Marine Pollution Bulletin 223 (2026) 119036

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Physiological and biochemical indicators of *Posidonia oceanica* transplantation success

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ARTICLE INFO

Keywords:
Seagrass
Restoration
Nutrients
Physiology
Photosynthetic activity
Carbohydrates

ABSTRACT

Evaluating the success of *Posidonia oceanica* transplantation is challenging due to the species' slow growth and delayed structural responses. This three-year study in Calvi Bay (Corsica) examined how transplantation method (iron staples, coconut fiber mats, BESE elements), donor source (donor meadow vs. storm-fragments), transplantation depth (20 m vs. 28 m) and time post transplantation influence the physiological and biochemical parameters of transplanted cuttings. Plant responses were assessed through photosynthetic activity, leaf elemental concentrations (C, N, P, S), and rhizome carbohydrate reserves. Transplanting depth and transplantation method had limited effects on the measured parameters. The transplanting method, influencing root development, suggests distinct strategies for resource acquisition without altering physiological parameters. In contrast, donor source emerged as the main driver of variability: cuttings from donor meadows consistently showed higher nitrogen and phosphorus concentrations, lower C:N ratios, and faster convergence towards natural meadow trait profiles than storm-fragments. Multivariate analyses revealed early convergence between donor meadow cuttings and natural meadows, whereas storm-fragments remained distinct. By 36 months, both donor types again diverged from reference conditions. These results demonstrate the value of trait-based approaches, particularly eco-physiological indicators, as sensitive, early measures of transplantation success, complementing traditional structural metrics. We recommend including C, N, P, and starch concentrations as key biochemical indicators in restoration monitoring programs, as they provide integrative and early signals of seagrass metabolic status and recovery potential. Full convergence with reference meadows appears to be a long-term process, emphasizing the importance of extended monitoring and careful donor selection to improve seagrass restoration outcomes.

1. Introduction

Seagrasses form underwater meadows in the photic zones of temperate and tropical coastlines and are widely recognized as foundational habitat-forming species (Den Hartog and Kuo, 2006; Larkum et al., 2006). These highly diverse and productive ecosystems (Vieira et al., 2024) fulfil important services such as carbon sequestration (Fourqurean et al., 2012) and protection against coastal erosion (Ganthy et al., 2015). Seagrasses display considerable variability in morphology and life-history traits. Some species, such as Cymodoceaceae, produce short-lived shoots with rapid growth and decay cycles, whereas others, like Posidoniaceae, are slow-growing and long-lived (Larkum et al., 2006). Despite their polyphyletic origins and morphological diversity, all seagrasses share a suite of adaptations to the marine environment (Larkum et al., 2006; Jackson et al., 2009). Among these, their photosynthetic machinery exhibits remarkable plasticity, enabling acclimation to variable light conditions, providing protection from photoinhibition in clear tropical waters and enhancing light capture in dimmer temperate environments (Cummings and Zimmerman, 2003; Ralph et al., 2002). However, seagrasses require light intensities 10–20 times higher than many marine autotrophs, making them particularly vulnerable to habitat disturbances, often driven by human activity

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https://doi.org/10.1016/j.marpolbul.2025.119036
Received 15 October 2025; Received in revised form 6 November 2025; Accepted 19 November 2025

(Duarte, 1991; Short and Wyllie-Echeverria, 1996). Over recent decades, seagrass meadows have faced extensive declines (Waycott et al., 2009). Key drivers include sediment and nutrient runoff, coastal development, marine heatwaves, dredging, trawling, anchoring, and disease (Turschwell et al., 2021). The degradation and loss of seagrass meadows undermine the vital ecosystem services they provide, prompting increasing global effort to conserve and restore them (Unsworth et al., 2022, 2024). Ecological restoration is defined as the process of intentionally assisting the recovery of a degraded or destroyed ecosystem (SER, 2004), and is now widely implemented as a management tool to promote recovery of impacted seagrass meadows, and safeguard ecosystem functions and services (Descamp et al., 2025; Rezek et al., 2019; van Katwijk et al., 2016).

Assessing restoration success, however, remains challenging. Various ecological, physiological, and biochemical parameters have been used to assess the progress and success of seagrass restoration. More recently, soundscape analysis has emerged as a promising complementary tool to assess the early success of marine habitat restoration, including seagrass meadows, coral reefs, sponge-dominated habitats, and oyster reefs (Butler et al., 2016; Lamont et al., 2022; La Manna et al., 2024). Monitoring has traditionally relied on simple metrics such as transplant survival, but this binary measure provides limited insight into plant health or functional recovery. Comparisons with reference meadows are rarely included, and changes in shoot condition are often overlooked (Pansini et al., 2022). Moreover structural indicators (e.g., shoot density, biomass) often fail to effectively monitor recovery processes after disturbances or restoration actions, especially for larger, slow-growing seagrass species like *Enhalus* or *Posidonia* spp. (Marbà and Duarte, 1998; Roca et al., 2016). In contrast, physiological and biochemical indicators can reveal stress responses and functional adjustments at earlier stages, offering sensitive tools for evaluating restoration outcomes (Cooke and Suski, 2008; Roca et al., 2016). These metrics capture the organism's regulatory capacity to cope with new environmental conditions, critical for both degradation and restoration contexts (Adolph, 1956; Horn et al., 2009), and can guide donor selection by identifying populations best suited for transplantation (Cooke and Suski, 2008).

Here, *Posidonia oceanica* (L.) Delile, a slow-growing, Mediterranean-endemic seagrass characterized by high morphological and physiological plasticity (Hemminga and Duarte, 2000), was used as a model species to evaluate the role of physiological and biochemical traits in transplantation methods and donor sources performance. The availability of donor material for transplanting is one of the main constraint in *P. oceanica* restoration endeavours. The use of germinated seedlings from floating or beach-stranded seeds is challenging (Mancini et al., 2024; Sutera et al., 2024), as episodes of mass flowering are unpredictable, irregular in space and time (Diaz-Almela et al., 2006; Marín-Guirao et al., 2019; Montefalcone et al., 2013; Stipcich et al., 2024a, 2024b). Another option is using fragments of *P. oceanica* rhizomes either extracted from donor meadows, which is a destructive harvesting impacting natural meadows, or collected from naturally detached fragments of unknown origin. A significant amount of seagrass fragments (later on referred to as storm-fragments) are dislodged during storms and accumulate in natural storage areas (Abadie et al., 2015; Boulenger et al., 2025a). Although storm-fragments provide a good opportunity as donor material for transplantation while minimizing the impact on the surrounding natural meadows, there remain uncertainties in the performance of those fragments compared to cuttings manually excised from healthy meadows. Indeed, as the origin and life-history of the storm-fragments are unknown, their ability to survive for extended periods of time is uncertain (Balestri et al., 2011). Moreover, shading and sediment deposition, as well as the lack of belowground nutrients absorption by the roots (Lepoint et al., 2004) could conversely affect their growth rates, metabolism and carbohydrates storage (Lai et al., 2020; Kraemer and Alberte, 1995).

In this study, transplantation trials were conducted in *P. oceanica* dead matte resulting from meadows degraded by boat anchoring, testing multiple transplantation methods and donor sources at two different depths. Three transplantation methods were tested: individual fixation (iron staples), soft three-dimensional structures (coconut fiber mats), and rigid three-dimensional structures (BESE elements). As recommended by Roca et al. (2016), a multi-trait approach combining indicators of photosynthetic activity (Fv/Fm, α, rETRmax, Ek), leaf elemental compositions (C, N, S, P), and rhizome carbohydrates reserves (sucrose, starch, total carbohydrates) was applied. The study aimed to: (1) assess the influence of transplantation method, donor origin, and transplantation depth on physiological and biochemical traits of *P. oceanica* cuttings; (2) determine whether certain donor sources develop trait profiles resembling those of reference meadows and evaluate the timescales over which such convergence occurs.

