Marine Pollution Bulletin 233 (2026) 120063

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Invasive macroalgae exert stronger effects than elevated CO_2 on seagrass (*Posidonia oceanica*) seedling performance and associated microbiomes

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ARTICLE INFO

Keywords:
Early life stage
Marine plant
Marine invasions
CO_2 supply
Global change
Ocean acidification
Lophocladia trichoclados
Caulerpa cylindracea

ABSTRACT

Seagrass seedlings are key to meadow recovery under global change, as they enable recolonization of degraded areas and provide genetic variability needed for adaptation. While invasive macroalgae increasingly threaten seagrass communities, elevated CO_2 has been proposed to enhance seagrass performance and potentially buffer other stressors. Here, we conducted a mesocosm experiment to test the combined effects of two invasive macroalgae (*Lophocladia trichoclados* and *Caulerpa cylindracea*) and elevated CO_2 on *Posidonia oceanica* seedlings. CO_2 enrichment increased carbohydrate reserves in rhizomes and induced subtle shifts in root-associated microbiomes. In contrast, invasive macroalgae had consistently negative effects on seedling development and physiology and strongly altered both above- and belowground microbial communities. Despite its potential to stimulate seagrass productivity, elevated CO_2 did not mitigate the detrimental impacts of invasive macroalgae. These findings indicate that future CO_2 conditions may not offset invasion-driven stress at the recruitment stage, highlighting the need for targeted management efforts to limit macroalgal proliferation and support seagrass meadow regeneration.

1. Introduction

Seagrasses are ecosystem engineers that support many important services including the provision of nursery habitat, offering coastal protection, and acting as carbon sinks (do Amaral Camara Lima et al., 2023; Koch et al., 2006; Mazarrasa et al., 2021; Nordlund et al., 2018). While their ecological importance is well recognized, studies generally warn that seagrass meadows are declining at an alarming rate (Duarte, 2002; Orth et al., 2006; Waycott et al., 2009), although a trend reversal has been reported for some European seagrass species, the overall status of *Posidonia oceanica* (L.) Delile remains of concern (de los Santos et al., 2019). The resilience and recovery of these meadows is partly determined by the health and survival of seagrass seeds and seedlings as they are sources of genetic diversity and provide opportunities for dispersal and (re)colonization (Kendrick et al., 2012). Meadow health can also be closely coupled to the microbial communities associated with the seagrasses (e.g., Beatty et al., 2022; Cúcio et al., 2016; Cúcio et al., 2018). Bacteria in these communities can benefit seedlings by aiding nutrient uptake from the sediment (Mensch et al., 2016; Mohr et al., 2021; Wang et al., 2021) and providing plant hormones (Crump et al., 2018). Therefore, understanding how early life stages of seagrasses and their microbiomes respond to stressors under altered environmental conditions can better help predict the future status and management of these meadows.

Biological invasions are a major cause of biodiversity loss worldwide (Bellard et al., 2022; Simberloff et al., 2013) and are an important threat to seagrasses (Orth et al., 2006; Williams, 2007). In particular, invasive macrophytes (i.e., macroalgae and other seagrasses) can significantly alter native ecosystems and they generally have harmful effects on native seagrasses (Mancuso et al., 2023; Thomsen et al., 2009). Effects of

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https://doi.org/10.1016/j.marpolbul.2026.120063
Received 3 February 2026; Received in revised form 11 May 2026; Accepted 23 June 2026

invasive macrophytes on seagrasses can occur at multiple levels, ranging from impacts on their physiology (e.g., changes in nutrient and/or organic compounds content; Firth et al., 2024; but see Pereda-Briones et al., 2019), their growth (e.g., decrease in leaf biomass; Ballesteros et al., 2007), to plant abundance (Ballesteros et al., 2007; Ceccherelli and Cinelli, 1997), and to eventually species replacement (Willette and Ambrose, 2012).

On the other hand, rising atmospheric pCO_2 increases the availability of dissolved CO_2 and HCO_3^- in marine environments (IPCC 2023, 2023), which can enhance the productivity of primary producers that use either of these carbon species as substrates for photosynthesis (Koch et al., 2013). Photosynthesis of seagrasses has been observed to increase under CO_2 enrichment (e.g., Alexandre et al., 2012; Cox et al., 2016; Invers et al., 2002; Palacios and Zimmerman, 2007), which can ultimately enhance the storage of carbohydrate reserves (Campbell and Fourqurean, 2013; Palacios and Zimmerman, 2007). However, increased photosynthesis rates do not always lead to higher leaf growth rates (Alexandre et al., 2012) and not always a positive effect on photosynthesis is observed (Hendriks et al., 2017). Recent studies likewise report variable responses, with CO_2-driven physiological or microbial changes (Berlinghof et al., 2024) but limited or inconsistent growth enhancement (Lee et al., 2022), suggesting that potential benefits may be constrained by other limiting factors such as nutrients or light. In particular, for *P. oceanica* seedlings, photosynthetic enhancement under elevated CO_2 has been reported only during the earliest developmental stages, with effects diminishing as seedlings age (Hernán et al., 2016). In contrast, most marine macroalgae are currently saturated in their dissolved inorganic carbon (DIC) use and thus increased CO_2 availability may not have profound effects on macroalgae (Koch et al., 2013). This has led to the hypothesis that seagrasses may benefit from enriched CO_2 conditions in the ocean (Koch et al., 2013), potentially improving their competitive abilities over macroalgae. Importantly, studies examining the effects of CO_2 increases on seagrass seedlings are scant, but suggest that responses may be weaker in early life stages and related to the role of seeds as a main storage of carbohydrate reserves (Durako and Sackett, 1993; Hernán et al., 2016; Pansini et al., 2023).

Changes in the conditions in seagrass meadows can also change the bacterial communities associated with marine macrophytes (Kardish and Stachowicz, 2023; Wang et al., 2021). Shifts in composition and/or structure of microbiomes have consequences for overall health and ecological success of macrophytes (Aires et al., 2016; Marzinelli et al., 2015, 2018; Zozaya-Valdes et al., 2015). These changes are usually associated with the weakening of the host's immune system rendering them more susceptible to pathogens (Campbell et al., 2011). Acidification experiments have shown that the effects on associated bacteria varied from limited on *Sargassum muticum* and *Fucus vesiculosus* (Aires et al., 2018; Mensch et al., 2016, 2020) to drastic and harmful in *Ecklonia radiata* individuals and their associated microbiome (Qiu et al., 2019). However, even in studies indicating limited effects, potential bacterial pathogens positively associated with CO_2 enrichment were identified. As an example, Hassenrück et al. (2015) found that seagrass (*Enhalus acoroides*) leaves had a more disease-related bacterial composition when exposed to natural acidification in CO_2-rich vents. In addition, the effects of invasive macrophytes on the microbiomes of native species remain underexplored. Invasive seaweeds can alter the bacterial communities in the surrounding environment (e.g., sediments), with consequences for native macrophytes (Aires et al., 2019; Callaway et al., 2004; Gribben et al., 2017), and potentially serve as reservoirs for pathogens (Aires et al., 2018; Rizzo et al., 2016). However, it remains unclear how those changes directly or indirectly affect native species' microbiomes.

To examine the possible effects of the presence of invasive macroalgae and interactions with high-CO_2 in a future scenario on seagrass seedlings, we performed an experiment where we monitored the physiologic responses and development of seedlings of the seagrass *P. oceanica* and the responses of their associated microbiome to enhanced pCO_2 and the presence of invasive algae. We hypothesized that enhanced availability of CO_2 would alleviate the negative impact of invasive algae on the physiology and development of seedlings. Furthermore, we expected that altered CO_2 conditions and the presence of invasive macrophytes may disrupt the natural seedling-associated microbiome. Because microbiome stability is tightly linked to host functioning, shifts toward less beneficial or potentially pathogenic bacterial assemblages could compromise nutrient uptake, stress resistance, and ultimately seedling performance.

2. Materials and methods

2.1. Study species and mesocosm setup

P. oceanica seeds were collected from beach cast fruits at the Bay of Palma (Mallorca, 39°31′36"N 2°33′06″E) in June 2015 and brought to the laboratory where they were left to germinate freely on the aquarium gravel substrate at 20 °C in UV-treated seawater, in aquaria equipped with aquarium pumps and bubble curtains and illuminated with white fluorescent lamps that provided 76 ± 2 μmol m^{-2} s^{-1} of photosynthetically active radiation in a 14:10 h light/dark photoperiod (see also Pereda-Briones et al., 2019). UV-filtered seawater was obtained from the aquarium of the Interpretative Centre of Cabrera Archipelago National Park (in Colonia de Sant Jordi) through a pipeline with an inlet away from shore. Seawater had a salinity of 37 ppt and an alkalinity of 2675 ± 8 μmol/kg μEq kg^{-1} (mean ± SD, $n = 3$). Around three months after germination, 240 seedlings of a similar size were randomly divided into sixteen 20 L-aquaria containing aquarium gravel (ca. 2–4 mm), with twenty seedlings placed in each of the eight Solitaire aquaria and ten seedlings in each of the eight Invaded aquaria. Aquaria were equipped with filter pumps with a maximum flow rate of 200 L/h (ReptiClear Compact Filter and MAGI Filtro Interior).

