

## Article

# Performance Assessment of *Posidonia oceanica* (L.) Delile Restoration Experiment on Dead *matte* Twelve Years after Planting—Structural and Functional Meadow Features

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**Abstract:** Following the restoration of natural conditions by reducing human pressures, reforestation is currently considered a possible option to accelerate the recovery of seagrass habitats. Long-term monitoring programs theoretically represent an ideal solution to assess whether a reforestation plan has produced the desired results. Here, we report on the performance of a 20 m<sup>2</sup> patch of *Posidonia oceanica* transplanted on dead *matte* twelve years after transplantation in the Gulf of Palermo, northwestern Sicily. Photo mosaic performed in the area allowed us to detect 23 transplanted patches of both regular and irregular shape, ranging from 0.1 to 2.7 m<sup>2</sup> and an overall surface close to 19 m<sup>2</sup>. Meadow density was 331.6 ± 17.7 shoot m<sup>-2</sup> (currently five times higher than the initial value of 66 shoots m<sup>-2</sup>), and it did not show statistical differences from a close by natural meadow (331.2 ± 14.9). Total primary production, estimated by lepidochronology, varied from 333.0 to 332.7 g dw m<sup>2</sup>/year, at the transplanted and natural stand, respectively. These results suggest that complete restoration of *P. oceanica* on dead *matte* is possible in a relatively short time (a decade), thus representing a good starting point for upscaling the recovery of the degraded meadows in the area.

**Keywords:** *Posidonia oceanica*; seagrass transplantation; dead *matte*; carbon stock; long-term monitoring; seabed mapping; habitat recovery



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## 1. Introduction

Seagrass habitats represent one of the most rapidly declining ecosystems on Earth [1]. As stated by [2], the rate of decline in known areas is 7% per year since 1990, and about 14% of all seagrass species currently show a high risk of extinction [3]. The decline is due to several factors: increasing human activities that affect water quality, turbidity and siltation in coastal areas, urban and industrial sewage, coastal development, trawling, aquaculture activities, anchorages, artisanal and recreational coastal fishing, global climate change and cumulative effects of multiple stressors at different temporal and spatial scales [4–13].

Approximately 10–15% of global oceanic organic carbon is stored in seagrass meadows [14,15]. The loss of seabed vegetation cover involves erosion of upper sediments [16], which may result in the degradation of blue carbon storage, increasing greenhouse gas emissions and accelerating global climate change [17].

In the Mediterranean, 13 to 50% of *Posidonia oceanica* (Linnaeus) Delile meadows have regressed considerably in terms of area and in some cases complete loss [12,13]. In the meantime, the remaining meadows have suffered a reduction in density and coverage in the last 20 years [12]. Hence, [12] estimate that the loss of *P. oceanica* meadows has caused

11–52% reduction in the carbon sink capacity of the entire Mediterranean. A recovery plan for such systems is, therefore, required to halt and reverse the expected decline of the species by year 2049  $\pm$  10 [18].

On the other hand, there is a lack of accurate data on *P. oceanica* distribution and quality for most of the Mediterranean Sea [10], and it is possible that the regression of seagrass meadows has been overstated [10,19,20]. However, *P. oceanica* meadows have regressed noticeably in the past decades in several coastal areas of the Mediterranean because of human pressures.

Currently, the main actions required to prevent and reduce the regression of *P. oceanica* meadows are focused on (i) reducing human pressure on marine ecosystems, (ii) increasing efforts to conserve existing meadows and (iii) promoting the resilience of the species by means of transplantation activities [12].

In recent decades, several rehabilitation measures for *P. oceanica* have been developed and implemented in many countries around the Mediterranean basin, namely:

- Urban and industrial wastewater treatment and wastewater disposal.
- Legislative instruments, based on EU Directives, aimed at environmental assessment of projects (Environmental Impact Assessment—E.I.A.), plans and programs (Strategic Environmental Assessment—S.E.A).
- Establishment of parks, marine protected areas and reserves along the coastal zone.

Due to their ecological role and the multiple ecosystem functions provided by seagrasses [21,22], the European Union, through the Habitats Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora, has included *P. oceanica* meadows among the priority habitats (Habitat code 1120\*). On 1 March 2019, the United Nations declared the UN Decade on Ecosystem Restoration 2021–2030, aimed at boosting existing efforts to restore degraded ecosystems and 350 million hectares of degraded ecosystems globally by 2030, including *P. oceanica* meadows. Furthermore, in May 2020, the European Union, within the framework of the EU Biodiversity Strategy for 2030 (COM(2020) 380 final) Bringing nature back into our lives, introduced a plan to restore ecosystems across land and sea, particularly those with high potential to capture and store carbon.

Following the restoration of natural conditions by reducing human pressures and removing the sources of degradation, reforestation is currently considered a possible option to accelerate the recovery of seagrass habitats [23].

However, restoration works have shown a high level of failure on a global scale [24], and the rate of success of all/most European recovery projects was very low [25].

It is well known that areas potentially suitable for restoration interventions may have low chances of rooting and development of transplanted plants [26]. Several studies have highlighted the importance of careful habitat selection for transplanting seagrasses [11,26–28]. Furthermore, most transplanting failures are attributed to plant detachment [29,30], and about 80% of *P. oceanica* transplantation losses are due to inappropriate plant anchoring systems [31]. Therefore, to optimize transplanting and restore degraded beds, it appears useful to combine multi-criteria models for optimal site selection and the use of appropriate anchorage systems [11,20].

Recently, models encompassing qualitative and quantitative data, including short-term monitoring of transplantation outcomes, have been developed to assess both the suitability of the area to be restored and the potential for success [11,32–34]. However, to establish whether a transplantation framework can really be considered successful, further temporal steps are clearly required in order to decide on whether a given a habitat is suitable for restoration. Long-term monitoring programs theoretically represent an ideal solution to assess whether a reforestation plan has actually produced the desired recovery effect [11,20,25,27], and [20] note that a “good outcome in the early stages does not necessarily correspond to real transplant success and, vice versa, low initial performance does not necessarily compromise a positive result in the future”.

Unfortunately, long-term monitoring of seagrass restoration to assess performance has been implemented in a limited number of cases [11], while short-term monitoring remains the norm (<1 year) [25].

A previous study aimed at testing the performance of a *P. oceanica* transplantation carried out in a dead *matte* area showed the short-medium term effect of the transplantation on some morphological and structural variables [11]. In particular, one year after the leaves of transplanted shoots were shorter than local natural ones, while six years after, the density was very low if compared with the expected values of a natural stand at same depth. However, considering that the seagrass is a slow-growing species, further extension of the monitoring period was clearly recommended to better assess the recovery of the system [11]. In this study, we returned to the same area in order to compare the transplanted shoots with those of a natural meadow 12 years after transplanting. Several metrics related to structural and functional descriptors collected at different hierarchical levels were analyzed for the evaluation in the longer term of the restoration process.

