Accepted Manuscript

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PII: S0304-3770(16)30059-6
DOI: <http://dx.doi.org/doi:10.1016/j.aquabot.2016.05.009>
Reference: AQBOT 2866

To appear in: *Aquatic Botany*

Received date: 29-10-2015
Revised date: 24-5-2016
Accepted date: 24-5-2016

Please cite this article as: Kaal, Joeri, Serrano, Oscar, Nierop, Klaas G.J., Schellekens, Judith, Martínez Cortizas, Antonio, Mateo, Miguel-Ángel, Molecular composition of plant parts and sediment organic matter in a Mediterranean seagrass (*Posidonia oceanica*) mat. *Aquatic Botany* <http://dx.doi.org/10.1016/j.aquabot.2016.05.009>

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Molecular composition of plant parts and sediment organic matter in a Mediterranean seagrass (*Posidonia oceanica*) mat

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Graphical abstract

Highlights

- Analytical pyrolysis applied to *Posidonia* seagrass organs and marine mat deposit
- *Posidonia* phenolic composition dominated by *p*-hydroxybenzoic acids
- Peat-like mat deposits composed predominantly of root, rhizome and sheath materials
- We suggest a link between *Posidonia* chemistry and C accumulation in mats

Abstract

Posidonia oceanica forms extensive peat-like deposits (mats) in Mediterranean coastal waters, which have a potential as carbon sinks and archives of environmental change. Nonetheless, the organic chemistry of both *P. oceanica* plant materials, as well as the environmental and diagenetic effects on the composition of its detritus, is poorly understood. We analyzed plant organs of *P. oceanica* and the coarse organic matter from a mat core spanning 750 yrs using pyrolysis techniques (PY-GC-MS and THM-GC-MS) to improve our understanding of their molecular properties and their preservation upon mat development. It appeared that leaf sheaths, roots and the outer parts of rhizomes were composed predominantly of phenolic constituents based on *p*-hydroxybenzoic acid (*p*-HBA), which is atypical for vascular plants, in addition to carbohydrates and lignin. The inner rhizome and leaf blades had a different composition, with predominance of carbohydrates. The seagrass detritus in the mat was composed mainly of *p*-HBA phenolic material and carbohydrates, confirming earlier studies showing that the coarse detritus in the mat originates largely from *Posidonia* sheath, root and rhizome debris. The intermolecular arrangement of the *p*-HBA remains unclear, as they seem to correspond to ester-bound phenols yet their persistence in the mat attributes them a refractory nature. Variations in molecular composition within the mat are probably associated with diagenetic alteration of *P. oceanica* detritus, such as the decomposition of minor amounts of fatty acids, chlorophyll and syringyl lignin, and selective preservation of *p*-HBA relative to carbohydrates. This work lays the foundations for a molecular understanding of carbon storage within the mats and the environmental changes recorded therein.

Keywords: seagrass, *Posidonia oceanica*, molecular composition, analytical pyrolysis

1. Introduction

Some terrestrial vascular plants living in coastal environments re-adapted to life in the sea more than 100 million years ago, evolving into marine phanerogams (seagrasses). Since then, seagrasses have inhabited shallow (<40–60 m depth) coastal areas worldwide, forming extensive and rich ecosystems along most of the world's coasts. Among the many services they provide (Orth et al. 2006), two have been recognized recently and are related to the accumulation of biogeochemical elements in the underlying sediments. Firstly, even though seagrasses only occupy 0.1% of the ocean surface, their ecosystems are considered a significant carbon (C) sink (Duarte et al. 2005, 2010; Mcleod et al. 2011). This can be explained by the fact that a few large seagrass species, mostly inhabiting protected and shallow environments, produces substantial accumulation of seagrass detritus buried in marine peat-like deposits. The most extraordinary example is exhibited by the Mediterranean endemic seagrass *Posidonia oceanica* L. (Delile) (Lavery et al. 2013), which forms up to several meters thick and several thousand years old deposits (Boudouresque et al. 1980; Mateo et al. 1997; Serrano et al. 2012, 2014) also known as 'mat' (or 'matte'; Boudouresque et al. 1980). The organic C stocks beneath *P. oceanica* meadows have been estimated to $0.5\text{--}12 \times 10^{15}$ g C for the Mediterranean Sea (Serrano et al. 2014). The role of *P. oceanica* in sequestering and storing CO₂ on centennial to millennial scales in the mats is being evaluated as a novel aspect of the global C cycle (Fourqurean et al. 2012; Duarte et al. 2013) and the recent focus on C trading has intensified the interest in quantifying the capacity of these ecosystems to store C (Lavery et al. 2013). Secondly, these mats form a repository of palaeo-environmental information (Mateo et al. 2002, 2010; López-Sáez et al. 2009; Serrano et al. 2011, 2012, 2013; Lopez-Merino et al. 2015), very much like terrestrial peat archives. Although a wealth of technical and scientific literature is available on the role of seagrass sediments as C sinks and palaeoarchives, these fields of research have critical knowledge gaps concerning some fundamental issues: (i) the origin of the accumulated C (i.e. the sources and composition of organic C retained in the sink), and (ii) the post-burial diagenetic alteration of *P. oceanica* mats (factors that control degradation/preservation dynamics).

Similar to other seagrasses, *P. oceanica* consists of long strap-like leaves connected to the rhizome through a leaf sheath (Fig. 1). Over the lifetime of an individual plant, leaves fall through abscission (by currents and waves, or during senescence), while the leaf sheaths remain

