

## Article

# Effectiveness of Eco-Engineering Structures in Salt Marsh Restoration: Using Benthic Macroinvertebrates as Indicators of Success

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**Abstract:** Salt marshes are vital coastal ecosystems, increasingly threatened by rising sea level and human pressures, that provide essential services, including coastal protection, habitat support, and carbon sequestration. This study examines the effectiveness of different eco-engineering structures in restoring salt marshes in the Mondego Estuary, Portugal, by assessing their impacts on benthic macroinvertebrate communities as bioindicators of ecosystem health. The experimental design included five experimental cells: wood palisade (Fence), geotextile fabric (Geotextile), geotextile bags filled with sand (Bags), a cell with autochthonous vegetation (Plants), and a Control cell with bare soil. Monitoring took place from 2019 to 2021, with both before and after intervention sampling to evaluate species composition, biomass, and density. Key ecological indices, such as the AZTI's Marine Biotic Index (AMBI), Shannon-Wiener Diversity, and Pielou's Evenness, were calculated alongside measurements of environmental variables. The results indicated minimal impacts on biodiversity, with observed variations primarily attributed to seasonal dynamics. While the wood palisade enhanced species richness and density, geotextile provided better community stability. The findings emphasize the importance of long-term monitoring, stakeholder engagement, and sustainable use of materials to optimize restoration efforts and better inform coastal management strategies in the face of climate change.

**Keywords:** eco-engineering; salt marsh restoration; benthic macroinvertebrates; environmental monitoring; coastal management



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## 1. Introduction

Coastal saltmarshes support diverse ecological services and are biodiversity hotspots that contribute substantially to carbon sequestration, provide coastal protection, improve water quality, nutrient cycling and sustain primary production [1–3]. While these areas act as natural buffers against storm surges [4,5] the current scenario of Sea Level Rise jeopardizes these vital ecosystems by saltwater intrusion [6], erosion [7], altered hydrology [8], loss of habitats and, consequently, a decline in biodiversity [9–11].

Restoration strategies have been employed as a mitigation solution [12,13] aiming the elevation of marsh and sedimentation improvement, promoting coastal protection by dissipating wave energy, enhancing habitat availability by sediment accretion, improving water quality, and providing relevant recreational and cultural benefits for the community [14].

The benefits of implementing man-made structures that mimic natural processes for coastal protection, such as sediment trapping to promote marsh accretion and using dredged material for marsh nourishment, are well documented [15]. However, the effectiveness of these different

eco-engineering approaches in stabilizing and protecting salt marshes can vary significantly. Various methods of salt marsh protection, such as the use of artificial structures, have shown mixed results in maintaining or enhancing sediment stability and marsh integrity. For example, the implementation of artificial structures in Chesapeake Bay, USA, successfully stabilized salt marshes but also altered the sediment composition by depleting organic carbon and nitrogen [16]. Similarly, diking for salt marsh protection in the Yangtze Estuary demonstrated structural success but resulted in changes to macrobenthos diversity, particularly affecting mollusks and polychaetes [17]. The short-term performance of sediment addition as a restoration strategy in a southern California salt marsh led to shifts in benthic community structure (from common marsh related groups like oligochaetes and polychaetes to insect larvae), which can serve as an indicator of ecosystem response and resilience [18].

In addition to playing an important role in nutrient cycling and organic matter decomposition, the abundance and diversity of benthic macroinvertebrates provide valuable insights into ecosystem health. These organisms act as indicators of ecosystem condition, reflecting changes in habitat, water quality and sediment composition. As bioindicators, they are especially useful for detecting anthropogenic pressures in aquatic ecosystems. Their sedentary nature and short life cycles make them ideal for spatial and temporal assessments of population changes and environmental impacts [19,20]. Understanding the effects of interventions aimed at restoring altered or threatened ecosystems on faunal communities, through changes in sedimentation and hydrology, is essential for evaluating restoration efforts and improving strategies to reduce biodiversity impacts [21,22].

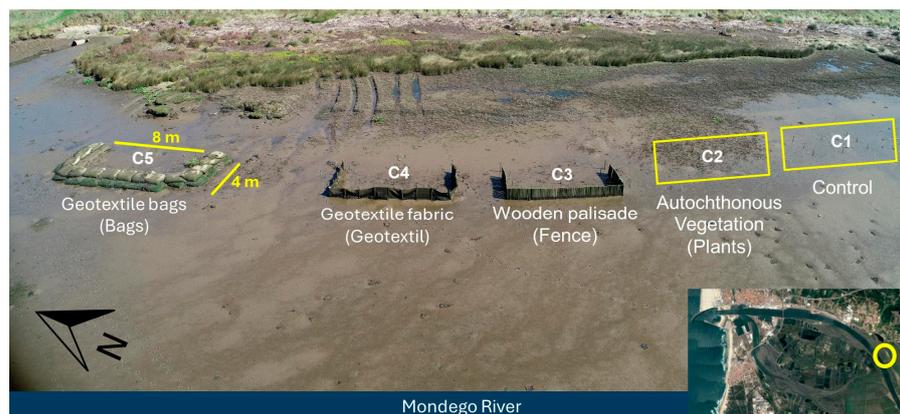
The main aim of this study was to evaluate the effectiveness of eco-engineering structures in restoring salt marsh areas using benthic macroinvertebrate communities as indicators of restoration success. By examining changes in species composition, abundance, and diversity, we assessed the spatial and temporal variability of these communities across different treatment and control sites. Additionally, by exploring the relationship between environmental variables and macroinvertebrate metrics, the study provides practical recommendations for designing and implementing eco-engineering interventions in salt marsh restoration, contributing to sustainable coastal management through the integration of ecological principles and engineering techniques.

## 2. Materials and Methods

### 2.1. Study Site and Experimental Design

The study was carried out in a salt marsh in the Mondego Estuary on the western coast of Portugal (40°08'01.3" N 8°48'05.8" W). The estuary is divided into two arms: the North, which is deeper and frequently dredged to accommodate large ships entering the port, and the South, which is shallower and less disturbed by navigation activities. The estuary covers an area of 9 km<sup>2</sup> and includes salt marshes (particularly in the southern arm) with a variety of halophytic vegetation, such as *Bolboschoenus maritimus*, *Halimione portulacoides* and *Aster tripolium*. It also features mudflats rich in macroinvertebrates, frequently visited by migratory birds, and *Zostera noltei* seagrass beds.

In this study, we tested the application of different structures aimed at protecting and restoring salt marsh areas, evaluating sedimentation rates in five experimental cells: (C1) Control—a bare-bottom area without plants or structures, (C2) Plants—an area with *Bolboschoenus maritimus*, (C3) Fence—wooden palisade on bare bottom, (C4) Geotextil—geotextil fabric on bare bottom, and (C5) Bags—geotextile bags filled with sand on bare bottom. The structures were positioned below the high-tide water level to facilitate the entry of water and suspended sediment, allowing the sedimentation process to occur (Figure 1). These structures were also designed to be permeable to facilitate the outflow that occurs between high and low tides. For example, with the palisade (cell C3), flow is facilitated by the spaces between the piles; with the geotextile bags filled with sand (cell C5), flow occurs through the voids between the bags; and with the geotextile fabric fixed to wooden supports (cell C4), percolation happens through the geotextile, which acts as a filter, allowing water to pass through while retaining soil particles.