## 2. Materials and Methods

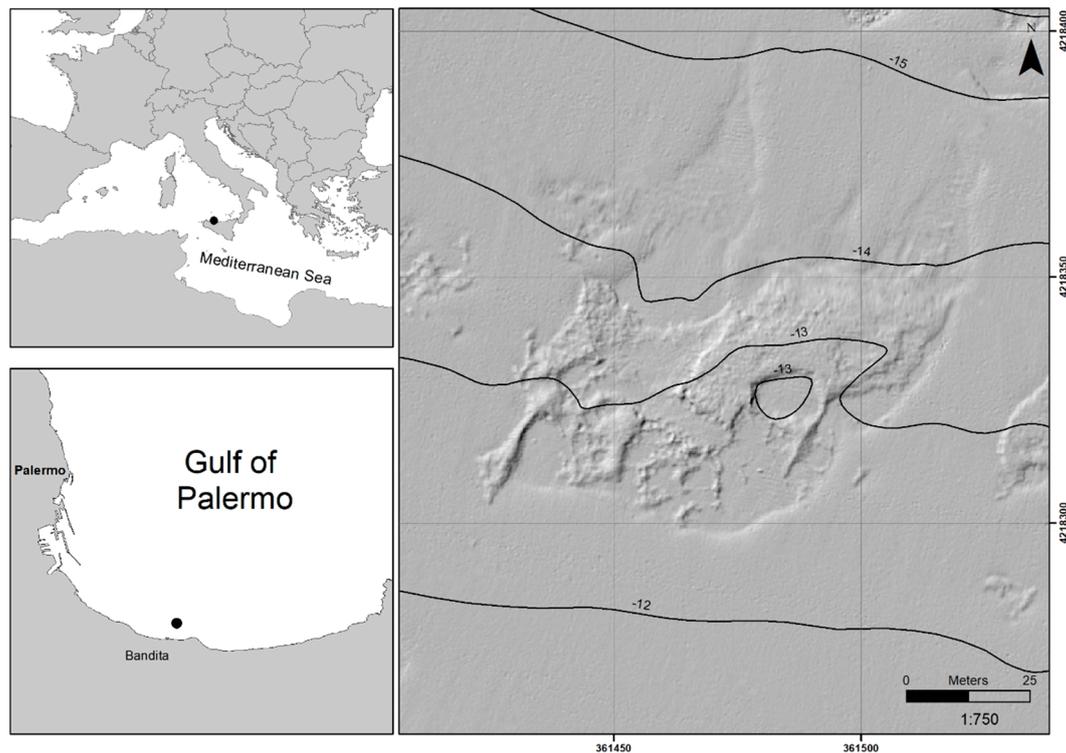
### 2.1. Study Area

The study area is located along the northwestern coast of Sicily (Figure 1), in a coastal area close to the urban center of the city of Palermo, which was exposed since the mid-20th century to multiple sources of pollution due to chaotic urban planning, improper waste disposal and untreated urban and industrial wastewater. Therefore, the Gulf of Palermo was affected in the past decades by severe trophic alteration of the coastal waters, and high concentrations of P-PO<sub>4</sub> (3–45 mmol L<sup>-1</sup>) and chlorophyll a (2–35 mmol L<sup>-1</sup>) were recorded during the summer season [35]. Consequently, significant modification of the structure and composition of the seabed (from mostly rocky to sandy and muddy) and the extensive *P. oceanica* meadow that grew in this area regressed and residual patches were detected at 7 to 21 m depth [36].

In recent decades—due to requalification of the metropolitan area mainly focused on the correct treatment and disposal of wastewater and reclamation, safety and restoration of the rubble landfills along the shoreline—there has been a clear improvement in water quality in the area, with TRophic IndeX levels [37] from good to high [38].

The transplanted site (Figure 1) that was selected using multi-criteria model [11] is located on a seabed characterized by dead *matte* structures [39]. The topography of the seafloor is irregular and includes dead *matte*, circular eroded structures delimited by *matte* walls up to 1 m high and areas with sandy bottoms, which sometimes cover dead *matte* structures and are, thus, invisible to visual inspection [39].

The transplantation dates from July 2008, when 20 metal grids (1 × 1 m), anchored to the substratum using iron spikes (~70 cm long), were placed in an area of about 40 m<sup>2</sup> at a depth of 14 m, through a quincunx spatial arrangement [11]. Twenty *P. oceanica* cuttings were fixed on each grid, carrying almost three shoots, with an initial mean density of 66 shoots m<sup>2</sup>. After six years of transplantation monitoring, the results showed a high loss of cuttings (61%) due mainly to fishing and anchoring activities, followed by an initial resilience process [11]. Then, monitoring was interrupted for three years, and when attempts were made to continue monitoring, the transplanted site was not found. It was only in April 2020 that the transplanted site was found again, after repeated surveys performed by scuba diving combined with resolution acoustic investigations.



**Figure 1.** Study area. In relief on the right dead matte structures surrounded by sandy bottoms (39), detected by Multibeam Echosounder MBES survey near “Bandita” (Gulf of Palermo), where the *P. oceanica* restoration site is located [11]. The black dot in the lower left corner identifies the location of the area covered by the MBSS survey.

## 2.2. Data Acquisitions, Field Measurements, Sampling and Laboratory Analysis

### 2.2.1. Image Acquisitions

In May 2020, a photogrammetric survey to obtain an ultra-high-resolution photo mosaic of the transplanted area was performed [20,40,41]. Before image acquisition, two  $50 \times 50$  cm squares were placed on the seabed, as scale reference to estimate the surfaces covered by the patches of the transplanted *P. oceanica*. A GoPro4 camera set to Multi-Shot shooting mode was used by scuba divers, swimming over the area at approximately 3 m from the seabed and performing sequential shooting every 2 s at 12-megapixel resolution with an image overlap of about 80%.

