# Combining nursery techniques and a novel planting tool from seagrass beach-wrack for improving the sustainability of coastal habitat restoration practices

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"Declarations of interest: none."

## ABSTRACT

One of the major challenges for restoration ecologists is re-establishing vegetation cover in degraded seagrass meadows and coastal dunes. Traditionally, revegetation methods involve the translocation of large amounts of plants from healthy populations to damaged sites, but the sustainability of such practice has recently been questioned. The transplantation of plants propagated in nurseries from seed is a less destructive, alternative approach. However, both methods may lead to high mortality rates and involve the use of structures to avoid plant dislodgment that are generally made of non-biodegradable materials with potential impact on receiving habitats.

To improve the environmental sustainability of restoration actions, a novel biodegradable growing container made of beach-cast seagrass wrack and a bio-based polymer was produced. The long-term performance of two seagrasses, *Cymodocea nodosa* and *Zostera noltei*, and two dune plants, *Euphorbia paralias* and *Thinopyrum junceum,* grown in nurseries using the bio-container or a non-biodegradable plastic container of equal size/form was investigated. The feasibility of using *C. nodosa* nursery-raised plants with bio-containers for restoration interventions was also evaluated, and the success of this new approach was compared to that of a traditional nursery-based method. The bio-container degraded slowly in seawater and in sand and lost its functionality after about three years. All the study species performed better when grown in bio-containers than in non-biodegradable containers in the nursery. Six months after transplanting into the field, 80% of the *C. nodosa* nursery-raised plants with bio-containers were alive and have colonized the surrounding substrate. Most of those raised according to the traditional method was lost. These results indicate that the environmental sustainability of future coastal restoration interventions could be effectively improved by using nursery techniques in combination with the new planting bio-container. This approach would also provide an opportunity for valorizing seagrass beach-cast material.

*Keywords:*

Biodegradable growing container
Dune plants
Ecological restoration
Plant nursery
Seagrasses
Seagrass beach-cast wrack

## 1. Introduction

Seagrass beds and coastal sand dunes are among the most productive and valuable natural habitats, providing a range of goods and ecosystem services on a global scale. However, large areas of these habitats have been lost worldwide due to the combined effect of climate change and anthropogenic pressure (Barbier et al., 2011; Bayraktarov et al., 2016; Nordstrom, 2008), and restoring these areas is one of the greatest challenges for managers and ecologists. A promising approach for promoting the recovery of damaged habitats and facilitating the re-establishment of ecosystem functions is the replanting of native “engineering” species (Christensen et al., 2004; Lithgow et al., 2013; Nordstrom, 2008; Teixeira et al., 2016). To date, a number of revegetation techniques have been established. However, their environmental sustainability has recently been questioned, and some obstacles still limit their application on a large scale (Balestri and Lardicci, 2012; Bull et al., 2004; van Katwijk et al., 2016). Indeed, most of these seagrass revegetation techniques involves the removal of adult plants (either shoots with bare roots or with intact sediment) from healthy populations and their translocation (transplanting) to damaged sites (van Katwijk et al., 2016). To avoid the dislodgment of transplants due to abiotic and/or biotic disturbances, they need to be anchored to the substrate with appropriate structures (Balestri and Lardicci, 2012; Balestri and Lardicci, 2014; Bayraktarov et al., 2016; Irving et al., 2010; van

Katwijk et al., 2016). Unfortunately, most of the anchoring structures used up to date are made of materials non-biodegradable or extraneous to the receiving ecosystems, such as iron, cement, plastics and geo-textiles, that can potentially impact natural habitats (Short and Coles, 2001). In addition, to ensure an adequate vegetative coverage and replenish plant losses due to transplant shock, i.e., the difficult of plants to adjust their growth once planted in a new environment, large amounts of planting material are necessary (Bessette et al., 2018; van Katwijk et al., 2016), especially for seagrass species with slow growth and low seedling establishment rates (Balestri et al., 1998; Balestri et al., 2015; Holbrook et al., 2002; Seddon, 2004). The removal of this material can cause fragmentation of donor beds making them more vulnerable to storm events, and this in turn can potentially exacerbate the original problem (Balestri et al., 2011; Bull et al., 2004). To minimize the environmental impact, alternative and more ecologically sustainable strategies have recently been developed (Balestri et al., 2010; Balestri et al., 2011; Balestri and Lardicci, 2012; Bird et al., 1994; Dawes and Meads, 2009; Marion and Orth, 2010; Zarranz et al., 2010). One of these strategies, namely nursery seagrass approach, involves the propagation of plants from seeds or cuttings in aquaculture facilities using plastic pots commercially available for terrestrial plants (Balestri and Lardicci, 2012; Balestri and Lardicci, 2014; Balestri et al., 2015). Before transplanting to damaged sites, the plants are removed from their container to allow root growth and fixed to the substrate with anchoring structures.

A similar plant nursery-based approach has largely been used in coastal dune stabilization programs. The planting material required for these actions is generally obtained from seeds and cuttings collected from existing populations and grown in nurseries using non-biodegradable containers, such as plastic pots, root trainers, rays/pots, tubes and planter bags, that are removed before out planting (Bachman and Whitwell, 1995; Kidd, 2001; Miller et al., 2018). However, very harsh environmental conditions and recurrent natural disturbances can exacerbate transplant shock causing large plant losses (Balestri and Lardicci, 2013; Maun 2009; Teixeira et al., 2016).