**Figure 1.** Study site and aerial view of the experimental area ( $40^{\circ}08'01.3''$  N  $8^{\circ}48'05.8''$  W), showing the different material cells and their orientation.

## 2.2. Sampling and Laboratory Procedures

Sampling was conducted both before and after the implementation of the experimental structures. For comparison, undisturbed plots included an adjacent area without vegetation and one with vegetation, allowing us to determine whether any ecological changes detected after the intervention were related to the implemented eco-engineering structures. Sampling was performed seasonally, with two campaigns per year. The first campaign, conducted before the intervention, took place in June 2019, and the first post-intervention sampling occurred in October 2019. Sampling was then repeated in May and October of the following two years (2020 and 2021). The number of sampling campaigns was limited to two per year to prevent trampling inside the experimental cells, and this frequency allowed us to understand intra-annual variation in these communities.

For the study of benthic macroinvertebrates, five sediment samples (replicates) were collected from each experimental cell during each sampling campaign. These samples were taken from five randomly selected locations using a corer with an area of  $0.0141\text{ m}^2$ , which was buried 25 cm into the soil. The collected sediment was washed in a water channel near the experimental area using a calibrated 0.5 mm mesh bag, and the remaining sediment was carefully placed in labelled cloth bags. To preserve the samples, they were placed in a 4% formalin solution, neutralized with sodium borate, and stained with Rose Bengal in plastic containers. During macroinvertebrate sampling, physical-chemical parameters (temperature, salinity, dissolved oxygen, pH and oxidation-reduction potential (ORP)) were measured at each experimental cell, in water pools, and in the main channel (North arm) using a multiparameter probe. In the laboratory, each sample was washed in running water through a series of sieves (2 mm, 1 mm and 0.5 mm) to facilitate the subsequent separation of biological material. The organisms were identified to the species level, whenever possible, using dichotomous keys for various groups and were counted. Biomass was estimated as ash free dry weight ( $\text{g AFDW m}^{-2}$ ) by drying until weight stabilization, followed by combustion at  $450\text{ }^{\circ}\text{C}$  for 8 h. Additionally, sediment samples were collected to determine organic matter content and grain size distribution. Sediment organic matter content was estimated as the weight loss after combustion, calculated as the difference between the dry sediment weight (obtained after drying at  $60\text{ }^{\circ}\text{C}$  for 72 h) and the weight after combustion at  $550\text{ }^{\circ}\text{C}$  for 4 h, expressed as a percentage of the total sample weight. Sediment grain size was determined by drying samples at  $60\text{ }^{\circ}\text{C}$  and weighing the fractions retained in a four "AFNOR" type sieves (0.063 mm, 0.250 mm, 0.500 mm, and 2.000 mm). Grain size was classified as gravel ( $>2\text{ mm}$ ), coarse sand (0.500–2.000 mm), medium sand (0.250–0.500 mm), fine sand (0.063–0.250 mm) and mud ( $<0.063\text{ mm}$ ), and the mean phi ( $\phi$ ) value for each sample was calculated, according to Folk and Ward (1957) [23].

## 2.3. Data Analysis

A Principal Component Analysis (PCA) of the environmental variables was conducted to identify patterns across locations (experimental cells) and years. To capture the maximum

variability in the data set, redundancy among variables was examined using Draftsman plots. Before the analysis, a resemblance matrix based on Euclidean distance was calculated, and all parameters were normalised. The redundant variables were removed from the analysis so that the first two axes account for the maximum variability in the data set. The variables that were retained in the model were acting as proxy for the ones that were eliminated.

A Permutational Multivariate Analysis of Variance (PERMANOVA) was performed to assess significant differences in macroinvertebrates species composition across experimental cells (spatial) and over time (temporal) based on density and biomass data. The two-way PERMANOVA experimental design included the fixed factors: “Local” (the five experimental cells: Control, Plants, Fence, Geotextile and Bags), and “Year” (2019, 2020, 2021).

Two-dimensional Principal Coordinates Analysis (PCO) plots were created to visualise community patterns between experimental cells and across years.

For both PCO and PERMANOVA analyses, the Bray–Curtis similarity coefficient was selected as the resemblance measure, and data were pre-treated using a square root transformation to reduce the influence of dominant species. The statistical significance of variance components was tested using 999 permutations of residuals under a reduced model, with a predetermined significance level (P) of 0.05. The multivariate analyses were performed using the PRIMER v7 statistical package [24], along with the PERMANOVA + PRIMER add-on package [25].

The relationship between environmental variables and the benthic community composition was explored by carrying out a Distance-Based Linear Model analysis (DistLM) with “Best” as selection procedure and “AIC” (Akaike Information Criterion) as selection criterion.

To further analyse the spatial and temporal differences in total macroinvertebrate biomass, density, number of species, and ecological indices (Pielou’s evenness index, Shannon-Wiener diversity index, and AMBI index), a Repeated Measures ANOVA was performed. Sphericity assumptions were tested using Mauchly’s test, and the degrees of freedom were adjusted with Greenhouse-Geisser corrections if necessary, using IBM SPSS Statistics version 29.0.0.0 (241).

The ecological indices used to assess macroinvertebrates diversity and environmental quality across experimental cells included the Shannon-Wiener Index, Pielou’s Evenness Index and AZTI’S Marine Biotic Index (AMBI) (see Table 1 for details). The AMBI 6.0 software and the May 2022 species list (freely available at <http://www.azti.es>, accessed on 16 July 2024) were used to calculate the AMBI [26], following the recommendations of Borja and Muxika (2020) [27] (Table 1). Diversity indicators (Shannon–Wiener and Pielou) were calculated from the benthic density data matrix using PRIMER v7 software package from Plymouth Marine Laboratory, UK [23].

**Table 1.** Ecological indices used to assess macroinvertebrates diversity and environmental quality across experimental cells and corresponding algorithm description and classification details.

Ecological Indicator	Algorithm	Classification
<b>Species as indicator</b>		
<b>AZTI’S Marine Biotic Index</b>	$AMBI = \left\{ \frac{(0 \times \%GI) + (1.5 \times \%GII) + (3 \times \%GIII) + (4.5 \times \%GIV) + (6 \times \%GV)}{100} \right\}$ <p>G I: Species very sensitive to organic enrichment and present under unpolluted conditions.            G II: Species indifferent to enrichment, always present in low densities with non-significant variations with time.            G III: Species tolerant to excess organic matter enrichment. These species may occur under normal conditions; however their populations are stimulated by organic enrichment.            G IV: Second-order opportunistic species, adapted to slight to pronounced unbalanced conditions.            G V: First-order opportunistic species, adapted to pronounced unbalanced situations</p>	<p>Normal 0–1.2            Slightly polluted 1.2–3.2            Moderately polluted 3.2–5.0            Highly Polluted 5.0–6.0            Azoic 6.0–7.0</p>

Table 1. Cont.

