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ORIGINAL PAPER

Exploring the anatomical and metabolomic variabilities in the seagrass *Posidonia oceanica* (L.) Delile fruits

Federico Pinna[1,2] · **Mauro Commisso**[3] · **Flavia Guzzo**[3] · **Simonetta Bagella**[2] · **Giulia Ceccherelli**[2] · **Giovanna Becca**[2] · **Maria Franca Usai**[2] · **Patrizia Stipcich**[2,4,5]

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Abstract

Anatomical and metabolomic variability in marine plants can provide valuable insights into species adaptive strategies and resilience to environmental changes. This study explores the anatomical characteristics and metabolomic profiles of *Posidonia oceanica* fruits, a key Mediterranean seagrass species, collected from three areas in Sardinia (Alghero, Oristano, and Olbia). Anatomical analyses of pericarps assessed cuticle thickness and intercellular spaces. Additionally, metabolomic analyses were performed using UPLC-HRMS on both the pericarps and the seeds. Significant differences in the percentage of intercellular spaces among pericarps from the different areas were found, with those from Alghero, exhibiting a higher percentage. Metabolomic analyses highlighted distinct profiles across areas, with Alghero pericarps showing greater metabolomics complexity, characterized by high levels of chicoric acid, hydroxycinnamate and hydroxybenzoate derivatives, while seeds exhibited an abundance of procyanidins, benzoic and caffeic acids and quercetins. Interestingly, seeds from Olbia presented a higher abundance of lipids. This study provides the first comprehensive metabolomic characterization of *P. oceanica* pericarps and seeds, highlighting significant variability that may influence seed dispersal, germination, and species adaptability. Our findings offer new perspectives for the conservation and management of this key species, which are particularly relevant in the context of global climate change.

Keywords Cuticle · Intercellular spaces · Mediterranean sea · Metabolites · Seagrass · Sexual reproduction

Introduction

Morphological and chemical traits in marine plants exhibit substantial variability across populations, environments, and life stages (Cornwell and Ackerly 2009; Dong et al. 2020). This variability can result from genetic differences, environmental influences during development and post-production processes such as transport and degradation (Orth et al. 2006; McMahon et al. 2014; Stipcich et al. 2023). Understanding trait variability is particularly crucial for foundation species such as seagrasses, which create complex habitats with significant ecological and economic impacts (Heck et al. 2003; Hughes et al. 2009). Their dense meadows support a wide range of fish species and invertebrates, offering valuable ecosystem services such as seabed stabilization, carbon storage and oxygen production (Terrados and Borum 2004; Cullen-Unsworth and Unsworth 2013), that might be affected by trait changes.

In the Mediterranean Sea, *Posidonia oceanica* (L.) Delile is the most widespread seagrass, forming extensive meadows from the surface down to about 40 m of depth, representing the most productive coastal ecosystem of the Basin (Campagne et al. 2015; Telesca et al. 2015). *P. oceanica* is a monoecious plant whose recruitment is either achieved by vegetative reproduction or sexual recruitment through

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✉ Federico Pinna
federico.pinna@szn.it

[1] Department of Integrative Marine Ecology Genova Marine Centre, Stazione Zoologica Anton Dohrn, Genova, Italy

[2] Department of Chemical Physical, Mathematical and Natural Sciences, University of Sassari, Sassari, Italy

[3] Department of Biotechnology, University of Verona, Verona, Italy

[4] Department of Biology, University of Naples Federico II, Naples, Italy

[5] National Biodiversity Future Centre, Palermo, Italy

flowering production, fruit dispersion, seed release, anchorage, stabilization and germination (Orsini et al. 2001; Badalamenti et al. 2015; Balestri et al. 2015; Guerrero-Meseguer et al. 2017).

P. oceanica sexual reproduction frequency has increased in the last decades, likely due to global warming (Diaz-Almela et al. 2006; Stipcich et al. 2024a, b) but it is not clear yet if the increase in flowering can be considered an evolutionary strategy to maximize survival under adverse settings (Marín-Guirao et al. 2019). Flower production generally begins at the end of August (Gobert et al. 2001), with fruits developing over the following months and reaching maturity during winter. Ripe fruits are released in spring: due to their buoyancy, they are transported on the sea surface following wind and current directions and travel long distances before releasing their seeds (Micheli et al. 2010; Ruocco et al. 2025). Many fruits strand on beaches, with some that return to the sea through wave actions (Sutera et al. 2024a, b). The stranding events provide a valuable opportunity for non-invasive fruit and seed collection, utilized in restoration efforts (e.g. Terrados et al. 2013; Pergent-Martini et al. 2024; Sutera et al. 2024a, b; Zenone et al. 2025), offering insights without disturbing in situ populations. However, the suitability of the seed for a restoration action may depend on the status of the fruit pericarp which protects the seed from desiccation (Sutera et al. 2024a, b).

Still, very little information is known about the structure of *P. oceanica* pericarp. To the best of our knowledge, the study by Guerrero-Meseguer et al. (2018) provides the only detailed anatomical description of the fruit pericarp, while other works have addressed only the morphology of fruits and seeds or broader aspects of the species reproductive ecology (e.g. Balestri and Cinelli 2003; Celdrán and Marín 2011; Sutera et al. 2024a, b). *P. oceanica* pericarps are characterized by an initial layer of epidermis, covered by a cuticle, and an inner layer of mesocarp that comprises large cells containing air lacunae that generally increase toward the internal mesophyll (Guerrero-Meseguer et al. 2018). This structure enables the pericarp to confer buoyancy while facilitating light transmission to the seeds, capable of performing photosynthetic activity and therefore producing oxygen (Guerrero-Meseguer et al. 2017). However, the variability of the marine conditions such as temperature, salinity, and light intensity can induce significant variations in the biological and physiological characteristics of the plants including metabolomic responses (Micheli et al. 2005; Bornette and Puijalon 2011; Sampaio et al. 2016; Duarte et al. 2018; Zayas-Santiago et al. 2020; Jung et al. 2023) and whether they can also affect the *P. oceanica* pericarp structure and its functionality remains unknown. In this context, traits of pericarps and seeds represent fascinating but relatively unexplored aspects of *P. oceanica*, being crucial for the dispersal, germination, and establishment of new seedlings (Guerrero-Meseguer et al. 2018), and thus directly influencing the resilience and expansion of *P. oceanica* meadows.

