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A six thousand-year record of climate and land-use change from Mediterranean seagrass mats

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Summary

1. The Mediterranean seagrass *Posidonia oceanica* maintains a biodiverse ecosystem and it is a world-wide important carbon sink. It grows for millennia, accumulating organic-rich soils (mats) beneath the meadows. This marine habitat is protected by the European Union; however, it is declining rapidly due to coastal development. Understanding its response to disturbances could inform habitat restoration, but many environmental impacts predate monitoring programs (<50 years).

2. This research explores the palaeoecological potential of *Posidonia* mats to reconstruct six thousand years of environmental change that could have affected *Posidonia* meadows and, in turn, left an imprint on the mats.

3. Palynological, microcharcoal, magnetic susceptibility and glomalin-related soil protein (GRSP) analyses on *Posidonia* mats enabled us to detect climate- and human-induced environmental processes impacting on the seagrass during the Late Holocene.

4. The pollen and microcharcoal records reconstructed anthropogenic disturbances attributed to agriculture. The record of GRSP shows that agrarian activities affected continental soil quality. Changes in magnetic susceptibility reveal that enhanced soil erosion was caused by both climate (major flooding events in the NW Mediterranean) and humans (cultivation) which impacted on the *Posidonia* mat. Finally, increased human impact is linked to eutrophication of coastal waters since Roman-Medieval times.

5. *Synthesis*. This study shows that climate and land-use changes in the western Mediterranean resulted in enhanced loadings of terrigenous material to the coastal zone since the Late Holocene, likely disturbing the *Posidonia* meadows and their mat carbon accumulation dynamics. Under the current global change scenario in which CO_2 emissions are projected to increase, restoring carbon sinks is a priority. Seagrass habitat restoration should consider not only the coastal perturbations, but also the continental ones at a catchment scale to preserve the socio-economic ecosystem services provided by seagrasses.

Key-words: ecosystem services, glomalin-related soil protein, magnetic susceptibility, microcharcoal, palaeoecology and land-use history, palynology, *Posidonia oceanica*, soil erosion

Introduction

Seagrass meadows maintain high levels of biodiversity and they are among the world's most important carbon sinks (Duarte, Middelburg & Caraco 2005), but they are also experiencing a world-wide decline becoming one of the most threatened ecosystems. Seagrass losses have been estimated at 29% of their global extent since 1880 with an average net decline in area of 7% annually since 1990 (Waycott *et al.* 2009). As a result of the loss of seagrass meadows, emissions of carbon dioxide (CO_2) have been estimated to have increased by up to 25% compared to land deforestation (Fourqurean *et al.* 2012). Thus, understanding the anthropogenic disturbances affecting seagrass ecosystem's dynamics is critical to prevent their decline and to implement conservation

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strategies to maintain their carbon sequestration capacity (Greiner *et al.* 2013; Marbà *et al.* 2015).

Posidonia oceanica (L.) Delile is a seagrass species endemic to the Mediterranean Sea, ranking among the slowest growing and longest-lived plants (Arnaud-Haond *et al.* 2012). This species plays a major role as a carbon sink, in water oxygenation and biomass production, as well as providing a breeding habitat for marine animals, and consolidating coastal sediments (Pergent *et al.* 2012). *Posidonia oceanica* meadows are a priority conservation habitat by the Habitat Directive of the European Union. However, the meadows are declining rapidly at an estimated rate of 5% annually due to human pressure on coastal areas, causing biodiversity loss with major natural and economic consequences (Marbà, Díaz-Almela & Duarte 2014). The concern is that while *P. oceanica* meadows have thrived for million years, their current rate of decline suggests they may no longer be able to adapt to the unprecedented rate of global change (Marbà & Duarte 2010). Research on the long-term dynamics of seagrass ecosystems in response to environmental threats is in its infancy, but it could provide a unique opportunity and a powerful tool to understand natural cycles and trends, reconstruct baselines, resilience and thresholds, and predict future responses to anthropogenic disturbances (Willis *et al.* 2007). This type of long-term ecological research could inform conservation strategies for maintaining seagrasses resilience (Serrano *et al.* 2016a).

Species of the genus *Posidonia* create mats which can be thousands of years old which form suitable sedimentary archives for a palaeoecological study (Mateo *et al.* 1997; Serrano *et al.* 2012). The study of trace metals in a ~4500 years old *P. oceanica* mat sediment core from the Portlligat Bay (NW Mediterranean Sea) revealed that metal fluxes peaked during Greek and Roman times, as well as more recently – a trend that concurs with other Iberian records (Serrano *et al.* 2011, 2013). The palynological study of the topmost part of the same core reconstructed the last twelve centuries of landscape change and showed land-use changes, the timing of which closely matches with other NE Iberian records (López-Sáez *et al.* 2009). López-Merino *et al.* (2015) compared the reconstructed land-use changes with the record of glomalin-related soil protein (GRSP), suggesting that the GRSP accumulated in the anoxic conditions of *Posidonia* mats can be used to unravel long-term trends in continental soil quality. A study of *Posidonia australis* Hook.f. mats from Oyster Harbour (SW Australia) reconstructed centennial metal fluxes (Serrano *et al.* 2016b) and provided information about the trajectories of estuarine ecosystems and associated regime shifts due to anthropogenic pressures since the European settlement in Australia (Serrano *et al.* 2016a). These studies demonstrate that the palaeoenvironmental information contained in *Posidonia* mats can be used to understand, predict and manage coastal ecosystems more comprehensively.

Therefore, the overarching goal of this research is to further evaluate the long-term ecological potential of *Posidonia* with the specific aim of identifying environmental stressors (climate- and/or human-induced) that have been archived in, and could have impacted on, the mat of *Posidonia*. Six millennia of environmental change have been reconstructed using a multi-proxy approach (palynological, microcharcoal, GRSP and magnetic susceptibility analyses) on *P. oceanica* mat cores from Portlligat Bay (Western Mediterranean).