Recent studies on crop and ornamental plants have shown that poor root allocation due to pot constraints and transplant shock can be minimized by planting nursery-raised seedlings and adults in soil together with bio-containers (i.e., biodegradable growing containers produced from natural fibers or bio-based polymers, Nambuthiri et al., 2015). To date, some attempts have been made to cultivate seagrasses in pots manufactured with natural fibers such as paper, cloth and peat (Bacci et al., 2014; Bird et al., 1994; Dawes and Meads, 2009; Kirkman, 1998; Seddon, 2004; Short and Coles, 2001), but their quick degradation in water (Bird et al., 1994; Irving et al., 2010) make them not suitable for long-term restoration programs. Instead, the biodegradability in marine environments of most of the bio-based polymers used to manufacturing bio-containers has not been proven or assessed (Nambuthiri et al., 2013; Volova et al., 2010). Given the need for coastal habitat restoration, and in consideration of the global policies recently adopted to reduce marine litter (Nazareth et al., 2019; Xanthos and Walker, 2017), the development of new, environmentally compatible tools for coastal restoration interventions is highly desirable.

In the present study, we designed and tested the feasibility of using a novel plant growing bio-container made of a seagrass wrack-based polymer composite (Seggiani et al., 2018) for seagrass meadow and sand dune restoration interventions. Composites of beach-cast seagrasses and bio-based polymers have recently been proven to possess good mechanical properties and to be potentially suitable for applications in marine environments (Seggiani et al., 2018). Moreover, the accumulation of seagrass wrack on beaches of some countries can cause management problems and it is a disposal challenge (Macreadie et al., 2017). For example, in Italy excessive accumulations of wrack of the seagrass *Posidonia oceanica* L. Delile, considered as a waste, are periodically removed from beaches, and part of this material transformed in compost (Legislative Decree no. 75, 2010). Specifically, we investigated in nursery experiments (i) the performance of seagrasses with different growth rates, slow (C*ymodocea nodosa* Ucria Asch.) vs. fast rate *(Zostera noltei* Hornem), and dune plants belonging to different classes, monocotyledons (*Thinopyrum junceum* (L.) Á. Löve) and dicotyledons (*Euphorbia paralias* L.), cultivated in the novel bio-container and (ii) the persistence of the bio-container over time. A plastic container of equal size and form, made of a non-biodegradable polymer commonly used for terrestrial pots manufacturing, was also tested. We assessed (i) whether the bio-container would support the growth of different plant species in culture and (ii) how long it would maintain the functionality before to degrade. We also evaluated in a field experiment the success, in terms of plant survival and growth, of the whole new method, i.e. propagating plants in nursery by cutting and planting nursery-raised plants *in situ* with their bio-container, using *C. nodosa* as a model. The success of this method was also compared to that achieved using the previously established nursery-based one to assess potential benefits. Our ecological approach is relevant in the context of environmental management as it would help in improving the sustainability of future coastal restoration interventions and providing a new route for valorizing seagrass beach-cast material.

## 2. Materials and methods

### *2.1. Bio-container design and manufacturing*

The newly designed bio-container was a bowl characterized by a height of 75 mm, a bottom diameter of 90 mm, a top diameter of 150 mm, a thickness of 1 mm and 1000 $cm^3$ volume (Fig. 1). The structural geometry of the container was reinforced with a wide curvature radius in the basal part to improve its stability in mobile sediments. Holes in the bottom and along the wall (35 mm below the border) of the container allowed water/nutrient exchanges between the container substrate and the surrounding environment. The small diameter of these holes (2 mm) prevented sand washing out from the container once watered or immersed in seawater. The composite used to manufacture the bio-container is biodegradable both in marine and terrestrial environment (Seggiani et al., 2017a, b; Seggiani et al., 2018), and it is not commercially available. It was produced by injection extrusion (Zefiro, Italy) of a mixture of *Posidonia oceanica* lignocellulosic fibers (10% of the total weight) derived from “egagropili”(i.e. agglomerates of residues of dead rhizomes and leaves, Cannon, 1979) and the thermoplastic matrix PHI002™ consisting of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV, Naturplast®, Caen, France), a polymer synthesized by a variety of microorganisms (Lee, 1996). The plasticizer acetyl tributyl citrate (10% of the total weight, Sigma Aldrich, St. Louis, MS, USA) and calcium carbonate (4% of total weight, OMNYA® Oftringen, Switzerland) with fine grain size (12 μm) were added to the mix to facilitate composite ductility and processability (Seggiani et al., 2018). The bio-container (thereafter referred to as PHBP container) was designed using computer-aided manufacturing and produced by injection-molding using standard industrial manufacturing machinery (Femto Engineering, Italy). A container with equal characteristics but made of a petroleum-based plastic (polypropylene) and iron oxide pigment (thereafter referred to as PP container) was also produced to serve as a reference since this polymer is used to manufacture most of the pots currently marketed for terrestrial plants.

