



Glomalin accumulated in seagrass sediments reveals past alterations in soil quality due to land-use change



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ABSTRACT

Arbuscular mycorrhizal fungi (AMF), symbionts with most terrestrial plants, produce glomalin-related soil protein (GRSP), which plays a major role in soil structure and quality. Both fungi hyphae and protein production in soils are affected by perturbations related to land-use changes, implying that GRSP is a sensitive indicator of soil quality. Unfortunately, GRSP degrades within years to decades in oxic environments, preventing its use as palaeoecological proxy. However, GRSP is transported to marine, near-shore anoxic sediments, where it accumulates and remains non-degraded, enabling the assessment of its potential as a palaeoecological proxy for soil ecosystem's health. Exploiting this fact, we have obtained for the first time a long-term record (c. 1250 years) of GRSP content using a *Posidonia oceanica* seagrass mat sediment core from the Western Mediterranean (Portlligat Bay, Spain). The trends in GRSP content matched well with land-use changes related to agrarian activities reconstructed by pollen analysis. In periods of cultivation, GRSP accumulation in the mat decreased. Given the role played by GRSP, the results suggest that agrarian intensification may have resulted in perturbations to soil quality. Thus, GRSP in seagrass mat sediments can be used to assess long-term trends in continental soil quality induced by human activities. These findings open new possibilities in long-term ecology research, as other anoxic environments could be potentially valid too. Testing them would open the possibility to identify long-term patterns in soil quality and other environmental stressors that could also affect AMF and GRSP production in soils.

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1. Introduction

Unravelling the potential of new palaeoecological proxies providing information on when a system has been disturbed is challenging. Past changes in land-use, i.e., agrarian practices, can be tracked back in time using palaeoecological techniques. Alterations in forest cover, hydrology and trophic status, as well as soil erosion, are the environmental consequences associated with the establishment of crops that are typically reconstructed in palaeoecological and palaeoenvironmental research (Dearing et al., 2006). However, other transformations that can be identified in present landscapes have passed unnoticed in the palaeoecological record, even when they could be useful to detect long-term variations in soil quality due to land-use change. One of them is the alteration of hyphae production

by arbuscular mycorrhizal fungi (AMF), which are symbionts with the roots of most terrestrial plants and play a significant role in soil ecosystems' functioning (Wright et al., 2006).

Glomalin-related soil protein (GRSP) is a recently discovered glycoprotein produced by AMF (Gadkar and Rillig, 2006). GRSP is a significant component of soil organic matter, playing an important role in nitrogen and carbon storage and turnover, soil aggregate stability and soil texture (Rillig and Steinberg, 2002; Rillig et al., 2001; Staddon et al., 2003; Wright and Upadhyaya, 1998). Concentrations of GRSP in soils have been linked to land-use change, indicating that its abundance responds to ecosystems' modifications. In the Delaware Wildlife Area (Ohio, US), agricultural (corn and soybean) and afforested soils had lower GRSP concentrations than native temperate deciduous forest soils (Rillig et al., 2003). The conversion of Mexican rainforest to pasture and agricultural land (corn and common bean) decreased soil GRSP concentrations (Violi et al., 2008). In soils from south Cameroon GRSP content declined from forest to fallow and field crops (groundnuts, corn cassava,

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plantain; Fokom et al., 2012). As land-use alters AMF communities, affecting GRSP production and its soil concentration (Wright et al., 1999), it is considered that the more GRSP in a soil the better the soil quality (Shrestha Vaidya et al., 2011), hence providing information on the health of a given soil ecosystem.

GRSP has a turnover time of years to decades in well-aerated soils (Rillig et al., 2001). Since it rapidly degrades with time in oxic environments, most terrestrial soils are not effective environmental archives for the reconstruction of long-term GRSP trends, preventing the use of GRSP as a palaeoecological proxy. However, GRSP has been detected in superficial sediments of intertidal *Posidonia australis* seagrass meadows and wetlands, and coastal coral reefs (Adame et al., 2010, 2012). Although AMF colonise most terrestrial plants' roots, the ability of AMF to colonise plants' roots is limited by salinity (Adame et al., 2012), and AMF colonisation of seagrass roots has not been observed (Nielsen et al., 1999). The GRSP produced by AMF is found outside of the fungal structures, indicating it is transferred to the environment (Driver et al., 2005). GRSP can leach or be washed from soils into streams, and it is detected downstream in floodplains and rivers (Harner et al., 2004). Rivers and groundwater transport terrestrial material containing GRSP that is deposited in accretion areas and coastal systems, evidencing a terrestrial-marine connectivity (Adame et al., 2010, 2012). The highly anoxic conditions of marine environments, such as seagrass sediments, would prevent the degradation of GRSP enabling the reconstruction of long-term records of GRSP change.

The GRSP found in the coastal marine environment is likely to reflect landscape-scale processes, as it is not produced locally and has been transported by watershed runoff towards the sea (Adame et al., 2012; Harner et al., 2004). Thus, it is worth comparing synchronous terrestrial landscape changes and variations in GRSP content in marine sediments. For this purpose, palynological studies provide landscape-scale information on when an area was transformed and the magnitude of that transformation. This comparison would allow the assessment of whether changes in GRSP content in marine sediments could be linked to terrestrial landscape changes (i.e., land-use change).

