Ecological recovery of *Zostera noltii* meadows: Benthic macrofauna as indicators

Cavalcante L. S.[1*], Oliveira, V.H.[1], Dolbeth M.[2], Crespo, D.[1], Brusch, J.[3,4], Misteli B.[3], Attermeyer K.[3,4], Rochera C.[5], Tropea C.[6], Bachi G.[6], Cabrera-Brufau M.[7], Carloni M.[6], Checcucci G.[6], Guerrazzi M.[6], Minaudo C.[7], Montes-Pérez J.J.[7], Obrador B.[7], Camacho-Santamans A.[7], Morant D.[5], Picazo A.[5], Sousa A. I.[1], Santinelli C.[6], Camacho A.[5], Lillebø A. I.[1], Coelho J. P.[1]

[1] *CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*
[2] *CIIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Novo Edifício do Terminal de Cruzeiros, Avenida General Norton de Matos S/N, 4450-208 Matosinhos, Portugal*
[3] *WasserCluster Lunz - Biological Station, Dr. Carl Kupelwieser-Prom. 5, 3293 Lunz am See, Austria*
[4] *Department of Functional and Evolutionary Ecology, Unit Limnology, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria*
[5] *Cavanilles Institute for Biodiversity and Evolutionary Biology, University of Valencia, E-46980 Paterna, Valencia, Spain.*
[6] *Consiglio Nazionale delle Ricerche (CNR), Istituto di Biofisica, Via Moruzzi 1, 56124 Pisa (PI), Italy*
[7] *Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Universitat de Barcelona (UB) Diagonal 643, 08028, Barcelona, Spain*

*Corresponding author: lamaracavalcante@ua.pt

Abstract

This study analyzed the benthic macrofauna associated with *Zostera noltii* (Hornemann, 1832) meadows. The objective was to evaluate the structure, diversity, and composition of the benthic macrofauna along a spatial gradient of restored, well-preserved, and altered meadows, and to assess the temporal evolution of species in the restored area over time. Temperature, salinity, dissolved oxygen, pH, sediment granulometry, and macrofauna and *Z. noltii* samples were collected in two campaigns (winter and summer of 2024). A total of 11 species were identified, belonging to three phyla: Mollusca (6 species), Crustacea (3 species), and Annelida (2 species). Mollusks were the most abundant group, and species such as *Peringia ulvae*, *Scrobicularia plana*, and *Hediste diversicolor* were identified as indicator species (IndVal = 0.916; p = 0.002). Diversity indices (Shannon-Wiener, Pielou, and Species Richness) were calculated and compared between areas and seasons. Restored and well-preserved areas showed high diversity and evenness, while altered areas showed low richness. Environmental parameters and biomass of *Z. noltii* differed significantly between locations, and showed a division between altered and vegetated areas, with a partial overlap between restored and well-preserved meadows. Temporal analysis of abundance and species in restored areas (2021 vs. 2024) showed an increase in richness (from 5 to 11 species) and a more balanced benthic composition (IndVal = 0.787; p = 0.005). These results indicate that the increase in *Z. noltii* biomass and benthic macrofauna diversity can be used as an indicator of restoration effectiveness and support efforts for coastal habitat restoration and nature conservation.

Keywords: ecological indicators, benthos, wetlands, restoration

1. INTRODUCTION

Coastal regions have historically supported human settlements and economic activity due to the abundance of resources and their geographic location. Anthropogenic pressures on these ecosystems have increased significantly since the 20th century, resulting in significant ecological changes over time (Neumann et al., 2015). These trends parallel those in terrestrial environments, such as the loss of biodiversity and essential ecosystem services, both of which are critical for maintaining ecosystem stability and supporting biodiversity (Blanchet et al., 2004; Cardoso et al., 2007; UNEP, 2020).

Seagrass meadows are some of the most at-risk habitats. They provide a myriad of ecosystem services, encompassing soil erosion prevention, pollutant filtration, and providing shelter for many bottom-dwelling species. These meadows also serve as feeding and nursery areas, including for species important to commercial fishing (Greiner et al., 2013; Short et al., 2017; Macreadie et al., 2019; Sousa et al., 2019).These habitats also capture and store blue carbon, which helps lessen the impacts of climate change (Bergstrom et al., 2019; Crespo et al., 2017; Koch et al., 2013).

Anthropogenic activities have been causing a global decline in seagrass meadows (de los Santos et al., 2019). Several stressors, such as habitat loss, pollution, and environmental imbalance, continue to threaten these habitats, with poor water quality accounting for 26% of these losses in Europe (Hughes et al., 2018; de los Santos et al., 2019). Ecological restoration has emerged as a fundamental component to reverse biodiversity loss and restore ecosystem functionality, which is a key indicator of restoration success (Sheaves et al., 2021).

Conservation policies are increasingly giving due ecological importance to seagrass habitats. In the United States, the Clean Water Act has contributed to increased protection of these habitats (Rezek et al., 2019). In Europe, the Habitats and Water Framework Directives use seagrasses as indicators of the health of coastal ecosystems (de los Santos et al., 2019; Krause-Jensen et al., 2005). The United Nations Decade on Ecosystem Restoration (2021-2030) and the EU Nature Restoration Regulation have enforced restoration to combat habitat loss and build ecological resilience (Buelow et al., 2022; Rezek et al., 2019). These actions include transplanting healthy seagrasses, regulating harmful activities such as dredging and bottom trawling, and promoting the improvement of environmental conditions (Cardoso et al., 2010; Hughes et al., 2018; Riemann et al., 2016). Although these efforts are still in their early stages, biodiversity has proven to be a reliable measure for assessing restoration progress, particularly in long-term projects with adaptive management and continuous monitoring (Bell et al., 2008; Rezek et al., 2019; van Katwijk et al., 2016).

In Europe, some studies have found that the seagrass *Zostera noltii* (Hornemann, 1832) is showing signs of recovery in several areas (de los Santos et al., 2019; Sousa et al., 2019). This recovery capacity stems mainly from its rhizome system and seed bank, which help it survive and regenerate even in adverse conditions (Zipperle et al., 2009). *Z. noltii* grows well in intertidal estuarine areas, where it supports a variety of benthic communities (Dolbeth et al., 2011), often dominated by small herbivorous gastropods such as *Peringia ulvae* (Blanchet et al., 2004; Cardoso et al., 2010; Crespo et al., 2017). This seagrass tolerates harsh environments, including high temperatures up to 37 °C (Massa et al., 2009) and a wide salinity range, from 7 to 41 (Charpentier et al., 2005; Sousa et al., 2017; Vermaat et al., 2000). It is also well adapted to turbid environments and can perform photosynthesis during low tide (Vermaat et al., 1996).

Zostera noltii is one of the most widespread seagrass species in European estuaries and coastal lagoons, including the Atlantic coast of Portugal. In the Ria de Aveiro, a coastal lagoon spanning 83 km² at high tide, *Z. noltii* meadows covered approximately 230 hectares in 2014 (Sousa et al., 2019). Seagrass conservation and restoration measures in the system encompass the validation of transplant methodologies and strategies to mitigate the effect of Non-Indigenous Species (Costa *et al*., 2022) and the use of seagrass transplantation as a nature-based solution for the restoration of historically contaminated mudflats (Oliveira *et al*., 2025) and the recovery of associated ecossystem functions (Fradoca *et al*., 2025; Oliveira *et al*., 2023).

This study, conducted in the Ria de Aveiro, was driven by the following research question: How do spatial and temporal gradients shape the structure, diversity, and composition of benthic macrofauna as restored seagrass meadows show improvements in environmental quality and seagrass biomass? The aim was to analyze the distribution of benthic macrofauna species in *Z. noltii* meadows in areas with different maturation stages, such as restored, well-preserved, and altered areas. Thus, we evaluated (i) differences in environmental conditions and seagrass biomass, (ii) spatial and seasonal patterns of benthic diversity and community composition, and (iii) temporal changes in restored areas to assess the response of benthic macrofauna to seagrass meadow restoration as ecological indicators of restoration success. It is expected that the results will contribute to advancing knowledge about the importance of biodiversity as a tool for monitoring the restoration of coastal habitats.

2. MATERIAL AND METHODS

2.1. STUDY AREA

The experimental procedures took place in *Zostera noltii* meadows of a shallow coastal lagoon (Ria de Aveiro), on the northwest coast of Portugal. (-40.63°N, -8.75°W) (Figure 1). The surface area of the lagoon is approximately 75 km², it is integrated into the Vouga River basin, inholding the Vouga River estuary, with a single connection to the Atlantic

Ocean. This system hosts a diversity of habitats and is part of the Natura 2000 network, classified as a Special Area of Protection and Conservation, and classified as a Site of Community Importance (EUNIS, 2020). It has a temperate climate, according to the Köppen classification, with dry summers and mild temperatures. The study by Sousa et al (2019) describes the evolution of seagrass meadows and the history of anthropogenic impacts in these habitats, which caused the loss of biodiversity and disappearance of subtidal meadows, and discusses the emergence of a state of passive habitat recovery, a period in which the meadows expand again and remain stable and healthy, colonizing other stretches and suggesting a natural adjustment of the ecosystem. For this study, two sites from each of three distinct health status were selected: well-preserved (mature *Zostera noltii* meadows), altered (bare-bottom areas formerly colonized by *Zostera noltii)*, and restored areas (areas subject to *Zostera noltii* re-colonization), for a total of 6 sampling locations, with the aim of representing a gradient of *Z. noltii* meadows health status (Figure 1). These locations reflect the structural and ecological diversity of the lagoon, ranging from healthy and preserved meadows to restored areas with transplanted *Z. noltii* at different maturity stages, and degraded habitats with reduced biodiversity and ecosystem functionality (Sousa et al, 2019; Crespo et al, 2023).