In marine plants, metabolomic approaches have proven valuable for characterizing biological variability and identifying chemical signatures that distinguish different populations or physiological states (Bundy et al. 2009; Kumar et al. 2016; Patel et al. 2021). Metabolites serve as intermediate and final products of biological processes, and they are essential for the survival, growth, and adaptation of plants to changing climatic and environmental conditions (Abdo et al. 2007; Bussell et al. 2008; Carreno-Quintero et al. 2013). Metabolite profiles in aquatic plants are shaped by a complex interplay of genetic factors and environmental conditions, which in turn influence their physiological and ecological dynamics. In particular, genetic factors influence metabolomic pathways that are vital for processes such as growth, development, and stress responses, allowing plants to adapt effectively to their surroundings (Alseekh et al. 2023; Mipeshwaree et al. 2023). Conversely, environmental conditions, including temperature, light, salinity, and nutrient availability significantly affect the biosynthesis of both primary and secondary metabolites, highlighting the importance of external stimuli (Sampaio et al. 2016).

Compared to terrestrial plants, relatively few studies of environmental metabolomics have been devoted to marine organisms (Bayona et al. 2022), focusing on sponges (Ivanisevic et al. 2011; Rohde et al. 2012), ascidians (López-Legentil et al. 2006), zoanthids (Cachet et al. 2015; Jaramillo et al. 2018), microalgae (Garcia-Perez et al. 2023), and corals (Slattery et al. 2001; Costa-Lotufo et al. 2018). In *P. oceanica* leaves, the metabolomic response has been studied in several context and it is considered as a good early warning indicator of environmental and anthropogenic stressors (Toniolo et al. 2018; Blanco-Murillo et al. 2024), but, despite their ecological and biological importance, there is a lack of studies on the metabolomic profiles of the fruit pericarps and seeds of *P. oceanica*. Understanding the metabolomic diversity of beach-cast fruits is crucial for conservation efforts, especially as climate change affects *P. oceanica* reproductive patterns and propagule dispersal (Diaz-Almela et al. 2006; Stipcich et al. 2024a, b). The combined approach of anatomical and metabolomic analyses might offer a unique opportunity to explore the variability of *P. oceanica* fruit pericarps and indirectly provides information on seed health that will be used in restoration works.

This study aims to bridge this gap by exploring the diversity in both anatomical traits, such as cuticle thickness and the percentage of intercellular spaces, and the metabolomic profile of *P. oceanica* pericarps and seeds collected from several beaches in Sardinia (Italy) located on different coastal sectors. By characterizing potential variations, results will enrich our understanding of the characteristics of *P. oceanica* pericarps and seeds from a chemical and structural perspective, contributing valuable information for the conservation of these key ecosystems. Furthermore, the metabolome of *P. oceanica* seed has been investigated here for the first time, since information on this subject is completely absent from the scientific literature, despite being essential for assessing seed characteristics for restoration purposes.

Materials and methods

Study areas and data collection

To study spatial variability in fruit pericarp structure and composition, fruits were collected in three different areas from Sardinia (Italy): Alghero (AHO, 40°34'N, 8°14'E), Oristano (ORI, 39°47'N, 8°31'E), and Olbia (OLB, 40°52'N, 9°39'E) (Fig. 1). Sampling was conducted in June 2023, when *P. oceanica* fruits were released. These three areas are located in different coastal sectors characterized by variable thermal regimes (Ceccherelli et al. 2020) influenced by seawater circulation patterns (Olita et al. 2013; Pinardi et al. 2015). The most significant disparity in sea surface temperatures (SST) between areas occurs during the summer, which impacts the structural composition of *P. oceanica* meadows (Pansini et al. 2021; Stipcich et al. 2022).

To standardize the maturation stage and minimize phenological variability, all fruits were collected within a small-time window (June 10–20, 2023). In each area, more than 25 fruits were collected from two beaches and immediately transported to the laboratory facilities of the University of Sassari in seawater. In the laboratory, fruits were inspected to be selected for the analyses. Selection criteria included intact pericarp and minimal signs of degradation. Fruits were characterized by the brownish to greenish coloration commonly observed in stranded specimens. Ten fruits ($n=10$) per area were thus chosen for anatomical analyses and fifteen ($n=15$) for metabolomic analyses (Table S1). Subsequently, some fruits were shipped to University of Verona for metabolomic analyses, where they arrived within 24 h. Upon arrival, samples were stored at +4 °C before further processing. It is worth highlighting that regardless of the visual selection applied, *P. oceanica* fleshy pericarps used in this study were at various levels of dehiscence, with samples from Oristano completely open and devoid to seeds.