attached to the rhizome. As such, various layers of sheaths can be identified on mature specimens, and confer a “hairy” appearance to the rhizome (Hemminga and Duarte, 2000). The root is often short but strong and grows adventitiously. The detritus of belowground organs (rhizomes, roots and sheaths), which constitute the vast majority of the plant’s dry weight (Alcoverro et al. 2001), are incorporated directly into the seabed, thereby protected against subsurface currents, wave action and herbivores. The aboveground organs (leaves), on the other hand, are largely eroded from the meadows and exported to adjacent systems including supra-littoral environments forming the so-called banquettes and agaeropili¹ (Mateo et al. 2002; Simeone and De Falco 2012). No coarse (>1 mm) leaf detritus had been found in seagrass sedimentary debris (Serrano et al. 2012). Knowledge on the *in situ* decomposition of *P. oceanica* detritus within the mats is limited. It involves an initial phase of abiotic leaching of fast-cycling components, consisting of low molecular weight (LMW) phenolic and carbohydrate compounds, followed by microbial and detritivorous breakdown of more refractory materials (Kenworthy and Thayer 1984; Harrison 1989; Peduzzi and Hernle 1991; Enríquez et al. 1993; Opsahl and Benner 1993; Mateo and Romero 1996). The second (biotic) decomposition phase is a much slower process (Romero et al. 1992), and allows the more refractory tissues to accumulate in mats. The mechanism behind this slow biotic decomposition after *P. oceanica* detritus enters the mat is largely unknown, but the likely candidates are anoxic conditions and biochemical stability of the organic matter (Mateo et al. 1997; Pedersen et al. 2011).

Most knowledge on the chemical composition of *P. oceanica* corresponds to foliar tissue. The leaves consist primarily of polysaccharides (roughly 60%, two thirds of which corresponds to holocellulose), and the remaining material is largely composed of various phenolic constituents, more specifically lignin, tannin and free and ester-bound phenolic acids (Zapata and McMillan 1979; Agostini et al. 1998; Arnold and Targett 2002; Torbatinejad et al. 2007). Reported lignin contents of *P. oceanica* leaves and agaeropili are in the range of 25–30% of their dry weight (Ncibi et al. 2009; Khiari et al. 2010; Bettaieb et al. 2015). However, this is operationally defined Klason lignin (acid-insoluble residues), only a small portion of which corresponds to “true” polyphenolic lignin (Klap et al. 2000). Tannins stored in tannin cells

¹ It is worth mentioning here that this *P. oceanica* beach material causes annoying flies, odours and beach size reduction in touristic areas along the Mediterranean coast, and associated expenditure on removal (Khiari et al. 2010; Simeone and De Falco 2012; Plis et al. 2014). On the other hand, it is a potential feedstock for biofuel, animal fodder, cellulose and activated charcoal (Torbatinejad et al. 2007; Ncibi et al. 2009; Coletti et al. 2013; Bettaieb et al. 2015), even though the ecological consequences of such usages have not been assessed. Knowledge on the molecular composition of *Posidonia* raw materials is obviously valuable to these potential future applications.

account for ca. 5–10% in *P. oceanica* leaves (Pergent et al. 2008). Roughly 2% of the dry weight of *P. oceanica* leaves corresponds to free or ester bound (water- or aqueous/organic solvent mixtures-extractable) phenols (Agostini et al. 1998), the molecular composition of which has been examined with relative scrutiny, as reviewed by Heglmeier and Zidorn (2010). The nature of the “remaining” phenols (total phenols minus lignin, tannin and extractable phenols), which account for an estimated 50% of the total phenols in *P. oceanica* leaves, is poorly understood. Other organic components in *P. oceanica* leaves include chlorophyll pigments, cutin in the cuticles and wax lipids. Molecular examinations of the composition of the rhizomes and particularly the roots are less frequent. Klap et al. (2000) demonstrated that the rhizome and roots have higher lignin contents than the leaves.

The molecular composition of seagrass materials can be determined using techniques such as nuclear magnetic resonance and infrared spectroscopy of bulk samples, and gas and liquid chromatography of extractable phenols. Other approaches rely on the chemical breakdown, usually by CuO oxidation (Hedges and Mann 1979; Opsahl and Benner 1993), or thermal breakdown, such as pyrolysis (Sáiz-Jiménez 1988), of the organic matter followed by gas chromatography and mass spectrometry (GC-MS) for identification of the products. Here we will focus on analytical pyrolysis techniques, i.e. conventional analytical pyrolysis (PY-GC-MS) and Thermally assisted Hydrolysis and Methylation (THM-GC-MS), the latter of which uses a derivatization agent simultaneously to thermal treatment to enhance the structural information obtained for polar aliphatic and phenolic materials (Wampler 1999; Challinor 2001; Shadkani and Helleur 2010). Only Gadel and Bruchet (1987) and Klap et al. (2000) studied *Posidonia oceanica* materials by PY-GC-MS, but they analyzed specific (and unquantified) fractions of humic/fulvic fractions and milled-wood-lignin preparations, respectively. As far as we know, THM-GC-MS has not been applied to *P. oceanica* materials yet. Kristensen et al. (2009) analyzed an undefined seagrass, while Maie et al. (2006) analyzed leachates from turtle grass (i.e. *Thalassia testudinum*) by this technique. Hence, contrary to the water- or aqueous/organic solvent mixtures-extractable fraction, detailed molecular information on the inextricable biocomponents, which constitute the vast majority of the plants’ dry weight, is very limited.

We analyzed different organs of *Posidonia oceanica* and their debris found along a 750-yr sedimentary record from the Mediterranean Sea by PY-GC-MS and THM-GC-MS. From the mat sediments, we analyzed the coarse (>1 mm) fraction of the organic matter (COM), which mainly consisted of sheath, root and rhizome debris (Serrano et al. 2012), to avoid, or at least

reduce to a minimum, the contribution from allochthonous sources (e.g. macroalgae and terrestrial detritus; Holmer et al. 2004) to the results obtained. The specific objectives were to determine the molecular composition of different organs of *P. oceanica*, compare that to the composition of the detritus in the mat core, and, ultimately start unraveling the processes involved in mat composition and diagenesis.

2. Materials and Methods

2.1 Study site, sampling and laboratory procedures

Plants of *P. oceanica* and a seagrass sediment core were sampled in Portlligat Bay (NW Mediterranean, Girona, Spain). Portlligat is a shallow bay (10 m deep) connected to the sea through a 213 m opening to the NE (Serrano et al. 2012). The seafloor is dominated by a consistent meadow (with some interspersed sandy bioclastic areas) that covers approximately 69% of the bay (94,315 m²). The sampling site (3 m depth) was chosen because it is part of a marine protected area and has a large *P. oceanica* meadow in a good state of preservation. Furthermore, the study site is located at a distance of only 2 m from a *P. oceanica* barrier formation, which ensured that there would be a thick, well developed *P. oceanica* mat (images provided in the digital map).