### 2.2.2. Acoustic Survey

High-resolution sonar data of the study area were collected by the University of Palermo Research Vessel “Antonino Borzi”. During the survey, morphometric and bathymetric data were acquired simultaneously by SSS Klein 3900 at 900 kHz and a pole mounted MBES ResonSeabat 8125 at 455 kHz, thus ensuring total coverage of the dead *matte* and seabed. The vessel was equipped with an RTK-GPS (Topcon GRS-1) System and a Kongsberg Seapath 130 with MRU 5+ that provided positioning data (centimetric accuracy) and altitude data ( $0.001^\circ$  accuracy) to the navigation and logging MBES data, PDS2000 software. A sound velocity profiler, Valeport SWIFT, was lowered through the water column in the survey area to obtain the velocity profile required for correct depth computation. The survey navigation lines run parallel to the bathymetric trend with an overlap of 60% between adjacent swaths to ensure total coverage of the SSS slant range. During the surveys, the boat speed was about 4 knots (about 2 m/s), and weather conditions were stable and characterized by high pressure and calm sea. All data were obtained continuously and stored in the shipboard computer.

### 2.2.3. Density Assessment and Shoots Sampling

Shoot density was determined in situ using 10 random quadrat counts ( $50 \times 50$  cm), both on the transplanted site and on the natural bed. Moreover, 18 *P. oceanica* orthotropic shoots connected to their horizontal axis were randomly collected from each bed. In addition, in November 2020, samples of the oldest leaves (most external) belonging to 18 shoots on each bed were taken (see below, Statistical Analysis). At the laboratory, two sets of biometric measures were taken: leaf biometry and datation [6]. Leaf biometry was carried out according to [42]. The total length and width of each leaf and the length of the base, when present, were measured. The leaves were separated into the various categories (adult, intermediate and juvenile) based on their length and the presence of a lignified base [43]. Datation was carried out by lepidochronology [44], which provides reliable estimates of seagrass growth performance. This dating technique allowed us to isolate and date rhizome segments corresponding to a 1-year period (lepidochronological year). Each lepidochronological year was dated starting from the rhizome apex (sampling year) downward and backdating the sequence of cycles with their corresponding rhizome segment, thus allowing shoot age determination [45,46]. This reiterative procedure was performed until the rhizome segment connected to the horizontal axis was reached, representing the year of shoot birth. For each segment, sheet number, length and dry weight were measured to estimate mean number of leaves produced, speed of growth and primary production of rhizomes, respectively. This method also made it possible to detect and date past flowering occurrence per shoot across time by finding floral stalk remains between the sheaths [47]. In addition, the integer sheet length was measured.

## 2.3. Data Processing and Statistical Analysis

### 2.3.1. Image and Acoustic Data

The frames obtained by photogrammetric survey were processed using Agisoft-Metashape software, which performs photogrammetric processing of digital images, generating a photo mosaic, eliminating errors due to the position of the shooting point, the inclination of the camera and the differences in height of the detected area, and allowing to extract information about the size, shape and distribution of *P. oceanica* transplanted patches.

The bathymetric data were processed using the PDS2000 software. Data de-spiking was carried out manually, without the application of automatic filters, with the aim to preserve data accuracy and resolution. Corrections for tide were applied to relate all data to the averaged sea level. Finally, a digital terrain model (DTM) with a 0.2 m grid cell size was produced. Sonograms obtained by SSS were processed by SeaView 2.8, a software package for smoothing navigation data, adjusting the time variable gain (TVG), and applied geometric and radiometric corrections [48].

All collected information (bathymetry, sidescan mosaic and photo mosaic) were joined and analyzed in a geographical information system (GIS), manually digitizing a seafloor map based on the recognized the different acoustic facies.

### 2.3.2. Biometry

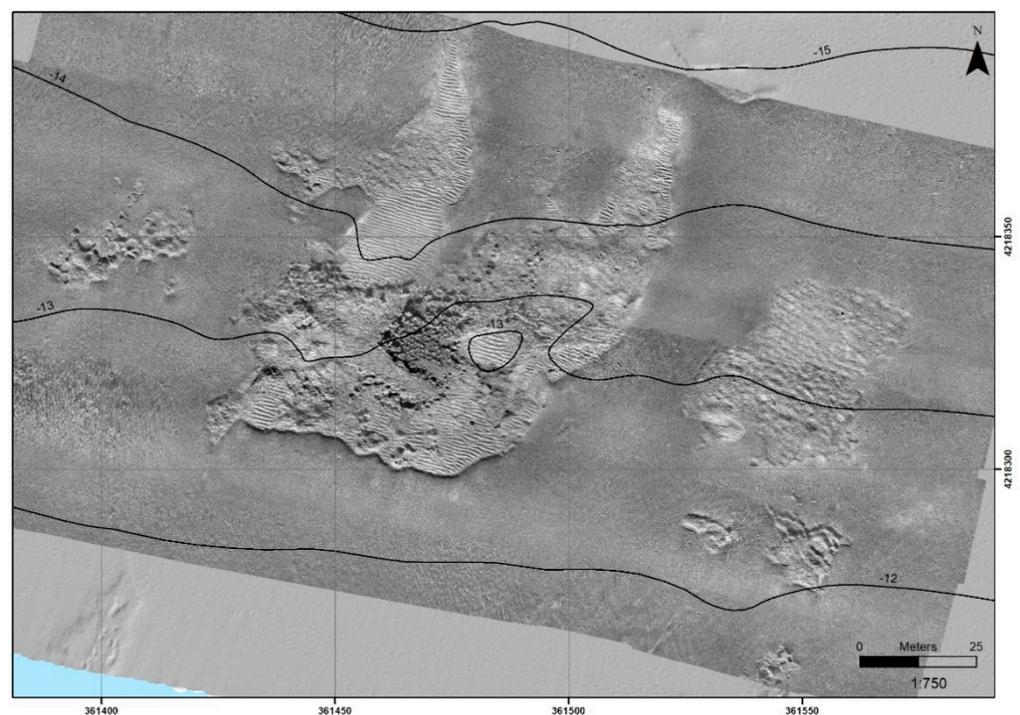
Differences in mean leaf biometry, shoot density, shoot age and production of leaves between beds were assessed by an independent *t*-test [49]. For the mean number of leaves produced, the value was obtained by averaging the last two lepidochronological years (2018 and 2019) in order to satisfy partially the assumptions of the statistical test [36]. Moreover, before performing all the analyses, the homogeneity of variance of the response variables was tested by Levene's test, and when necessary, data were transformed. Finally, the results were presented at the original scale to facilitate the interpretation of results. Instead, speed of growth and primary production of rhizomes were processed using the reference growth chart classification step by step procedure [46], to bypass the known confounding effect of shoot age [36,39,46,50–53]. In this case, the most recent annual rhizome segments were excluded from the analysis to allow comparison with the reference values corresponding to

each shoot age level (see [46] for further details). Finally, past primary leaf production (PI) was estimated by combining leaf biometry and datation [54], and applying the formula:  $PI = N \times D \times L$ , where:  $N$  = mean number of leaves produced annually (lepidochronological data);  $D$  = leaf density (phenological data) corresponding to leaf weight by unit of length;  $L$  = theoretical mean leaf length (lepidochronological data) estimated using the morphometric relationship between the length of integer sheath persisting on the rhizome as predictor variable. The slope and intercept of the morphometric relationship between sheath length and overall leaf length were estimated by least-squares linear regression applied to measurements obtained from the oldest leaves with their tips sampled during two seasons, as recommended [54], to maintain the error below 20%.