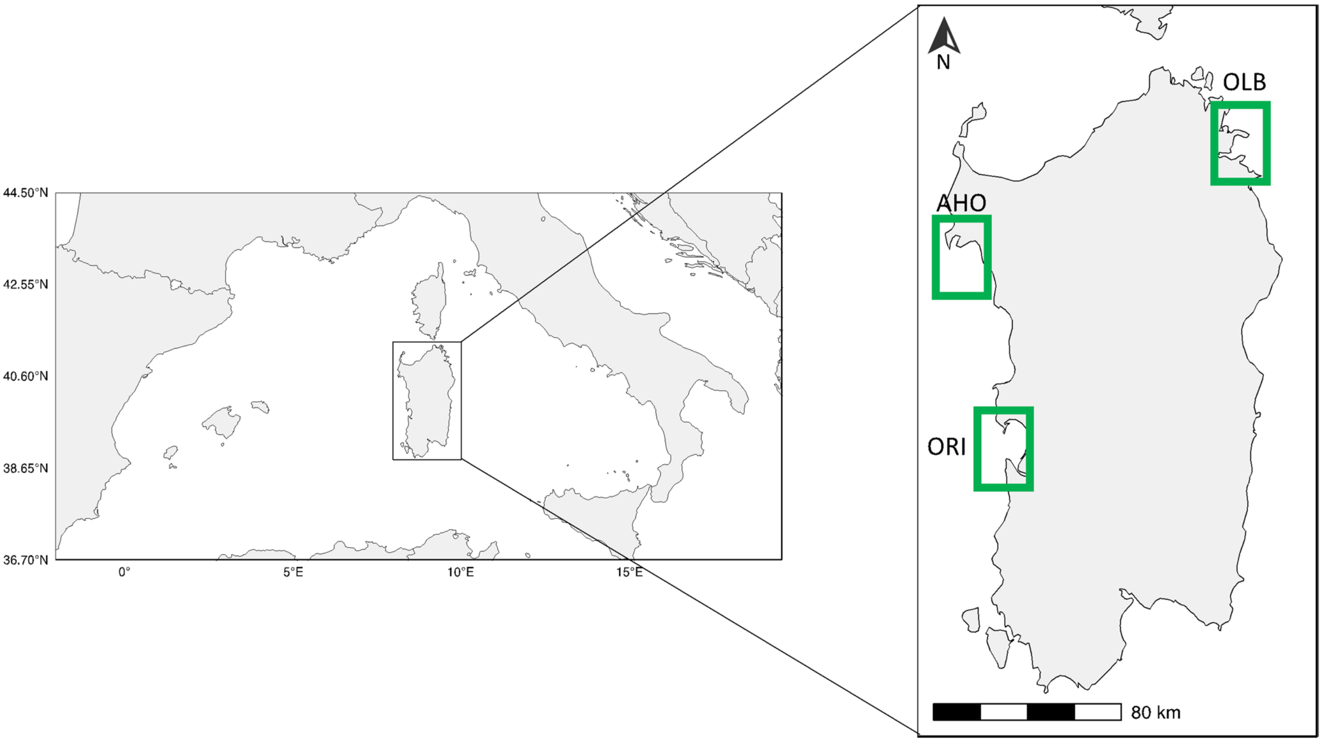

Fig. 1 Map showing the three areas of fruit collection in Sardinia (AHO, ORI, OLB). The green square indicates their location along the island's coast

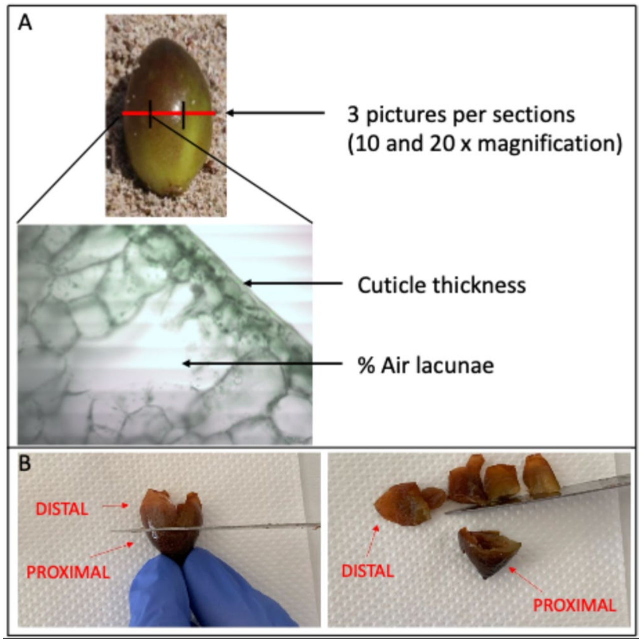

Fig. 2 (**A**) Conceptual design of the methodology used in the analysis, specifically referring to the transverse section. (**B**) Photographic image illustrating the identification of the distal and proximal portions of the fruit

Pericarp anatomy

For the anatomical analysis, thin sections were obtained from each pericarp ($n=10$) using a blade along the longitudinal and transverse axes. The longitudinal sections were cut along the major axis of the fruit from the proximal to the distal end, while transverse sections were cut perpendicular to this axis, creating circular cross-sections. These orientations were chosen to comprehensively assess the anatomical structures, particularly the distribution of intercellular spaces and cuticle thickness across different planes of the pericarp. Observations were performed using a Zeiss Axiophot optical microscope. Images were captured with an Infinity 1 camera, and the software used for image analysis was Infinity Analyze (Lumenera Corporation) (Fig. 2). Two different optical magnifications (i.e., 10x and 20x) were selected to facilitate image analysis. Specifically, the 10x magnification was used to analyze the percentage of intercellular spaces (Air lacunae), while the 20x magnification was used to analyze the thickness of the cuticle. The thickness of the cuticle and the percentage of intercellular spaces were estimated from the images of each section using ImageJ software (https://imagej.nih.gov, Fig. 3A, B). Three photographs were taken for each thin section. Along each photograph, a total of three measurements were made and an average was calculated to determine the cuticle thickness in each thin section. Similarly, the area of each intercellular space was calculated for each section and the percentage of intercellular spaces was obtained by relating the total area of the section to the area occupied by the spaces in the individual section. Each pericarp was considered a replicate ($n=10$).

Pericarp and seed metabolomics

P. oceanica fruits from Olbia, Oristano and Alghero were shipped in plastic bottles in seawater and were split in three groups, each composed of 15 fruits. Fruit pericarps were cut in two portions: one distal and the other proximal, considering the distal as the part of the fruit that was already open and the proximal as the part of the fruit that was still closed. This choice was also necessary to recover a more intact portion of the fruit (the proximal one) as opposed to the distal portion, which, especially after the opening of the fruit, was ruined and apparently oxidized. Seeds were