Posidonia oceanica (L.) Delile plants from Portlligat Bay were oven-dried and dissected into their main organs. The leaf sample was obtained from the middle section of ca. 20 cm long blades, avoiding sections with epiphyte populations. Three leaf sheath samples (inner, centre and outer sheaths) were obtained, with the inner sheaths (close to the active leaf position) corresponding to relatively recently abscised leaves. No attempt was made to determine the age of the sheaths by lepidochronology (Peirano 2002), but a rough indication of the ages of these materials is <1, 5–10 and 10–15 years, from the inner to the outer layers. The dark-coloured exterior (epidermis) of the rhizome and the reddish inner sections were also separated. Finally, no attempts were made to dissect the root materials, which had a homogenous appearance. We selected short (1 cm) and thick (1 mm) dark-coloured roots from the rhizome. Hence, even though the sampled plants were living specimens, the sheaths and

root materials correspond to dead plant fractions. The plant organ samples were ball-milled to powder before analysis.

The core was sampled from a floating drilling platform using a corer that consisted of a 150 cm long stainless steel pipe with an 88 mm (outer diameter) PVC pipe inside. The core was sealed at both ends, transported to the laboratory, and stored at 5 °C before processing. The core was cut into 1 cm slices and each slice/sample was weighed before and after oven drying to constant weight at 60 °C. The samples were re-suspended in seawater and wet-sieved (1 mm mesh). The coarse fraction (>1 mm) was then re-suspended in seawater and sorted into organic (coarse organic matter, COM, >1 mm) and inorganic fractions (Serrano et al. 2012). A total of 14 samples of COM along the core were selected for molecular characterization, focusing on the debris-rich surface layer (ca. 25 cm, 0-400 cal yrs BP) (Fig. 2). Serrano et al. (2012) determined that the vast majority of the organic matter in the bulk sediment samples (same samples as the ones studied here) consisted of COM (73 ± 11%; average and standard deviation) (Fig. 2). The COM consisted of sheath-, root- and rhizome-derived *P. oceanica* remains. The proportion of sheaths in the COM ranged from 4 to 33%, while root and rhizome contents varied from 34 to 95%, and from 2 to 43%, respectively (Fig. 2).

2.2 Analytical pyrolysis techniques (PY-GC-MS and THM-GC-MS)

Conventional PY-GC-MS was performed with a Pyroprobe 5000 (CDS Analytical) coupled to a 6890N GC and 5975B MSD (Agilent Technologies). Samples (recent and up to 750 yr-old plant organs stored in the mat) were embedded in glass wool-containing fire-polished quartz tubes. The samples were pyrolyzed at 650 °C for 10 seconds (heating rate 10 °C/ms). The interface and GC inlet were set at 325 °C and 320 °C, respectively. The GC-MS transfer line was held at 320 °C, the ion source (electron impact mode, 70 eV) at 230 °C and the quadrupole detector at 150 °C, measuring fragments in the m/z 45–500 range. The GC used was equipped with a (non-polar) HP-5MS 5% phenyl, 95% dimethylpolysiloxane column (length 30 m; internal diameter 0.25 mm; film thickness 0.25 µm). Helium was used as the carrier gas (constant gas flow, 1 ml/min). The oven of the GC was heated from 40 to 320 °C at 7 °C/min for the recent plant fractions and at 20 °C/min for the plant debris from the seagrass core.

The seven plant organs of *P. oceanica* and three selected samples from the mat sequence were also analysed by THM-GC-MS. The conditions were as those used for conventional PY-GC-MS (with the GC temperature program of the seagrass mat), but prior to the analysis a droplet of tetramethyl ammonium hydroxide (TMAH, 25% in water, Sigma-Aldrich) was added, allowed to impregnate into the sample for one hour, followed by solvent evaporation at 50 °C. The TMAH treatment was repeated to ensure full contact between the sample and excess TMAH reagent.

Compounds were identified using the NIST '05 library and available literature. Relative proportions were calculated as the percentage of the total quantified peak area (TQPA), using the main fragment ions (m/z) of each product, creating two separate datasets for PY-GC-MS (42 compounds) and THM-GC-MS (58 compounds). This is a semi-quantitative exercise that allows more detailed comparisons between samples than visual inspection of chromatograms alone.

3. Results and Discussion

3.1 Recent *Posidonia* material

3.1.1 Pyrolysis-GC-MS

The leaf sheath pyrolyzates were dominated by phenol (52–57% of TQPA), which was also the main pyrolysis product from the root (44%) and outer rhizome (37%), but presented lower abundance in the inner rhizome and leaf blade (7% and 11%, respectively) (Table 1; Figure 3; left column). Phenol and methylphenols (cresols) are potential pyrolysis products of many biopolymers, including carbohydrates, lignin and proteins. The sheath and root materials (3 sheath and 1 root sample, $n=4$) also produced significant proportions of 4-hydroxybenzoic acid ($4 \pm 3\%$), 4-hydroxybenzoic acid methyl ester (ME) ($0.8 \pm 0.7\%$) and traces of 4-methoxybenzoic acid ME (anisic acid) ($0.1 \pm 0.1\%$), which originate from *p*-HBAs. The significant proportions of these non-decarboxylated compounds, 4-hydroxybenzoic acid in particular, suggest that the phenols are probably largely *p*-HBA decarboxylation products (see scheme in Fig. 4). In addition, the presence of methylated carboxylic groups suggests that methylation already occurred within the plant (i.e. before burial) and therefore that at least part of the *p*-HBA are ester-bound.

Indeed, *p*-HBA were also detected among the extractable free and ester-bound phenols in previous studies on *P. oceanica* (Zapata and McMillan 1979; Cariello et al. 1979; Cuny et al. 1995; Agostini et al. 1998; Dumay et al. 2004; Pergent et al. 2008).