The aquaria were kept at constant temperature, salinity, and light regimes. Ambient temperature was set a 20 °C in a temperature-controlled room, and temperature variation in each aquarium was monitored with HOBO data loggers measuring every 30 min (Onset®) over an 8-day consecutive monitoring period toward the end of the experiment (Weeks 20–22). Water was replaced with new seawater every 2–3 weeks, salinity, ranging between 37 and 38 ppt, was measured weekly (YSI salinometer), and water loss from evaporation was corrected by replenishing depleted aquaria with distilled water. T5 lamps (Odyssea, 6500 K) for indoor aquaria with four 54-W fluorescent LEDs set to a photoperiod of 13:11 h (ca. reflecting daytime duration between April and August) delivered PAR light levels similar to the germination period. Light levels over the aquaria were stable, as measured at the onset and end of the experiment with a Licor-1400. The light intensity was representative of a seagrass meadow with light limitation, while remaining above the compensation irradiance of three-month-old *P. oceanica* seedlings of 12 μmol photons m^{-2} s^{-1} (Hernán et al., 2017).

2.2. Experimental treatments

Seedlings were separated into four treatments with four replicate aquaria each (16 total) and remained in the specific treatment condition for ca.22 weeks (Table S1). To represent current ("Ambient") and future ("High") atmospheric CO_2 levels (high-emission scenario RCP 8.5; (IPCC 2023, 2023), the aquaria were aerated with an air-CO_2 mixture with 380 or 1000 ppm CO_2, respectively. The resulting mean pH of 7.92 in the High CO2 treatment was not a deliberately targeted value but rather the emergent result of aerating aquaria with air at a targeted pCO2 of 1000 ppm, consistent with projected ocean pH values under high-emission scenarios by 2100 (IPCC 2023, 2023; SSP5–8.5, IPCC, 2021) and with values used in previous experimental studies on *P. oceanica* (Hernán et al., 2016; Cox et al., 2016). Atmospheric air obtained by a pump was first passed through a filter with soda lime to remove all CO_2 and

Table 1
Experimental conditions in aquaria. Mean pH is calculated over all available pH measurements per aquarium. Mean aquarium temperatures include day and night measurements. TA = Total Alkalinity, expressed as Equivalents of the concentration H^+ that can be neutralized ($n = 6$ per aquarium). pCO_2 and Ω_{arag} were calculated using CO2SYS using sampling intervals from TA.

Variable	Sampling frequency	Solitaire - Ambient	Solitaire - High	Invaded - Ambient	Invaded - High	*p*-value	*p*-value (Ambient vs. High)	*p*-value (Solitaire vs Invaded)
Temperature (°C)	Continuous day and night sensor measurements recorded over 8 consecutive days	20.97 ± 0.11	20.92 ± 0.14	20.42 ± 0.15	20.45 ± 0.12	**0.01**	0.95	**<0.001**
pH_{NBS}	Continuous pH sensor data (logged; analysed as fortnightly averages per aquaria)	8.51 ± 0.24	7.92 ± 0.09	8.55 ± 0.10	7.92 ± 0.06	**0.001**	**0.001**	0.93
Total Alkalinity (µmol/kg)	Every 2 weeks (discrete water samples)	2560.32 ± 25.95	2718.35 ± 52.77	2636.75 ± 31.38	2865.88 ± 46.86	**0.001**	**0.002**	0.10
pCO_2 *(µatm)*	Calculated from TA & pH every 2 weeks	327.88 ± 230.04	1012.68 ± 195.36	179.88 ± 49.85	1062.67 ± 210.17	**0.009**	**<0.001**	0.85
Ωarg	Calculated from TA & pH every 2 weeks	6.36 ± 1.68	2.38 ± 0.52	6.44 ± 0.83	2.41 ± 0.28	0.01	**0.002**	0.96
Nox (µmol/L)	Every 2 weeks (nutrient water samples)	28.91 ± 28.48	1.37 ± 0.38	7.50 ± 6.50	8.40 ± 7.28	0.60	0.38	0.64
NO_2 *(µmol/L)*	Every 2 weeks (nutrient water samples)	3.49 ± 3.30	0.45 ± 0.17	1.29 ± 1.12	1.76 ± 1.29	0.70	0.49	0.81
NO_3 *(µmol/L)*	Every 2 weeks (nutrient water samples)	25.43 ± 25.18	0.92 ± 0.43	6.21 ± 5.38	6.63 ± 6.00	0.59	0.37	0.62
NH_4 *(µmol/L)*	Every 2 weeks (nutrient water samples)	2.09 ± 1.09	6.10 ± 3.71	2.79 ± 1.64	0.77 ± 0.17	0.36	0.66	0.30
PO_4 *(µmol/L)*	Every 2 weeks (nutrient water samples)	2.54 ± 2.44	9.13 ± 6.90	0.70 ± 0.57	0.90 ± 0.30	0.36	0.39	0.20

then was mixed with pure CO_2 from a bottle to desired concentrations using mass flow controllers (MFCs; AALBORG GFC-17) in a mixing tube (1.5 L) filled with marbles to increase the surface area. The mixed gas was gently bubbled in replicate aquaria from the base of each aquarium (for more details in methodology see Hendriks et al., 2017). Both pCO_2 treatments were divided into two growing conditions, either without ("Solitaire") or with ("Invaded") invasive macroalgae. The introduced invasive macroalgae were *Lophocladia trichoclados* (previously mistakenly identified as L. *lallemandii*; Golo et al., 2024) and *Caulerpa cylindracea*, which are two dominant invaders threatening *P. oceanica* (e.g., Ballesteros et al., 2007; Klein and Verlaque, 2008). *C. cylindracea* rhizoids were buried in the gravel substrate, while L. *trichoclados* was maintained freely on the bottom; biomass added was ca. 5 g and 7.5 g wet weight per aquarium, respectively (Sureda et al., 2008; Holmer et al., 2009). To balance the total biomass load across the aquaria, twenty seagrass seedlings were placed in the Solitaire treatment aquaria and ten seedlings were placed in the Invaded treatment aquaria. Although these densities do not reflect natural field conditions, they were chosen to balance total biomass load across aquaria, as the Invaded treatment already contained macroalgal biomass, and to ensure comparable conditions for assessing seedling physiological responses. Macroalgae were present for the first ~15 weeks of the experiment (Table S1), during which the seedlings in all aquaria were kept clean of epiphytes by gently rubbing the leaves every fortnight Epiphyte removal by gentle rubbing was discontinued in all aquaria from Week 16 onwards, coinciding with the start of the final measurement period.

2.3. The carbonate system in the aquaria

To monitor the pCO_2 levels for the different treatments, we assessed the carbonate system in the water throughout the experiment (Table 1). pH was measured with pH sensors (Omega engineering PHE-1411), calibrated periodically using pH_{NBS} standards and connected to a D130 or D230 data logger (Consort), or by pH sensors from IKS Aquastar, connected to the system in measuring mode. Since not all 16 replicates of the aquaria could be measured simultaneously due to sensor shortage, 14 aquaria were measured for a period of time of two weeks (November 15 to December 21; Table S1) after which the pH electrodes were recalibrated and inserted into other aquaria. Every two weeks, water samples for alkalinity and nutrients were taken from each aquarium and fixed with 0.02 mL of saturate solution $HgCl_2$ (Merck, Analar). Total alkalinity (TA) was determined from these samples by open cell titration as described in Sop6b (Dickson et al., 2007) with a Metrohm Titrando 808 and Tiamo™ titration software 2.2. Certified CO_2 seawater reference material (CRM Batch 136) from the Andrew Dickson lab at UC San Diego was used to evaluate the accuracy of the results.

Stability and variation in pH through time were assessed by fortnightly averages from sensor data between 15/10/2015 to 28/01/2016 (16 weeks; Table S1). This produced seven pH averaging intervals: six periods of 14 days each and one final period of 21 days (Table 1). Alkalinity samples were matched to each two-week period of pH measurements. The remaining parameters of the carbonate system (i.e., bicarbonate, carbonate, and aragonite saturation state) were calculated in the Excel version of CO2SYS (Pierrot et al., 2006), using measured temperature, pH_{NBS} TA values, and dissociation constants K1 and K2 from Mehrbach et al. (1973) refit by Dickson and Millero, 1987 and $KHSO_4$ dissociation as formulated by Dickson (1990) (see Table 1.).

2.4. Seedling photosynthesis and productivity

After 16 weeks of experimental manipulations, and prior to the destructive sampling procedures carried out during weeks 20–22 (oxygen incubations, microbiome sampling and final harvest; Table S1), non-destructive measurements of photosynthetic variables were taken from seven seedlings per aquarium to ensure seedling integrity was maintained for subsequent measurements.PI-curves were estimated from Rapid Light Curves (RLCs) with a diving PAM (Walz, Germany). Measurements were taken in the mornings (<11 am) of four consecutive days. Diving PAM clips were used to control the distance of the PAM optic fibre sensor to the leaf and to exclude external light. The clips were placed on the 2nd youngest leaf at 2 cm from the meristem (Hernán et al., 2016; Cox et al., 2016). The studied variables were: (i) Alpha (ΔF/fm'), (ii) Yield (photons m^{-2} s^{-1}), (iii) E_k (saturation irradiance, µmol photons m^{-2} s^{-1}) and (iv) ETR_{max} (µmol electrons m^{-2} s^{-1}) (for more detailed information see Supplementary Materials: Appendix A1).