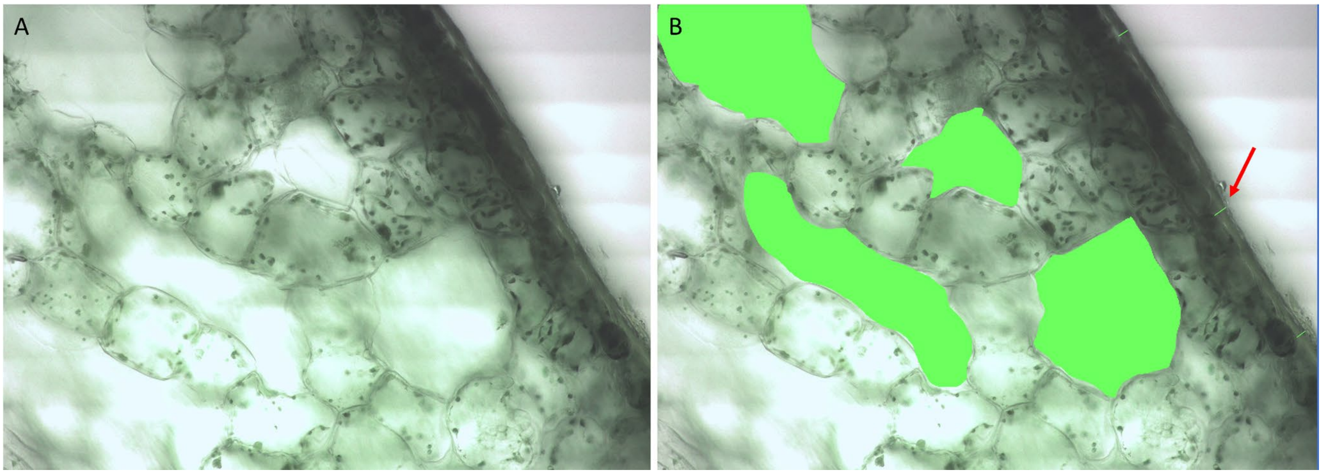

Fig. 3 Transverse section of the pericarp before (**A**) and after (**B**) image analysis for calculating the percentage of air lacunae and the cuticle thickness. The red arrow indicates one of the three measurements of the cuticle thickness per thin section portion

retrieved only from fruits sampled in Olbia and Alghero. As for the pericarps, seeds from a single region were split in three replicates, each composed of nine seeds, to perform a minimal statistical analysis. Both pericarps and seeds were washed with double-distilled water three times and dried at each step. Fruits were later frozen in liquid nitrogen, ground using an A11 basic analytical mill (IKA-Werke, Staufen, Germany) and reduced to powder. The frozen powders were stored at −80 °C.

Approximately 100 mg of powder was weighed and 10 volumes of 100% methanol (w/v; LC-MS grade, Honeywell) were used for extraction. Samples were vortexed for 30 s, sonicated at 40-kHz for 15 min in an ice ultrasonic bath (SOLTEC, Milano, Italy) and centrifuged at 4 °C, 13,000 rpm, for 10 min (De Soricellis et al. 2024). Supernatants were collected and then diluted 1:10 with water LC-MS grade (Honeywell), filtered through 0.22 µm Minisart filters (Sartorius-Stedim Biotech, Göttingen, Germany) and injected into the UPLC-HRMS system (5 µl) (De Soricellis et al. 2024).

The metabolite identification was performed by comparing the accurate mass, retention time and fragmentation pattern of *P. oceanica* metabolites against those included in our internal library of commercial standards or with data presented in literature or in public databases (for example MoNA (https://mona.fiehnlab.ucdavis.edu/) and MassBank (https://massbank.eu/MassBank/Search) (De Soricellis et al. 2024).

Data analyses

The differences in anatomical traits between areas were assessed by one-way ANOVAs, considering Area with three levels (Alghero = AHO, Oristano = ORI and Olbia = OLB). Response variables were cuticle thickness in both longitudinal and transverse sections and the percentage of intercellular spaces in longitudinal and transverse sections. ANOVA assumptions for normal distribution and equality of variance were assessed by using Shapiro-Wilks and Levene's test, respectively. Post hoc comparisons were conducted using Tukey's test. Anatomical analyses and graphics were performed in R using version 4.2.2 (R Core Team 2021).

The UPLC-HRMS raw data files generated by the analyses of Olbia and Alghero seeds extracts in negative ionization mode were processed by using Progenesis QI (http://www.nonlinear.com/) to get a data matrix including rt/mz features and the relative abundances. The metabolite peak areas were normalized on the total signal of each sample and the data matrix was submitted to multivariate statistical analysis by using SIMCA 13 (Umetrics). The metabolite abundances were mean-centered, and Pareto scaled before the unsupervised principal component analysis (PCA) and the supervised orthogonal partial least square discriminant analysis (OPLS-DA), which model was validated by performing 200 permutations on a PLS-DA model having the same components and a CV-ANOVA test ($p < 0.05$).

Results

No differences among areas in the cuticle thickness were detected for the transverse section (Table 1; Fig. 4A), while a difference in the percentage of the intercellular spaces was found in the same section, with a higher percentage observed in Alghero (Table 1; Fig. 4B). No differences in

Table 1 Results of ANOVA for the four variables considered (cuticle thickness, longitudinal section; cuticle thickness, transverse section; percentage of intercellular spaces, longitudinal section; percentage of intercellular spaces, transverse section) along with Tukey test on area factor. In bold, statistically significative results

Source of variation	Df	Mean Sq	F value	Pr(>F)
Longitudinal section				
Cuticle thickness				
Area	2	1.1E-05	2.234	0.1270
Residuals	27	5.1E-06		
Air lacunae %				
Area	2	37.200	1.669	0.2070
Residuals	27	22.290		
Transverse section				
Cuticle thickness				
Area	2	1.8E-07	0.013	0.9870
Residuals	27	1.4E-05		
Air lacunae %				
Area	2	44.57	5.930	**0.0073**
Residuals	27	7.52		
Air lacunae % Tukey test on Area				
AHO > ORI = OLB				

