frontiers

Supplementary Material

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

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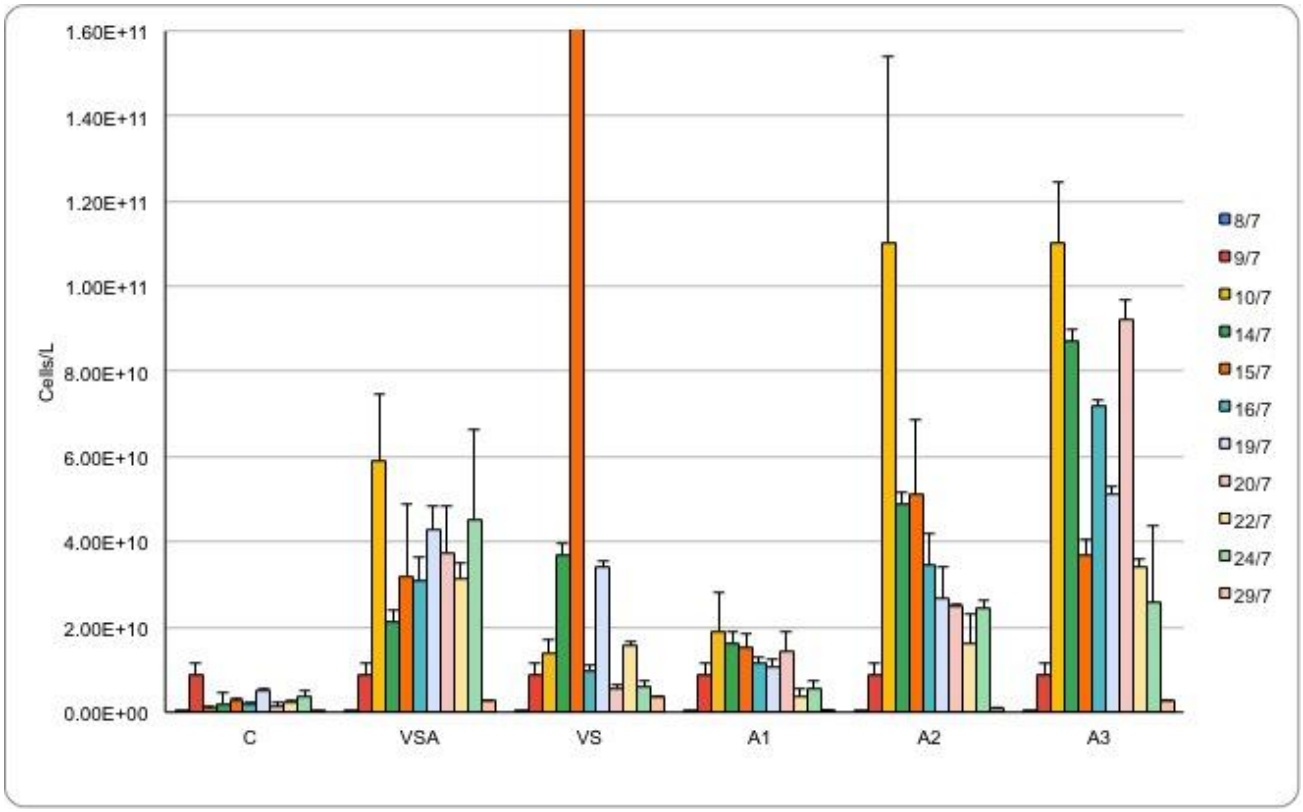
1. Supplementary Data and Figures

Flow Cytometry analysis

Liquid media were sampled prior to water change in each treatment on 8th, 9th, 10th, 14th, 15th, 16th, 19th, 20th 22nd, 24th and 29th of July 2021. Algatron solution was also tested for the presence of microbes at the flow cytometry. Briefly, the liquid samples were fixed with GTA at a final concentration of 5% and frozen at 80^{o}C prior analysis at BD FACS CANTO II (BD Biosciences) equipped with a 488-nm laser excitation and the standard filter setup. Samples were diluted 1:10 in 1xTE (pH 8) and stained with SYBRGREEN ™ I (final dilution 1:4) for 10 min in the dark following the protocol of Gasol and Del Giorgio, 2000. Heterotrophic prokaryotes were discriminated from other particles or background based on their green fluorescence from SYBR GREEN I (FL1) and side scatter (SSC). Data acquisition was performed using BD FACSDiva software (BD Biosciences) while data analysis using FCS Express 6 Flow v 6.06.0025 software (DeNovo Software).