Materials and methods

SETTING AND CORING

Portlligat Bay (42°17′ 32″ N; 3°17′ 28″ E) is a small (0·14 km^2) and shallow (<10 m deep) inlet located in Cape Creus (NE Spain, western Mediterranean; Fig. 1). The area has a Mediterranean climate, with mild winters and warm, dry summers. The annual precipitation ranges between 500 and 800 mm and mainly falls from October to December (Franquesa i Codinach 1995). The current landscape has been influenced by a long history of human activities, mostly related to farming (Franquesa i Codinach 1995). However, traditional dry-land farming and fisheries have recently been replaced by tourism, resulting in the abandonment of terraced vineyards (*Vitis vinifera* L.) and pasturelands. Currently, Mediterranean shrubland dominates the landscape and mainly comprises *Cistus monspeliensis* L., *Cistus albidus* L., *Lavandula stoechas* L., *Calicotome spinosa* (L.) Link, *Ulex parviflorus* Pourr, *Pistacia lentiscus* L., *Juniperus oxycedrus* L., *Arbutus unedo* L., *Quercus coccifera* L. and *Erica arborea* L. Forest cover is sparse: some small areas are covered by cork trees (*Quercus suber* L.) and pines (*Pinus halepensis* Miller, *Pinus pinea* L.), while *Quercus ilex* L. and *Quercus pubescens* Willd. occur in sheltered areas. In riparian areas, *Ulmus minor* Mill., *Fraxinus angustifolia* Vahl, *Alnus glutinosa* (L.) Gaertn., *Corylus avellana* L. and *Salix* sp. can be found (Franquesa i Codinach 1995).

The bay receives freshwater from a temporary stream that flows from its NE shore. *Posidonia oceanica* meadows cover about 68% of the bottom of the bay. A 498-cm long mat sediment core ('core 2006') was sampled in the central part of the bay (Fig. 1) in 2006, at a water depth of 3 m. The core was taken using a petrol drill and breaker (Combi Cobra, Atlas Copco) operated with a crane from a 10-m boat (see details in Serrano *et al.* 2012). The length of the core barrel inserted into the sedimentary deposit and the length of retrieved seagrass mat were recorded in order to correct the core lengths for compression effects and all variables studied here are referenced to the corrected, uncompressed depths. This new sediment core ('core 2006') is close to the already studied 'core 2000' (Fig. 1; López-Sáez *et al.* 2009; Mateo, Renom & Michener 2010; Serrano *et al.* 2011, 2012, 2013; López-Merino *et al.* 2015).

RADIOCARBON DATING AND AGE-DEPTH MODEL OF 'CORE 2006'

Thirteen samples from the 498-cm long *P. oceanica* mat core were radiocarbon dated by AMS at three different laboratories (NOSAMS Facility-Woods Hole, USA; Direct AMS-Radiocarbon Business Unit, Accium Biosciences, USA; and the ^{14}Chrono Dating Laboratory at Queen's University Belfast, UK). With the exception of the basal radiocarbon date that was performed on gastropod shells, the other radiocarbon dates were obtained from *P. oceanica* sheath remains (Table 1). The ^{14}C dates were calibrated using the marine13 radiocarbon age calibration curve (Reimer *et al.* 2013) taking into account a local Delta R of 23 ± 71 years (Siani *et al.* 2000) (Table 1). An

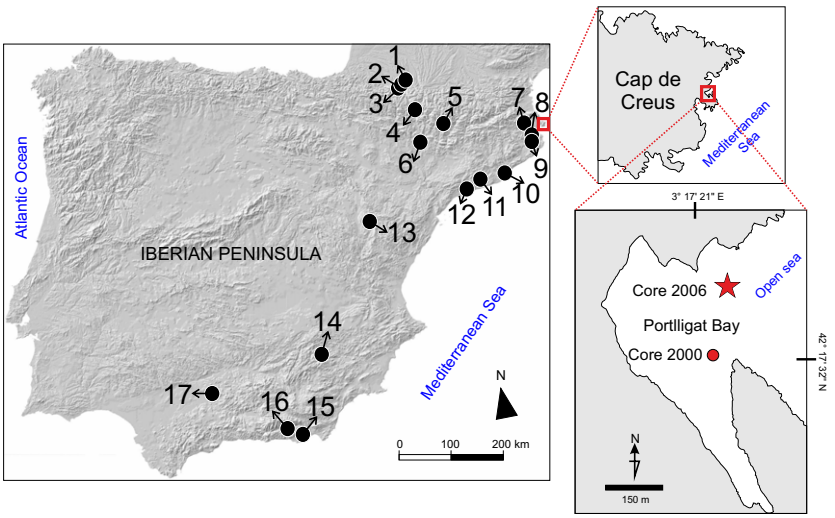


Fig. 1. Location of the drilling point of 'core 2006' (red star) in the *Posidonia oceanica* bed in the NW Mediterranean Sea (Portlligat Bay, Cape Creus, Spain). The location of the previously studied 'core 2000' (red dot; López-Sáez *et al.* 2009; López-Merino *et al.* 2015) and other palaeoenvironmental records cited in the text are also indicated: (1) Biscaye bog (Rius *et al.* 2011), (2) Lake Lourdes (Rius *et al.* 2011), (3) Col d'Ech (Rius, Vannière & Galop 2012), (4) Basa de la Mora (Pérez-Sanz *et al.* 2013), (5) Montcortès Lake (Rull *et al.* 2011), (6) Estanya Lake (Riera, Wansard & Julià 2004), (7) Castelló d'Empúries (Burjachs *et al.* 2005; Ejarque *et al.* 2016), (8) Sobrestany (Parra, van Campo & Otto 2005), (9) Ullastret (Riera & Esteban 1994), (10) Besós (Riera & Esteban 1994), (11) Cubelles (Riera & Esteban 1994), (12) Creixell (Burjachs & Schulte 2003), (13) Villarquemado (Aranbarri *et al.* 2014), (14) El Sabinar (Carrión *et al.* 2004), (15) San Rafael (Pantaleón-Cano *et al.* 2003), (16) Gádor (Carrión *et al.* 2003), (17) Zoñar Lake (Martín-Puertas *et al.* 2008). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1. Radiocarbon dates in the *Posidonia oceanica* mat sediment 'core 2006'

Laboratory code	Material type	Sample	Depth (cm)	AMS ^{14}C date BP	cal. yr BP (2σ range)*	Probability (%)	Median probability
OS-59949	*Posidonia* sheaths	I-20	23·84	645 ± 25	0–4	0·3	261
					60–434	99·7	
OS-59950	*Posidonia* sheaths	I-60	71·52	1150 ± 30	540–846	100	687
OS-59952	*Posidonia* sheaths	I-90	107·28	1710 ± 25	1064–1384	100	1241
OS-59954	*Posidonia* sheaths	I-126	150·19	2010 ± 65	1331–1782	100	1550
OS-59955	*Posidonia* sheaths	II-50	173·64	2220 ± 50	1566–1993	100	1792
OS-59956	*Posidonia* sheaths	II-100	234·43	2860 ± 30	2359–2739	100	2580
OS-59953	*Posidonia* sheaths	II-156	301·19	3550 ± 30	3218–3602	100	3416
UBA-25344	*Posidonia* sheaths	III-119	329·06	3825 ± 29	3552–3957	100	3747
OS-59981	*Posidonia* sheaths	III-156	379·26	4320 ± 35	4166–4651	99·9	4419
					4669–4671	0·1	
D-AMS 009118	*Posidonia* sheaths	IV-98	413·6	4638 ± 29	4592–5041	100	4836
D-AMS 009119	*Posidonia* sheaths	IV-113	436·37	5017 ± 30	5117–5555	100	5349
UBA-25345	*Posidonia* sheaths	IV-123	450·6	5087 ± 31	5266–5581	100	5418
OS-59925	Gastropod shells	IV-126	497·56	5370 ± 30	5574–5886	100	5717

