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Microbe-assisted seedling crop improvement by a seaweed extract to address fucalean forest restoration

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In the Mediterranean, *Cystoseira sensu lato* (*s.l.*) (Phaeophyceae) forests have sharply declined and restoration measures are needed to compensate for the loss. Assisted regeneration through the outplanting of seedlings grown *ex-situ* has proven to be a sustainable option. Optimizing mesocosm culture can maximize survival of the most critical embryonic stages and reduce long-term maintenance costs. Host-microbiome interactions could also play a crucial role in seedling development and welfare. In this context, we aimed to advance a cultivation protocol that stimulates the growth and fitness of *Ericaria amentacea* (Phaeophyceae) seedlings and identify the associated microbial biofilm communities. Seedlings were cultured in 6 treatments [i.e., filtered seawater (SW, C, Control), von Stoch-enriched SW (VS), VS + algal extract (VSA), algal extract-enriched SW: A1< A2< A3]. After the field, A2 seedlings had the highest cover (1372 $\pm$ 53.66 mm^2), which was 1.8 and 1.9 times greater than in VS and VSA, respectively. The addition of the algal extract and nutrients significantly affected the structure and composition of the microbial community that shifted over time in each culture medium. We identified a treatment-specific microbial fingerprint. After the mesocosm phase, A2 was characterized by 4 unique taxa: *Postechiella*, *Winogradskyella, Roseovarius* and *Arenibacter* (Bacteria). Given the success of A2 seedlings, we propose the probiotic consortia candidates characterized by the unique treatment-taxa in conjunction with the shared taxon *Psychroserpens* (Bacteria, present in A1, A2, VSA, VS) and the reminder community. Within the holobiont concept, the effect of algal extract or nutrients on the algae and/or biofilm could have important consequences for tuning the overall interaction networks. Our study has shown that macroalgal restoration could benefit from both the use of commercial algal extract and tailored nutrient enrichment in *ex-situ* cultures and the identification of probiotic consortia candidates that promote seedling growth.

1 Introduction

In the Mediterranean Sea, *Cystoseira sensu lato* (*s.l.*) (Phaeophyceae) form extensive forests, which are among the most productive habitats in the coastal zone (Fabbrizzi et al., 2020). In recent decades, *Cystoseira s.l.* canopies have declined significantly in many coastal regions and are now critically threatened (e.g. Mangialajo et al., 2008; Falace et al., 2010; Vergés et al., 2014; Thibaut et al., 2015; Blanfuné et al., 2016; Valdazo et al., 2017), due to multiple stressors such as overgrazing, eutrophication, habitat fragmentation and climate change (e.g Thibaut et al., 2005; Mangialajo et al., 2008; Thibaut et al., 2015; Blanfuné et al., 2016; Mancuso et al., 2018; Bevilacqua et al., 2019; Falace et al., 2021). There is little evidence of natural recovery of damaged populations, hampered by low zygote and embryo dispersal (Clayton, 1990; Buonomo et al., 2017), so proactive actions are needed to support the regeneration of such populations. Restoration is increasingly recognized as useful tool to trigger the recovery of degraded coastal habitats (Abelson et al., 2020), as stated by the UN Decade of Ecosystem Restoration (2021-2030; https://www.decadeonrestoration.org/). Several techniques have been advanced for the restoration of *Cystoseira s.l.*: transplantation of adult thalli (Falace et al., 2006; Susini et al., 2007), *in situ* deployment of receptacles (Verdura et al., 2018; Medrano et al., 2020) and outplantation of seedlings grown *ex situ* (Falace et al., 2006; Sales et al., 2011; Falace et al., 2018a; Verdura et al., 2018; De La Fuente et al., 2019; Orlando-Bonaca et al., 2021; Savonitto et al., 2021; Lardi et al., 2022; Orlando-Bonaca et al., 2022). The latter two techniques are preferable to avoid further depletion of donor populations (De La Fuente et al., 2019).

Optimizing growth conditions can maximize zygote settlement and survival of the most critical embryonic stages and reduce the cost of long-term maintenance (Savonitto et al., 2021; Clausing et al., 2022; Orlando-Bonaca et al., 2022). Nevertheless, overgrowth of bacteria, epiphytic diatoms and filamentous algae could strongly limit growth and development of zygotes (Orlando-Bonaca et al., 2021; Clausing et al., 2022; Lardi et al., 2022). To overcome these issues, commercially manufactured extracts of a variety of seaweeds have recently been used to reduce epiphyte attachment (Jiksing et al., 2022) and as biostimulants to increase survival, growth and stress tolerance of macroalgae, including brown algae (Hurtado and Critchley, 2018; Umanzor et al., 2019; Hurtado and Critchley, 2020; Umanzor et al., 2020a; Umanzor et al., 2020b; Ali et al., 2021; Han et al., 2022; Jiksing et al., 2022; Umanzor et al., 2022). Macroalgae-derived biostimulants are also used as a sustainable option to improve agronomic production, plant growth and health (Crouch and Van Staden, 1993; Battacharyya et al., 2015; Hurtado and Critchley, 2020; Samuels et al., 2022), thanks to their broad spectrum of constituents (i.e., macro- and microelements, amino acids, hormones, phenolic compounds and saccharides) (Khan et al., 2009; Ali et al., 2021; Sujeeth et al., 2022). Biostimulants are defined as substances and materials, excluding pesticides and nutrients, that can modify the physiological processes of plants improving their growth, development and/or responses to stress (du Jardin, 2012; Rouphael and Colla, 2020). In particular, they promote natural processes for efficient nutrient uptake and utilization,

chlorophyll content and photosynthesis, stress resistance, root development and also trigger early flowering and seed germination (Blunden and Wildgoose, 1977; Crouch and Van Staden, 1993; Van Oosten et al., 2017; Ali et al., 2021; Shukla et al., 2021; Sujeeth et al., 2022).

The interactions between host and microbiome can also play a crucial role in development and welfare in the early life stage, as has been shown for corals and sponges (Taylor et al., 2007; Cleary et al., 2022; Peixoto et al., 2022; Thatcher et al., 2022). In the marine field, microbe-assisted crop improvement is still in its infancy compared to agriculture, where plants benefit from mixtures of diverse microbes to protect them from pests or pathogens, improve nutrient supply and directly promote growth through microbial antagonistic interactions (Berg et al., 2021). A growing body of literature (reviewed in Florez et al., 2017 and Duarte et al., 2018) on microbe-algae highlights the paramount importance of the tiny unseen majority in morphological development (Matsuo et al., 2003; Grueneberg et al., 2016), antimicrobial activity and control of microbial colonization *via* quorum sensing (Manefield et al., 1999; Holmström et al., 2002; Rao et al., 2005; Wang et al., 2009) and algal status under healthy and stressful conditions across the lifespan (Ivanova et al., 2002; Mancuso et al., 2016; Minich et al., 2018; Juhmani et al., 2020). The microbial biofilm forms an outer layer over the host epidermis that plays a functional role between the host and the environment, and is almost considered a new and functionally different "tissue" (Steinberg et al., 2011). The microbes living on the algal surface have an abundance of 10^6 to 10^9 bacteria per cm^2 and a high phylogenetic diversity (Martin et al., 2014), and differ from the surrounding seawater community (Bengtsson et al., 2012; Michelou et al., 2013; Florez et al., 2017). The algae provide a rich substrate for the microorganisms to thrive, and antagonistic relationships can strongly shape the community alongside algal physiology. As the macroalgal surface is highly dynamic, the functioning of the microbial interface intimately depends on the complex relationships between the host and all-associated organisms (Trevathan-Tackett et al., 2019; Dittami et al., 2021). Hence it is useful to view it as a network of interactions forming a functional synergistic unit called a holobiont (Zilber-Rosenberg and Rosenberg, 2008). The associated microbes play a crucial role in the biology, ecology, and well-being of their hosts, and therefore altering these associations can lead to a pathological state within the holobiont (Egan and Gardiner, 2016; Pitlik and Koren, 2017; Sullivan et al., 2018; Longford et al., 2019; van der Loos et al., 2019).

In the context of *ex-situ* restoration, it may therefore be relevant to investigate the microbial biofilm associated with the algae in mesocosm cultures to identify possibly probiotic communities with beneficial effects on the algal fitness once they are outplanted in the field. As the application of algal-derived biostimulants in ecological restoration also needs to be explored (Hurtado and Critchley, 2020; Jiksing et al., 2022), in the present study, we used a commercial extract derived from *Macrocystis pyrifera* (Linnaeus) C. Agardh (Laminariales, Ochrophyta) as an additive to culture media for the cultivation of *Ericaria amentacea* (C.Agardh) Molinari & Guiry (Fucales, Ochrophyta) seedlings.