Algatron medium presented an abundance of heterotrophic bacteria in the order of a few hundreds 10^{8} cells/L. The biostimulant is expected to be non-axenic since it is a cold extract from a brown alga. Overall, heterotrophic bacteria abundance over the experiment in bulk water varied greatly over time, Supplementary Figure 1. Cwas < than A2, A3, VSA and VS. This difference was statistically significant (ANOVA, with Tukey's multiple comparison test, $p<0.01$, $p<0.0001$, $p<0.05$ and $p< 0.01$). A1<A3 and this was statistically significant ($p<0.005$). Overall, VSA, VS, A2 and A3 were not statistically significant to each other. It seems that



Supplementary Figure 1: Heterotrophic bacterial abundance in the six treatments over time in the bulk water. Units are cells/L, values are average with standard deviations.

Gasol, J. M., & Del Giorgio, P. A. (2000). Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Scientia Marina, 64(2), 197-224.

INDEX SPECIES ANALYSIS

Multilevel pattern analysis

Association function: r.g

Significance level (alpha): 0.05

999 permutations

Total number of species: 144

Selected number of species: 99

Number of species associated to 1 group: 51

Number of species associated to 2 groups: 37

Number of species associated to 3 groups: 7
Number of species associated to 4 groups: 3
Number of species associated to 5 groups: 1
Number of species associated to 6 groups: 0

List of species associated to each combination:

Group A1 #sps. 3

	stat	p.value
Agarivorans	0.539	0.010 **
Antarctobacter	0.484	0.023 *
Vibrio.14	0.462	0.030 *

Group A2 #sps. 4

	stat	p.value
Postechiella	0.642	0.002 **
Roseovarius	0.556	0.009 **
Arenibacter	0.494	0.019 *
Winogradskyella.2	0.479	0.036 *

Group A3 #sps. 3

	stat	p.value
Wenyingzhuangia	0.566	0.008 **
Yangia	0.483	0.033 *
Arcobacteraceae	0.459	0.045 *

Group C #sps. 12

	stat	p.value
Tateyamaria	0.773	0.001 ***
Actibacterium	0.705	0.001 ***
Thalassobaculum	0.692	0.001 ***
Rhodobacteraceae..Uncultured	0.692	0.003 **
Thalassotalea	0.660	0.003 **
Rhodobacteraceae.1	0.644	0.005 **
Tropicibacter	0.584	0.005 **
N.A.1	0.571	0.011 *
Glaciecola	0.535	0.018 *
Alteromonas.3	0.530	0.016 *
Micavibrionaceae.1	0.505	0.025 *

Lentilitoribacter	0.492	0.025	*

Group THALLI #sps. 17

	stat	p.value	
Pleurocapsa.PCC.7319	0.991	0.001	***
Rubidimonas	0.969	0.001	***
Saprospiraceae.3	0.967	0.001	***
Lewinella	0.961	0.001	***
N.A.3	0.959	0.001	***
Candidatus.Thiodiazotropha	0.956	0.001	***
Saprospiraceae.2	0.940	0.001	***
Saprospiraceae	0.932	0.001	***
N.A.5	0.928	0.001	***
N.A.4	0.924	0.001	***
Rubidimonas.1	0.924	0.001	***
Saprospiraceae.1	0.908	0.001	***
Granulosicoccus	0.881	0.001	***
Lewinella.1	0.849	0.001	***
Schizothrix	0.842	0.001	***
Schizothrix.1	0.819	0.001	***
N.A.2	0.749	0.001	***

Group VS #sps. 7

	stat	p.value	
Sulfitobacter.2	0.821	0.001	***
Sedimentitalea	0.803	0.002	**
Roseobacter.1	0.754	0.003	**
Thalassococcus	0.711	0.003	**
Pseudophaeobacter.2	0.687	0.006	**
Haliea	0.667	0.002	**
Shimia	0.582	0.007	**

Group VSA #sps. 5

	stat	p.value	
Nautella	0.633	0.005	**
Neptuniibacter.2	0.619	0.004	**
Leisingera	0.546	0.014	*
Neptuniibacter	0.543	0.006	**