2. Material and methods

2.1. Study area and seagrass transplantation

This study was carried out between May 2022 and May 2025 in Alga Bay, a sub-bay of Calvi Bay (8°43′52″ E; 42°34′20″ N) located in front of the STARESO oceanographic research station (Calvi, NW Corsica, France). The bay hosts an extensive *P. oceanica* meadow, covering approximately 0.78 km^2 and extending from 3 to 37 m in depth (Abadie et al., 2016). Intensive anchoring activity has caused significant seagrass decline (Fullgrabe et al., 2022) and limited natural recolonisation (Boulenger et al., 2025a), prompting restoration efforts. Dead matte patches (average size of 191.5 m^2) were selected as experimental sites; three at 20 m and four at 28 m depth. In spring 2022, a total of 693 *P. oceanica* fragments (i.e., a living plagiotropic rhizome with a couple of orthotropic shoots; with 99 fragments per site) were transplanted as part of a pilot restoration project designed to test transplantation methods prior to upscaling (see Boulenger et al., 2025b). Both storm-fragments and cuttings extracted from *P. oceanica* meadows were used as donor sources in this study to test their physiological and biochemical performance three years after transplantation. Among the 693 fragments, a total of 462 storm-fragments were collected by SCUBA divers near the STARESO at depths of 6–28 m, while 231 cuttings were manually harvested from the erosion edge of a natural sandy intermatte at 15 m depth (Gobert et al., 2016). Harvesting from eroding edges was chosen to minimize disturbance to intact donor meadows, as these zones naturally produce fragments when matte structure degrade (Gobert et al., 2016). Furthermore, shoots obtained from erosion edges exhibit similar photosynthetic efficiency, leaf surface area, and biomass to those from undisturbed meadows at the same depth (Abadie et al., 2017; Lapeyra et al., 2016). All harvested material was stored in outdoor flow-through seawater aquaria until biometric measurements were performed. Only cuttings with at least three shoots and a plagiotropic rhizome of at least 15 cm in length were retained, while those with severe leaf necrosis were discarded. After initial biometric measurements, selected cuttings were transplanted into the experimental sites using three different biodegradable materials: (i) iron staples, (ii) biodegradable mat in natural coconut fiber woven mesh (referred to as coconut fiber mat), and (iii) BESE elements (BESE Ecosystem Restoration Products, Culemborg, The Netherlands). BESE elements are biodegradable sheets made of potato-waste-derived Solanyl C1104M (Rodenburg Biopolymers, Oosterhout, the Netherlands) stacked together to form a 6-cm high 3D honeycomb-shaped matrix. For each biodegradable material/transplantation method at each experimental site, 33 cuttings were attached using cable ties, consisting of 22 storm-fragments and 11 intermatte cuttings.

2.2. Sampling strategy

Seven field campaigns were conducted between May 2022 (initial transplantation) and May 2025. Six post-transplantation monitoring campaigns were carried out at 3, 12, 15, 24, 27, and 36 months. These included three spring surveys (12, 24, 36 months; April–June), and three fall surveys (2, 15, 27 months; September–October). Prior to transplantation, 20 *P. oceanica* fragments were collected from nearby reference meadows at 20 and 28 m depth, along with 20 cuttings, comprising both storm-fragments and cuttings from intermattes, set aside for physiological and biochemical analyses. At each monitoring campaign, in situ survival and shoot production were assessed (see Boulenger et al., 2025b). There were no significant differences in both variables between the two donor sources 36 months after transplantation, and the overall survival rate was 67.2 % (Boulenger et al., 2025b). Considering that transplantation success is commonly defined as a survival rate of at least 50 % after three years (Danovaro et al., 2025; Molenaar and Meinesz, 1995), this project can therefore be regarded as a successful transplantation effort. Foliar shoots were sampled twice annually using the Non-Destructive Shoot sampling Method (NDSM; Gobert et al., 2020). At each site ($n = 7$), 12 foliar shoots were sampled, resulting in 84 sampled shoots per campaign, plus 10 control shoots from reference meadows at 20 m and 28 m depth. Whole cuttings (rhizome with foliar shoots) were sampled annually during spring to assess rhizome carbohydrate storage. Six rhizomes per site were sampled, along with 10 complete fragments from reference meadows at 20 and 28 m depth.

2.3. Photosynthetic activity measurements

After sampling, leaves from both transplanted and control plants were transported to the laboratory under shaded conditions. Chlorophyll *a* fluorescence analysis was used to assess photo-physiological performance, as it provides sensitive indicators of plant stress and acclimation (Gera et al., 2012; Larkum et al., 2007; Madonia et al., 2021). Photosynthetic activity was measured with a Pulse-Amplitude-Modulated (PAM) chlorophyll fluorometer, widely applied tool for seagrass health assessment (Belshe et al., 2007; Gobert et al., 2015; Madonia et al., 2021). Four parameters were recorded using a DIVING-PAM-I (Heinz Walz GmbH; hereafter referred to as PAM device): maximum photochemical quantum yield (Fv/Fm), maximum relative electron transport rate (rETRmax), photosynthetic efficiency (α), and saturation irradiance (Ek). Measurement protocols and instrument settings followed Boulenger et al. (2024).

2.4. Nutrients' concentrations in leaves, rhizomes and roots

Following photosynthetic activity measurements, epiphytes were scraped from all sampled leaves using a ceramic scalpel blade (Dauby and Poulicek, 1995). Leaves were weighed fresh, oven-dried at 60 °C for 48 h and reweighed to determine dry biomass. For phosphorus analysis, dried leaves were shredded with ceramic scissors to facilitate homogenization during the mineralization process. Approximately 100 mg of dried powders per sample was digested in Teflon bombs using a closed microwave digestion system (Ethos D, Milestone Inc.) with nitric acid-hydrogen peroxide (HNO_3/H_2O_2; suprapur grade, Merck), following Richir and Gobert (2014). Ten analytical blanks were prepared to establish detection (LD) and quantification limits (LQ). The quantity of material placed in each bomb varied between 80 and 120 mg, depending on the quantity of available dried leaves powder for each sample. Phosphorus concentrations in the samples were determined using Inductively Coupled Plasma Mass Spectrometry with the Dynamic Reaction Cell technique (ICP-MS ELAN DRC II, Perkin Elmer), following the method described by Richir and Gobert (2014). Accuracy was verified using a Certified Reference Material (GBW 07603 bush branches and leaves). The calculated LDs and LQs were based on the measurement distribution over their respective blanks, following the recommendations of Currie (1999). Remaining coarse powders were ground to fine consistency and analysed for carbon, nitrogen and sulfur concentrations with a C-N-S elemental analyser (VarioMicro, Elementar, Germany). Results are expressed in % of dry weight.

2.5. Carbohydrates content in the rhizomes

The rhizome were cleaned of scales, frozen at −20 °C, and sent to MicroPolluants Technology SA (Saint Julien Les Metz, France) for carbohydrate analysis, following the protocol described in Boulenger et al. (2024). Results are expressed as total carbohydrate reserves (TCR), sucrose and starch, with an accuracy of 1 %.