Within this framework, the aims of this study were: (i) to investigate the effects of different culture media on the survival,

growth and photosynthetic efficiency of *E. amentacea* seedlings both in culture and in the field, (ii) to identify the microbial biofilm associated with *E. amentacea* seedlings during an *ex-situ* experiment and (iii) to create conditions to promote potentially beneficial microbes by using a seaweed extract.

2 Materials and methods

2.1 Fieldwork

Fertile apices of *E. amentacea* were collected in July 2021 at Bogliasco in the Ligurian Sea (NW Italy, 44°22′31.4″ N, 9°04′35.2″ E) and transported to the facilities of the University of Trieste in the dark and at 4°C.

2.2 Effects of culture media on seedling survival and growth

2.2.1 Culture in mesocosms

Apices were rinsed with filtered seawater, carefully cleared of epiphytes and stored in the dark at 4°C for 24 hours to promote gamete release.

Six culture media were tested: *i*) autoclaved filtered seawater (SW) (0.22 μm Durapore membrane philtre, Merck millipore Ltd) as control (**C**); *ii*) SW enriched with Von Stosch's solution (VS) (Von Stosch, 1963; Guiry and Cunningham, 1984; Falace et al., 2018a); *iii*) SW enriched with the commercial macroalgal extract AlgatronCifo® [Cifo S.p.A., San Giorgio di Piano, Bologna, Italy]

(here after Algatron) at three concentrations (i.e., A1 = 2 mL L^{-1}; A2 = 4.5 mL L^{-1}; A3 = 9 mL L^{-1}). The concentrations of the algal extract for solutions A2 and A3 correspond to the manufacturer's recommendations for foliar and soil application, respectively, while concentration A1 was chosen to test whether *E. amentacea* could benefit from a lower concentration; *iv*) VS solution containing 4.5 mL L^{-1} Algatron as in A2 (VSA). Algatron contains N, P, alginic acid, mannitol, other carbohydrates, polyphenols and essential amino acids (Prisa, 2021). Information on the culture media and N:P ratio are reported in Supplementary Table 1.

To test the effects of the different culture media on the microbial biofilm communities associated with the seedlings and the overall performance of the culture, we set up tanks containing 2 L of culture medium (n= 3 tanks per treatment), and 11 clay tiles (4.5 cm diameter with a central hole of 0.8 cm diameter) (Figure 1), each containing four randomly assigned fertile apices. The tiles were sterilized by autoclaving and the tanks were cleaned with 1% HCl. The apices were removed after 24 hours and the tiles left undisturbed for another 24 hours to ensure attachment of the zygotes.

Seedlings were grown for three weeks in environmentally controlled rooms under optimal conditions as previously determined by Falace et al. (2018a). The temperature was 20°C, the light intensity 125 μmol photons m^{-2} s^{-1} (LED lamps AM366 Sicce USA Inc., Knoxville, USA) with 15:9 h light:dark photoperiod. Air temperature was automatically set by the room controller and water temperature was measured daily with a thermometer in each aquarium to ensure it was constant. The culture medium was changed every four days to avoid nutrient depletion and the tanks
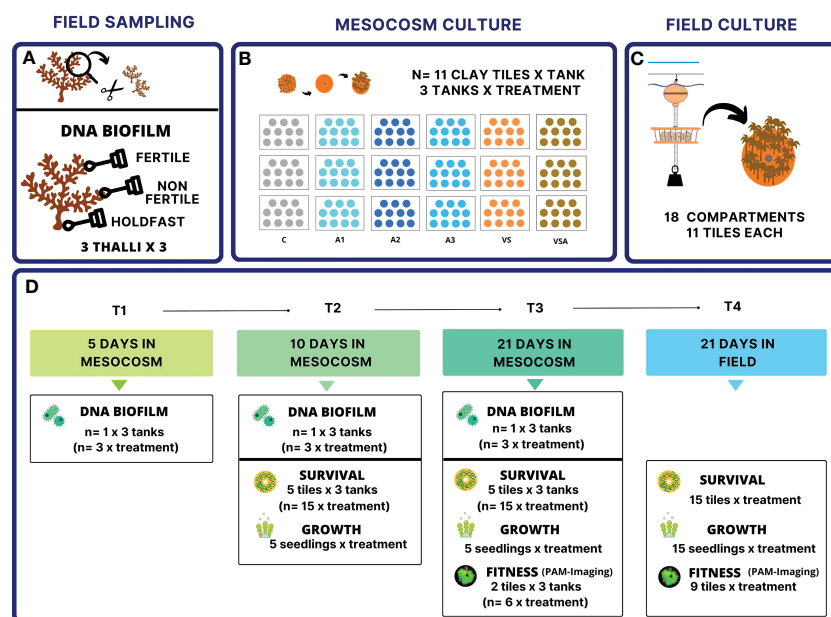
FIGURE 1
Experimental design. **(A)** field sampling, **(B)** mesocosm culture, **(C)** field culture. Culture media: A1 = 2 mL L^{-1} Algatron; A2 = 4.5 mL L^{-1} Algatron; A3 = 9 mL L^{-1} Algatron; C= filtered seawater; VS, SW enriched with Von Stosch's solution; VSA, VS solution with 4.5 mL L^{-1} Algatron. **(D)**. Summary of collected data. The days (T) are counted from the release of the zygotes (Supplementary Table 1). the tiles from the same tank are considered pseudo-replicates.

were cleaned accordingly with 1% HCl. The tanks were oxygenated using air pumps and bubblers. Treatment aquaria as well as tiles within the aquaria were randomly repositioned each time the medium was changed to control for positional variations in light and temperature intensity.

The following data were collected (Figure 1):

- *Seedling survival* (i.e., tile area covered by seedlings, mm^2) was measured on day 10 (T2) and 21 (T3) post fertilization (PF). The area was estimated from images (Pentax k5 MarkII, Pentax, Tokyo, Japan). At each time point, n= 5 tiles per tank (n= 15 per treatment) were randomly selected for measurements. Images were processed using ImageJ software (Schneider et al., 2012).

- *Seedling growth* (i.e., length, mm) was measured at T2 and T3 with an inverted microscope (Leica, DM IL LED) and photographed with a Canon Powershot G9. At each time point, n= 5 seedlings per treatment were randomly selected from the tiles in the tanks. The limited replication for this variable was necessary because the measure implies destructive sampling.

- *Seedling fitness* (i.e., F_v/F_m); *in vivo* chlorophyll-a fluorescence (ChlaF) of the photosystem II (PSII) was measured at T3 on 2 tiles per tank (n= 6 per treatment) using the PAM-Imaging Fluorometer Open FluorCam (Photon Systems Instruments, Brno, Czech Republic). Shutter time and sensitivity of the charge-coupled device (CCD) camera were set to 1 and 10, respectively. Each tile was placed at a constant distance of 17.5 cm below the camera objective, and the lamps were placed at an angle of 45° to the center of the measurement area. Prior to measurements, the tiles with seedlings were dark adapted for 20 min to allow complete oxidation of the PSII reaction centers prior to measurements, then the basal fluorescence (F_0) was measured (Krause and Weis, 1984). Then a saturating pulse of actinic light (4040 μmol m^{-2} s^{-1}, 0.8 s) was administered to induce maximum fluorescence (F_m). The maximum quantum yield of PSII was calculated as follows: $F_v/F_m = [F_m - F_0]/F_m$ (Maxwell and Johnson, 2000).

2.2.2 Cultivation in the field

After cultivation in the mesocosms, all the tiles were transferred outdoors for the next phase of cultivation in the sea. The tiles were attached to a hanging structure in a way that they could surface regularly, as the species naturally does in the intertidal. The hanging structure consisted of 18 contiguous compartments; each set of 11 tiles (i.e., one set for each tank of each treatment) was labeled and randomly assigned to one of the 18 compartments.

After 21 days (T4), the tiles were returned to the laboratory and measured as in the first phase (Figure 1):

- *Seedling survival* was estimated from images for which 5 tiles per compartment (n= 15 per treatment) had been randomly selected.

- *Seedling growth* was measured from 5 randomly selected seedlings per compartment (n= 15 per treatment).

- *Seedling fitness* was measured on 3 randomly selected tiles per compartment (n= 9 per treatment).

2.2.3 Statistical analyses

Analysis of Variance (ANOVA, R Core Team, 2022) was performed to test the effects of culture medium and exposure time on seedling survival and growth (i.e., cover, length) and fitness (i.e., Fv/Fm). For cover, the design for the analysis consisted of the two factors Time (Ti, fixed, 2 levels), and Medium (Me, fixed, 6 levels, crossed), with n=3. For Fv/Fm, the design included only factor Me (as this variable was measured at the end of the lab experiment, at T3), with n=3. For these two variables, the average values measured in the three tanks were used as independent replicates for each treatment and each time (Millar and Anderson, 2004). For seedling length, measurements were made on a total of n=5 seedlings across tanks to avoid excessive tile manipulation (see above). In this case, the design for ANOVA included the factors Ti and Me, with n=5 seedlings randomly selected across all tanks for each treatment (i.e., medium culture) and sampling time.