Mesoflavibacter.1	0.520	0.011 *

Group A1+C #sps. 10

	stat	p.value
Vibrio.15	0.730	0.001 ***
Vibrio.8	0.716	0.002 **
Labrenzia	0.643	0.002 **
Alteromonas.4	0.630	0.006 **
Alteromonas	0.584	0.009 **
Alteromonas.1	0.577	0.010 **
Alteromonas.9	0.577	0.007 **
Alteromonas.7	0.561	0.011 *
Chitinophagales	0.548	0.012 *
Alteromonas.5	0.498	0.025 *

Group A1+VS #sps. 4

	stat	p.value
Phaeobacter	0.628	0.008 **
Pseudophaeobacter.1	0.583	0.011 *
Dokdonia	0.550	0.012 *
Marivita	0.505	0.021 *

Group A2+A3 #sps. 10

	stat	p.value
Cobetia	0.792	0.001 ***
Epibacterium	0.782	0.001 ***
Cobetia.1	0.750	0.001 ***
Epibacterium.1	0.745	0.001 ***
Sulfitobacter.1	0.672	0.002 **
Photobacterium	0.657	0.005 **
Winogradskyella.3	0.647	0.001 ***
Paraglaciecola	0.590	0.005 **
Polaribacter	0.490	0.027 *
Marinomonas	0.448	0.042 *

Group A2+VS #sps. 1

	stat	p.value	
Roseibacterium	0.492	0.029	*

Group A2+VSA #sps. 1

	stat	p.value	
Rubritalea	0.512	0.018	*

Group A3+VSA #sps. 4

	stat	p.value	
Olleya	0.606	0.004	**
Mesoflavibacter	0.605	0.009	**
Mesoflavibacter.2	0.598	0.004	**
Lacinutrix	0.497	0.033	*

Group C+VS #sps. 6

	stat	p.value	
Bradymonadales	0.699	0.002	**
Seohaeicola	0.685	0.002	**
Labrenzia.1	0.596	0.004	**
Phycisphaeraceae	0.589	0.009	**
N.A	0.462	0.041	*
Vibrio.10	0.461	0.037	*

