

Metabolomics and Microbiomics Insights into Differential Surface Fouling of Three Macroalgal Species of *Fucus* (Fucales, Phaeophyceae) that Co-exist in the German Baltic Sea

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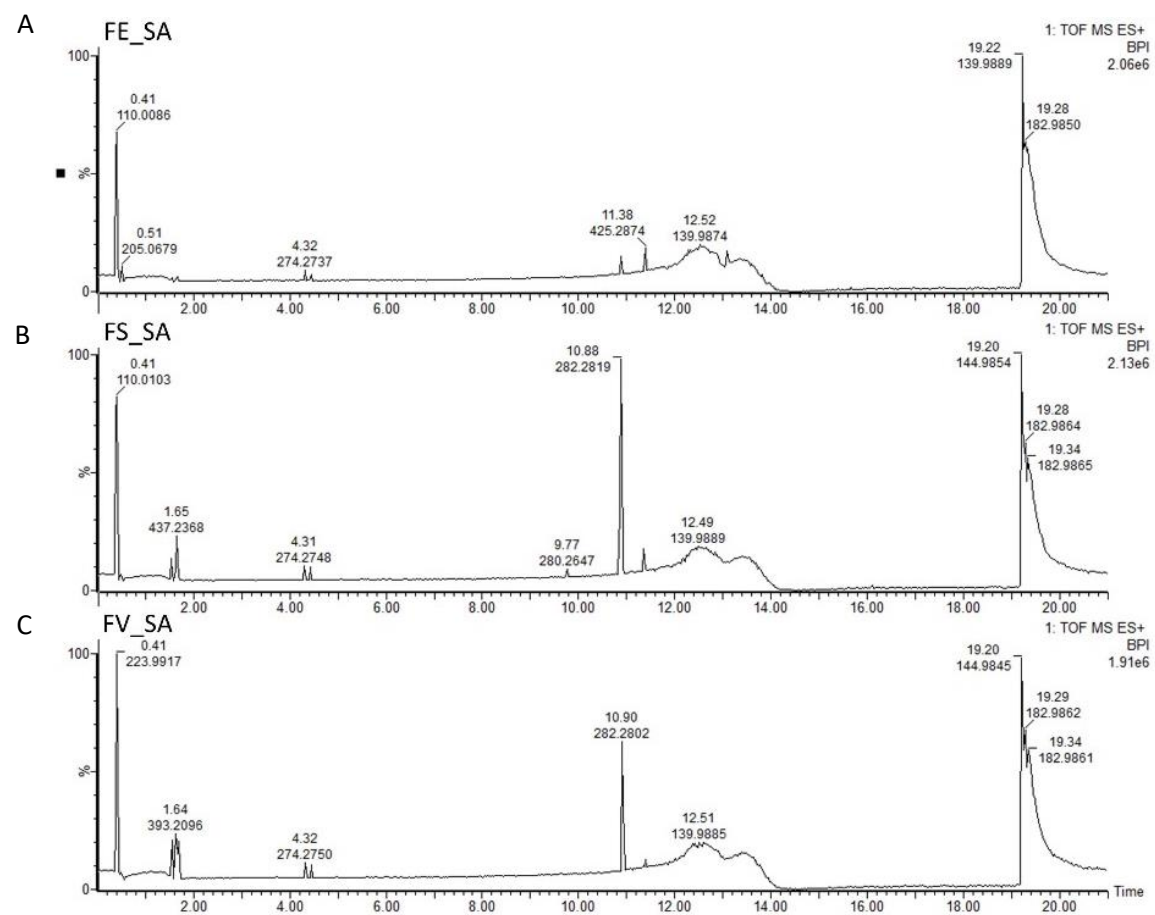


Figure S1. UPLC-MS(+) base peak chromatograms of Surface Adsorption (SA) extracts of A) FE, B) FS and C) FV

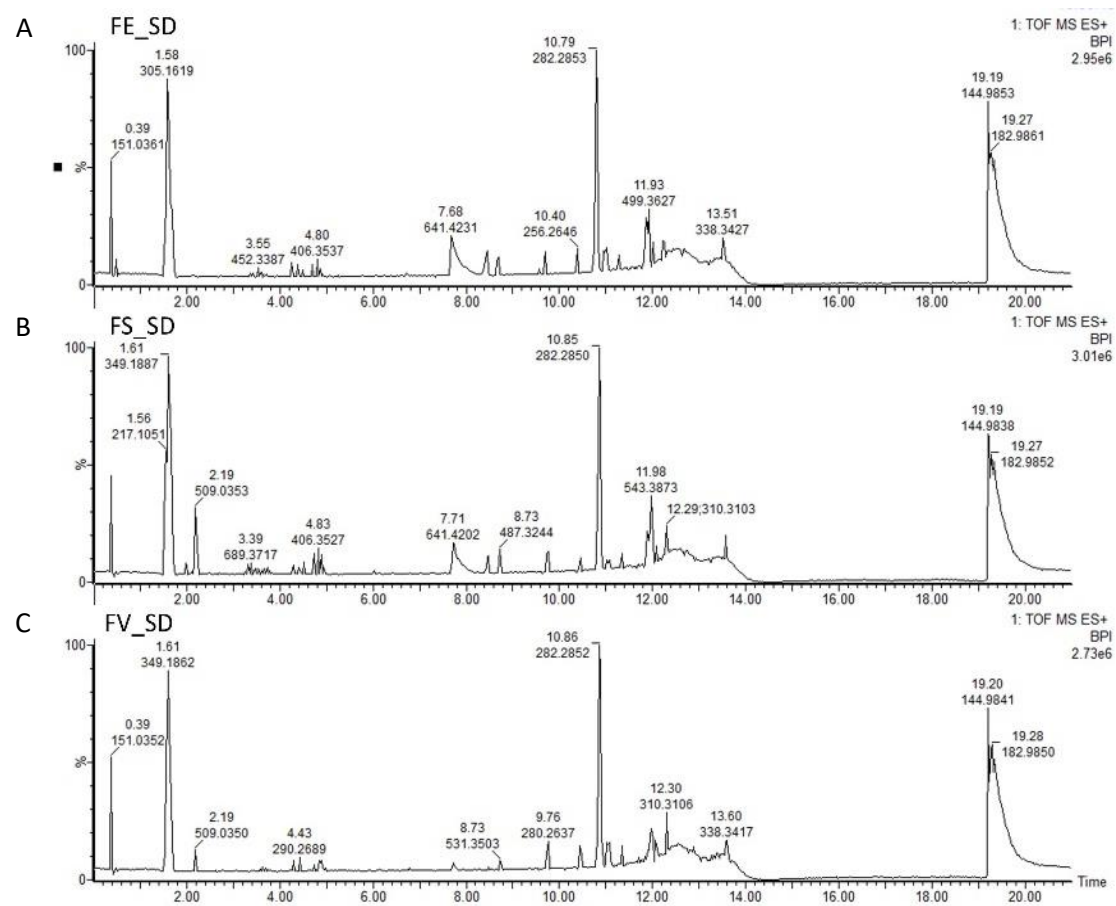


Figure S2. UPLC-MS(+) base peak chromatograms of Solvent Dipping (SD) extracts of A) FE, B) FS and C) FV