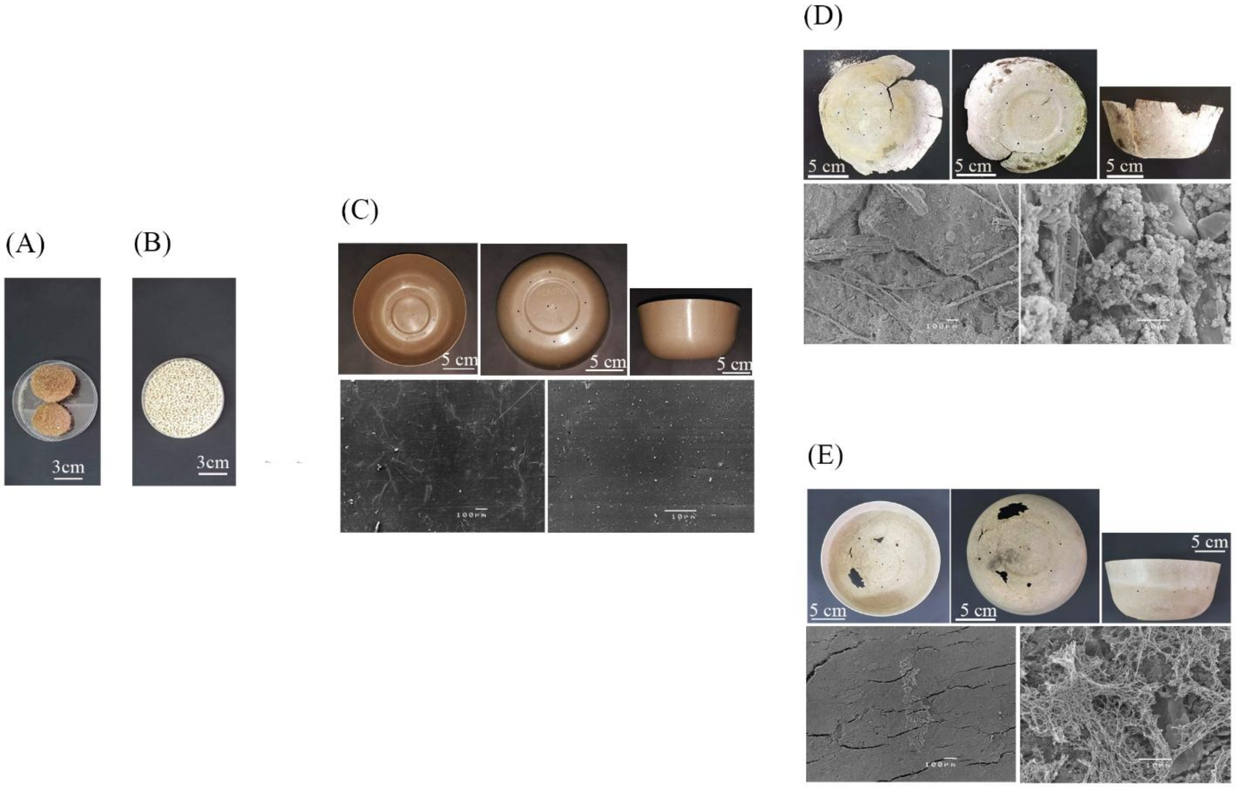

**Fig. 1.** *Posidonia oceanica* “egagropili” (A) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV, B) used to manufacture the bio-container, details and SEM images of the virgin bio-

container (July 2016) (C) and the same container 26 months after planting (September 2018) in the seagrass (D) and dune nursery experiments (E).

1.5-column fitting image

Before the start of the experiments, a test was conducted to examine both the buoyancy and the water retention capability of the two types of container. To this end, containers (n = 5 per container type) were weighted and then immersed in distilled water at 25°C. After 24 h of immersion they were extracted from water, dried with paper and weighted. Water retention capability was calculated as difference between final and initial container weight. The PHBP container was negatively buoyant and the average net gain in weight after water immersion was 2.69 g (± 0.16 SE), corresponding to an increase of about 5% of initial weigh. Instead, the PP container was positively buoyant and did not retain water.

### *2.2. Set up of plant nursery experiments*

To evaluate the suitability of the bio-container in sustaining seagrass and dune plant growth and the bio-container functionality over time, in terms of maintenance of physical integrity, two separate long-term nursery experiments were conducted at the INVE Aquaculture Research Center located in a back-dune area at Rosignano Solvay (Italy). The seagrass nursery consisted of outdoor tanks (7000 L) equipped following a protocol previously established for growing seagrasses (Balestri and Lardicci, 2012). The seawater level in the tanks was maintained at 0.5 m by providing continuous natural seawater supply. Seawater temperature ranged from 11 to 28 °C, pH was 8.0-8.2, and salinity varied between 37.6 and 38.2 over the experimental period. The dune nursery consisted of an outdoor area with an artificial dune (1.50 x 1.50 m, h 0.30 m) created with local beach sand. Air mean temperature ranged from 4.6 to 29.8 °C during the whole study period. Before the start of the planting experiments, all the containers were weighted and filled with commercially available silica sand (1 mm diameter, organic matter < 0.01%). A controlled-release fertilizer (Cifo Italy, N:P:K 20:10:10; six months) was added (1 g $L^{-1}$ of sand) in each container to facilitate plant establishment.

### *2.2.1. Seagrass nursery experiment*

In July 2016, 28 containers (14 PHBP and 14 PP containers) with sand were placed in a completely randomized design in an aquaculture tank and attributed to one of two species, *Cymodocea nodosa* and *Zostera noltei.* Initial planting materials (cuttings) were obtained from two-year old plants grown in a previously established seagrass nursery (Fig. 2, Balestri and Lardicci, 2012). For each container type, there were in total 14 cuttings (seven cuttings per species). Each *C. nodosa* cutting consisted of a 7.5 cm rhizome fragment and three shoots while each *Z. noltei* cutting consisted of a 4.5 cm rhizome fragment and two shoots. Plants were left undisturbed for 26 months, and the number of alive plants and the number of shoots per plant were recoded every two months. The containers were monthly inspected over the experimental period to detect conformational changes and signs of degradation, such as discoloration and presence of physical openings in the container walls. At the end of the experiment, the plants were extracted from the containers, and all the containers were transferred to the laboratory where they were carefully cleaned, washed with tap water, dried at 30 °C for five days and then weighted to determine the extent of their degradation. Container degradation was determined as weight loss and expressed as a percentage of the initial dry weight. In addition, samples of the PHBP containers were fixed in 2% OsO4, dehydrated in ethanol and, after critical point drying, coated with gold and observed with a JEOL/JSM-5410 scanning electron microscope to visualize texture alterations of their wall. Samples from virgin PHBP containers were also collected and examined for comparison.