Group VS+VSA #sps. 1

	stat	p.value	
Dokdonia.1	0.508	0.018	*

Group A1+A2+A3 #sps. 1

	stat	p.value	
Vibrio.4	0.48	0.045	*

Group A1+A2+C #sps. 1

	stat	p.value	
Alteromonas.2	0.504	0.015	*

Group A1+C+VS #sps. 1

	stat	p.value	
Nisaea	0.606	0.005	**

Group A2+A3+VS #sps. 1

	stat	p.value
Winogradskyella.1	0.473	0.036 *

Group A2+A3+VSA #sps. 2

	stat	p.value
Oceanobacterium	0.679	0.003 **
Mesoflavibacter.3	0.596	0.002 **

Group A3+VS+VSA #sps. 1

	stat	p.value
Pseudophaeobacter	0.591	0.004 **

Group A1+A2+A3+C #sps. 1

	stat	p.value
Neiella	0.55	0.013 *

Group A1+A2+A3+VS #sps. 1

	stat	p.value
Roseobacter.clade.CHAB.I.5.lineage	0.455	0.044 *

Group A1+A2+VS+VSA #sps. 1

	stat	p.value
Psychroserpens	0.486	0.027 *

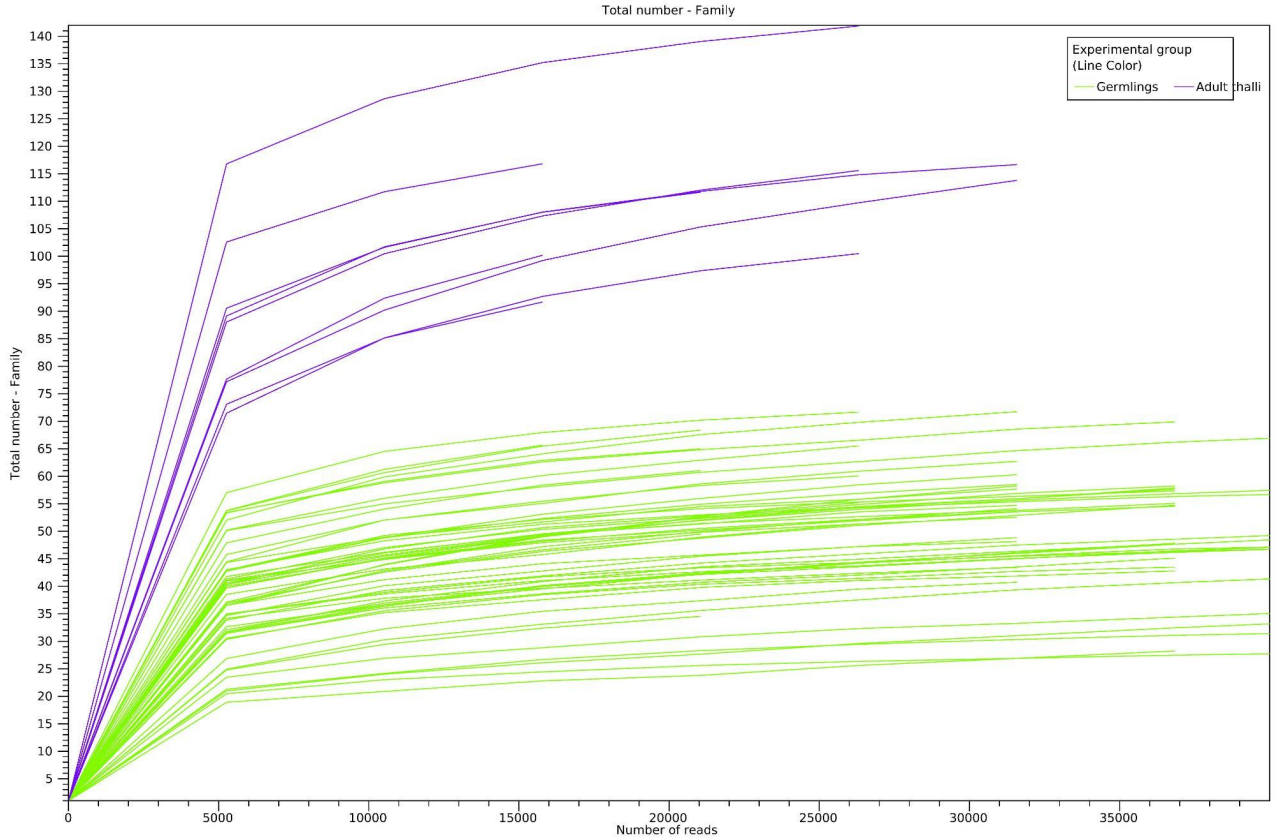
Group A2+A3+C+VS+VSA #sps. 1

	stat	p.value
Leisingera.1	0.47	0.035 *

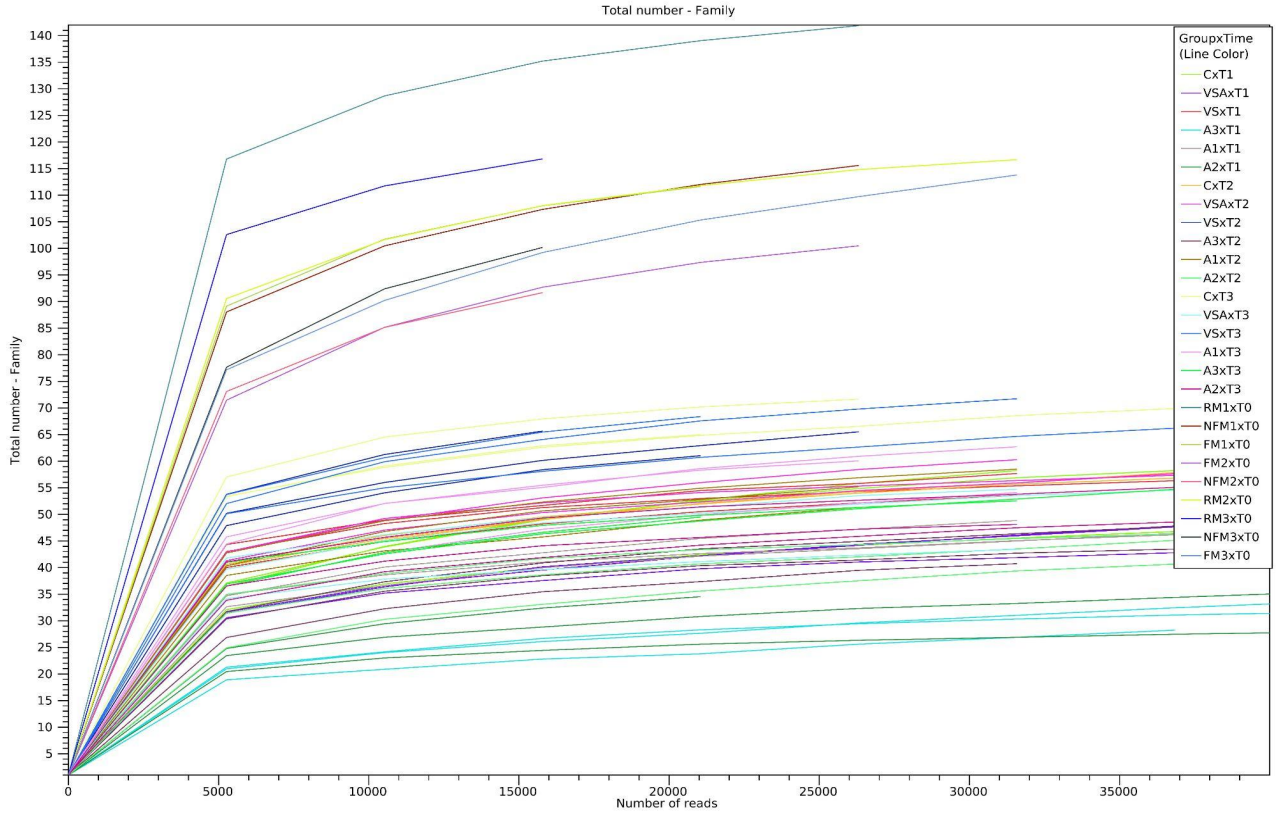
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Microbial diversity and community structure