2.5. Net and gross primary production and respiration

To complement non-destructive PAM measurements and account for variability in photosynthetic estimates, oxygen incubations were performed at week 20 (Table S1) to estimate seedling productivity. Briefly, the second leaf of three seedlings per aquarium ($n = 3$) was placed in 5.14 mL incubation chambers filled with aquarium water and equipped

with Oxy-4 Mini oxygen sensors (PreSens) that recorded oxygen concentration every 15 s. Separate light (70 μmol photons m − 2 s − 1) and dark incubations of ca. 50 min each were performed, with chamber water replaced between incubations, until oxygen concentration had stabilized.

Changes in O_2 concentration were standardized to the fresh weight of the seedling leaf (g) and chamber volume (5.14 mL) and converted to hourly rates (mmol O_2 h-1 gfw-1). Net oxygen production in the light and oxygen consumption in the dark were used to calculate net primary production (NPP) and respiration (R), respectively. Gross primary production (GPP) was calculated as NPP plus dark respiration, assuming equal respiration rates in light and dark conditions.

2.6. Seedling physiological responses

For chemical analyses of the separate tissues, the dried mass samples for roots, rhizome and young leaves (i.e., leaves 0–2) from all seedlings within each aquarium were pooled together ($n = 10$ seedlings in Solitaire aquaria and $n = 7$ in Invaded aquaria) and finely ground. Part of the pooled sample was used to determine sucrose and non-structural carbohydrate reserves (NSC; i.e., sucrose and starch), measured following the methodology described by Invers et al. (2004). Another part was used to assess the C and N content and C:N ratio by dynamic combustion in a FlashEA1110 elemental analyser (ThermoFinnigan).

2.7. Seedling development

Since *P. oceanica* seedlings were of similar size when initially assigned to their respective aquaria — selected by eye for comparable leaf number, leaf length and root development, and further supported by initial morphological measurements from a parallel experiment conducted simultaneously with the same seedling cohort (Pereda-Briones et al., 2019) —, any differences between measurements obtained at the end of the experiment were considered to reflect true treatment effects. The morphologic parameters measured per seedling were: number of leaves, width of 2nd leaf (cm), maximum leaf length (cm), total leaf area (width 2nd leaf multiplied by the sum of all leaf lengths, cm^2), number of roots, and total root length (sum of all root lengths, cm). Rhizome length was not included as a morphological parameter as, at this early seedling stage, the rhizome is extremely short, curved and three-dimensional, making accurate length measurements without destroying the seedling impractical and highly variable; rhizome dry mass was therefore quantified instead as a more reliable indicator of belowground size and carbon storage ($n = 10$ and $n = 7$ seedlings per aquarium in Solitaire and Invaded treatments, respectively). After measurements were taken, seedlings were rinsed with distilled water and frozen in liquid nitrogen prior to storage in −80 °C, and after 1 week of storage they were freeze dried (−50 °C, <0.1 bar, 48 h). The dry weight (dw; g) of the separate tissue compartments (roots, rhizome, leaves) was quantified.

2.8. Microbial community analysis

From each aquarium, three *P. oceanica* seedlings were collected at the end of the experiment (Table S1). The roots and young leaves were taken for microbiome analysis. These samples were rinsed with biologically sterile seawater (passed through a 0.2 μm biological filter) and preserved in separate tubes containing 0.4 mL of stabilization solution (Xpedition™ Lysis/Stabilization Solution, Zymo Research®). Microbial DNA was extracted using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) following the MO BIO Vortex Adapter protocol.

Extracted DNA was sent to Molecular Research (MR DNA www.mrdnalab.com, Shallowater, TX, USA). Prior to sequencing, modified primers 799F (5'-AACMGGATTAGATACCCKG-3′), which minimize contamination from plastid DNA (Hunter et al., 2010), and 1193r (5'-ACGTCATCCCCACCTTCC- 3′) (Bodenhausen et al., 2013) with barcode on the forward primer, were used to amplify the V5, V6 and V7 regions of the 16S rRNA in a 30-cycle PCR (5 cycle used on PCR products) using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94 °C for 180 s, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 60 s, after which a final elongation step at 72 °C for 300 s was performed. After amplification, the PCR products were checked on a 2% (*w/v*) agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads and used to prepare a DNA library by following the Illumina TruSeq DNA library preparation protocol. Sequencing was performed on an Illumina MiSeq technology (2 × 300 bp) following the manufacturer's guidelines.

Analyses of the 16S Amplicon sequences were performed using the QIIME 1.8.0 pipeline (Caporaso et al., 2010). High-quality sequences were clustered into Operational Taxonomic Units (OTUs) within reads using the denovo approach. Representative sequences for each OTU were selected using the "most-abundant" method and OTU sequence alignment was carried out using Pynast (Caporaso et al., 2010). Taxonomic assignments were done using the UCLUST method with a 97% confidence threshold. To assign each OTU to the closest matching described taxon, searches were performed against the Greengenes database (McDonald et al., 2012) and sequences were putatively assigned to a described taxon with a minimum threshold of 0.001 (default value). Sequences with the best match for organelles (i.e., chloroplasts and mitochondria), as well as unassigned sequences, were excluded from the OTU table in the downstream analyses.

2.9. Statistical analysis

To assess environmental conditions throughout the experimental period, we statistically examined whether physicochemical parameters of the seawater (i.e., temperature, pH, total alkalinity, nutrients) remained stable across treatments. Specifically, for each environmental variable we used One-way ANOVAs with "treatment combinations" as the fixed factor, corresponding to the four experimental groups: Ambient-Solitaire, Ambient-Invaded, High-Solitaire, and High-Invaded. This approach was deliberately chosen to verify that non-manipulated environmental conditions remained stable and comparable across all treatment combinations, rather than to partition variance attributable to individual experimental factors, which was the approach used for all biological response variables (GLMs and GLMMs; see below). These analyses were performed on the entire time series of measurements available for each variable. To verify that experimental treatments did not differ in non-manipulated environmental conditions, we analysed monthly averages of continuous pH sensor data, biweekly measurements of total alkalinity and nutrients, and temperature time series collected toward the end of the experiment.

To evaluate the effects of CO_2 supply, macroalgal invasion, and their interaction on *Posidonia oceanica* seedlings, we used Generalized Linear Mixed Models (GLMMs) or Generalized Linear Models (GLMs) depending on the response variable. Photosynthetic parameters (Alpha, Yield, E_k, and ETR_{max}) and metabolic rates derived from oxygen incubations (Net primary production, Gross primary production, and Respiration) were analysed using GLMMs. Because multiple seedlings were measured within each aquarium, aquarium was included as a random effect to account for non-independence at the tank level. GLMMs were fitted using the lmer function in the lme4 package (Bates et al., 2015). For photosynthetic parameters approximating normality (Alpha, Yield, Ek and ETRmax), linear mixed models (LMMs) with Gaussian distribution were fitted using the lmer function. For variables showing positive skewness and heteroscedasticity (NPP, GPP and respiration), a Gamma distribution with an inverse link function was applied using the glmer function, as this distribution is appropriate for continuous positive data