To evaluate the performance of plants, the total number of alive vs. dead plants of both the species grown with the PHBP bio-container or the PP container was compared using the Fisher exact one-tailed test. Data on the maximum number of plant shoots were analyzed using two-way analysis of variance (ANOVA) with container type and species as fixed orthogonal factors to test for possible variations in plant growth response due to different architecture and growth rate. Prior to the analyses, data were assessed for normality and homogeneity of variance using Shapiro-Wilk test and Cochran's C test (α = 0.05), respectively. The maximum number of shoots was log x transformed to meet ANOVA assumptions. All the analyses were run using STATISTICA 6.0 (Statsoft, Inc).

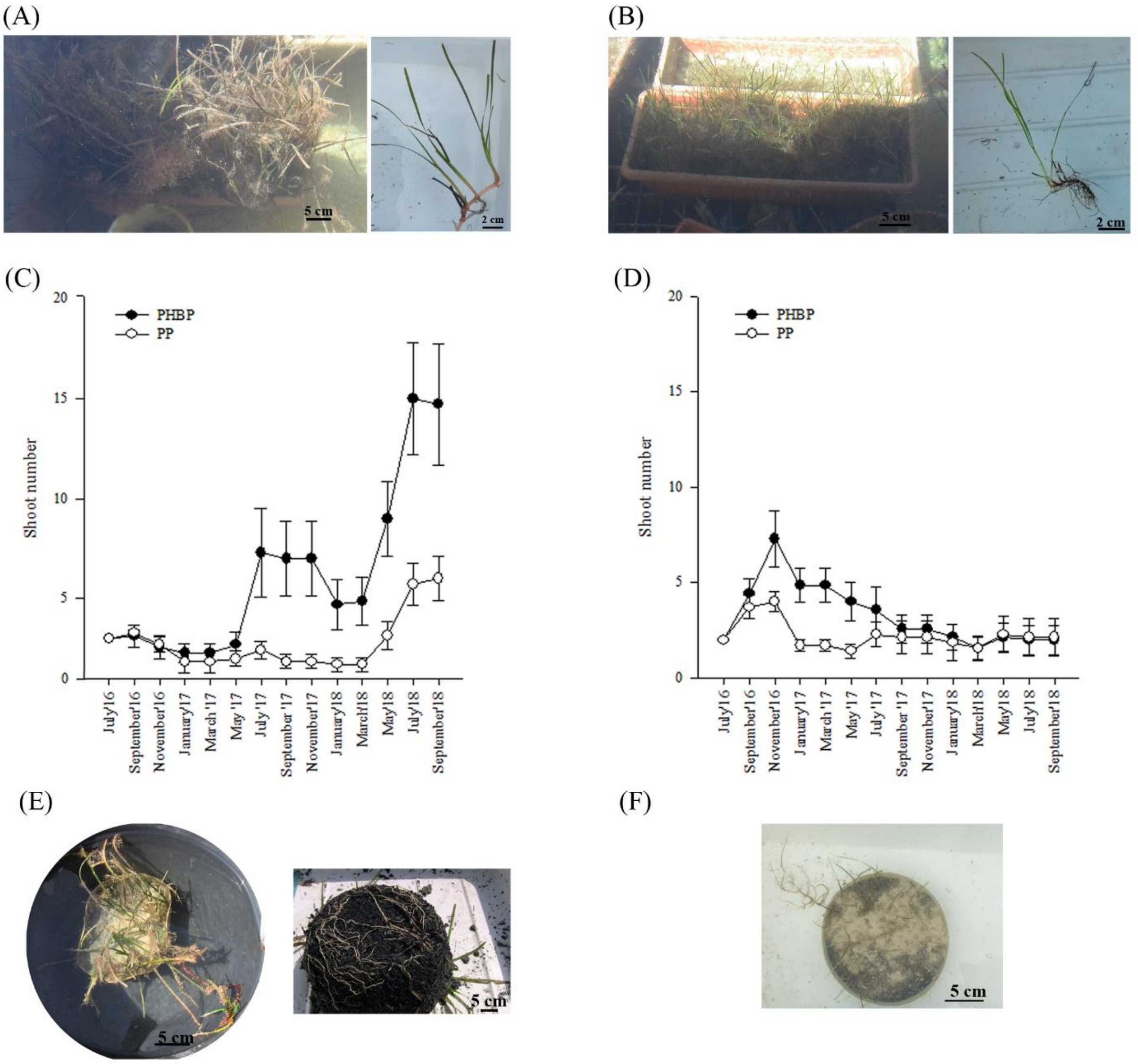

**Fig. 2.** Mother plants and cuttings of *Cymodocea nodosa* (A) and *Zostera noltei* (B) before the start of the seagrass nursery experiment, number of alive shoots recorded in *C. nodosa* (C) and *Z. noltei* (D) plants grown in PHBP and PP containers over the experimental period, and *C. nodosa* (E) and

*Z. noltei* (F) plants with rhizomes extending out the container substrate in the nursery. Data are means ± SE.