Ecological Indicator	Algorithm	Classification	
<b>Diversity</b>			
<b>Shannon-Wiener Index (Shannon and Weaver, 1963)</b>	$H' = -\sum p_i \log_2 p_i$ $p_i$ : proportion of abundance of species $i$ in a community were species proportions are $p_1, p_2, p_3 \dots p_n$	Low diversity	0
		High diversity	$\geq 5$
<b>Pielou Evenness Index (Pielou, 1969)</b>	$J' = H' / H'_{\max} = H' / \log S$ $H'_{\max}$ : maximum possible value of the Shannon diversity	Low diversity	0
		High diversity	1

### 3. Results

#### 3.1. Environmental Variables

The Principal Component Analysis (PCA) of environmental variables revealed that the first two principal components accounted for 77.2% of the total variation across the sample sites, with PC1 and PC2 explaining 43.5% and 33.7% of the variance, respectively. Salinity and sand content (%) were the environmental variables that contributed most to PC1, effectively differentiating samples across sites. The second axis (PC2) primarily separated samples based on oxygen levels, along with organic matter (OM%) and silt-clay content, which were colinear variables. Other variables, such as temperature and oxidation-reduction potential (ORP), had a smaller influence on the observed variation. These results suggest that salinity, sand content, and oxygen levels play a key role in shaping the environmental conditions at each site. When samples were grouped by “Local” (Figure 2), the PCA revealed distinct clustering, reflecting spatial differences in environmental conditions. Samples with higher salinity and sand content clustered together, while those with higher oxygen levels and organic matter content formed a separate group. In contrast, when grouped by “Year” (Figure 3), the PCA highlighted temporal variation in environmental characteristics, with salinity and sand content driving differences among years. Samples from 2019 were associated with higher salinity and sand content, while 2021 samples showed higher organic matter and lower oxygen levels. Overall, despite temporal changes, spatial differences among locations remained more prominent.

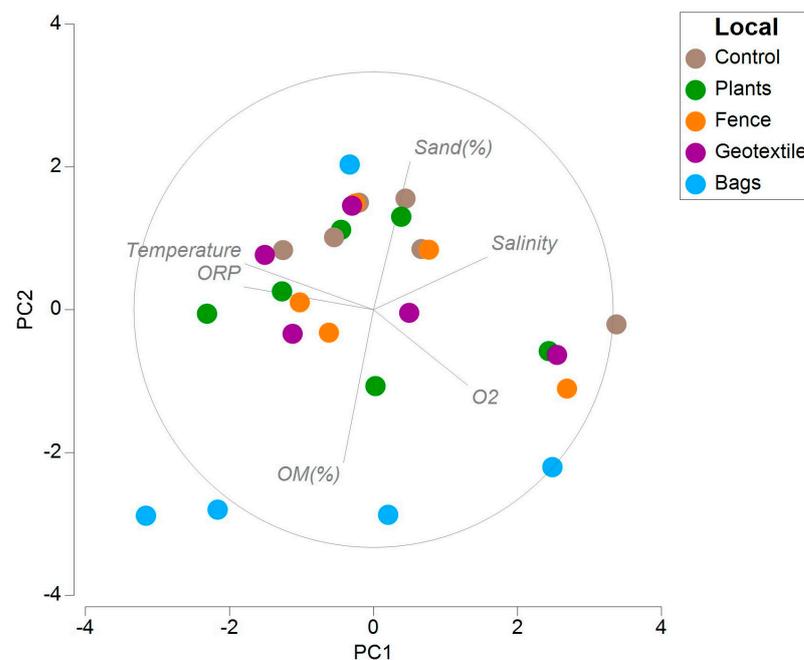
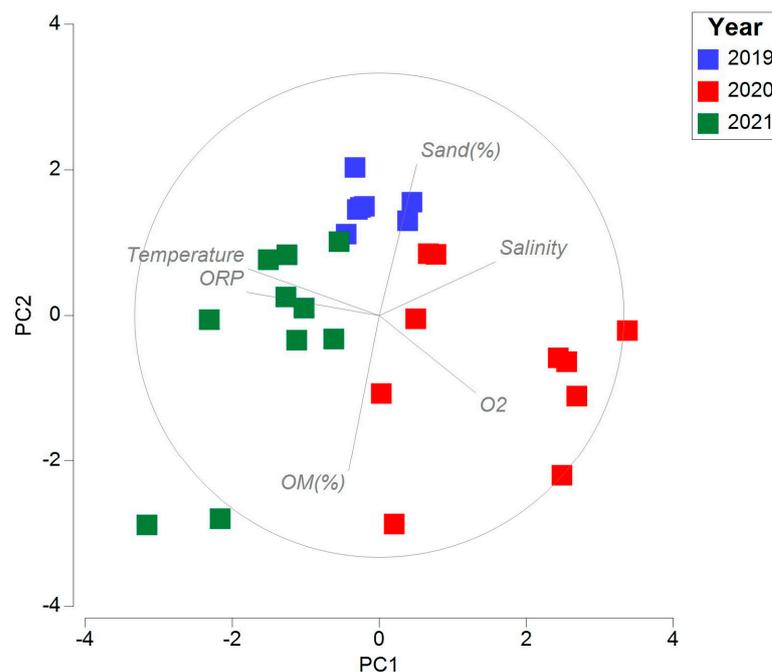


Figure 2. Principal Component Analysis (PCA) ordination diagram of environmental variables (Temperature, Salinity, ORP, Organic Matter (OM), Sand and O<sub>2</sub>), with data points labelled by “Local” (Experimental cells: Control, Plants, Fence, Geotextile, and Bags).



**Figure 3.** Principal Component Analysis (PCA) ordination diagram of environmental variables (Temperature, Salinity, ORP, Organic Matter (OM), Sand and O<sub>2</sub>), with data points labelled by “Year” (2019, 2020 and 2021).