(FIGURE 1)

2.2. DATA COLLECTION

The study was conducted in two seasonal sampling campaigns, one in January 2024 (winter) and the other in July 2024 (summer). At each sampling moment and site, five samples were collected using a circular bottom corer (11.5 cm², ~20 cm depth). The samples were washed in situ using a 500 µm mesh and transported in thermal boxes to the laboratory, where they were frozen (−20 °C) until further processing. To minimize impacts on the restored meadow, removed samples were replaced with new patches from the original meadow. The physicochemical parameters of the water (water temperature, salinity, and pH) were also measured using a properly calibrated portable multiparameter probe (WTW), and sediment samples were collected in each sampling site for granulometry and biomass analysis of *Z. noltii*.

In the laboratory, the samples were washed in sieves with 2 mm, 1 mm and 0.5 mm meshes, and the benthic macrofauna species were selected and identified to the smallest possible taxon, using identification guides and specialized literature. The biomass in ash free dry weight (AFDW) of the benthic species was estimated after drying in an oven at 60 °C for 72 h and incineration in a flame loss ignition muffle furnace at 450 °C for 8 h. After combustion, samples were let to cool down in an exicator and then weighed on an analytical balance. Finally, the leaves, roots, and rhizomes of *Z. noltii* were separated during sample processing and dried in a forced-air oven at 60 °C for 48 h. Dry biomass was determined in g/m^2.

Sediment grain size distribution was characterized through Laser Diffraction Particle Size Analysis, according to Coblentz *et al*. (2015). Sediment organic matter was first removed from the dried samples using hydrogen peroxide, as particle size distribution only refers to mineral particles. 3 ml of 30% H_2O_2 were added to 0.3 g of dry sediment in a petri dish. Additionally, 1.5 ml of sodium hexametaphosphate $(NaPO_3)_6$ with a concentration of 2 g/L were added as a deflocculant for silt and clay particles. The samples were left to react for at least 72 hours. The sediments were transferred into 15 ml centrifuge tubes and diluted with distilled water (Sartorius water). They were then centrifuged to take off excess water using Avanti® J-26XP 20.1 (Beckman Coulter, Brea, CA, USA) for 10 minutes (8000 rpm, 20 °C). The pretreated samples were suspended in 1.5 ml of Sartorius water and sonicated for 60 seconds before measurement. The ULM of the Particle Size

Analyzer was loaded with a few drops to 1.5 ml of the sample solution to reach a target loading of 8 ± 2%. Each loaded sample was measured three times.

2.3. DATA ANALYSIS

The analyses were performed to test the differences between environmental, sedimentary, and biological parameters and the relationship between vegetated habitat and benthic macrofauna in the ecological recovery of restored meadows over time. The data were processed and analyzed in the RStudio statistical environment (R Core Team, 2025, version 4.4.2). We used the readxl, dplyr, and tidyr packages for importing and organizing spreadsheets, the ggplot2 and patchwork packages for creating graphs and panels, and stats for descriptive calculations (mean, standard deviation, minimum and maximum values) for all biotic and abiotic variables between the season (summer and winter) and areas (A1, A2, P1, P2, R1, R2). The assumptions of normality and homogeneity of variances on the raw data were verified using the Shapiro–Wilk test ($p \leq 0.05$) and the Levene (Brown-Forsythe) test. After verifying that the data were not normally distributed, it was decided to test the difference between the factors using the non-parametric Kruskal-Wallis test. Ecological diversity indices (richness, Shannon–Wiener, Simpson, and Pielou's evenness) were calculated using the vegan package (Oksanen et al., 2024), which was also used in multivariate analyses of the benthic community, including nMDS and cluster analyses based on Bray–Curtis distance.

The community composition was described by analyzing the relative abundance of species (proportion of the number of individuals of each species in relation to the total per sample), calculated with the dplyr and tidyr packages, and indicator species were identified using the indicspecies package through the IndVal analysis (Dufrêne &

Legendre, 1997). The IndVal index combines a frequency component for the species within a group of locations, and another component measuring the degree of exclusivity of that species to that group in relation to others, resulting in a value ranging from 0 to 1 (0–100%). A temporal analysis focused on the restored area was also carried out, comparing data on benthic species collected in 2024 with data from 2021, collected in the same areas and described by Crespo et al. (2023). This comparison allowed for the evaluation of changes in community structure throughout the restoration process and for the identification of common species in the different stages of recovery, considering their frequency of occurrence, percentage contribution to the community, and temporal consistency. The methodology adopted was based on a combination of the approaches used by Rodil et al. (2021, Basyuni et al. (2021), Steinfurth et al. (2022) and Crespo et al. (2023), adapted to the context of seagrass meadows in the Ria de Aveiro and allowing the interpretation of benthic macrofauna as a functional indicator in the restoration process.

3. RESULTS

3.1. Environmental variables and site characterization

Analysis of the physicochemical variables and biomass of *Z. noltii* showed differences according to the altered–restored–preserved gradient (**Figure 2**). Water temperature showed higher values in summer 21.93 ± 0.39 °C (restored) and lower values in winter, 12.78 ± 0.05 °C (altered). The Kruskal–Wallis test did not indicate significant differences ($p = 0.199$) between sites. pH varied between 7.73 ± 0.09 (restored, summer) and 7.98 ± 0.08 (preserved, summer) with no significant differences ($p = 0.167$). Salinity ($p = 0.0161$), dissolved oxygen ($p = 0.00745$), and *Z. noltii* biomass ($p = 0.0064$) showed significant differences, with the highest average values in preserved and restored areas in summer, and the lowest in altered areas in both seasons. These results show that the differences between altered, restored, and preserved areas are mainly related to salinity and oxygenation conditions of the water and to vegetation growth, factors more related to the ecological functionality and to the health status of the habitats (Supplementary Material - Table S1).

(FIGURE 2)

The sediment granulometric parameters did not show statistically significant differences (**Figure 3**). These results indicated that the type of sediment is relatively similar between the areas and with low variation in the average values of D10, D50, D90 and StdDev, with the finer sediments (D10 = 18.36 ± 4.03 winter) in the preserved areas, while the coarser fraction of the sediment (D90) was observed in the restored area during the summer (D90 = 436.64 ± 101.30). Regarding grain size (StDev), the most homogeneous sediments occurred in the preserved areas during the summer (149.98 ± 33.23), while the most heterogeneous were observed in the summer, in the restored areas (186.24 ± 34.04) and in the altered areas (175.33 ± 31.24). (Supplementary Material – Table S2).

(**FIGURE 3**)

3.2. Ecological structure of benthic macrofauna

Eleven species belonging to three phyla, Mollusca, Crustacea, and Annelida, were identified. Mollusks were the most numerous, with two species of bivalves and four gastropods. Among the crustaceans, three species were identified, and one species of polychaete from the phylum Annelida (**Supplementary Material - Table S3**). Considering the total number of species recorded, the preserved areas had 11 species, the restored areas eight, and the altered areas three. The density among the species (ind. m^{-2}) was higher in the preserved and restored areas. The bivalve *S. plana* had the highest average densities in the restored area during the summer (206 ± 9) and (165 ± 15) in the preserved area during the winter. The species *H. diversicolor* (150 ± 89, preserved-winter), and the gastropod *P. ulvae* (142 ± 103, preserved-summer) also presented the highest densities, these being the three most abundant species in all areas and seasons. The other species presented lower densities, with the bivalve *C. edule*, the isopods *C. carinata* and *Idotea chelipes*, and the crab *Carcinus maenas* present in preserved and restored areas, and the gastropods *Bittium reticulatum* and *Phorcus lineatus* recorded only in preserved meadows (**Supplementary Material - Table S 4**).

Regarding diversity indices, the highest values were observed in both restored and preserved areas (**Figure 4**). Species richness (S), which represents the total number of taxa present, was higher during the summer in the preserved area ($S = 4.60 \pm 1.58$), and in the restored area (3.50 ± 1.18). The altered areas showed low richness in both seasons ($S = 1.40 \pm 1.07$ in summer; 1.30 ± 1.06 in winter), indicating the dominance of a few taxa tolerant to degraded environments. Although no significant difference was observed in relation to seasonality (summer and winter) ($p = 0.448$), this pattern was evidenced by a significant difference between the sites (Kruskal–Wallis: $p < 0.001$) and Dunn's post-hoc test, which indicated that altered differed from well-preserved ($p_{adj} < 0.001$) and restored ($p_{adj} < 0.001$), while well-preserved and restored showed no differences ($p_{adj} = 0.195$).