*Calibration dataset: marine13.14C, Delta R = 23 ± 71 years.

age-depth model was built using the dates and the year the core was collected was added as the age of the sediment-water interface with an error of ±5 years. This age-depth model was generated using a Bayesian approach with the software *Bacon* 2.2 (Blaauw & Christen 2011) and indicates that the *P. oceanica* mat sediment core encompasses the last ~5800 years (Fig. 2).

PALYNOLOGICAL, MICROCHARCOAL AND MAGNETIC SUSCEPTIBILITY ANALYSES IN 'CORE 2006'

The 'core 2006' was sliced every 1 cm and samples were oven-dried at 70 °C at the CEAB-CSIC (Blanes, Spain). Magnetic susceptibility was measured using a Bartington MS2 susceptibility meter with the MS2B sensor in 119 samples packaged in 10 cm^3 plastic pots at room temperature at Brunel University London (UK). The measurements were conducted at low frequency and on the 0·1 sensitivity setting. Each sample was measured twice, in non-consecutive measurements, with air readings before and after each measurement to adjust for drift correction. The average of the two corrected measurements was taken as the final value (κ). The low-field κ values were normalised with respect to sample mass (χ in m^3 kg^{-1}).

A total of 53 samples were taken for palynological analysis. The analysis was performed at Brunel University London (UK). The average sample volume used was 3·4 cm^3. *Lycopodium* tablets were added at the beginning of the chemical treatment (Stockmarr 1971). The analysis was performed following standard procedures (Fægri &

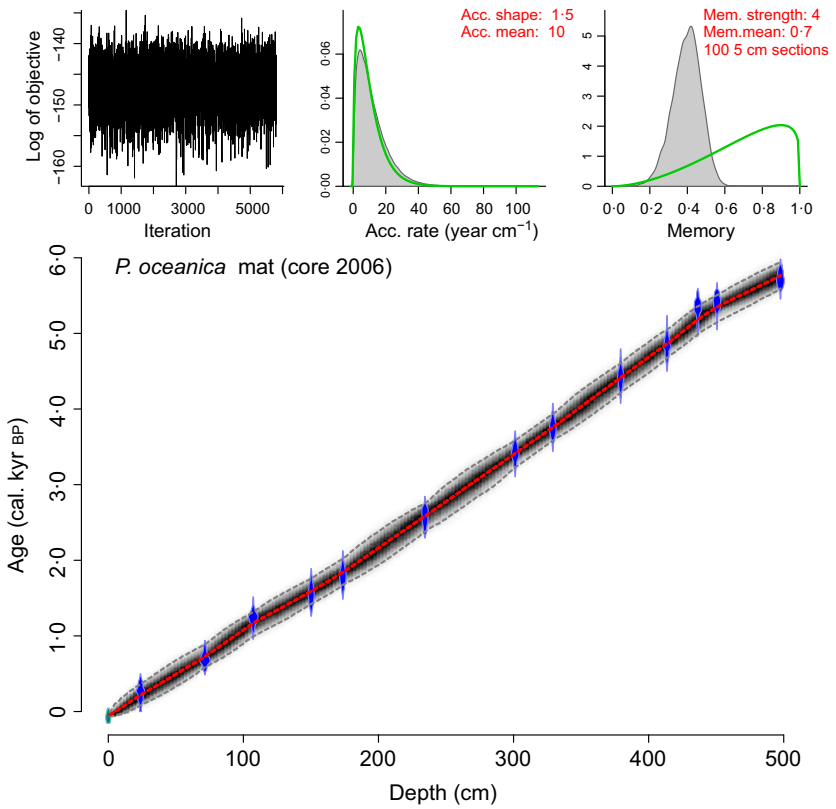


Fig. 2. Age-depth model for the *Posidonia oceanica* mat sediment 'core 2006'. Upper left: Markov Chain Monte Carlo iterations, showing a stationary distribution with little structure among iterations. Upper middle: prior (green curve) and posterior (filled grey curve) distribution of accumulation rates. The mean accumulation rate was set to 10 year cm^{-1}. Upper right: the prior and posterior probability distributions for memory (i.e. autocorrelation strength). Lower plot: *Bacon* age-depth model. Individual radiocarbon dates are shown in probability density functions of calibrated ages. The grey area indicates the uncertainty envelope of the age model with grey dashed curves indicating 95% confidence intervals and the red dashed curve shows the 'best' model based on the weighted mean age for each depth. [Colour figure can be viewed at wileyonlinelibrary.com]

Iversen 1989). Residual fractions were sieved through 125 and 10 μm nylon mesh sieves and final residues were mounted on slides in glycerol. Palynological identification and counting were completed at ×400 on an Olympus BX40 light microscope (London, UK), and at ×1000 using immersion oil for more delicate identifications, supported by the Brunel pollen reference collection and atlases (Reille 1992, 1995, 1998). Terrestrial pollen sum consisted of a minimum of 300 non-reworked pollen grains per sample when possible (average = 296; median = 306), excluding fern, fungal and algal spores as well as other non-pollen palynomorphs. Reworked pollen grains (broken, corroded, degraded and/or crumpled) were also counted. Palynological zones were identified by stratigraphically constrained cluster analysis by sum-of-squares (CONISS) including the terrestrial pollen types with percentages larger than 2·5%. Percentages were recalculated and square-root transformed prior to analysis. Diagrams were plotted and CONISS analysis performed with Psimpoll 4.27 (Bennett 2009).

Microcharcoal counts were performed on the same slides used for palynological analysis. Microcharcoal particles (10–125 μm) were identified following Turner, Roberts & Jones (2008), measuring their longest axis (Mooney & Tinner 2011), and counted until at least 200 items (sum of microcharcoal particles and *Lycopodium* spores) were reached (Finsinger & Tinner 2005). Charcoal accumulation rates (CHAR, number of particles cm^{-2} year^{-1}) were then calculated.

