, this variation in the canopy structure is considered the main adaptive mechanisms of this and other *Posidonia* species to offset depth-related light reductions (Olesen et al. 2002; Ralph et al. 2007; Collier et al. 2008).

Evidence provided by this study strongly suggests that the low light levels reported within the seagrass canopy are more limiting for *C. cylindracea* growth and survival than those recorded outside. In general, fronds growing inside the meadow margin, showed photoacclimatory responses already described for plants growing outside together with other typical acclimation adjustments of marine macrophytes to low light conditions, such as an increase in chlorophyll content and in the chlorophyll b/a ratio, aimed at enhancing the efficiency of light absorption (e.g. Kirk 1994; Falkowski and Raven 2007; Raniello et al. 2004). Raniello et al. (2004) reported increments in Chl *b* and other complementary pigments (e.g. siphonoxantin) in *C. cylindracea* growing under dense *C. nodosa* canopies, which could be linked to a more efficient exploitation of green light, which is the dominant light under seagrass canopies (e.g. *P. oceanica*; Dalla Via et al. 1998). In the summer sampling (T2), the increment in pigment content and Chl *b*/*a* ratio could explain the maintenance of photosynthetic efficiencies (α) very similar to those plants growing outside the seagrass canopy. Further to this, the inhibition of respiration (up to 89 % with respect to the fronds outside) likely allowed *C. cylindracea* to attain positive carbon balances within the seagrass canopy at the three sites, despite the fact that the daily saturation periods were less than 4 h (i.e. 64–72 % lower than for plants growing outside the canopy). The high correlation of this variable with the first PCA axis (Fig. 4) suggests that the inhibition of respiratory rates represent one of the most important photoacclimatory mechanisms of *C. cylindracea*, not only within the severe shading created by the canopy, but also (as explained above) outside the canopy in winter. Although this response might also reflect low temperature effects on algal metabolism in winter conditions (Flagella et al. 2008; Robledo and Freile-Pelegrín 2005; Terrados and Ros 1992), it has been recognized to be a common physiological strategy to minimize carbon losses and allow seaweed survival under low light regimes (Littler et al. 1986; Lüning et al. 1990; Markager and Sand-Jensen 1994; Pérez-Lloréns et al. 1996; Bernardeau-Esteller et al. 2011). In addition, low respiration rates are indicative of limited growth (Kirk 1994; Pérez-Lloréns et al. 1996), which could further explain the large differences in algal biomass between the inner and outer stands observed at the deepest sites (CT and CB) during the summer, despite their almost identical carbon balances.

Light climate within the canopy in the winter represented the most extreme condition for the alga, since it showed a very limited photoacclimatory capacity unable to maintain H_c values and achieve H_k daily periods longer than 1.1 h at the shallower site (IG) and of zero h at the deeper sites. As a result, carbon balances were negative and hence light availability in these conditions must be below the minimum requirements for algal growth and survival (Dennison and Alberte 1982; Gómez et al. 1997). In agreement with this, and based only on water-K_d values (Table 1) and the Beer-Lambert equation (Kirk 1994), light levels recorded within the canopy are equivalent to the range of maximum distributional depths reported in the Western Mediterranean basin (35–60 m: Piazzi et al. 2005; Klein and Verlaque 2008; Ruiz et al. 2011). These results are also consistent with those obtained in the PCA analysis (Fig. 4), in which the ordination of the objects (i.e. measurements obtained in each combination of position, location and time) along the PC1 axis was highly correlated with light availability and mainly represented the integration of all photoacclimative responses in each case. With respect to the objects during the winter time, it should be noted that measurement made at the inner position at the shallow site were very close to those at the outer position of the deepest site. This simple observation suggests that the extreme low light conditions observed beneath the seagrass meadow in winter have overcome, or are close to, the limit of the photosynthetic plasticity of *C. cylindracea*. For instance, it can be seen that, particularly in deepest sites, values of P_{max} and R_d values (and E_c and E_k) of *C. cylindracea* plants growing inside the seagrass canopy did not differ from those showed by plants growing outside, suggesting a limit for the plasticity of these variables under more limiting light conditions. In such a situation, the maintenance of algal biomass observed underneath the seagrass canopy in winter is only possible by using carbon storage reserves during summer, when the carbon balance was shown to be positive and growth arrested. This is a strategy to survive light-limiting periods previously reported for this (e.g. Terrados and Ros 1992; Robledo and Freile-Pelegrín 2005) and other seaweed species (e.g. Rosemberg and Ramus 1982; Gagne et al. 1982; Dunton and Shell 1986; Gómez and Wiencke 1998; Lobban and Harrison 1997). Other possible mechanisms of *C. cylindracea* survival beyond its photoautotrophic limits might be carbon acquisition by heterotrophy, as reported for the congenerous *C. taxifolia* (Chisholm and Jaubert 1997), or sharing of resources between shaded and illuminated parts of the coenocytic stolons (Collado-Vides and Robledo 1999; De Senerpont Domis et al. 2003).

Our results show that the development of *C. cylindracea* biomass is consistently limited inside the *P. oceanica* canopy, irrespective the sampling site and time considered in this study. A monitoring study performed at the same sampling sites (unpubl. data) has demonstrated that this sharp biomass gradient is stable over years (i.e. 2007–2013) without any symptoms of seagrass meadow deterioration. In fact, this is consistent with the observation that *P. oceanica* meadows are one of the least-invaded habitats elsewhere and the idea that *P. oceanica* can be considered as an effective "ecological barrier" against the spread of this highly invasive alien species. Nonetheless, these types of generalizations must be subjected to future evaluations of possible long-term interactions between the alga and the seagrass through, for instance, phytotoxic allelochemical effects (Dumay et al. 2002; Raniello et al. 2007) or deterioration of substrate conditions (Holmer et al. 2009). This study provides extensive and consistent evidence supporting the hypothesis that light plays a key role in explaining the high resilience of the *P. oceanica* meadow with regard to the *C. cylindracea* bioinvasion. As reported in this and other studies using similar ecophysiological approaches, *C. cylindracea* has a great physiological and vegetative plasticity allowing it to adapt to a wide range of environmental conditions and light climates (Raniello et al. 2004, 2006; Bernardeau-Esteller et al. 2011), which in turn is one of the traits contributing to explain its highly invasive character (Klein and Verlaque 2008). However, results obtained in this study has shown that the extremely low light levels within *P. oceanica* meadows can be below the minimum light requirements for *C. cylindracea* growth, surpassing its plastic capacity to acclimate to further light reductions. However, much more research must be done before attaining some robust conclusions about this topic. First of all, other factors could be involved, or interact with light availability, that should be investigated. In the case of this particular study, the substrate type was the same at both sides of the seagrass meadow edge (i.e. *P. oceanica* "matte"), and hence other kind of factors such as nutrients, sedimentation, water movement or space limitation should be considered. Secondly, given the experimental design used in this study, results obtained here must be corroborated with similar studies in other regions and using complementary experimental work in the field and in the laboratory. Furthermore, other basic aspects should be addressed, such as the potential of early recruitment phases (e.g. spores) to colonize the seagrass meadows, in addition to the acclimation capacity of the adult stages. Regardless the factors involved, the apparent ecological resistance of *P. oceanica* meadows seem to be linked to its complex canopy structure. Therefore, and considering that recovery of damaged *P. oceanica* meadows is a very slow process (Duarte et al. 2006), the conservation of its integrity against anthropogenic disturbances must be a priority of environmental policies concerned with the control of bioinvasions in the Mediterranean Sea, such as the Marine Strategy EU Directive or the Ecosystem Approach.

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