For the field experiment, ANOVA was used to test for differences among treatments on seedling survival, growth and welfare. In this case, the design for the analysis included only factor Me with n=3 replicates per each level of the factors. Analogously to the analysis of laboratory data, the average values measured in the three sections corresponding to the original laboratory tanks were used as independent replicates for each level of factor Me.

The assumption of normality of response variables was tested using the Shapiro-Wilk test, while Cochran's C-test (Underwood, 1997) was used to test the assumption of homogeneity of variances prior to analysis. For all response variables, since non-normality and/or variance heterogeneity remained after data transformation, untransformed data were analyzed with a permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) based on Euclidean distance with 5,000 permutations. Significant interaction terms involving the factor Me or its main effect were examined by *post hoc* pairwise t-tests. PERMANOVA makes no assumptions about the data distribution and is robust to variance heterogeneity in experiments with balanced designs (Anderson, 2014), as in our case.

2.3 Microbiome: DNA extraction, PCR conditions and sequencing

On the day of sampling, microbial biofilms associated with three whole thalli were collected in triplicate by rubbing three areas with sterile cotton swabs: basal holdfast, non-fertile and fertile apices. Thalli that were overgrown with epiphytic algae or animals were avoided. The swabs were immediately placed in sterile 1.5 mL Eppendorf tubes and stored in DNA-RNA shield solution (Zymo Research). Samples were transported at -20°C in the dark and stored at -80°C until DNA extraction.

During the mesocosm experiment, microbial biofilm was sampled in triplicate per treatment with sterile cotton swabs from independent tiles of *Ericaria amentacea* seedlings at T1 (i.e., 5 days post fertilization, PF), T2 (i.e., 10 days PF) and T3 (i.e., 21 days PF, Figure 1). We followed the microbial abundance in the water compartment over the experiment. Samples were analyzed at the BD FacsCanto cytometer after SYBRGreenTM I staining according to Gasol and Del Giorgio (2000) (Supplementary Data Sheet). It was beyond the scope of this work to investigate the free-living microbial community in the bulk-water during the mesocosm phase. We used the E.Z.N.A Soil DNA Kit (Omega Bio-Tek) to extract DNA from the swabs. Extracted DNA was quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). We targeted the V4 hypervariable region of the 16S rRNA gene with 515F primer (Caporaso et al., 2011), and a mix of 802R (Claesson et al., 2009) and 806R (Caporaso et al., 2011) primers. Primers were tailed with two different GC rich sequences enabling barcoding with a second amplification step. We run three technical replicates for each sample in 20 μL of volume reaction containing 10 μL AccuStartII PCR ToughMix 2X (Quanta Bio), 1 μL EvaGreen™ 20X (Biotium), 0.8 μL 515 F (10 μM— 5' modified with unitail 1-CAGGACCAGGGTACGGTG-), 0.4 μL 802 R (10 μM— 5' modified with unitail 2-CGCAGAGAGGCTCCGTG-), 0.4 μL 806 R (10 μM— 5' modified with initial 2-CGCAGAGAGGCTCCGTG-), and 50 ng of DNA template. We used a CFX 96™ PCR System (Bio-Rad) with a real-time limited number of cycles (94°C for 20 s, 55°C for 20 s, 72°C for 60 s). The second PCR amplification (outer PCR) was required to uniquely label each sample. We used a forward primer composed of the 'A' adaptor, a sample-specific 10 bp barcode and the tail 1 of the primary PCR primers, and a reverse primer composed of the P1 adaptor sequence and the tail 2. We followed the protocol of Giglio et al. (2021) for creating the library and setting the sequence reaction with Ion 316 chip (Life Technologies) in the Ion Torrent PGM System.

2.3.1 Microbiome bioinformatics pipeline and analyses

The CLC Microbial Genomics Module as part of CLC Genomics Workbench 20.0 (QIAGEN Digital Insights, Aarhus, Denmark) was used to analyze alpha and beta diversity, and bacterial community composition. Raw sequencing reads were imported into the CLC environment, and we performed quality control, primers and adapter sequences removal and minimum size cut-off of 150 bp, chloroplast, mitochondrial, unassigned and *Eukarya* sequences were removed from the database. Operational Taxonomic Units, OTUs, were picked by mapping sequences against the SILVA 16S v138 97% database (Quast et al., 2013) at the same identity percentage to observe OTU at the species level. We focused on the most abundant top twenty OTUs. We computed the alpha diversity and then performed the Kruskal-Wallis H test to assess statistical significance Results with q-value < 0.05 were considered significant. Alpha diversity, the diversity within the sample (Whittaker, 1972) based on OTUs, was computed among the three adult thalli and seedlings using the Chao 1 index. A principal coordinate analysis (PCoA) of the beta diversity distance

matrices was performed. We used the Bray–Curtis distance where we considered the abundance of each OTU balanced with the cladistic information (weighted UniFrac index). We generated a heat-map by clustering the z-score normalized relative abundance OTU data using the Euclidean distance and the average linkage to identify the shared taxa present in adult thalli and in the seedlings. We ran indicator species analysis using the R package indicspecies (De Cáceres and Legendre, 2009) to identify associations among OTUs and treatments with statistical significance. 148 OTUs at the genus level with a relative abundance >1% were selected and uniquely associated with one or more treatment combinations.

3 Results

3.1 Effects of culture media on seedling survival and growth

Seedling survival was significantly affected by the interaction of treatment and time during the mesocosm culture (p-value=0.0036) and by treatment after the field period (p-value=0.0002) (Supplementary Table 2).

At T2, significant differences were observed between the culture media in terms of survival (i.e., tile coverage) (Figure 2A). A2 seedlings had the highest survival (85.31 ± 6.43 mm^2), while C and A3 had the lowest survival (28.99 ± 4.40 and 22.43 ± 1.77 mm^2, respectively). The differences between treatments increased with time. At T3, the seedlings cultured in seawater (C) and A3 had the lowest survival (5.09 ± 0.90 mm^2 and 8.23 ± 1.49 mm^2 respectively) (Figure 2A). Seedling survival in A1, A2, VS and VSA was comparable at T3 (Figure 2A), with the highest values for VSA (201.00 ± 30.26 mm^2) and A2 (187.00 ± 21.29 mm^2).

The lowest growth was observed at T3 for the C condition (length= 0.45 ± 0.03 mm), while it was highest for VSA (length= 2.30 ± 0.21 mm) and VS (length= 2.24 ± 0.15 mm). However, seedlings growing in VSA and VS had the lowest photosynthetic efficiency (i.e., F_v/F_m 0.51 ± 0.02 and 0.46 ± 0.02, respectively) (Figure 3A). In contrast, seedlings cultivated in Algatron (i.e., A1, A2, A3) and in C showed optimal photophysiological fitness (F_v/F_m > 0.69) (Figure 3A). At T3, tiles and seedlings cultivated in VS and VSA were covered by epiphytes, while they were absent in the Algatron and seawater treatments (Figure 4).

After the seedlings were exposed to the field (T4), there were still significant differences in survival between treatments (Supplementary Table 2). Seedlings cultured in seawater (C) did not survive, while seedlings in A2 had the highest coverage (1372.00 ± 53.66 mm^2), which was 1.8 and 1.9 times higher than seedlings from VS and VSA, respectively (Figure 2B).

At T4, seedling length was comparable in all treatments (Supplementary Table 2). Seedlings from A3 showed the lowest growth (length= 3.53 ± 0.17 mm), while the highest growth was recorded in seedlings from VSA (4.69 ± 0.20 mm) and A2 (4.45 ± 0.20 mm).

The F_v/F_m values of all treatments were within the range of values indicating healthy PSII (Figure 3B), although a significant
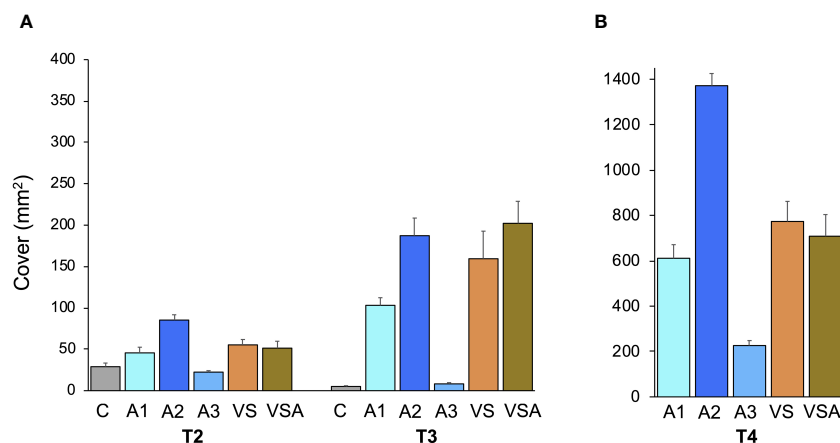
FIGURE 2
Mean values (± SE) of tile area covered by *Ericaria amentacea* seedlings: **(A)** in mesocosm at T2 (10 days post fertilization) and T3 (21 days post fertilization); **(B)** after 3 weeks in field culture (T4). C (seawater, SW); A1 (SW with Algatron 2 mL L^{-1}); A2 (SW with Algatron 4.5 mL L^{-1}); A3 (SW with Algatron 9 mL L^{-1}); VS (SW with addition of von Stoch); VSA (SW with addition of von Stoch and Algatron 4.5 mL L^{-1}). At T4, no seedlings could be measured for C, as the tiles were free of seedlings.

difference was observed between A1 and the other treatments (Supplementary Table 2).