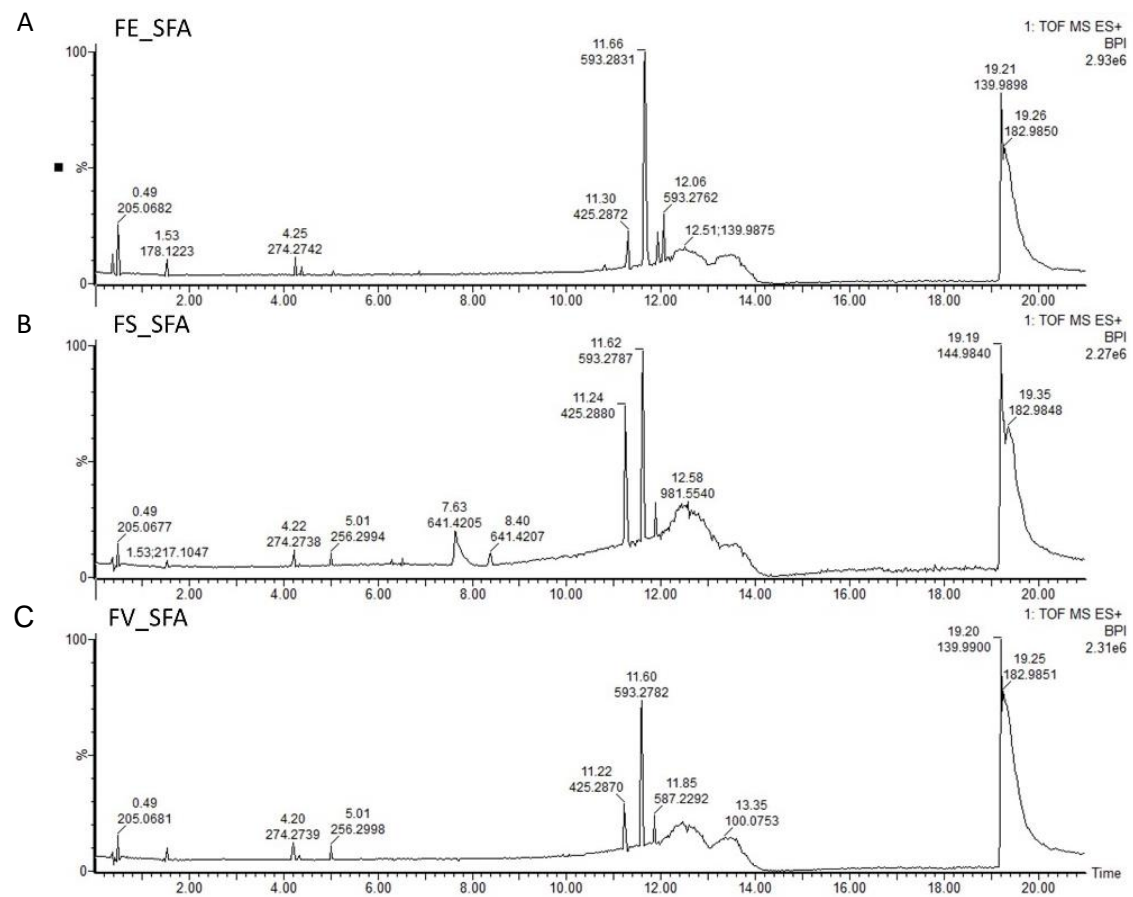


Figure S3. UPLC-MS(+) base peak chromatograms of Surface-free after adsorption (SFA) extracts of A) FE, B) FS and C) FV