GLOMALIN-RELATED SOIL PROTEIN ANALYSIS IN 'CORE 2000'

Glomalin-related soil protein extraction was undertaken on 29 samples of 'core 2000' at The University of Queensland (Australia) to extend the previously published ~1250 year old record (23 samples; López-Merino *et al.* 2015) back to ~4500 years ago. Samples were oven-dried at 60 °C and the GRSP content was analysed following the procedures described by Wright & Upadhyaya (1996, 1998) to obtain Bradford-reactive soil protein (BRSP) and immunoreactive soil protein (IRSP) pools. Samples were extracted with 100 mM of $Na_4O_7P_2$ (pH = 9) for 1 h at 120 °C for BRSP analysis, while samples were extracted with 50 mM of $Na_3C_6H_5O_7$ (pH = 8) for 30 min at 120 °C for IRSP analysis. Bradford-reactive soil protein concentrations were determined using the Bradford dye-binding assay with protein dye reagent (Bio-Rad Laboratories) and bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) as the standard and they were read at 595 nm in a spectrophotometer (Model 680; Bio-Rad Laboratories, Philadelphia, PA, USA). Based on BRSP concentrations, solutions containing 0·02 μg of protein per well (Dynex 96 well polyvinyl chloride u-bottom plates; Dynex Technologies, Chantilly, VA, USA) were prepared for IRSP analysis. Immunoreactive soil protein was analysed with an indirect enzyme-linked immunosorbent assay with a monoclonal antibody MAb 32B11 against spores of arbuscular mycorrhizal fungi (AMF; Wright & Upadhyaya 1996). Due to the lack of a commercial standard, IRSP values are shown as unit-less measurements, providing a comparative mean to test the relative amount of GRSP from different soils tested in a similar way, using the same standard curve (Adame *et al.* 2012).

Results

Four palynological zones (P-1 to P-4) summarise the Portlligat Bay vegetation history (Fig. 3). Palynological (Fig. 3), CHAR, magnetic susceptibility and GRSP (Fig. 4) data are described following the palynological zones.

Zone P-1 (480−368·5 cm, ~5·6−4·3 cal. kyr BP): *Pinus* occurs in low values (10–20%). Deciduous *Quercus* is the mesophyte with the largest percentages (15–35%). Among the

Fig. 3.

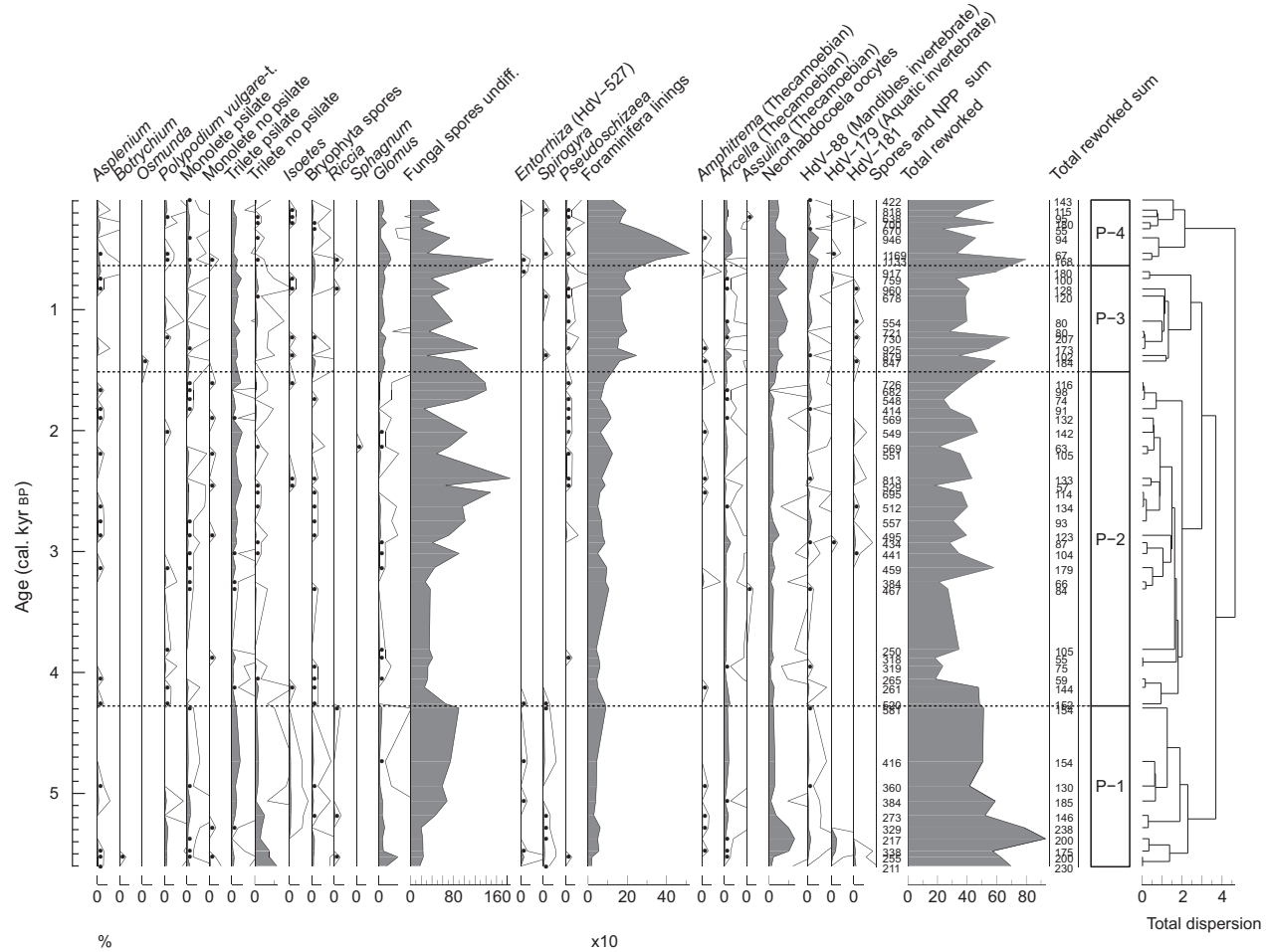


Fig. 3. Percentage palynological diagram of the Portlligat Bay *Posidonia oceanica* mat sediment 'core 2006' plotted against age. The filled silhouettes show the percentage curves of the taxa, while the white silhouettes show the ×10 exaggeration curves. Dots represent percentages below 0·5%. The constrained cluster analysis zonation is also included. Note that the Fungal spores undiff., Foraminifera linings and Total reworked curves have different scales than the other palynological taxa.