3.2 Microbiome: structure and dynamics

The analysis of the 16S rRNA genes allowed the identification of the most abundant microbial taxa associated with the seedlings and adult thalli of *E. amentacea*. A grand total of 2373362 sequences were generated and 97.5% were assigned to OTUs. The rarefaction curves showed that the sequencing effort sufficed sample biodiversity (Supplementary Data Sheet). Seedling microbial biofilm replicates were significantly correlated within treatment ($p < 0.05$). Alpha diversity analysis (Chao 1 index, Figure 5) showed that the whole thalli were more diverse than the seedlings. The alpha diversity index of the microbial biofilm among the different treatments for the total number of families (Figure 6) showed that A2 and A3 were not significantly different, while A2 was different from the other treatments (see p-values in Figure 6), and had the less diverse biofilm communities. Over time, diversity increased in all other treatments.
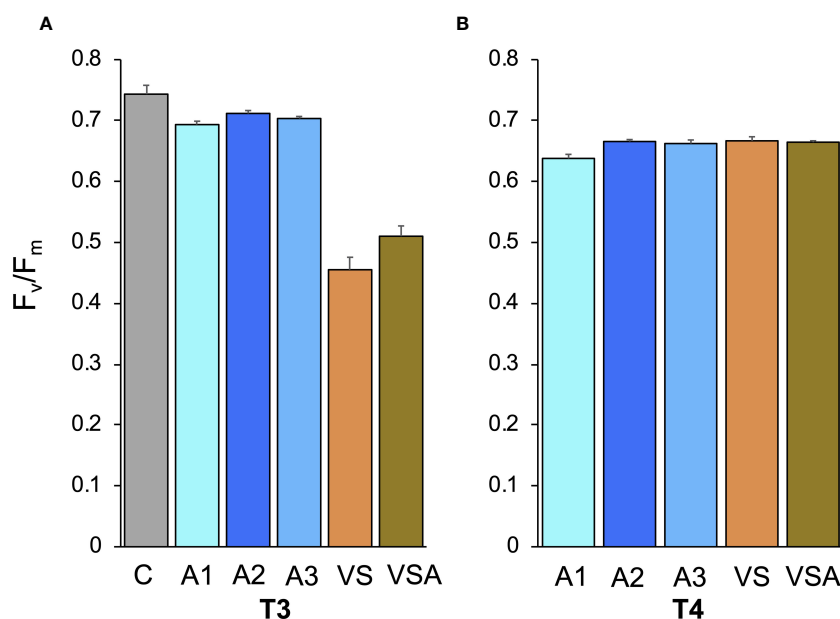

FIGURE 3
Mean values (± SE) of the maximum quantum yield of PSII (F$_v$/F$_m$) of *Ericaria amentacea* seedlings; **(A)** at the end of laboratory culture (T3); **(B)** after 3 weeks of culture in the field (T4). C (seawater, SW); A1 (SW with Algatron 2 mL L^{-1}); A2 (SW with Algatron 4.5 mL L^{-1}); A3 (SW with Algatron 9 mL L^{-1}); VS (SW with addition of von Stoch); VSA (SW with addition of von Stoch and Algatron 4.5 mL L^{-1}). At T4 C-treatment tiles were free of seedlings.
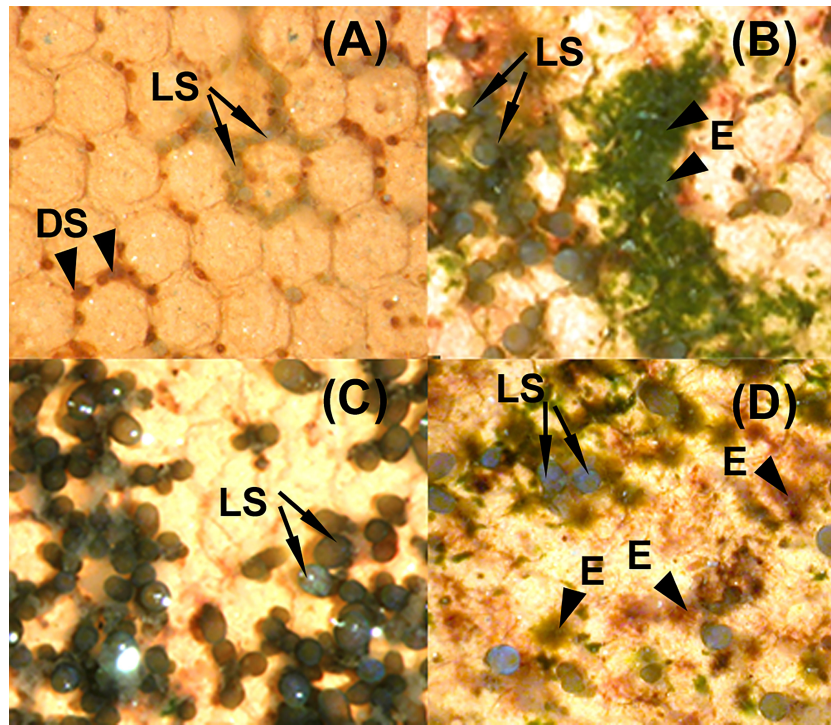
FIGURE 4

Clay tiles with *Ericaria amentacea* seedlings at the end of the laboratory culture (T3) under different culture media: **(A)** C (seawater, SW) with living seedlings (LS, arrows) and dead seedlings (DS, arrowheads). **(B)** VS (SW with addition of von Stoch) with living seedlings (LS, arrows) and epiphytes (E, arrowheads). **(C)** A2 (SW with Algatron 4.5 mL L^{-1}) with living seedlings (LS, arrows). **(D)** VSA (SW with addition of von Stoch and Algatron 4.5 mL L^{-1}) with living seedlings (LS, arrows) and epiphytes (E, arrowheads).

Beta diversity showed the differences among microbial communities when comparing adult thalli *vs* seedlings (Figure 7) and treatment *vs* treatment (Figure 8). PCoA showed that the adult thalli were separated from the seedlings (Figure 7). PCoA showed that the treatments had their own trajectories over time (Figure 8). At T3, C (i.e., the seawater control) was separate from the others, supporting the hypothesis that the treatments affected beta-diversity. We also noted a gradient, along the PCo2 axis according to Algatron concentration.

Phylogenetic characterization identified 303 families (Bacteria) in the seedling microbial biofilm communities (Figure 9) and only 32 families had at least one OTU scoring >1% of the relative abundance in a treatment. In general, T1 was characterized by the dominance of four families: Rhodobacteraceae, Flavobacteriaceae, Alteromonadaceae and Vibrionaceae. This fingerprint changed at T3, where only the Rhodobacteraceae and Flavobacteriaceae emerged as major groups. The twenty most abundant families
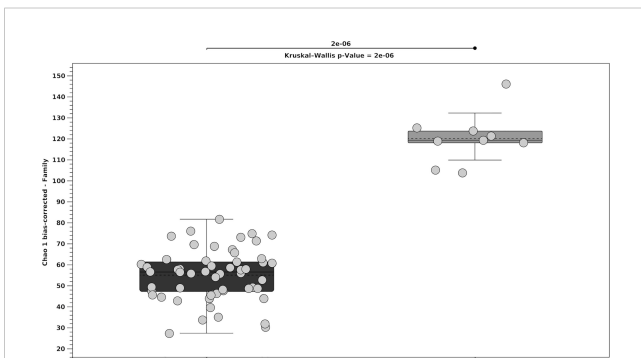

FIGURE 5

Median value 25th and 75th percentiles of alpha diversity for the microbial biofilms (total number of families) for the total seedlings in black and the three adult whole thalli in gray. Circles indicate treatments and time in seedlings whereas positions (i.e., basal holdfast, fertile and not fertile apices) in adult thalli.

