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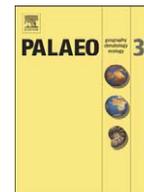
Long-term stability in the production of a NW Mediterranean *Posidonia oceanica* (L.) Delile meadow

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ABSTRACT

Long-term changes in leaf net production of the seagrass *Posidonia oceanica* (L.) Delile, and in the irradiance arriving at the meadow canopy, were investigated in a NW Mediterranean meadow using a palaeoecological approach. We conducted *in situ* shade experiments to find the relationships between the carbon stable isotope ratio ($\delta^{13}C$) of plant tissues, leaf net production, and ambient irradiance. The relationships were highly significant and fitted the Michaelis–Menten equation and power functions. These functions were used to reconstruct light and net production using the $\delta^{13}C$ of *P. oceanica* detritus as a palaeoecological proxy along a 150 cm core of *P. oceanica* matte (a peat-like deposit formed by this endemic seagrass species). The $\delta^{13}C$ values of sheath detritus along the core (i.e., the net leaf production) showed a weak but significant decrease towards the present time ($R = 0.308$; $P = 0.02$) probably as a result of (1) an increase in DIC availability, and/or (2) a progressive change in the carbon isotopic signature of DIC, both of which are consistent with a rise in anthropogenic atmospheric CO_2. The canopy irradiance, reconstructed for the last 1200 years, showed a mean value of 128 $\mu E \, m^{-2} \, s^{-1}$ with a range of 12.5–280 $\mu E \, m^{-2} \, s^{-1}$. The reconstruction of the net leaf production for the same period yielded a mean value of 2.5 mg dw day^{-1} shoot^{-1} with a range of 2.0–2.7 mg dw day^{-1} shoot^{-1}. Both ranges are within the values reported in the literature for present day studies. The absence of significant fluctuations or sudden changes through time suggests remarkable ecosystem stability during the last millennium.

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1. Introduction

Seagrass beds are among the most productive and complex oceanic ecosystems (McRoy and McMillan, 1977). This high productivity and complexity is the result of an overall annual positive carbon balance at the ecosystem level that renders a substantial surplus of energy available to feed a high diversity of communities (Cebrián, 2002; Mateo et al., 2006). Perturbations, either natural or anthropogenic, at local or regional levels, either strong and episodic or weaker but persistent in time, can potentially compromise meadow gains leading to meadow decline or disappearance (Marbà et al., 1996). This is particularly critical for climax, slow-growing seagrass species such as *Posidonia oceanica*. Therefore elucidating the range and time scales of variability in climax seagrass ecosystem productivity, the frequency and magnitude of perturbations, and species capacity to maintain an overall stability or to recover from perturbations, should constitute a priority for ecologists and environmental managers when elaborating conservation action plans for seagrass-dominated ecosystems (Short and Wyllie-Echeverria, 1996). Because these processes and phenomena take place at a very long time scale, the study of seagrass-dominated ecosystems is difficult and usually has to rely on relatively short time-series of variables and models (Duarte et al., 20062).

Palaeoenvironmental reconstructions are of interest as they provide data about longer-term ecosystem dynamics such as natural fluctuations and succession (Indermuhle et al., 1999; Petit et al., 1999; Arnand, 2000). At the shorter temporal scale of centuries, these studies have useful applications, especially within the framework of recent global environmental change (Crutzen and Stoermer, 2000). In fact, the palaeoecological approach can be considered a short-cut to fulfilling some of the objectives of costly monitoring plans (i.e. identifying general trends and potential future scenarios). The possibility of creating such reconstructions critically depends upon the availability of adequate palaeo-records and proxies.

Records may be abiotic (lacustrine or oceanic sediments, stalagmites and icecaps), biotic (peat bogs, corals, tree-rings and shells) or a mix of these two. Their quality usually depends on adequate long-term preservation of their chronological and chemical structure after formation. Such conditions are rarely encountered in temperate coastal ecosystems, even in well-protected bays, because of the characteristic tension at the interface between marine and terrestrial systems (hydrodynamism and bioturbation). One of the exceptions is the peat-like sediments accumulated by the endemic Mediterranean seagrass *Posidonia oceanica* (L.) Delile. These sediments, known as "mat" or "matte", constitute a unique bio-construction formed by debris from the

belowground organs of the plant (sheaths and roots). Carbon dating has revealed that they span several thousand years (Boudouresque et al., 1980; Romero et al., 1994; Lo Iacono et al., 2008) and that they accurately record chronology of deposition (Mateo et al., 1997; Mateo et al., 2002). Hence, the detailed analysis of cores from these deposits may provide a new way to study local and regional broad changes in key environmental and biological variables during the Holocene.

Palaeoecological transfer functions are empirically derived equations for making quantitative estimates of past environmental conditions from palaeontological data (Sachs et al., 1977). They are generally obtained by calibration against datasets of extensive modern sample sets (Birks, 1998; Sejrup et al., 2004). However, when the gradient of data sampled under natural conditions is not wide enough, or when the relationship between past environment and palaeontologial data is poor, it is necessary to take an experimental approach (Tilman et al., 1994).

The recognised relationship between carbon stable isotopes and primary production (Craig, 1954a; Park and Epstein, 1960; O'Leary, 1981; Farquhar et al., 1982), together with its conservative nature through time, has allowed its use as a proxy of past production in planktonic organisms (Rau et al., 1989; Singer and Shemesh, 1995), corals (Knutson et al., 1972; Erez, 1978; Reynaud-Vaganay et al., 2001) and trees (Craig, 1954b; Pearman et al., 1976; Francey and Farquhar, 1982; McCornac et al., 1994; Trimborn et al., 1995). To our knowledge, only one attempt has been made to use $\delta^{13}C$ to link seagrass ecology to palaeoecology at the decadal scale (i.e., lepidochronology; Mateo et al., 2000) with no information available for longer temporal scales.

The central hypothesis of this work is that the climax seagrass *P. oceanica*, through a high dynamism that combines processes that operate at various spatial and temporal scales, and in the absence of severe catastrophic natural or human-induced disturbances, can maintain one of its key processes, i.e. primary production, rather stable during centuries or millennia. The present paper reports a first attempt to couple experimental field ecology and palaeoecology for seagrass ecosystems. *In situ* shading experiments and isotope analysis have been used to calibrate a palaeoecological proxy trough the development of transfer functions that, in turn, allowed the reconstruction of changes in light reaching the canopy and the net leaf production of *P. oceanica* over the last thousand years. Despite of this mechanistic approach, at this stage our work does not aim at providing a detailed quantitative description of how were those parameters in the past. We propose that *P. oceanica* mattes hold information susceptible to palaeo-reconstruction methods which, by outlining past scenarios, may provide key knowledge to better understand present and future climate and ecosystem dynamics. Although our work is focussed on a Mediterranean seagrass, the principles unravelled from this species may be valid for other low-turnover seagrass species of the world accumulating refractory organic matter.