In this study we report the last c. 1250 years of changes in GRSP content, detected in a Western Mediterranean seagrass *Posidonia oceanica*

mat core (Portlligat bay, Spain, Fig. 1). The GRSP record is compared with landscape vegetation changes, including agriculture indicators, obtained from a previous palynological study performed on the same mat core (López-Sáez et al., 2009) (see Supplementary Information). Fluctuations in GRSP content in the seagrass mat and in terrestrial landscape composition and structure inferred by pollen data (i.e., turnover, PCA results, richness and evenness) were characterised using change-point modelling (Gallagher et al., 2011) to statistically detect synchronous events. The goal was to evaluate whether GRSP accumulated in coastal anoxic environments could be used as a palaeoecological proxy and to assess whether long-term GRSP variations in coastal environments accurately reflect soil alterations in quality related to past changes in land-use.

2. Methods

2.1. Study site and sampling

The Portlligat Bay ($42^{\circ}17'32''$ N; $3^{\circ}17'28''$ E) is a small (0.14 km²) and shallow (<10 m deep) inlet located in NE Spain, in the Cape Creus, connected to the sea through a 213 m wide opening to the NW (Fig. 1). Living meadows of *P. oceanica* cover about 68% of the bottom of the bay. The bay receives freshwater input from a typical Mediterranean temporary stream that flows into it from its NE shore. The Cape Creus itself (eastern Pyrenees) is a geological formation originated 400 million years ago. This area presents a Mediterranean climate, with warm summers, mild winters and summer drought. The landscape has had an intense human fingerprint for a long time. Traditional activities, such as dry-land farming and fisheries, have been replaced by tourism, leaving the area occupied by abandoned terraced vineyards and pasturelands. Mediterranean scrubland dominated by *Pistacia lentiscus*, *Juniperus oxycedrus*, *Calicotome spinosa* and species of *Erica* and *Cistus* are important. Forest cover is sparse, with *Pinus halepensis* and *Quercus suber* as the most abundant tree species, while in sheltered areas *Quercus ilex* and *Quercus pubescens*, and in riparian areas *Ulmus minor*, *Fraxinus angustifolia*, *Alnus glutinosa*, *Corylus avellana* and *Salix* sp. appear (Franquesa I Codinach, 1995).

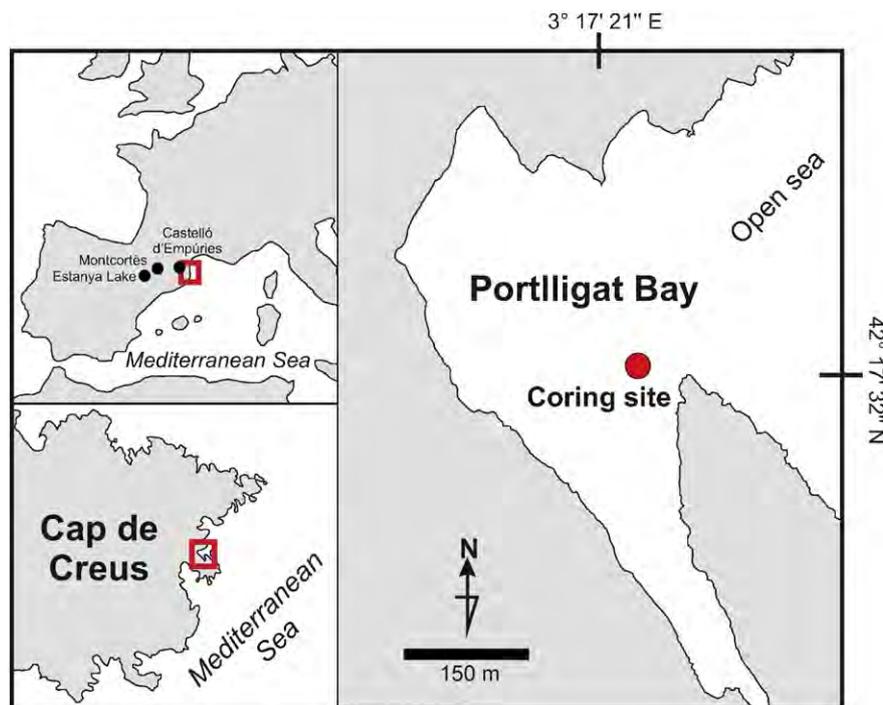


Fig. 1. Location of the drilling point of core 2000 in the *P. oceanica* bed at the north-western part of the Mediterranean Sea (Portlligat Bay, Cape Creus, Spain). Other sites cited in the text are also located in the map.

A 475 cm-long core (core 2000) was sampled in a *P. oceanica* meadow, at a water depth of 3 m, in the Portlligat bay using a floating drilling platform that combined pneumatic percussion and rotation. A detailed description of the study site, coring procedures and mat characteristics can be found in Serrano et al. (2012).

2.2. Previous palaeoenvironmental studies on the same seagrass mat

Reliable palaeoecological studies are only possible with consistent chronologies. Radiocarbon dating revealed that the 475 cm-long core mat (core 2000) was c. 4500 years old (Serrano et al., 2012). The records of trace metals matched well with phases of European mining and metalworking, confirming the accuracy of the chronology (Serrano et al., 2011, 2013). The palynological study of the uppermost section of the same mat core and spanning the last c. 1250 years (López-Sáez et al., 2009) showed that the Portlligat bay catchment area experienced farming activities. The timing of the changes agrees with other NE Iberian reconstructions (Burjachs et al., 2005; Riera et al., 2004; Rull and Vegas-Vilarrúbia, 2015; Rull et al., 2011), demonstrating the reliability of pollen studies on coastal *P. oceanica* mats in reconstructing terrestrial landscapes. We have updated the chronological model provided in López-Sáez et al. (2009) to the most accurate one developed by Serrano et al. (2012) (see Supplementary Information).