Cinnamyl structures were recognized by 4-vinylphenol ($n=7$, $0.4 \pm 0.5\%$), 4-vinylguaiacol ($1.3 \pm 0.8\%$) and 4-vinylsyringol ($0.3 \pm 0.2\%$), which are produced after decarboxylation of *p*-coumaric, ferulic and sinapic acids, respectively (Ralph and Hatfield 1991; Martín et al. 1995) (Fig. 4). The leaf had larger proportions of these compounds, particularly of 4-vinylphenol and -guaiacol. The other guaiacols ($5 \pm 2\%$) and syringols ($0.8 \pm 0.3\%$) largely originate from “true” lignin moieties (Fig. 4). Guaiacyl (G) products were more abundant than syringyl (S) products, and the S/G ratio ranged from 0.11 (outer rhizome) to 0.29 (inner sheath). Catechol (1,2-dihydroxybenzene) was more abundant in the rhizome materials (10%) than in the other plant parts ($2 \pm 1\%$). Even though demethylation of methoxy groups in degraded lignin may produce catechols (Filley et al. 2002), this compound is often associated with the presence of tannins (Galletti and Reeves 1992), which explains their large proportion in extant rhizomes. The sum of all phenol derivatives (phenols, *p*-hydroxy benzoic acids, cinnamyl, guaiacyl and syringyl lignin, and catechol) was $58 \pm 21\%$.

Carbohydrate products (Table 1) were the second most abundant group of pyrolysis products ($39 \pm 18\%$). These products were particularly abundant in the leaf blade (56%) and inner rhizome (72%). Levoglucosan (1,6-anhydro- β -D-glucopyranose) was the most abundant product ($17 \pm 10\%$), while the other carbohydrate products were mainly furans, furaldehydes, pyrans and levoglucosenone. A product with *m/z* 57, 69, 70 and 85 was tentatively identified as 5-hydroxymethyl-2-dihydrofuraldehyde-3-one, similar to the tetrahydro-analogue (M^+ 144) reported by Pouwels et al. (1989). Carbohydrates were more abundant in leaves and inner rhizomes than in sheaths, outer rhizomes and roots.

Leaf blade material gave significant peaks for phytadienes (8% of TQPA) from chlorophyll's phytol chain (Van de Meent et al. 1980), not detected in any other fraction. This confirms that the sheaths do not contain chlorophyll (Haznedaroğlu and Akarsu 2009). The only N-containing pyrolysis products identified were indole and diketodipyrrole. Indole probably originates from tryptophane in proteinaceous biomass (Tsuge and Matsubara 1985), and was enriched in the leaf material (2.5%) and scarce in the other samples ($0.1 \pm 0.1\%$), suggesting that proteins are relatively abundant in the leaf blade compared to other plant organs. Diketodipyrrole (from hydroxyproline) had a similar distribution: 0.5% in leaf and $0.1 \pm 0.1\%$ in

other tissues. C₁₆-fatty acid (palmitic acid) is also concentrated in the leaf (1.5%, $0.6 \pm 0.5\%$ in the other tissues). Finally, two pyrolysis products (#33, #42, Table 1) remained unidentified ($0.3 \pm 0.2\%$), one of which might correspond to an alkylcyclohexane (#42).

3.1.2 Thermally assisted hydrolysis and methylation (THM-GC-MS)

The THM products of most tissues were extraordinarily dominated by the methylated derivative of 4-hydroxybenzoic acid (4-methoxy benzoic acid ME); especially the sheath (94–98% of TQPA) and root (92%) samples, but also the outer rhizome (68%) (Table 2; Figure 3; right column). Leaf blade (10%) and inner rhizome (3%) produced minor proportions of this compound. There is a strong correlation between the abundance of 4-methoxy benzoic acid ME (THM-GC-MS) and the sum of phenol, *p*-cresol and *p*-HBAs (PY-GC-MS) ($r^2=0.92$, $P<0.001$), strongly suggesting that they originate from the same precursor and hence that the phenols from PY-GC-MS are indeed decarboxylation products of *p*-HBA (as illustrated in Fig. 4). The similar link between high phenol proportions by PY-GC-MS and *p*-HBA derivatives by THM-GC-MS was reported by Rencoret et al. (2013) in coconut shell fibers (coirs), even though the *p*-HBA proportion was much lower and the lignin content much higher than observed here for *P. oceanica*. Hence, the exceptional dominance of *p*-HBA is an idiosyncratic feature of *P. oceanica*. Note that the *p*-HBA structures refer exclusively to compounds based on 4-hydroxybenzoic acid, and not other *p*-hydroxyphenyl compounds such as 4-hydroxyacetophenone or 4-hydroxy- β -[carboxymethyl]-cinnamic acid (sphagnum acid) (Killops and Killops 2005; Abbott et al. 2013).

The most abundant THM product in the inner rhizome was a product with characteristic *m/z* 101, 111, 187 and 219 (74% of TQPA). A similar mass spectrum was presented by Yeloff et al. (2008) and Spaccini et al. (2013) in *Juncus* seed coats and soil organic matter from agricultural sites, respectively. This compound was tentatively identified as a tetramethyl-substituted glucopyranoside derivative (Yeloff et al. 2008). The NIST '05 library gave a very good correlation with phenyl-O-2,3,4,6-tetra-O-methyl- α -D-glucopyranoside as well, which implies that this compound possibly corresponds to an aromatic glycoside, with a structure similar to that of phloridzin, present in *Posidonia* leaf extracts (Cuny et al. 1995). It was abundant in the outer rhizome (16%) and significant in the leaf blade (2%) as well. The molecular structures of the other carbohydrate products with specific *m/z* 75, 129 and 161 (Spaccini et al. 2013) and *m/z* 142, 57, 71, 127 (Schwarzingier et al. 2002) have not been elucidated yet. They were relatively

abundant in the leaf material. There was a strong discrepancy between the sum of polysaccharide products as identified by PY-GC-MS and THM-GC-MS. We believe that the unidentified glucopyranoside compound reflects only the LMW carbohydrates, which do not require efficient hydrolysis in order to become methylated by TMAH, and that the majority of the structural polysaccharides were underrepresented because of inefficient hydrolysis under strongly alkaline conditions (Van der Kaaden et al. 1984).