Shannon diversity (H′), which combines the number of species and their relative proportion, was always higher in the restored area ($H' = 0.99 \pm 0.26$ in winter and 0.90 ± 0.23 in summer). In the altered areas, the values were lower (0.30 ± 0.40 in summer; 0.32 ± 0.36 in winter), evidencing low diversity and strong dominance of a few species. The statistical test indicated that Shannon (H′) also differed between sites ($p < 0.001$), altered and well-preserved ($p_{adj} = 0.00184$) and altered x restored ($p_{adj} = 2.93 \times 10^{-5}$), with no difference between well-preserved and restored ($p\_adj = 0.234$) and between seasons ($p = 0.625$). Pielou's evenness (J′) showed a significant difference between sites ($p = 0.028$) and Dunn's post-hoc test indicated a difference between altered and restored ($p\_adj = 0.042$), while altered and well-preserved did not differ ($p\_adj = 0.953$) and the well-preserved and restored areas showed a trend ($p\_adj = 0.0559$), but did not reach significance. Between seasons, there was no significant difference for Pielou (J′) ($p = 0.063$). Simpson's index (1–D), which reflects diversity (higher values indicate lower dominance), also had the highest values in the restored areas in winter ($1–D = 0.59 \pm 0.09$) and summer (0.51 ± 0.10), followed by the preserved areas (~0.4). However, this index did not show a significant difference between sites ($p = 0.160$), and nor between seasons ($p = 0.108$). Total benthic biomass showed a similar pattern, with higher values in preserved (9.4 ± 2.2 $g \cdot m^{-2}$ in summer; 8.1 ± 2.1 $g \cdot m^{-2}$ in winter) and restored areas (7.5 ± 2.3 $g \cdot m^{-2}$ in summer) with significant differences. between sites ($p < 0.001$) and between all pairs ($p\_adj < 0.001$). The post-hoc test indicated significant differences between altered and well-preserved ($p\_adj = 9.40 \times 10^{-9}$), altered vs. restored ($p\_adj = 0.000625$), and well-preserved vs. restored ($p\_adj = 0.000938$). Furthermore, biomass was significantly different between seasons (Kruskal–Wallis: $p < 0.001$) (Supplementary Material - Table S5).

(**FIGURE 4**)

The results of the Kruskal–Wallis statistical test indicated significant differences between areas for the species *S. plana* ($p = 0.0019$), *P. ulvae* ($p < 0.0001$), *H. diversicolor* ($p < 0.0001$), *C. multisetosum* ($p = 0.0008$), *C. maenas* ($p = 0.0101$), and *I. chelipes* ($p = 0.0066$). Dunn's post-hoc test confirmed that the altered areas presented significantly different densities from the preserved and restored areas, with adjusted p-values less than

0.01, but no significant difference was observed between the preserved and restored areas ($p > 0.05$), and no significant seasonal differences were observed; only *C. maenas* ($p = 0.0106$) and *I. chelipes* ($p = 0.0207$) showed significant differences between winter and summer, with higher values observed in the latter. The results of the non-metric multidimensional ordination analysis (nMDS) indicated a stress value (Stress = 0.0116) with clear patterns in the benthic community structure among the different areas studied (**Figure 5**). Samples from the Altered area are more widespread in multidimensional space, indicating greater dispersion, and samples from the restored and preserved areas show partial overlap. The similarity dendrogram (based on the Bray–Curtis index) formed three large clusters corresponding to the ecological states of the areas: restored and preserved samples in the same branch, suggesting ecological convergence between these zones, while altered samples formed a separate group, reflecting low similarity with the other areas.

(**FIGURE 5**)

The composition of the benthic community showed evident variations between areas and seasonal periods (Figure 6). In figure A, the stacked bars show the relative abundance of the main species throughout the different sampled months, and figure B shows the indicator species test (Indicator Value Analysis, IndVal = 0.916; $p = 0.002$), which indicates that all sites are mainly dominated by the species *P. ulvae*, *S. plana*, and *H. diversicolor*, and that it presents high specificity and fidelity in relation to vegetated areas, especially in preserved and restored areas. When we highlight the composition between the years (2024 x 2021), considering only the restored areas, we observe the same dominant species present in both years (*P. ulvae*, *H. diversicolor*, and *S. plana*) and only 5 species in common overall (Figure 7). The comparison between years showed that in

2024, the community also presents a statistically significant association in relation to 2021 (IndVal = 0.787, p = 0.005). Typical species from more consolidated sediments, such as *S. plana*, showed a more balanced distribution among the main taxa, and a greater increase in number of species was observed in 2024, resulting in a taxonomically richer community that contributes to greater stability of the community structure.

(FIGURE 6)

(FIGURE 7)

4. DISCUSSION

The results obtained in this study highlight the clear relationship between habitat structure and macrofauna composition along the restored, well-preserved, and altered gradient. The conservation status and quality of these habitats is a determining factor for the ecological functionality of these ecosystems (Heck et al., 2003). The degradation and recovery processes of meadows habitats result in significant changes in the associated biodiversity, relating these factors as indicators of habitat health status (Waycott et al., 2009). The significant differences observed in salinity, dissolved oxygen, and biomass of *Z. noltii* showed that these factors are directly associated with the ecological functionality of the habitat, confirming that vegetated areas tend to maintain environmental conditions favorable to macrofauna development, as also observed by Sousa et al. (2019) and Crespo et al. (2023). While the effect of sediment granulometry, namely grain size coefficient of variation, on benthic taxonomic richness is well documented (Coblentz *et al*. 2015), the absence of marked differences in this parameter (as well as other such as pH) suggests that the ecological recovery observed in restored areas is more related to biological and biophysical improvements than to substrate modification, a pattern observed by Basyuni et al. (2021).

The structure of the benthic community reflected this gradient of ecological habitat status. The dominance of some taxa in habitats with greater coverage of *Z. noltii* indicates these species as positive indicators of stability and productivity in seagrass meadows and intertidal zones (Dong et al., 2021; Gamito & Furtado, 2009). On the other hand, the low richness and diversity in altered areas evidence the structural impoverishment and loss of trophic complexity typical of degraded ecosystems, in which only generalist and tolerant species persist (Morrisey et al., 2010; Simboura & Reizopoulou, 2008).

The restored areas showed Shannon diversity and Pielou's evenness values similar to those of the preserved areas. These patterns indicate a community undergoing restructuring, with increased uniformity and stability in population composition. The higher the diversity and evenness values, the more balanced and functional the habitats are (Rodill et al., 2021). Furthermore, the vegetation cover of *Z. noltii* influences the greater species richness, showing the importance of structured habitat in maintaining communities (Gintowt et al., 2025). The presence of a more structured vegetation cover favors the abundance and diversity of benthic macrofauna compared to non-vegetated or disturbed areas (Colvin & Snelgrove, 2025).

These patterns indicate a community in the process of restructuring, with an increase in the uniformity and stability of the populations. Similar results were observed by Basyuni et al. (2021) and Sousa et al. (2019), who describe gradual increases in benthic diversity and biomass throughout the natural recovery of the vegetation. The presence of species characteristic of more consolidated sediments, such as *S. plana*, and the relative increase in deposit-feeding mollusks reinforce that benthic recolonization accompanies the structural maturation of the seagrass meadows.

The significant differences between areas for the main species (*S. plana*, *P. ulvae*,; *H. diversicolor*; *C. multisetosum*,) indicate that thestatus of the habitat influences the distribution and relative abundance of the dominant species. In addition, small seasonal variations observed for *I. chelipes* and *C. maenas* reflect natural fluctuations in temperature and food availability, without altering the general spatial pattern. The clear separation of samples in the nMDS graph confirms a strong differentiation in community structure between altered and vegetated areas, while restored and preserved areas showed greater overlap, suggesting ecological convergence, indicator of success in restoration processes (Crespo et al., 2023; Basyuni et al., 2021).

The indicator species test reinforced that dominant species exhibit greater fidelity and specificity to seagrass meadows, acting as indicators of functional habitat recovery. The applied temporal analysis showed that five species were common among the restored areas between 2021 and 2024. In 2024, the number of species increased to 11. The IndVal indicates that, in 2024, there is a pattern of maintenance of key species accompanied by an increase in diversity and community stability, a phenomenon recognized as a sign of progressive ecological success in restoration projects (Sousa et al., 2022; Crespo et al., 2023; Gamito & Furtado, 2009).

Our study observed that the structural and functional recovery of the restored areas follows a trajectory of ecological convergence towards the state of preserved areas, supported by physicochemical improvements, increased biomass of *Z. noltii*, and rebalancing of the benthic community. The combination of multiple metrics, diversity indices, and multivariate analyses has proven effective in detecting benthic macrofauna as an indicator of the effectiveness of habitat restoration processes in coastal areas of tropical and temperate ecosystems (Basyuni et al., 2021; Dong et al., 2021; Sousa et al., 2022; Crespo et al., 2023).