FIGURE 6

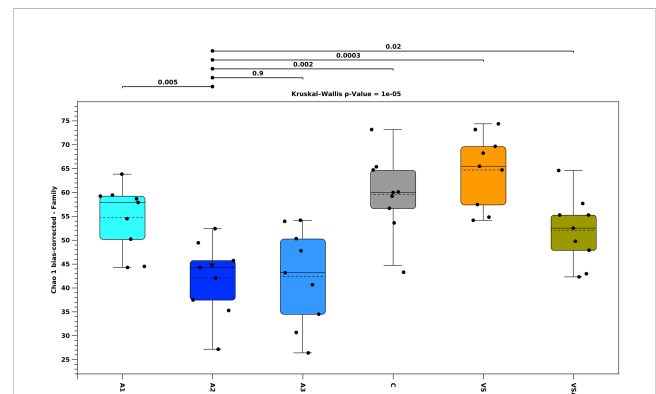Median value 25th and 75th percentiles of alpha diversity for the microbial biofilm (total number of families), measured in 6 different treatments: Algatron (A1), Algatron (A2), Algatron and (A3), Control (C), von Stosch (VS), von Stosch with Algatron (VSA) for the entire dataset. C (Control, gray), VSA (brown), VS (orange), A1 (baby-blue), A2 (blue), A3 (navy-blue). Circles indicate replicates over time.
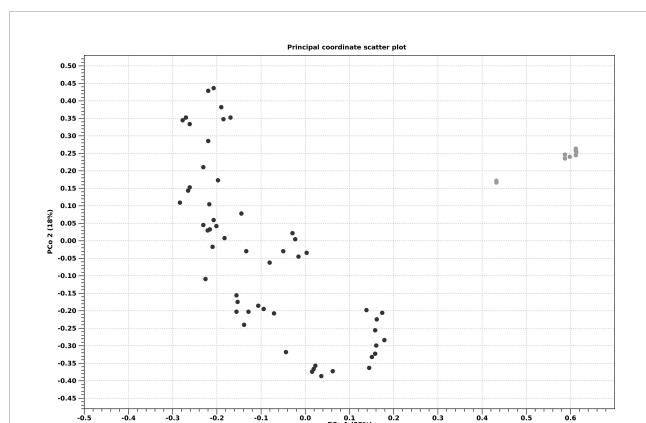
FIGURE 7
Principal coordinate analysis ordination (PCoA) of a Bray-Curtis dissimilarity matrix for adult thalli (gray) and seedlings (black).
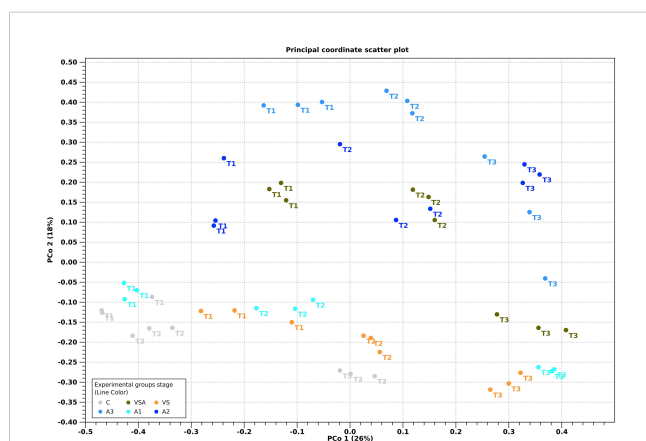

FIGURE 8
Principal coordinate analysis ordination (PCoA) of a Bray-Curtis dissimilarity matrix for the treatment over time. Treatments are color coded: C (Control, gray), VSA (brown), VS (orange), A1 (baby-blue), A2 (blue), A3 (navy-blue).

account for more than 95% of OTU abundance. The biofilm communities of adult thalli are discussed in SI.

On *E. amentacea* seedlings, the 20 most dominant families were: Rhodobacteraceae, Flavobacteriaceae, Alteromonadacea, Vibrionaceae, Halomonadaceae, Nitricolaceae, Acrobacteriaceae, Puniceicoccaceae, Phormidesmiaceae (Cyanobacteria), a family belonging to Synechoccales (Cyanobacteria), a family belonging to the Chitinophagales, Rubriraleaceae, Stappiaceae, Micavibrionaceae, a family belonging to the Bradymonadales, Bacteriovoraceae, Saprospiraceae, Psychromonadaceae and Marinomonadaceae (Figure 10).

In the seawater treatment (C), the microbial biofilm structure was characterized over time by an increase in Rhodobacteraceae (T1-T3: from 5.8% to 39.9%), Flavobacteriaceae (T1-T3: from 1.8% to 12.6%), an uncultured family of Chitinofagales (T1-T3: <1% to 4.7%) and Stappiaceae (T1-T3: from <1% to 2.5%), Micavibrionaceae (T1-T3: from <1% to 5.0%), Saprospirareae (T1-T3: from <1% to >1%), Phycisphaeraceae (T1-T3: from <1% to 2.2%), Nisaeceacea, Rhizobiaceae and Talassobaculaceae (T1-

from <1% to >1%). Over time, the microbial biofilm structure in C was characterized by the decrease of Alteromonadaceae (T1-T3: from 47.0% to 13.0%), Vibrionaceae (T1-T3: from 12.6% to 3%), Acrobacteraceae (T1-T3: from 19.8% to < 1%).

In VS, the microbial biofilm structure was characterized over time by the increase in Flavobacteriaceae (T1-T3: from 16.0% to 30.4%), Phormidesmiaceae (Cyanobacteria, T1-T3: from <1% to 11.4%), an uncultured family of Synechococcales (Cyanobacteria, T1-T3: from <1% to 11.0%), an uncultured family of Chitinofagales (T1-T3: from <1% to 2.1%), Phycisphaeraceae (T1-T3: from <1% to >1%). Furthermore, the microbial biofilm structure in VS was characterized over time by the decrease in Rhodobacteraceae (T1-T3: from 36.6% to 29.1%), Alteromonadaceae (T1-T3: from 18.6% to 1.4%), Vibrionaceae (T1-T3: from 11.0% to < 1%), Nitricolaceae (T1-T3: from 5.2% to < 1%).

In VSA, the microbial biofilm structure was characterized over time by an increase in Rhodobacteraceae (T1-T3: from 10.5% to 28.9%), Vibrionaceae (T1-T3: from 6.7% to 36.1%), an uncultured family of Synechococcales (Cyanobacteria, T1-T3: from <1% to 2.1%), Rubritaleaceae (T1-T3: from <1% to 3.0%). Furthermore, the VSA microbial biofilm structure was characterized by a decrease of Flavobacteriaceae (T1-T3: from 27.9% to 18.8%), Alteronoadeaecae (T1-T3: from 17.3% to <1%), Halomonadaceae (T1-T3: from 4.4% to 1.2%), Nitrincolaceae (T1-T3: from 29.2% to <1%).

In A1, over time, the microbial biofilm structure was characterized by an increase of Rhodobacteraceae (T1-T3: from 13.0% to 60.9%), Flavobacteriaceae (T1-T3: from 10.5% to 22.3%), Puniceicoccaceae (T1-T3: from <1% to 2.4%), Rubritaleaceae (T1-T3: from <1% to 1.2%), Saprospiraceae (T1-T3: from <1% to 2.6%) and NS9 marine group of the Flavobacteriales (T1-T3: from <1% to 1.6%). Furthermore over time, the A1 microbial biofilm structure was characterized by a decrease of Alteromonadaceae (T1-T3: from 47.7% to < 1%), Vibrionaceae (T1-T3: from 17.5% to 3.4%), Arcobacteraceae (T1-T3: from 3.7% to < 1%), Psychoromonadaceae (T1-T3: from 1.1% to < 1%).

In A2, over time, the microbial biofilm structure was characterized by an increase of Rhodobacteraceae (T1-T3: from 14.1% to 27.2%), Flavobacteriaceae (T1-T3: from 17.5% to 23.0%), Halomonadaceae (T1-T3: from 11.2% to 21.6%), Puniceicoccaceae (T1-T3: from <1% to 9.2%), Rubritaleaceae (T1-T3: from <1% to 3.2%) and Micavibrionaceae (T1-T3: from <1% to 1.1%). Furthermore over time, the A2 microbial biofilm structure was characterized by a decrease of Alteromonadaceae (T1-T3: from 37.9% to 1.2%), Vibrionaceae (T1-T3: from 13.3% to 6.0%), Marinomonadaceae (T1-T3: from 1.6% to < 1%), Pseudoalteromonadaceae (T1-T3: from 1.2% to < 1%).