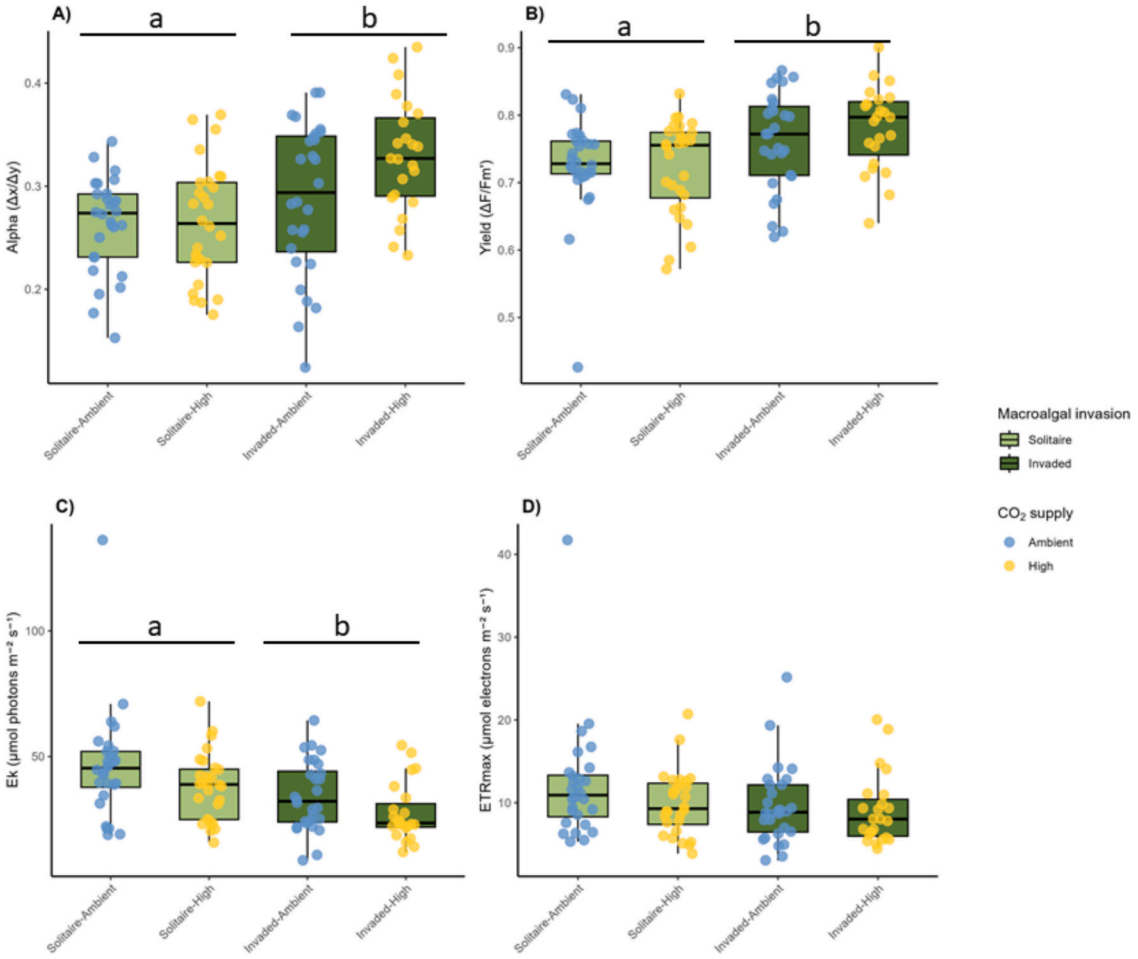

Fig. 1. *Posidonia oceanica* seedling photosynthesis and productivity: A) Alpha (Δx/Δy), B) Yield (ΔF/fm'), C) E_k (saturation irradiance, µmol photons/m^2 s), D) ETR_{max} (µmol electrons/m^2 s). Boxes represent interquartile ranges (IQR), horizontal lines indicate the median, whiskers show variability outside the upper and lower quartiles. Green box colours represent the macroalgal invasion factor (light green = Solitaire, n = 56; dark green = Invaded, n = 56), while point colours represent the CO_2 supply (yellow = Ambient, blue = High). Lower-case letters indicate significant difference between treatments ($p \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

where variance scales with the mean.

By contrast, GLMs were used for carbohydrate reserves (starch and sucrose content) and elemental composition (%C, %N, C:N ratio) as well as morphological and biomass-related traits (number of leaves, leaf area index, rhizome and leaf dry weight). GLMs were fitted using a Gamma distribution with a log-link function for carbohydrate reserves and biomass-related traits, and a Gaussian distribution for elemental composition variables (%C, %N, C:N ratio), based on visual inspection of response variable distributions. All GLMs included two fixed factors: CO_2 level (Ambient, High) and macroalgal treatment (Solitaire, Invaded), as well as their interaction. For each response variable, three model structures were compared using Wald tests: (1) CO_2 supply alone, (2) macroalgal invasion alone, and (3) both factors with their interaction, with the significance of individual terms assessed via the summary () function in R. When CO_2 supply showed no significant main effect ($p > 0.05$) on a given response variable, data from Ambient and High CO_2 treatments were pooled to specifically isolate the effect of macroalgal invasion on seedling responses.

Microbial α-diversity (Chao1 richness and Shannon diversity) was calculated in QIIME. Treatment effects were evaluated using univariate PERMANOVAs on Euclidean distance matrices. β-diversity patterns were assessed using a three-way PERMANOVA on Bray–Curtis dissimilarities (square-root-transformed OTU tables), with Tissue type (leaves vs. roots), CO_2 level (Ambient vs. High), and macroalgal invasion (Solitaire vs. Invaded) as fixed factors and their interactions, and visualised with canonical analysis of principal coordinates (CAP). Tissue type was included as a factor in all microbiome analyses, as roots and leaves harbour distinct bacterial communities and responses to experimental treatments may differ between tissues, in contrast to plant response variables for which measurements were performed on specific tissues separately. Homogeneity of multivariate dispersion was tested using PERMDISP. All multivariate analyses were performed in PRIMER-E + PERMANOVA. When significant effects were detected, post-hoc pairwise comparisons were performed using the emmeans package (Lenth, 2024) to identify specific differences between treatment combinations.

Differential abundance patterns were explored using ANOVA/*t*-tests in MicrobiomeAnalyst, and confirmed with ManyGLM models (negative binomial distribution) using the mvabund package in R. Indicator taxa were identified using the indicspecies package in R.

All statistical analyses were carried out in R version 4.4.0.

3. Results

3.1. Environmental conditions

Environmental conditions were stable, although a statistically significant difference was detected in mean temperature between Solitaire (20.95 ± 0.23 °C) and Invaded (20.44 ± 0.26 °C) aquaria ($p < 0.001$, Table 1), probably due to aquarium placement in the controlled-temperature room. This difference of ~0.5 °C is considered

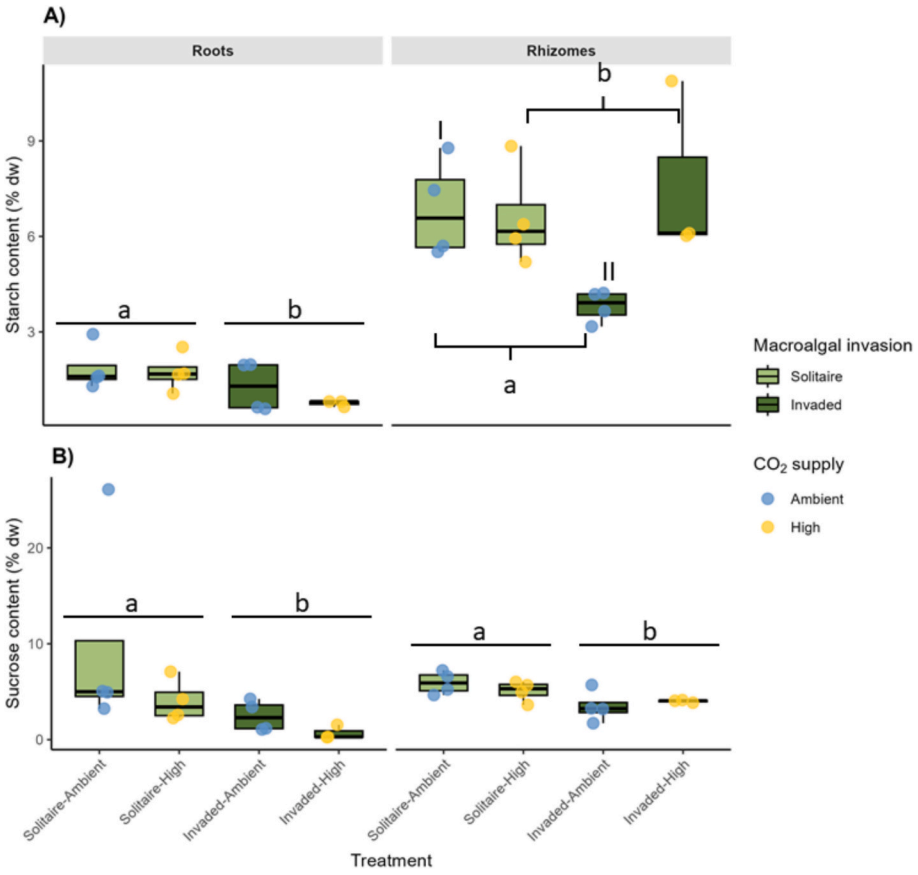

Fig. 2. *Posidonia oceanica* seedling carbohydrate reserves: A) Starch, and B) Sucrose content (% dw) in roots and rhizomes. Boxes represent interquartile range (IQR), horizontal lines indicate the median, whiskers show variability outside the upper and lower quartiles. Green box colours represent the macroalgal invasion factor (light green = Solitaire, $n = 8$ aquaria; dark green = Invaded, n = 8 aquaria Invaded), while point colours represent the CO_2 supply (yellow = Ambient, blue = High). Lower-case letters and roman numerals indicate significant differences between treatments ($p \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

biologically negligible, as negative physiological effects on *P. oceanica* seedlings have been reported only above 27 °C (Guerrero-Meseguer et al., 2017), approximately 6–7 °C above the temperatures recorded in our experiment, and is therefore unlikely to have confounded the observed treatment effects. Furthermore, nutrient concentrations (NO_2, NO_3, NH_4, PO_4), did not exhibit significant differences across treatments ($p > 0.05$) confirming consistency in water chemistry (Table 1).

Treatment-related environmental variables, like total alkalinity (TA ranging between 2560 and 2865 μmol/kg) were slightly higher in High CO_2 supply treatments ($p = 0.001$), but did not differ between Solitaire and Invaded treatments (Table 1). Bubbling air with different pCO_2 concentrations led to pCO_2 of between 180 and 328 μatm in the ambient treatments and 1013–1063 μatm in the High pCO_2 treatments. pH variability reflected diurnal CO_2 uptake and nightly respiration while pH averages where 8.55 in the ambient treatments and 7.92 in the High CO_2 treatments ($p = 0.001$).

3.2. Seedling photosynthesis and productivity

CO_2 enrichment did not result in increased photosynthesis of seedlings based on PAM fluorometry measurements (Alpha, Yield, E_k, and ETR_{max}). Therefore, CO_2 treatments were pooled to analyse effect of the presence of invasive macroalgae on seedling photosynthesis. Seedlings in the Invaded treatment exhibited significantly higher Alpha and Yield than Solitaire (Alpha: 0.31 ± 0.02 vs. 0.28 ± 0.01, t(14.3) = −2.42, $p = 0.05$; Yield: 0.76 ± 0.01 vs. 0.72 ± 0.01, t(13.9) = −3.42, $p = 0.004$; Table S2, Fig. 1A, B). In contrast, E_k was significantly lower in Invaded than in Solitaire seedlings (31.49 ± 2.09 vs. 42.29 ± 3.82 μmol photons m^{-2} s^{-1}, t(14.9) = 2.50, $p = 0.03$; Table S2, Fig. 1C), indicating that seedlings in the presence of invasive macroalgae reached photosynthetic saturation at lower irradiance. ETR_{max} did not differ significantly between invasion treatments or CO_2 levels ($t = 1.31$, $p = 0.210$, Table S2, Fig. 1D).