The rarefaction curves showed the sequencing effort was sufficient to detect the microbial diversity in adult thalli and seedlings. The adult thalli presented a higher number of families relative than the seedlings. The triplicate samples of seedlings had a comparable number of families (Supplementary Figure 2a,b).

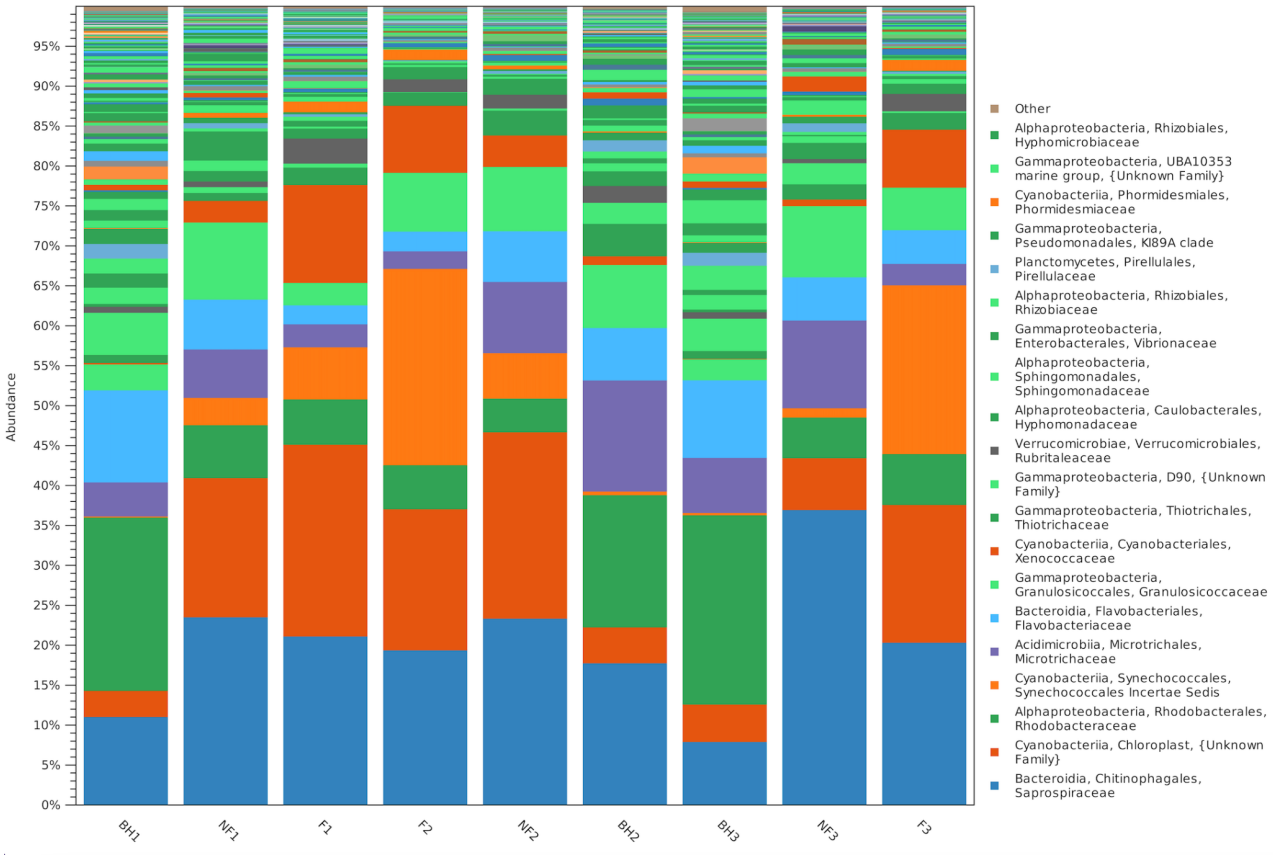


Supplementary Figure 2a: Rarefaction curve of adult thalli (purple) and seedlings (green).



Supplementary Figure 2b: Rarefaction curve of adult thalli and seedlings color coded per treatment and time. Basal holdfast is labeled as RM, not fertile apices as NFM and fertile apices as FM.