2.6. Data analysis

2.6.1. Univariate

The data analysis rationale followed the same approach as that used for *P. oceanica* transplant morphological traits in Boulenger et al. (2025b). To assess the effects of the different experimental treatments on the physiological and biochemical traits of *P. oceanica* transplants, Generalized Linear Mixed Models (GLMMs) were used. The analyses included physiological variables (Fv/Fm, α, rETRmax, Ek) and biochemical variables (C, N, P, S concentrations, C:N, C:P, N:P, sucrose, starch, and total carbohydrate reserves). Fixed factors included in the GLMMs were 'Transplantation method' (three levels: iron staple, coconut fiber mat, and BESE element), 'Donor source' (two levels: intermatte cutting and storm-fragment), 'Transplantation depth' (two levels: shallow and deep. As all traits exhibited strong temporal variability consistent with well-known seasonal dynamics, 'Months post-transplantation' was included as a random factor to account for temporal autocorrelation rather than as a fixed effect. Because the experimental sites were nested within the transplantation depth levels, resulting in a nested random structure (1|Transplantation depth/Site) + (1|Months post-transplantation). A Gamma distribution with a log link function was used for all traits. GLMMs were built using the *glm* function in RStudio software version 4.3.2 (RStudio Inc., Boston, MA, USA). Model selection was guided by Akaike's Information Criterion (AIC), progressively removing non-significant terms based on statistical criteria until no further variables could be eliminated. Overdispersion was assessed by comparing the residual deviance to the residual degrees of freedom. To test the statistical significance of differences between treatments, estimated marginal means (EMMs) were computed using the *emmeans* function in RStudio, applying Bonferroni correction to adjust *p*-values for multiple comparisons.

Univariate statistical analyses were performed to assess whether specific donor sources promote a temporal convergence of physiological and biochemical traits towards values observed in reference control meadows. Transplantation depth and transplantation method were initially included in the full design but were later excluded from the final analyses because they showed very few significant effects or interactions. Their removal simplified the model structure and allowed for a clearer interpretation of the results, focusing on the main biological drivers of interest. Given that the data did not meet the assumptions required for parametric tests, two-way permutational analyses of variance (PERMANOVA; Anderson, 2001) were performed. The PERMANOVA design included the fixed factor 'Donor source' (three levels: intermatte cutting, storm-fragment, and control meadow), and 'Months post-transplanting' (seven levels: 0, 3, 12, 15, 24, 27, and 36 months). All main effects and interactions among these factors were tested. Prior to analysis, a resemblance matrix based on Euclidean distances was constructed using untransformed data. The influence of each factor on the response variables was assessed through permutation tests on the residuals of a reduced model, using Type III partial sums of squares. A total of 999 permutations were used, and Monte Carlo p-values were calculated when the number of unique permutations was less than 100 (Anderson et al., 2008). Pairwise post-hoc tests were conducted when significant main effects were detected.

2.6.2. Multivariate

Non-metric multidimensional scaling (nMDS) based on a Bray–Curtis dissimilarity matrix was used to visualize annual changes in the combined physiological and biochemical traits of *P. oceanica* transplants and control meadows during spring months only. The stress value of each ordination was used as a measure of the reliability of the two-dimensional representation. A PERMANOVA was performed on all physiological and biochemical variables to test for the effects of donor source, months post-transplantation, and their interaction on the multivariate trait structure. Prior the PERMANOVA analysis, a resemblance matrix based on Euclidean distances was constructed using normalized data. Pairwise post-hoc tests were conducted when significant main effects were detected. Finally, a similarity percentage (SIMPER) analysis was used to identify the physiological and biochemical traits that contributed most to the observed dissimilarities among donor sources and between donor sources and control meadows at each time point. nMDS and SIMPER analysis were performed using Rstudio software.

All PERMANOVA analyses were carried out using PRIMER-E with PERMANOVA+ software (version 7.0.24; PRIMER-E, Auckland, New Zealand). Statistical significance was set at $p < 0.05$, and all reported values are presented as mean ± standard error.

3. Results

3.1. *Effects of transplantation method, donor source, and transplantation depth on the physiological and biochemical traits of P. oceanica transplants*

GLMMs were used to test the effects of transplantation method, donor source, and transplantation depth on the physiological and biochemical traits of *P. oceanica* transplants. Most traits (Fv/Fm, alpha, carbon concentration, sulfur concentration, total carbohydrate reserves, sucrose, and starch) were not significantly affected by any factor or their interactions. Among photosynthetic parameters, rETRmax was significantly influenced by donor source and transplantation depth (Table S1). Post-hoc tests showed that intermatte cuttings had significantly higher values than storm-fragments, and higher values for the deep sites compared to the shallow sites. Ek was significantly affected by the interaction between donor source and transplantation depth (Table S1). Post-hoc tests showed that, at the deepest sites, intermatte cutting had higher Ek values than storm-fragments ($p = 0.001$), while no difference was observed at the shallowest sites. For leaf elemental concentrations, both nitrogen and phosphorus concentrations were significantly influenced by donor source (Table S1). Intermatte cuttings showed significantly higher nitrogen and phosphorus concentrations compared to storm-fragments. The C:N ratio was significantly affected by both donor source and transplantation method (Table S1). Storm-fragments showed higher C:N ratios compared to intermatte cuttings. Post-hoc comparisons for transplantation method revealed that BESE elements had significantly higher C:N ratios than coconut fiber mats and iron staples, while no significant difference was found between the latter two transplantation methods (Table S1). Finally, the C:P and N:P ratios were significantly influenced by the interaction between transplantation method and transplantation depth (Table S1). At shallow sites, BESE elements showed significantly higher C:P ratios than coconut fiber mats and iron staples. For the N:P ratio, BESE elements had significantly higher values compared to coconut fiber mats, but not compared to iron staples. At the deepest sites, no significant differences in C:P or N:P ratios were detected among transplantation methods (Table S1).

3.2. *Temporal dynamics of individual physiological and biochemical traits in P. oceanica transplants and control meadows*

3.2.1. *Photosynthetic activity*

Donor source, months post-transplantation, and their interaction significantly influenced all photosynthetic parameters, with the exception of donor source for α (Table S2).

For Fv/Fm, intermatte cuttings initially (0 month) had lower values than storm-fragments and the controls. This difference progressively disappeared, although control meadows generally maintained higher values than transplants throughout the study (Fig. 1A; Table S3). No consistent patterns were found for α, rETRmax, and Ek with respect to the interaction between donor source and months post-transplantation (Fig. 1B–D; Table S3). Differences between controls and transplants were sometimes observed (e.g., 12, 24 months) but did not persist, and by 36 months, no significant differences remained (Fig. 1B–D; Table S3). At transplantation (0 month) intermatte cuttings displayed distinct behaviour. They had higher rETRmax and Ek values than both storm-fragments and controls, and α values higher than controls (Fig. 1B–D; Table S3). These initial differences diminished over time, converging with the other groups.