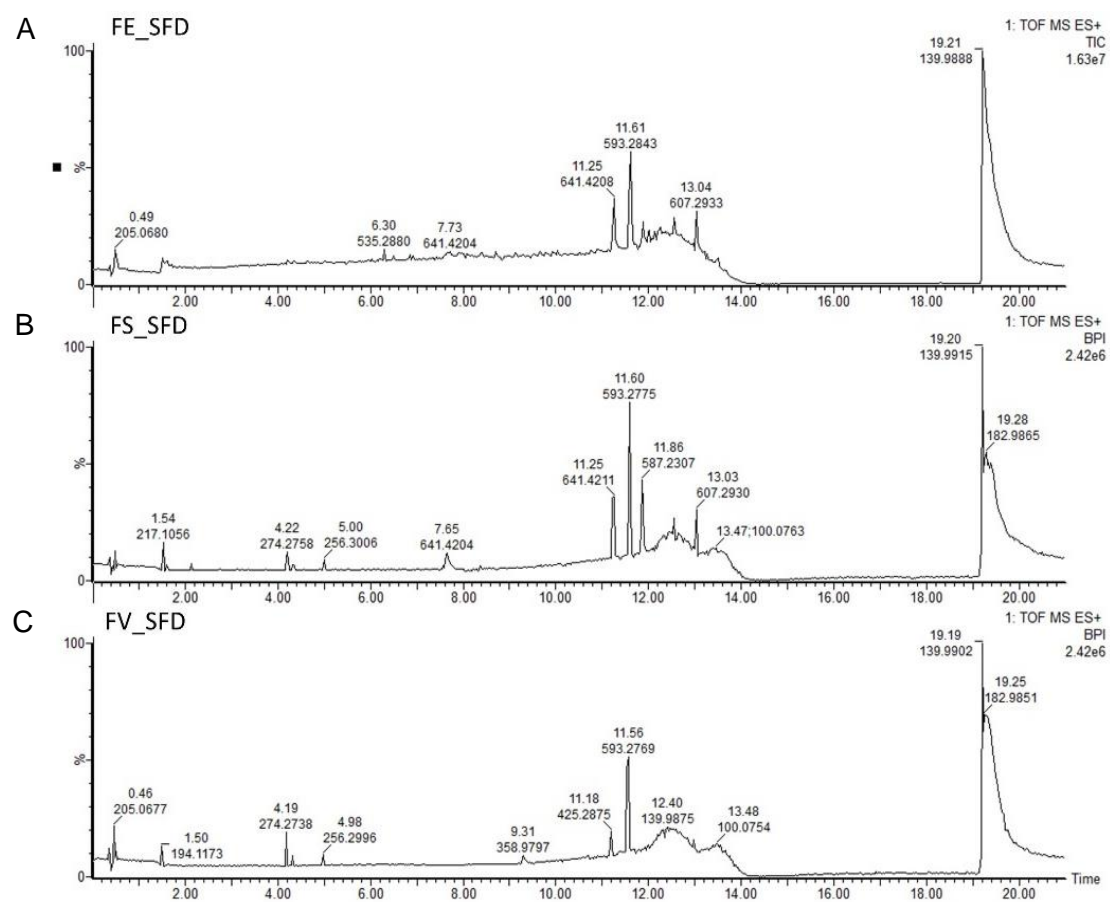


Figure S4. UPLC-MS(+) base peak chromatograms of Surface-free after dipping (SFD) extracts of A) FE, B) FS and C) FV

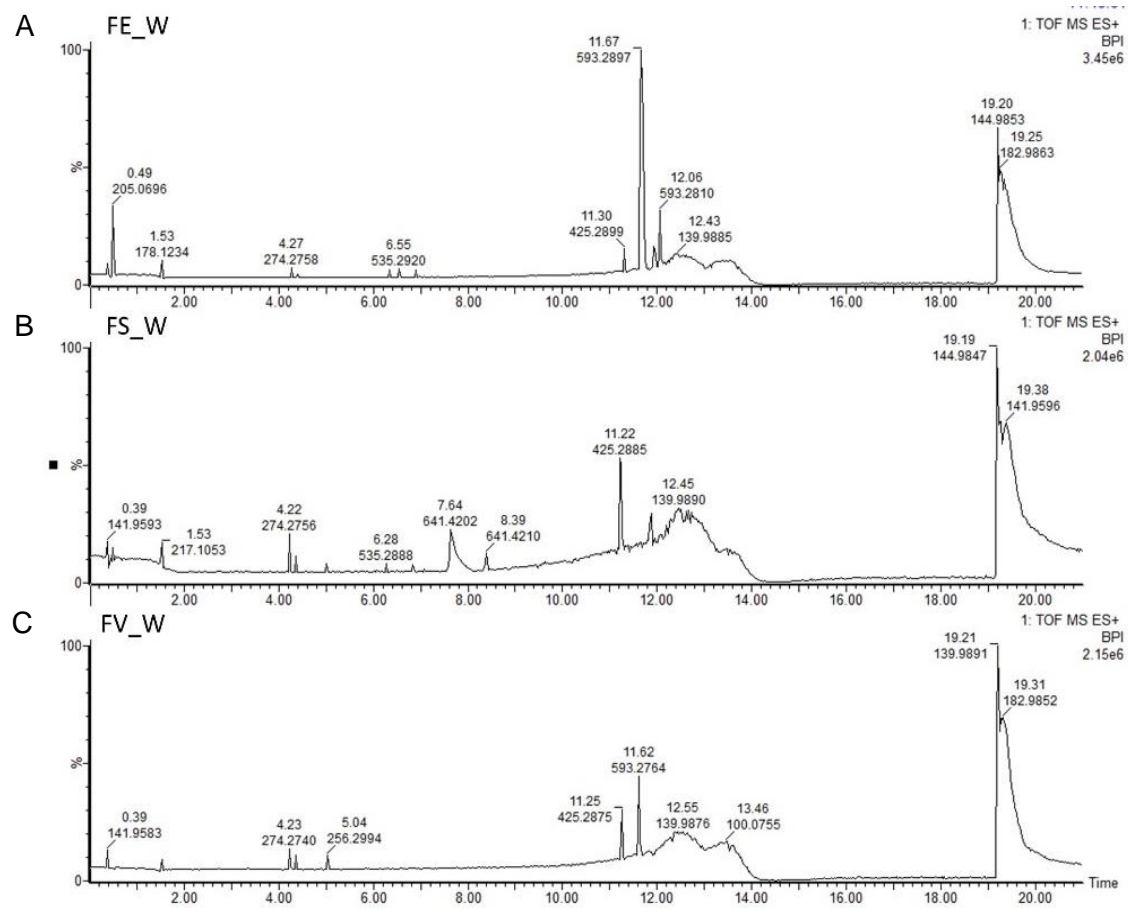


Figure S5. UPLC-MS(+) base peak chromatograms of Whole (W) extracts of A) FE, B) FS and C) FV