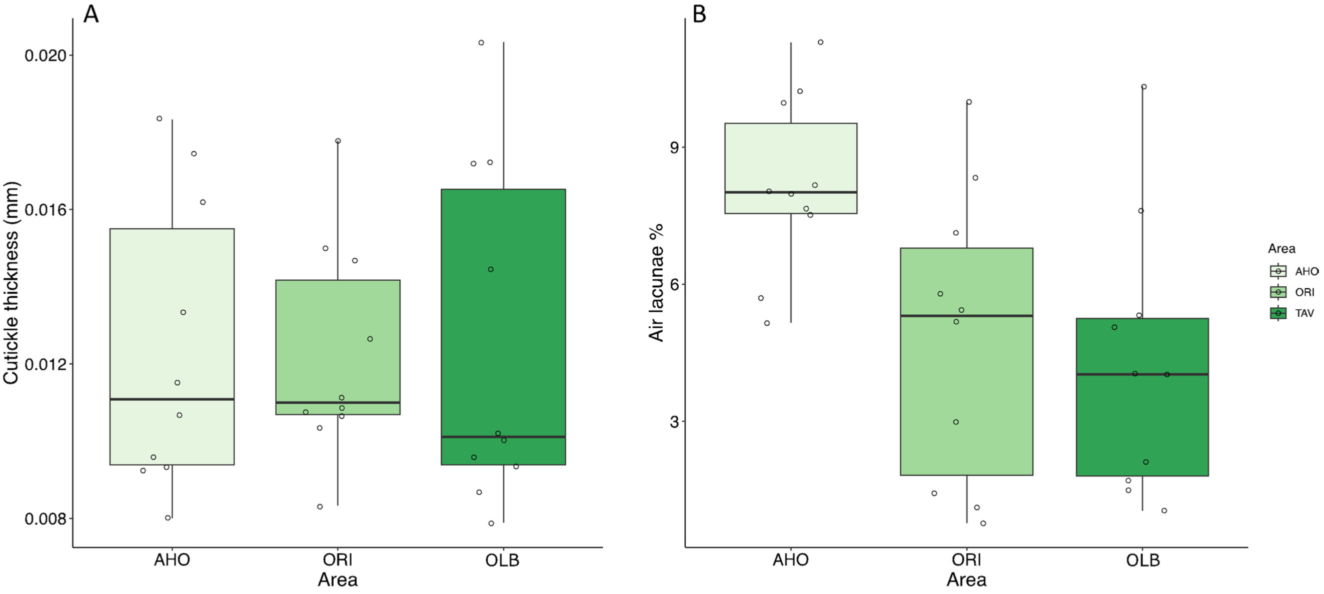

Fig. 4 Box Plot of anatomical variables (cuticle thickness and percentage of air lacunae) across areas (AHO, ORI, OLB) calculated in the transverse section

the longitudinal section were observed for the anatomical response variables considered.

The phytochemical profiles of the distal and proximal portions of pericarp and seed extracts from *P. oceanica* were investigated by following an untargeted metabolomics approach. The UPLC-HRMS analyses resulted in chromatograms (Fig. 5) and in 57 detected and putatively identified metabolites (listed in Supplementary Table 1). Pericarps collected in Alghero presented a more complex metabolic profile than those collected in Oristano and Olbia (Fig. 5A and B): the distal and proximal portions of these pericarps included a greater number of abundant metabolites, such as two isoforms of chicoric acid, which were the most accumulated metabolites, hydroxycinnamate and hydroxybenzoate derivatives, procyanidins, and a glycosylated methylated flavonol (isorhamentin).

The chromatograms of pericarps collected in Oristano and Olbia showed the two isoforms of chicoric acid as still the most abundant metabolites, but with reduced intensity levels compared to those found in Alghero fruits. Moreover, new peaks appeared especially in Oristano pericarps and consisted of valeric acid and valerolactone derivatives, metabolites that might derive from the biological degradation of procyanidins as suggested in previous works (Kutschera et al. 2011; Said et al. 2020).

The chromatograms of seeds (Fig. 5C) showed peaks of chicoric acid, with the isoform 1 as the most abundant metabolite, hydroxycinnamate and hydroxybenzoate derivatives, flavan-3-ols (procyanidins), methyl guaiacol derivatives, a glycosylated quercetin and phospholipids. The PCA multivariate statistical analysis of the whole feature datamatrix (with both the putatively identified and unidentified metabolites) revealed a clear separation between seeds from Olbia and Alghero (Fig. 6A), with the first two principal components explaining ~ 87% of the total variance among samples.

The OPLS-DA analysis allowed to discriminate which metabolites (p) correlated with a specific class (q), i.e. seeds from Olbia or Alghero pericarps. The S-loading plot (Fig. 6B) highlighted that seeds from Alghero and Olbia were better characterized by flavan-3-ols (highly polymerized procyanidins) and phospholipids, respectively.

Discussion

Understanding the anatomical and metabolomic variability of beached *P. oceanica* fruits provides essential baseline data for seagrass conservation and restoration. This study provide the first comprehensive analysis of anatomical and metabolomic diversity in beached *P. oceanica* fruits. Results revealed a variability in the metabolomic profile of fruits from several Sardinian beaches. This variability likely reflects the complex interplay of multiple processes, such diverse source meadows contributing to each beach, environmental conditions that might affect the fruit traits, different timing of fruit release and site-specific degradation conditions. In addition, the variability observed may also arise from intrinsic intraspecific differences, such as variations in physiological status, maturity stage, or post-detachment degradation. Similar intra-specific variation in secondary metabolites has been reported for terrestrial