### 3.2. Macroinvertebrate Communities’ Structure

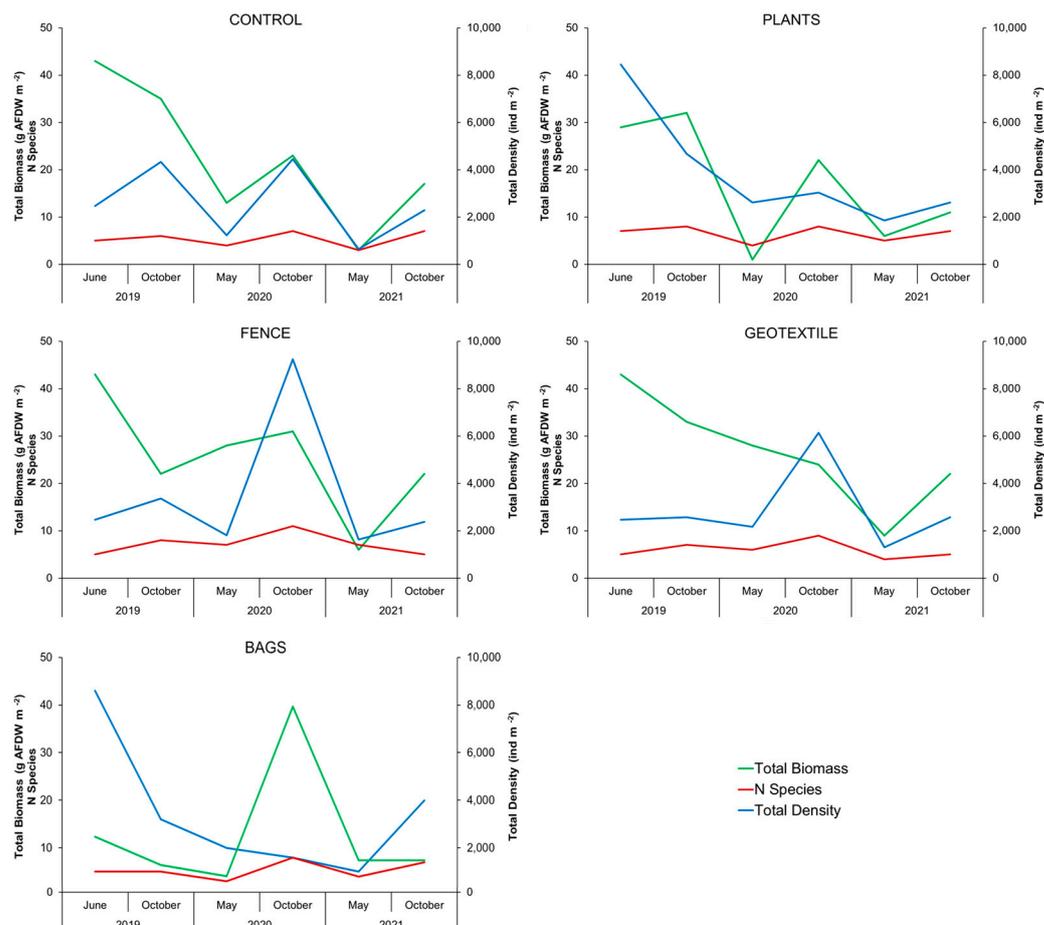
Table 2 lists the 15 benthic macroinvertebrate taxa identified in all experimental and control cells from the pre-intervention sampling in June 2019 to October 2021. Several species were consistently present throughout the sampling period, but only two species were found in all sampled areas during the entire study: the isopod *Cyathura carinata* (Krøyer, 1847) and the polychaete *Hediste diversicolor* (O.F. Müller, 1776). The most represented groups were arthropods (8 taxa), followed by annelids (4 taxa) and mollusks (3 taxa).

The number of species (Figure 4) recorded in the Control cell fluctuated, reaching a low of 3 species in May 2021 and peaking at 7 species in October 2020 and October 2021. The Plants cell showed a higher number of species, peaking at 8 species in October 2019 and October 2020, and a low of 4 species in May 2020. The Fence cell showed the greatest variability, with a peak of 11 species in October 2020 and a drop to 5 species in October 2021. The Geotextile cell peaked at 9 species in October 2020, with a low of 4 species in May and October 2021. The Bags cell experienced a significant decline to 3 species in May 2020, followed by a recovery to 7 species by October 2021. Overall, the Fence cell had the highest species count (11), while the Control and Bags cells had the lowest (3).

The total density (Figure 4) recorded for the different experimental cells fluctuated in the Control cell, with peaks in October 2019 (4326 ind m<sup>-2</sup>) and October 2020 (4454 ind m<sup>-2</sup>), and a low in May 2021 (638 ind m<sup>-2</sup>). The Plants cell exhibited the highest overall densities, peaking in June 2019 (pre-intervention; 8454 ind m<sup>-2</sup>) and declining to a low in May 2021 (1858 ind m<sup>-2</sup>). The Fence cell showed significance variation, peaking in October 2020 (9234 ind m<sup>-2</sup>) and dropping to 1631 ind m<sup>-2</sup> in May 2021. The Geotextile cell peaked in October 2020 (6142 ind m<sup>-2</sup>) and reached a low in May 2021 (1305 ind m<sup>-2</sup>). The Bags cell had its lowest density in May 2020 (809 ind m<sup>-2</sup>), with a peak in October 2020 (7929 ind m<sup>-2</sup>).

**Table 2.** List of benthic macroinvertebrates taxa present in each sample and number of individuals, categorized by year (2019 to 2021) and experimental cell (Control (C), Plants (P), Fence (F), Geotextile (G) and Bags (B)). “JUN 2019” represents the pre- intervention sample.

Taxa	JUN 2019					OCT 2019					MAY 2020					OCT 2020					MAY 2021					OCT 2021				
	C	P	F	G	B	C	P	F	G	B	C	P	F	G	B	C	P	F	G	B	C	P	F	G	B	C	P	F	G	B
<i>Alkmaria romijni</i> (Horst, 1919)	7	3	7	7	7	3	1	4					11			6		6	8			4	3			2	2	3		
<i>Carcinus maenas</i> (Linnaeus, 1758)		1					1	1					1			3	1	4	7	1		2	1		1	2	7		11	2
<i>Cerastoderma edule</i> (Linnaeus, 1758)									1																					
Chironomidae		6						2	4	3	1	13	1	6	4	19		2	1	32	1	7	8	2	15	2	2			9
<i>Corophium multisetosum</i> (Stock, 1952)										6		1	2					3		86										3
<i>Crangon crangon</i> (Linnaeus, 1758)							4	1																						
<i>Cyathura carinata</i> (Kroyer, 1847)	22	44	22	22	22	44	34	46	32	16	11	11	12	11	2	68	29	39	26	6	8	7	19	15	12	66	37	37	31	12
<i>Echinogammarus</i> sp.							10										2	12	4											
<i>Hediste diversicolor</i> (O.F. Müller, 1776)	70	165	70	70	70	95	120	81	70	47	63	159	73	64	52	30	86	84	48	26	36	111	72	64	76	57	113	84	104	53
<i>Lekanesphaera levii</i> (Argano & Ponticelli, 1981)							1																							
<i>Melita palmata</i> (Montagu, 1804)									1																		6	16	1	
<i>Nephtys caeca</i> (Fabricius, 1780)																						1								
<i>Peringia ulvae</i> (Pennant, 1777)																108	4	309	206	358										
<i>Scrobicularia plana</i> (da Costa, 1778)	59	55	59	59	59	105	147	56	72	19	11		23	47	3	80	60	102	80	34		11	11		31	18	37	19	24	
<i>Streblospio shrubsolii</i> (Buchanan, 1890)	16	322	16	16	16	54	24	46	1				7	24			31	88	53	16					1	5				