2. Materials and methods

2.1. Sampling site

A 150 cm core of *P. oceanica* matte was obtained in the summer of 2000 in Portlligat Bay, Natural Parc of Cap de Creus (NW Mediterranean, Girona, Spain; Fig. 1). From ortho-photo images of the bay (316-82 and 316-83, 1:5000, issued by the Institut Cartogràfic de Catalunya) and direct observations using SCUBA it has been estimated that the bottom of the bay is ca. 68% covered in by living *P. oceanica* meadows (ca. 9 ha) with shoot densities ranging from 100 to 900 shoots m^{-2}; dead *P. oceanica* matte is found in small areas adding up around 4% of the bottom of the bay (ca. 5 ha). In some areas
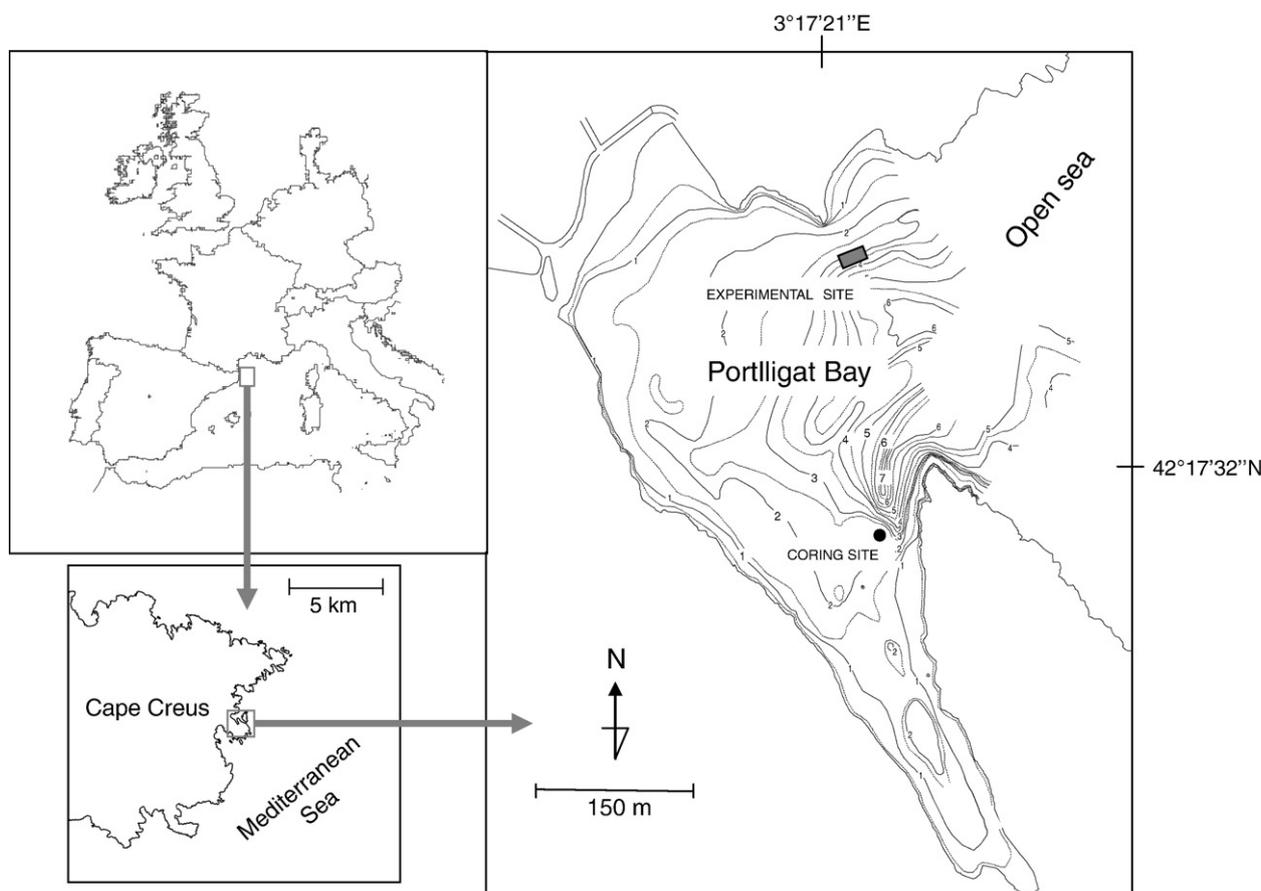

Fig. 1. Location of the study area, Portlligat Bay, Girona, Northwestern Mediterranean. The experimental area occupied around 200 m². Plots were situated at 3.5–4.5 m depth.

of the bay, the bottom is irregular and presents several forms of erosion that evidence thick *P. oceanica* mattes (up to 2 m).

In the area where the core was sampled, the meadow grows at ca. 3 m of depth and presents between 350 and 650 shoots m^{-2}.

2.2. Field works

2.2.1. Matte coring

A light floating drilling rig (Eijkelkamp) was placed over the selected point and safely anchored to the bottom. A stainless steel barrel 105 mm (outer diameter), 150 cm long was pushed down into the matte by means of a roto-percutor self-powered pneumatic hammer (Cobra, Atlas-Copco). The barrel accommodated an 88 mm (inner diameter), 150 mm long PVC pipe. The effective size of the mat core obtained using the coring system described above was 156×8.5 cm (length×diameter). The core was transported the same day to the laboratory and stored in the darkness at 5 °C before processing.

2.2.2. Field experiments

We exposed various plots of *P. oceanica* at the study site to different light regimes by placing in situ 1.5×1.5 m screens made of a commercial polyester net with a known percentage of light filtration (Polysack Europa, S.L.) mounted on PVC-pipe frames. The screens were maintained at around 50 cm above the bottom. Four irradiance levels were established: control (no net), 50%, 70%, and 99% shading. In situ light measurements under the screens were made to check for effective shading using a spherical underwater quantum sensor (Li-Cor). Three screens for each treatment (excluding controls) were placed at random over the meadow during 4 months (March–June 2004; Fig. 1). All screens were brush-cleaned 2 or 3 times a month to eliminate fouling. The average light reaching the canopy was recorded every 10 min by placing one underwater light logger (StowAway LI, Onset Computer Corporation) in the centre of one plot of each light condition. Loggers were either cleaned or replaced every two weeks by SCUBA diving to prevent bio-fouling and for data downloading. Footcandles were transformed into photon flux density units (μmol photons m^{-2} s^{-1}) applying an empirical regression using data obtained from simultaneous records of StowAway and Li-Cor (underwater PAR spherical quantum sensor) placed aside at the experimental site during three different periods during the course of previous works (Renom et al, in preparation): 29–30th of November 2000, 10–11th of January 2001 and 2–4th of April 2001 ($n = 567$ simultaneous readings; $r^2 = 0.95$). Net leaf production (obtained as in Zieman, 1974; $N=8$–12 per plot) was measured at increasing time intervals (weekly, fortnightly and monthly) during the course of the experiment. Plants were sampled near the centre of the plot to avoid any border effects.

Water temperature in Portlligat Bay was recorded at around midday at 3 m depth using 3 mercury thermometers between March 2004 and March 2005 with variable periodicity (Fig. 2).

2.3. Laboratory procedures

The first section of the core was cut into 1 cm slices and 62 of these samples (approximately every other cm) were used for processing. *P. oceanica* sheath debris >1 mm were selected from the bulk sample and oven-dried at 70 °C until constant weight.