Mediterranean taxa, *Q. suber*, evergreen *Quercus* and *Pistacia* have large values (~10% each), while *Phillyrea* and *Olea* present lower values. The most important shrub taxa are *Erica*-t. (15–20%) and *Cistus*-t. (2–10%). Herbs are dominated by Poaceae, Cichorioideae, Amaranthaceae, Cyperaceae, Asteroideae, *Plantago* spp. and Urticaceae (10–30%). Charcoal accumulation rates values are first high and they drop at ~5·3 cal. kyr BP. Magnetic susceptibility values are low in the oldest samples, although they rise and peak at the end of the zone. A similar pattern is found in the fungal spore record. Neorhabdocoela oocytes and *Spirogyra* are present. Percentages of reworked pollen are high (40–90%).

Zone P-2 (368·5−142·5 cm, ~4·3−1·5 cal. kyr BP): *Pinus* occurs in larger values (20–50%) but gradually decreases from ~2·0 cal. kyr BP onwards. Deciduous *Quercus* percentages also decrease slightly (10–20%). *Q. suber*, *Pistacia* and *Phillyrea* have lower values, while evergreen *Quercus* presents higher ones. *Erica*-t. dominates the shrub component (15–20%). Herbaceous percentages are low (<10%), although Cichorioideae, *Plantago* and *Thalictrum* values increase from ~2·0 cal. kyr BP. The GRSP record shows three periods with lower values coeval with the presence of *Olea*, *Cerealia*-t., *Cannabis/Humulus*-t. and *Juglans* pollen. CHAR values are low, although they rise from ~2·0 cal. kyr BP onwards peaking at ~1·8–1·6 cal. kyr BP. Magnetic susceptibility values show peaking values. Fungal spore abundance drops from the onset of zone P-2 and remains low until ~3·0 cal. kyr BP (265 cm), when they increase and show a see-saw pattern. Neorhabdocoela oocytes and *Spirogyra* occur in trace amounts. Percentages of reworked pollen are relatively lower (30–60%).

Zone P-3 (142·5−63 cm, ~1·5−0·6 cal. kyr BP): *Pinus* has lower values compared to zone P-2 (10–30%). Deciduous *Quercus* values decrease (~15%). Evergreen *Quercus* and *Q. suber* have lower percentages, while *Pistacia* are higher. The shrub component increases, with *Erica*-t. the most dominant taxa (~20%), along with *Cistus*-t. (5–10%), *Cytisus/Genista*-t., *Helianthemum*-t. and *Calluna*. Herbs percentages increase (10–30%), with Poaceae, Amaranthaceae, Cichorioideae, *Artemisia*, Asteroideae, *Plantago*, Urticaceae and *Thalictrum* as the main types. The GRSP record shows two periods of decreasing values coeval with the presence of *Olea*, Cerealia-t., *Cannabis/Humulus*-t., *Castanea*, *Juglans*

and *Vitis* pollen. CHAR values have a see-saw pattern. Magnetic susceptibility values and foraminifera linings percentages follow increasing trends. Fungal spore abundance is high. Neorhabdocoela oocytes and *Spirogyra* presence increase. Percentages of reworked pollen also increase.

Zone P-4 (63−13 cm, <~0·6 cal. kyr BP): *Pinus* abundance is high (40–45%), while deciduous *Quercus* values are low (~10%). Evergreen *Quercus*, *Q. suber*, *Phillyrea* and *Pistacia* are lower or at a similar value compared with those in zone P-3. The shrub component decreases, *Erica*-t. in particular (<10%). Herbs are well represented (~25%) with Amaranthaceae, Poaceae, Cichorioideae, *Plantago*, Urticaceae, *Artemisia*, Cardueae, *Centaurea jacea*-t., Brassicaceae, *Thalictrum* and Caryophyllaceae as the main components. *Olea*, *Castanea*, *Juglans*, *Vitis*, *Cannabis*/*Humulus*-t. and Cerealia-t. have an important presence, and GRSP values are lower when they peak. CHAR values are relatively high. Magnetic susceptibility peaks at the onset of the zone. Foraminifera linings and fungal spore values are high and also peak at the beginning of the zone. Neorhabdocoela oocytes and *Spirogyra* percentages are similar to those recorded in zone P-3.

Discussion

CONTINENTAL LANDSCAPE AND FIRE HISTORY

Evidence from the western Mediterranean basin between 40°N and 45°N latitudes shows an increase in fires from ~8 cal. kyr BP up to around 5 cal. kyr BP, when a drop in the frequency of fires occurred (Vannière *et al.* 2011). In the Portlligat record, CHAR values are high from the onset of the record up to ~5·3 cal. kyr BP (Fig. 4). This phase with fires is also detected regionally in the Pyrenees and has been linked to the increased summer temperature during the Holocene Thermal Maximum (Rius *et al.* 2011; Rius, Vannière & Galop 2012), and to the development of the mesophytic forest with low amounts of *Pinus* and high lake levels (Pérez-Sanz *et al.* 2013). Neorhabdocoela oocytes and *Spirogyra*, indicative of freshwater, occur during this phase of the Portlligat record (Fig. 3), pointing to increased freshwater discharge in the bay due to the more humid conditions. The high values of reworked pollen found in this interval could be linked to it as well, as larger river discharge would deliver higher amounts of damaged pollen (Fig. 4).

In the Portlligat record, CHAR is low from 5·3 to ~2·0–1·8 cal. kyr BP which concurs with other regional records (Rius *et al.* 2011; Rius, Vannière & Galop 2012; Pérez-Sanz *et al.* 2013). The inferred landscape is also characterised by the higher representation of *Pinus* (Fig. 3), suggestive of increased aridity. The shift from *Q. suber* to evergreen *Quercus*, along with the lower values of freshwater indicators, supports this interpretation (Fig. 3). The nearby Sobrestany pollen record also shows an increase in *Pinus* and evergreen *Quercus* and a decrease in *Q. suber* during this time (Parra, van Campo & Otto 2005). The aridity trend is also recorded in other Iberian records by the expansion of *Pinus*, sclerophyllous and/or steppe taxa (Carrión *et al.* 2003, 2004;