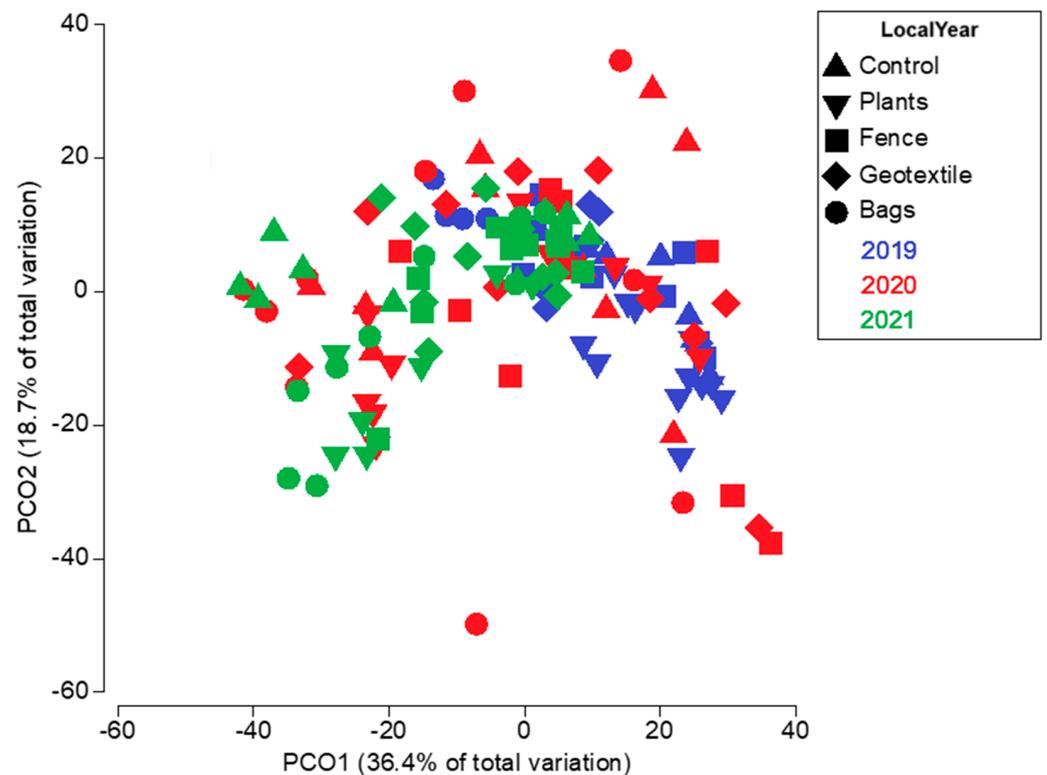
**Figure 4.** Number of species (N Species), Total Density and Total Biomass recorded for the five experimental cells: Control, Plants, Fence, Geotextile, and Bags, over the 3-year study period (2019 to 2021).

The total biomass (Figure 4) recorded for the different experimental cells showed that the Control peaked in June 2019 ( $43 \text{ g AFDW m}^{-2}$ ) and October 2019 ( $35 \text{ g AFDW m}^{-2}$ ), with a low in May 2021 ( $3 \text{ g AFDW m}^{-2}$ ). The Plants cell generally had lower biomass, peaking in October 2019 ( $32 \text{ g AFDW m}^{-2}$ ) and hitting a low in May 2020 ( $1$ ), with a slight recovery by October 2021 ( $11 \text{ g AFDW m}^{-2}$ ). The Fence cell exhibited high biomass, with peaks in June 2019 (pre-intervention,  $43 \text{ g AFDW m}^{-2}$ ) and October 2020 ( $31 \text{ g AFDW m}^{-2}$ ), and a low in May 2021 ( $6 \text{ g AFDW m}^{-2}$ ). The Geotextile cell also peaked before the intervention in June 2019 ( $43 \text{ g AFDW m}^{-2}$ ) and again in May 2020 ( $28 \text{ g AFDW m}^{-2}$ ), maintaining a moderate level through October 2021 ( $22 \text{ g AFDW m}^{-2}$ ). The Bags cell peaked pre-intervention ( $43 \text{ g AFDW m}^{-2}$ ), with a low in May 2020 ( $10 \text{ g AFDW m}^{-2}$ ), and recovered by October 2021 ( $20 \text{ g AFDW m}^{-2}$ ).

The PERMANOVA analysis of community composition, using the density data, indicated a significant interaction ( $\text{Pseudo-}F_{\text{Lo} \times \text{YE}} = 1.4457, p = 0.036$ ) between the 'Local' and 'Year' factors. This indicates that the effect of the different eco-engineering structures on community composition varied across years, and conversely, the impact of the years on community composition was not consistent across the different experimental cells. The pair-wise tests revealed significant differences as follows: In 2019, significant differences were found between multiple pairs—Control vs. Plants, Control vs. Bags, Plants vs. Fence, Plants vs. Geotextile, and Plants vs. Bags. In 2020, fewer significant differences were observed, with only Plants vs. Bags showing significance ( $p < 0.05$ ). In 2021, only Geotextile was not significantly different from Control and Fence.

The Principal Coordinates Analysis (PCO) plot of density data, with PCO1 and PCO2 explaining 55.1% of the total variation (36.4% and 18.7%, respectively), showed some

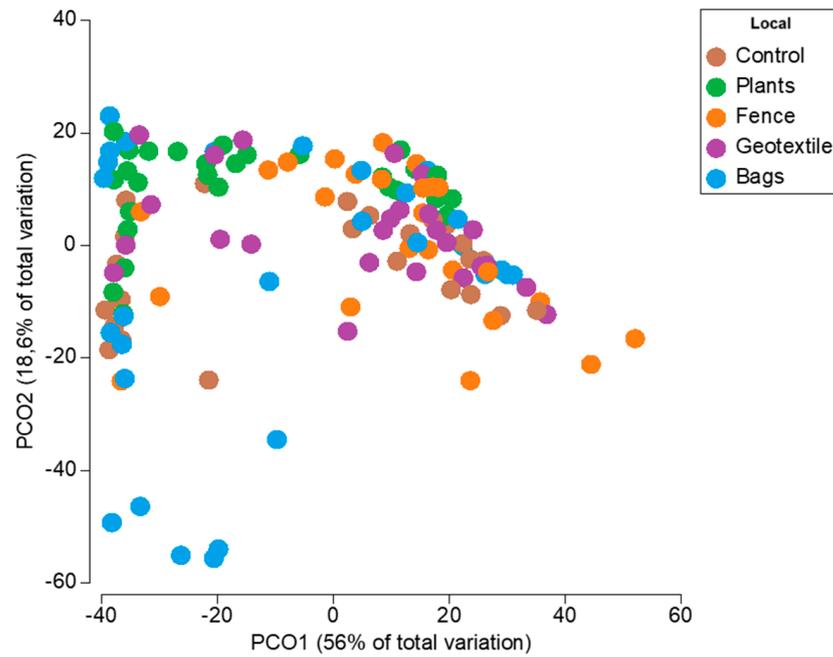
separation between 2019 and the other years. This separation is particularly evident in the Plants experimental cell, which exhibited more pronounced differences across years, suggesting greater sensitivity to changes over time (Figure 5).



**Figure 5.** Principal Coordinates Analysis (PCO) plot of density data illustrating the significant interaction between experimental cells (Control, Plants, Fence, Geotextile, and Bags represented by symbols) and years (2019, 2020, and 2021, represented by colors).