2.3. Glomalin-related soil protein extraction

Twenty three samples from the topmost 150 cm of core 2000, sampled every c. 2–10 cm and covering the last c. 1250 years, were analysed for their GRSP content. The samples were oven-dried at 60 °C before analysis, which was carried out using the procedures described by Wright and Upadhyaya (1996, 1998). Two extractions were performed to obtain two different glomalin pools: Bradford-reactive soil protein (BRSP) and immunoreactive soil protein (IRSP).

For BRSP, samples were extracted in sodium pyrophosphate, pH 9 at 120 °C by autoclaving for 60 min. Multiple extractions were performed (2–3) until the supernatant presented none of the red-brown colour typical of GRSP. For IRSP, samples were extracted in sodium citrate, pH 8.0 by autoclaving at 120 °C for 30 min. After each extraction, samples were centrifuged at 3220 g for 10 min. Once the process was complete, the total volume of the supernatants was recorded and sub-samples were centrifuged at 10,000 g. The Bradford assay was first used to determine the concentration of BRSP using bovine serum albumin as a standard. Based on BRSP concentrations, solutions containing 0.02 µg of protein were set for IRSP analysis. IRSP was analysed with an indirect enzyme-linked immunosorbent assay (ELISA) using the monoclonal antibody MAb 32B11 developed against spores of arbuscular mycorrhizal fungi (Wright and Upadhyaya, 1996). Values were compared with a standard curve calculated by Adame et al. (2012). Due to the lack of available commercial standards, IRSP values are shown as unit-less measurements, providing a comparative mean to test the relative amount of GRSP in different samples using the same standard curve. Immunoreactivity (IR%) was calculated as the IRSP/BRSP ratio and refers to the ability of the protein to attach to the antibody, informative of the decomposition state of GRSP (Lovelock et al., 2004; Wright et al., 1996).

2.4. Numerical analyses

The previously published palynological analysis performed on the same *P. oceanica* mat core (López-Sáez et al., 2009) is available at the European Pollen Database. The palynological data-set has been explored numerically to detect past landscape changes and to compare them with GRSP trends. Pollen and fern spores were considered for the calculation of the percentages and the diagram plotted using the most updated chronology developed by Serrano et al. (2012) (see Supplementary Information).

Palynological richness was estimated by rarefaction analysis, a measure of richness that standardises sample sizes to a common sum (Birks and Line, 1992), usually the lowest sum (here 213). Evenness was calculated using the Buzas and Gibson's index, providing information about the homogeneity in the distribution of the taxa abundances in the assemblages. Taxonomical turnover was assessed by detrended correspondence analysis (DCA). Percentages were squared-root transformed to stabilise variances. Detrending was by segments, a non-linear rescaling was applied and rare taxa were not down-weighted. Thus, samples scores are scaled in standard deviation (SD) units of compositional change (Birks et al., 2012). DCA gradient lengths were <2.5 SD, indicating monotonic responses and that a linear approach is applicable (Birks et al., 2012). Thence, principal component analysis (PCA) was used to summarize temporal trends in the palynological dataset. Only taxa with percentages >2% were considered, and *Juglans*, *Vitis*, *Cannabis/Humulus* t. and *Cerealia* were grouped as "Crops". Percentages were squared-root transformed, and the covariance matrix was chosen (Birks and Gordon, 1985).

Because palynological and GRSP analyses were performed at different depths/ages, change-point modelling was applied to both GRSP content and palynological numerical results (turnover, PC1 and PC2 factor scores, richness and evenness) to detect statistically periods of common changes in trends. The approach used here is based on Bayesian transdimensional Markov chain Monte Carlo (Gallagher et al., 2011) and it is the first time it has been used in a multi-proxy record.

Palynological richness and evenness were calculated using PAST 3.01 (Hammer et al., 2001), DCA and PCA performed with Psimpoll 4.27 (Bennett, 2009), and change-point modelling computed with the CPQtr1.0.3 statistical package (Gallagher et al., 2011).

3. Results

3.1. Glomalin-related soil protein record in the seagrass mat

GRSP had an average content of 0.080 ± 0.005 (unit-less), with minimum and maximum values of 0.027 and 0.161 respectively. GRSP content and immunoreactivity (IR%) had a similar pattern, except for the surface sample that showed the highest IR% (Fig. 2a). This could suggest that GRSP may have lost part of its immunoreactivity after burial, although quickly reaching a post-burial stabilisation. We did not find a decreasing trend of GRSP content and IR% with depth (hence age) (Fig. 2b), which indicates that variations in GRSP might be driven by

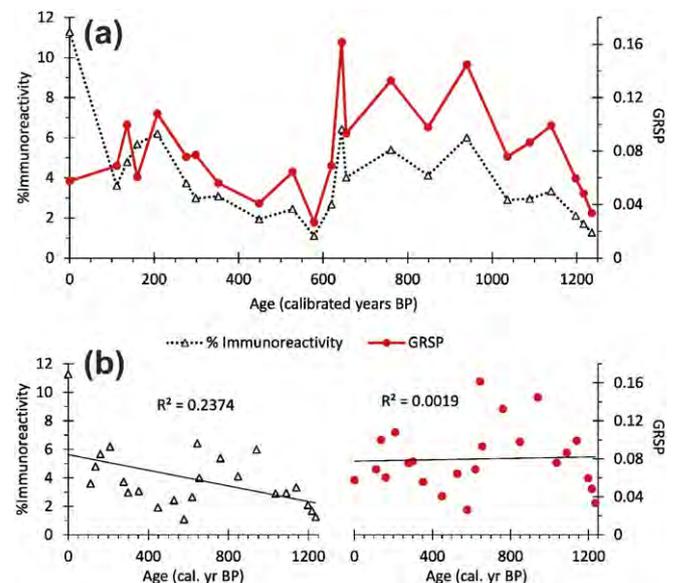


Fig. 2. Glomalin-related soil protein (GRSP) results obtained for the studied *P. oceanica* mat (core 2000). (a) GRSP relative content and immunoreactivity (IR%) for the last 1200 years. (b) Age is not significantly correlated either with GRSP or IR%.

factors other than post-burial degradation kinetics. GRSP content increased from c. 1235 to 650 cal. yr BP. A sudden decrease (in about 25 years) took place at c. 580 cal. yr BP remaining low until c. 250 cal. yr BP and partially increasing afterwards.