In A3, over time, the microbial biofilm structure was characterized by an increase of Rhodobacteraceae (T1-T3: from 5.3% to 45.5%), Flavobacteriaceae (T1-T3: from 16.9% to 21.5%), Puniceicoccaceae (T1-T3: from <1% to 1.3%) and Rubritaleaceae (T1-T3: from <1% to 1.8.%). Vibrionaceae stayed stable from T1 to T3, accounting for 4%. Furthermore, over time, the A3 microbial biofilm structure was characterized by a decrease of Alteromonadaceae (T1-T3: from 16.7% to 3.7%), Halomonadaceae (T1-T3: from 43.0% to 15.3%), Acrobacteriaceae (T1-T3: from 9.8% to < 1%) and Marinomonadaceae (T1-T3: from 1.1% to < 1%).
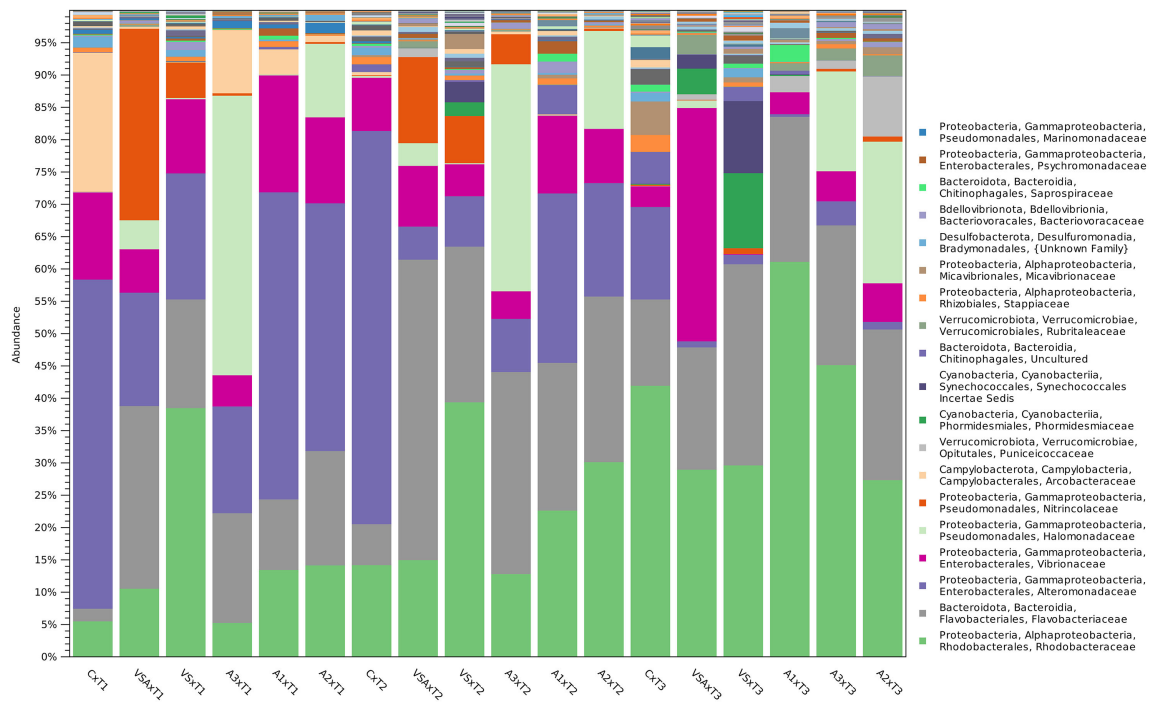
FIGURE 9

OTU relative abundance (20 most abundant families) aggregated per treatment over time of seedlings microbial biofilm. Bar height is proportional to taxa abundance.
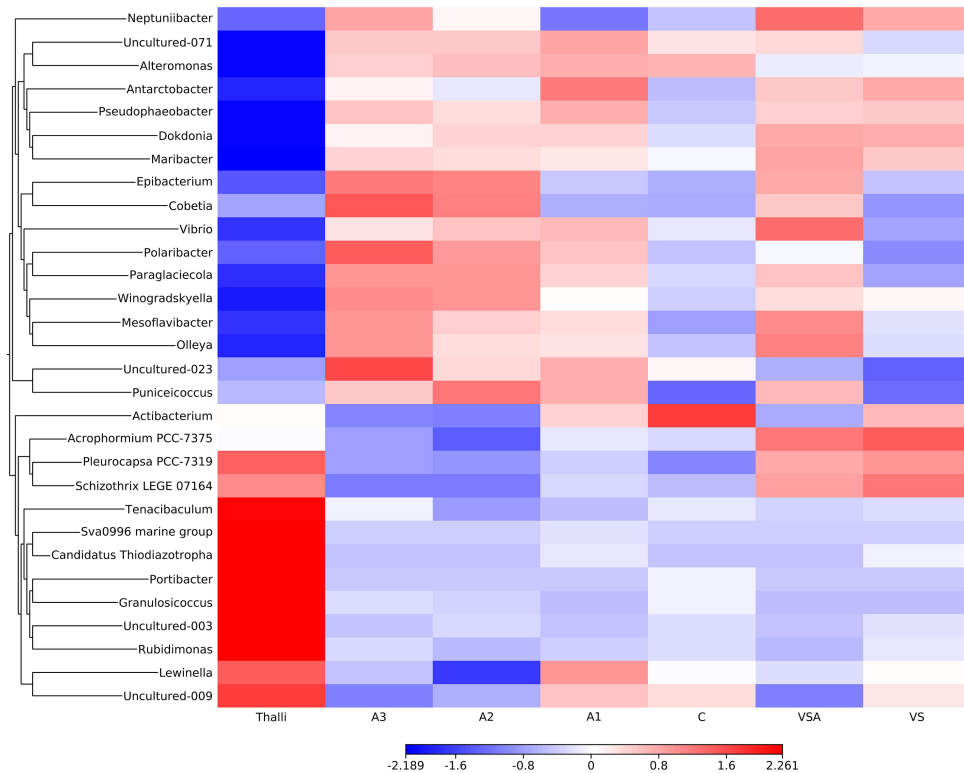

FIGURE 10

Heat-map of OTU relative abundance in the adult thalli and the seedlings community. Z score bar indicates enrichment (red) or depletion (blue) factor in comparison to the mean OTU value.

The most abundant taxa of adult thalli decreased during the cultivation phase in the seedling (Figure 10). The adult thalli were characterized by *Pleurocapsa* (Cyanobacteria), *Schizotrhrix* (Cyanobacteria), and Bacteria: *Tenacibaculum*, uncultured OTU from SVa0996 marine group, *Candidatus Thiodiaxotropha*, *Portibacter, Ganulosicoccus, Rudimonas* and *Lewinella*. On the other hand, the most abundant taxa in the seedlings were less abundant on the adult thalli (Figure 11).

The A series were characterized by *Neptuniibacter, Alteromonas, Antarctobacter, Pseudophaeobacter, Dokdonia, Maribacter, Epibacterium, Cobetia, Vibrio, Polaribacter, Paraglaciecola, Winogradskyella, Mesoflavibacter, Olleya* and *Puniceicoccus*.

The indicator species analysis depicted no statistically significant shared OTUs in both the adult thalli and seedling microbial biofilm communities (Figure 11; Supplementary Data Sheet). Each treatment was characterized by specific OTUs at the genus level, respectively: C by 12, VS by 7, VSA by 5, A1 by 3, A2 by 4, A3 by 3, and adult thalli by 17. Adult thalli were not sharing unique taxa within the seedling communities. C shared OTUs with A1(10) and VS (6) uniquely. A2 was uniquely characterized by *Postechiella, Roseovarius, Winogradskyella.2* and *Arenibacter*. A1, A2, VS, VSA shared *Psychroserpens*.

Whereas, A2 shared only with VS *Roseibacterium*; with VSA *Rubritalea* and with A3 10 OTUs (*Cobetia, Epibacterium, Cobetia.1, Epibacterium.1, Sulfitobacter.1, Photobacterium, Winogradskyella.3, Paraglaciecola, Polaribacter* and *Marinomonas*). A1, A2 and A3 shared *Vibrio* 4 and *Amylibacter*. A1, A2, A3 and VS shared *Roseobacter*.clade.CHAB.I.5.lineage. A2, A3, VSA shared *Oceanobacterium* and *Mesoflavibacter*.3. Finally, VS and VSA shared *Dokdonia 1*. A1 was uniquely characterized by *Agarivorans, Antarctobacter* and *Vibrio 14*. VS was uniquely characterized by *Sulfitobacter*.2, *Sedimentitalea, Roseobacter.1*,

Thalassococcus, Pseudophaeobacter.2, Haliea and *Shimia*. VSA was uniquely characterized by *Nautella, Neptuniibacter.2* and 1, *Leisingera, Neptuniibacter* and *Mesoflavibacter*.1.

4 Discussion

Our results show that the commercial seaweed extract AlgatronCifo® and von Stoch medium (VS) influenced seedling survival and growth as well as microbial biofilm communities. Overall, the A2 culture survived best in the field. VS, VSA and A1 were the second- best treatments, A3 performed poorly, while C seedlings did not survive in the field.