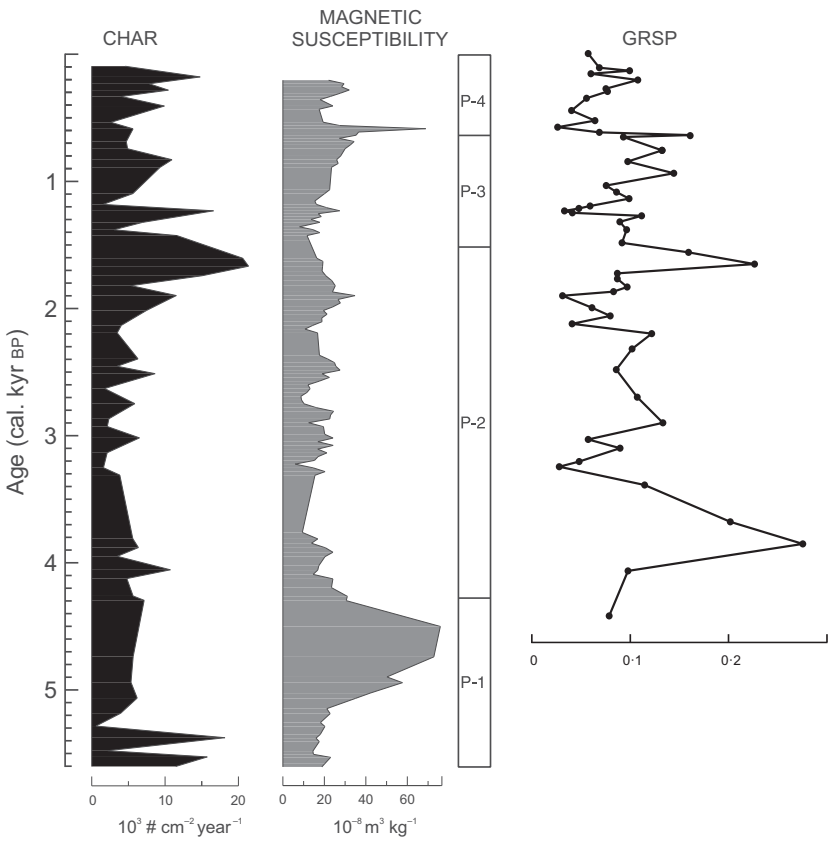


Fig. 4. Charcoal accumulation rate (CHAR) and magnetic susceptibility values from the *Posidonia oceanica* mat sediment 'core 2006' plotted against age. The last ~4·5 cal. kyr BP record of GRSP from the *P. oceanica* mat sediment 'core 2000' is also shown. The chronology of 'core 2000' was developed by Serrano *et al.* (2012). The topmost part of the GRSP record (last ~1250 years) was published in López-Merino *et al.* (2015).

Pantaleón-Cano *et al.* 2003; Martín-Puertas *et al.* 2008; Pérez-Sanz *et al.* 2013; Aranbarri *et al.* 2014).

The main period of cultivation apparently occurred in Roman (~2·2 cal. kyr BP) and especially from Medieval times (~1·5 cal. kyr BP) onwards, with *Olea*, Cerealia-t., *Cannabis*/*Humulus*-t., *Juglans*, *Castanea* and *Vitis* presenting highest percentages at Portlligat (Fig. 3). The development of crops is coeval with high CHAR values (Fig. 4), suggesting that human-induced fires were used to open up the landscape and expand the area of cultivated land. Deciduous and evergreen *Quercus* were the most impacted taxa (Fig. 3). The rise in crops from the Roman period onwards is also a common occurrence in other pollen records in the region (Riera & Esteban 1994; Riera, Wansard & Julià 2004; Burjachs *et al.* 2005; Parra, van Campo & Otto 2005; López-Sáez *et al.* 2009; Rull *et al.* 2011; Ejarque *et al.* 2016). The presence of freshwater indicators, together with higher values of reworked pollen (Figs. 3 and 4), may indicate moister conditions again.

LAND-USE CHANGE AND ITS IMPACT ON THE CONTINENTAL LANDSCAPE

Emran, Gispert & Pardini (2012) measured present GRSP levels under different terrestrial vegetation communities in the study area where the *Posidonia* mat cores were taken (Cape Creus, NE Spain, Fig. 1). They found that GRSP production was lower in cultivated soils (under vines and olive groves) than in non-cultivated soils. By the last twelve centuries of land-use change with the GRSP record from the *P. oceanica* mat 'core 2000', López-Merino *et al.* (2015) found that when indicators of crops (e.g. Cerealia-t., *Cannabis*/*Humulus*-t., *Vitis*, *Juglans*, *Castanea* and *Olea*) increased, the GRSP content decreased accordingly. The GRSP perturbation linked to anthropogenic activities is related to the impact of cultivation practices on the AMF, symbionts with the roots of most terrestrial plants that produce GRSP. Arbuscular mycorrhizal fungi have a significant role in continental soil ecosystems' functioning and quality (e.g. Wright & Upadhyaya 1998; Rillig *et al.* 2001; Rillig & Steinberg 2002). López-Merino *et al.* (2015) suggested the use of GRSP measured in the anoxic seagrass mat as a palaeo-proxy of continental soil quality. In this new study, the GRSP record of 'core 2000' is extended down to ~4·5 cal. kyr BP, and compared with the palynological indicators of potential crops detected in 'core 2006'. Notwithstanding the chronological uncertainties of comparing two cores with independent age-based chronologies, drops in the GRSP record match with increasing indicators of potential crops (Fig. 5). Six periods with decreasing trends in GRSP and increasing land-use change indicators are identified at ~3·4–2·9 (Late Bronze Age), ~2·6–2·3 (Iron Age), ~2·2–1·7 (Roman times), ~1·5–1·0 (Visigothic times), ~0·9–0·7 (Medieval times), and <~0·6 (Late Medieval to present times) cal. kyr BP (Fig. 5).