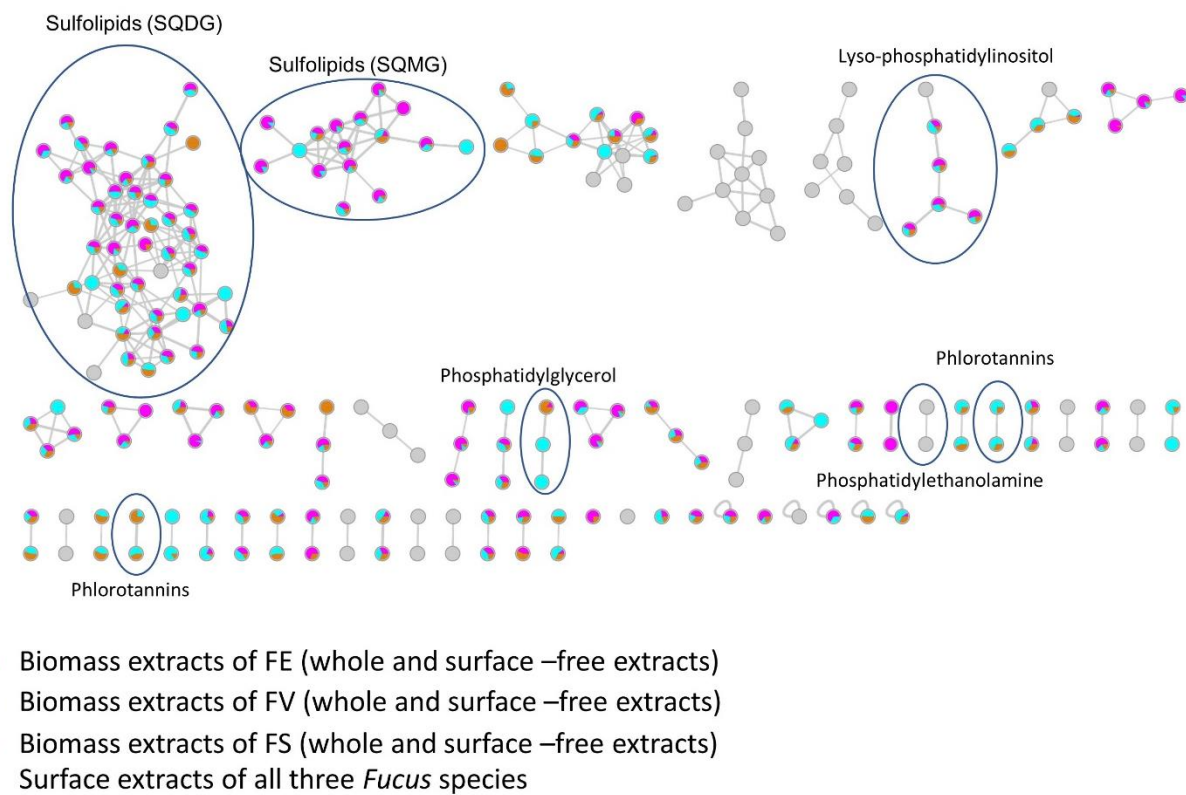


Figure S6. MN generated from UPLC(-)-ESI-MS/MS data of all extracts of *Fucus* spp. in the negative ion mode. Node colour represents the source of the ion.

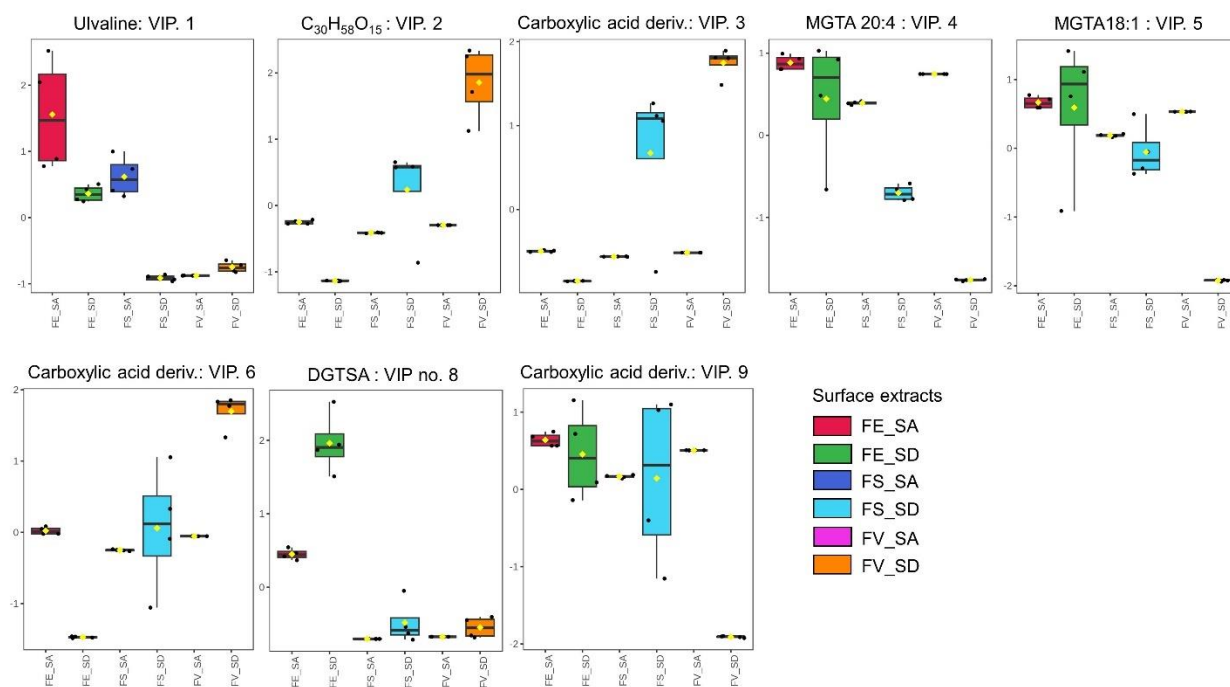


Figure S7. Variation of the most significant discriminatory metabolite markers (VIP > 1.8) on the surfaces of the three *Fucus* spp. Normalized intensities are presented on the y-axis.