5. CONCLUSIONS

This study addressed the question of how spatial and temporal gradients shape the structure, diversity, and composition of benthic macrofauna, as well as the extent to which restored seagrass meadows exhibit an ecological trend toward preserved conditions as environmental quality and seagrass biomass improve.

The diversity indices and community composition revealed patterns of ecological convergence, with restored areas exhibiting diversity and evenness values similar to those of preserved areas, indicating a progressive reorganization of populations, associated with improved habitat structure and increased vegetation biomass.

These results indicate that restoration efforts are promoting conditions that support a more balanced and diverse benthic community, expressed by taxonomic diversity, which showed improvements between 2021 and 2024, indicating a continuous process of ecological recovery associated with increased biomass and structural maturation of *Z. noltii* vegetation.

Taken together, results demonstrate that benthic macrofauna is a sensitive and functional indicator of the effectiveness of coastal restoration actions, and a suitable tool to monitor the ecological evolution of seagrass meadows over time.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT to improve the language and readability of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research has received funding from the project RESTORE4Cs - Modelling RESTORation of wEtlands for Carbon pathways, Climate Change mitigation and adaptation, ecosystem services, and biodiversity, Co-benefits (DOI: 10.3030/101056782), co-funded by the European Union under the Horizon Europe research and innovation programme (Grant Agreement ID: 101056782). The views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union or the granting authority. Neither the European Union nor the granting authority can be held responsible for them.

Acknowledgements

UAveiro team acknowledge the support of CESAM-Centro de Estudos do Ambiente e do Mar, funded by national funds through FCT - Fundação para a Ciência e a Tecnologia I.P., under the project references UID/50017/2025 (doi.org/10.54499/UID/50017/2025) and LA/P/0094/2020 (doi.org/10.54499/LA/P/0094/2020)

6. REFERENCES

Basyuni, M., Sulistiyono, N., Fitri, A., Wati, R., Slamet, B., & Koshiba, T. (2021). Macrozoobenthic community assemblage as key indicator for mangrove restoration. Restoration Ecology, 29(4), e13352. https://doi.org/10.1111/rec.13352

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., & Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. Ecological Monographs, 81(2), 169–193.

Bell, S. S., Tewfik, A., Hall, M. O., & Fonseca, M. S. (2008). Evaluation of seagrass planting and monitoring techniques: Implications for assessing restoration success and habitat equivalency. Restoration Ecology, 16(3), 407–416.

Blanchet, H., de Montaudouin, X., Lucas, A., & Chardy, P. (2004). Heterogeneity of macrozoobenthic assemblages within a Zostera noltii seagrass bed: Diversity, abundance, and productivity. Estuarine, Coastal and Shelf Science, 61(1), 111–123.

Bueno-Pardo, J., Almeida, C., Costa, J. L., & Chainho, P. (2018). Mercury contamination in the Ria de Aveiro lagoon (Portugal): A review. Marine Pollution Bulletin, 127, 390–403.

Cardoso, P. G., Pardal, M. Â., Lillebø, A. I., Ferreira, S. M., Raffaelli, D., & Marques, J. C. (2007). Resilience of Hydrobia ulvae populations to anthropogenic and natural disturbances. Marine Ecology Progress Series, 289, 191–199.

Charpentier, A., Grillas, P., & Lescuyer, F. (2005). Spatio-temporal dynamics of submerged macrophyte populations in temporary Mediterranean wetlands. Aquatic Botany, 83(3), 163–174.

Coblentz, K. E., Henkel, J. R., Sigel, B. J., & Taylor, C. M. (2015). The use of laser diffraction particle size analyzers for inference on infauna-sediment relationships. Estuaries and Coasts, 38, 699-702.

Colvin, T.J. and Snelgrove, P.V.R. (2025) Role of Seagrass Physical Structure in Macrofaunal Biodiversity-Ecosystem Functioning Relationships. Marine Ecology Progress Series, 754, 35-49. https://doi.org/10.3354/meps14774

Costa, J. L., Chainho, P., Almeida, C., & Domingos, I. (2022). Seagrass restoration in Europe: Challenges and opportunities. Frontiers in Marine Science, 9, 1–15.

Crespo, D., Dolbeth, M., Leston, S., Sousa, R., Pardal, M. Â., & Marques, J. C. (2017). Distribution of benthic macrofauna in estuarine seagrass meadows: The influence of habitat, season, and environmental conditions. Marine Environmental Research, 131, 1–14.

Crespo, D., Dolbeth, M., Leston, S., Sousa, R., & Pardal, M. A. (2023). Restoration of seagrass beds enhances macrofaunal communities in estuarine ecosystems. Estuarine, Coastal and Shelf Science, 284, 108412. https://doi.org/10.1016/j.ecss.2023.108412

de los Santos, C. B., Krause-Jensen, D., Alcoverro, T., Marbà, N., Duarte, C. M., van Katwijk, M. M., & Pérez, M. (2019). Recent trend reversal for declining European seagrass meadows. Nature Communications, 10(1), 1–8.

Dolbeth, M., Cardoso, P. G., Ferreira, S. M., Verdelhos, T., & Pardal, M. Â. (2011). Long-term changes in the production by estuarine macrobenthos affected by multiple stressors. Estuarine, Coastal and Shelf Science, 92(1), 10–18.

Duarte, C. M. (2002). The future of seagrass meadows. Environmental Conservation, 29(2), 192–206.

Fradoca R, Oliveira VH, Fonte BA, Sousa AI, Marques B, Lillebø AI, Coelho JP (2025) The effect of Zostera noltei recolonization on N and P fluxes at the sediment/water interface. Marine Pollution Bulletin 215:117901. doi:https://doi.org/10.1016/j.marpolbul.2025.117901

FOLK R. L.; WARD W. C. Brazos River bar: a study in the significance of grain size parameters. Journal of Sedimentary Petrology, v. 27, n.1, p. 3-26. 1957.

Greiner, J. T., McGlathery, K. J., Gunnell, J., & McKee, B. A. (2013). Seagrass restoration enhances "blue carbon" sequestration in coastal waters. PLoS ONE, 8(8), e72469.

Gintowt, N.A.; Kendzierska, H.; Janas, U. Seasonal Dynamics of Benthic Infauna Communities in Zostera marina Meadows: Effects of Plant Density Gradients. Biology 2025, 14, 153. https://doi.org/10.3390/biology14020153.

Gräfnings, M., Infantes, E., & Moksnes, P. O. (2024). Seagrass restoration supports the recovery of ecosystem functions and biodiversity. Marine Environmental Research, 196, 106312. https://doi.org/10.1016/j.marenvres.2023.106312

Heck Jr KL, Hays G, Orth RJ (2003) Critical evaluation of the nursery role hypothesisfor seagrass meadows. Marine Ecology Progress Series 253: 123–136. https://doi.org/10.3354/meps253123.

Hughes, A. R., Williams, S. L., Duarte, C. M., Heck, K. L., & Waycott, M. (2018). Associations of concern: Declining seagrasses and threatened dependent species. Frontiers in Ecology and the Environment, 6(5), 242–246.

INSTITUTE for Nature Conservation and Forests (Portugal). Ria de Aveiro Special Protection Zone (PTZPE0004). Lisbon: ICNF, 2020. Available at: https://eunis.eea.europa.eu/sites/PTZPE0004. Accessed on: October 8, 2025.

Janousek, C. N., Folger, C. L., & Blum, L. K. (2007). Plant–soil interactions in a tidal freshwater marsh: implications for community structure and nutrient dynamics. Wetlands, 27(1), 200–214. https://doi.org/10.1672/0277-5212(2007)27[200:PIIATF]2.0.CO;2

Koch, M. S., Bowes, G., Ross, C., & Zhang, X. H. (2013). Climate change and seagrass: The interactive effects of light and temperature on seagrass photosynthesis. Journal of Experimental Marine Biology and Ecology, 441, 28–36.

Krause-Jensen, D., Greve, T. M., & Nielsen, K. (2005). Eelgrass as a bioindicator under the European Water Framework Directive. Water Resources Management, 19(1), 63–75.

Macreadie, P. I., Costa, M. D. P., Atwood, T. B., Friess, D. A., Kelleway, J. J., Kennedy, H., & Duarte, C. M. (2019). Blue carbon as a natural climate solution. Nature Reviews Earth & Environment, 1(1), 1–12.

Magurran, A. E. (2013). Measuring biological diversity. John Wiley & Sons.
Marín, C., van Katwijk, M. M., Bouma, T. J., van der Heide, T., & Orth, R. J. (2024). Pathways to recovery: Seagrass restoration in the Wadden Sea. Journal of Applied Ecology, 61(2), 230–242. https://doi.org/10.1111/1365-2664.14570

Massa, S. I., Paulino, C. M., Serrao, E. A., & Duarte, C. M. (2009). Temperature tolerance and survival of seagrass populations under extreme heat stress. Marine Ecology Progress Series, 390, 67–75.