3.2. Numerical analyses of terrestrial landscape changes

Change-point modelling applied to GRSP and records of landscape change obtained from palynological data (turnover, PC1 and PC2 scores, richness and evenness) enabled the identification of six statistically significant periods of change (change-points CP-1 to 6) (Fig. 3). CP-5, the most abrupt of all of them, is contemporary with the main drop in GRSP content and important changes in the composition and structure of the terrestrial landscape vegetation. Richness and turnover, that presented similar trends ($R^2 = 0.78$), showed an abrupt increase during this event and a decrease during CP-6 (Fig. 3). Evenness showed a see-saw pattern. The principal component analysis distinguished two principal components (PC) (Fig. 4). The first principal component (PC1) accounted for 33.6% of the variance, while the second principal component (PC2) explained 13.1%. In PC1, *Q. suber*, *Erica*, *Cistus*, *Betula* and deciduous *Quercus* presented positive loadings, while evergreen *Quercus*, "Crops" and Cyperaceae had negative loadings. PC1 is interpreted as an agrarian gradient from lower (positive scores) to larger agrarian impact (negative scores). PC1 showed positive scores before

CP-5, with a shift to negative scores from CP-5 to CP-6 and a return to positive scores after CP-6. In PC2, Cichorioideae and *Pinus* presented positive loadings, while *Erica* had a negative one. All samples scores for PC2 were between 0.3 and -0.3 except for one sample. Such sample presented anomalous high percentages of Cichorioideae and low values of *Erica*, and a very positive sample score, which is probably related to a punctual perturbation. For the rest of the samples, PC2 could be indicating a larger contribution of heathlands when scores are negative and of pine woodlands when scores are positive (Fig. 4).

4. Discussion

4.1. Land-use change affects the accumulation of GRSP in seagrass sediments

The most significant terrestrial landscape change (CP-5) occurred abruptly at c. 585–560 cal. yr BP and was related to the start of a large agrarian perturbation with increasing percentages of *Cerealia*, *Cannabis/Humulus* t., *Vitis* and *Juglans*, and also a significant presence of *Castanea* and *Olea* (Fig. 5). Richness and turnover increased during this event, indicating deep transformations in landscape composition involving a fast, abrupt replacement of woodlands and scrublands by crop fields and associated weeds and other anthropogenic indicators (see Supplementary Information). The end of this intensive agrarian