The leaf material was characterized by a large proportion of C₁₆-alkanoic acid ME (54%), which originates from either cutin in the cuticles or free palmitic acid (Viso et al. 1993). Minor peaks for C₁₄ and C₁₇-C₂₈ fatty acid MEs and C₂₂-C₂₈ ω -methoxy fatty acid MEs, with strong even-over-odd carbon numbers, were found as well (8% in leaf, 1 $\pm$ 1% in the other samples). The long chain fatty acid MEs and ω -methoxy fatty acid MEs were more abundant in the root and outer rhizome, which is in agreement with a suberin origin (Kolattukudy 2001; Nierop and Verstraten 2004). Traces of non-methylated phytadienes, the same compounds as detected after PY-GC-MS, were found among the THM products of the leaf material only (0.4%). Furthermore, the THM-GC-MS fingerprints consist of numerous guaiacyl (3 $\pm$ 2%) and syringyl (1 $\pm$ 1%) products, including *threo/erythro* 1,2,3-trimethoxypropyl-3,4-dimethoxybenzenes and -3,4,5-trimethoxybenzenes, which originate from particularly intact “true” lignin. Finally, 1,3,5-trimethoxybenzene and 2,4,6-trimethoxytoluene, which are markers of the A rings in condensed tannins (Nierop et al. 2005), were relatively abundant amongst the THM products of the rhizome (0.25-0.33%) and leaf blade (0.15%). Absence of gallic acid derivatives is in agreement with the prevalence of condensed tannins, not hydrolyzable ones, in seagrasses (Arnold and Targett 2002). This supports that tannin is the most likely origin of catechol from conventional PY-GC-MS (relation between catechol from PY-GC-MS and the sum of A products from THM-GC-MS; $r^2 = 0.83$; $P < 0.001$). Even though THM-GC-MS allows for an unequivocal identification of condensed tannins, their proportion cannot be estimated as the condensed tannin B rings cannot be distinguished from guaiacyl and syringyl lignin unless ¹³C enriched TMAH is used (Nierop and Filley 2008).

It is concluded that (1) the sheath and root are strongly dominated by *p*-HBA-based substances, with significant amounts of lignin, tannin and polysaccharides, (2) the outer rhizome is also composed primarily of *p*-HBA but has larger proportions of tannin and polysaccharides, (3) leaf material is composed primarily of free or cutin-derived *n*-alkanoic acids and polysaccharides, with smaller proportions of tannin and phytol, and (4) the inner rhizome is

composed primarily of polysaccharides, with minor proportions of tannin, *p*-HBA and lignin. The differences in relative proportions of these constituents in the different plant organs analyzed were in excellent agreement for the two techniques, but there were large discrepancies between the percentages obtained. It is clear that in these phenol-dominated samples THM-GC-MS has a better structure-resolving potential than PY-GC-MS, but the former seems to be strongly biased towards the *p*-HBA and low molecular weight carbohydrates, underestimating the abundance of lignin, tannin and structural high molecular weight polysaccharides.

3.2 *Posidonia* mat PY-GC-MS and THM-GC-MS

From the seagrass mat deposit, 14 COM samples were analyzed by PY-GC-MS. Only three samples were analyzed by THM-GC-MS, intended for support. Figure 5 shows example chromatograms of PY-GC-MS and the chromatograms obtained by THM-GC-MS. The large proportions of phenol (PY-GC-MS) and 4-methoxybenzoic acid ME (THM-GC-MS) show that the COM fraction of the debris stored in the mat is composed predominantly of *p*-HBAs-dominated detritus, which strongly suggests that it originates predominantly of sheath, root and outer rhizome tissues. This in agreement with Serrano et al. (2012) who reached the same conclusion based on the quantification of plant organs within the COM fraction (Fig. 2). The proportion of the glucopyranoside compound (THM-GC-MS) of the seabed materials is below 0.01% of TQPA, which is lower than even the least carbohydrate-containing plant tissues (Fig. 5). This probably corresponds to a selective degradation of the low molecular weight carbohydrate precursor of this compound, as this was not observed for the other carbohydrate products. Peduzzi and Herndl (1991) showed that the initial decay of the seagrass *Cymodocea* was characterized by an abiotic leaching of monomeric carbohydrates. Such a process may occur in the *Posidonia* mat detritus as well, as roughly one-sixth of the carbohydrates is water-soluble (Torbatinejad et al. 2007), that may leach from the material after cell wall disruption, while the structural polysaccharides would be unaffected. The slightly larger proportions of suberin-derived long-chain ω -methoxy fatty acid MEs in the THM products of the sample at ca. 690 yr can be explained by a combination of the good preservation of suberin and the high suberin content in roots, which dominate the COM fraction of this sample (Serrano et al. 2012).

The only PY-GC-MS product that could not be identified in any plant material but was present in all samples of the core was ferulic acid ME ($0.03 \pm 0.01\%$), which might originate from the rhizome (traces detected in milled-wood-lignin extracts; Klap et al. 2000) or, alternatively, from graminoid debris (Schellekens et al. 2015), which would imply a minor terrestrial source. In addition, a large discrepancy was found between the relatively high proportions of 4-hydroxy-2-methoxycinnamaldehyde in the mat samples ($0.2 \pm 0.1\%$) compared to the traces (0.006%) tentatively identified in the root sample. Nonetheless, we consider a significant contribution of detritus from other sources unlikely, because (1) the *p*-HBA-dominance is a peculiar property of *P. oceanica* that would not be recognized as such if the COM were to be a mixture of different plant species, and (2) because visual examination of the COM fraction allowed it to be quantitatively subdivided into the remains of *P. oceanica* sheaths, roots and rhizomes (Serrano et al. 2012).