2-column fitting image

### 2.2.2. Dune nursery experiment

In July 2016, seeds of *Thinopyrum junceum* and *Euphorbia paralias* were collected from local populations and sown in an outdoor seedbed. In May 2017, one-month-old seedlings (Fig. 3) were individually transplanted into 20 containers (10 PHPB and 10 PP containers). There were five seedlings per species and container type. Seedlings were left to grow in their container for about one year. At the end of the experiment (March 2018), the status of plants (alive vs. dead), the maximum height of aboveground organs and the number of erect stems of *T. junceum* and *E. paralias* in each container were recorded.

In July 2016, additional (six) PHBP containers were individually inserted into holes made in sand in randomly chosen positions in the artificial dune to investigate their functionality over time. In March 2017, five seeds of the dune plant *Glaucium flavum* Crantz collected from a local population in the previous summer were planted into each container to provide vegetative cover. Only one seedling was left in each container. At the end of this experiment (September 2018), the containers were removed from sand and transferred to the laboratory where they were dried and individually weighted to calculate the percentage of weight loss. Samples of virgin and environmentally exposed PHBP containers were collected and processed as described in the previous section for SEM analyses.

To evaluate the performance of plants, the total number of alive vs. dead plants for each container type was analyzed using the Fisher exact one-tailed test. Data on plant height and number of stems of each species were analyzed using two-way ANOVA with container type and species as fixed orthogonal factors to test for species variations in growth response. Prior to the analyses, data were checked for normality and homogeneity of variance using Shapiro-Wilk test and Cochran's C test (α = 0.05), respectively, and no transformation was necessary. All the analyses were run using STATISTICA 6.0 (Statsoft, Inc).

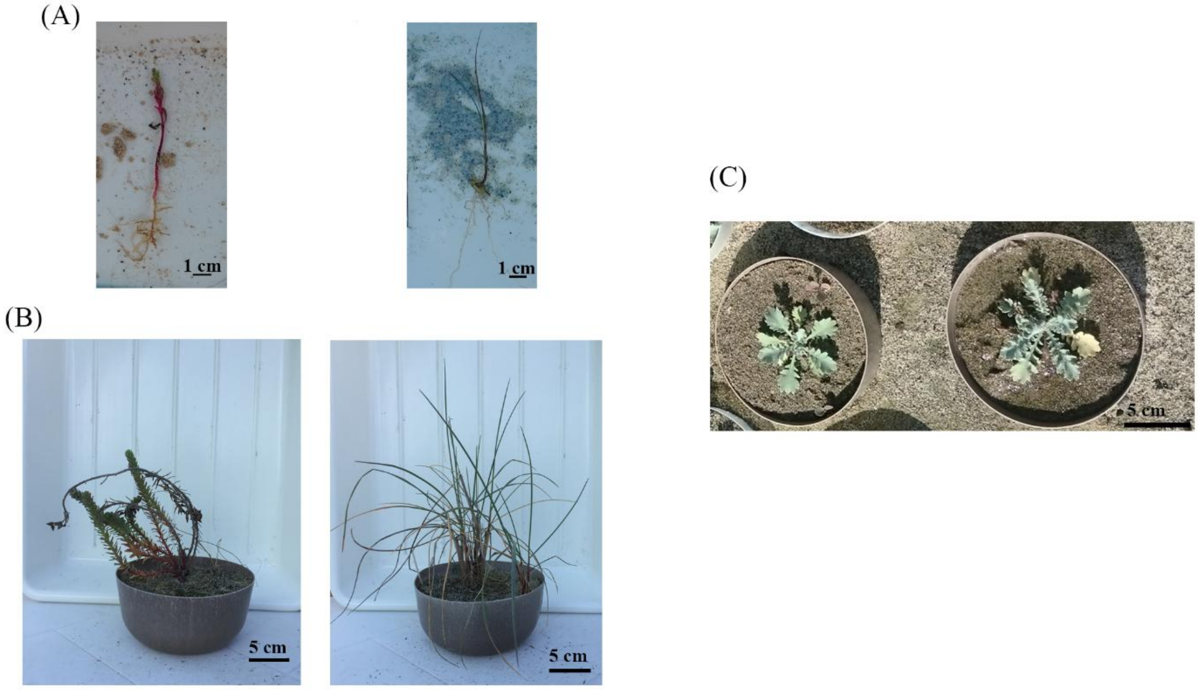

**Fig. 3. S**eedlings of *Euphorbia paralias* (left side) and *Thinopyrum junceum* (right side) before (A) and one year after planting (B) in the dune nursery. Bio-containers (PHBP) buried in sand (C).

1.5-column fitting image

### *2.3. Field seagrass experiment*

To evaluate the feasibility of restoring seagrass meadows by using the new nursery/bio-container-approach, the performance of nursery-raised plants transplanted with their PHBP container was examined in the field. The success of this new approach was also compared to that achieved by using the previously developed nursery-based approach, i.e. the out planting of nursery-raised plants removed from non-biodegradable plastic containers (Balestri et al., 2012). In

April 2017, similar-sized cuttings (20) of *Cymodocea nodosa,* excised from nursery-grown mother plants (Fig. 3), were individually planted in PHBP and PP containers. Plants were left undisturbed for about one year in the nursery as described above to allow root development. At the start of the second growing season, just prior to the beginning of container degradation (March 2018), the plants with their containers were loaded in trays with seawater covering and transferred to a relatively sheltered coastal area near to Rosignano Solvay. This area had experienced human impact and had a history of having seagrasses; the substrate was composed of fine carbonate sand and recolonized by *C. nodosa* (Fig. 4). The containers with plants were distributed along the edge of the *C. nodosa* meadow (at 0.5 m depth). To place plants at the same substrate level as local naturally established plants, twenty holes were made in the substrate with a spade in randomly positions within an area of about 50 m$^2$. PHBP containers were directly inserted into holes, while PP containers were removed and the plants with their growing substrate were inserted into holes and fixed with iron staples as described by Balestri et al. (2014). Containers and plants were weekly monitored over the first transplanting season. At the end of the experiment (October 2018), the number of plants still in place was counted. Seawater temperature ranged from 15 to 29.8 °C during the experimental period. Plant development was non-destructively evaluated by counting *in situ* the number of survived plants and the number of alive shoots per plant. The number of plants with rhizome expanding out of their original growing substrate was also recorded to determine the colonization extent.