The age of the matte was determined in 5 sheath samples using accelerator mass spectrometry (AMS) at the National Ocean Sciences AMS Facility (Woods Hole Oceanographic Institution, USA) following standard procedures (Stuiver and Polach, 1977; Stuiver and Braziunas, 1993; Table 1).

A correction for the marine reservoir effect of the carbon dissolved in marine waters was made considering the intersection of the fitted linear function with the *x*-axes of the uncorrected Age vs. Depth plot
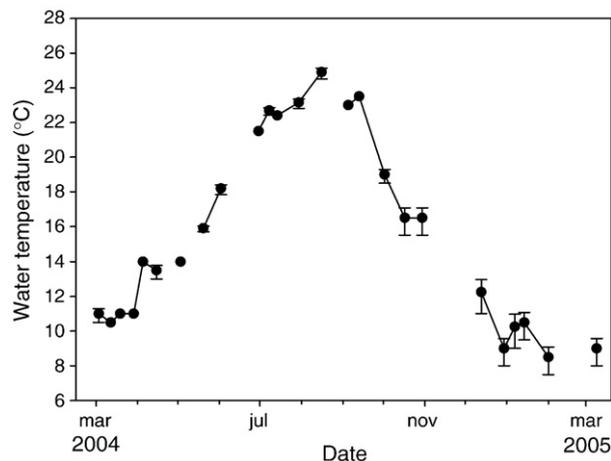

Fig. 2. Seasonal water temperature changes in Portlligat Bay during 2004. Error bars represent the standard error of the mean ($N=3$).

as the regionally-specific age offset, i.e., subtracting 313 years to raw laboratory radiocarbon ages.

Elemental and isotopic composition was determined for the gases evolved from a single combustion using a Finnigan Delta S isotope ratio mass spectrometer (Conflo II interface) at the Scientific–Technical Services of the University of Barcelona. Isotopic values are reported in the δ_{VPDB} notation relative to the standards Vienna Pee Dee Belemnite ($\delta_{sample} = 1000[(R_{sample}/R_{standard}) - 1]$, $R = {}^{13}C/{}^{12}C$). Analytical precision based on the standard deviation of internal standards (atropine, IAEA CH3, CH6, CH7, and USGS40—analytical grade L-glutamic acid) ranged from 0.11‰ and 0.06‰ (mean = 0.09‰).

2.4. Numerical procedures

Prior to the analyses, data were tested for normality (Kolmogorov–Smirnov test) and Cochran's *C* test was used to check for the assumptions of homogeneity of variance. Non-linear regressions were used to explore the relationship between $\delta^{13}C$ and (1) light at canopy, and (2) net leaf production. We adopted an inverse palaeoecological approach (Birks, 1998), by regressing the biological variable over the environmental variable. The isotopic values of the living sheaths at the end of the experimental shading period were used in the regressions. Average values of leaf production and light at canopy from the entire period were used for regressions against sheath $\delta^{13}C$ values.

Fitting of the variables to non-linear models was achieved using the Quasi-Newton minimal squares method. Two-way ANOVA was used to test differences in $\delta^{13}C$, carbon content, and net leaf production as affected by light and sampling date. The resulting fitted functions were used to infer past meadow light environments and plant leaf production. All statistical procedures were performed using STATISTICA (Kernel release 5.5, '99 Edition, STATSOFT, Inc. OK, USA).

Table 1
Details on radiocarbon dating of the *P. oceanica* sheath samples from the core. The accession laboratory sample number assigned by NOSAMS is indicated. The reservoir effect (RE) affecting the ages was estimated in 313 years. BP stands for 'before present'.

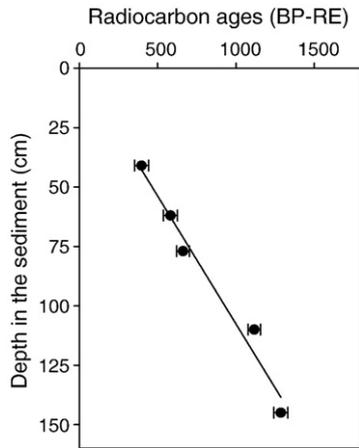| Depth (cm)	NOSAMS #	Raw age (y BP)	Age error +/−	Corrected age (y BP-RE)
41	OS-29665	710	45	397
62	OS-29666	895	45	582
77	OS-29667	975	40	662
110	OS-29668	1430	40	1117
145	OS-29651	1600	45	1287

Fig. 3. *Posidonia oceanica* sheath ages along the matte core (as years BP corrected for the reservoir effect, RE). Equation fitted: $y = 0.107 \times - 33.685$; adjusted $R^2 = 0.993$; $N = 5$.

3. Results

3.1. Matte age

Radiocarbon dating revealed a continuous organic record formed as a consequence of ca. 1200 years of matte accumulation (Fig. 3). The ages and the stratigraphic position of the samples allowed determining the matte formation rate (seafloor elevation) in 1.1 mm y^{-1} ($y = 0.107 \times - 33.685$; adjusted $R^2 = 0.993$; $N = 5$).

3.2. Shading experiments

In situ light measurements revealed important discrepancies with respect to the expected light filtration efficiency of the shading screens. Effective filtration was actually 80 and 90% for the 50% and 70% screens respectively, probably as a result of algal growth on the screens. A clear increment in the incident light was recorded after mid-March, especially in the controls and to a lesser degree in the rest of the treatments (Fig. 4).

The evolution of net leaf production reflected the changes in incident light (Fig. 5 top). The carbon content in living sheaths did not show any marked seasonal pattern throughout the experiment (Fig. 5 middle). The overall mean value was 38% with a range of 29–46%; the highest values corresponded to the sheaths growing in the control plots. The carbon isotopic composition of living sheaths progressively and significantly increased following light increase with the season in the control plots (Fig. 5 bottom). Conversely, the δ^{13}C of sheaths from the shaded plots became increasingly negative. Both sheaths from shaded and control plots did not begin to show significant differences in δ^{13}C values but 40–50 days after the beginning of the experiment.