3.3. Net and gross primary production and respiration

The production and consumption of oxygen by seedling leaf fragments was not affected by CO_2 enrichment, so these treatments were also pooled to analyse differences related to macroalgae addition (Fig. S1). Respiration rates did not differ between Invaded treatments (overall mean ± SE: −0.47 ± 0.04 mmol O_2 h^{-1} gfw^{-1}; z = −1.05, $p = 0.294$; Table S2; Fig. S1B). In contrast, both Net primary production (NPP) and Gross primary production (GPP) were consistently higher in the Solitaire treatment compared to seedlings exposed to invasive macroalgae (Fig. S1A, C). On average, seedlings growing without macroalgae had ~1.8-fold higher NPP (z = −2.63, $p = 0.003$) and ~ 1.7-fold higher GPP (z = −2.49, p = 0.003) than those in Invaded conditions (Table S2). NPP also exhibited a significant interaction between macroalgal invasion and CO_2 supply, driven by lower NPP in the Invaded–Ambient treatment compared with Solitaire–High (post-hoc contrast, z = −2.42, $p = 0.04$).

3.4. Seedling physiological responses

The CO_2 supply treatment only affected one physiological response, with rhizome starch content tending to be higher (1.3 times) in the

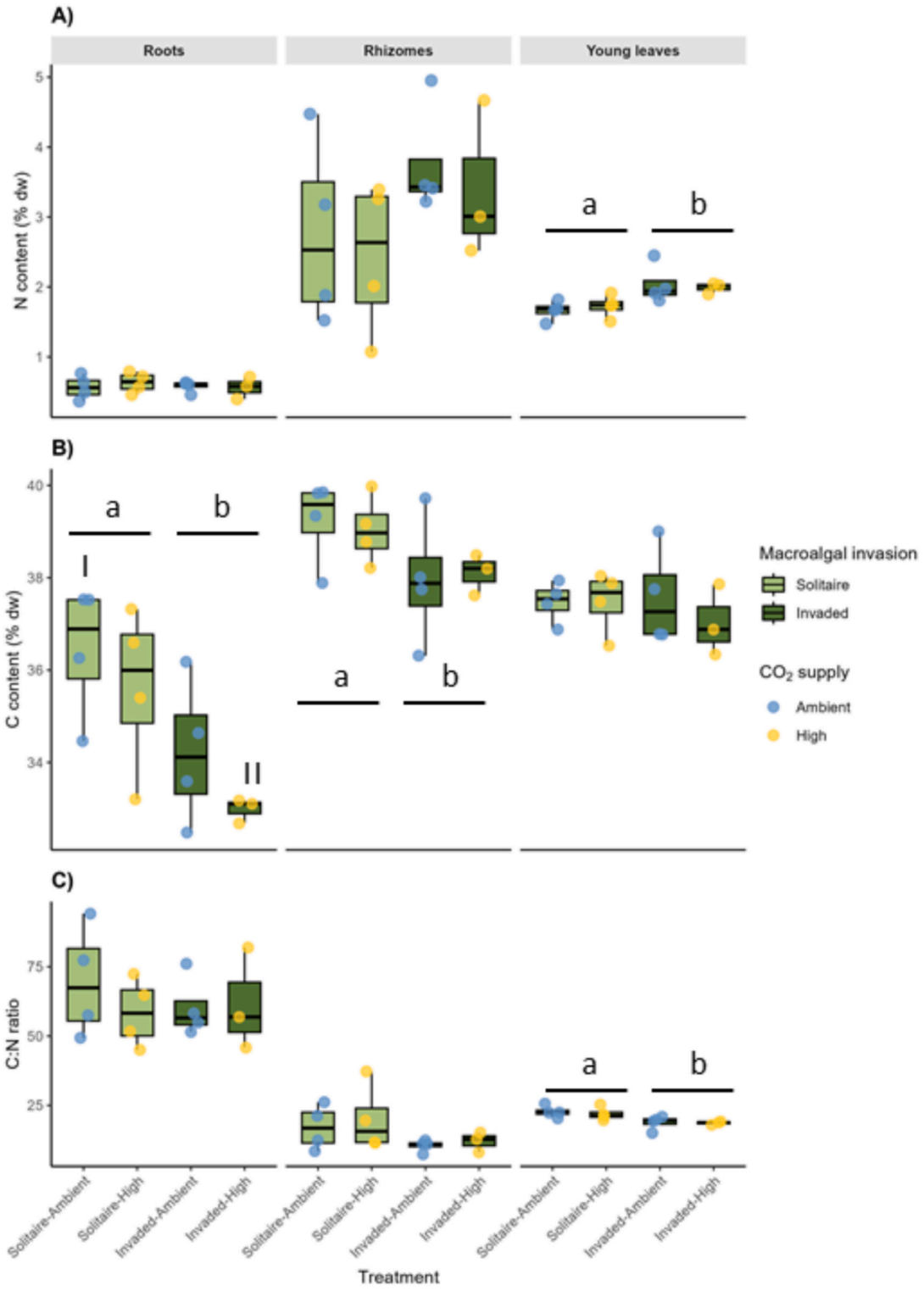

Fig. 3. *Posidonia oceanica* seedling elemental composition parameters content: A) N, B) C content (% dw) and C) C:N ratio in roots, rhizome, and young leaves. Boxes represent interquartile range (IQR), horizontal lines indicate the median, whiskers show variability outside the upper and lower quartiles. Green box colours represent the macroalgal invasion factor (light green = Solitaire, n = 8 aquaria; dark green = Invaded, n = 8 aquaria), while point colours represent the CO_2 supply (yellow = Ambient, blue = High). Lower-case letters and roman numerals indicate significant difference between treatments ($p \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

enriched CO_2 treatment compared to the ambient ($t(11) = 3.02$, $p = 0.05$; Fig. 2A and Table S2). Therefore, Ambient and High CO_2 treatments were pooled to assess the effect of invasive macroalgae on the other physiological responses. In the presence of invasive macroalgae, NSC reserves in seedling roots were markedly reduced. Root starch and sucrose contents were significantly higher in Solitaire seedlings (1.8-fold and 4.4-fold higher respectively; root starch: $t(13) = 2.29$, $p = 0.05$; root sucrose: $t(13) = 1.71$, $p = 0.01$; Fig. 2A, B, Table S2). A similar pattern occurred in rhizomes, where sucrose content was lower under Invaded conditions (1.5-fold reduction, $t(13) = 2.96$, $p = 0.01$; Fig. 2A, B,