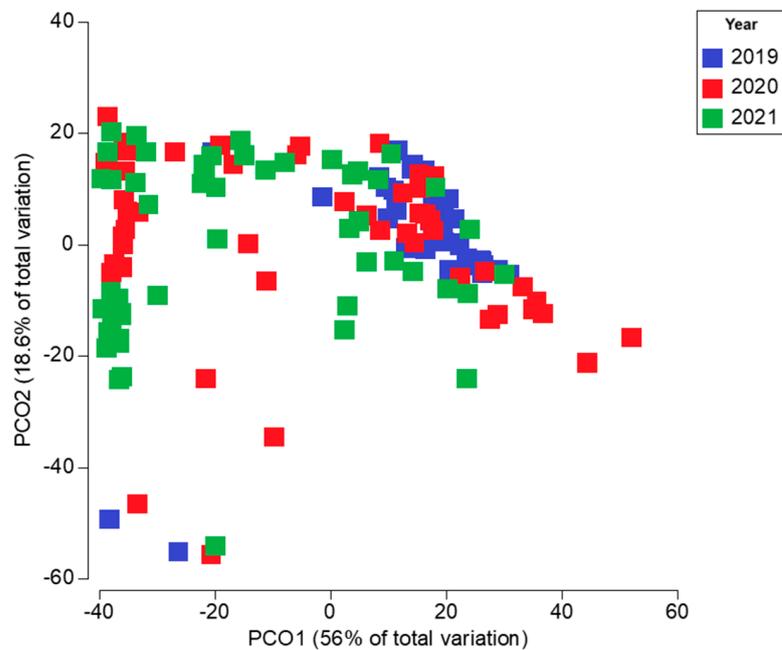
The two-way PERMANOVA test using biomass data revealed significant effects for both the factor “Local” ( $P_{\text{pseudo-F}_{\text{Local}}} = 6.77, p = 0.001$ ) and the factor “Year” ( $P_{\text{pseudo-F}_{\text{Year}}} = 17.61, p = 0.001$ ), but no significant interaction between these factors. This indicates that community composition varied significantly among experimental cells and across years, but the effect of one factor did not depend on the level of the other. Specifically, differences in community composition were consistent across experimental cells regardless of the year, and variations across years were evident irrespective of the experimental cell. The lack of significant interaction suggests that the impact of the eco-engineering structures on community composition was stable over time, and temporal changes in community composition were consistent across different experimental setups. According to the pairwise test results, for the factor “Local”, all experimental cells were significantly different ( $p < 0.05$ ), except Geotextile, which was similar to both Control and Fence ( $p > 0.05$ ). For the factor “Year”, significant differences were observed between all years, indicating temporal changes in biomass composition.

In the biomass data PCO plot for the factor “Local” (Figure 6), PCO1 (56%) and PCO2 (18.6%) together explain 74.6% of the total variation. All experimental cells displayed a high degree of overlap, with the main variation in biomass observed in the Bags experimental cell, where samples showed greater dispersion.



**Figure 6.** Principal Coordinates Analysis (PCO) plot of biomass data, color-coded for the factor “Local” (Control, Plants, Fence, Geotextile, and Bags).

In the biomass data PCO plot for the factor “Year” (Figure 7), PCO1 (56%) and PCO2 (18.8%) together explain 74.8% of the total variation. The tight cluster of the 2019 points reflected the initial conditions of the experiment, where biomass values were similar across experimental sites. The second year, with intermediate spread, represents a transitional phase, during which the experimental cells began to show results that became more stable by the end of the study in 2021.



**Figure 7.** Principal Coordinates Analysis (PCO) plot of biomass data, color-coded for the factor “Year” (2019, 2020 and 2021).

The DISTLM analysis showed that the overall best solution for explaining the variation in the benthic community included five environmental variables: temperature, dissolved

oxygen, organic matter, sand, and silt+clay content (%) ( $R^2 = 0.234$ , with an  $AIC = 957.38$ ), indicating that these variables together accounted for 23.5% of the variation in benthic community composition. These results highlight the importance of both sediment characteristics (OM, sand, and silt+clay content) and water chemistry (temperature and oxygen) in shaping the benthic macroinvertebrate communities in the study area.

Results from the repeated measures ANOVA indicated no statistically significant differences in mean biomass ( $F_{(4, 20)} = 2.82$ ,  $p = 0.05$ ), mean number of species ( $F_{(4, 20)} = 2.55$ ,  $p = 0.07$ ), or mean densities ( $F_{(1.37, 6.86)} = 0.71$ ,  $p = 0.47$ ) across the experimental cells. Post hoc analysis with Bonferroni adjustment further confirmed no significant differences in biomass, number of species, or densities between any of the installed structures.