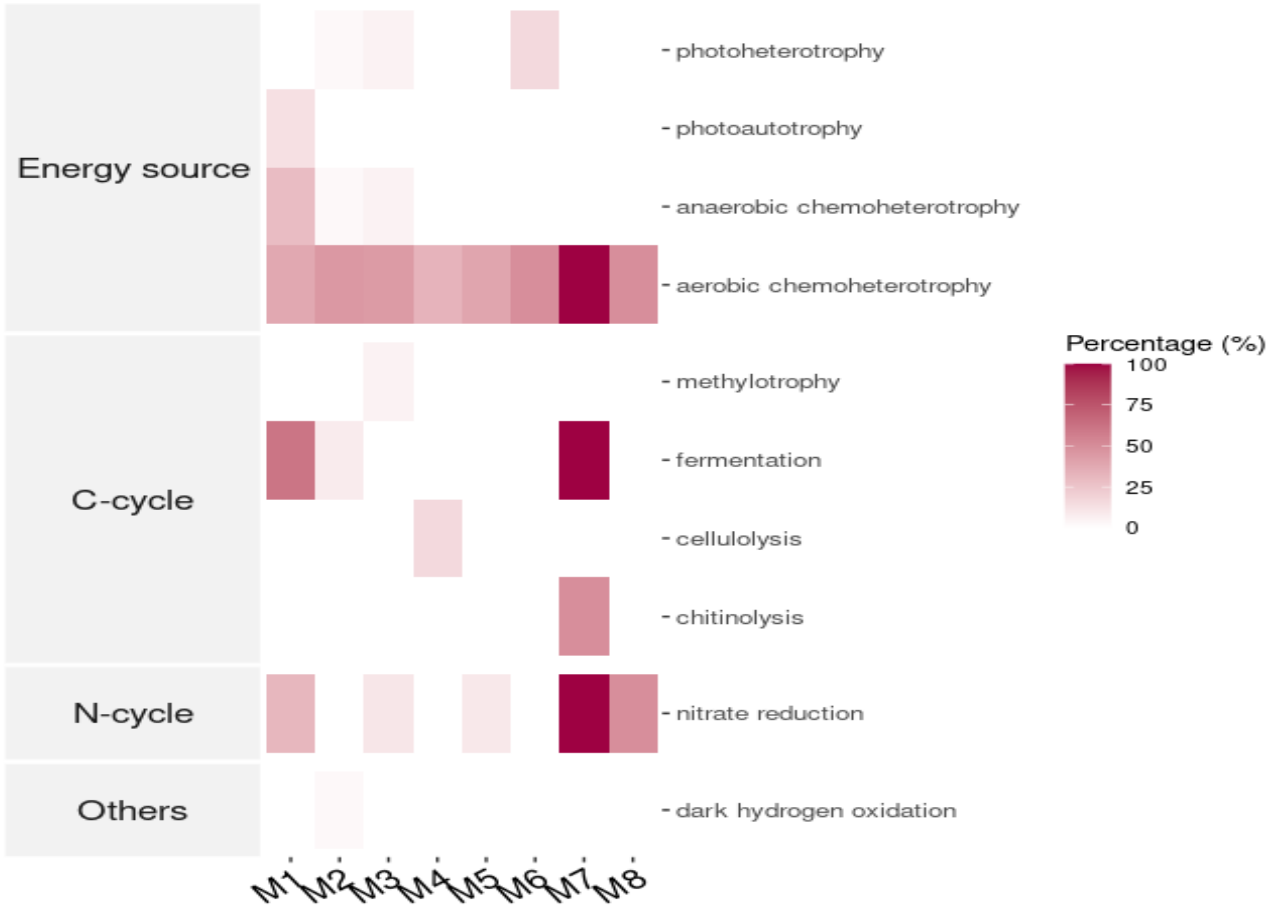
Adult thalli were characterized by the dominance of Saprospiraceae, three distinct families of Cyanobacteriia, Rhodobacteraceae, Microtrichaceae, Flavobacteriaceae, Granulosicoccaceae and Thiotrichaceae Supplementary Figure 3). These families represented up to 85% of relative OTU abundance for fertile and not fertile apices, whereas only 55% for the holdfasts. Holdfast communities were more diverse than fertile and not-fertile apices ones (Supplementary Figure 2b).