3.2.2. *Leaves' elemental concentrations*

Carbon (C) concentration was significantly affected by donor source, months post-transplantation, and their interaction (Fig. 2A; Table S2). Significant differences were observed between the control meadow and the transplants for up to two years, but diminished thereafter (Fig. 2A; Table S2). No significant differences in C concentration were detected between storm fragments and intermatte cuttings after transplantation (i.e., beyond the initial measurements at 0 months following post-transplanting) (Fig. 2A; Table S3). Nitrogen (N) concentration was also significantly influenced by donor source, months post-transplantation, and their interaction (Fig. 2B; Table S2). From 12 to 24 months, storm fragments exhibited significantly lower N concentration than both control meadows and intermatte cuttings (Fig. 2B; Table S3). At 27 months, storm-fragments and intermatte cuttings did not differ, although N concentration in storm-fragments remained significantly lower than in control meadows. By 36 months, control meadows still displayed significantly higher N concentration than both transplant types (Fig. 2B; Table S3). Phosphorus (P) concentration was significantly affected by donor source and months post-transplantation, but not their interaction (Fig. 2C; Table S2). Sulfur (S) concentration was

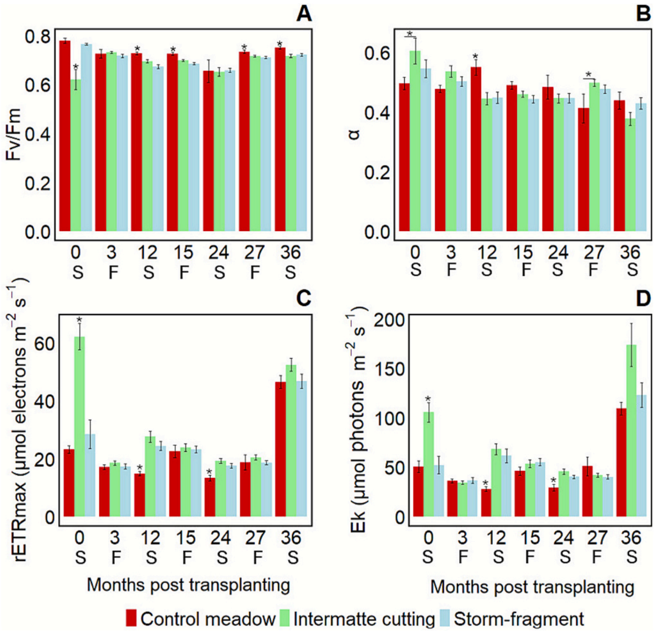

Fig. 1. Temporal dynamics of photosynthetic parameters in *P. oceanica* transplants (storm-fragments and intermatte cuttings) compared to control meadow: (A) maximum photochemical quantum yield (Fv/Fm), (B) photosynthetic efficiency (α), (C) maximum relative electron transport rate (rETRmax), and (D) saturating light intensity (Ek). The letters below the months post transplanting indicate the sampling season (S = spring and F = fall). Error bars indicate standard error. Significant differences ($p < 0.05$) are marked with an asterisk (*).

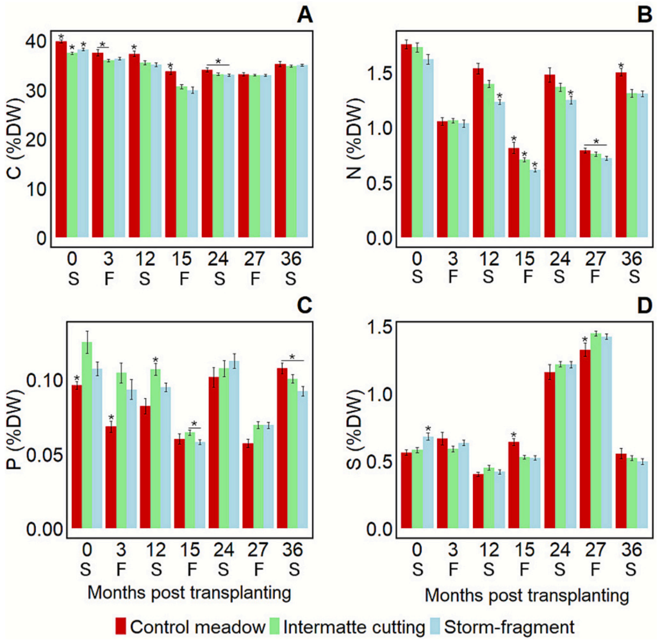

Fig. 2. Temporal dynamics of leaves' elemental concentration in *P. oceanica* transplants (storm-fragments and intermatte cuttings) compared to control meadow: (A) carbon concentration, (B), nitrogen concentration, (C), phosphorus concentration, and (D) sulfur concentration. The letters below the months post transplanting indicate the sampling season (S = spring and F = fall). Error bars indicate standard error. Significant differences ($p < 0.05$) are marked with an asterisk (*).

not significantly affected by donor source but was significantly influenced by months post-transplantation, and its interaction with donor source (Fig. 2D; Table S2). Marked temporal variability was observed throughout the study period (Fig. 2D).

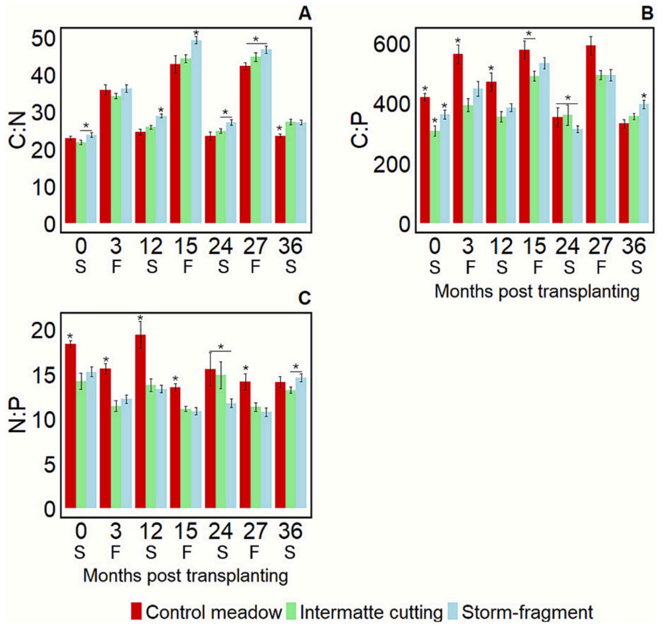

Fig. 3. Temporal dynamics of leaves' elemental ratios in *P. oceanica* transplants (storm-fragments and intermatte cuttings) compared to control meadow: (A) C/N, (B) C/P, and (C) N/P. The letters below the months post transplanting indicate the sampling season (S = spring and F = fall). Error bars indicate standard error. Significant differences ($p < 0.05$) are marked with an asterisk (*).

The three elemental ratios (C:N, C:P, and N:P) were significantly affected by donor source and months post transplantation (Fig. 3; Table S2). C:N ratios were consistently higher in storm-fragments compared to both control meadows and intermatte cuttings (Fig. 3A; Table S3). C:P ratios were highest in control meadows, followed by storm-fragments and then intermatte cuttings (Fig. 3B; Table S3). Finally, N:P ratios were significantly higher in control meadows than in either transplant type (Fig. 3C; Table S3).

3.2.3. *Carbohydrate storage*

Total carbohydrate reserves were significantly influenced by donor source, months post-transplantation, and their interaction (Fig. 4A; Table S2). Initially, intermatte cuttings contained the highest carbohydrate and starch contents, but these differences disappeared after 12–24 months. By 36 months, control meadows exhibited significantly higher reserves than both transplant types (Fig. 4A; Table S3). Sucrose content was significantly affected by donor source and months post-transplantation (Table S2). Sucrose content fluctuated markedly through time, with a general decline at 24 months across all groups. Control meadows maintained higher sucrose levels than transplants at 24–36 months (Fig. 4B; Table S3). Starch content was significantly influenced only by the donor source (Table S2). Its temporal dynamics (Fig. 4C) closely mirrored those of total carbohydrate content (Fig. 4A). Intermatte cuttings initially contained significantly more starch than control meadow and storm-fragments, but this difference disappeared over time (Fig. 4C; Table S3). As with total carbohydrates, no differences were observed between donor sources at 12 and 24 months, while control meadows showed significantly higher starch content than transplants at 36 months (Fig. 4C; Table S3).