### 3. Results

#### 3.1. High-Resolution Sonar Data and Photo Mosaic

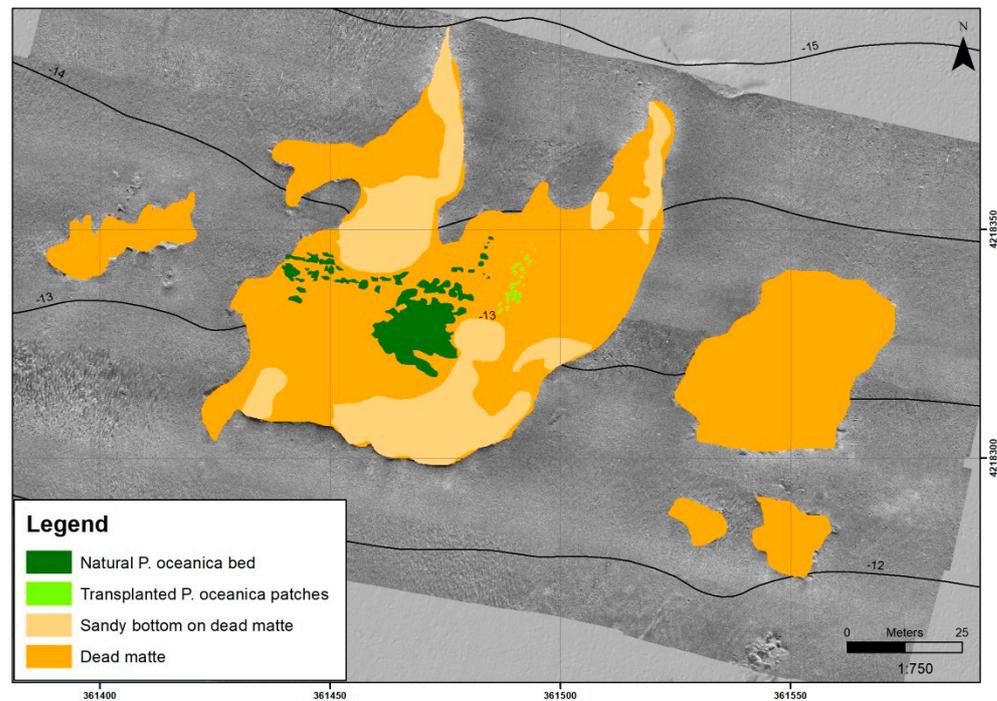
The area investigated by Multibeam is located at a depth of 12.4–15.2 m and covers a surface of about 45,000 m<sup>2</sup> (Figure 1), mainly represented by sandy bottoms partly covering with dead matte structures [39]. A preliminary acoustic (Side Scan Sonar) and underwater scuba diver's survey highlighted the presence of dead matte, both emerging (about 4850 m<sup>2</sup>) and partially buried and hidden by ripple marks (about 1350 m<sup>2</sup>), surrounded by sandy bottoms of different granulometry (Figure 2). Moreover, patches of natural meadow (about 300 m<sup>2</sup>) and transplanted meadow on the dead matte are recognizable by SSS (Figure 3).



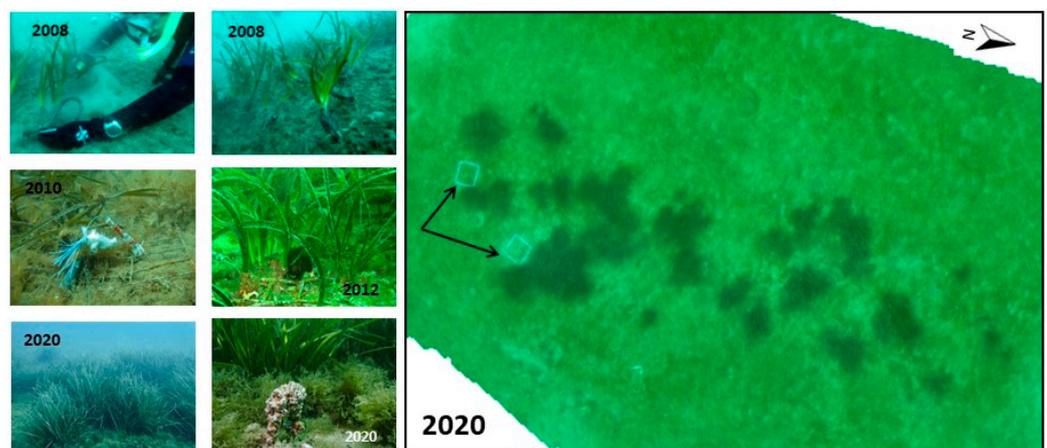
**Figure 2.** Side Scan Sonar mosaic. There are sandy bottoms with different granulometry, ripple marks covering dead matte and dead matte outcrops where natural and transplanted *P. oceanica* patches are established (see Figure 3).

The photo mosaic provides an overall picture of the area, showing the distribution of transplanted patches and several spikes encrusted with bio-fouling; these spikes were used for fixing the initial metal grids on the seabed (Figure 4). The metal grids on which the cuttings were anchored are not visible because they were completely destroyed. It was possible to detect 23 patches of both regular and irregular shape. The patch sizes range from 0.1 to 2.7 m<sup>2</sup>, covering a bottom surface close to 19 m<sup>2</sup>. The frequency histogram of transplanted patches shows a positive skewness distribution (Figure 5), with the most frequent class (69.6%) between 0.1–0.99 m<sup>2</sup>. Moreover, 13.0% of patches range between

1.0–1.49 m<sup>2</sup> and 8.7% between 1.5–1.99 m<sup>2</sup>. The frequency classes between 1.5–1.99 m<sup>2</sup> and 2.0–2.7 m<sup>2</sup> include only two patches (8.7%) per class, respectively. Larger patches (~30% of cases) have a more irregular shape, probably due to the fusion of several nearby patches.



**Figure 3.** Distribution map of *P. oceanica* meadows (natural and transplanted) on dead matte surrounded by sandy bottoms.