There were significant differences in sheath δ^{13}C, carbon content and net leaf production, which were affected by both shading treatment and exposure time. Except for carbon content, the other two variables showed different trends in each shading treatment; this determined the significant interaction between the factors time and shading. The main source of variance for δ^{13}C and net leaf production
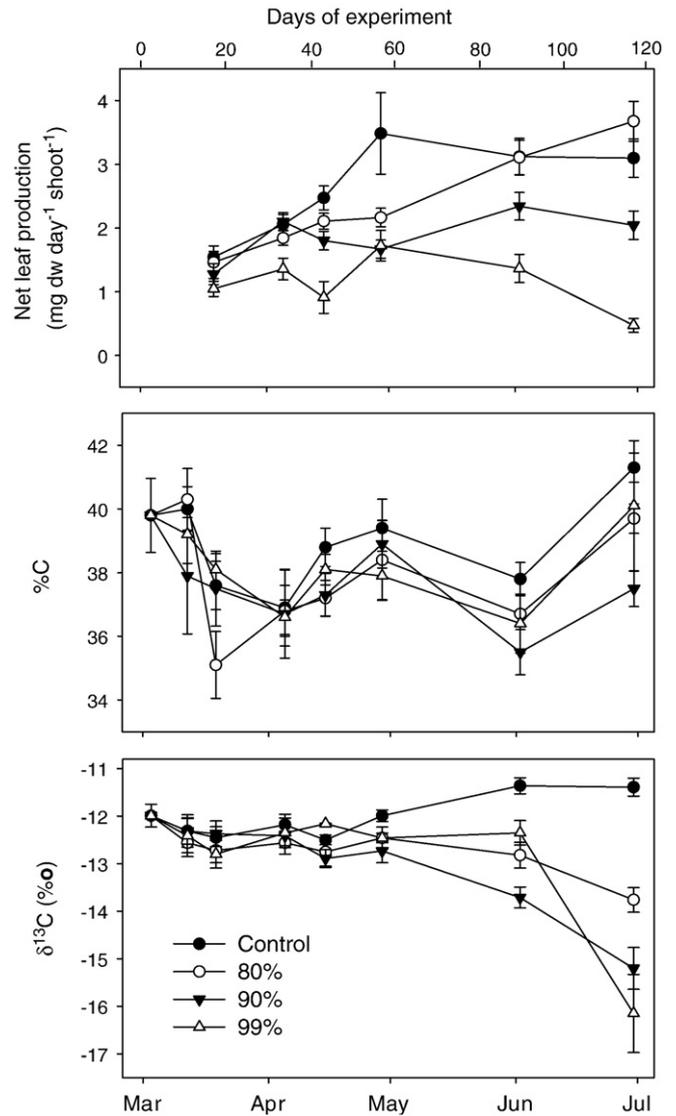

Fig. 5. Evolution of net leaf production (top), % C (middle), and δ^{13}C values (bottom) of living sheaths throughout the experiment under the four shading treatments. Error bars represent the standard error of the mean (net prod. $N = 15$–32; % C and δ^{13}C $N = 2$–9).

was the shading treatment while for carbon content it was time, as revealed by the MS values (Table 2).

Net leaf production fitted Michaelis–Menten dynamics well when related to light at canopy ($r = 0.941$; $n = 4$; Fig. 5 top). The relationship between sheath carbon isotopic ratios and average light at canopy was well described by a power function ($r = 0.996$; $n = 4$; Fig. 5 middle), whereas the relationship between δ^{13}C and average net leaf production fit the Michaelis–Menten function ($r = 0.932$; $n = 4$; Fig. 5 bottom).
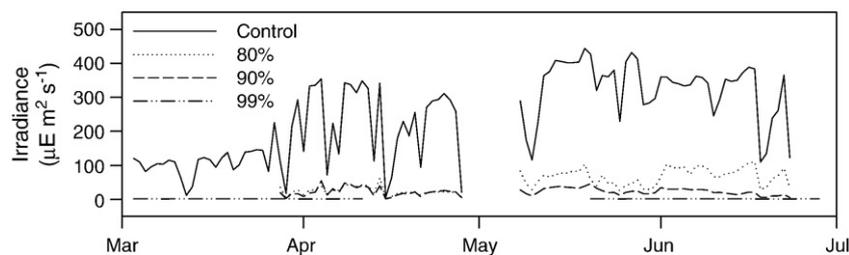

Fig. 4. Daily average irradiance recorded at the canopy under the four shading treatments.

Table 2
Two-way ANOVA of δ^{13}C, carbon content and net leaf production in shading treatments with time. Shading: 0, 80, 90 and 99%; Time: 8, 15, 32, 42, 56, 90, and 118 days after starting the experiment. ns: non-significant; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Variable	Factor	df	MS	F	P
δ^{13}C (‰)	Shading (Sh)	3	13.222	22.674	***
	Time (t)	6	10.569	18.124	***
	Sh*t	18	4.408	7.559	***
	Error	200	0.583		
Carbon content (%)	Shading (Sh)	3	24.380	3.441	**
	Time (t)	6	46.617	6.579	***
	Sh*t	18	5.801	0.819	ns
	Error	200	7.086		
Net leaf production (mg dw day^{-1} shoot^{-1})	Shading (Sh)	3	67.723	42.528	***
	Time (t)	5	18.977	11.917	***
	Sh*t	15	7.604	4.775	***
	Error	634	1.592		

The power fitting yielded better regression coefficients than any other model and matched the expected patterns better: (1) small variations in light at the canopy near its lowest range causes noticeable differences in productivity, and also in the isotopic signal, in relation to mid or upper range variations and (2) the isotopic values tend to approach a maximum in spite of further increases in incident light (Grice et al., 1996; Mateo et al., 2000). We used a Michaelis–Menten model for describing the observed relationship between δ^{13}C and net leaf production because physiological processes involved in carbon fixation (and thus carbon discrimination) are driven by enzyme-mediated reactions (especially RUBISCO) that can be well described by a Michaelis–Menten model (Farquhar et al., 1989).

The carbon content of sheath debris from the matte core was of the same order as that in the living material, showing a mean value of 39% and a range of 28–47%. A slight but significant net carbon gain was observed towards the present time ($R=0.378$; $P<0.005$; $N=57$). The carbon isotopic ratio of sheath debris fluctuated between -11.00 and -15.00‰ and showed a weak but significant depletion towards the present time ($R=0.309$; $P<0.05$; $N=58$; Fig. 7 top).

After applying the corresponding transfer function, the reconstructed light at canopy for the last 1200 years yielded a mean value of 128 µE m^{-2} s^{-1} (from 12.5 to 280 µE m^{-2} s^{-1}). The reconstructed net leaf production was 2.5 mg dw day^{-1} shoot^{-1} on average (range = 2.0–2.65 mg dw day^{-1} shoot^{-1}).

4. Discussion

Combining experimental theory and the carbon isotopic information stored in the millenary detritus of the *P. oceanica* matte of Portlligat, it was possible to reconstruct plausible scenarios of seagrass productivity, irradiance and C utilization over the last 1200 years. The palaeo-series reconstructed are strongly supportive of a time period of remarkable stasis. An increase in atmospheric CO_2 seemed to be reflected by a steady increase in the carbon fixed by the seagrass and by a decrease in its stable carbon isotopic signature.