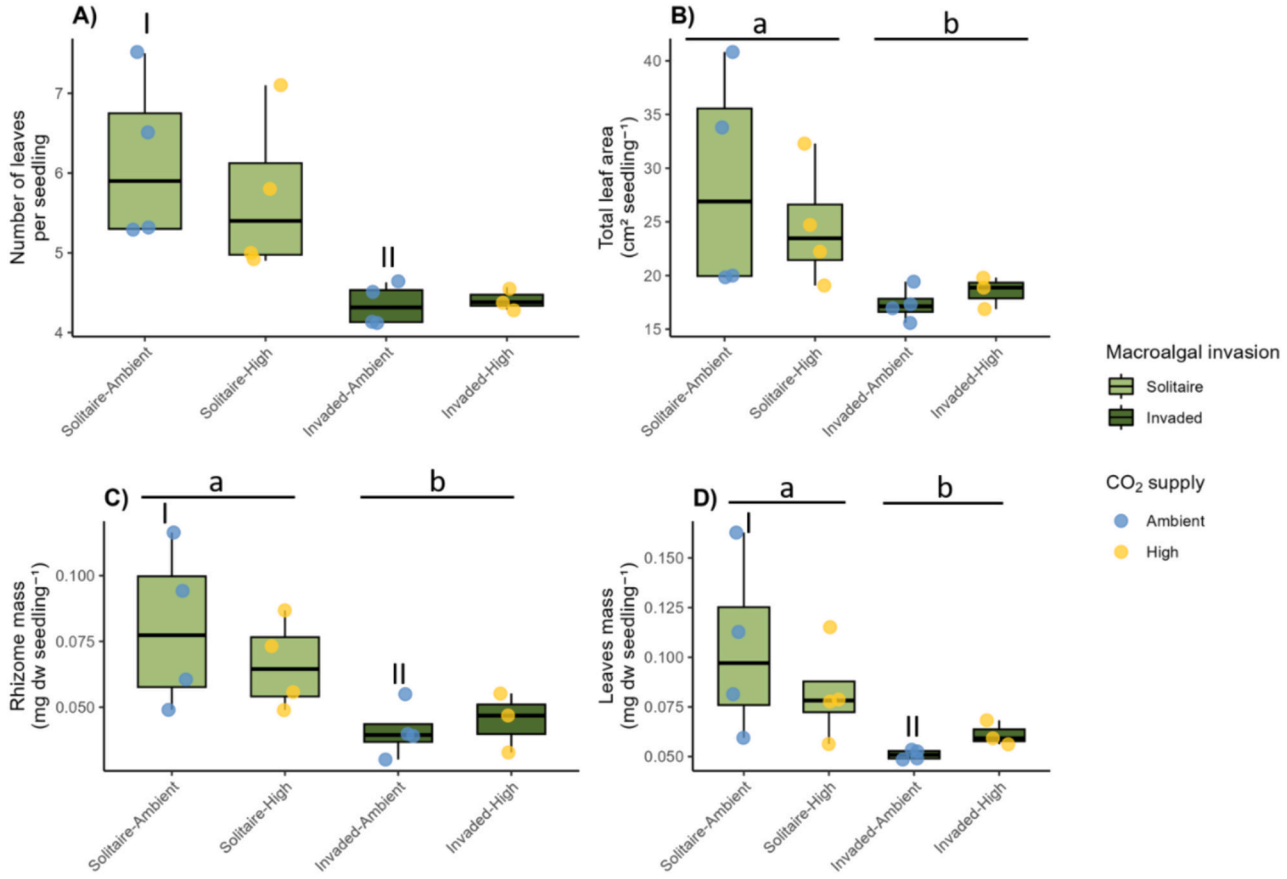

Fig. 4. *Posidonia oceanica* seedling development and growth responses: A) Number of leaves per seedling, B) Total leaf area (cm^2/seedling), C) Rhizome, and D) Leaves mass (mg dw/seedling). Boxes represent interquartile range (IQR), horizontal lines indicate the median, whiskers show variability outside the upper and lower quartiles. Green box colours represent the macroalgal invasion factor (light green = Solitaire, $n = 80$; dark green = Invaded, n = 56), while point colours represent the CO_2 supply (yellow = Ambient, blue = High). Lower-case letters and roman numerals indicate significant difference between treatments ($p \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table S2). The presence of invasive macroalgae significantly altered leaf nutrient content, with higher nitrogen concentration and lower C:N ratio in Invaded seedlings compared to Solitaire plants. Nitrogen concentration in young leaves was higher in Invaded seedlings compared to Solitaire plants ($t(13) = -3.50$, $p = 0.004$; Fig. 3A, Table S2), and did not differ in terms of %C (37.4 ± 0.4%C; $p = 0.72$; Fig. 3B), resulting in a lower C:N ratio (1.2-fold decrease, $t(13) = 3.43$, $p = 0.004$; Fig. 3C, Table S2). In contrast, carbon content in roots and rhizomes was slightly but consistently higher in Solitaire than in Invaded seedlings (roots: $t(13) = 3.11$, $p = 0.04$; rhizomes: $t(13) = 2.39$, $p = 0.03$; Fig. 3B, Table S2).

3.5. Seedling development

No significant effects of pCO_2 enrichment were detected for plant development responses (Table S2., all $p < 0.05$). Pooling CO_2 treatments to analyse effects of invasive macroalgae revealed that seedlings in Invaded aquaria developed less leaves (1.35× lower, $t(16.0) = 4.25$, $p = 0.03$; Fig. 4A and Table S2) and also had lower total leaf area, (1.48× smaller, $z = -2.60$, $p = 0.001$; Fig. 4B and Table S2) compared to seedlings in the Solitaire treatment. The presence of invasive macroalgae had no effect on the other seedling characteristics [i.e., width of the 2nd leaf (0.6 ± 0.02 cm), maximum leaf length (10.8 ± 0.9 cm), total root length (20.5 ± 2.4 cm), or number of roots per seedling (4.4 ± 0.3)]. Seedling rhizome and leaves mass were 1.4× and 1.5× times smaller, respectively, in the presence of invasive macroalgae compared to Solitaire plants (rhizome: $z = -3.10$, $p = 0.002$; leaves: $z = -2.75$, $p = 0.006$; Fig. 4C, D and Table S2). The invasive macroalgae treatments did not affect root mass (0.14 ± 0.02 g, $z = -0.47$, $p = 0.638$; Table S2).

3.6. Microbial community responses

The composition of bacterial communities differed significantly between roots and leaves ($p = 0.001$; Table S3, Fig. 5). Root-associated communities were 2–10 times more diverse than leaves communities (Fig. 5A, Table S3). Diversity and richness were not affected by high CO_2 supply independently ($p = 0.86$ and 0.747; Table S3) or in interaction with the Invaded treatment ($p = 0.994$ and 0.747; Table S3). However, both diversity indices in root-associated communities were significantly lower in the Invaded treatment ($p = 0.021$ and 0.003 for Tissue × Invaded interaction; Table S3, Fig. 5A), with post-hoc tests confirming this effect was root-specific ($p = 0.043$ and 0.016 for Solitaire vs. Invaded within roots; Table S4) while leaves remained unaffected ($p = 0.507$ and 0.110; Table S4).

High CO_2 supply did not affect *Posidonia*-associated bacterial community composition ($p > 0.05$). However, visual inspection revealed CO_2 effects were most apparent in root communities under Solitaire conditions (AS vs. HS), with clear sample separation, while Invaded root communities (AI vs. HI) exhibited overlapping patterns (Fig. 5B).

Indicator species analysis identified 7 and 5 genera for High CO_2 supply treatment leaves and root communities, respectively. Leaves indicators included Alteromonadaceae members (Microbulbifer, Glaciecola), Hyphomonadaceae and Piscirickettsiaceae. Root indicators comprised diverse taxa including Legionellaceae, Methylocystaceae, Caulobacterales, Marinicella and Maribacter. Differential abundance analysis confirmed enrichment of Alteromonadales, Rhizobiales,

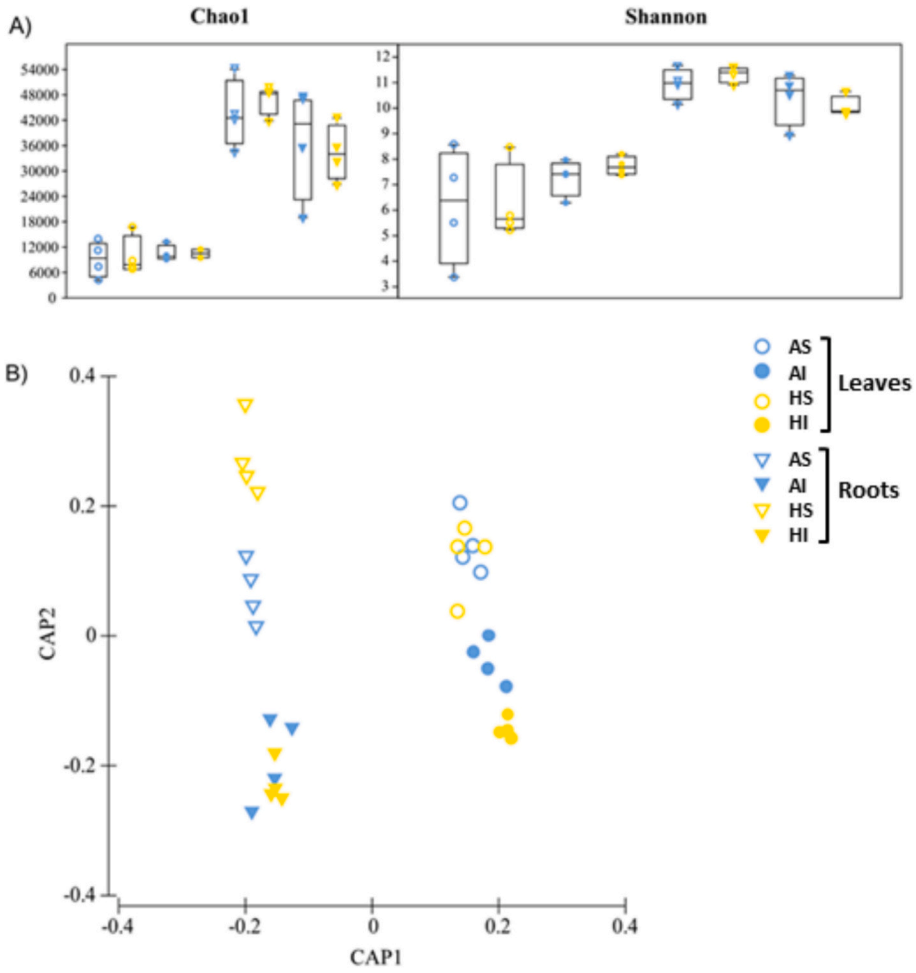

Fig. 5. Microbial α- and β- diversity associated to *P. oceanica* seedlings. A) Boxplots showing Chao1 richness and Shannon diversity indices across treatments and tissues. B) Constrained analysis of principal coordinates (CAP) based on Bray-Curtis dissimilarities. Treatments are coded as AS = Ambient–Solitaire, HS = High-Solitaire, AI = Ambient-Invaded, HI = High-Invaded. Circles represent leaves and inverted triangles represent roots. Colours indicate CO_2 supply conditions (Ambient vs High), while open symbols correspond to Solitaire (n = 24) and filled symbols to Invaded (n = 24) seedlings.

Rhodothermaceae, Kordiimonadaceae, Gammaproteobacteria and Thiotrichales under High CO_2 (Fig. 6).

Macroalgal invasion significantly affected bacterial composition in both tissues (leaves $p = 0.039$, roots $p = 0.030$; Table S5, Fig. 7). The Invaded treatment was enriched with Chromatiales, EC214, Pseudomonas and Phyllobacteriaceae.

4. Discussion

pCO_2 enrichment had almost no effects on plant responses, including photosynthesis, metabolic rates, development, growth, and physiology, with only a tendency toward higher starch content in rhizomes. In addition, the overall microbiome was not strongly influenced by CO_2 enrichment, with only a few bacterial OTUs being differentially abundant in seedlings under this treatment. In contrast, the presence of invasive macroalgae affected the seedlings in numerous, and mostly negative, ways (i.e., plant development, growth and physiologic responses), including an impact at the microbial community level.