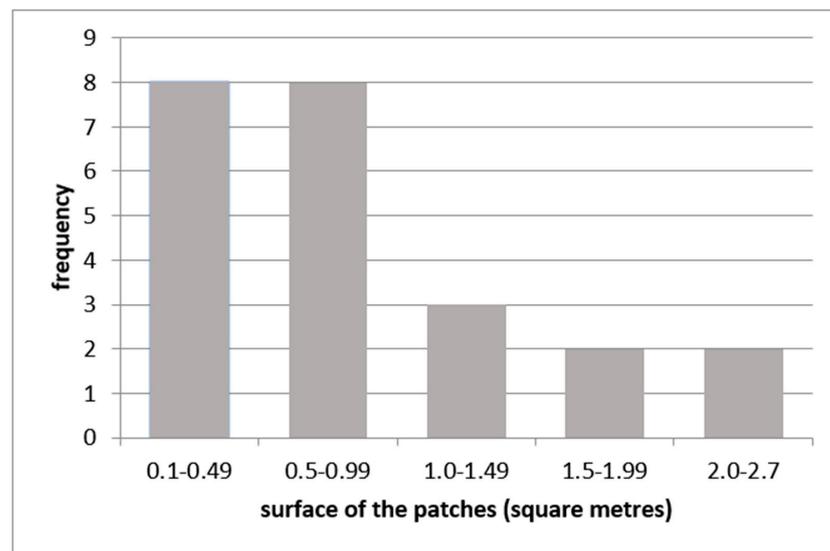


**Figure 4.** Photo mosaic of the reforestation pilot plant carried out on 11 May 2020 (on the right); black arrows indicate white  $0.5 \times 0.5$  m squares. On the left, performance of the plant from 2008 to 2020. The below right photo shows the head of one of the several iron spikes in the foreground, used to anchor the metal grids in 2008 (above left photo), fully encrusted by biofouling. The 2010 photo highlights an additional source of disturbance (gear for octopus fishing trapped in the mesh of an implant grid) for pilot transplantation project [11].

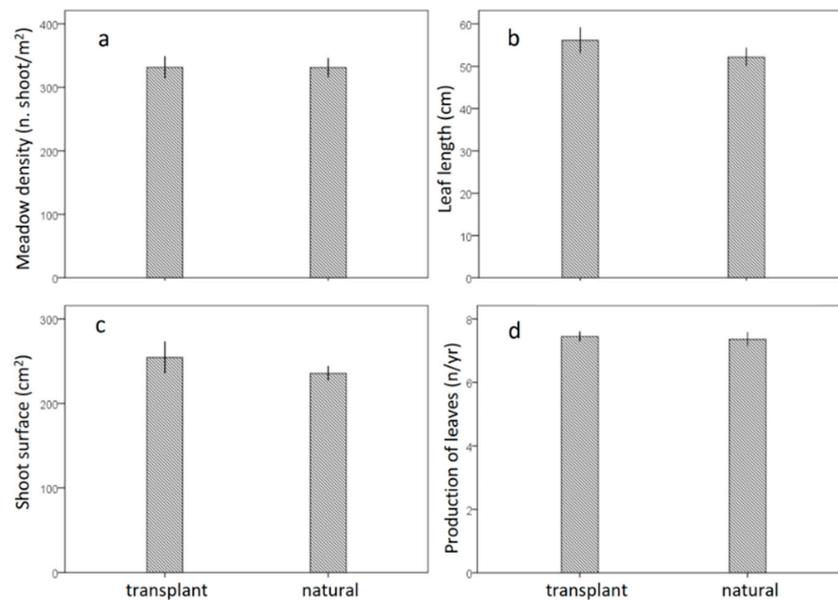
### 3.2. Leaf Biometry

Average shoot density varies from  $331.6 \pm 17.7$  to  $331.2 \pm 14.9$  shoots m<sup>-2</sup> (Table 1; Figure 6a), and differences between natural and transplanted beds are not significant ( $p > 0.05$ ). Leaf length varies from  $56.1 \pm 3.1$  to  $52.2 \pm 2.2$  cm and shoot surface from

254.4 ± 18.7 to 235.6 ± 8.7 cm<sup>2</sup> (Figure 6a,b). Considering both variables, non-significant differences are detected (Table 1).



**Figure 5.** Frequency histogram of *P. oceanica* patches in relation to the area covered in the transplanted area.



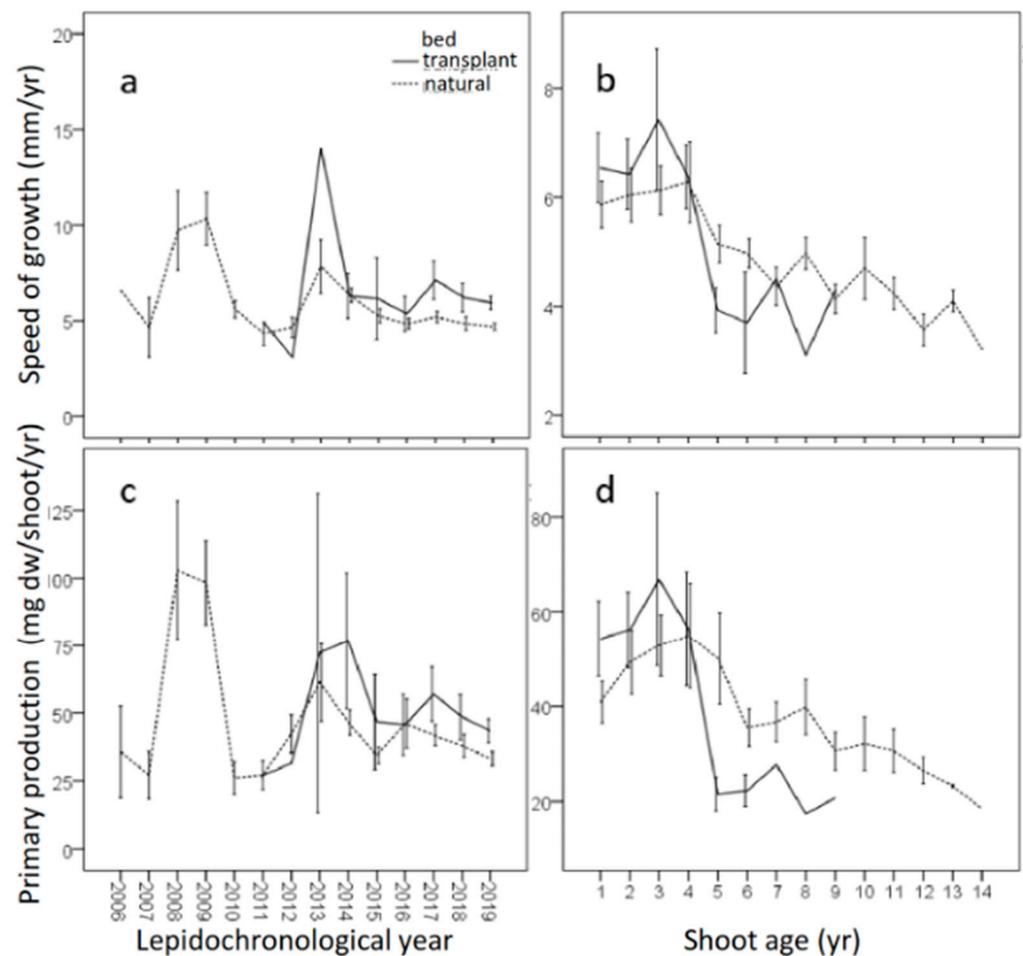
**Figure 6.** Mean values (SE) of meadow density (a), leaf length (b), shoot surface (c) and number of leaves produced (d) in transplanted and natural beds.