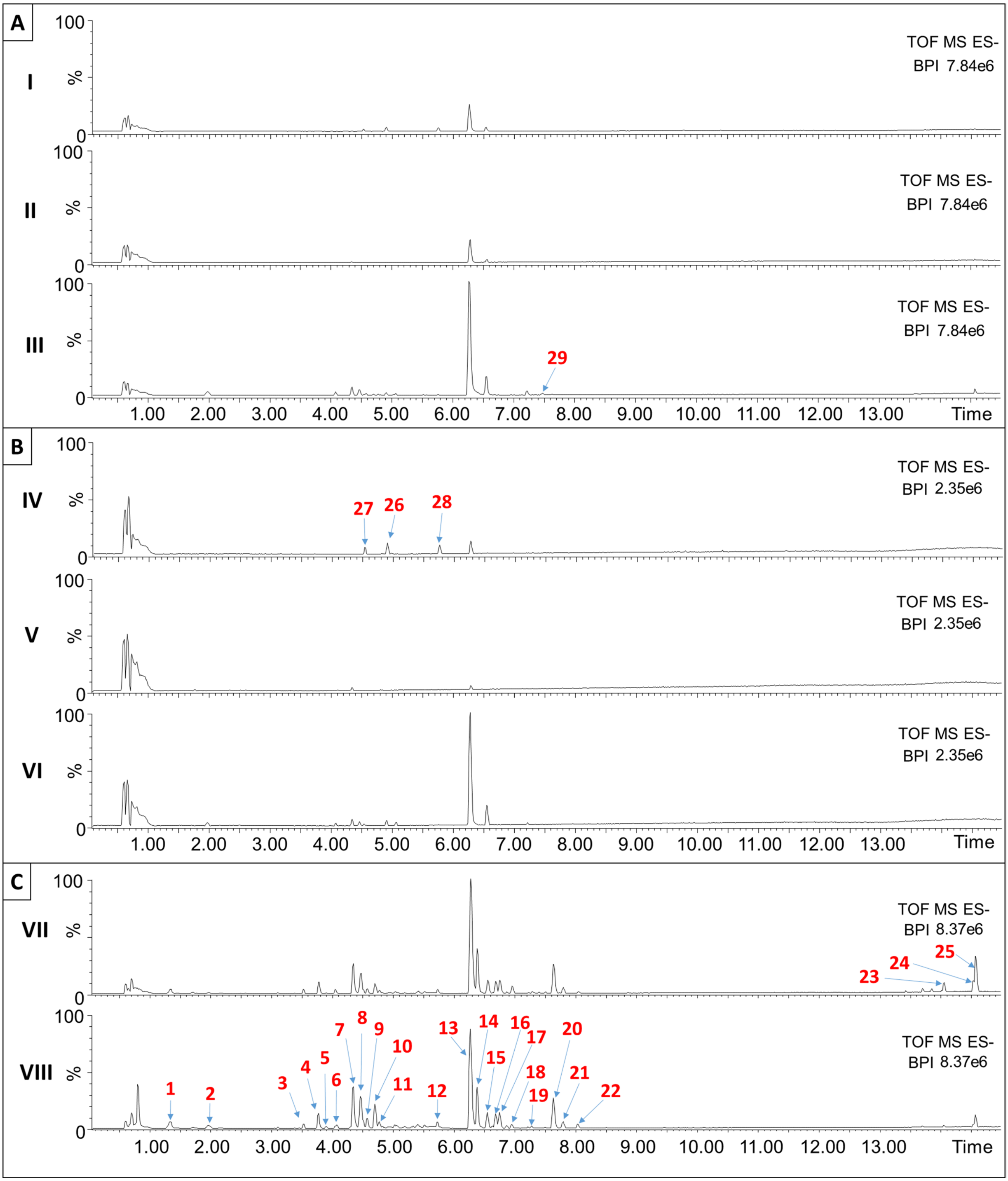

Fig. 5 UPLC-HRMS Base Peak Ion (BPI) chromatograms of fruits and seeds of *P. oceanica* extracts ionized in negative mode. (**A**) Chromatograms of the distal part of the fruits collected from Oristano (I), Olbia (II) and Alghero (III); (**B**) Chromatograms of the proximal part of the fruits collected from Oristano (IV), Olbia (V) and Alghero (VI); (**C**) Chromatograms of seeds collected from Olbia (VII) and Alghero (VIII). Numbers indicate the putatively identified metabolite listed in Supplementary Table 2