Neumann, B., Vafeidis, A. T., Zimmermann, J., & Nicholls, R. J. (2015). Future coastal population growth and exposure to sea-level rise and coastal flooding: A global assessment. PLoS ONE, 10(3), e0118571.

Oliveira VH, Fonte BA, Costa F, Sousa AI, Henriques B, Pereira E, Dolbeth M, Díez S, Coelho JP (2023) The effect of Zostera noltei recolonization on the sediment mercury vertical profiles of a recovering coastal lagoon. Chemosphere 345. doi:10.1016/j.chemosphere.2023.140438

Oliveira, M., Almeida, C., & Chainho, P. (2022). Mercury contamination in sediments and its effects on benthic communities in the Ria de Aveiro. Marine Pollution Bulletin, 174, 113–123.

Rodil, I.F., Lohrer, A.M., Attard, K.M. *et al.* Macrofauna communities across a seascape of seagrass meadows: environmental drivers, biodiversity patterns and conservation implications. *Biodivers Conserv* 30, 3023–3043 (2021). https://doi.org/10.1007/s10531-021-02234-3

Rezek, R. J., Furman, B. T., Jung, R. P., & Heck, K. L. (2019). Seagrass restoration success depends on site characteristics and planting protocols: A meta-analysis. Marine Ecology Progress Series, 620, 1–17.

Serrano, O., Lavery, P. S., Duarte, C. M., & Kendrick, G. A. (2019). Seagrass sediments reveal the long-term role of ecosystem engineers in blue carbon storage. Global Change Biology, 25(2), 419–432.

Sheaves, M., Baker, R., Nagelkerken, I., & Connolly, R. M. (2021). Nature-based solutions in coastal restoration: The role of biodiversity in ecosystem recovery. Frontiers in Marine Science, 8, 1–12.

Short, F. T., Polidoro, B., Livingstone, S. R., Carpenter, K. E., Bandeira, S., Bujang, J. S., & Zieman, J. C. (2017). Extinction risk assessment of the world's seagrass species. Biological Conservation, 144(7), 1961–1971.

Sousa, A. I., Lillebø, A. I., Pardal, M. Â., & Marques, J. C. (2017). Resilience of *Zostera noltei* meadows to environmental changes: A review. Marine Pollution Bulletin, 114(1), 5–15.

UNEP-WCMC and IUCN (2020). *Marine Protected Planet: [The World Database on Protected Areas (WDPA)].* Cambridge: UNEP-WCMC.

van Katwijk, M. M., Thorhaug, A., Marbà, N., Orth, R. J., Duarte, C. M., Kendrick, G. A., & Althuizen, I. H. (2016). Global analysis of seagrass restoration: The importance of large-scale planting. Journal of Applied Ecology, 53(2), 567–578.

Vermaat, J. E., Verhagen, F. C. A., & Lindenburg, D. (1996). Photosynthesis, light and carbon balance in a Zostera noltei meadow. Marine Ecology Progress Series, 142, 253–261.

Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., & Watson, R. (2006). Impacts of biodiversity loss on ocean ecosystem services. Science, 314(5800), 787–790.

Society for Ecological Restoration International Science & Policy Working Group (SERI). (2004). The SER International Primer on Ecological Restoration. Tucson, Arizona: Society for Ecological Restoration International.

Suding, K. N., Higgs, E., Palmer, M., Callicott, J. B., Anderson, C. B., Baker, M., … & Schwartz, K. Z. S. (2015). Committing to ecological restoration. Science, 348(6235), 638–640. https://doi.org/10.1126/science.aaa4216

Waycott, M., Duarte, C. M., Carruthers, T. J. B., Orth, R. J.,Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean,J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A.,Kenworthy, W. J., Short, F. T., & Williams, S. L. (2009).Accelerating loss of seagrasses across the globe threatenscoastal ecosystems. Proceedings of the National Academyof Sciences, 106(30), 12377–12381. https://doi.org/10.1073/pnas.0905620106

Ye, C., Zhang, L., Wang, Y., Li, C., & Zhou, J. (2024). Rapid response of benthic macroinvertebrates to Spartina alterniflora removal in a subtropical estuary. Data in Brief, 56, 110788. https://doi.org/10.1016/j.dib.2024.110788

Zipperle, A. M., Cabaço, S., & Santos, R. (2009). Seagrass resilience to disturbance: The role of rhizome storage and seed banks. Marine Ecology Progress Series, 377, 93–103.

Figure Captions

Figure 1. Location map of the Ria de Aveiro coastal lagoon, Portugal. Highlighted are the sampling sites categorized into three habitat types: well-preserved areas (Well Preserved 1 and Well Preserved 2), restored areas (Restored 1 and Restored 2), and altered areas (Altered 1 and Altered 2).

Figure 2. Variation of the physicochemical variables of water and seagrass biomass in the studied areas. The graphs show the means and standard errors for temperature (T), salinity, dissolved oxygen (DO), pH, and biomass of *Z. noltii* ($g \cdot m^{-2}$).

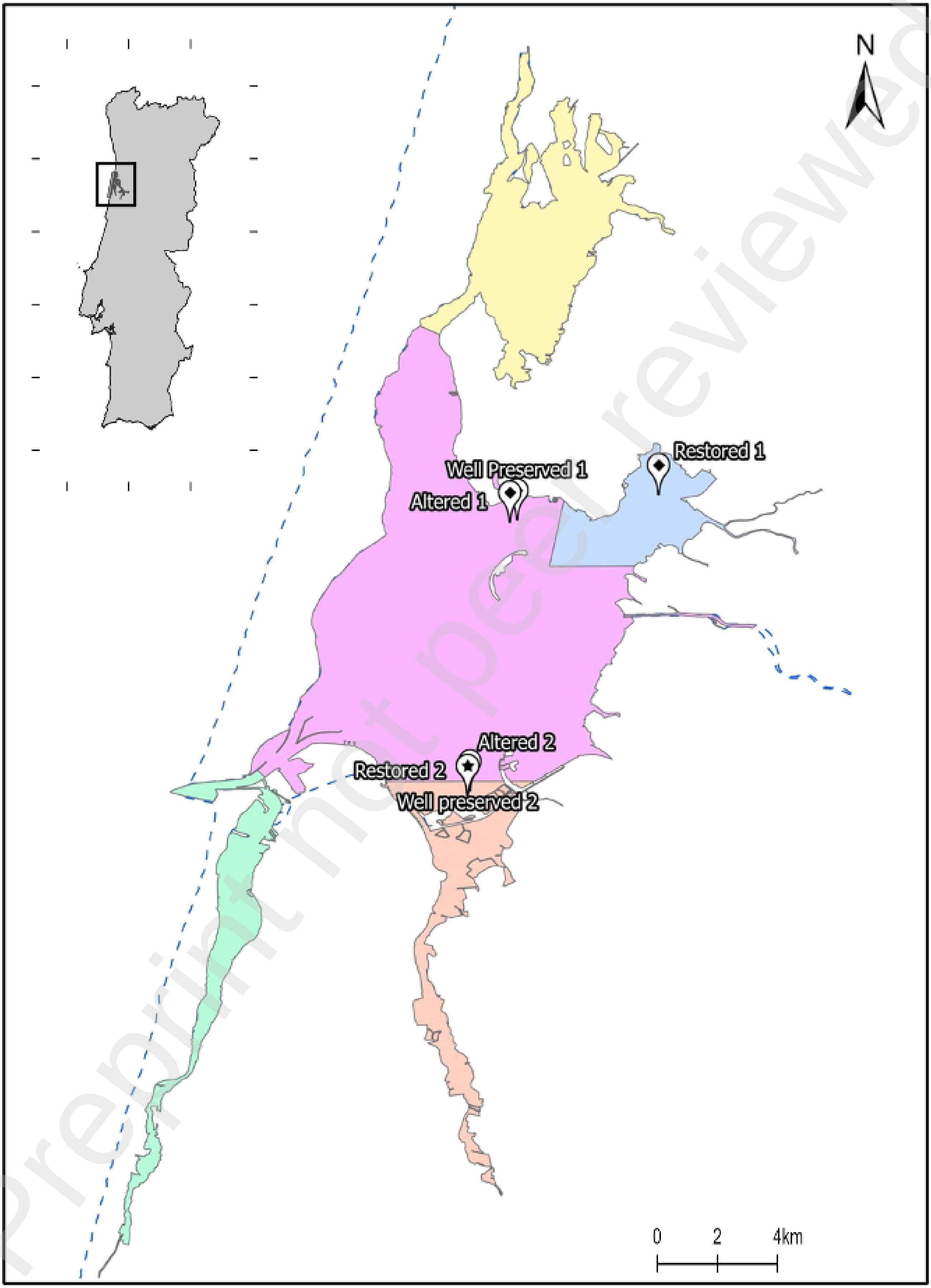
Figure 3. Granulometric characteristics of sediment in sampling sites. The graphs present the mean and standard error for the D50, D10, and D90 fractions (µm), skewness, and standard deviation (StDev), which represent the distribution and heterogeneity of sediment grains.