**Table 1.** Comparison outcomes for density, leaf biometry and dating between beds. Significance: \*\*\*  $p < 0.001$ ; ns  $p > 0.05$ . Standard error in brackets. (-) Statistical test not performed (see Methods for details).

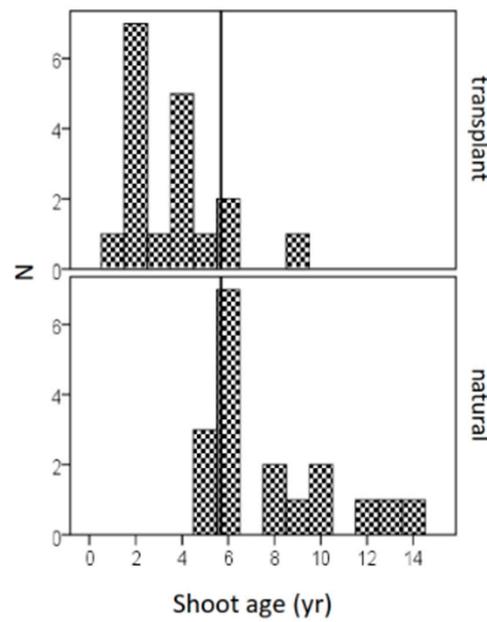
Variables	Bed		<i>t</i>	<i>p</i>
	Transplant	Natural		
Density (n shoot/m <sup>2</sup> )	331.6 (17.7)	331.2 (14.9)	0.2	ns
Leaf length (cm)	56.1 (3.1)	52.2 (2.2)	0.1	ns
Shoot surface (cm <sup>2</sup> )	254.4 (18.7)	235.6 (8.7)	0.9	ns
Shoot age (year)	3.6 (0.5)	7.8 (0.7)	5.1	***
Production of leaves (n/shoot/year)	7.4 (0.2)	7.3 (0.2)	0.3	ns
Rhizome primary production (mg dw/shoot/year)	58.7 (8.5)	47.5 (3.2)	-	-
Speed of growth (mm/shoot/year)	6.4 (0.6)	5.7 (0.2)	-	-

### 3.3. Dating Measures

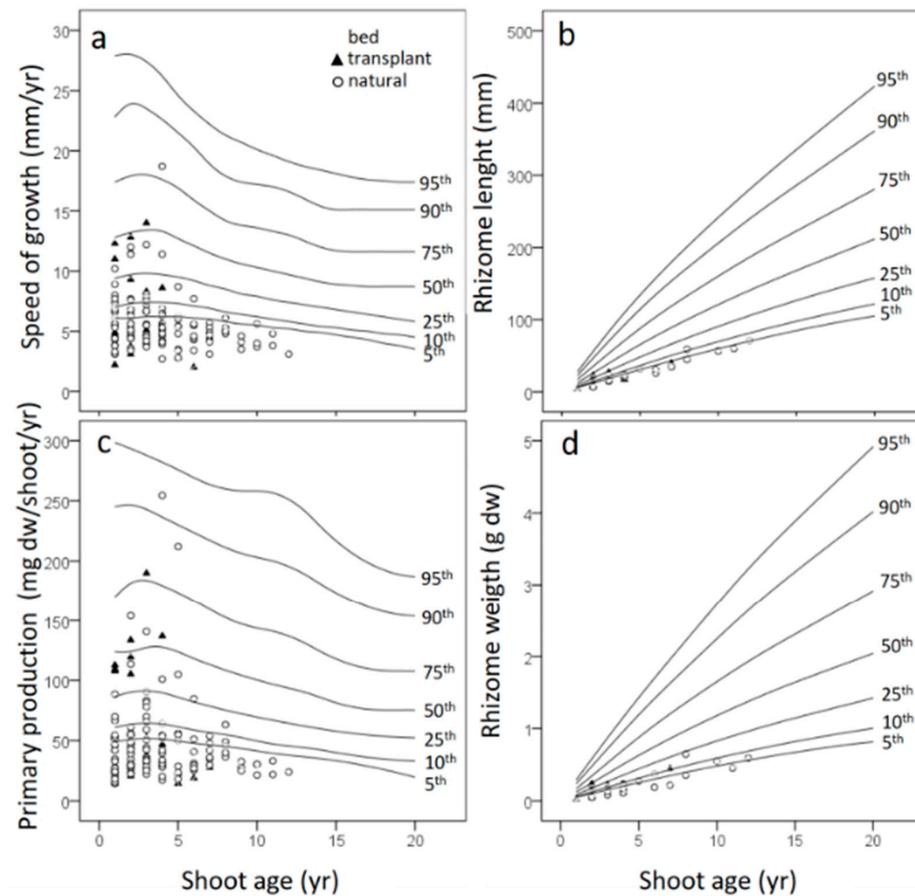
Dating measures allowed reconstruction of growth performance within temporal ranges from 2006 to 2019 and 2010 to 2019 in natural and transplanted beds, respectively, with interannual mean fluctuations progressively quite synchronous between beds towards the end of the time series (Figure 7a,c). Speed of growth shows mean values ranging from  $6.4 \pm 0.6$  mm/shoot/year (transplanted) to  $5.7 \pm 0.2$  (natural). Rhizome primary production varies from  $58.7 \pm 8.5$  mg dw/shoot/year (transplanted) to  $47.5 \pm 3.2$  (natural). Inspection of the patterns across age reveals the presence of natural age-induced growth variation, exhibiting nonlinear growth trends characterized by an initial phase of increasing speed of growth of rhizomes and production until 3–4 years, followed by a deceleration with aging (Figure 7b,d). Shoot age varies between 1 and 14 years, with an average of 5.7 years (Table 1). The frequency histograms of age (Figure 3) show clear heterogeneity in age distribution. Shoots sampled in the transplanted area display a modal peak at the age of 2 years and an average value of  $3.6 \pm 0.5$  years (Table 1). On the other hand, the mean age of shoots sampled from natural beds was significantly higher (Figure 8; Table 1). No floral stalk remains were found across the time series explored. According to reference growth chart classification, speed of growth and primary production values fell mostly below the 50th percentile, exhibiting very similar growth performance between beds with about 60% of values (the mode) below the 5th percentile (Figure 9a,c). Additionally, cumulative growth performance values corresponding to the total length and weight of rhizomes were below the 50th percentile with the modal class again below the 5th percentile (Figure 9b,d).