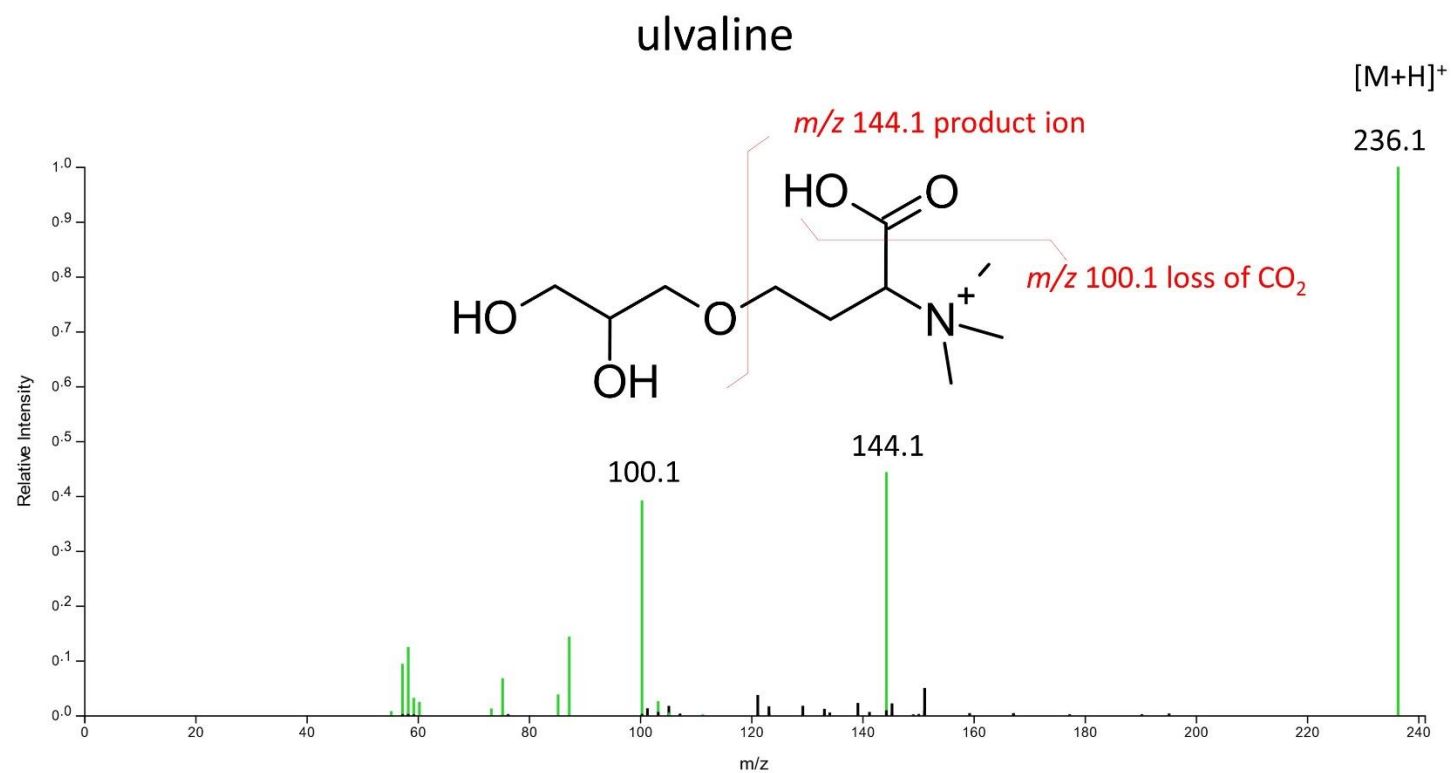


Figure S8. MS/MS spectra of $[\text{M} + \text{H}]^+$ ion of ulvaline at m/z 236.1 displaying the typical product ions.

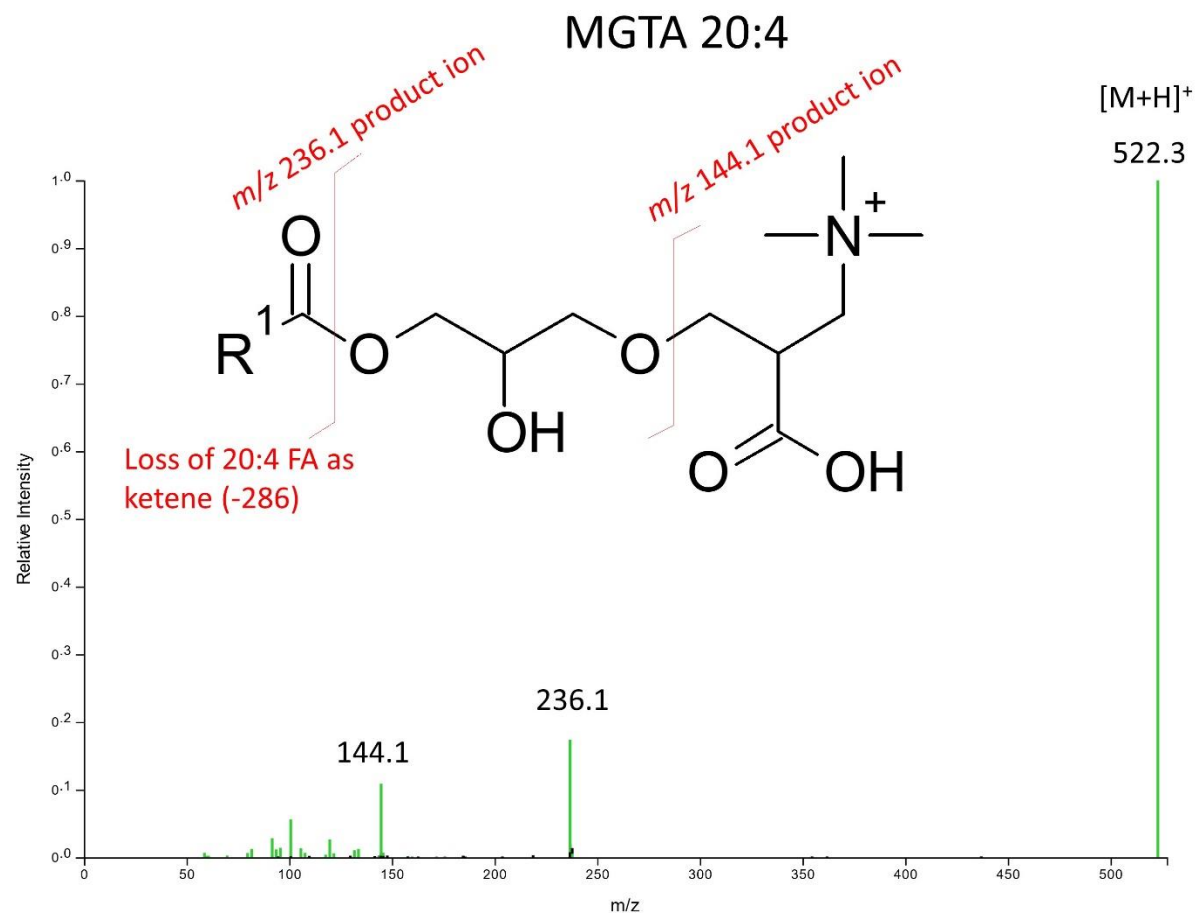


Figure S9. MS/MS spectra of $[M + H]^+$ ion of MGTA 20:4 at m/z 522.3 displaying the typical product ions.