The lack of major effects from CO_2 increase on plant responses may be related to seedling age and incubation time. For instance, for *P. oceanica* seedlings, Hernán et al. (2016) observed that photosynthetic enhancement under High CO_2 conditions occurred primarily during early seedling development (in their case, for seedlings not more than 3 months old), whereas we worked with seedlings with a starting age of 3 months and for which we measured photosynthetic responses when they were ca. 6.5 months old (after 22 weeks of experimental treatment; Table S1). Consistently, a field study on *P. oceanica* seedlings of similar age (5–7 months) also reported that acidification limits rather than stimulates morphological development (Pansini et al., 2023), further supporting the idea that CO_2 enrichment effects may be constrained at this life stage. In our experiment, it is possible that the higher starch content in rhizomes detected under elevated CO_2 reflects increased photosynthesis and carbon allocation during earlier developmental stages, or alternatively, reduced consumption of reserves, with photosynthetic effects ceasing toward the end of the experiment. Studies in other seagrass species reveal similarly variable and context-dependent responses, such as responses ranging from enhanced photosynthesis without growth increase (as in *Cymodocea serrulate*; Ow et al., 2015; Repolho et al., 2017) to coupled increases in both productivity and biomass (as in *Halodule uninervis* and *Thalassia hemprichii*), with responses changing with exposure duration (with effects appearing within 2 weeks but potentially diminishing over longer periods), species-specific carbon allocation strategies (Ow et al., 2015), and with the interactive effects of other environmental stressors such as warming (Repolho et al., 2017).

In contrast to the limited CO_2 effects, the presence of invasive macroalgae significantly altered photosynthetic parameters. Seedlings growing with invasive macroalgae exhibited higher photosynthetic efficiency at low irradiance (increased Alpha), higher Yield, and lower light saturation (E_k). These changes are characteristic of shade

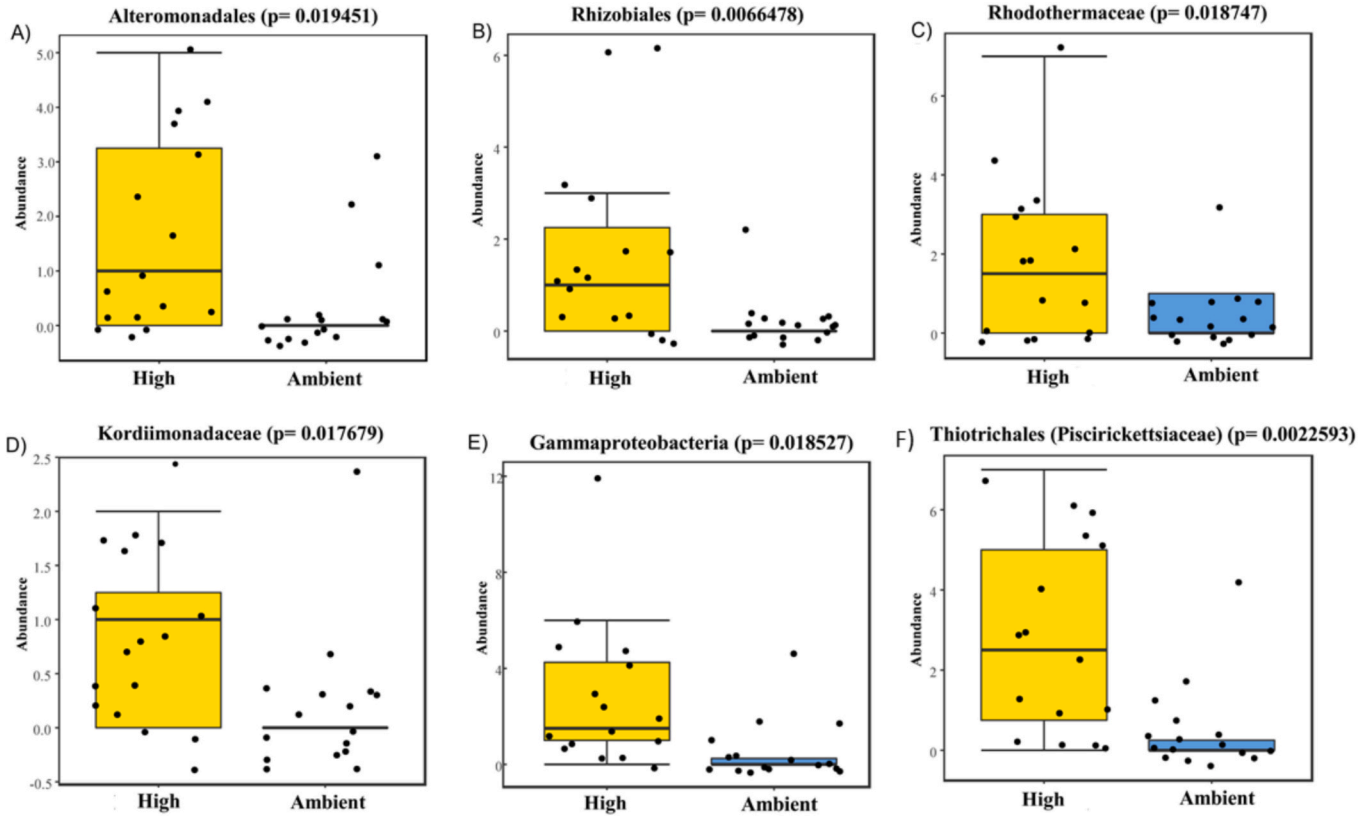

Fig. 6. Boxplots showing taxa significantly affected by CO_2 conditions according to univariate analysis at the feature level: A) Alteromonadales, B) Rhizobiales, C) Rhodothermacea, D) Kordiimonadaceae, E) Gammaproteobacteria, and F) Thiotrichales (Piscirickettsiaceae). Comparisons are shown for High CO_2 (yellow; n = 24) and Ambient CO2 (blue; n = 24) supply conditions, irrespective of tissue and association with invasive seaweeds. Only taxa with higher relative abundance under High CO_2 supply are represented. *p*-values are indicated for each taxon (significance thresholds set at $p > 0.02$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

acclimation responses, whereby plants adjust their photosynthetic apparatus to maximize light capture and utilization under reduced or altered light conditions (Falkowski and Raven, 2007). The presence of the macroalgal canopies likely reduced both light quantity and quality reaching the seedlings, triggering adjustments in photosystem composition, chlorophyll content, or reaction centre efficiency to maintain carbon gain under sub-optimal light (Longstaff et al., 1999; Ralph et al., 2007). Notably, similar shade acclimation responses have been observed in *P. oceanica* seedlings growing beneath the canopy of adult conspecifics, where light limitation inside dense meadows also constrains seedling photosynthesis and growth (Stipcich et al., 2025), suggesting that light limitation is a general bottleneck for *P. oceanica* seedling development regardless of its source. The fact that ETR_{max} remained unchanged suggests that the maximum photosynthetic capacity was not compromised, but rather that seedlings shifted their light utilization strategy toward greater efficiency at lower irradiances.

Although seedlings increased their efficiency to capture C and did not exhibit lower ETR_{max} under the presence of invasive macroalgae, they did display markedly lower NPP and GPP, indicating that shade acclimation could not compensate for the overall negative effects of macroalgal presence. Reduced light availability beneath macroalgal canopies—both in intensity and in the red wavelengths essential for seagrass photosynthesis— often limits total carbon fixation despite increased efficiency (Choice et al., 2014; Cummings and Zimmerman, 2003; Drake1 et al., 2003; Tait et al., 2014). In addition to light limitation, invasion may have also triggered allelopathic interactions, further constraining metabolism and growth (Oliva et al., 2024; Raniello et al., 2007). The energetic costs associated with maintaining shade-acclimated photosystems and coping with chronic invasion-related stress may also have diverted resources away from growth and storage, creating a negative feedback in which acclimation becomes metabolically expensive (Ruiz and Romero, 2001; Yaakub et al., 2014).

Indeed, we detected a dramatic depletion of NSC reserves in rhizome seedlings exposed to invasive macroalgae. Given that these early life stages are highly sensitive to resource limitation and disturbance, and seedlings that exhaust their reserves or fail to build belowground NSC stocks have low probabilities of persistent establishment and survivorship (Balestri et al., 2009; Fernández-Torquemada and Sánchez-Lizaso, 2013; Govers et al., 2015; Pereda-Briones et al., 2020; Statton et al., 2017), the presence of invasive macroalgae may strongly compromise seedling recruitment success. In accordance with limited capacity to store NSC, we also observed lower C content in roots and rhizomes of Invaded seedlings, further suggesting that seedlings may have been carbon limited, not only in their ability to store soluble carbohydrates but also structural carbon compounds (cell walls, lignin; Duarte, 1990), potentially compromising their structural integrity.