Supplementary Figure 3: Community structure of the three whole thalli. Relative abundance of OTUs at the family level. BH, Basal Holdfast, NF, non-fertile apices, F fertile apices.

We run Functional Annotation of Prokaryotic Taxa (FAPROTAX v1.1) software to predict functional annotations based on microbial community (Louca et al. 2016). We match the taxonomic data (used for indicator species analysis) of the prokaryotes against this database to predict the functions of the prokaryotes for their biogeochemical function. We obtained putative functional assignment for 144 prokaryotic OTUs (>90% of the global dataset), which we also used for the indicator species analysis and classified into 23 functional groups (Supplementary Table 3, Supplementary Figure 4, 5). We

have identified 8 network modules formed by OTUs that were sharing the same functional annotation (Supplementary Figure 4, 5). M1 contained 44 OTUs (33.58% of total OTUs), M2 contained 35 OTUs (26.71% of total OTUs), M3 contained 18 OTUs (13.74% of total OTUs), M4 contained 12 OTUS (9.16% of total OTUs), M5 contained 10 OTUs (7.63% of total OTUs), M6 contained 6 OTUs (4.58% of total OTUs), M7 contained 4 OTUs (3.05% of total OTUs) and M8 contained 2 OTUs (1.52% of total OTUs).

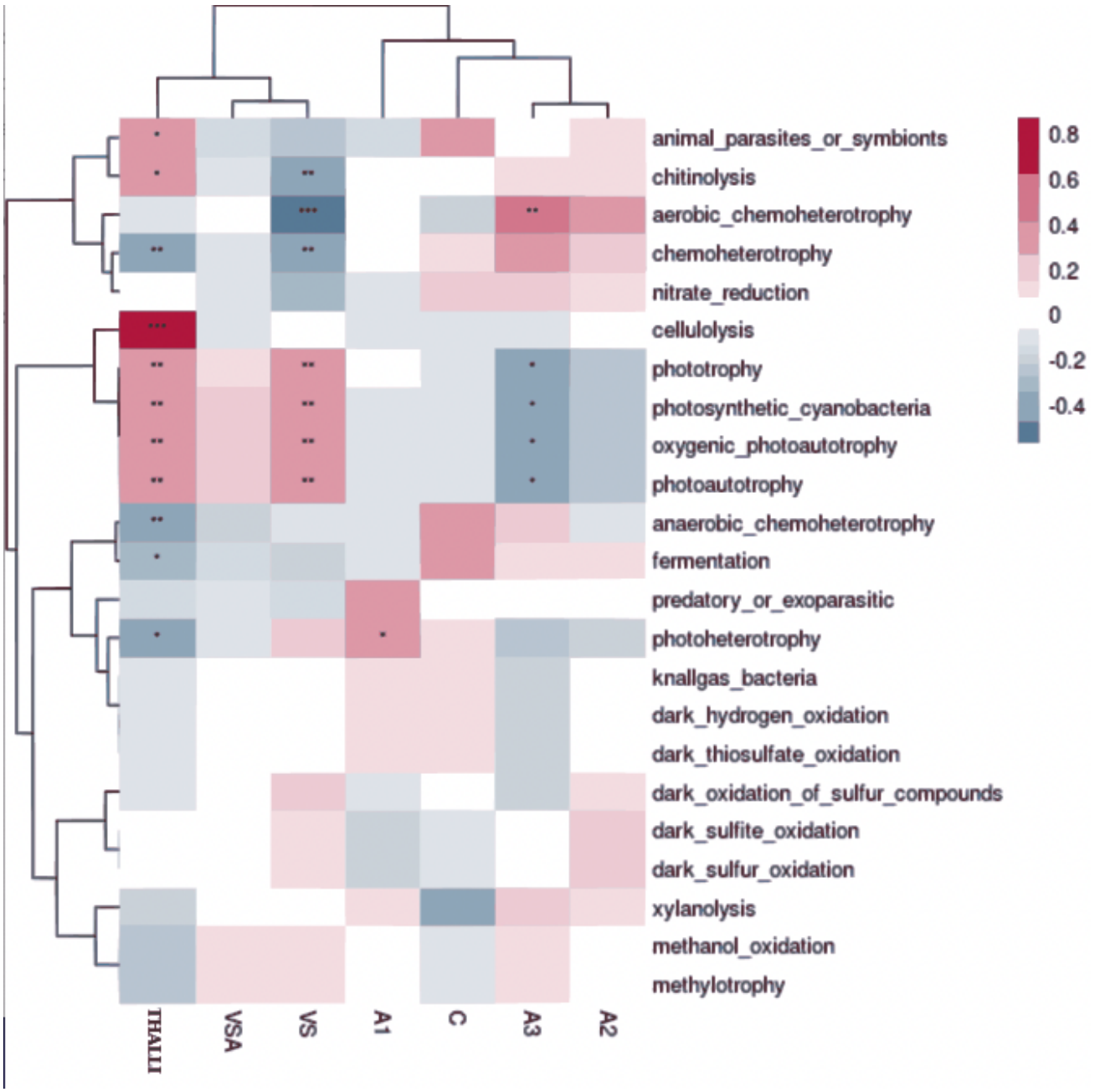


Supplementary Figure 4: Percentages of the OTUs for each functional group in the 8 network modules.

M1 OTU network was characterized by photoautrotophy, anaerobic and aerobic chemoheterotrophy, fermentation and nitrate reduction. M2 OTU network was characterized by photoheterotrophy, anaerobic and aerobic chemoheterotrophy, fermentation and dark hydrogen oxidation. M3 OTU network was characterized by photoheterotrophy, anaerobic and aerobic chemoheterotrophy and nitrate reduction. M4 OTU network was characterized by aerobic chemoheterotrophy and cellulolysis. M5 OTU network was characterized by aerobic chemoheterotrophy and nitrate reduction. M6 OTU network was characterized by photoheterotrophy and aerobic chemoheterotrophy. M7 OTU network was characterized by aerobic chemoheterotrophy, fermentation, chitinolysis and nitrate reduction. M8 OTU was characterized by chemoheterotrophy and nitrate reduction.

The most abundant functional groups were aerobic chemoheterotrophy, nitrate reduction and fermentation. aerobic Aerobic chemoheterotrophy was present in all the modules (100% in M7, 50% in M6, 50% in M8, 45.77% in M2, 44,44% in M3, 38,64% in M1 and 33.33% in M4. Nitrate reduction was present in 5 modules (100% in M7, 50% in M8, 31.82% in M1, 11.11% in M3 and 10% in M5). Fermentation was present in 3 modules (100% in M7, 61.36% in M1 and 8.57% in M2).

FAPROTAX analysis (Supplementary Figure 5) identified two groups: the first was formed by adult thalli, VSA and VS and the second by A1, C, A2 and A3. Adult thalli, VSA and VS were characterized by phototrophy, whereas chemoheterotrophy and fermentation were positively correlated with C, A2 and A3. Cellulolysis and chitinolysis were strongly correlated with adult thalli. Nitrate reduction was positively correlated with C, A2 and A3 and negatively correlated with VSA and VS.



Supplementary Figure 5: Heatmap of predicted functions based on the FAPROTAX database correlated with Spearman to treatments and adult thalli. The 23 categories presented here correspond to 148 prokaryotic OTUs of the indicator species analysis. Asterisks indicate Spearman's correlation coefficient (*p < 0.05; **p < 0.01; ***p < 0.001). Cold to warm color scale indicates value of Spearman correlation index.

Louca, S., Parfrey, L. W., & Doebeli, M. (2016). Decoupling function and taxonomy in the global ocean microbiome. *Science*, *353*(6305), 1272-1277.