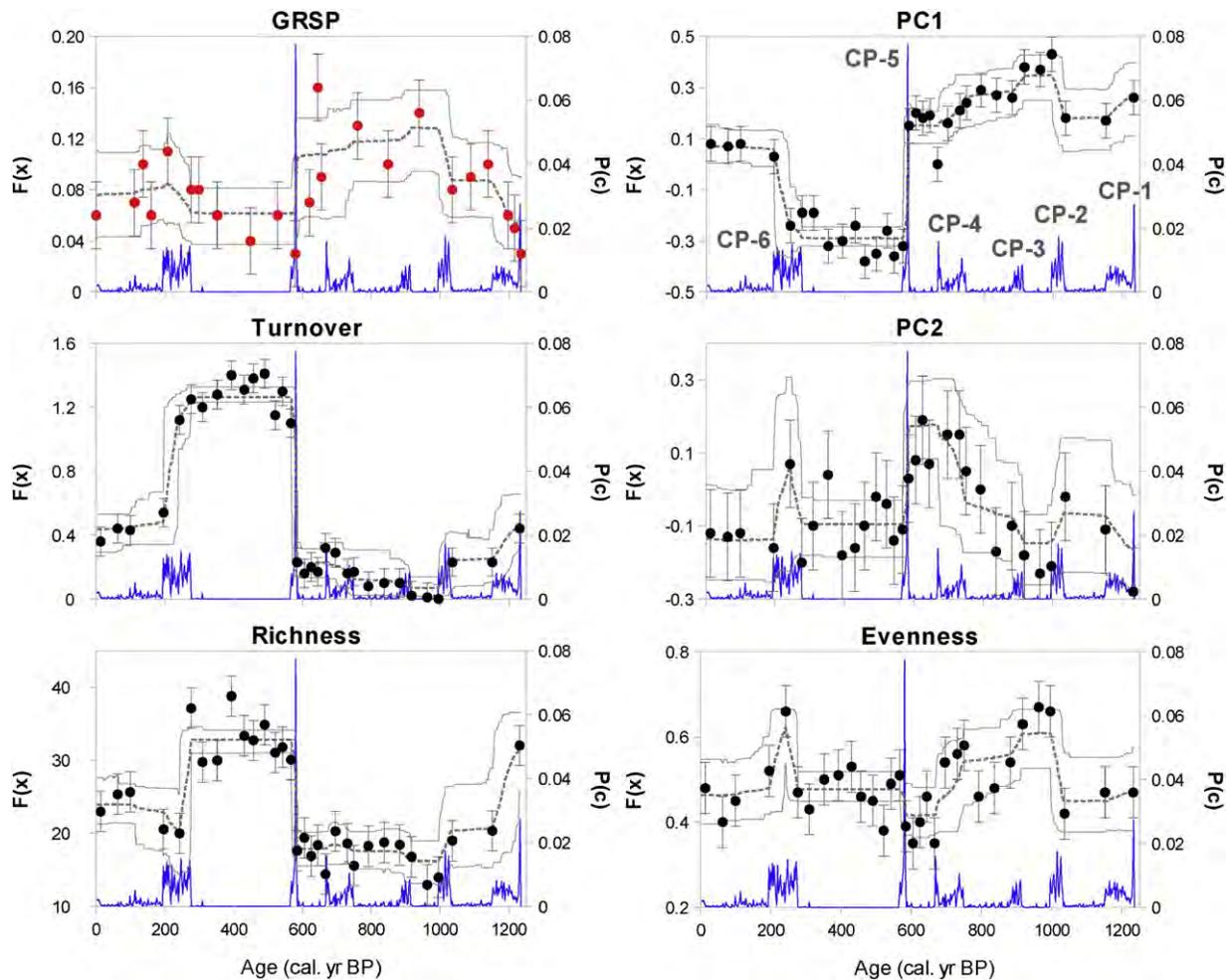


Fig. 3. Change-point functions for GRSP, turnover, PC1 and PC2 scores, richness and evenness. Dots indicate the original data, and vertical lines are the noise estimation. Thick intermittent grey curves are the average inferred functions $[F(x)]$ after 120,000 functions, while thinner grey lines define the 95% interval ranges. Blue curves show the probability of change-point $[P(c)]$, indicating that CP-5 was the most abrupt change for all the variables, while the other detected change-points were less abrupt.

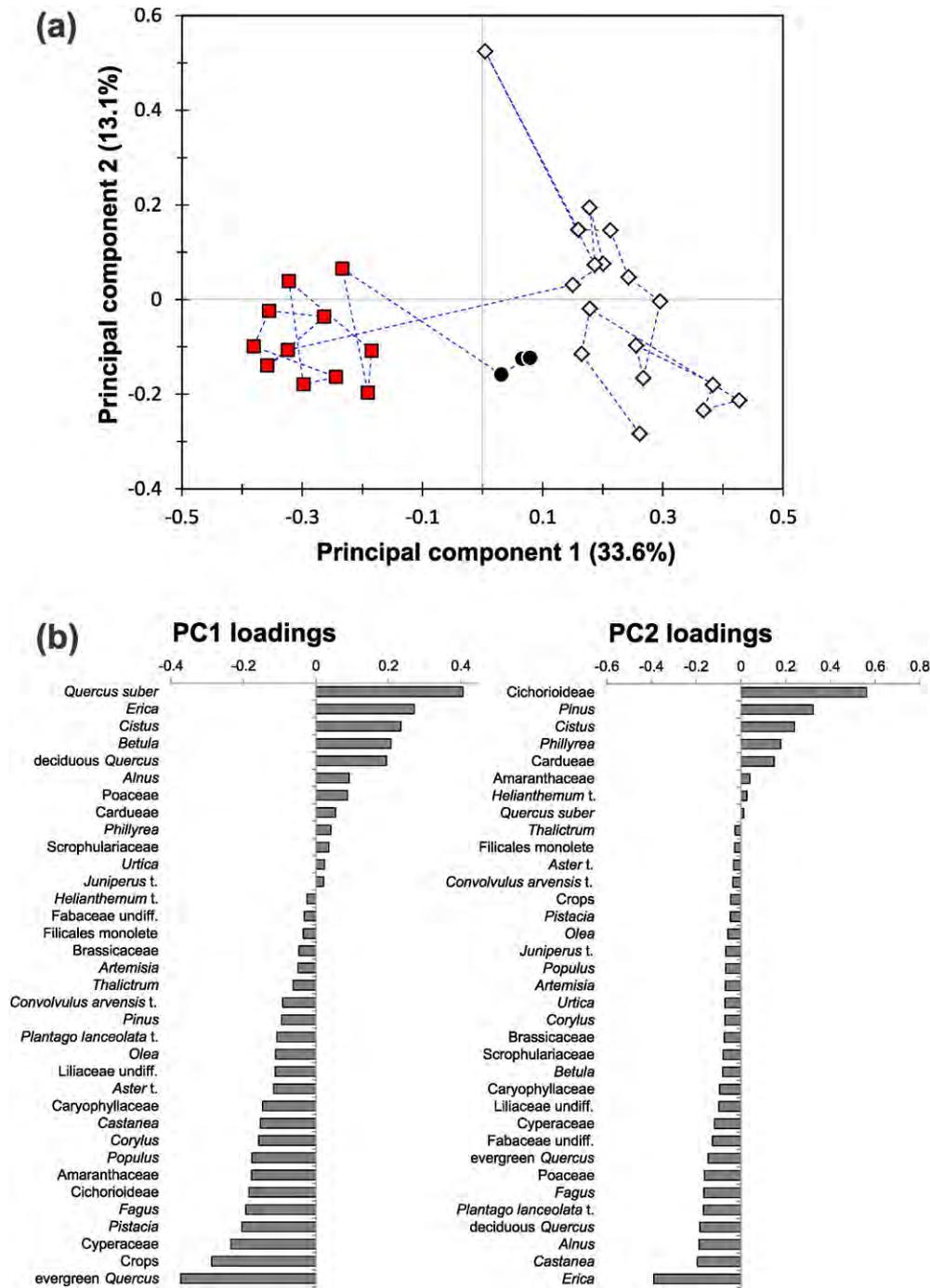


Fig. 4. Principal component analysis results of core 2000 palynological data (data from López-Sáez et al. (2009) and available at the European Pollen Database). a) PCA biplot of samples scores along the first and second principal component axes. Sample points are connected in chronological order, with the base (oldest samples) of the sequence in the lower right. White squares indicate samples before CP-5, when the main compositional change occurred. Red squares indicate samples between CP-5 and CP-6, the period in which agrarian activities were more extensive, changing the landscape structure, and a drop in GRSP content is detected. Black dots indicate samples after CP-6. b) Species loadings for the two principal components. “Crops” refers to the sum of *Juglans*, *Vitis*, *Cannabis/Humulus* t. and Cerealia.