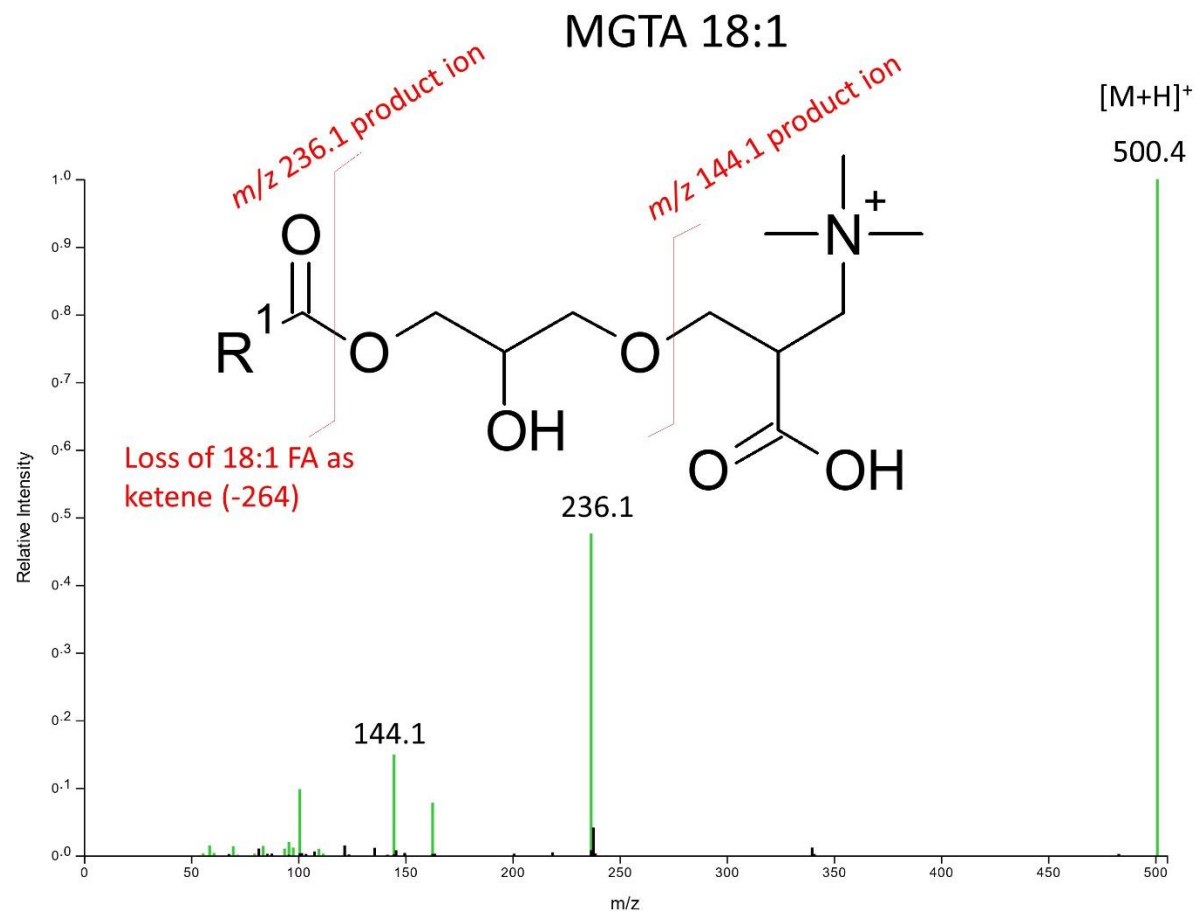


Figure S10. MS/MS spectra of $[M + H]^+$ ion of MGTA 18:1 at m/z 500.4 displaying the typical product ions.

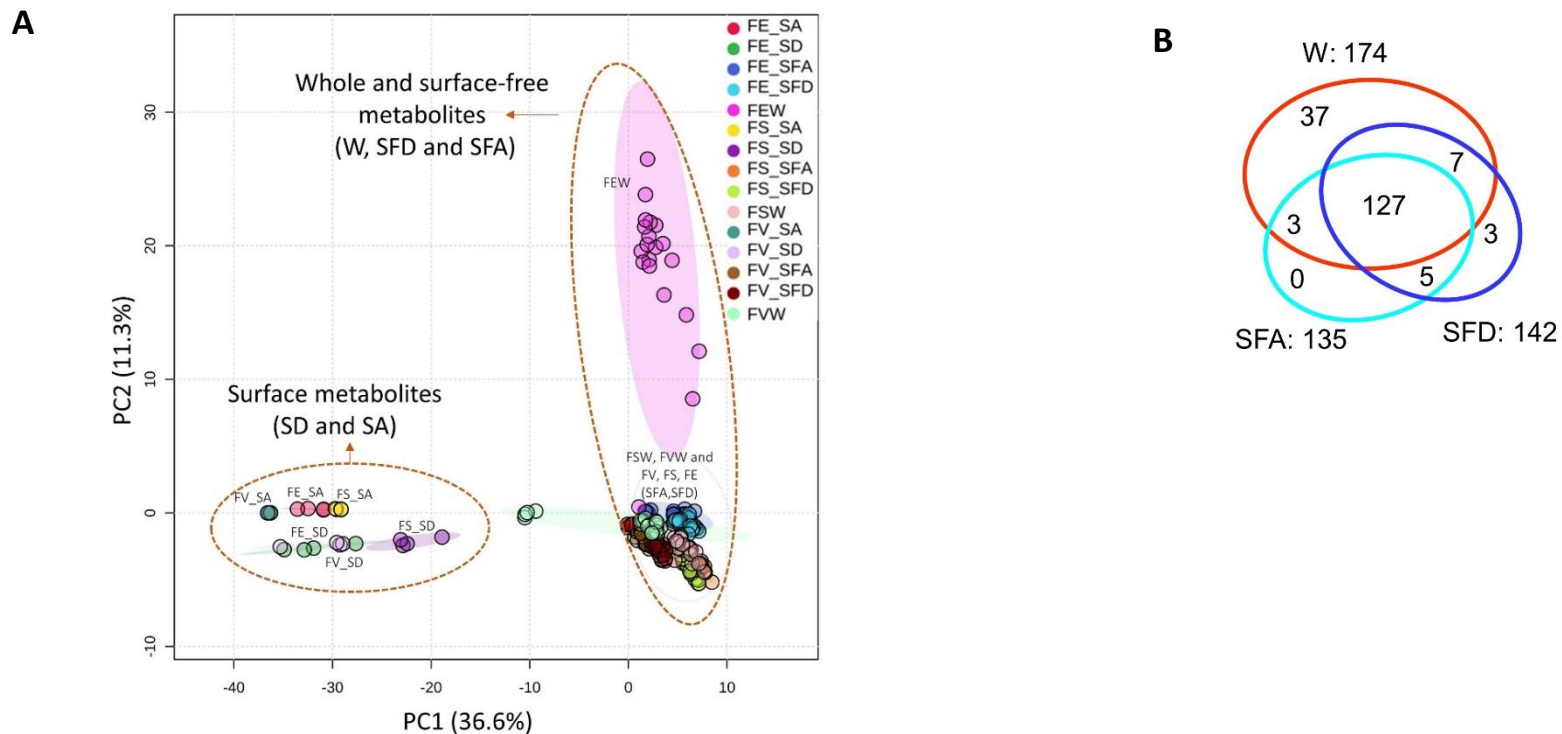


Figure S11. PCA scores plot generated from UPLC-(+)-ESI-MS data of all extracts showing a clear discrimination between surface metabolites from surface free and whole metabolome. Algal species: FV: *F. vesiculosus*, FS: *F. serratus*, FE: *F. distichus* subsp. *evanescens*, Extracts: SA: surface adsorption, SD: solvent dipping, SFA: surface-free after adsorption, SFD: surface-free after dipping, W: whole, untreated algae (B) Venn diagram showing distribution of nodes among the whole extracts (W), surface-free extract after adsorption (SFA) and dipping (SFD).