3.3. *Temporal dynamics of combined physiological and biochemical traits in P. oceanica transplants and control meadows*

The nMDS ordinations revealed strong dissimilarities among control meadows, storm-fragments, and intermatte cuttings prior transplantation, with the greatest separation observed between control meadows and intermatte cuttings (Fig. 5). PERMANOVA confirmed that donor source, months post-transplantation, and their interaction significantly influenced the multivariate structure of physiological and biochemical traits throughout the study period. Post-hoc tests supported the nMDS results, showing significant differences among all three groups before transplantation (Table S4). At 12 months, dissimilarity between storm-fragments and intermatte cuttings largely disappeared, although both donor sources remained distinct from control meadows (Fig. 5; Table S5). Across 0 and 12 months, six traits consistently explained more than 90 % of group dissimilarities: C:P, TCR, starch, sucrose, Ek, and rETRmax (Table S6). By 24 months, intermatte cuttings and storm-fragments showed high similarity and no longer formed isolated clusters, while differences with control meadows had further decreased (Fig. 5). Post-hoc tests detected no significant differences between intermatte cuttings and control meadows, though storm-fragments remained distinct (Table S5). SIMPER analysis identified C:P, TCR, starch, and Ek as the primary contributors (>85 %) to dissimilarities, with C:N and N:P distinguishing storm-fragments and intermatte cuttings, and sucrose and N:P differentiating both donor sources from control meadows (Table S6). By 36 months, control meadows again formed a distinct cluster, while intermatte cuttings and storm-fragments overlapped partially but remained somewhat separated (Fig. 5). Post-hoc tests confirmed significant differences among between controls and transplants, but no significant differences are found between storm-fragments and intermatte cuttings (Table S5). As at 0 and 12 months, the same six traits (C:P, TCR, starch, sucrose, Ek, rETRmax) explained more than 90 % of observed dissimilarities (Table S6).

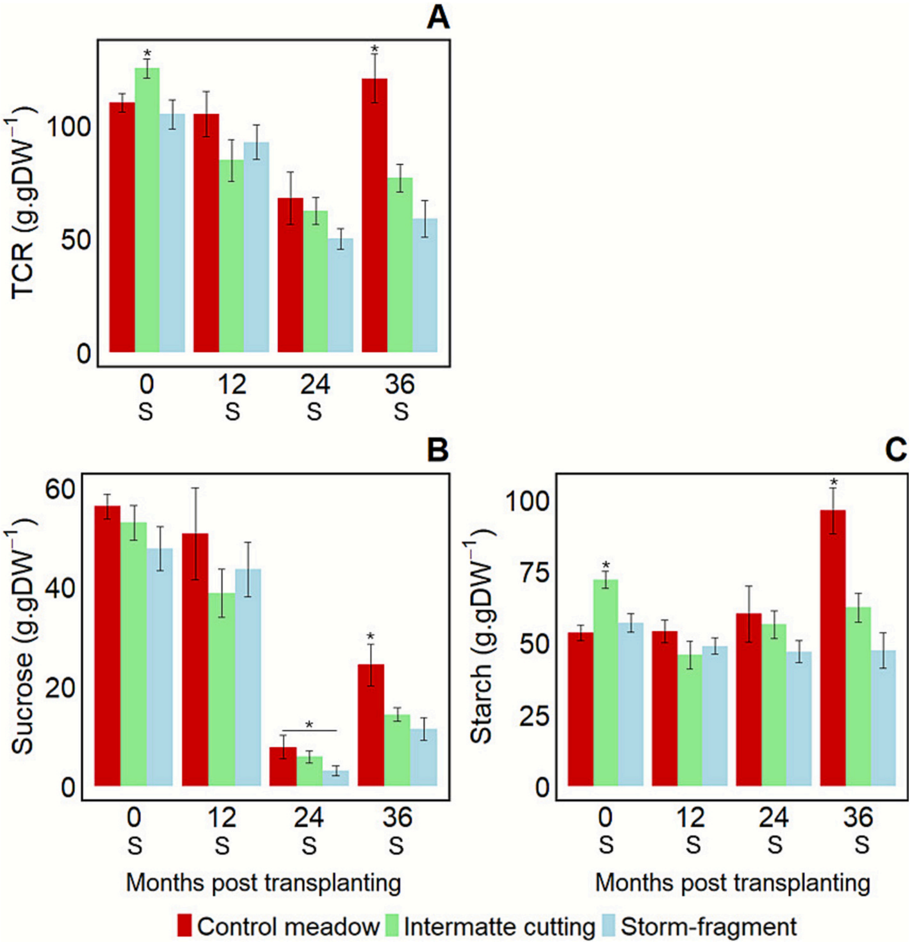

Fig. 4. Temporal dynamics of (A) total carbohydrate reserve (TCR), (B) sucrose, and (C) starch content in *P. oceanica* transplants (storm-fragments and intermatte cuttings) compared to control meadow. The letters below the months post transplanting indicate the sampling season (S = spring). Error bars indicate standard error. Significant differences ($p < 0.05$) are marked with an asterisk (*).

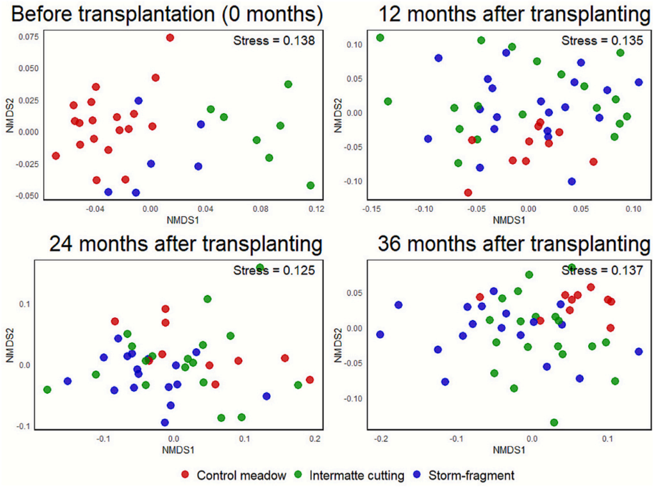

Fig. 5. Non metric multidimensional scaling (nMDS) ordination plots illustrating time series of multivariate physiological and biochemical traits' shifts as a function of donor source from pre-transplantation (0 months) to post-transplantation (12, 24 and 36 months).

4. Discussion

Assessing the success of *P. oceanica* restoration efforts remains challenging due to the slow growth and delayed structural responses of this foundational seagrass species. Traditional metrics such as shoot density or coverage often fail to capture early signs of recovery, especially over the short timescales of most restoration projects (Cooke and Suski, 2008; Horn et al., 2009; Pansini et al., 2022). In this context, physiological and biochemical indicators offer a valuable alternative, as they can respond more rapidly and specifically to environmental conditions (Roca et al., 2016). Their integration into monitoring frameworks is therefore essential to monitor transplantation success and identify the underlying drivers influencing restoration outcomes (Pansini et al., 2022; Roca et al., 2016). Over a three-year monitoring period, the effects of transplantation method, donor source, and transplantation depth on the physiological (photosynthetic activity) and biochemical (elemental nutrient concentration and carbohydrate storage) traits of *P. oceanica* transplants were evaluated. The study specifically aimed to determine whether certain donor sources were more likely to develop trait profiles that progressively converged with those of natural meadows, thereby offering greater potential for long-term restoration success.