Furthermore, seedlings exposed to macroalgal invasion exhibited 2–3 times lower biomass of both rhizomes and leaves, and 20–40% fewer leaves and substantially reduced leaf area compared to Solitaire seedlings. In fact, the higher %N content observed in leaves of Invaded seedlings likely reflects a limitation use of such nitrogen for growth (Invers et al., 2004; Touchette and Burkholder, 2000). This pattern may partly reflect invasion-driven shifts in the root-associated microbiome, as microbial associates of *P. oceanica* are key contributors to ammonification and nitrogen supply to the host (Pfister et al., 2023). Disruption of these microbial communities by invasive macroalgae could alter nitrogen cycling in the rhizosphere, potentially increasing nitrogen availability to seedling leaves while simultaneously impairing overall plant growth. These dramatic reductions in development and biomass accumulation further highlight that invasive macroalgae may severely

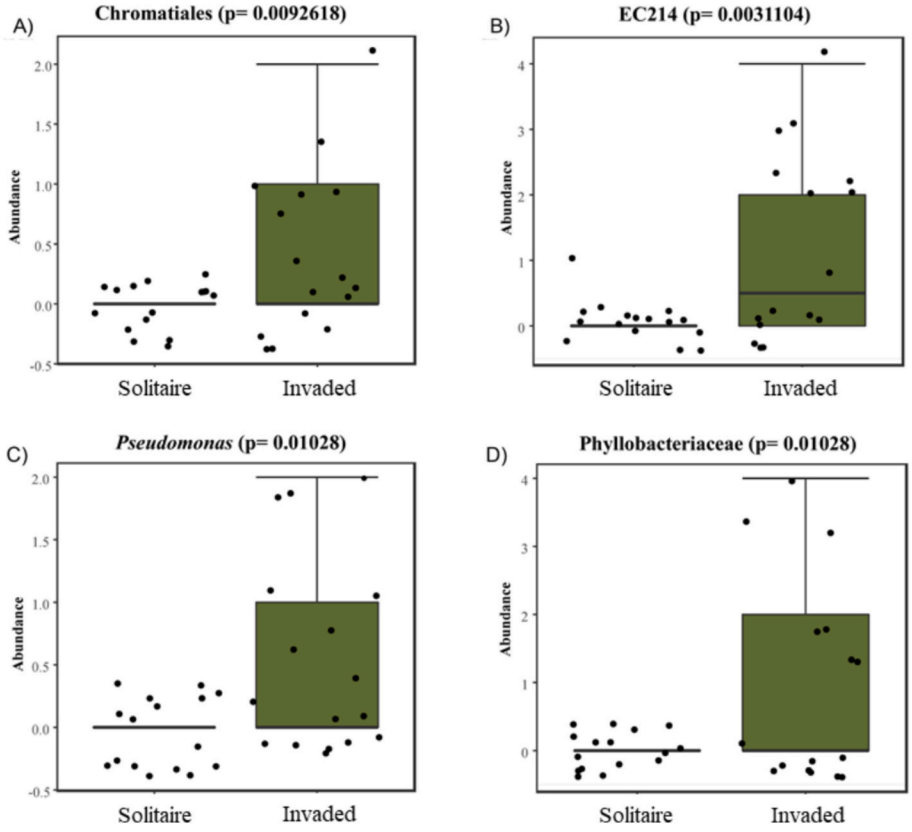

Fig. 7. Boxplots showing taxa significantly affected by treatment according to univariate analysis at the feature level: A) Chromatiales, B) EC214, C) *Pseudomonas*, and D) Phyllobacteriaceae. Comparisons are shown for Solitaire (light green; n = 24) and Invaded (dark green; n = 24) treatments, irrespective of tissue type and CO_2 supply condition. Only taxa with higher abundance in Invaded treatments are represented. *p*-values are indicated for each taxon (significance thresholds set at $p > 0.002$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compromise the establishment potential of *P. oceanica* recruits, with critical implications for meadow regeneration and long-term resilience. However, experimental work by Pereda-Briones et al. (2019) indicates that, at least when cultured separately, *L. trichoclados* and *C. cylindracea* do not have strong negative effects on *P. oceanica* seedlings, which suggests that the detrimental impacts observed here may result from additive or synergistic interactions when both species co-occur. Since our Invaded treatment included both species simultaneously, we cannot disentangle the relative contribution of each species to the observed effects, which represents a limitation of our study. Interestingly, a field study using one-year-old seedlings reported that *C. cylindracea* alone can even promote root development and enhance seedling establishment (Pereda-Briones et al., 2018), contrasting with our findings. This discrepancy may reflect differences in seedling age — older seedlings with more developed root systems may be more resilient to macroalgal competition — as well as the combined pressure of two invasive species in our mesocosm experiment, which likely produced stronger competitive and allelopathic effects than a single species in field conditions.

In addition, changes in nutrient dynamics may have also been influenced through microbially-mediated processes. For instance, leaves in *P. oceanica* strongly contribute to N_2 fixation (Agawin et al., 2017), which is accelerated under elevated CO_2 (Berlinghof et al. 2024) Other studies likewise highlight the important role of associated microbes in nitrogen cycling and nutrient supply to seagrasses, including rhizosphere diazotrophs that mobilize ammonium along plant-driven redox gradients (Brodersen et al., 2024), warming-induced microbiome shifts that alter N and S cycling pathways (Aires et al., 2024), and nutrient-driven enrichment of N- and S-cycling bacteria in root communities (Wang et al., 2020). Our altered %N and C:N ratios in Invaded seedlings may therefore reflect similar microbe-driven changes in nitrogen cycling triggered by macroalgal presence. In particular, invasion-associated changes in root microbiome composition observed here may have disrupted ammonification pathways, altering nitrogen supply to the host and contributing to the elevated leaf %N detected in Invaded seedlings (Pfister et al., 2023).

At the microbiome level, our results show that root-associated communities were inherently richer and more diverse than those on leaves, yet this diversity declined markedly under invasion while remaining largely unaffected by CO_2 enrichment. Such patterns align with growing evidence that seagrass microbiomes tend to be taxonomically resilient to ocean acidification but highly sensitive to local biotic pressures (Banister et al., 2022; Berlinghof et al., 2024; Pfister et al., 2023). In our case, elevated CO_2 produced only subtle shifts—such as enrichments in Alteromonadales, Rhizobiales and Rhodothermaceae—whereas invasion favoured taxa commonly linked to organic loading or potential pathogenicity (e.g., EC214, Pseudomonas, Phyllobacteriaceae). The occurrence of Pseudomonas is noteworthy: while this genus includes many known plant pathogens (Hirano and Upper, 2000), some species are also involved in nutrient cycling processes such as denitrification and phosphate solubilization (Das Mohapatra et al., 2024). Regardless of their specific functional role, the key point is that the overall shift in community structure under invasion likely has functional consequences for the host, potentially both increasing disease susceptibility and altering nutrient dynamics available to the seedlings.

Our results suggest that macroalgal invasions create a complex stress environment where both carbon limitation (via shading and reduced photosynthesis) and altered nutrient dynamics (via competition and microbially-mediated processes) interact to shift seedling physiology, development and stoichiometry. Critically, elevated CO_2 had no effect

on seedling elemental composition and did not mitigate the negative effects of invasive macroalgae, which were consistently expressed at both physiological and microbial levels. This indicates that CO_2 enrichment alone is unlikely to enhance the resilience of *P. oceanica* seedlings under invasion pressure, and that negative macroalgae–seagrass interactions may already operate at the recruitment stage, preventing seedling establishment and thereby limiting meadow regeneration. These findings highlight that future ocean conditions may not provide the anticipated competitive benefit for seagrasses if biological invasions persist, underscoring the need for local management actions—such as controlling invasive species—to safeguard seagrass recovery and persistence.

CRediT authorship contribution statement

Iris E. Hendriks: Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Julia Máñez-Crespo:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation. **Clea N. van de Ven:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **R. Sean Fitzpatrick:** Methodology, Investigation, Conceptualization. **Alberto V. Borges:** Writing – review & editing, Validation, Conceptualization. **Willy Champenois:** Writing – review & editing, Validation. **Tania Aires:** Writing – review & editing, Visualization, Validation, Resources, Formal analysis, Data curation, Conceptualization. **Aschwin H. Engelen:** Writing – review & editing, Visualization, Resources. **Gerard Muyzer:** Writing – review & editing, Validation, Resources, Conceptualization. **J. Arie Vonk:** Writing – review & editing, Validation, Conceptualization. **Fiona Tomas:** Writing – original draft, Validation, Resources, Methodology, Investigation, Conceptualization.

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

During the preparation of this work, the author used ChatGPT (OpenAI) in order to improve the quality of the English language and to assist in resolving coding issues with R. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the published article.

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.

Acknowledgements

The authors want to thank Laura Pereda-Briones for help with seed collection and maintenance, and the Cabrera National Park Visitor Centre for providing seawater. This work was funded by the Ramon y Cajal Program to FT, the Fulbright Scholar Program to RSF and the Spanish Ministry of Economy and Competitiveness (Project MEDSHIFT, CGL2015-71809-P) to IEH. AVB is a research director at the Fonds National de la Recherche Scientifique (FNRS). TA was funded by a scholarship from the Portuguese Foundation for Science and Technology with the reference SFRH/BPD/116774/2016. FT was partly funded by the Subprograma Estatal de Formación, Atracción y Retención del Talento en el marco del PEICTI 2024, from the Spanish Ministry of Science, Innovation and Universities within the program "Estancias de movilidad en centros extranjeros de enseñanza superior e investigación" (PRX24/00396). GM was supported by the RESTORESEAS project, funded by the Dutch Ministry of Agriculture, Nature, and Food Quality, and by the University of Amsterdam's Research Priority Area 'Systems Biology'. This study received Portuguese national funds from FCT - Foundation for Science and Technology through contracts UID/04326/2025, UID/PRR/04326/2025 and LA/P/0101/2020 (DOI:10.54499/LA/P/0101/2020).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2026.120063.

Data availability

Data will be made available on request.

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