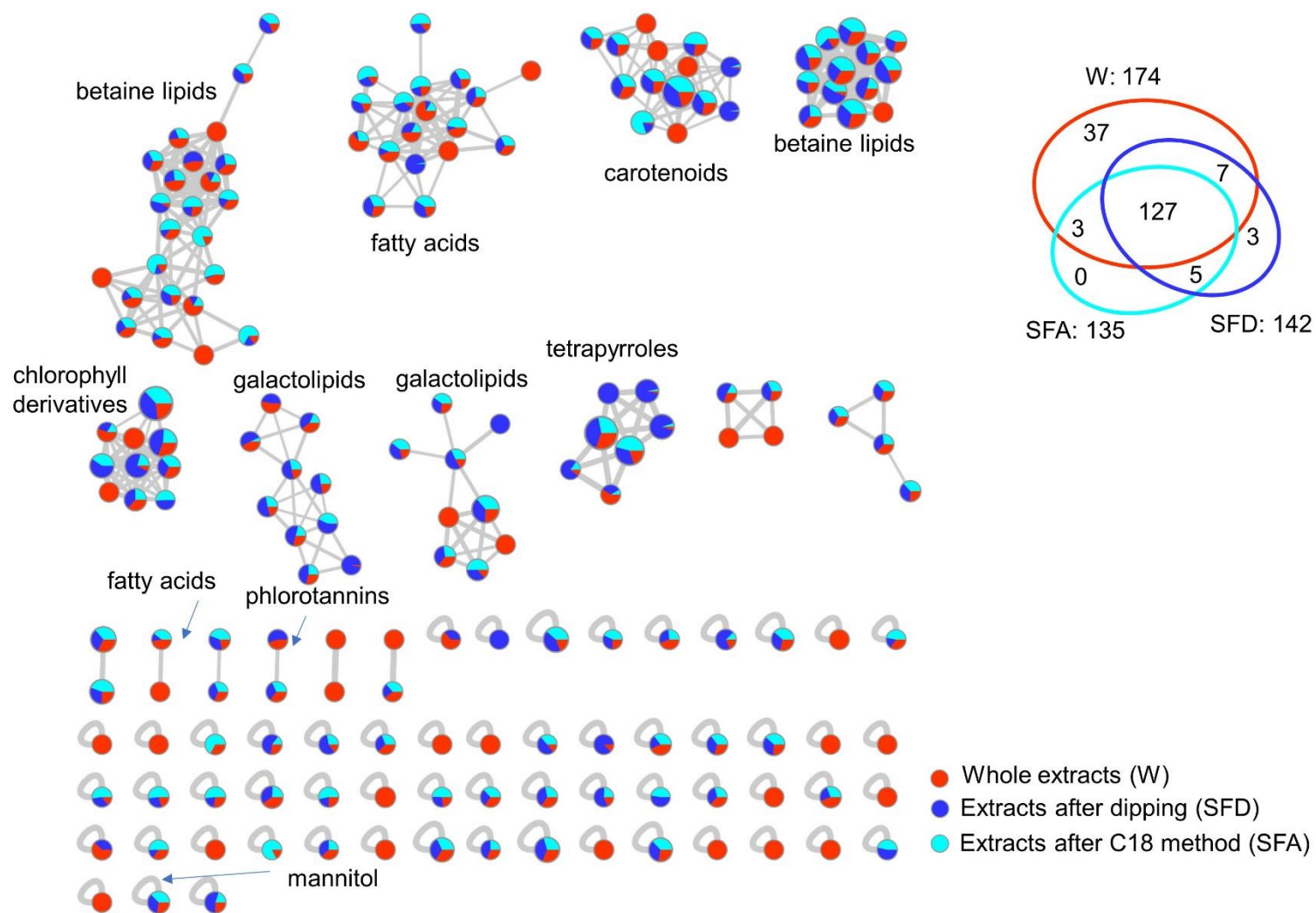


Figure S12. MN generated from UPLC-(+)-ESI-MS/MS data of all surface-free and whole extracts. Node sizes are modulated according to the sum of intensities of the ions in all extracts while the colors in the pie chart of each node represents the relative quantity of the ion from whole extracts (red), surface-free after dipping (blue) and surface-free after C18 adsorption (light blue)

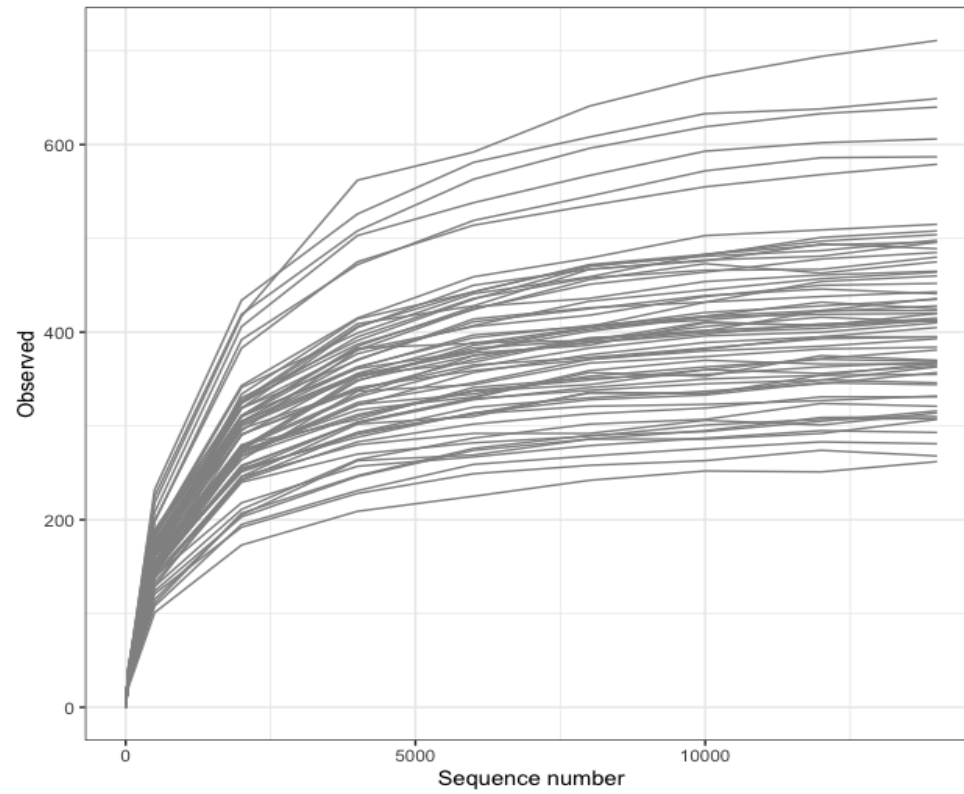


Figure S13. Rarefaction curves of bacterial V3/V4 region amplicon sequences from all 66 samples.