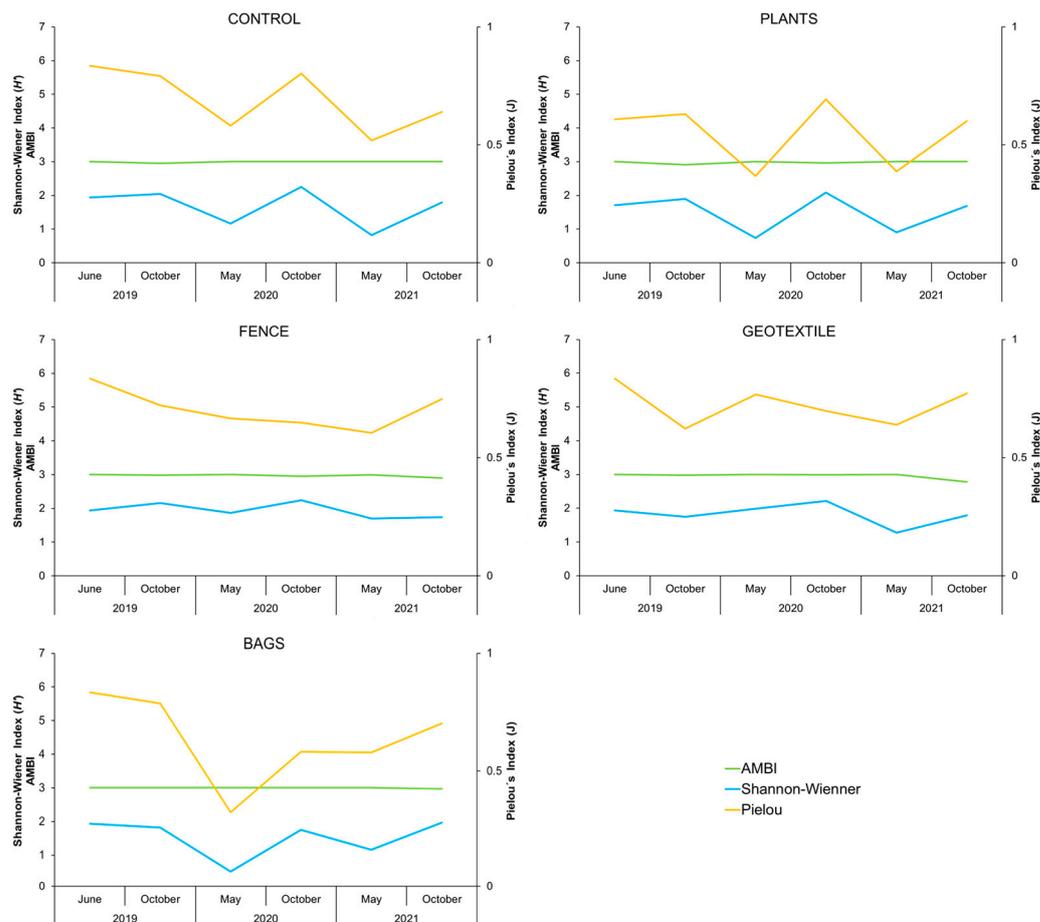
### 3.3. Ecological Indices

The Shannon-Wiener diversity index (Figure 8) for the different cells showed that the Control and Plants cells experienced a decline in May 2020 and May 2021, with a peak in October 2020. The Fence cell remained stable, maintaining high diversity values, particularly in October 2019 and 2020. The Geotextile cell showed an initial decline, peaked in October 2020, and then experienced moderate fluctuations. The Bags cell dropped in May 2020 but recovered by October 2021.

Pielou's evenness index for the experimental cells (Figure 8) showed that the Control cell value dropped in May 2020 and May 2021, with partial recovery by October 2021. The Plants cell had lower indices overall compared to the other cells, reaching its lowest value in May 2020, followed by an increase in October 2020 and recovery by October 2021. The Fence cell maintained relatively high evenness, experiencing a slight decline over time but increasing again in October 2021. The Geotextile cell exhibited high evenness, peaking in May 2020 and remaining elevated through October 2021. The Bags cell experienced a drop in May 2020, followed by a gradual recovery by October 2021.

The data from the Marine Biotic Index—AMBI (Figure 8) revealed reduced levels of disturbance, typical of estuarine environments, and a predominance of relatively tolerant species, with no clear distinction between the pre- and post-intervention sampling. This suggests that the intervention did not significantly influence the conservation status of the local benthic macroinvertebrate communities. It is also important to highlight that the AMBI may not be sufficiently sensitive or suitable for capturing changes in benthic community dynamics within saltmarsh ecosystems. The index is traditionally more effective in areas with distinct disturbance gradients, whereas its application in highly dynamic estuarine systems, such as saltmarshes, may not adequately reflect the more subtle changes resulting from the intervention.

Results from the repeated measures ANOVA showed no statistically significant differences in the mean Shannon-Wiener index between experimental cells ( $F_{(4, 20)} = 2.51$ ,  $p = 0.07$ ). Similarly, the Greenhouse-Geisser correction indicated no significant differences in mean AMBI scores ( $F_{(1.26, 6.29)} = 0.75$ ,  $p = 0.45$ ). However, Pielou's Evenness Index did show significant variation between experimental structures ( $F_{(4, 20)} = 3.40$ ,  $p = 0.03$ ), with the Control having a significantly higher evenness compared to the Plants (mean difference 0.15, 95% CI [0.01 to 0.28],  $p = 0.04$ ). In summary, the only significant difference was found in Pielou's Evenness Index, where the Control exhibited higher evenness than the Plants. Shannon-Wiener and AMBI scores showed no significant differences across the experimental cells.



**Figure 8.** Ecological indices (Shannon-Wiener, Pielou and AMBI) for the five experimental cells (Control, Plants, Fence, Geotextile, and Bags) over the three-year study period (2019, 2020, and 2021).

## 4. Discussion

### 4.1. Macroinvertebrates Responses to Eco-Engineering Structures

This study provided insights into the effectiveness of eco-engineering structures in salt marsh restoration by evaluating changes in benthic macroinvertebrate communities, focusing on species composition, biomass and diversity. It contributed to a better understanding of restoration strategies that can be applied to similar estuarine environments affected by sea-level rise and anthropogenic pressures.

The results indicated that the interventions led to some responses in the benthic macroinvertebrate community, but the overall impact was minimal. This suggests that the use of such structures for protection and restoration of impacted areas does not negatively affect benthic communities. The biodiversity present in the ecosystem depends on multiple factors [28], including the site's environmental conditions at a given time and the type of structures applied. For instance, a previous study demonstrated that benthic macroinvertebrates responded positively to restoration efforts, with community composition, diversity, density and biomass significantly recovering three years after dike removal on the Nisqually River Delta, Washington, USA [29]. Similarly, a wetland restoration in Northern New York found that taxa numbers in restored areas were comparable to those in natural sites during a three-year study [30].

The macroinvertebrate community varied across experimental cells and, over time, showed an inconsistent response to the interventions. This variability may be influenced by the dynamic nature of salt marsh habitats, which are subject to seasonal changes and fluctuations in abiotic conditions [31–33]. Seasonal variations in macroinvertebrate communities are well-documented in estuarine systems, including the Mondego Estuary. As

reported by Teixeira et al. (2008) and Veríssimo et al. (2012), these communities typically experience seasonal shifts in abundance, diversity, and composition due to changes in temperature, salinity, food availability, and reproductive cycles [20,34]. During warmer months, species abundance tends to increase due to higher productivity, while colder months are characterized by a reduction in diversity, with more resilient species dominating. These natural fluctuations in community structure and species life cycles must be considered when interpreting the effects of restoration interventions. While seasonal variations may have influenced community responses, the data also highlights the role of site-specific factors and intervention types in shaping macroinvertebrate communities. In this study, seasonal changes were particularly evident in the second year (2020), which acted as a transition period when community composition began to diverge from its initial state. By the third year (2021), stabilization started to occur, suggesting that these communities may require at least two years to adjust to new conditions. While this 3-year periodicity may be partially tied to the life span and life cycles of the species within the macroinvertebrate community, as recovery and adjustment times often reflect the biological rhythms and reproductive strategies of organisms, caution is needed before generalizing this timeline across ecosystems or taxa. Community responses can vary significantly based on the specific species introduced or removed during the intervention. Keystone species or those with strong interspecific interactions, for example, may disproportionately influence recovery dynamics. Additionally, studies from other ecosystems, such as rocky shore manipulations reported by O'Connor et al., emphasize how community composition and functional roles drive differing responses to restoration efforts [35]. This variability highlights the importance of long-term monitoring to capture delayed responses and underscores the need to account for species-specific traits (e.g., Beauchard et al.) and ecosystem contexts in the design and evaluation of restoration practices [36–39].

The lack of significant differences in density, biomass and number of species across experimental cells suggests that the eco-engineering structures did not cause notable changes in these metrics. In the Plants and Bags experimental cells, the more pronounced variations in the Shannon-Wiener diversity and Pielou's evenness indices seemed to be more influenced by seasonal environmental changes than by the type of intervention. However, the relatively short duration of post-intervention monitoring (two years) may have been insufficient to fully capture the macroinvertebrate community's responses. The limited impact of the eco-engineering structures on biomass and diversity is further supported by the AMBI index, which points to the dominance of tolerant species in all the experimental cells, indicating a slightly disturbed environment that restricts the colonization of more sensitive species. Macroinvertebrates communities in estuarine salt marshes are strongly shaped by environmental variables, with salinity being a critical factor influencing species distribution and abundance, supporting the dominance of salt-tolerant species [32,40]. The AMBI index, while providing some insights, may lack the sensitivity needed to detect the subtle shifts in community dynamics within saltmarsh ecosystems, where disturbances are often less pronounced and occur more gradually. Our findings also suggest that site-specific factors, such as local environmental conditions, played a more significant role in determining community structure than temporal variation alone.