Data on the recorded number of plants still in place or lost with the new vs. the previous method were analyzed using the Fisher exact one-tailed test. Prior to the analysis, data were assessed for normality using Shapiro-Wilk test. Since most of the seagrasses obtained by using the previous method and transplanted without the container was lost during the experiment**,** data on shoot number and colonization extent were not analyzed. All the analyses were run using STATISTICA 6.0 (Statsoft, Inc).

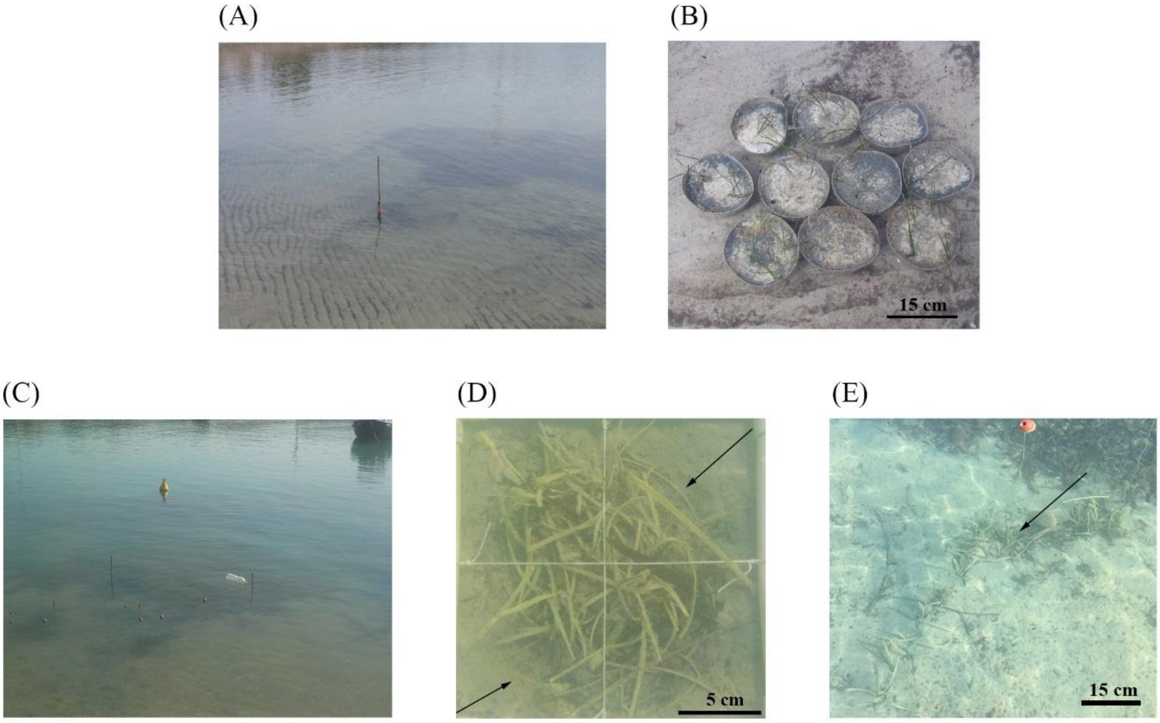

**Fig. 4.** Transplantation site (A), one-year old *C. nodosa* plants grown PHBP (B) containers in the marine nursery before their transplantation, and plants still in place six months after transplantation with PHBP containers (C, D). Arrows indicate the borders of a buried container (D) and the rhizomes of plants extended out the original container substrate (E).

2-column fitting image

## 3. Results

### *3.1. Seagrass nursery experiment*

During the experimental period, both seagrass species exhibited the typical growth cycle observed in the Mediterranean Sea with cessation of rhizome growth and shoot production in winter (Kraemer and Mazzella, 1992). The number of shoots produced by *C. nodosa* plants grown in the PHBP container (Fig. 2) increased over time and peaked in the middle of July of the second growing season (14 ± 2.7 shoots per plant). Instead, the highest number of *Z. noltei* shoots (7.3 ±

1.4 shoots per plant) was observed at the end of the first growing season (Fig. 2). Subsequently, the number of *Z. noltei* shoots decreased due to the detachment of rhizome portions extending out the substrate container. At the end of the experiment, all *C. nodosa* plants were alive and showed rhizomes extending out the container substrate (Fig. 2) while some *Z. noltei* plants were died (Table 1). No significant effect of container type was detected on the total number of survivors (p = 0.50). For both species, the maximum number of newly produced shoots was significantly higher in PHBP containers than in PP containers (Table 2). Plants grown in PHBP containers also showed a well-developed root system with white actively growing root tips. When extracted from their bio-container, the root mass held all of the sand (Fig. 2), a behavior typic of healthy root systems.