Despite of the limited number of samples analyzed, some information can be deduced from the differences in relative abundances of the main PY-GC-MS product groups between (1) plant materials and the mat, and (2) variations with depth/age within the mat. The following discussion is based on seven main components (Fig. 6), calculated from the sum of individual pyrolysis products corresponding to each of them, and combined account for $93 \pm 1\%$ of TQPA. Note that within these groups individual products may have different depth trends. The proportion of phenols (see group labels in Table 1) in the seagrass debris stored in the mat fluctuated between 35 and 55%, which is in the same range of recent sheath, outer rhizome and root materials (Fig. 6a). There was a significant increase in the proportion of phenols in the seagrass debris preserved in the mat over the first 400 yr after burial ($n = 12$; $r^2 = 0.59$; $P < 0.005$), which corresponds to the organic debris-rich section of the deposit (Fig. 2), which might reflect selective preservation of their main precursor, i.e., *p*-HBA, during diagenesis. Furthermore, the proportions of *p*-HBA derivatives in the seagrass detritus in the mat exceeded that of the plant organs (Fig. 6b), suggesting that it is selectively preserved. On the contrary, the sum of carbohydrates, which was in the same range as the plant organs (Fig. 6c), decreased slightly during the first 400 yrs ($r^2 = 0.42$; $P < 0.05$), possibly indicative of selective degradation of polysaccharides during senescence. Note that two samples (estimated age 95 and 129 cal yrs BP) show deviations from the trends in both phenols and carbohydrates proportions (more carbohydrates, less phenols). Contrary to the main depth trend, which reflects mainly diagenesis, this kind of minor fluctuations may be useful to study the variations in source

materials and degradation/preservation state (Buurman et al. 2006) in seabed mat records, and their link to ecological controls, but a higher resolution analytical exercise would be required for such purposes.

Guaiacols ($7.0 \pm 1.4\%$; excluding 4-vinylguaiacol) and syringols ($1.3 \pm 0.3\%$; excluding 4-vinylsyringol) were relatively abundant in the mat (Fig. 6d and 6e) in comparison with recent plant organs. Interestingly, selective preservation of the lignin moieties with intact C₃-side chains may be reflected by the increase with age of *trans* 4-propenylphenol ($n=14$; $r^2=0.80$; $P<0.001$), *cis* 4-propenylguaiacol ($r^2=0.68$; $P<0.001$) and *trans* 4-propenylguaiacol ($r^2=0.49$; $P<0.01$), even though a source effect cannot be ruled out. However, the syringols, and the S/G ratio (not shown), decreased significantly during the first 400 yrs after burial ($n=12$; $r=0.74$; $P<0.001$, and $r^2=0.62$, $P<0.005$, respectively). This implies that guaiacyl lignin is probably better preserved in the mat deposit than syringyl lignin. Also, the proportion of syringols decreased from the inner sheaths (i.e. younger) towards the outer sheaths (i.e. older), suggesting that degradation of syringyl lignin occurs already during senescence in the plant.

As aforementioned, the phytadienes from chlorophyll were detected only in the leaf material. However, traces of these compounds could also be identified in the superficial samples of the seabed core (up to 0.08%), and up to ca. 200 yr after burial (Fig. 6f). Considering that leaf-derived COM was not found (Serrano et al. 2012), small proportions of leaf particles may be absorbed in macroscopic *P. oceanica* debris, but chlorophyll may also have algae or terrestrial origin. Anyhow, the chlorophyll was rapidly depleted upon burial, probably because of chemical decomposition and perhaps further physical fragmentation. Indeed, preferential chemical degradation is likely because chlorophyll is among the most labile constituents of plants (e.g. Carpenter et al. 1986), while physical fragmentation is evidenced by the relative proportion of coarse and fine organic matter (Fig. 2): COM accounts for $81 \pm 3\%$ of total organic matter in samples up to 200 yr ($n=7$) and for $65 \pm 1\%$ between 200 and 800 yr ($n=6$), which is significant ($P<0.005$) evidence of physical fragmentation processes (Serrano et al. 2012).

Only traces of C₁₆-fatty acid ($0.06 \pm 0.02\%$) (Fig. 6g) were detected in the mat. Decomposition or fragmentation of leaf material can only partially explain this discrepancy, as all plant materials have larger proportions of this compound than the maximum value in the mat (at the surface), suggesting that it was rapidly depleted from other plant organs through degradation as well. The proportion of C₁₆-fatty acid declined sharply during the first 400 yrs ($r^2=0.78$; $P<0.001$). Similar to the syringols, C₁₆-fatty acid is higher in the living plant organs

(leaves and inner rhizome) and the younger inner sheaths, which could be indicative of continued degradation of this compound during diagenesis.

Serrano et al. (2012) showed that there are large variations in the relative proportions of sheath, root and rhizome fractions in the COM along the core (Fig. 2), which should theoretically be reflected by the PY-GC-MS signals obtained if decay would not efface such signal. However, only two significant correlations were found between these proportions and the pyrolysis fingerprints: i) guaiacol in the COM of the mat detritus was positively correlated to the abundance of sheath debris ($n=13$, $r^2=0.41$; $P<0.05$), and ii) syringol ($r^2=0.47$; $P<0.01$), 4-vinylsyringol ($r^2=0.60$; $P<0.005$) and 4-formylsyringol ($r^2=0.40$; $P<0.05$) were positively correlated to the abundance of rhizome debris. The lack of closer relations with respect to the abundance of the main constituents, i.e. polysaccharides and *p*-HBA-based phenolic fibres, might be explained by the relatively similar pyrolysis fingerprints between the sheath, root and outer rhizome materials, perhaps in combination with diagenetic effects.

4. Final remarks and conclusions

The coarse organic matter (COM; <1 mm) in the mat was composed predominantly of *p*-HBA-type phenolic compounds, carbohydrates and smaller proportions of lignin and tannin. The similarity in PY-GC-MS and THM-GC-MS fingerprints of several plant organs (sheaths, roots, outer rhizomes) and the COM from the mat suggested that the vast majority of the COM originated from these organs of *P. oceanica*. This is in agreement with Serrano et al. (2012) who physically separated the COM into sheath, root and rhizome materials. The COM fraction constituted $73 \pm 11\%$ of the total organic matter found in bulk sediments, suggesting that the molecular properties of the COM probably resemble the properties of the bulk organic matter. Nonetheless, future studies should focus on the analysis of bulk organic matter samples though, not COM, i) to pursue a more complete characterization of the long-term C sink, ii) to better understand the fragmentation/degradation of the *P. oceanica* debris over decadal to millennial time scales and iii) to assess the incorporation of debris from remains of other organisms than *P. oceanica* (other seagrasses, algae, phytoplankton, bacteria and terrestrial matter). Use of bulk organic matter samples will probably enhance the signals of chemical degradation in the PY-GC-MS data as well, and perhaps allow to establish a link between molecular composition,

variations in source materials, intensity of diagenetic processes, and their environmental controls.