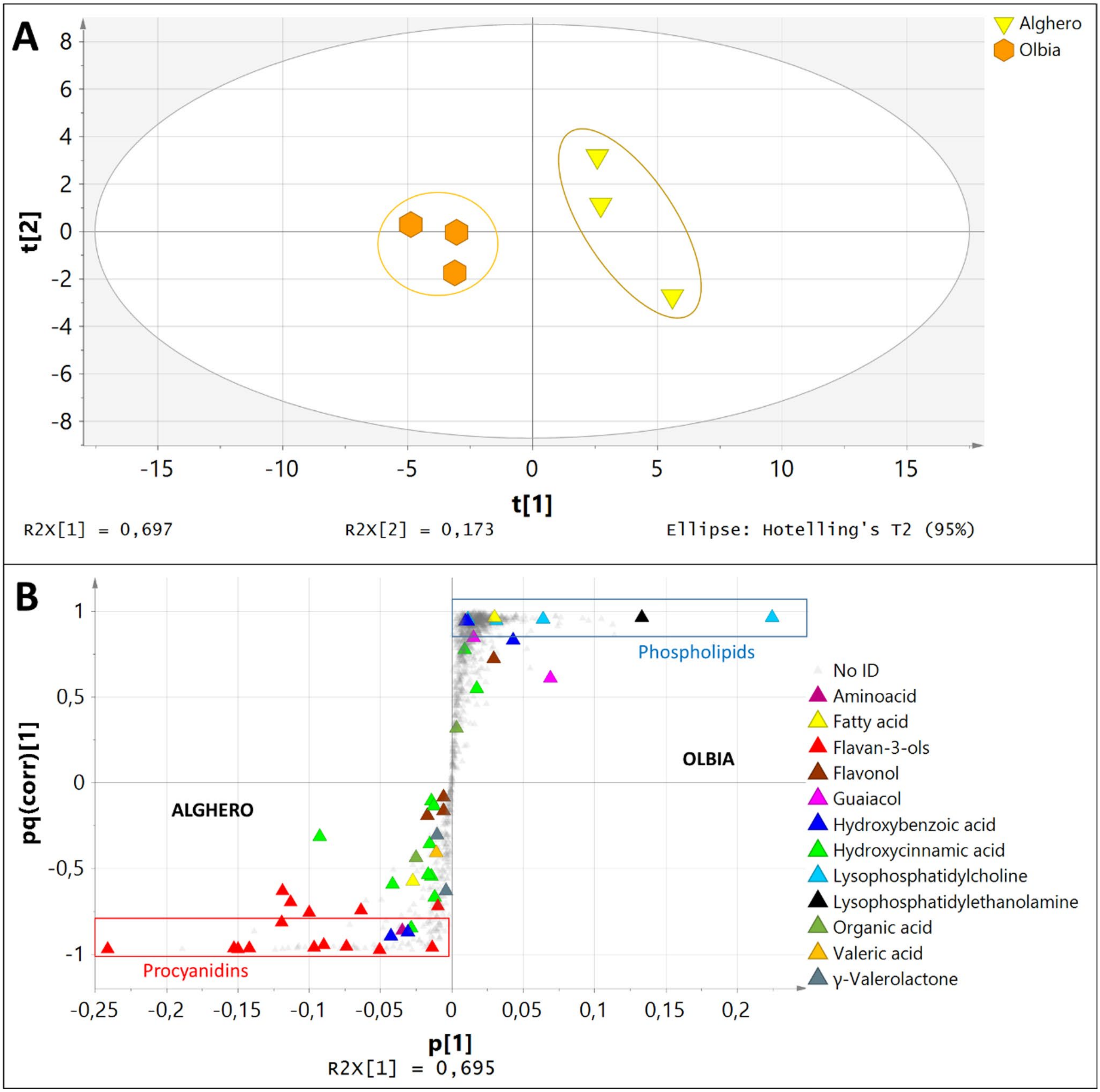

Fig. 6 multivariate statistical analysis of seed extracts achieved from Alghero and Olbia fruits. (**A**) PCA score scatter plot showing a net separation between Alghero (yellow inverted triangles) and Olbia (orange hexagons) samples. (**B**) OPLS-DA S-loading plot showing the metabolites correlating with Alghero or Olbia samples. The triangles are the metabolites and colours represent the metabolite classes

plants, where differences in phenolic acids and flavonoids are associated with developmental and maturation stages (Yan et al. 2022; Yang et al. 2025). Moreover, studies on other species have linked metabolic heterogeneity to distinct physiological or biochemical conditions among individuals (Chen et al. 2024). These findings suggest that the heterogeneity detected in *P. oceanica* propagules could reflect comparable intrinsic processes. Importantly, the variability observed among areas indicates that stranded propagules do not represent a uniform resource but instead encompass a mixture of different histories of dispersal, accumulation and degradation. This heterogeneity has clear ecological and applied implications as it highlights that the propagules available for restoration may differ substantially in their metabolomic traits and thus in their potential contribution to recruitment success. Further steps are necessary to understand exactly what are the factors that mainly affect the fruits metabolomic and how this variability can affect the viability of the seeds and therefore the success of the sexual reproduction, since flowering events in *P. oceanica* have intensified in recent decades, likely due to the rising sea temperatures, leading to a higher number of beached fruits (Diaz-Almela et al. 2006; Stipcich et al. 2024a, b).

The outer pericarp cuticle plays a crucial role in protecting the fruit and seed from dehydration and mechanical damage during dispersal at sea (Belzunce et al. 2005; Kuo and Hartog 2006). In our case, no differences in cuticle thickness between areas were detected, indicating substantial uniformity in the structure of this component in the Sardinian fruits examined. On the other hand, the higher percentage of intercellular spaces observed in fruits from Alghero represents a novel and potentially relevant result. These structures have been associated with multiple functions, including buoyancy of the fruit, thermal insulation, and diffusion of gases (Belzunce et al. 2005; Kuo and Hartog 2006). In addition, Guerrero-Meseguer et al. (2018) suggested that air lacunae could promote light transmission to the seed, allowing it to maintain photosynthetic activity even within the fruit.

The presence of extensive aerenchyma has also been associated with improved oxygen transport and storage in aquatic plants (Justin and Armstrong 1987; Colmer 2003). Interestingly, these aerenchyma structures could theoretically confer advantages for long-distance dispersal. Fruits with more extensive air spaces might remain buoyant and viable during extended journeys at sea, suggesting the possibility that the Alghero assemblage includes propagules that have travelled longer distances. However, we lack direct evidence, and additional data would be needed to explore this scenario. The structural differences we observed could result from fruits originating from genetically distinct meadows with inherent structural variations (Procaccini et al. 2023), from different residence times in water or on beaches affecting tissue structure, and/or from different fruit maturation stages when released from parent meadows. Future studies tracking fruits from known source meadows would be valuable for understanding the drivers of pericarp structural diversity.

The metabolomics analysis of fruits from Oristano and Olbia showed metabolomics profiles compatible with an advanced rotting state (samples from Oristano were completely open with no seeds and samples from Olbia were also in an advanced rotting status), with a lower level of typical fruit metabolites and the presence of bacterial metabolites, namely (dihydroxyphenyl)-g-valerolactone and 4-hydroxy-5-(dihydroxyphenyl) valeric acid. The latter metabolites are known to be produced from dietary flav-3-ols catechin and epicatechin by human gut microbiota (Kutschera et al. 2011; Said et al. 2020). The presence of these metabolites in Olbia and Oristano fruits, together with the lower phytochemical complexity of these extracts, suggests that a degradative process was probably underway when samples from Olbia and Oristano were collected. On the other hand, *P. oceanica* fruit tissues were very rich in catechin/epicatechin oligomers, the so-called condensed tannins (Figure S1). This class of molecule is known to be generally toxic to many microorganisms and to retard the rate of decomposition of organic plant-based matter in soil (Bhat et al. 1998) and only some specific bacteria are able to degrade them (Bhat et al. 1998). Since the fleshy pericarp rotting is probably to be involved in the fruit dehiscence and seed release, and that only specific bacteria are able to degrade fruit procyanidins, this important finding raises the question whether specific seawater bacteria are involved in fruit opening. This hypothesis arises from the fact that certain *P. oceanica* associated microorganisms (microbiota) are involved in different physiological processes, such as promoting plant growth and fitness (Celdran et al. 2012; Khaled and Mohamed 2025), as well as in the production of enzymes that degrade cell wall components (Rubio-Portillo et al. 2021; Souii et al. 2025). This latter finding suggests that the pericarp tissues integrity might be affected by the microbiota, thus potentially facilitating the dehiscence process. Indeed, although observed in a different species, the artificial inoculation of *Panax ginseng* fruits with specific microbes has been shown to affect endocarp dehiscence (Kim 2025). However, it is still unknown if the different metabolomic profile and the presence of microbial metabolites in fruits from different areas can be related to the different stages of fruit maturation, considering also that some fruits were in an advanced rotting status, or if the presence of different bacterial communities in fruits from different area may have accelerated the degradation process.