4.1. Sheath δ^{13}C dynamics (field experiments)

Significant changes in leaf production and carbon isotopic ratios were observed only at 5 and 8 weeks, respectively, after the establishment of the shading treatments (Fig. 5 bottom). This suggests the existence of certain homeostatic mechanisms in the plant that make these two variables temporarily independent from external factors. A plausible hypothesis may be linked to the use of plant reserves stored during the previous season (Alcoverro et al., 2001). This would explain the observed absence of a significant response to light availability, and the constancy of the isotopic ratios (the plant would be using carbon from internal pools). A negligible metabolic carbon isotopic discrimination during transformation and transport of
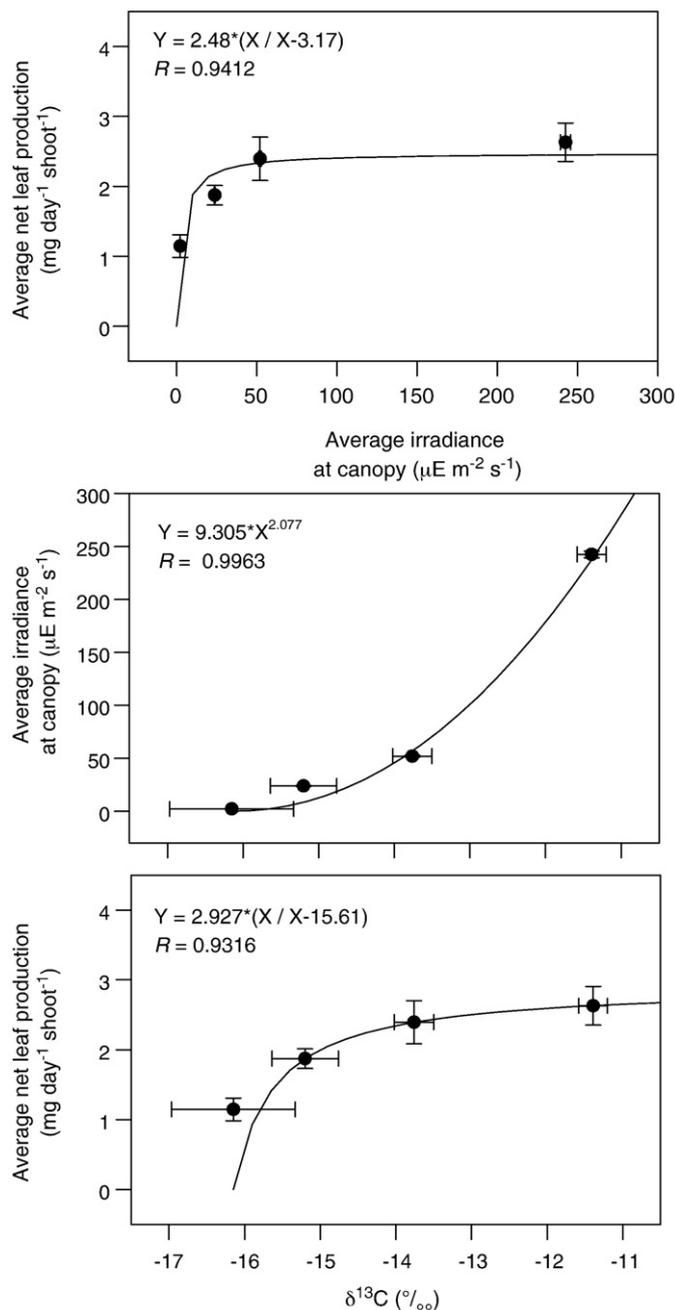

Fig. 6. Non-linear regression of average light at the canopy with average net leaf production (top), δ^{13}C of living sheaths with average light at the canopy (middle), and δ^{13}C of living sheaths with average net leaf production (bottom).

the stored materials should be assumed. In late spring, the exhaustion of reserves leads to the use of carbon from ambient pools (dissolved inorganic carbon, DIC). The photosynthetic demand of the plant increases in response to increasing irradiance resulting in higher net leaf productivity. The increased activity of the enzyme responsible for carboxylation (RUBISCO) may determine a lower discrimination against the heavy carbon isotope (Farquhar et al., 1989) and, therefore, increase the δ^{13}C values of the sheaths (Fig. 5 bottom).

However, complete information about δ^{13}C dynamics is still lacking. The results found on sheath carbon isotopic dynamics would probably reflect a complex model that would include the use of stored material, a gradual change from stored to ambient energy and finally the exclusive use of ambient pools of carbon. Additional experiments should be done at other times of the year to complete the model.
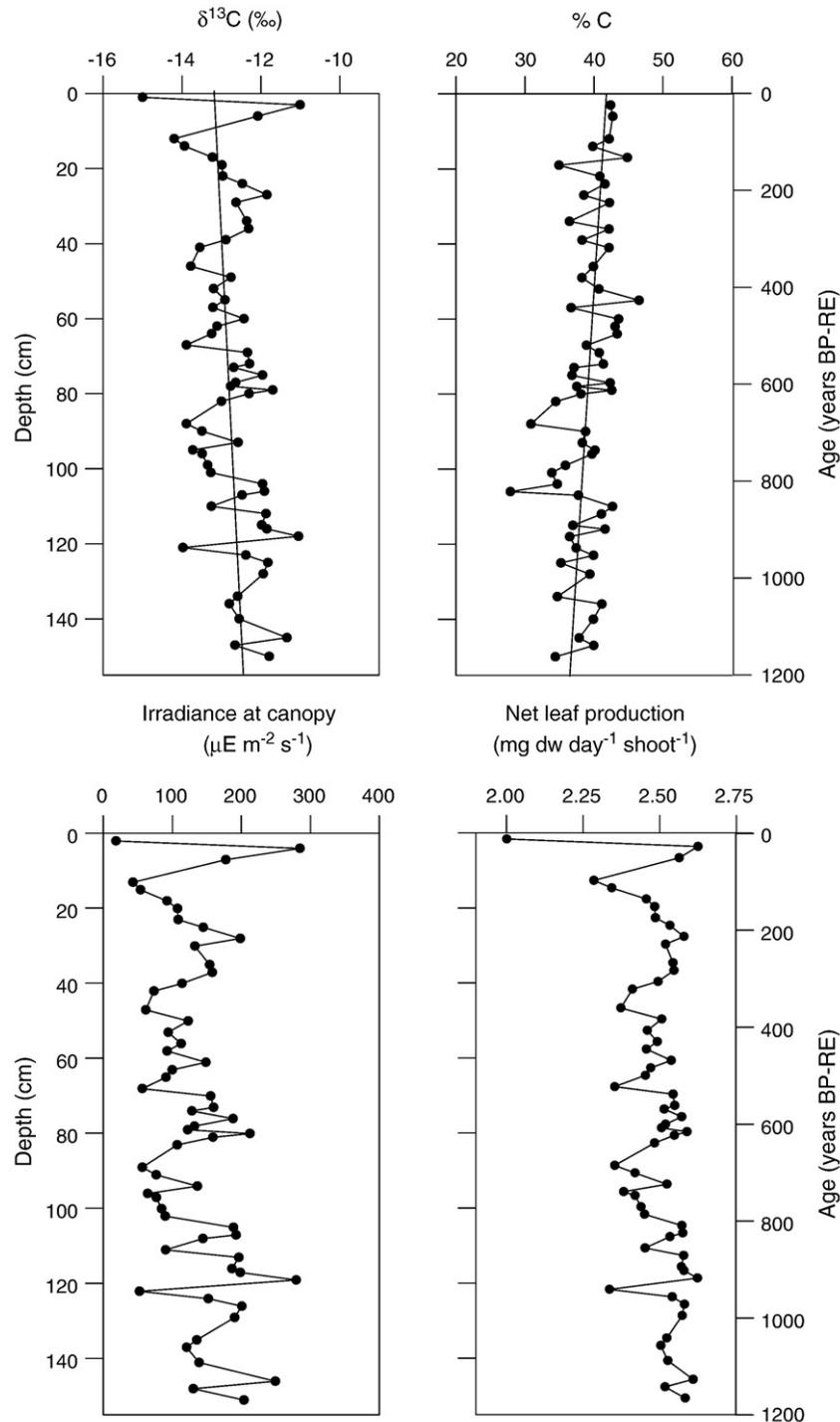
Fig. 7. Top: profiles of δ^{13}C and % C of sheath debris obtained along the core, fitted to linear regressions. Bottom: reconstructed profiles of light at the canopy and net leaf production along the core, using the transfer functions derived from the shading experiment (Fig. 6). Linear fittings: δ^{13}C, $Y = 0.01*X - 13.17$; $R = 0.31$; % C, $Y = -0.03*X + 41.58$; $R = 0.38$.