THM-GC-MS is a useful tool for structural elucidation of the polar phenolic and aliphatic fractions of *Posidonia* debris, providing more detailed information than PY-GC-MS because of less intense secondary rearrangements, but PY-GC-MS is probably the more reliable tool for environmental reconstructions, as it provides more reliable information on structural polysaccharides and N-containing moieties. This may be particularly important in the analysis of bulk organic matter samples (contrary to the COM fraction), where microbial and humified materials accumulate in the fine fraction (Gadel and Bruichet 1987).

Finally, even though part of the *p*-HBA phenolic compounds may have been lost by leaching during initial decay, the vast majority of the *p*-HBA tissues survived centennial storage in the mat. They are probably ester-bound phenolic compounds with a fairly low molecular weight (for example <1000 amu; Maie et al. 2006), but not as low as to enable efficient leaching as was postulated for the LMW carbohydrates (this study). Bitam et al. (2012) recently detected several “neolignans” (IUPAC 2000; Apers et al. 2003) based on oligomers of 4-hydroxybenzoic acid groups esterified to guaiacyl groups in *P. oceanica* (they only analyzed rhizomes). This raises the suspicion that such *p*-HBA-based neolignan fibres may accumulate in *P. oceanica* mats, forming a key ingredient of the long-term C sink.

Acknowledgements

The pyrolysis experiments and J.K. were funded by the project CGL2010-20662 (Plan Nacional de I+D+i, Ministerio de Economía y Competitividad, España) and grant GPC2014/009 (Xunta de Galicia). O.S. was supported by the ECU Faculty Research Grant Scheme and the CSIRO Flagship Marine & Coastal Carbon Biogeochemical Cluster (Coastal Carbon Cluster). The field work and sample preparation were funded by the SUMILEN project “Advances in sampling techniques, biogeochemical characterization, and quantification of the millenary deposits of seagrasses” (Plan Estatal CTM2013- 47728-R) and PALEOPARK project “Millenary changes in the insular Spanish National Parks: perturbations, resilience and trends after the seagrass archives” (Parques Nacionales 1104/2014). This is a publication of the Group of Benthic Ecology 2014 SGR 120.

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Tables

Table 1 PY-GC-MS compound list. Group codes (see text): CARB=carbohydrate, FA16=palmitic acid, G=guaiacyl phenols, HBA=4-hydroxy benzoic acids, PHEN=phenols, PHYT=phytadienes, S=syringol phenols. ME=methyl ester.

Peak label	Pyrolysis product	m/z^a	Group	RT (min) ^b	RT (min) ^c
1	2-methylfuran	82+53	CARB	2.432	1.579
2	3/2-furaldehyde	95+96	CARB	3.456	1.902
3	phenol	94+66	PHEN	5.308	3.113
4	4-hydroxy-5,6-dihydro-(2H)-pyran-2-one	114+58	CARB	5.510	2.903
5	3-methyl-1,2-cyclopentanedione	112+55	CARB	5.931	3.430
6	2-methylphenol	108+107	PHEN	6.327	3.737
7	3/4-methylphenol	108+107	PHEN	6.639	3.893
8	guaiacol	109+124	G	6.763	3.945
9	levoglucosenone	98+68	CARB	7.174	3.982
10	3-hydroxy-2-methyl-(2H)-pyran-4-one	126	CARB	7.298	4.133
11	5-hydroxymethyl-2-dihydrofuraldehyde-3-one	57+70	CARB	8.224	4.751
12	4-methylguaiacol	123+138	G	8.381	4.928
13	1,4:3,6-dianhydro- α -D-glucose	69+57	CARB	8.599	5.068
14	4-vinylphenol	91+120		8.825	5.188
15	catechol	110+64		9.009	5.209
16	methoxycatechol compound	140+125	G	9.505	5.588
17	4-ethylguaiacol	137+152	G	9.709	5.708
18	indole	117+90		9.774	5.994
19	4-vinylguaiacol	150+135		9.961	6.004
20	syringol	154+139	S	10.486	6.238
21	<i>trans</i> 4-propenylphenol	133+134		10.638	6.254
22	<i>cis</i> 4-propenylguaiacol	164+149	G	10.849	6.378
23	4-methoxybenzoic acid ME	135+166	HBA	10.026	6.427
24	4-formylguaiacol	151+152	G	10.211	6.612
25	<i>trans</i> 4-propenylguaiacol	164+149	G	10.752	7.153
26	<i>p</i> -hydroxybenzoic acid ME	121+152	HBA	10.867	7.268
27	C ₃ H ₃ -guaiacol	147+162	G	10.929	7.330
28	4-(propan-2-one)guaiacol	137+180	G	11.298	7.699
29	4-vinylsyringol	180+165		11.615	8.016
30	4-hydroxybenzoic acid	121+138	HBA	12.042	8.443
31	levoglucosan	60+73	CARB	12.893	8.557
32	4-formylsyringol	182+181	S	13.007	8.671
33	unidentified terpenoid	131+174		13.111	8.775
34	diketodipyrrole	186+93		13.325	8.989
35	<i>trans</i> 4-propenylsyringol	194+179	S	14.682	9.046
36	4-hydroxy-3-methoxycinnamaldehyde	178+135	G	14.895	9.259
37	4-(propan-2-one)syringol	167+210	S	15.113	9.477
38	ferulic acid ME	208+177		15.602	9.966
39	phytadiene 1	68+95	PHYT	15.971	10.278
40	phytadiene 2	82+81	PHYT	16.408	10.559
41	C ₁₆ -fatty acid	60+73	FA16	17.287	10.970
42	alkylcyclohexane (C ₂₀ H ₄₀)	83+280		20.620	13.158

^aMass-to-charge ratio of fragment ions

^bRetention time in recent plant materials

^cRetention time in plant detritus samples from the seagrass core

Table 2 THM-GC-MS compound list. ME=methyl ester, FAME=fatty acid methyl ester, DAME=diacid dimethyl ester.