**Figure 7.** Mean values (SE) of speed of growth (a), primary production (c) across lepidochronological years and shoot age (b,d).



**Figure 8.** Frequency of shoot age histograms for both beds. The vertical line represents the general mean shoot age.



**Figure 9.** Growth performance measurements plotted on reference growth charts. The distribution of speed of growth of rhizomes (a), rhizome length (b), primary production (c) and weight (d) of the two beds are compared with the expected percentile curves at different ages.

### 3.4. Leaf Primary Production

The morphometric relationship between the length of integer sheath and total length of *P. oceanica* leaves was  $y = 22.5x - 37.9$  ( $R^2 = 0.58$ ,  $p < 0.001$ ). Based on this equation applied to average sheet length, past mean leaf lengths of 39.3 and 48.1 cm were estimated at transplanted and natural stands, respectively (Table 2). Leaf density (blades plus sheaths) ranges from  $4.5 \pm 0.2$  mg dw/cm (transplanted) to  $4.2 \pm 0.1$  mg dw/cm (natural). Estimates of past primary production of leaves per shoot range from 1.3 g dw/shoot/year (transplanted) to 1.5 g dw/shoot/year (natural) (Table 2). Total primary production of meadows (leaves + rhizomes) shows mean values ranging from 333.0 to 332.7 g dw/m<sup>2</sup>/year at transplanted and natural stands, respectively (Table 2). Given the transplanted meadow coverage and its primary production per unit area, it was possible to calculate global primary production amounting to 6.3 g dw/year (Table 2).

**Table 2.** Primary production values estimated in two beds.

Variables	Bed	
	Transplant	Natural
Leaf density (mg dw/cm)	4.5 (0.22)	4.2 (0.12)
Production of leaves (n/shoot/year)	7.4	7.3
Past sheet length (cm)	3.43 (0.9)	3.82 (0.8)
Theoretical past oldest leaf length (cm)	39.3	48.1
Leaf primary production (gr dw/shoot/year)	1.3	1.5
Rhizome primary production (g dw/shoot/year)	0.06	0.05
Total primary production (g dw/shoot/year)	1.4	1.5
Meadow density (n shoots/m <sup>2</sup> )	331.6	331.2
Covering (m <sup>2</sup> )	24.0	-

## 4. Discussion

The long-term monitoring project presented in this study showed that the 12-year-old transplanted *P. oceanica* displays certain structural and functional characteristics that are very similar to those of a natural meadow.

The dating method allowed reconstruction of the history of *P. oceanica* shoots involving different growth performance measures taken from a natural bed and, for the first time, a transplanted one. The demography of shoots sampled from the transplant bed clearly showed a younger structure dominated by two-year-old shoots and generally not exceeding six years, except in one case where maximum age was nine years. Shoot age structure of living shoots of *P. oceanica* may show large variation among and within locations [55,56]. However, when meadows exhibit low maximal shoot age and youngest modal age, it is expected that shoot mortality starts to operate at their earlier life span stages [55]. This is what occurred in this case. The shoots chosen for the initial transplantation were very young [11], and if they were still alive today, after twelve years, they should have been at least older than this age, on average. This is consistent with previous observations made on the transplanted bed under study, which showed a high loss of cuttings (> 50%), mainly due to the intense impact of fishing [11]. The speed of growth and primary production of rhizomes in the transplanted bed was slightly higher than in the natural one, although it should be noted that this difference was affected by shoot age confounding, since the shoots between beds were of uneven-aged [36]. In the presence of such confounding, it should be possible to discern whether *P. oceanica* growth reflects variations in temporal or spatial exogenous factors or simply unbalanced shoot age structure among meadows, to avoid misleading interpretation of results. The distribution of the annual and cumulative growth measurements displayed and then converted into growth chart centile ranges allowed us to compare bed performance, taking into account the age differences found. The annual or cumulative growth measures of both beds exhibited very similar positions within centile curves, thus demonstrating that natural or transplanted shoots belonged to the same population in terms of growth performance. Moreover, as regards aging in both

beds, *P. oceanica* growth followed a similar pattern confirming the typical non monotonic relationships of growth performance vs. age with an initial increase reaching a maximum of 2–4 years followed by a decrease in both stands [46]. Nevertheless, it is worth noting neither stand exhibited “normal growth”, since the dominant values recorded were far below the 25th centile expected for Sicilian undisturbed meadows at the same depth. The primary leaf production estimated in the transplanted bed was negligibly different compared to natural beds, and consequently, this important ecological function has now been re-established on the site of the destroyed. At hierarchical shoot level, this value is consistent with the estimates reported for several Mediterranean Sea meadows [54,57]. However, the low growth and production of vertical rhizomes values estimated here suggest that the carbon fixed by photosynthesis might have been more oriented towards the complex and very expensive process of storage, translocation and use of resources within the *P. oceanica* networks, which are necessary to sustain branching and clonal expansion [46,58,59]. On the contrary, non-detection of sexual reproduction events suggests that additional carbon demand required for inflorescence elaboration and seed production might have been difficult to achieve, given that for a large seagrass species such as *P. oceanica*, it might become considerable compared to the life span of the shoots [50,60,61]. Undoubtedly, the results of the branching process are evident. The current average density value of the transplanted shoots is five times higher than the initial value (331 vs. 66 shoots/m<sup>2</sup>), but ten times higher than the density achieved three years after transplanting (331 vs. 35 shoots/m<sup>2</sup>) [11]. Moreover, by the fourth year after transplantation, there were signs of a progressive increase in density [11]. Illegal coastal fishery—which led to the loss of 61% of cuttings and 53% of shoots 3 years after transplanting—and the initial quincunx spatial configuration [11] both explain the current fragmentation of distribution and patch coverage. It has been shown that the distribution and size of seagrass patches can influence the growth dynamics of shoot populations. Spatially explicit models [62] have provided evidence that patch expansion does not proceed at a constant rate. In particular, it has been simulated that the radial growth rate increases progressively with increasing patch size; based on these models, it is, therefore, expected that the fragmentation present in the transplant zone determines a different rate of lateral expansion with higher values in larger patches. However, further repeated measurements over time will be needed to verify this prediction.