The Plants cell, composed of autochthonous vegetation, supported a higher number of species, particularly in the initial period after the intervention, and exhibited greater species richness and density. This suggests that the presence of natural vegetation enhances the habitat, providing better conditions for a broader range of species [41]. However, the decline in biomass and density by the end of the study period indicates that vegetation alone is insufficient to maintain a stable community without additional support or restoration measures. The Fence cell displayed the highest species count and density peaks, indicating a positive effect on the macroinvertebrate community. However, the pronounced variability in density and biomass suggests that the community in this cell was more sensitive to environmental fluctuations. In contrast, species richness and biomass tended to be more stable in the Geotextile cell. The Bags cell initially had the lowest values for species richness

and density but showed strong recovery by the end of the study period. This suggests a stabilization of environmental conditions and sediment characteristics over time, leading to a recovery of the macroinvertebrate community, which eventually reached levels similar to those in the other experimental cells.

Considering the responses of benthic macroinvertebrates across all experimental cells, the Fence (wood palisade) appears to be the most effective in promoting species richness and density over time, while the Geotextile promotes a more stable community. Therefore, a combined approach using both the Fence and Geotextile methods may provide the most effective strategy for salt marsh restoration projects. As both seemed effective approaches, it is important to consider the advantages and drawbacks of each material, especially from a potential large-scale perspective. The wood palisade is made from a sustainable, biodegradable material that can withstand the forces of water flow between tides. Unlike the easily maintained and repaired wood fence, geotextile fabric is more difficult to repair and is usually made from synthetic fibers.

The temporal variations recorded highlight the importance of long-term monitoring of indicators, such as fauna, in a restoration project [42]. Short-term measurements may not capture all the changes that occur in a highly dynamic ecosystem like an estuarine salt marsh, potentially leading to misleading conclusions about the effectiveness of restoration efforts. Therefore, future studies should extend post-intervention monitoring to assess any delayed ecological responses and evaluate the long-term effectiveness of eco-engineering approaches [43].

#### *4.2. Practical Recommendations for Designing and Implementing Eco-Engineering Interventions*

The findings of this study offer valuable insights into optimizing eco-engineering interventions for salt marsh restoration, with a key recommendation being the strategic combination of wood palisades (Fence) and geotextile materials. The Fence was shown to enhance species richness and density, while the Geotextile fosters macroinvertebrate community stability. Therefore, future restoration projects should adapt these methods to their specific ecological goals, whether improving biodiversity or promoting community stability. In addition, incorporating habitat heterogeneity into restoration designs is essential. Varying substrates, elevations, and plant species can create diverse microhabitats that support a wider range of species, making the ecosystem more resilient to environmental changes [44]. This approach mimics the natural complexity of estuarine environments and provides better long-term conditions for diverse benthic communities.

In the face of increasing anthropogenic threats to marine and estuarine ecosystems, long-term monitoring is crucial for capturing the full impact of eco-engineering interventions [45,46]. Given the delayed ecological responses observed in this study, a monitoring period of at least three years should be included in projects to track changes in species composition, biomass, and diversity over time. This will provide a clearer understanding of the effectiveness of the interventions.

To better adapt to the dynamic nature of estuarine ecosystems, real-time monitoring of key environmental parameters, such as salinity and oxygen levels, is also recommended. This would allow for adaptive management, where interventions can be adjusted in response to sudden changes in environmental conditions, helping to safeguard the restored habitats.

Lastly, the sustainability of the materials used for eco-engineering structures [47] must be considered. Wood palisades, made from biodegradable and renewable materials, offer an eco-friendly option that can withstand tidal forces. However, while geotextile materials are effective, they are typically made from synthetic fibers and are harder to repair. Careful selection of materials that balance durability, ease of maintenance, and environmental impact is essential for large-scale restoration efforts.

#### *4.3. Integration into Sustainable Coastal Management Practices*

The recommendations derived from this study can significantly contribute to sustainable coastal management practices. Eco-engineering interventions, such as those tested

here, align with the principles of nature-based solutions (NbS), which aim to enhance natural processes while addressing environmental challenges like sea-level rise and coastal erosion [48]. By promoting habitat heterogeneity and using natural materials, these interventions can help increase the resilience of salt marshes and other coastal ecosystems against the impacts of climate change.

Effective eco-engineering requires the integration of scientific research with policy-making to establish clear standards and guidelines [49]. Policymakers can promote the use of eco-engineering strategies in coastal restoration by setting regulations that encourage the use of sustainable, site-specific interventions. For widespread adoption, these strategies should be incorporated into national and regional coastal management policies and planning frameworks.

Engagement with local communities, government agencies, and conservation groups will also be crucial for the success of these eco-engineering projects [50]. Stakeholders must be educated about the benefits of such interventions and the importance of ongoing monitoring. Additionally, capacity-building initiatives should be developed to train local practitioners [51] in effectively implementing and maintaining these solutions.

Coastal management strategies that incorporate eco-engineering methods will enhance the adaptive capacity of vulnerable habitats, making them more resilient to future changes. Ultimately, eco-engineering solutions should be viewed as part of a broader, holistic approach to coastal zone management, to balance ecological, social, and economic factors [52]. By addressing site-specific environmental variables and using sustainable materials, these interventions can maintain biodiversity, support fisheries, and protect coastal communities from natural hazards.

## 5. Conclusions

This study provided valuable insights into the role of eco-engineering in salt marsh restoration, particularly its impact on benthic macroinvertebrate communities. Although the overall short-term effects of the tested structures were minimal, the research highlighted the potential of methods like wood palisades (Fence) and geotextile materials to enhance species richness, density, and community stability over time. The variability in responses across experimental cells underscored the dynamic nature of estuarine ecosystems and the need for long-term monitoring to fully assess restoration efforts. The results suggested that combining approaches such as Fence and Geotextile may better promote biodiversity and ecological stability. The study also emphasizes the importance of site-specific factors in shaping macroinvertebrate communities, indicating that eco-engineering strategies must be adapted to the local environmental conditions.

The findings have broader implications for sustainable coastal management. Integrating eco-engineering solutions into coastal policies can enhance resilience to climate change, while promoting biodiversity and ecosystem health. Long-term monitoring, coupled with stakeholder engagement, will be key to ensuring the success and sustainability of these interventions. Thoughtfully applied and monitored over time, eco-engineering solutions can provide significant benefits for salt marsh restoration and coastal ecosystem management, supporting both ecological integrity and human well-being.

Future research should focus on understanding the ecological processes behind these outcomes and exploring how successful restoration methods can be adapted to different geographic regions and environments. This will contribute to more resilient ecosystems and help establish best practices for salt marsh and estuarine restoration.

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