The metabolome of green, well conserved and partially germinating seeds was very rich in procyanidins, benzoic acid derivatives, caffeic and coumaric acid derivatives, guaiacol derivatives and quercetins. While in literature, LC-MS-based phytochemical composition of *P. oceanica* leaves, roots, rhizomes, and even Neptune balls and freshly beached residues are reported (Barletta et al. 2015; Cornara et al. 2018; Farid et al. 2018; Astudillo-Pascual et al. 2021), to the best of our knowledge, information on fruit and seed phytochemicals are completely missing.

Besides procyanidins and the unreported bacterial metabolites of procyanidins discussed above, the other major classes of metabolites found in fruits and seeds were, hydroxycinnamic acids, hydroxybenzoic acids, flavonoids and lipids. The procyanidin, as well as its monomers catechin and epicatechin, have been previously reported in leaves, beached leaves residues, root, rhizomes and even Neptune balls (Barletta et al. 2015; Cornara et al. 2018; Farid et al. 2018; Astudillo-Pascual et al. 2021). However, the procyanidins profiles of fruits and seeds appeared more complex, with (epi)catechin trimers, tetramers, pentamers and heptamers not reported in the other investigated part of the seagrass.

Hydroxybenzoic acids, gallic and salicylic acids, which were observed in leaves and Neptune balls (Barletta et al. 2015; Farid et al. 2018), were not detected in fruits and seeds. Vanillic acid was observed in leaves and Neptune balls (Farid et al. 2018), while fruits and seeds also contained also vanillic acid glycosides and other derivatives. The flavonoid profile of fruits and seeds, on the contrary, was simpler compared with those reported for other organs, being represented by isorhamnetin and quercetin, glycosilated and partially acetylated. Luteolin, kaempferol, naringenin, pinocembrin and others, reported in other organs, were not detected in fruits and seeds (Astudillo-Pascual et al. 2021). Finally, the hydroxycinnamic acid profiles of fruits and seeds was similar to those reported for other part of the plant, and included various coumaric, caffeic and feulic acid derivatives, mainly esterified with tartaric ad quinic acid (Barletta et al. 2015; Farid et al. 2018; Astudillo-Pascual et al. 2021). LC-MS metabolomics analysis revealed also the presence of various guaiacol derivatives in the seeds. Guaiacol and various guaiacol metabolites have already been shown also in the other investigated *P. oceanica* portion by GC-MS analysis (Rencoret et al. 2020). Interestingly, LC-MS analysis of seeds revealed also the presence of various lipids, mainly lysophospathidylcholine esters with various fatty acids, much more abundant in samples from Olbia.

The presence of various lipids in *P. oceanica* seeds and a high percentage of air lacunae in the pericarp warrants further exploration regarding their ecological functions. In particular, lipid-rich layers in the pericarp may form a semi-impermeable barrier that slows water entrance and microbial degradation, likely delaying decomposition and prolonging buoyancy. Moreover, lipids contribute to the structural integrity of the fruit by preventing moisture loss and decay a feature particularly important in marine environments where saltwater can accelerate tissue degradation (Du et al. 2021) thus helping to maintain seed viability during transport. Lipids and air lacunae, which positively increase the fruit buoyancy, may play a critical role in extending the period during which seeds remain suspended in the water column, thereby enhancing their potential for wide dispersal through marine currents. Such a mechanism would increase the likelihood of seeds reaching suitable habitats for germination, thus contributing to the spatial resilience and expansion of *P. oceanica* meadows. Beyond their role in buoyancy in fruit pericarps, lipids in seeds serve as vital energy reserves, particularly important during the early stages of seed germination (Belzunce et al. 2005). A similar role has been observed in zoospores of the giant kelp *Macrocystis pyrifera*, where lipids act as primary energy sources during active swimming and dispersal (Brzezinski et al. 1993).

The high abundance of lysophosphatidylcholine esters identified in seeds from the Olbia region represents an interesting pattern in the metabolomic diversity of beached propagules. While the ecological significance of this variation remains to be determined, these compounds could reflect an adaptive strategy to ensure a sufficient energy supply for successful seedling establishment, especially under the variable environmental conditions typical of marine ecosystems. The relationship between fatty acid composition and germination has been reported for many terrestrial species such as sunflowers (Murcia et al. 2006) and cotton seedlings (Bartkowski et al. 1977). Although available studies on the effect of oil fatty acid composition on germination capacity are scarce and sometimes contradictory (Izquierdo et al. 2017), in the marine environment they are completely absent.

The interpretation of our results should consider certain methodological aspects. While we cannot provide absolute assurance of local seed origin, we acknowledge that beached fruits can originate from multiple unknown sources, potentially including meadows hundreds of kilometers away (Ruocco et al. 2025). The complex current patterns in the Mediterranean and the buoyancy of *P. oceanica* fruits make it impossible to determine their precise origins, however this inherent uncertainty remains a fundamental constraint of working with beached propagules. Additionally, the different conservation states of fruits, particularly evident in those from Oristano in advanced dehiscence, might have influenced our analyses. The natural stranding process results in fruits at various stages, and while we minimized potential bias through careful selection criteria and adequate replication, we provided baseline data representative of actual field conditions.

Overall, this study represents the first metabolomics report on the fruits of *P. oceanica*, expanding the understanding of the metabolomic and anatomical diversity of this species. Results highlight the importance of considering natural variability and the conservation status of fruit pericarps for conservation and restoration efforts of *P. oceanica* seagrass and open many questions to be further investigated. Future research could explore specific metabolites involved in stress resistance and plant health maintenance to develop more targeted conservation interventions. Understanding the natural range of variation in fruit characteristics provides essential information for monitoring future changes in *P. oceanica* reproductive ecology. The increased frequency of *P. oceanica* sexual reproduction events, already linked to global warming (Diaz-Almela et al. 2006; Stipcich et al.

2024a, b), makes understanding fruit and seed characteristics increasingly important.

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Data availability The data that support the findings of this study are available on request from the corresponding author (FP).

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics approval This article does not contain any studies with animals performed by any of the authors.

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