| Container type | No. alive/dead plants per species | | Total number of alive/dead plants |
|---|---|---|---|
| a) | *C. nodosa* | *Z. noltei* | |
| PHBP | 7/0 | 5/2 | 12/2 |
| PP | 7/0 | 4/3 | 11/3 |
| b) | *T. junceum* | *E. paralias* | |
| PHBP | 5/0 | 5/0 | 10/0 |
| PP | 5/0 | 4/1 | 9/1 |
| c) | *C. nodosa* | | |
| PHBP | 8/2 | | 8/2 |
| PP | 1/9 | | 1/9 |

**Table 1**

Number of survived plants grown in the new bio-container (PHBP) and in an equal container made of a non-biodegradable polymer (PP) in the seagrass (a) and (b) the dune nursery. The number of survived *C. nodosa* plants in the field (c) using the novel PHBP container-nursery method and the nursery approach based on PP containers was also reported.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Container type = C | 1 | 2.0878 | 10.05 | **0.004** |
| Species = S | 1 | 0.7364 | 3.55 | 0.071 |
| CXS | 1 | 0.0003 | 0.00 | 0.968 |
| Residual | 24 | 0.2076 | | |
| Total | 27 | | | |
| | | | | |
| Log x transformation | | | | |
| SNK test | PHBP > PP | | | |

**Table 2**

Results of two-way ANOVA on the maximum number of shoots produced by *Cymodocea nodosa* and *Zostera noltei* plants grown in the novel biodegradable container (PHBP) and in the non-biodegradable container (PP) in the seagrass nursery. Results of SNK test were also reported. Significant values are in bold.

All PHBP containers were still intact and retained their functionality one year after seawater immersion. Conformational changes of the bio-container and signs indicators of undergoing degradation processes, such as discoloration and presence of breaks in container walls, were observed by the start of the second planting year (Fig. 1). The mean weight of virgin PHPB and PP containers was 54.83 g (± 0.03) and 38.56 g (± 0.07), respectively. At the end of the experiment, PP containers retained their integrity and did not exhibit any variation of weight as expected. Instead, the wall of PHBP containers was entirely colonized by macroalgae and other marine organisms, and the containers resulted extremely fragile (Fig. 1) making their handling difficult. The extent of their degradation, in term of percentage of weight loss, was 19.42 % (± 1.18). Considerable texture changes also occurred on PHBP containers (Fig. 1). SEM observations revealed that their surface became totally heterogeneous and rough with holes and fissures. The same surface was almost completely colonized by a thick and well-developed biofilm, including diatoms, bacteria and fungi probably involved in the biodegradation process (Fig. 1).

### *3.2. Dune nursery experiment*

At the end of the experiment, all *T. junceum* plants were still alive and only one out of five *E. paralias* planted was died (Table 1). The total number of survivors was not affected by the type of container ($p = 0.50$). Significant differences in plant height (Table 3) were observed between container types and species (Table 3). On average, plants grown in PHBP containers were significantly taller than those raised in PP ones (Table 3), and *T. junceum* was taller than *E. paralias* (Fig. 3, Table 3) irrespectively of container type. No significant effect of container type, species or their interaction was observed for the number of stems per plant (Table 3).

PHBP containers extracted from sand at the end of the experiment still retained their original shape but showed signs of degradation such as the presence of holes, especially in their basal part (Fig. 3). On average, the containers have lost 9.72 % (± 1.48) of their initial weight. At SEM visualization, the surface of the planted PHBP containers was fissured and deeply uneven. A discontinuous biofilm with a spotted distribution (Fig. 1) was present and bacteria were also singly scattered all over the surface.

a)

| Container type | Plant height (cm) | | No. of stems | |
|---|---|---|---|---|
| | *T. junceum* | *E. paralias* | *T. junceum* | *E. paralias* |
| PHBP | 35.3 ± 0.8 | 27.4 ± 3.7 | 10 ± 2.7 | 8.4 ± 0.6 |
| PP | 27.4 ± 3.7 | 20.2 ± 5.4 | 11.2 ± 2.1 | 4.6 ± 1.1 |

b)

| Source | df | MS | F | P | MS | F | P |
|---|---|---|---|---|---|---|---|
| | | Plant height | | | Number of stems | | |
| Container type = C | 1 | 270.8480 | 4.53 | **0.04** | 11.2500 | 0.65 | 0.43 |
| Species = S | 1 | 301.0880 | 5.04 | **0.03** | 76.0500 | 4.40 | 0.05 |
| CXS | 1 | 0.0980 | 0.00 | 0.96 | 36.4500 | 2.11 | 0.16 |
| Residual | 16 | 59.7793 | | | 17.3000 | | |
| Total | 19 | | | | | | |
| | | | | | | | |
| SNK test | | PHBP > PP | | | | | |
| | | *T. junceum* > *E. paralias* | | | | | |

**Table 3**

Results of two-way ANOVA on plant height and number of stems produced by *Thinopyrum junceum* and *Euphorbia paralias* plants grown in the novel biodegradable container (PHBP) and in the non-biodegradable container (PP) in the dune nursery. Results of SNK test were also reported. Significant values are in bold.

### *3.3. Field seagrass experiment*

During the first month of transplantation, a severe storm caused the dislodgment of some plants from the substrate. At the end of the first transplanting season, the number of alive plants (Table 1) raised in PP containers and transplanted with iron tools was significantly lower than that of those planted with the PHBP container (p = 0.002). These latter plants had produced from 4 to 31 new shoots (mean 14.5 ± 3.3), and five of them showed at least one horizontal rhizome (runner) extending out to the substrate container (Fig. 4). The plant raised in the PP container still present at the end of the study period showed 18 new shoots and two runners.