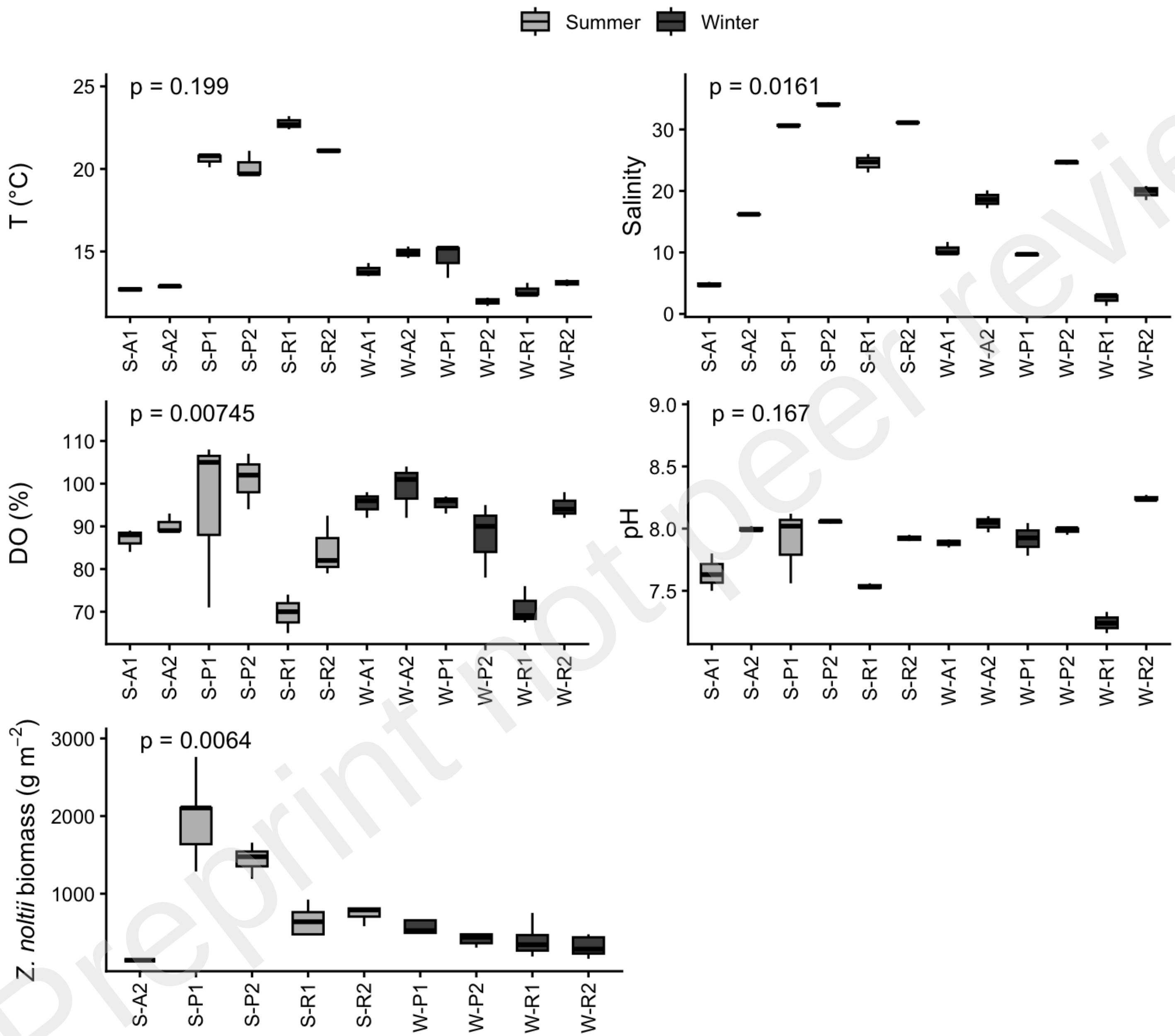
Figure 4. Diversity and biomass indices by area and season of macrozoobentofauna for three health status areas (Altered, Preserved, Restored) and two seasons (Summer, Winter). Panels: Pielou's evenness (J′), Species richness (S), Shannon (H′), Simpson (1–D), and Biomass ($g\ m^{-2}$) (AFDW - Ash Free Dry Weight). Sample sizes (N): Altered (n = 8 summer; 7 winter), Preserved (n = 10; 10), and Restored (n = 10; 10).

Figure 5. Similarity and composition of the benthic macroinvertebrate community in *Zostera noltii* seagrass areas. Figure (A) The hierarchical clustering dendrogram (Bray–Curtis coefficient) shows the degree of similarity between samples based on species composition. Figure (B), non-metric ordination analysis (nMDS) presents the distribution of samples in two dimensions (MDS1 and MDS2), representing the differences in community composition between areas and seasons.

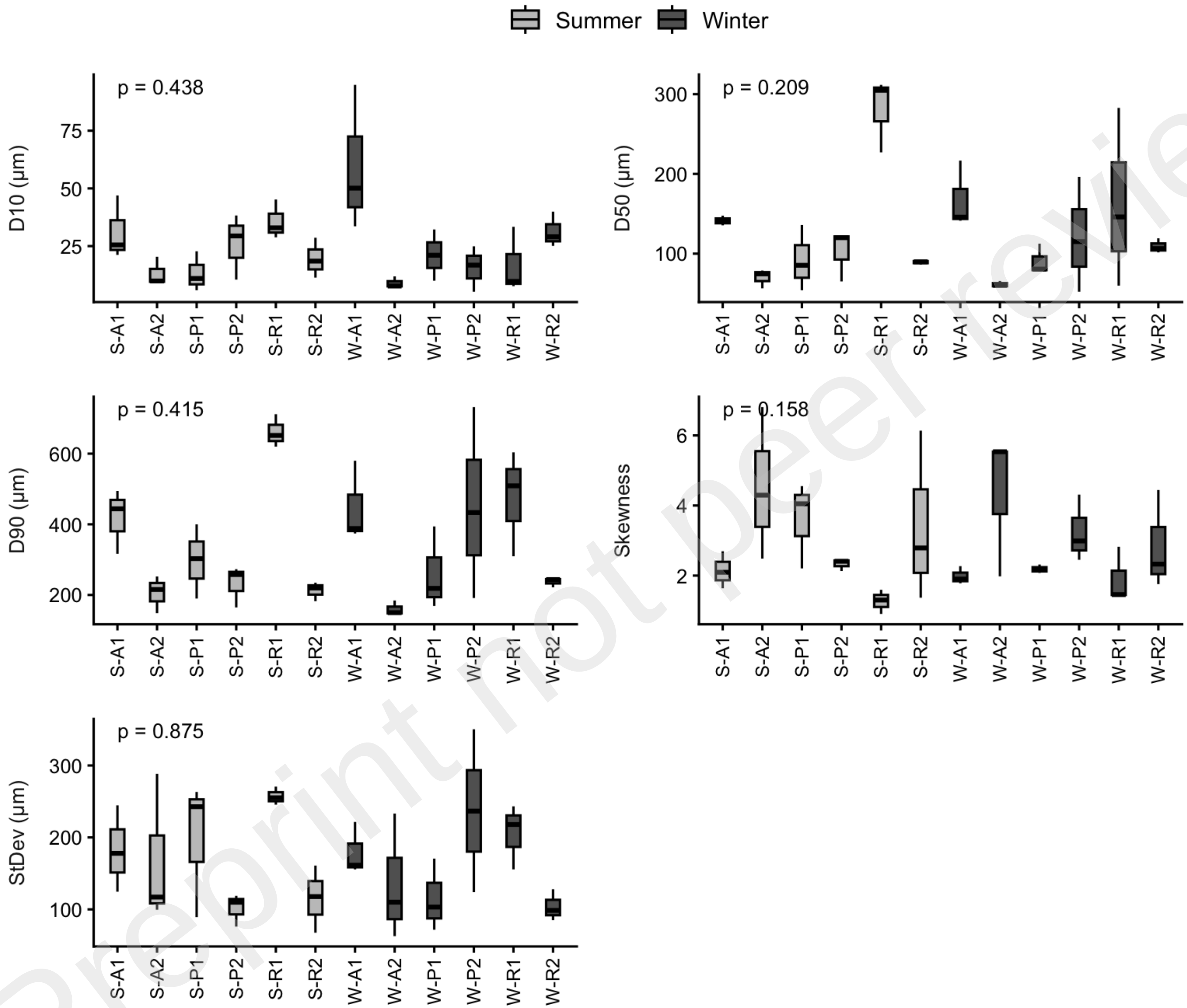
Figure *6*. Relative composition of the benthic macroinvertebrate community associated with *Zostera noltii* seagrass areas, between winter (JAN) and summer (JUL) 2024. (A) Is the relative proportion of abundance among all recorded species and (B) shows only the indicator species identified by the IndValtest. The abbreviations correspond to the species: SP = Scrobicularia plana, CE = *Cerastoderma edule*, CC = *Cyathura carinata*, PU = *Peringia ulvae*, BR = *Bittium reticulatum*, PL = *Phorcus lineatus*, HD = *Hediste diversicolor*, CM = *Corophium multisetosum*, CO = *Calliostoma occidentale*, CMA = *Carcinusmaenas* and IC = *Idotea chelipes*.