period, indicated by CP-6 (c. 280–190 cal. yr BP), was less abrupt than the previous event and meant a partial increase in the GRSP content in the mat (Fig. 5). The impact that agrarian activities had on the structure of the landscape is also evident in the PCA biplot, as the analysis clearly differentiated samples older than CP-5, samples between CP-5 and CP-6, and samples younger than CP-6 (Fig. 4). This period of intensive farming (CP-5 to CP-6) was coeval with the phase of low GRSP content

in the mat (Fig. 5), supporting the idea that agrarian activities negatively affect AMF communities and the production of GRSP in soils (Fokom et al., 2012; Rillig et al., 2003; Violi et al., 2008).

Even though the period from c. 585–560 to 280–190 cal. yr BP showed the maximal expansion of cultivated land for the last c. 1250 cal. yr BP with a clear drop in GRSP content, two other short-term agricultural events were also identified. The first short-term

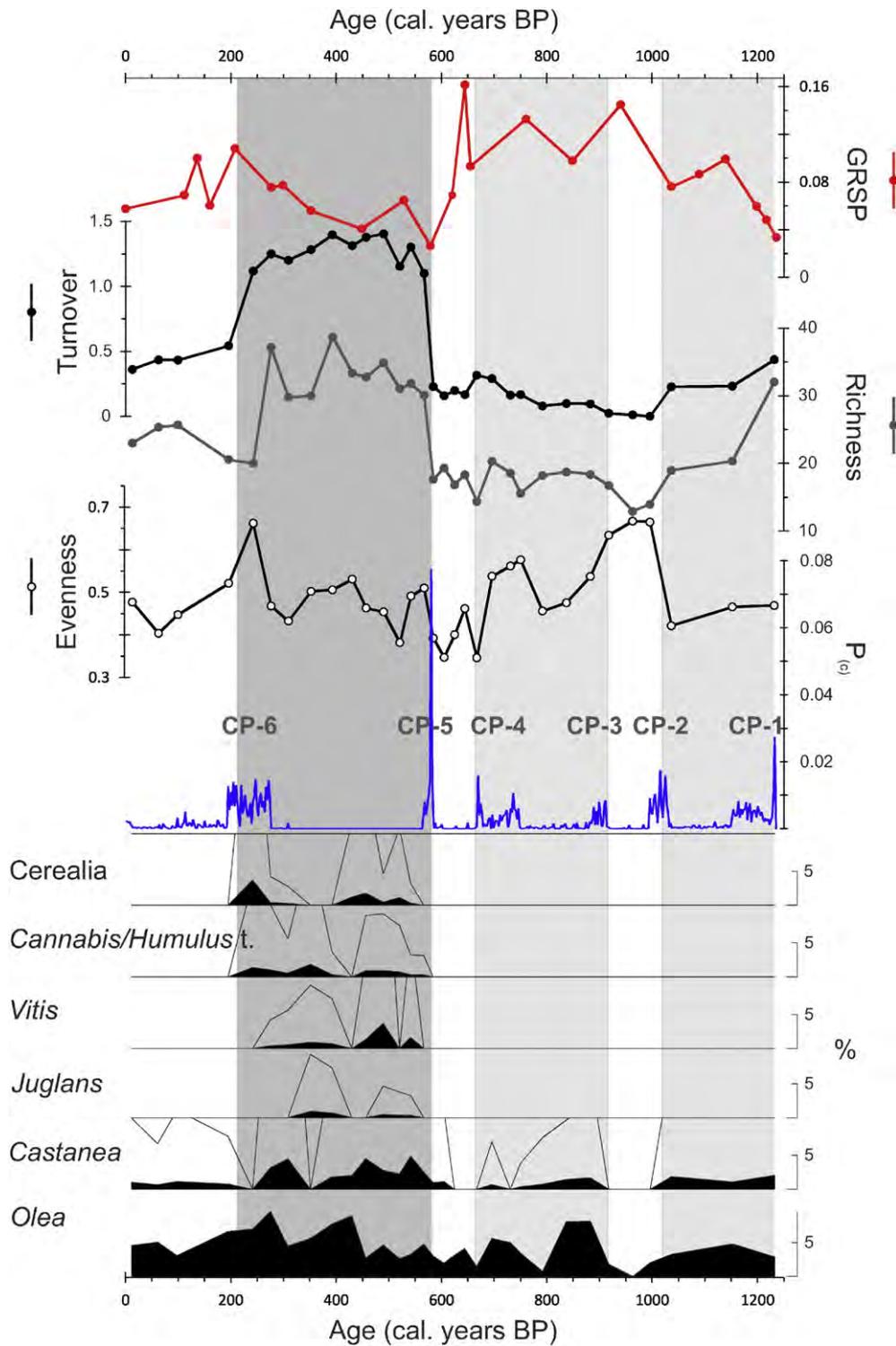


Fig. 5. Synthesis of the last 1200 years of landscape change detected after the study of a *P. oceanica* mat (core 2000, Portlligat bay, NE Spain). Richness, evenness and turnover are compared with GRSP content and palynological indicators of crops (data from López-Sáez et al. (2009) with the chronology of Serrano et al. (2012)). The filled silhouettes show the percentage curves of the taxa, the unfilled silhouettes show the $\times 10$ exaggeration curves. Probability of change-point indicates significant, common events of change in GRSP contents and landscape structure. Vertical grey bands highlight phases with an importance of agrarian activities (the dark grey band for the largest period, and the two light grey bands for the two short-term periods).