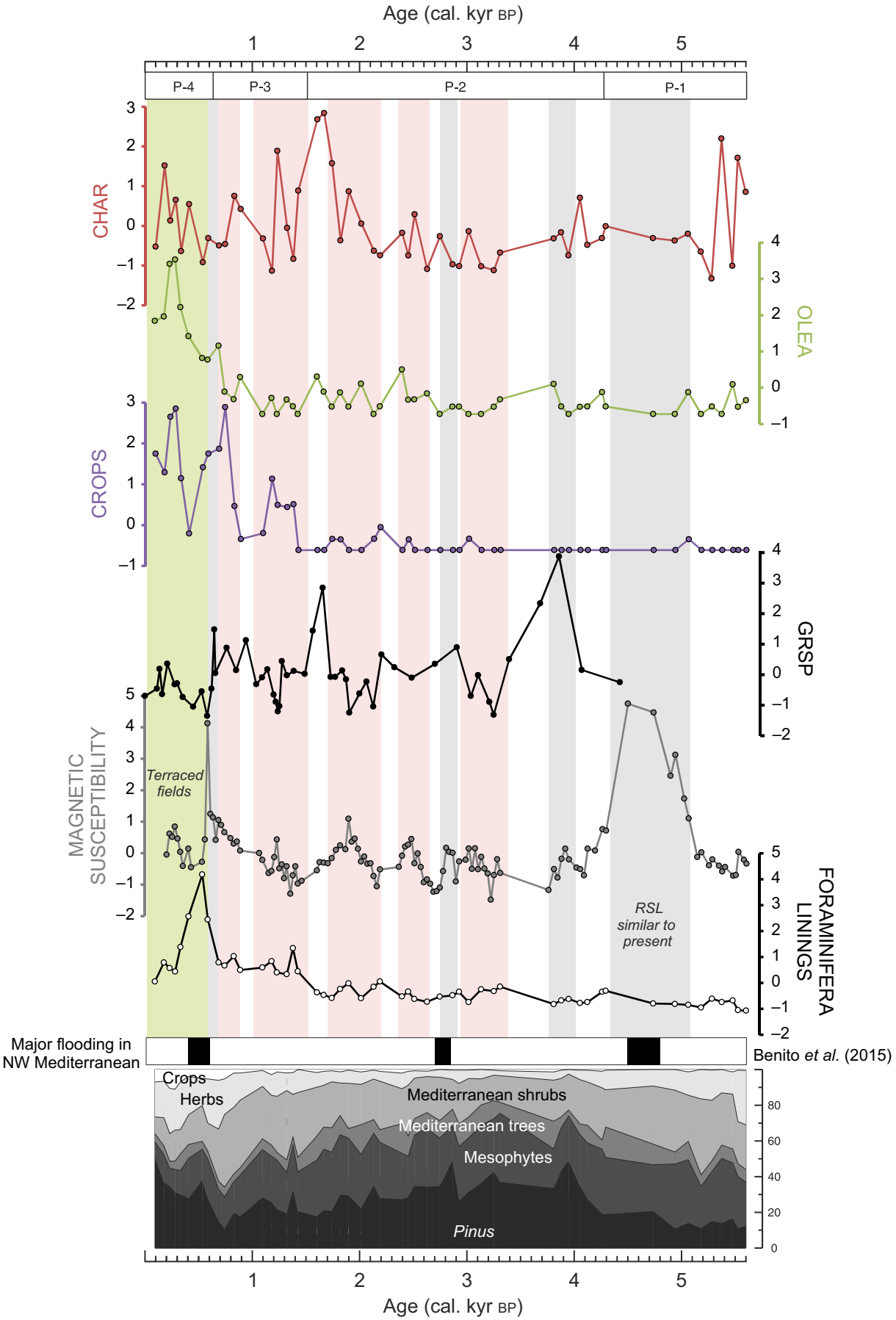
Importantly, GRSP represents a sizeable portion of the terrestrial carbon pool (4–8% of soil carbon; Rillig *et al.* 2003); therefore alterations in the AMF hyphae and the production of GRSP have a direct effect on the terrestrial carbon storage (e.g. Rillig *et al.* 2001; Wilson *et al.* 2009). This new study provides further evidence of the long-term impact of anthropogenic activities on important soil ecological features that may play a key role under the rising CO_2 atmospheric levels (Treseder & Allen 2000).

IMPACT OF CLIMATE AND LAND-USE CHANGE ON THE MARINE POSIDONIA MEADOWS

The establishment of crops had an impact on the continental soil quality and terrestrial carbon storage during the Late Holocene (Fig. 5). Considering that the seagrass ecosystem is an important carbon sink located in coastal areas, did agriculture and/or other environmental changes have an effect on the functioning of the *P. oceanica* system? Many human pressures have been linked to the recent decline of *P. oceanica* meadows in the Mediterranean coastal zone (Marbà, Díaz-Almela & Duarte 2014). Most of these anthropogenic impacts have a recent origin or their intensification have occurred after the Industrial Revolution. Adding a palaeoecological perspective to reconstruct environmental impacts on seagrasses could inform the restoration and management of the very sensitive seagrass meadows.

Magnetic susceptibility in a sample depends on its mineralogical composition, and its increases are related to the presence of ferromagnetic minerals (Thompson & Oldfield 1986). In *Posidonia* mats, due to their marine location, diamagnetic materials

Fig. 5. Linking environmental changes (climate- and human-induced) with perturbations on the terrestrial and coastal systems for the last ~5·6 cal. kyr BP in the Portlligat Bay (western Mediterranean) inferred by the palaeoecological study of *Posidonia oceanica* mats. From top to bottom: CHAR represents charcoal accumulation rates related to fire activity; *Olea* and crops (Cerealia-t., *Cannabis*/*Humulus*-t., *Juglans*, *Castanea* and *Vitis*) are palynological indicators of potential crops that account for land-use changes; GRSP (glomalin-related soil protein) content is a proxy for changes in the continental soil quality; magnetic susceptibility indicates changes in the seagrass mat organic/inorganic content, higher values interpreted as enhanced loadings of terrigenous material; Foraminifera linings account for eutrophication of the bay waters due to the extensification of the agrarian activities. 'Red bands' highlight periods with increased land-use change and fire activity that affected the continental soil quality (decreases in GRSP) and the coastal seagrass mat sediments (increases in magnetic susceptibility). The most recent 'green band' highlights also a moment in which land-use change is detected, disturbing the continental soil quality; however, the seagrass mat does not reflect an increase in magnetic susceptibility. This fact is linked to the onset of the olive tree and vine terrace farming in the area (Franquesa i Codinach 1995). 'Grey bands' highlight four phases in which higher values of magnetic susceptibility are not linked to land-use change and decreasing trends in GRSP. Three of them are likely related to major flooding events in the NW Mediterranean area (Benito *et al.* 2015), although one of them remains unexplained. CHAR, *Olea*, crops, GRSP, magnetic susceptibility and Foraminifera linings values have been standardised (Z-scores) for comparison purposes. CHAR, palynological and magnetic susceptibility data are from 'core 2006', while GRSP is from 'core 2000'. [Colour figure can be viewed at wileyonlinelibrary.com]



(carbonates and organic matter) are the main components of the mat (Serrano *et al.* 2012). Hence, increases in magnetic susceptibility may be indicative of higher loadings of ferromagnetic minerals derived from soil erosion. When comparing the magnetic susceptibility record with the palynological indicators of potential crops and the record of GRSP, it could be seen that for the last ~3·4 cal. kyr BP increases in magnetic susceptibility are coeval with five of the six above mentioned inferred periods of land-use change: ~3·4–2·9 (Late Bronze Age), ~2·6–2·3 (Iron Age), ~2·2–1·7 (Roman times), ~1·5–1·0 (Visigothic times) and ~0·9–0·7 (Medieval times) cal. kyr BP (Fig. 5). This means that soil erosion triggered by anthropogenic activities was most likely responsible for the detected changes in the magnetic susceptibility. Interestingly, the most recent period of inferred land-use change (<~0·6 cal. kyr BP, Medieval to present times) is not linked to higher magnetic susceptibility values despite being the phase with the highest presence of crops. This could be related to the olive tree and vine terrace farming in the area (Franquesa i Codinach 1995), as terraced fields limit the amount of soil erosion (Montgomery 2007).