Brown algae extract with biostimulant properties have recently been used to improve harvest in aquaculture (Hurtado and Critchley, 2018; Umanzor et al., 2019; Hurtado and Critchley, 2020; Umanzor et al., 2020a; Umanzor et al., 2020b; Han et al., 2022; Jiksing et al., 2022; Umanzor et al., 2022). The priming effect is likely due to their complex composition and the presence of compounds with biostimulatory activities like polysaccharides (Shukla et al., 2021) and secondary metabolites. Phenolic compounds (Stirk et al., 2020; Sujeeth et al., 2022) play a crucial role in cell wall formation and early development of brown algae (Schoenwaelder, 2002), help adapt to environmental stressors and defend against biological pressures such as grazers, pathogens and epiphytes (Stiger-Pouvreau et al., 2014; Generalić Mekinić et al., 2019). In plants, algal-derived extracts have been shown to induce changes in the microbial community and inhibit plant pathogens (see references in Ali et al., 2021; Shukla et al., 2021).

In our study, the culture media (i.e., VS) and the addition of Algatron had a significant effect on the structure and composition of the biofilm communities associated with the seedlings (Figure 11). The biofilm communities shifted in distinct ways
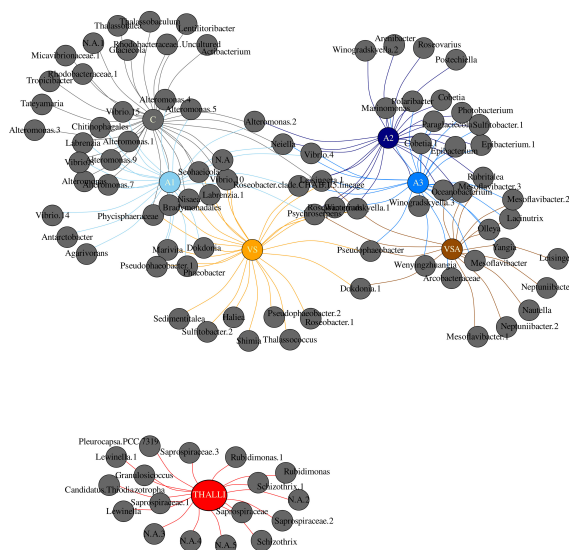

FIGURE 11
Indicator species analysis based on OTU > 1% abundance at the genus level of the adult thalli (red) and seedlings in each treatment. Treatments are color coded: C-gray, A1-baby blue, A2-blue, A3-navy blue, VS-orange, VSA-brown.

over time, with an overall decrease in the diversity of OTUs compared to adult thalli. This could be due to the more controlled and less variable culture environment compared to the intertidal zone where *E. amentacea* thrives, where desiccation, wave dynamics, temperature stress and ever-changing seawater conditions (i.e., nutrients, salinity, pH, pollution, etc.) strongly influence the algae and their biofilm.

In our experimental setting, we cannot unambiguously distinguish between the effect of the additive on algal physiology, which in turn modified the microbes (indirect effect of biostimulant and nutrients, *via* algal metabolism, Ren et al., 2022) and the direct effect of biostimulant/nutrients on microbial growth and community structure.

At the end of the mesocosm culture (T3), the treatments with VS (i.e., VS, VSA) and A1 and A2 showed comparable seedling coverage. However, A2 and A1 also showed optimal photophysiological fitness (F_v/F_m > 0.69). The VS and VSA treatments had the lowest F_v/F_m values (0.51 ± 0.02 and 0.46 ± 0.02, respectively), indicating stress to the photosynthetic apparatus (Celis-Plá et al., 2014; Smolina et al., 2016; Falace et al., 2018b; Savva et al., 2018; Verdura et al., 2021). After the field period, all treatments showed good photosynthetic efficiency, with A2 having the highest coverage, being 1.8, 1.9, 2.2 and 6.0 times higher than VS, VSA, A1 and A3, respectively.

We hypothesize that the higher survival is due to the interplay of nutrients, presence/absence of epiphytes and their antagonistic or beneficial relationships with the *E. amentacea* seedlings and finally priming by the algal extract.

While *E. amentacea* is mainly restricted to oligotrophic waters and is sensitive to eutrophication (Pinedo et al., 2007; Mangialajo et al., 2008; Thibaut et al., 2014), seedlings may have a higher nutrient uptake capacity than adult thalli, as observed in other Fucales (Thomas et al., 1985; Sánchez de Pedro et al., 2022). Although culture media were frequently renewed, C-culture seedlings likely suffered from nutrient limitation (Supplementary Table 1), which may have affected many processes like photosynthetic capacity, embryonic development and growth (Duarte, 1992; Roleda and Hurd, 2019). Lower growth of *E. amentacea* seedlings in seawater compared to VS was also observed by Susini (2006). The lowest performance in terms of survival and growth of C seedlings was followed by total failure in the field. We found that the C biofilm differed the most from the other treatments (in the PCoA plot) despite the high alpha diversity. This treatment shared fewer OTUs with the adult thalli (13/64 scoring > 1% relative abundance) than the other treatments with nutrient addition, indicating the importance of microbial interactions and algal growth in co-shaping the microbial biofilm, as reported in *Sargassum* (Hervé et al., 2021) and *Macrocystis* (Michelou et al., 2013).

The VS and VSA treatments had high nutrient concentrations, with the highest nitrate (NO_3^-) and phosphate (PO_4^{3-}) levels of all treatments (Supplementary Table 1), which were 5.25 and 5.32 fold and 26.5 and 32.3 fold higher than the C-treatment, respectively. NO_3^- is one of the two main sources of nitrogen (N), which is essential for macroalgal growth as it is a major component of the photosynthetic apparatus, amino acids and cellular enzymes, and thus can be a critical limiting factor in the marine environment (Hurd

et al., 2014). As well, phosphorus (P) is a vital nutrient for macroalgae, involved in algal photosynthesis and respiration, particularly energy transfer through the synthesis of adenosine triphosphate (ATP) and other energy-rich compounds (Lobban and Harrison, 1994). The nutrient-rich conditions, characterized by a lower N:P ratio in VS and VSA (10.29 and 65.26 respectively, Supplementary Table 1) which fall within the optimal range for seaweeds growth (i.e., from 10:1 to 80:1; Suthar et al., 2019), simultaneously promoted the highest growth of seedlings, but also, as expected, of epiphytes. Indeed, fast-growing opportunistic epiphytes (i.e., filamentous algae, diatoms, cyanobacteria) have a competitive advantage at high nutrient concentrations by maximizing rapid nutrient uptake and photosynthetic efficiency (Fujita, 1985; Carpenter, 1990; Duarte, 1995). Furthermore, when growing on the outer surface of seedlings, they compete for light (Pang et al., 2011) and act as a barrier to carbon uptake (Sand-Jensen, 1977), resulting in lower photosynthetic efficiency of their hosts. In algal cultures, epiphyte infestation has been shown to impair algal productivity (Lüning and Pang, 2003; Ward et al., 2020) and is one of the main barriers to *ex situ* culture of *Cystoseira s.l.* (e.g. Orlando-Bonaca et al., 2021; Clausing et al., 2022; Lardi et al., 2022). In addition, epiphytes can in some cases damage host tissues (Hayashi et al., 2010) and facilitate colonization by opportunistic bacteria (Vairappan et al., 2008; Jiksing et al., 2022) that cause disease.

Contrary to the treatments with VS (i.e., VS, VSA), in the Algatron treatment, the main source of N was ammonium (NH_4^+) (Supplementary Table 1), which may be a more efficient form of N fertilizer for brown algal growth (Dā Costa Braga and Yoneshigue-Valentin, 1996; Smart et al., 2022). Among these treatments, A3 showed the lowest performance in terms of growth and survival. Seedlings cultivated in A3 treatment were exposed to the highest ammonium concentration (NH_4^+) (Supplementary Table 1), which might have been the cause of the low cover despite the good Fv/Fm. In an indoor culture of *Sargassum* spp. seedlings, NH_4^+ was shown to effectively promote their growth (Han et al., 2018) and increase photosynthesis (Hong et al., 2021), but a too high ammonium content (i.e., 900 µmol·L^{-1}) can negatively affect growth (Hong et al., 2021). It has also been noted that algal extracts can have both positive and negative effects depending on the concentration (Kapoore et al., 2021). A1 and A2 seedlings had on average the same F_v/F_m, with A2 seedlings showing higher growth and survival, indicating a more suitable concentration than A1. In treatments A1 and A2, NH_4^+ was supplied at concentrations 4.5 and 2 times lower, respectively, than in A3 (Supplementary Table 1), which promoted the growth of *E. amentacea* without favoring the development of epiphytes. Apart from high NH_4^+ availability, other constituents of the algal extract Algatron may have induced changes in seedlings metabolism and signaling pathways specifically related to nutrient uptake and/or nutrient translocation improving nutrient use efficiency, as observed in plants (Jannin et al., 2013; Saa et al., 2015; Sujeeth et al., 2022). Nevertheless, in our experimental settings we cannot unambiguously assess the effect of the biostimulant versus the ammonium (NH_4^+) per se. The toxicity of A3 medium to the seedlings could be due to high ammonium concentration. On the other hand, the lower cover (%) of seedlings under A1 than in A2 media showed indeed an effect of biostimulant

because nutrients (i.e., nitrate and ammonium) were not limiting in the A1 medium. Further studies are required to disentangle a possible biostimulant action from the NH_4^+ effect on the seedling growth. Moreover, complementary studies using -omics techniques (i.e., metabolomics and/or transcriptomics) could identify the modes of actions and the metabolic pathways by which the complex extract might improve seedlings performance.