Figure 7. Relative composition of the benthic macroinvertebrate community associated with restored Zostera noltii areas compared between 2021 and 2024. (A) Is the relative proportion of abundance among all species recorded in the samples and (B) shows only the indicator species identified by the IndValtest. The abbreviations correspond to the species: CMA = *Carcinus maenas*, CC = *C. carinata*, HD = *Hediste diversicolor*, PU = Pe*ringia ulvae,* SP = *Scrobicularia plana.*

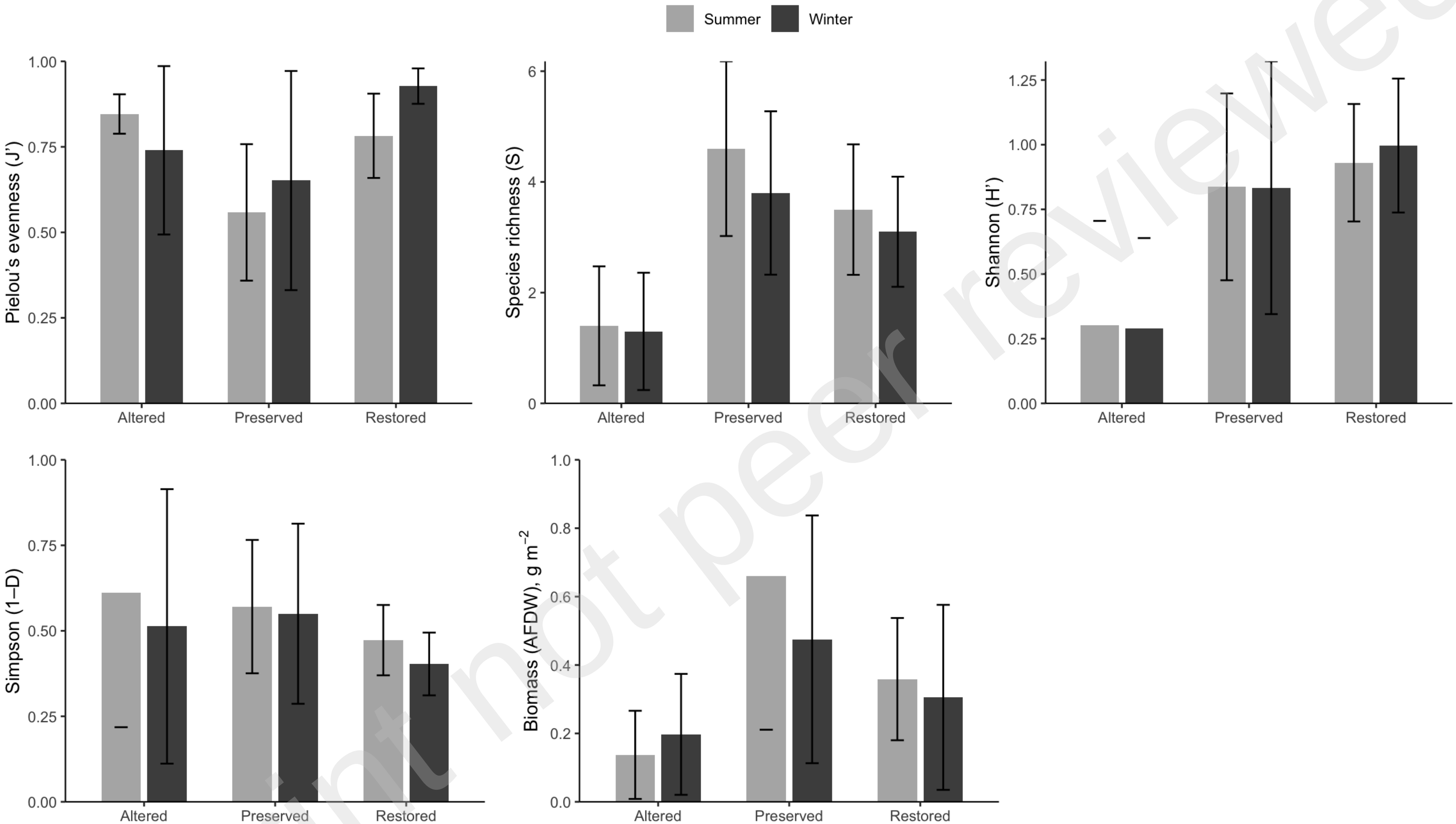

N
Well Preserved 1
Altered 1
Restored 1
Altered 2
Restored 2
Well preserved 2
0
2
4km



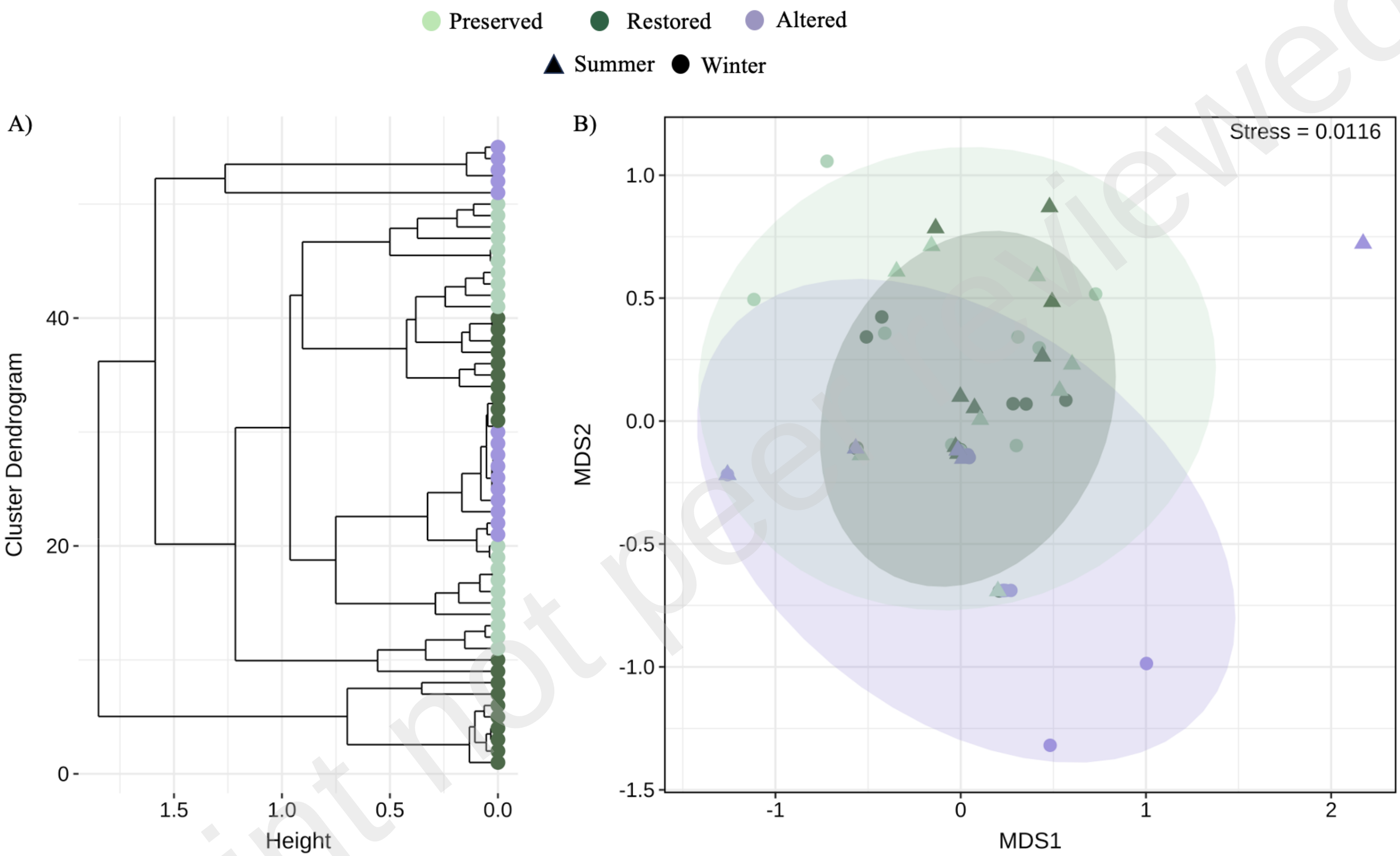
Summer
Winter
p = 0.199
T (°C)
p = 0.0161
Salinity
p = 0.00745
DO (%)
p = 0.167
pH
p = 0.0064
Z. noltii biomass (g m^{-2})
S-A1 S-A2 S-P1 S-P2 S-R1 S-R2 W-A1 W-A2 W-P1 W-P2 W-R1 W-R2