An effect of temperature on the final isotopic signatures used for the model should not be discarded. During the experimental period the plants experienced a ca. 8 °C range of temperature variation in water temperature. Although not directly addressed for aquatic plants (as far as we know), it is since long known that temperature plays a role in carbon isotopic fractionation as it controls the kinetics of enzymatic reaction rates at reaction sites (see e.g. Bernacchi et al, 2004 and references therein). So temperature may have affected the isotopic discrimination as it changed seasonally and across treatments (if shadowing of seagrass plots may have had an effect on the temperature at canopy level — not measured). Surprisingly, in their pioneering and key paper, Farquhar et

al. (1989) did not even mention temperature as a factor affecting plant isotopic composition and focused their attention in water availability, salinity, air pollution and irradiance. They found that the increase of this last factor is, in general, accompanied by an increase in δ^{13}C.

Hemminga and Mateo (1996) reviewed the role of temperature in the variability observed in the carbon isotopic composition of seagrasses. At that time the authors already identified a gap in knowledge on information concerning temperature effects on carbon isotopic composition of seagrasses. Since then, no significant information has been added. They reported that studies for plankton in the South Atlantic Ocean showed that changes in isotopic fractionation

were basically attributable to the effect of temperature on CO_2 solubility and, hence, concentration in seawater. DIC increases with decreasing temperatures, being ca. 2.5 times higher at 60°S than near the equator (Rau et al., 1989). A higher availability of DIC determines a higher isotopic discrimination and, hence, lower $\delta^{13}C$ values. In mesocosms experiments with the seagrass *Thalassia testudinum*, Durako and Sackett (1991) also observed that fractionation of stable carbon isotopes generally increased with increasing DIC. Consistently, Hemminga and Mateo (1996), found a $\delta^{13}C$ decrease in ca. 4‰ in a 60° of latitude increase (what represents a ca. 15 °C difference in annual sea surface mean temperature).

On the other hand, laboratory experiments with phytoplankton cultures failed to reproduce such patterns although much wider temperature ranges were used (Thompson and Calvert, 1994, 1995). Furthermore, macroalgae grown at a temperature range of 0 to 25 °C did not show continuous isotope changes (Wiencke and Fischer, 1990).

It is also to be mentioned that lower temperatures would tend to increase the $\delta^{13}C$ of DIC in seawater as discrimination against ^{13}C between atmospheric CO_2 and DIC in the seawater increases with falling temperatures (ca 1‰ per 10 °C; Mook et al., 1974; Zhang et al., 1995), due to diffusion-associated fractionation.

In our field experiments, taking place from the beginning of March to the end of June, temperature experienced a ca 8 °C increase (Fig. 2). Following the observations by Hemminga and Mateo (1996), this should be enough to explain a 2‰ increase in the carbon isotopic signature of *P. oceanica* sheaths in the control treatment. An additional 1‰ increase could be expected due to diffusion discrimination. The effective change, on the contrary, was a hardly 1‰ increase in the control plots and a decrease in the other ones. These results strongly suggest a dominant effect of irradiance instead of temperature to explain the observed trends. And the most plausible explanation for this is the simple fact that the shallow meadows at Portlligat are probably never limited by carbon and therefore changes in carbon availability do not make any difference for plant's carbon economy. Only the increase of the photosynthetic demand as irradiance increases towards summer has a significant effect on the final plant carbon isotopic composition through an increase in RUBISCO's activity.

So together with irradiance, the effect of temperature on dissolved CO_2 availability in seawater and on the RUBISCO activity, could be a potentially but largely unexplored relevant factor contributing to explaining some of the observed seasonal changes in the *Posidonia oceanica* carbon isotopic composition. Daylength may also play a role (Durako and Sackett, 1991). However we believe that our results are showing that irradiance is playing a dominant role. A detailed study on the contribution of each one of the above factors is, to the best of our knowledge, currently unknown and we propose it as an exciting field for further research.

Concerning the palaeo-reconstruction, where the issue of temperature would have the highest impact, it cannot be discarded that part of the reconstructed patterns of irradiance and productivity (Fig. 7 bottom) could be ascribed to the effect of water temperature on the carbon isotopic signature and therefore could be partially masking the real irradiance and productivity driven patterns. But as long as temperature has been recognised not to have varied substantially during the period reconstructed (Holocene stasis; see further), and that the meadow may not have been carbon limited during that period, it seems reasonable to consider that the reconstructed productivity scenarios based upon an irradiance-based model, may be meaningful. Further experimental and field research is required to specifically address the combined effect of temperature, DIC availability, productivity and isotopic composition of seagrasses. Such a research would be most useful to improve the goodness of the reconstructed palaeo-series of productivity and irradiance.

The range of $\delta^{13}C$ variation at the end of the experiment measured between the two extreme treatments (control and 99% shading) was approximately 5‰. Depth gradients of 40 m have been shown to be necessary to represent the same range of variation (Cooper and DeNiro, 1989). Other studies have reported wider natural ranges of $\delta^{13}C$ variation for *P. oceanica* up to 8.9‰ (Lepoint et al., 2003) associated with depth. Compiling data of different works from shallow (1.5 m) to deep meadows (20–32 m), the range may extend up to 10.9‰, including the maximum (-8.8‰; Vizzini et al., 2003) and the minimum (-19.7‰; Lepoint et al., 2003) $\delta^{13}C$ values ever recorded. In our experiments, the lowest $\delta^{13}C$ value was -17‰, recorded at the 99% shading treatment. This is a direct consequence of the very small photosynthetic demand experienced by the plants during the experiment. These light conditions led to the death of more than half of the initial number of shoots (control plots: 390 ± 54 shoots m^{-2}; 99% shading plots: 161 ± 35 shoots m^{-2}). The $\delta^{13}C$ average value for plant sheaths from the 90% shading treatment was -15‰. From values reported in the literature and from our results, it seems reasonable that $\delta^{13}C$ values below -14‰ are to be considered indicative of a poor carbon balance condition. Control plot $\delta^{13}C$ values approached -11‰. The results of the two extreme light treatments are comparable to light conditions recorded for shallow and deep meadows and are very likely to correspond to the photosynthetic light conditions of saturation and compensation (or even beyond compensation), respectively. Therefore, our experimental light range was likely wide enough to cover most of the possible natural irradiance levels, production, and $\delta^{13}C$ ranges. This justifies the use of the relationships between them for palaeo-reconstruction purposes.