The lower occurrence of opportunistic epiphytes can also be attributed to the lower PO_4^{3-} concentrations compared to VS and VSA, since fast-growing species have much higher P-demands than slower growing algae (Pedersen et al., 2010). According to the N:P ratio at which nutrients were supplied in Algatron treatments (A1 = 278.5; A2 = 324.58; A3 = 350.93, respectively), there would be an external P-limitation also for seedlings development, nevertheless the culture media were suitable for seedling growth, especially in A2, where the PO_4^{3-} was 5.85 times higher than in the C treatment despite a relatively more balanced N:P ratio in the latest (i.e., 60.1) (Supplementary Table 1). Indeed, to avoid misuse, the N:P ratio should be interpreted along with the absolute amounts of each nutrient (Dodds, 2003).

The good performance in terms of growth, survival and photophysiological fitness of the seedlings in A2 treatment might have played an important role in the success of this treatment in the field. In previous *ex-situ* outplantings, high detachment of *E. amentacea* seedlings was documented after transfer to the field (De La Fuente et al., 2019; Clausing et al., 2022). The high survival of A2 seedlings in the field might be related to the ability of the seedlings to anchor to the tiles due to the alginate-enriched algal extract, which can act as a metabolic stimulant and trigger favorable physiological responses (Briceño-Domínguez et al., 2014; Shukla et al., 2021). Although the synthetic pathways of alginates are not fully understood, it is thought that alginates are first synthesized as a polymer of mannuronic acid (a component of alginic acid) by alginate synthase and that some of the mannuronic acid is converted to guluronic acid (Michel et al., 2010). Alginates can influence the mechanical properties of cell walls in the rhizoids of developing zygotes of Fucales (Linardic, 2018; Yonamine et al., 2021) and provide for a stronger rhizoid system. We can assume that the A2 seedlings had stronger rhizoids that allowed them to adhere more firmly to the tiles. Considering that *E. amentacea* thrives in wave-exposed sites (Agardh, 1842; Boudouresque, 1971; Thibaut et al., 2014), it follows that well-developed rhizoids could promote better seedling survival.

The 20 most dominant families we found on the seedlings and adult thalli of *E. amentacea* were also identified as the most abundant on other brown algae (Florez et al., 2017 and reference), as Fucales and Laminariales. This suggests a great microbial plasticity that allows them to thrive with ecologically diverse algae. Within these families, there are many multitudes of microbial adaptive strategies for growth, nutrient uptake, and antagonistic interactions. From an algal perspective (Florez et al., 2017), studies have shown that Rhodobacteraceae, Flavobacteriaceae, Alteromonadaceae, Vibrionaceae, and Halomonadaceae can induce morphogenesis and degrade algal compounds. Rhodobacteraceae, Flavobacteriaceae, Alteromonadaceae, Vibrionaceae, Halomonadaceae Bacteriovoraceae, Saprospiraceae may be related to pathogenesis and antibacterial activity. Furthermore,

microbially induced diseases have been identified in *Laminaria*, such as hole-rotten disease, red spot disease, spot-wounded fronds and swollen gametophytes and filamentous fading caused by *Pseudomonas*, *Vibrio*, *Halomonas* and *Alteromonas* strains (Egan et al., 2014). It follows that biofilm-associated microbes can become opportunistic pathogens when the host is stressed by temperature, UV and nutrient stress, eutrophication or other anthropogenic induced challenges (Egan et al., 2014; Duarte et al., 2018; van der Loos et al., 2019).

Overall, the adult thalli have structured seedlings biofilm community composition. Taxa that were dominant in the adult thalli decreased in the seedlings and *vice versa*. Taxa that were dominant in the seedlings were copiotrophs, particle- or biofilm-associated (Lauro et al., 2009; Heins and Harder, 2023) and high-efficient organic matter degraders (Cottrell and Kirchman, 2000). Given these features, we can surmise that these microbes were closely associated with algal growth. The seedling biofilm communities were characterized by metabolisms predicted by the FAPROTAX annotation, which primarily used chemical energy released by breaking chemical bonds to degrade organic carbon from the primary production of algae and alginates contained in the algal extract Algatron.

Index species analysis based on pre-infield biofilm communities revealed that the best performing treatment, A2, was uniquely characterized by 5 Gram-negative aerobic genera *Postechiella*, *Roseovarius, Winogradskyella* and *Arenibacter*. Many strains from these genera have been isolated from diverse marine systems, indicating the huge metabolic flexibility (Lee et al., 2012; Luo and Moran, 2014; Zhuang and Luo, 2020). Furthermore, these unique taxa have been reported as easy to cultivate and purify, in relation to the fact that not more than 1% of the microbes in every environment can be cultivated. This is an extremely important and desirable feature for planning future probiotic-focused bacterial strain cultivation efforts. *Postechiella, Winogradskyella* and *Arenibacter* (Flavobacteriaceae) have been shown to be able to degrade agar, DNA, starch and many diverse sugars beside proteins and lipids (Lee et al., 2012; Gutierrez et al., 2014; Kurilenko et al., 2019). The microbial degradative activities could be important for supplying the algae with nutrients from the carbohydrate, protein and lipid pools of the algal extract, which can then be used for primary production. Another possible function is the control of biofilm structure and dynamics by DNase and sugar and protein hydrolysis activities (Whitchurch et al., 2002; Wang et al., 2004). *Arenibacter* is also able to degrade polycyclic aromatic hydrocarbon compounds. This is interesting given that *E. amentacea*, like the other brown algae, produces polyphenols (Generalić Mekinić et al., 2019) and Algatron contains these compounds. Polyphenols are important for chemical defense against herbivory (i.e., non palatability) and microbes, and for protection against oxidative stress by absorbing harmful UV and excessive irradiation. Thus, for microbes being able to degrade a toxic substance as phenols could therefore be beneficial for more efficient microbial competition for space and nutrients. The *Roseovarius* (family Rhodobacteraceae) genome presents pathways for the production of thiamine and cobalamin (Luo and Moran, 2014). Thiamine and cobalamin are important micronutrients for algae (Croft et al., 2006), playing key roles in their central

metabolism. Many genera belonging to the Rhodobacteraceae produce these vitamins and share them with primary producers within the virtuous cycle of organic matter production and remineralization (Bertrand and Allen, 2012; Luo and Moran, 2014).

A2, A1, VSA and VS shared the taxon *Psychroserpens* (Flavobacteriaceae, Ping et al., 2022), which is able to degrade alginate and casein, suggesting a role in degrading sugar-rich and phospho-protein rich compounds within the biofilm. Interestingly, some strains require vitamins for growth thus being a competitor for these micronutrients in the algal biofilm.

A few studies have found that secondary metabolites from macroalgal extracts have strong effects on microbial surface colonization, altering the bacterial biofilm formation and community composition under laboratory and field conditions (Sneed and Pohnert, 2011; Egan et al., 2013; Lachnit et al., 2013).

Further experiments should elucidate the direct or indirect effect of nutrients and biostimulants in promoting a probiotic microbial community by investigating the gene expression of algae and microbes along the diverse treatments and their degree of interdependence. It would be important to track O_2 evolution using optode technique in the key treatments to guide microbial sampling when changes in algal performance occur. Such an approach will allow coupling algal performance with microbial community response and fill the gap in the functional role of microbes in macroalgal holobiont health (Duarte et al., 2018).

Given the seedling success of A2, we propose treatment tailored probiotic consortia candidates characterized by the unique treatment-taxa (A2: *Postechiella*, *Roseovarius*, *Winogradskyella* and *Arenibacter*) and the reminder community. At this stage, we cannot disentangle the net contribution of the unique vs. shared vs. reminder community in relation to the overall seedling fitness. Within the holobiont concept, we can hypothesize that the nature and intensity of interactions might be important in defining probiotic consortia. Furthermore, the effect of algal extract or nutrients on the algae and/or biofilm could have important consequences for tuning the overall interaction network. Our study has shown that *ex-situ* restoration of macroalgae could benefit from both the use of commercial algal extracts in *ex-situ* cultures and the identification of probiotic consortia candidates that promote seedling growth and optimal protection against biotic and abiotic stressors.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: BioProject, PRJNA922753.

Author contributions

AF, SK, FM designed the study. LA, FM, SK, AS, CB, FG, AF performed the laboratory measurements. AP, FM, SB, AS, SN performed the formal data analysis. FM, SK, AF wrote the original draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1181685/full#supplementary-material

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