event occurred from CP-1 (c. 1235–1150 cal. yr BP) to CP-2 (c. 1030–990 cal. yr BP), while the second one was framed between CP-3 (c. 915–880 cal. yr BP) and CP-4 (c. 750–665 cal. yr BP) (Fig. 5). These events presented increasing percentages of *Castanea* and *Olea* that, although did not completely change the structure of the landscape, were accompanied by small decreases in the content of GRSP in the

mat (Fig. 5). Richness values were slightly higher during such events, although not at the level detected during the largest agrarian disturbance. Evenness, on the contrary, tended to be lower, pointing out that land-use change towards agriculture involved a more uneven landscape (Fig. 5). Our results indicate that soil quality at the Portlligat bay catchment area has varied significantly during the last twelve centuries.

In particular, soil functions were affected during agrarian periods, and partially recovered when crops were no longer abundant, showing some degree of resilience.

4.2. Factors determining the GRSP terrestrial-marine transport/accumulation

After GRSP was found in surface sediments from intertidal seagrass meadows and wetlands, and coastal coral reefs, a conceptual model of the possible factors affecting the terrestrial-marine connectivity driving its transfer to the marine environment was proposed (Adame et al., 2012). Climate (mainly rainfall), runoff potential (mainly land cover

and associated soil erosion) and distance of the marine environments from the shore were proposed as the main factors (Adame et al., 2012). Based on single modern sampling points distributed along different coastal settings, it was pointed out that higher content of GRSP in marine sediments should be expected when extensive forest clearance occurred, as the associated soil erosion would increase sediment runoff, enhancing the connectivity (Adame et al., 2012). However, in our long-term study, periods with lower GRSP content in the mat were coeval with declines in natural tree and shrub cover, prompted by the introduction of crops (Fig. 5). It was also proposed that the higher the rainfall, the larger the GRSP transfer to the marine environment