4.1. Influence of transplantation methods on *P. oceanica* transplants' physiological and biochemical traits

The three transplantation methods tested in this study (i.e. iron staples, coconut fiber mats, and BESE elements) had a very limited overall influence on the physiological and biochemical traits of *P. oceanica* transplants. Among the few significant differences, the C:N ratio in leaf tissues was notably affected by the transplantation method, with higher values observed in transplants using BESE elements compared to those using coconut fiber mats or iron staples. The C:N ratio is a complex indicator, as it can respond to both changes in nutrient availability and light limitation (Roca et al., 2016). However, since planting densities were standardized across all transplantation methods, the hypothesis of self-shading effects can be ruled out. Although no significant differences were observed in C or N concentrations among transplantation methods, higher C:N ratios in BESE elements suggests reduced nitrogen availability or uptake, possibly due to limited root development (Boulenger et al., 2025b; de Boer, 2007; Lepoint et al., 2004; Udy and Dennison, 1997). Microenvironmental constraints associated with BESE elements' biodegradation (Nitsch et al., 2021), may also underlie this pattern; the release of dissolved organic carbon (DOC) from BESE elements (Nitsch et al., 2021) could further influence microbial or redox dynamics in the underlying sediment (Tu et al., 2025), indirectly affecting nitrogen cycling (Pedersen et al., 1999). However, the absence of significant differences in N concentration between transplantation methods does not suggest a pronounced nutrient limitation. Furthermore, no significant differences were observed in elemental nutrient concentrations (C, N, S, P) or in rhizomes' carbohydrate reserves (TCR, sucrose, starch) among the three transplantation methods.

However, root development was markedly reduced in cuttings transplanted using BESE elements and coconut fiber mats compared to those fixed with iron staples three years after transplantation (Boulenger et al., 2025b). This unexpected result may reflect the existence of distinct resource-use strategies between individual fixation methods (i.e., iron staples) and three-dimensional transplantation structures (i.e., coconut fiber mats and BESE elements) (Fig. 6). In BESE elements and coconut fiber mats, the absence of roots may be linked to the lack of direct contact with the sediment, which limits the release of root exudates into the sediment. Such exudates promote microbial colonization via chemotaxis and attract key microbial partners that enhance plant fitness within the seagrass rhizosphere (Crump et al., 2018; Sogin et al., 2022; Zhang et al., 2024). Consequently, only foliar nutrient uptake and internal nitrogen recycling occur in these treatments (Fig. 6) (Alcoverro et al., 2000; Lepoint et al., 2002; Vangeluwe et al., 2004), and nitrogen is not invested in root system development. By contrast, cuttings attached with iron staples can also absorb nutrients from the sediment porewater through their roots (Lepoint et al., 2002). This enhanced nutrient availability likely supported root growth, creating a positive feedback loop that reinforced both belowground development and nutrient assimilation. This sedimentary nitrogen may be directly allocated to continued root system development, which would explain why higher foliar nitrogen concentrations are not observed with this transplantation method (Fig. 6). As a result, while iron staple transplants may rely on active nutrient uptake for root system development, those on BESE elements and coconut fiber mats may adopt a more conservative survival strategy, characterized by reduced root development and a tighter regulation of internal resource use. In comparison, natural meadows exhibit higher foliar nitrogen concentration than transplants because their root systems are already fully developed. As a result, the retranslocation of nitrogen from leaves and rhizomes to the roots is no longer necessary (Fig. 6) (Lepoint et al., 2004). Furthermore, Kraemer et al. (1997) hypothesized that the activity of leaf glutamine synthetase, the key enzyme responsible for converting inorganic nitrogen into organic forms, may be upregulated as a compensatory metabolic adjustment when root system is reduced. This enzymatic response reflects a form of metabolic plasticity that enables plants to maintain nitrogen assimilation and support the de novo synthesis of nitrogen-containing organic compounds, even in the absence of an efficient root system (Kraemer et al., 1997). Such contrasting strategies, root-supported nutrient uptake versus foliar metabolic compensation, may help explain why nutrient concentrations and carbohydrate reserves remained comparable across the three transplantation methods, despite underlying differences in root system development.

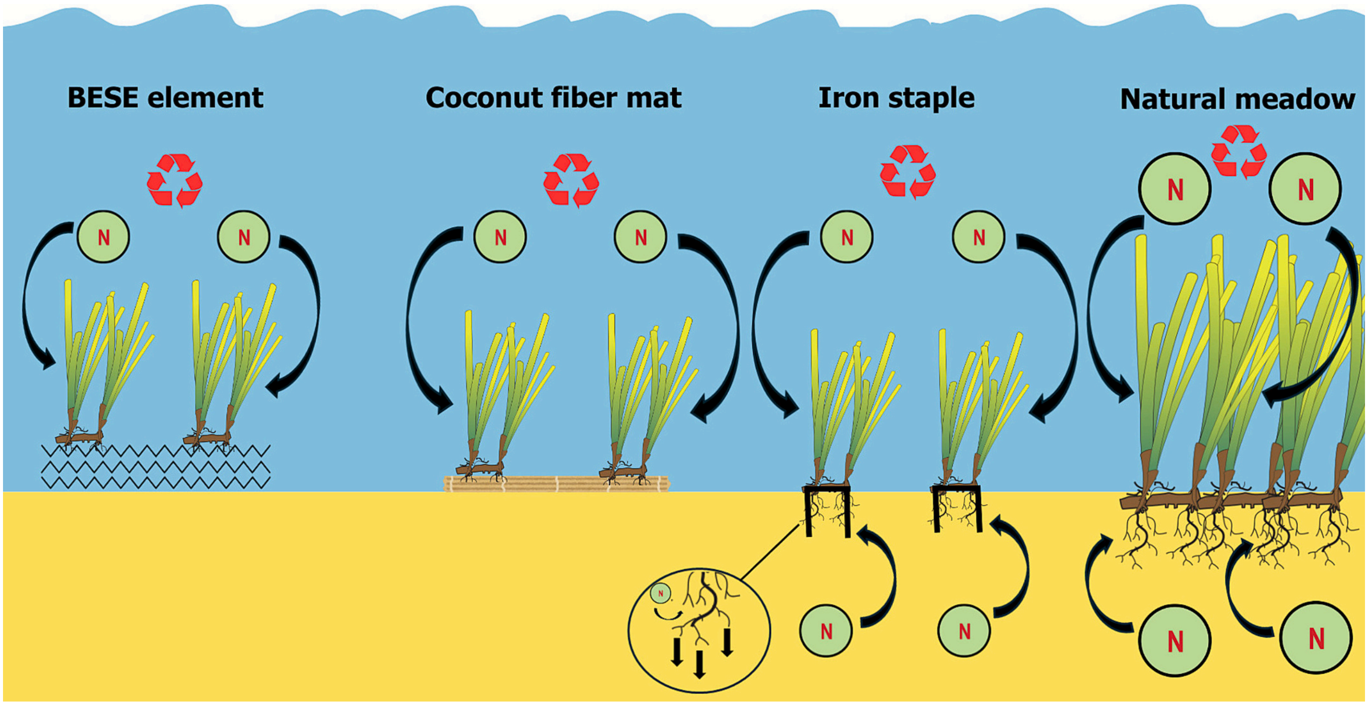

Fig. 6. Conceptual figure of nitrogen uptake and allocation strategies in *P. oceanica* cuttings under different transplantation methods.