4.2. Palaeoecological reconstruction

Alterations in the accretion dynamics of *P. oceanica* mattes by erosion and re-deposition of layers or by changes in the sedimentary regime of the area may result in abrupt or incoherent changes in sample age and isotopic values, which hampers reconstruction efforts. Because the matte core used here did not present any chronological incoherence, from this point of view we consider the present palaeo-reconstruction a very robust one. The $\delta^{13}C$ decrease shown by sheath debris towards the present time may be explained by a progressive change in the trade-off between availability of and demand for carbon by the plant or by an equally progressive change in the carbon isotopic signature of the carbon source. The first hypothesis could be ascribed to either an increase of carbon availability or a reduction in carbon demand as a consequence of light reduction, and/or nutrient limitation. We compared the time-series of the $\delta^{13}C$ values for the *P. oceanica* sheath debris from the Portlligat core with some available time-series of total solar radiation. For series encompassing the last four centuries (Lean et al., 1995; Lean and Rind, 1999; Solanki and Fligge, 2000), we found no concordance with our data. When compared to a time-series obtained by Bard et al. (2000) for the total solar radiation arriving at the earth during the last one thousand years, (http://www.cerege.fr/), we found no correlation. The comprehensiveness of the time-series provided by Bard et al. (2000) allowed us to perform some more detailed statistical analysis. We performed a conventional correlation and a scale-dependent correlation analysis [SDC] (Rodríguez-Arias and Rodó, 2004) between both time-series (total solar radiation and $\delta^{13}C$ of sheath debris). The SDC is an improvement of the floating window correlation analysis that calculates the correlation between all possible synchronous and shifted pairs of segments of a given length extracted from two time-series. However, neither the correlation nor SDC showed any significant link between solar radiation and the carbon isotope signatures along the core.

A sustained increase in water turbidity leading to local light reduction is not likely to have taken place during the last millennium. Water turbidity depends on the concentration of suspended particles and nutrients supplied by river input, and therefore, a complex relationship exists between precipitation, soil composition and, for most of the Holocene (Ruddiman, 2003), human activity. Precipitation and human activity were not significant factors during the last millennium (Burjachs et al., 2005; Parra et al., 2005).

A sustained increase in water turbidity at the regional scale is not likely to have occurred. The hydrological regime of the river Rhône, the largest river of the NW Mediterranean, which influences NE Spanish coasts, does not show any increase in turbidity for the past thousands of years (Arnaud-Fassetta et al., 2000; Teles et al., 2001). Despite the uncertainty of long-term trends in nutrient dynamics, it is widely recognised, at least for the last four decades, that the Mediterranean Sea is undergoing a process of eutrophication that could have significant effects on $\delta^{13}C$. A weak eutrophication would stimulate plant production (theoretically leading to ^{13}C enrichment), while an intense eutrophication would stimulate planktonic production, reducing both water transparency and benthic plant production (leading to ^{13}C depletion). Finally, we have not considered the palaeomeadow depth. However, if the ancient meadow grew deeper in the past than at present, less light would have reached the plants and $\delta^{13}C$ would have been more negative than it is presently. The evolution of $\delta^{13}C$ shows just the opposite tendency (Fig. 7 top). This tendency towards more $\delta^{13}C$-depleted plant tissues would have probably been more accentuated if the palaeo-meadow depth had exerted a significant effect.

As a consequence, the observed trend could reflect: (1) a progressive increase in the availability of carbon in marine waters (DIC) resulting from atmospheric CO_2 rise. The slight but significant increase in carbon content of sheath debris towards the present time could be taken as supporting evidence of this hypothesis. (2) A progressive change in the carbon isotopic signature of DIC. This could be the result of a decrease in atmospheric $\delta^{13}C$ and later, a decrease in marine $\delta^{13}C$ (Raven and Falkowski, 1999) derived from deforestation (Indermuhle et al., 1999), and more recently from the burning of fossil-fuel (the Suess effect; Keeling et al., 1979). The same interpretation has been given for similar tendencies observed in planktonic and benthic foraminifera records sampled from deep waters in the Eastern Mediterranean (Schilman et al., 2001a; Schilman et al., 2001b).

To what extent the carbon isotopic signature of *P. oceanica* tissues remains constant after plant death is another major question, which is still a matter of controversy since different research shows contrasting results (Dean et al., 1986; Sackett, 1986; Calvert et al., 1992; Fontugne and Calvert, 1992; Zieman et al., 1984). In light of this, we cannot discard that the temporal trend may be a consequence of a gradual diagenetic effect of aging on the isotopic ratios of *P. oceanica* sheath debris. The use of seagrass-specific recalcitrant compounds for isotopic analyses in further studies should be taken into consideration as an alternative to bulk material measurements (e.g., seagrass sheath lignin; Klap, 1997; Klap et al., 2000). Also, specific experiments addressing the long-term stability of the isotopic signatures of detrital material should be encouraged.

Changes in the patterns of terrestrial organic inputs into the Portlligat Bay could be a source of 'non-physiological' changes in plant isotopic signatures. This aspect was not considered in this work although we are conscious of its potential importance. In future studies, this potential "noise" in the isotopic signatures of the plants may be filtered to a greater extent by measuring the carbon isotopic signatures of biogenic carbonates (e.g., shells, foraminifera, ostracods, etc.) trapped in the matte. The $\delta^{13}C$ of these carbonates should reflect the DIC of the surrounding waters (Wefer and Berger, 1991).

The reconstructed light time-series at the canopy level for the last millennium, lies within the range of natural light levels currently measured in NW Mediterranean coastal waters (Renom et al, in preparation; Table 3). The range for the reconstructed production is also in good agreement with the values reported in the literature (Table 4). In those and other studies, the production variability for *P. oceanica* has been related specifically with light (Bay, 1984; Libes, 1986; Romero, 1989; Alcoverro et al., 1995; Dalla Via et al., 1998; Alcoverro et al., 1998; Ruiz and Romero, 2001; Olesen et al., 2002), temperature (Zupo et al., 1997), nutrients (Kraemer and Mazzella, 1996; Alcoverro

Table 3
Comparison of irradiance measured in the present study from March to June 2004 with irradiance measured with the same sensors in other localities of the Catalan Coast (NW Mediterranean) during the whole year 2000. Differences in sampling size (n) are caused by gaps in the record; SEM: standard error of the mean [data from Renom et al, in preparation].