Peak label	THM product	<i>m/z</i>
1	methoxybenzene (P1)	78+108 (65)
2	4-methyl-1-methoxybenzene (P2)	122+77
3	1,2-dimethoxybenzene (G1)	95+138 (123)
4	1,4-dimethoxybenzene	123+138 (95)
5	unidentified carbohydrate	142 (57,71,127)
6	4-methoxybenzaldehyde (P4)	135+136
7	C ₆ -metasaccharinic acid ME	129 (75,161)
8	C ₆ -metasaccharinic acid ME	129 (75,161)
9	3-methoxybenzoic acid ME	135+166
10	4-methoxybenzoic acid ME (P6)	135+166
11	1,2,3-trimethoxybenzene (S1)	139+168 (153)
12	1,3,5-trimethoxybenzene	139+168
13	unidentified compound	59 (75,173,191)
14	unidentified carbohydrate	71+72 (101,218)
15	C ₆ -metasaccharinic acid ME	129 (75,161)
16	1,2,3-trimethoxytoluene (S2)	182+153 (167)
17	2,4,6-trimethoxytoluene	182+153
18	3,4-dimethoxybenzaldehyde (G4)	166+165 (95)
19	C ₆ -metasaccharinic acid ME	129 (75,161)
20	DAME C ₉	55+59 (152,185)
21	3,4-dimethoxybenzoic acid ME (G6)	165+196
22	3,4,5-trimethoxybenzaldehyde (S4)	196+181
23	<i>cis</i> 2-methoxyethenyl-3,4-dimethoxybenzene (G7)	151+194 (179)
24	<i>trans</i> 2-methoxyethenyl-3,4-dimethoxybenzene (G8)	151+194 (179)
25	<i>trans</i> 3-methoxyprop-1-ene-3,4-dimethoxyphenyl (G13)	208+193 (165)
26	3-methoxypropyl-3,4-dimethoxybenzene	210+195
27	FAME C ₁₄	74+87
28	3,4,5-trimethoxybenzoic acid ME (S6)	226+211
29	<i>cis</i> 2-methoxyethenyl-3,4,5-trimethoxybenzene (S7)	209+224
30	threo/erythro-1,2,3-trimethoxypropyl-3,4-dimethoxybenzene (G14)	181 (270)
31	<i>trans</i> 2-methoxyethenyl-3,4,5-trimethoxybenzene (S8)	209+224
32	threo/erythro-1,2,3-trimethoxypropyl-3,4-dimethoxybenzene (G15)	181 (270)
33	phytadiene	67+68
34	phytadiene	81+82 (95,268)
35	<i>trans</i> 3-methoxyprop-1-ene-3,4,5-trimethoxybenzene (S13)	238 (195, 207)
36	<i>trans</i> 2-propenoic acid-3,4-dimethoxybenzene ME (G18)	222+191
37	threo/erythro-1,2,3-trimethoxypropyl-3,4,5-trimethoxybenzene (S14)	211 (300)
38	FAME C ₁₆	74+87
39	threo/erythro-1,2,3-trimethoxypropyl-3,4,5-trimethoxybenzene (S15)	211 (300)
40	FAME C ₁₇	74+87
41	FAME C ₁₈	74+87
42	FAME C ₁₉	74+87
43	methylated glucopyranoside oligomer	101+111
44	FAME C ₂₀	74+87
45	FAME C ₂₁	74+87

46	FAME C ₂₂	74+87
47	FAME C ₂₃	74+87
48	FAME C ₂₄	74+87
49	ω -methoxy FAME C ₂₂	55+74 (69,84)
50	FAME C ₂₅	74+87
51	FAME C ₂₆	74+87
52	ω -methoxy FAME C ₂₄	55+74 (69,84)
53	FAME C ₂₇	74+87
54	DAME C ₂₄	74+98 (55)
55	FAME C ₂₈	74+87
56	ω -methoxy FAME C ₂₆	55+74 (69,84)
57	ω -methoxy FAME C ₂₈	55+74 (69,84)
58	<i>trans</i> 1-propenoic acid-4-methoxybenzene ME (P18)	161+192 (133)

Figure captions

FIGURE 1. Anatomy of *Posidonia oceanica* with indications of the plant sections analyzed in this study. Drawn by K. Inostroza.

FIGURE 2. a) Relative proportion of coarse seagrass-derived organic matter (COM) as a percentage of total organic matter and the relative contributions of sheath, root and rhizome materials to the COM fraction (from Serrano et al., 2012), and a photo of the corresponding Port Lligat seabed mat core. b) Detail of the upper 25 cm of the core. Photo credit: M.A. Mateo

FIGURE 3. Example total ion current chromatograms from the *Posidonia* plant materials obtained by PY-GC-MS (left column) and THM-GC-MS (right column). Peak labels correspond to peak labels in Table 1 (PY-GC-MS) and Table 2 (THM-GC-MS). * = contamination.

FIGURE 4. Building blocks of the main groups of phenolic compounds and main analytical pyrolysis products. PY = PY-GC-MS, THM = THM-GC-MS.

FIGURE 5. Example total ion current chromatograms from the *Posidonia* seabed deposit obtained by PY-GC-MS (left column) and THM-GC-MS (right column). Peak labels correspond to peak labels in Table 1 (PY-GC-MS) and Table 2 (THM-GC-MS). * = contamination.

FIGURE 6. Relative proportions of selected pyrolysis (PY-GC-MS) products in the recent plant organs (bar graphs) and in the plant debris from the mat core (line graph). a) Phenols (phenol, C₁-phenols), b) *p*-hydroxybenzoic acids (*p*-hydroxybenzoic acid, *p*-hydroxybenzoic acid methyl ester), c) carbohydrates (all carbohydrate products: cyclopentenones, furans, furaldehydes, pyrans and anhydrosugars), d) guaiacols (all guaiacols except for 4-vinylguaiacol), e) syringols (all syringols except for 4-vinylsyringol), f) phytadienes (both compounds) and g) C₁₆-fatty acid. Note the scale difference in f and g.

PY-GC-MS

THM-GC-MS

PY-GC-MS

THM-GC-MS