4.2. Influence of transplantation depth on P. oceanica transplants' physiological and biochemical traits

When *P. oceanica* cuttings are used for restoration purposes, accounting for light intensity is critical to avoid excessive mortality caused by levels falling outside the species' tolerance range (Stipcich et al., 2023). In the present study, as with transplantation method, the two transplantation depths tested (20 m vs. 28 m) had only a limited influence on the physiological and biochemical traits of *P. oceanica* transplants. It is important to note that most cuttings were transplanted to sites deeper than their original location. In particular, intermatte cuttings were harvested at 15 m depth, while storm-derived fragments originated from a broader depth range, spanning 6 m to 28 m. Although it was not possible to precisely assign storm-fragments to their original depths, this variability represents an additional source of uncertainty that could partly explain the observed heterogeneity among treatments. Previous studies have shown that transplanting cuttings deeper than their original depth can compromise survival, photosynthetic performance, and carbohydrate storage (Genot et al., 1994; Molenaar and Meinesz, 1992). However, the results of this study align with more recent studies suggesting that *P. oceanica* may acclimate to different depth-related light environments through physiological buffering and morphological plasticity (Dattolo et al., 2017; Ismael et al., 2023; Ruiz and Romero, 2003; Stipcich et al., 2023). Interestingly, studies reporting reduced survival and physiological performance typically used orthotropic rhizomes (Genot et al., 1994; Molenaar and Meinesz, 1992), while those observing minimal or no impact of transplantation depth relied on plagiotropic rhizomes (Dattolo et al., 2017; Mancini et al., 2022; Stipcich et al., 2023).

The limited physiological and biochemical traits' responses may result from insufficient irradiance contrast between the shallowest (20 m) and the deepest (28 m) sites to exceed a critical acclimation threshold (Ruiz and Romero, 2003). Ismael et al. (2023) showed that *P. oceanica* can maintain photosynthetic activity and carbon allocation even under low-light conditions in deep waters, partly through starch mobilization in rhizomes and possibly enhanced amylase activity. In contrast, shallow-water plants may produce more carbohydrates due to higher irradiance but also face greater stress (e.g., epiphytes, oxidative bursts), leading to increased carbohydrate consumption (Costa et al., 2015; Sureda et al., 2008). These compensatory mechanisms could explain the lack of significant differences in carbon concentrations and carbohydrate reserves between the two transplantation depths. Interestingly, while depth alone did not significantly alter photosynthetic activity parameters, significantly higher Ek (i.e. saturating irradiance) values were observed in intermatte cuttings compared to storm-fragments, but only at deep sites. The significantly higher Ek values observed in intermatte cuttings suggest that they have a greater capacity for light utilization in deep conditions. However, these differences in Ek did not translate into significant variations in other photosynthetic activity parameters, carbon concentration or carbohydrate reserves. Finally, these results indicate that, while transplanting cuttings at similar depths may facilitate acclimation through pre-existing physiological adaptations, this condition is not essential, especially when using plagiotropic rhizomes and when donor and transplantation sites share similar environmental conditions within the same coastal area.

4.3. Influence of donor source on P. oceanica transplants' physiological and biochemical traits

P. oceanica restoration projects have typically used rhizome fragments from two main donor sources: either cuttings harvested directly from natural *P. oceanica* meadows (e.g., Bacci et al., 2024; Calvo et al., 2021; De Luca et al., 2024; Pirrotta et al., 2015), or naturally detached storm-fragments (e.g., Castejón-Silvo and Terrados, 2021; Mancini et al., 2021; Piazzi et al., 2021). This study aimed to experimentally compare the physiological and biochemical traits of *P. oceanica* transplants derived from these two donor sources. Such comparisons are essential, as restored populations may develop altered physiological traits, the ecological consequences of which remain largely uncertain (Cooke and Suski, 2008). The exact time since detachment of storm-derived fragments could not be determined, which may have influenced their initial physiological condition. Prolonged drifting before collection can lead to nutrient depletion and stress accumulation, yet empirical data on fragment survival and viability during the floating phase remain scarce (Balestri et al., 2011). Available studies on other seagrass species indicate that detached fragments of *Halophila johnsonii* degrade within 4–8 days (Hall et al., 2006), whereas *Zostera marina* and *Halodule wrightii* can remain viable for up to 12 weeks, although their re-establishment capacity declines markedly after 6 weeks (Ewanchuk and Williams, 1996).

By identifying trait-specific differences related to donor source, this study contributes to a better understanding of the biological performance and restoration potential of each donor source. Among these traits, rETRmax, N, and P concentrations were significantly higher in intermatte cuttings compared to storm-fragments. The C:N ratio was also significantly influenced by donor origin, with higher values observed in storm-fragments than in intermatte cuttings.

Temporal dynamics of these traits revealed that rETRmax differed significantly between intermatte cuttings and storm-fragments only prior to transplantation. This difference is likely attributable to varying light exposures in their original environments (Dattolo et al., 2014; Horn et al., 2009; Major and Dunton, 2002), as discussed in the preceding section. Nitrogen concentrations exhibited longer-lasting effects, with significantly higher N concentrations in intermatte cuttings compared to storm-fragments at 12, 15, and 24 months post-transplantation. Symbiotic N_2-fixing microorganisms play a critical role in nitrogen assimilation by eukaryotes in nitrogen-limited environments (Poole et al., 2018). At 24 months post transplanting, the same samples in the study of Boulenger et al. (2025c) showed that the roots of intermatte cuttings had a notably higher abundance of the bacterial order *Chromatiales*, particularly the genus *Candidatus* Thiodiazotropha, than those of storm-fragments. *Candidatus* Thiodiazotropha has been identified as a key endosymbiont in the coastal cordgrass *Spartina alterniflora* (Rolando et al., 2024). Given the pivotal role of this genus in sulfur oxidation and nitrogen fixation processes (Martin et al., 2020; Rolando et al., 2024), further research is warranted to determine whether the higher abundance of *Candidatus* Thiodiazotropha in intermatte cuttings could contribute to improved plant performance, for example through increased nitrogen concentration in transplanted seagrass tissues (Mohr et al., 2021; Zhou et al., 2024). Higher nitrogen concentration in the intermatte cuttings explains why the C:N ratio was significantly higher in the storm-fragments.

4.4. Temporal convergence and divergence of physiological and biochemical traits between natural meadows and transplants

Survival of transplanted *P. oceanica* cuttings can be influenced by a number of different factors: organic matter content of the sediment (Boulenger et al., 2025a; Cancemi et al., 2003), surrounding algal community (Pereda-Briones et al., 2018), nutrients' uptake (Lepoint et al., 2004; Vangeluwe et al., 2004), and carbohydrate reserves (Genot et al., 1994). Assessing the nutrient concentrations of transplanted cuttings in comparison with that of shoots from the surrounding natural meadows can provide insight into whether nutrient uptake in the transplants is sufficient to meet these requirements (Castejón-Silvo and Terrados, 2021). The three-year monitoring of nutrient concentrations in the leaves of transplanted and natural *P. oceanica* meadows highlights an expected natural seasonal dynamic (Gobert et al., 2005a, 2005b; Lepoint et al., 2002). Previous transplantation experiments with *P. oceanica* cuttings in the Bay of Calvi have shown that cuttings are unable to meet their nutrient requirements for growth, exhibiting lower phosphorus (Gobert et al., 2005a, 2005b; Vangeluwe et al., 2004) and nitrogen concentrations (Gobert et al., 2005a, 2005b; Lepoint et al., 2004; Vangeluwe et al., 2004) in their shoots compared with those from natural meadows. Regarding phosphorus, substantial temporal variability has been observed, with P concentrations generally higher in transplants than in natural meadows, as noted by Castejón-Silvo and Terrados (2021). However, 36 months after transplantation, natural meadows display higher P concentration than transplants, in agreement with the results reported by Vangeluwe (2006).