## 4. Discussion

The propagation of seagrasses and dune plants under controlled conditions is a promising alternative method for improving the sustainability of restoration actions, enabling restoration practitioners to achieve large supply of planting material with limited impact on natural populations. However, effective eco-compatible tools for propagating and transplanting plants in restoration sites are not still developed. Indeed, most of the bio-containers currently marked for long term use in plant nursery and greenhouse production are designed to degrade at the end of the cultivation cycle in industrial facilities (Schrader et al., 2017). Since the polymers used for manufacturing these bio-containers do not degrade or degrade very slowly when planted in aquatic environments (Balestri et al., 2017; Nazareth et al., 2019), they are not recommended for coastal revegetation interventions. Very few types of containers, for example those made of bio-based polymers such as polyhydroxyalkanoates, can degrade in soils and thus can be considered as plantable (Nambuthiri et al., 2013; Schrader et al., 2017).

The present study is the first aimed at developing a new plantable bio-container made of natural fibers from marine plants specifically designed for growing and transplanting nursery-raised coastal plants. The released of these fibers during bio-container degradation do not constitute a hazardous material for the receiving ecosystems being themselves a natural coastal habitat component. The polymer polyhydroxyalkanoate (PHBV) was chosen as a matrix because it is one of the few polymers proven to biodegrade both in marine habitats (Nambuthiri et al., 2013; Volova et al., 2010). The incorporation of *P. oceanica* leaf fibers into the PHBV matrix enhanced bio-composite flexibility (Seggiani et al., 2018), and the poor fiber adhesion to the matrix and high cellulose fiber content have found to promote polymer degradation by increasing water uptake (Ferrero et al., 2015; Khiari et al., 2011; Le Duigou et al., 2014; Seggiani et al., 2018).

Results of our study demonstrate that the bio-container degraded after about three years in seawater and dune sand, a period long enough to obtain well-developed plants in nurseries. The bio-container also resulted plantable in sandy substrates using standard sediment extracting tools. All the investigated species cultivated in the PHBP containers performed better than those grown in equal containers made of polypropylene, a conventional polymer commonly used to manufacture pots for terrestrial plants. The persistence and the development of *Cymodocea nodosa* plants in the nursery indicates that this species could be maintained in culture for long periods (years) before the transplantation in the field. Instead, the decline in shoot number in *Z. noltei* suggests that this species would be preferentially transplanted during the first months of culture. A decline in *Z. noltei* shoot number over time was also reported in previous experiments (Suykerbuyk et al., 2016). The observed higher survival rate of *C. nodosa* plants recorded in the field using the bio-container-based approach compared to that of the previously established approach, i.e., without the bio-container, and their quick colonization of the surrounding substrate by centrifugal spread, indicates that this method would be effective for restoration interventions. The number of shoots produced by container-grown plants during the first transplanting season was about two folds that recorded in a previous study on bare *C. nodosa* cuttings obtained from nursery-raised mother plants and transplanted in a restoration site with iron anchoring tools (Balestri and Lardicci, 2014). Here, the presence of the bio-container might have favored the establishment and spread of plants in coastal environments by minimizing transplant shock and enhancing the capacity of plants to resist to physical disturbances such as those due to storm events. However, the positive effects of the bio-container on shoot production during the nursery growth period before transplanting could also play an important role. These findings suggest that by applying simple propagation techniques in combination with the bio-container, restoration practitioners could be able to produce seagrasses in nurseries established permanently or temporary, depending upon the biology of target species and duration of the interventions, preferentially close to restoration sites, and then directly planted nursery-grown plants with the bio-container in these sites during the most favorable period.

The planting of nursery-raised dune plant species is already an indispensable part of dune stabilization projects (Bachman and Whitwell, 1995; Miller et al., 2018; Siyag, 2014). However, dune plants generally require the application of rooting hormones and fertilizers to grow (Balestri et al., 2012; Thetford and Miller, 2002), and they must be acclimatized to harsh dune conditions for several months before being transplanted into the field to increase the chance of a successful establishment (Miller et al., 2018; Siyag, 2014). In addition, repeated watering is required during and after planting because water is not long retained by sand. Our results suggest that planting of nursery-grown plants directly with our bio-container in the field could not only minimize transplant shock but also reduce the frequency of water applications owing to the capacity of the container to absorb and retain water.

Given the relatively small size, our bio-container is probably best suited for transplanting rooted cuttings and seedlings of small or relatively fast-growing species. However, the bio-container could be useful even for sowing seeds of some species. Seeds could be readily germinated in the nursery and grown in the bio-containers before planting, thereby minimizing the negative impacts of harvesting of plants from donor meadows. The design of the bio-container could be also adjusted to achieve greater substrate capabilities and support the growth of larger plants. The lifetime span of the bio-container could be either enhanced by increasing wall thickness to be employed in long-term restoration projects or reduced by increasing the fiber content of the composite up to 20% (Seggiani et al., 2018) for application in short-term restoration projects. In addition, leaf fibers of other seagrasses, and in particular *Zostera marina* L, could be used as an alternative to *P. oceanica* ones as they have been found to be a suitable material for polymer reinforcement (Davies et al., 2007).

Overall, the findings of this study indicate that our novel conceptual and methodological approach could be useful for restoring different coastal habitats, such as seagrass beds, foredunes, estuaries and coastal lagoons. Importantly, it could offer considerable ecological and practical benefits by simultaneously alleviating some limitations to the success of current restoration practices, including the impact of plant collection, transplant shock and pollution of receiving natural habitats. Lastly, the bio-container is relatively inexpensive (about 0.20 €-container) and could provide a new opportunity for valorizing seagrass beach-cast material.

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