Four peaks in the magnetic susceptibility record are not linked to land-use change and other factors apart from human-induced soil erosion due to farming have to be found. These increases in magnetic susceptibility are detected at ~5·1–4·3, ~4·0–3·8, ~2·9–2·8 and ~0·6 cal. kyr BP (Fig. 5). Major events of flooding in the NW Mediterranean area during the Late Holocene have been reconstructed at ~4·8–4·5, ~2·75 and ~0·5 cal. kyr BP (Benito *et al.* 2015), and three of the four peaks in the magnetic susceptibility values are likely to reflect these major flooding events that would mean a loading of terrigenous material due to hydrological changes. The higher values at ~4·0–3·8 cal. kyr BP remain, however, unexplained. In addition, the larger peak in magnetic susceptibility detected at ~5·1–4·3 cal. kyr BP is contemporaneous not only to a major flooding event but also to the stabilisation of the relative sea-level (RSL). Relative sea-level rose continuously throughout the Holocene with a deceleration after ~5·0–4·0 cal. kyr BP, when the RSL approached the present level (Vacchi *et al.* 2016). The coupling of two environmental forcings might explain the enhanced soil erosion triggering the largest increase in the magnetic susceptibility. This sequence of events warn what may happen in the near future if several impacts combine together to initiate ecological shifts in seagrass-dominated ecosystems.

At the *Posidonia* meadows scale, higher loading of terrigenous materials would most likely cause a decrease in the health of the meadows, as the material would dilute the biogenic carbonates deposited and potentially lead to a decline in the assemblage of calcifying organisms sustained by the meadows, a decline in seagrass productivity and a loss in biodiversity (Short & Wyllie-Echeverria 1996; Montefalcone *et al.* 2015). Thus, since the Late Holocene, soil erosion induced by major flooding events and land-use change in the NW Mediterranean area has probably disturbed *Posidonia* meadows at Portlligat Bay, potentially affecting the meadows' health and, hence, disturbing the mat carbon accumulation dynamics.

Finally, the extensification of agrarian activities from Roman and Medieval times onwards is not only reflected in the mat-inferred continental soil erosion record, but also in the coastal environment with the rise in the abundance of foraminifera linings. It is plausible that species tolerant to low oxygen concentrations and high organic fluxes dominated the benthic assemblages (Thibodeau, de Vernal & Mucci 2006), likely indicating significant changes in bottom water conditions (Fig. 5) due to river-induced eutrophication and anoxia (Brasier 1995). However, the establishment of terraced fields reduced not only the terrigenous loading to the coastal environment but also the eutrophication levels, as the abundance of foraminifera linings has fallen in the last few centuries (Fig. 5).

THE IMPORTANCE OF A PALAEOECOLOGICAL PERSPECTIVE FOR SEAGRASS ECOSYSTEM MANAGEMENT

On the one hand, this work provides a detailed record of land-use and climate change derived from seagrass mats of *P. oceanica*. Therefore, coastal landscapes, which have few or no on-land palaeoecological sites, could be reconstructed by investigating adjacent shallow marine seagrass mats (López-Sáez *et al.* 2009). On the other hand, the proposed combination of palaeoecological proxies have delivered long-term data-series that provide useful information to test the palaeoecological potential of *Posidonia* mats by detecting long-term environmental dynamics that affected *P. oceanica* system, and thus providing clues for habitat restoration at a coastal marine scale. First, the microcharcoal record agrees with regional trends, showing the reliability of *Posidonia* mats for the reconstruction of regional fire history. Furthermore, palynological data have provided a detailed reconstruction of the landscape, identifying periods of enhanced agrarian activity, especially from Roman and Medieval times (Fig. 5). Second, we provide evidence to suggest that the impact of long-term land-use changes on the continental soil AMF microbiota affecting soil quality can be identified in the GRSP analysis in *Posidonia* mats. Third, phases with higher anthropogenic and climate stressors at continental scale are mirrored in the *Posidonia* mat archives. On one hand, the coastal environment is affected by agrarian-induced eutrophication since Roman and Medieval times. On the other hand, enlarged coverage of crops (Fig. 5) and major flooding events reconstructed for the NW Mediterranean area (Benito *et al.* 2015) resulted in increased levels of soil erosion – reconstructed by the increasing values in magnetic susceptibility – that impacted on the *Posidonia* mat inorganic content and most probably on mat carbon accumulation dynamics.

The threats to *P. oceanica* meadows, accelerating loss rates, are mainly related to chemical (e.g. urban waste discharges and eutrophication) and mechanical (e.g. fish farming, trawl fishing, anchoring and coastal development) processes (e.g. Waycott *et al.* 2009). However, this research shows that land-use and climate changes, although not being the main factors inducing seagrass meadows disappearance, have affected the properties of the *Posidonia* mat, likely impacting

the meadow health and their carbon accumulation dynamics during the Late Holocene. Further research is required to fully understand long-term seagrass ecosystem dynamics in response to Holocene environmental disturbances. As CO_2 emissions are projected to increase dramatically and coastal development in the Mediterranean continues, any single step in restoring seagrass meadows and their carbon sink capacity is important. This new research recommends taking into account the catchment scale (e.g. reducing soil run-off) in the Mediterranean coastal areas in order to manage and protect the important socio-economic ecosystem services that seagrass meadows provide.

Authors' contributions

L.L.M., O.S., A.M.C. and M.A.M. conceived the ideas and designed the methodology; O.S. and M.A.M. performed the fieldwork; L.L.M., N.R.C.R. and M.F.A. collected the data; L.L.M. and A.M.C. analysed the data; L.L.M. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data collected in this study (palynology, microcharcoal, magnetic susceptibility and glomalin-related soil protein) are available at figshare, https://doi.org/10.17633/rd.brunel.4210185.v1 (López-Merino *et al.* 2016).

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