Nitrogen acquisition by *P. oceanica* transplants has long been considered a critical factor for the long-term success of seagrass restoration projects (Lepoint et al., 2004; Pansini et al., 2024; Pergent-Martini et al., 2024). Previous studies have reported highly contrasting results, making it difficult to generalize this process in transplanted cuttings: pronounced temporal variability without a clear pattern (Pansini et al., 2024), higher N concentrations in transplants than in natural meadows (Castejón-Silvo and Terrados, 2021), and the opposite outcome, with higher concentrations in natural meadows than in transplants (Gobert et al., 2005a, 2005b; Lepoint et al., 2004; Vangeluwe et al., 2004). The higher nitrogen concentration in transplants compared to control meadows observed by Castejón-Silvo and Terrados (2021) may be explained by the storage of transplants in mesocosms for several months prior to transplantation. Indeed, their results show that the transplants had significantly higher nitrogen concentrations when maintained in mesocosms than before their storage. Moreover, the water circulating in their tanks exhibited strong variations in nitrate concentrations (0.66 ± 0.25 μM–4.20 ± 0.38 μM), with very high values recorded in summer (4.20 ± 0.38 μM) (Castejón-Silvo and Terrados, 2021). In contrast, the water column in the Bay of Calvi is much more oligotrophic, with monthly mean nitrate concentrations never exceeding 1 μM and with higher values in winter than in summer (Fullgrabe et al., 2020; Lepoint et al., 2002). The results of the present study are consistent with earlier work conducted in the Bay of Calvi (Gobert et al., 2005a, 2005b; Lepoint et al., 2004; Vangeluwe et al., 2004), showing overall higher nitrogen concentrations in natural meadows compared to transplants, with these differences persisting for up to three years after transplantation.

Akey parameter driving seagrass growth and survival is the internal carbohydrate reserves, especially starch (Govers et al., 2015), stored in rhizomes as they can strongly influence biomass production (Alcoverro et al., 1995) and play a critical role in the overwintering capacity of seagrasses when photosynthetic activity is reduced (Alcoverro et al., 2001; Govers et al., 2015). Unlike natural meadows, transplanted cuttings are not physiologically integrated into an extensive rhizome network and therefore cannot translocate resources over long distances (Alcoverro et al., 2000; Marbà et al., 2002), which may limit their ability to buffer environmental stress and sustain growth (Castejón-Silvo and Terrados, 2021). Before transplantation, the higher TCR and starch concentration in intermatte cuttings compared to storm-fragments and control meadow can be explained by their greater light exposure on the eroding edges of intermattes (Genot et al., 1994; Gera et al., 2013). TCR and starch content remained relatively stable in both transplants and control meadows at 12 and 24 months after transplantation. However, a marked increase in starch content was observed in control meadows at 36 months, whereas transplants maintained similar values throughout the three-year monitoring period. The constant starch levels in transplants suggest that they are able to maintain and replenish their starch reserves, with no differences detected between donor sources.

The temporal analysis of multivariate trait structure revealed the recovery dynamics of physiological and biochemical traits in transplanted cuttings. Such indicators are known to be particularly effective in capturing recovery processes in large seagrass species (Roca et al., 2016). Nevertheless, because their responses are highly stress-specific, they should be interpreted in combination with other complementary indicators (Roca et al., 2016). Overall, the combined trait analysis indicated that transplants from both donor sources acclimated to their local environments, as evidenced by the convergence of their physiological and biochemical traits after 12 months post-transplantation. At 24 months, intermatte cuttings converged towards trait values comparable to those of natural meadows, whereas storm-fragments consistently displayed traits distinct from natural meadows. These results suggest that the intermatte cuttings show a faster convergence than the storm-fragments, potentially linked to their different life histories (Pergent-Martini et al., 2024). However, 36 months post-transplanting, divergences re-emerged between control meadows and transplants. A general decline in transplants performance after 36 months appears unlikely in this case, as physiological and biochemical individual indicators remained stable over time. These differences more likely reflect interannual environmental variability, to which well-established control meadows may respond more strongly under favorable conditions (e.g., light, temperature, nutrient concentrations in the water column). These findings indicate that the time elapsed since intervention can strongly influence the assessment of restoration success (Pansini et al., 2024). Further long-term research is needed to determine when the cuttings reach a stable state, with complete convergence of traits between transplants and natural meadows, thereby reflecting the full recovery of physiological and biochemical traits in *P. oceanica* transplants.

5. Conclusion

This study demonstrates that the physiological and biochemical parameters of *P. oceanica* transplants are strongly influenced by temporal variation, consistent with the well-documented seasonal dynamics of the species. They are also primarily affected by donor source, whereas transplantation method and depth exerted comparatively minor effects. While iron staples, coconut fiber mats, and BESE elements produced comparable nutrient concentrations and carbohydrate reserves, differences in root system development suggest distinct resource acquisition strategies. Depth-related light variation within the tested bathymetric range had minimal effects on transplant performance, indicating a capacity for physiological acclimation. Donor origin emerged as a key driver of recovery trajectories, with intermatte cuttings exhibiting consistently higher nitrogen concentration and faster convergence towards natural meadow trait profiles than storm-fragments. These differences are likely linked to pre-transplant light history, nutrient assimilation capacity, and associated microbial partners. Despite convergence of physiological and biochemical traits between intermatte cuttings and control meadows two years after transplantation, divergences reappeared by the third year, highlighting the importance of long-term monitoring to capture non-linear recovery patterns. Overall, this work emphasizes the importance of integrating physiological and biochemical indicators into restoration assessment frameworks, as they provide early, sensitive insights into transplant performance. Among these, C, N, P, and starch concentrations are recommended as priority variables for inclusion in restoration monitoring programs, given their central roles in seagrass transplants survival and growth. Achieving full recovery in *P. oceanica* transplants is a long-term process, and future studies should aim to identify the time thresholds at which physiological and biochemical trait convergence with natural meadows becomes stable and sustained. Such knowledge will refine restoration strategies, optimize donor material selection, and improve the long-term success of seagrass restoration projects.

CRediT authorship contribution statement

Arnaud Boulenger: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Ana Lucía Azul Acuna:** Writing – original draft, Investigation, Formal analysis. **Stéphane Roberty:** Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Gilles Lepoint:** Writing – original draft, Methodology, Investigation, Formal analysis. **Michel Marengo:** Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Sylvie Gobert:**

Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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.

Acknowledgments

This work was supported by the University of Liege (grant FSR2021 from UR FOCUS) and the Fonds National de la Recherche Scientifique—FNRS (grants ASP 40006932 and CDR J.0076.23). This study is part of the STARECAPMED (STAtion of Reference and rEsearch on Change of local and global Anthropogenic Pressures on Mediterranean Ecosystems Drifts) project funded by the Territorial Collectivity of Corsica and by the Rhone-Mediterranean and Corsican Water Agency. The authors are grateful to STARESO for facilities and field assistance. Authors are grateful to Cédric Delforge for field work and samples processing assistance, as well as ICP-MS analyses. The authors have no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2025.119036.

Data availability

Data will be made available on request.

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