	% of shading	Depth (m)	Daily average	Irradiance ($\mu E\ m^{-2}\ s^{-1}$) SEM	max	min	n
Portlligat	0	3.5–4.5	241.9	12.0	443.6	6.9	104
Portlligat	80	3.5–4.5	51.2	3.3	110.6	2.4	79
Portlligat	90	3.5–4.5	23.7	1.3	55.4	2.0	79
Portlligat	99	3.5–4.5	2.2	0.0	2.7	1.6	40
Medes Islands	0	5	113.9	5.2	374.9	8.0	266
Medes Islands	0	19	39.0	1.7	144.0	1.3	343
Mataró	0	19	24.7	1.1	87.2	0.5	364
Vilanova	0	19	20.7	1.01	125.2	0.2	366

et al., 1997; Alcoverro et al., 2000; Invers et al., 2002a), CO_2 (Invers et al., 2002b), salinity (Fernández-Torquemada and Sánchez-Lizaso, 2005), flowering episodes (Balestri and Vallerini, 2003), competition with macroalgae (Devillele and Verlaque, 1995; Dumay et al., 2002), competition with epiphytes (Alcoverro et al., 2004), herbivory (Cebrián et al., 1998; Tomas et al., 2005), terrigenous discharges (Guidetti and Fabiano, 2000), fish farming and coastal construction (Ruiz et al., 2001; Ruiz and Romero, 2003), urban and industrial pollution (Balestri et al., 2004) and cropping (Wittmann and Ott, 1982). Apart from light, a minor contribution to the variability in the reconstructed net leaf production may be attributable to most of the natural factors mentioned but unfortunately, there are no data available for such factors during the time period considered here. However, complementary palaeoecological approaches based on our organic record may be proposed for assessing some of the phenomena mentioned above. For instance, the pressure of herbivores could be partially estimated by the quantification of sea urchin populations through the presence of abundant spines and plates of sea urchins along the core. Similarly, detailed granulometric analysis could provide information on the intensity and the frequency of terrigenous discharges. As well, the $\delta^{18}O$ and $\delta^{13}C$ of shells and the $\delta^{15}N$ of sheath debris may provide information on the evolution of temperature, salinity and nutrients through time.

As derived from matte accretion values, the theoretical temporal resolution of this palaeo-reconstruction is 9.3 y cm^{-1}. But the overall error associated to AMS radiocarbon dating (± 45 years) and the variability of accretion rates along the core sets the range of such resolution from ca. 5 to 150 years. This inherent resolution limitation of many palaeo-records together with the uncertainties around the goodness of the proxy used, have to be taken into account when interpreting the palaeo-series reconstructed here. The series of light and production should only be considered as a palaeoecological supporting evidence of a certain overall environmental and biological stability in the *P. oceanica* ecosystem during the last millennium and of the lack of clear and/or marked trends. Hence, we believe that the small changes along the reconstructed series are probably meaningless and should not be interpreted as light-driven effects. The scenario provided by the matte record is in accordance with the known stasis of the recent Holocene in the Mediterranean. This period is characterised by high climatic stability, especially when compared with the Pleistocene (Kallel et al., 2004; Sbaffi et al., 2004), as revealed by several regional studies using dendrochronology (Creus et al., 1996), palinology (Riera et al., 2004; Burjachs et al., 2005; Parra et al., 2005), anthracology (Terral and Mengüal, 1999; Burjachs et al., 2005) and historic climatology (Barriendos and Martin-Vide, 1998; Camuffo et al., 2000; Barriendos and Llasat, 2003).

We have reported here a first attempt in reconstructing long-term time-series of biological and environmental variables in a seagrass-dominated ecosystem using the exceptional organic record formed by *P.*

Table 4

Compilation of net leaf production for *Posidonia oceanica* found in the literature, chronologically ordered. Some units were transformed from elongation (mm or cm^2) into dry weight (mg) using wide spread equivalencies. Maximal value from Bay (1984) was excluded when computing averages since considered an out layer. The range of production does not necessarily correspond to the range of depth.

Leaf production (mg dw shoot^{-1} day^{-1})	Depth (m)	Location	Method	Source
0.2–10.6	4	Ischia (Gulf of Naples, Italy)	Zieman	Ott (1980)
7.41–10.86	4	Ischia (Gulf of Naples, Italy)	Zieman	Wittmann and Ott (1982)
3.45–39.66	10 and 30	Calvi Bay (Corsica)	Tagging and harvesting	Bay (1984)
0.02–0.17	5 and 13	Medes Islands (NE Spain)	Zieman	Romero (1989)
1.4–5.6	0.5–6	Valencian Coast (E Spain)	Zieman	Esteban et al. (1990)
1.24–7.6	4, 12.5 and 19	Tabarca (Alicante, SE Spain)	Zieman and lepidochronology	Sánchez-Lizaso (1993)
0.85–4.22	0.7–32	Corsica, France, Italy, Sardinia and Turkey	Lepidochronology	Pergent-Martini et al. (1994)
3.15–4.67	5 and 13	Catalan Coast (NE Spain)	Zieman	Alcoverro et al. (1995)
1.02–6.84	5.0–10	Spanish Mediterranean Coast	Lepidochronology and plastochrone	Marbà et al. (1996)
3.43–3.94	5	Ischia (Gulf of Naples, Italy)	Zieman	Kraemer and Mazzella (1996)
1.0–4.0	22	Ischia (Gulf of Naples, Italy)	Zieman	Zupo et al. (1997)
1.84–3.01	10–18.0	Marseilles (France) and Ischia (Italy)	Lepidochronology	Pergent et al. (1997)
5.78	4	Cala Jonquet (NE Spain)	Lepidochronology and plastochrone	Cebrián et al. (1997)
2–7.9	5	Medes Islands (NE Spain)	Zieman	Alcoverro et al. (2001)
3.62–3.75	6–8.0	Ligurian Coast (NW Italy)	Lepidochronology and plastochrone	Guidetti (2000)
3.0–8.0	8	El Hornillo Bay (SE Spain)	Zieman	Ruiz et al. (2001)
3.0–7.5	8	El Hornillo Bay (SE Spain)	Zieman	Ruiz and Romero (2001)
2.75–6.60	5	Medes Islands (NE Spain)	Zieman	Invers et al. (2002a)
3.0–9.0	10	Levante Bay (SE Spain)	Zieman	Ruiz and Romero (2003)
2.52–5.70	5–6.0	Medes Islands (NE Spain)	Zieman	Tomas et al. (2005)
0.04–0.18	4–8.0	Alacant (SE Spain)	Zieman	Fernández-Torquemada and Sánchez-Lizaso (2005)
3.15	5	Portlligat (NE Spain)	Zieman	Alcoverro et al. (1995)
1.72	5	Portlligat (NE Spain)	Lepidochronology and plastochrone	Marbà et al. (1996)
1.89–2.66	0.7–15.6	Portlligat (NE Spain)	Plastochrone	Olesen et al. (2002)

Mean: 3.87 + 0.43
Range: 2.4–5.6

oceanica. Although this was not the case, it is presumable that other cores may present discontinuities or sharp changes which will be extremely useful for elucidating meadow key issues like the frequency of perturbations, the capacity for meadow recovery and recolonization, etc. The new approach presented here is likely to be applied to other *P. oceanica* meadows in the Mediterranean as well as to other matte forming seagrasses, such as the Australian relatives of the *Posidonia* genus (Shepherd and Sprigg, 1976), and to the tropical *Thalassodendron ciliatum* (Lipkin, 1979).

In a scenario of generalized decline of *P. oceanica* meadows in the Mediterranean, the unprecedented long-term perspective recorded in *P. oceanica* matte may represent a valuable contribution to help forecasting potential future changes of seagrass dynamics in a rapidly changing environment.

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