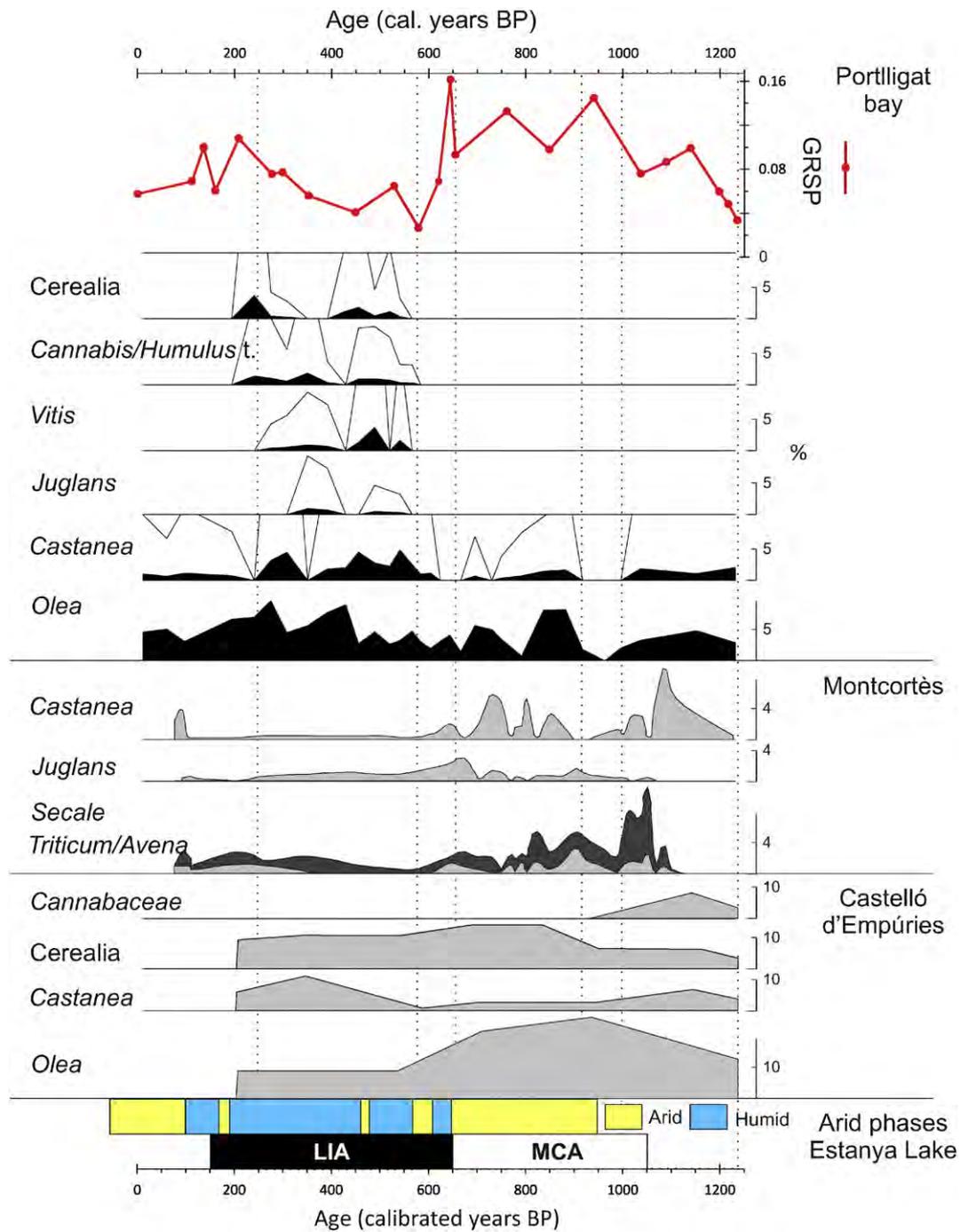


Fig. 6. Comparison of the changes detected in GRSP content and palynological indicators of agrarian activities from the core 2000 (*Posidonia* mat at Portlligat bay) with some palynological indicators from other sequences located in the area: Montcortès (Rull and Vegas-Vilarrúbia, 2015) and Castelló d'Empúries (Burjachs et al., 2005). The arid and humid phases reconstructed in the Estanya Lake by Morellón et al. (2011) are also included, as well as the chronology of the Little Ice Age and the Medieval Climate Anomaly. Fig. 1 shows the location of the cited records.

(Adame et al., 2012). The reconstructed arid and humid phases in the nearby Estanya Lake and the precipitation patterns for NE Iberia (Morellón et al., 2011, 2012) highlight a more humid Little Ice Age (c. 650–150 cal. yr BP) than the Mediaeval Climate Anomaly (c. 1050–650 cal. yr BP) (Fig. 6). We have not detected higher GRSP content in the mat during the Little Ice Age, which partially overlaps with the period of extensive agrarian activities and lowest GRSP contents in the mat (Fig. 6). Additionally, the agrarian history detected by pollen analysis in the Portlligat bay coheres with the histories reconstructed regionally (Fig. 6), supporting that the GRSP signal recorded in the sediment core from the *Posidonia* mat has a regional character. Our findings, hence, suggest that one of the most important environmental factors driving GRSP content changes in the marine environment (i.e., *P. oceanica* mat) was the production of GRSP in the continental soils, apparently overriding the importance of soil erosion and rainfall.

Finally, although the speed of the GRSP transference and deposition from the continent to the marine environment is unknown, it appears to be fast. Firstly, due to the fast degradation of the protein, as if the GRSP transference and deposition was slow, no GRSP would have been detected in the *Posidonia* mat. Secondly, as we did not find any significant decoupling between changes in the structure of the landscape inferred by pollen analysis and changes in GRSP content in the mat (Figs. 5 and 6), confirming what ecological and soil studies state: agrarian activities negatively affect GRSP production, hence affecting soil quality (i.e., Rillig et al., 2003; Violi et al., 2008; Fokom et al., 2012).

4.3. A new palaeoenvironmental tool to evaluate soil quality

The negative effects that anthropogenic disturbances, especially agriculture, and habitat loss have on soils via the decrease of AMF association with roots, and the suggestion that GRSP produced by AMF can be used as indicator of soil quality, has boosted research from both farming and conservation point of views. For example, Bedini et al. (2013) showed that GRSP content was higher in organically-managed fields than in conventionally-managed ones. Moreover, Turrini and Giovannetti (2012) make an interesting evaluation of the importance of AMF preservation in protected areas worldwide, as they play a central role in the maintenance of ecosystem services, soil functions (as nutrient cycling) and plant community dynamics. These microscopic environmental changes, which can go unnoticed, are ecologically important, and provide invaluable information about the health of a given soil ecosystem.

Seagrass mat sediments have proven to be valid for unravelling past patterns in GRSP, although it is likely that other anoxic, accreting environments could be also valid. The lack of oxygen is the main feature that the palaeoenvironmental archives have to have in order to avoid the degradation of GRSP and its proper long-term accumulation for a reliable palaeoenvironmental reconstruction. However, a further understanding of the archives that are of potential use is required as changes in GRSP content could be related to other environmental stressors apart from regional land-use change. The marine coastal setting of *Posidonia* mat is ideal because there is not local signal (such as in situ plants with AMF in their roots producing GRSP). Hence, the information obtained reflects the regional landscape-scale signal that can be comparable with other landscape-scale indicators such as pollen of continental plants. However, plants growing in the fresh-water aquatic environments of peatlands or lakes do form symbiotic associations with AMF (Chaubal et al., 1982; Clayton and Bagyaraj, 1984; Bagyaraj et al., 1979; Beck-Nielsen and Madsen, 2001; Bohrer et al., 2004), so regional and local signals could be mixed.

Unravelling past patterns and processes of the AMF community via changes in GRSP could constitute a valuable tool to analyse past trends in soil quality, establish soils' ecological baselines, and assess whether changes in GRSP concentration are driven not only by changes in land-use but also by other environmental stressors. Testing more

archives could open the possibility to assess past changes in soil quality at different parts of the world that faced dissimilar land-use scenarios.

5. Conclusions

Exploring the previously reported terrestrial-marine connectivity suggested by the accumulation of GRSP in seagrass mat sediments, this study reports the first attempt to use changes in GRSP content in a *P. oceanica* mat to reconstruct long-term changes in continental soil's quality. Unlike terrestrial soils, our findings demonstrate that the anoxic environment created in seagrass mats prevents the complete degradation of GRSP once it is buried, allowing a palaeoecological approach to be employed. Our results also suggest that the negative impact of past intensification of land-use related to agrarian activities on the AMF communities is captured in the seagrass mat via changes in GRSP content. It is likely that, apart from seagrass mat sediments, other marine sediments and continental anoxic environments such as lake sediments could be also of use. Their study could open the opportunity to reconstruct patterns and processes in past changes in soil quality in many areas, which is of interest to both conservation and the farming community.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.gloplacha.2015.08.004>.

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