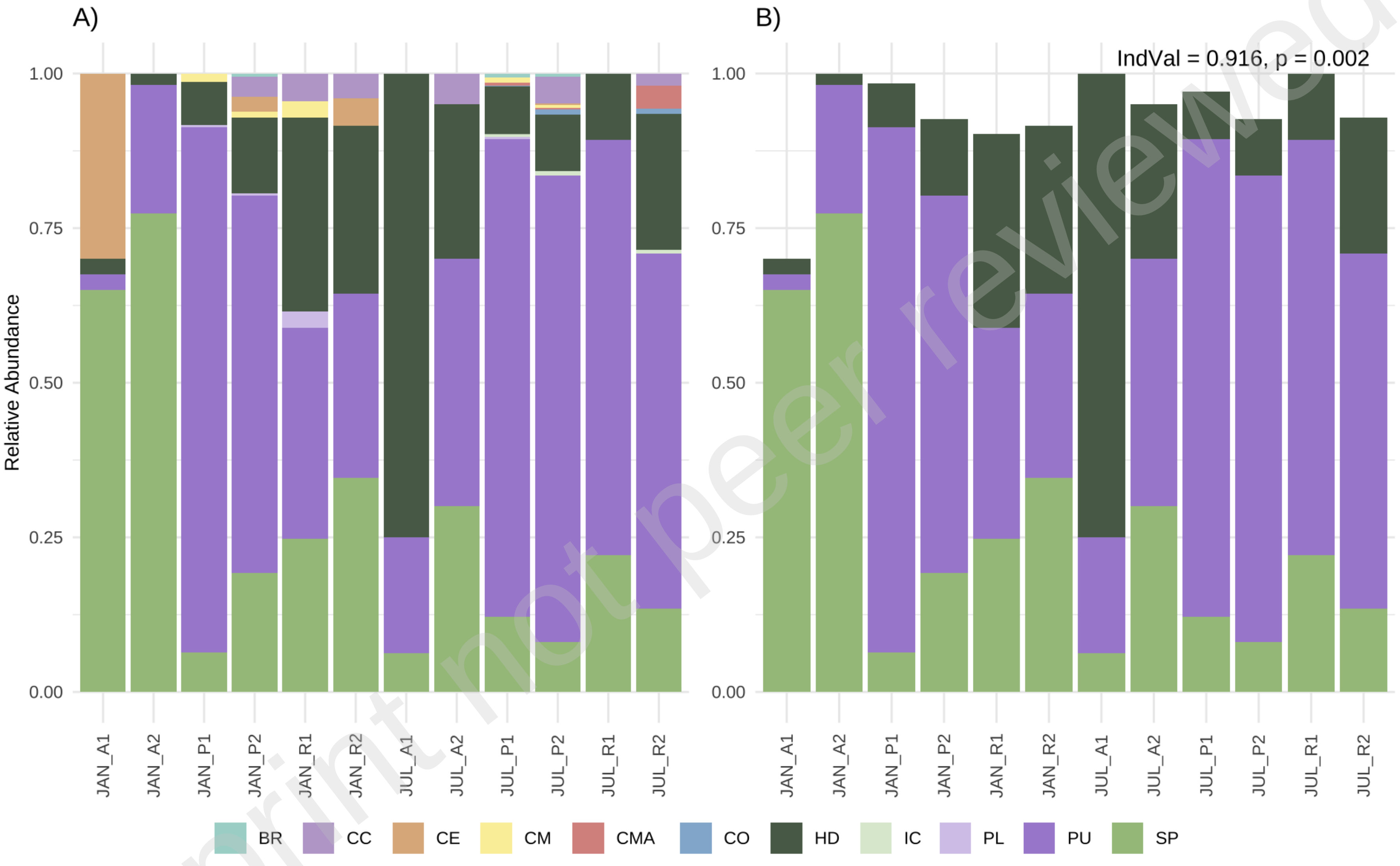
Summer
Winter
p = 0.438
D10 (µm)
p = 0.209
D50 (µm)
p = 0.415
D90 (µm)
p = 0.158
Skewness
p = 0.875
StDev (µm)
S-A1
S-A2
S-P1
S-P2
S-R1
S-R2
W-A1
W-A2
W-P1
W-P2
W-R1
W-R2

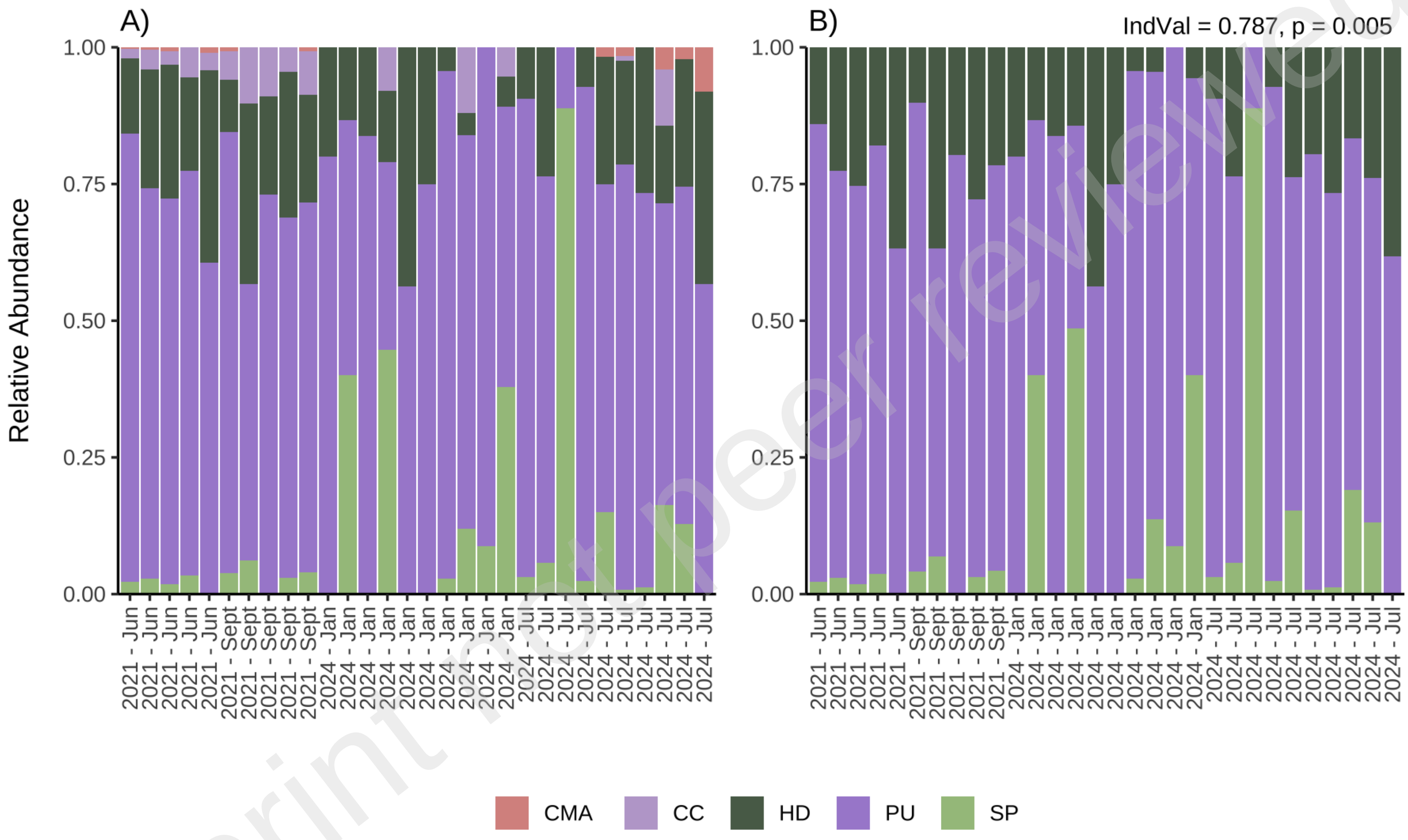


Summer
Winter
Pielou's evenness (J')
Species richness (S)
Shannon (H')
Simpson (1−D)
Biomass (AFDW), g m−2
Altered
Preserved
Restored



Preserved
Restored
Altered
Summer
Winter
A)
Cluster Dendrogram
Height
B)
Stress = 0.0116
MDS1
MDS2


A)
B)
IndVal = 0.916, p = 0.002
Relative Abundance
1.00
0.75
0.50
0.25
0.00
JAN_A1
JAN_A2
JAN_P1
JAN_P2
JAN_R1
JAN_R2
JUL_A1
JUL_A2
JUL_P1
JUL_P2
JUL_R1
JUL_R2
BR
CC
CE
CM
CMA
CO
HD
IC
PL
PU
SP


A)
B)
IndVal = 0.787, p = 0.005
Relative Abundance
1.00
0.75
0.50
0.25
0.00
CMA
CC
HD
PU
SP