The transplanting site showed an evident shoot population increment in the following 9 years. The density values have increased by one order of magnitude, currently achieving “normal density” according to the standardized classification [63], providing evidence that this framework restored not only the meadow’s density but also its resilience [64]. Considering average surface ( $0.82 \pm 0.14/\text{m}^2$ ) and radius (0.52 m) values of the patches observed in the planting area, we estimated the horizontal growth rate of transplanted meadow on dead matte (4.26 cm/year), which is slightly higher than the natural regeneration rate of the meadow (3.7 cm/year) destroyed by a bomb in Villefranche Bay [65], but lower when compared to the growth rate of plagiotropic rhizomes (5–10 cm/year) [66].

The results show that the vegetative reproduction process observed compensated for the losses due to the initial mechanical damage caused by fishing activities. Although coastal fishing is regulated by national laws, also aimed to protect seagrasses, illegal fishing continues throughout the year in the area by unlicensed fishermen. Recently, two main categories of seagrass meadow increase have been defined: natural recovery or colonization and management intervention including removal/reduction in direct impacts, while restoration was poorly considered [67]. In our opinion, active restoration should be included as an additional measure for seagrass expansion. In any case, we believe that anchorage and fishing represent two of the main threats to reforestation, at an early stage in particular [11], since (i) the root system has not had sufficient time to fully develop and anchor the cuttings tenaciously on the substrate; (ii) shoot density has not reached the typical maximum growth phase of the logistic curve able to compensate faster for any possible losses. These long-term monitoring results further confirm that

monitoring period should last at least 4–6 years for correct evaluation of the effectiveness of restoration projects [11]. The use of very high-resolution acoustic systems (MBS and SSS) and photogrammetric surveys helped to evaluate the performance of the intervention with greater accuracy and provide a detailed description of the state of the art at the time considered. These tools are useful for accurate documentation and describing changes occurring in transplanted areas.

The ability of *P. oceanica* to colonize/recolonize all types of substrate is documented and described in several papers (for example, see [68–73]). It is a characteristic of the meadow's natural dynamics, namely, alternate shoot death and natural recolonization by vegetative fragments and/or clonal expansion [65,74,75]. Therefore, according to other authors [20,65], the results achieved clearly show that dead *matte* is also a substrate on which vegetative fragments of *P. oceanica* can settle and colonize, even immediately after an acute event leading to disappearance [65], without requiring a long transitional phase or structuring of the substrate by other phanerogams (*Cymodocea* or *Zostera*).

## 5. Conclusions

The *P. oceanica* meadows of the Mediterranean Sea constitute extraordinary ecosystems fragile and extremely vulnerable to natural and anthropogenic pressures, and protection and conservation are, thus, justified not only by their very great heritage value but also for economic reasons [68]. In particular, *P. oceanica* tanatocenoses (dead *matte*) store and preserve a vast long-term carbon stock that has accumulated over millennia within the *matte* [76,77]. Therefore, in the absence of restoration actions, these degraded meadows will be left as a heritage to future generations, due to slow natural recovery [10].

It is known that when *P. oceanica* regresses, the leaf canopy disappears, and the underlying *matte* of shallow meadows is no longer protected from high-energy wave erosion. Consequently, the carbon stock stored for millennia inside the dead *matte* could become a sort of “time-bomb”, rapidly switching from sink in source [77]. Hence, after removing or reducing the causes that led to the disappearance of a meadow, dead *matte* should be considered a substrate where *P. oceanica* meadows should potentially and primarily be restored, thus also contributing to climate change mitigation [20]. In addition, this agrees with the Conference of the Parties (COP 21) to the Climate Change Convention [78] that noted the importance to ensure the integrity of all ecosystems, including the oceans, to conserve and enhance sinks and reservoirs of greenhouse gases, thus improving the Tokyo Protocol, focusing on forest-based sinks.

The transplantation results obtained in the Gulf of Palermo, although concerning a small plant, show that complete restoration of a *P. oceanica* meadow on degraded seabed is possible in a relatively short time (a decade), compared to the time required to achieve the same results under natural recolonization (from centuries to millennia). The results obtained so far are very promising after 12 years and, therefore, represent a good starting point for planning a larger-scale planting effort, in the context of new perspectives for the recovery of degraded meadows in the area.

Under this scenario, in analogy to the proposal of Piano [79,80] regarding the recovery of fragile and degraded urban suburbs by means of “mending” actions, we are about to carry out a new larger pilot restoration project (~1000 square meters) in the Gulf of Palermo on a fragmented *P. oceanica* meadow, within the framework of the Marine Hazard PON03PE\_00203\_1 project, supported by the Italian Ministry of Education, University and Research (MIUR). Within this framework, the “mending” approach will be tested by transplanting patches of *P. oceanica* (“small drops” *sensu* Renzo Piano) within a mosaic of natural meadow and dead *matte*. Therefore, it will be possible to recognize the dynamics of the patches as natural phenomena and to emulate the natural restoration potential of the meadows [25] by means of the seed, seedling and vegetative fragment (rhizomes and cuttings) dispersal. The final goals will be to “fertilize” the degraded ecosystem through the catalyzing function of *P. oceanica* patches, minimize environmental impact on the habitat to be restored, optimize work and costs and rehabilitate the seabed in a

reasonable time (~10–12 years), in view of improving the connectivity between populations and reconstructing the underwater landscape [25].

Hence, we believe that it is very important to implement education and awareness campaigns on the role and importance of marine phanerogams, involving as many stakeholders as possible (politicians, regulators, managers, NGOs, fishermen, students and citizens) [25]. Their involvement and cooperation can promote the protection and preservation of existing meadows, by preventing further damage and avoiding the most relevant forms of disturbance (recreational and artisanal fishing, anchoring of pleasure boats, etc.), which represent the main causes of physical damage to reforestation plants.

Finally, to improve the sustainability of plant material samples to be used for transplanting activities, we suggest searching for and identifying areas normally found along the coast, where rhizomes and cuttings accumulate during fall/winter and can be collected easily in large numbers [81].

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