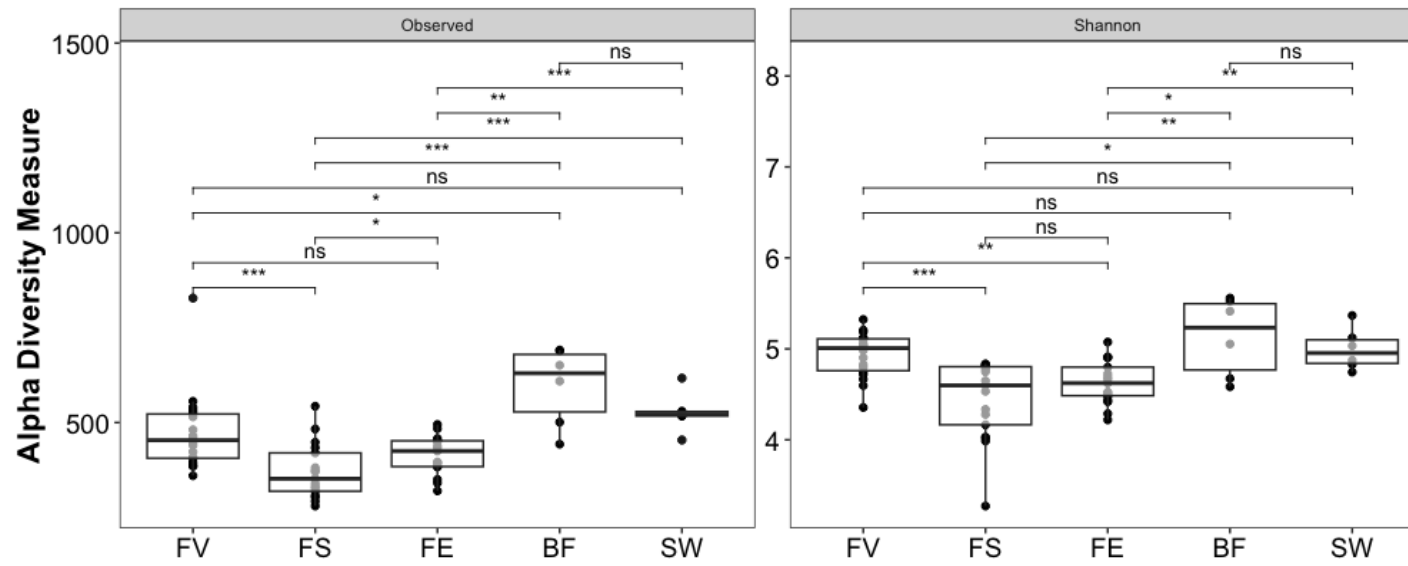


Figure S14. Alpha diversity (ASV-Observed vs. Shannon) of bacterial epiphytic community with regard to sample source. FV: *F. vesiculosus*, FS: *F. serratus*, FE: *F. distichus* subsp. *evanescens*, BF: biofilm on stone, SW: seawater. Significance levels: >0.0001: ***, >0.001: **, 0.01: *, >0.05: ns)

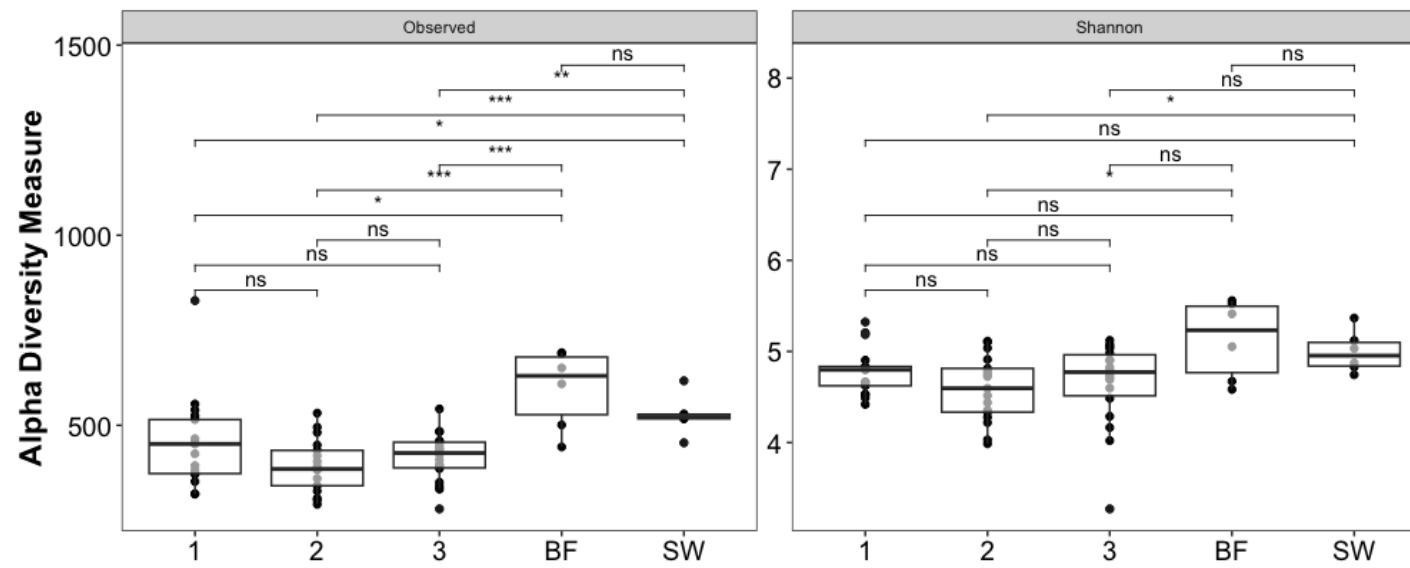


Figure S15. Alpha diversity (ASV-Observed vs. Shannon) of bacterial epiphytic community with regard to individual. 1: individual 1, 2: individual 2, 3: individual 3, BF: biofilm on stone, SW: seawater. Significance levels: >0.0001: ***, >0.001: **, 0.01: *, >0.05: ns)

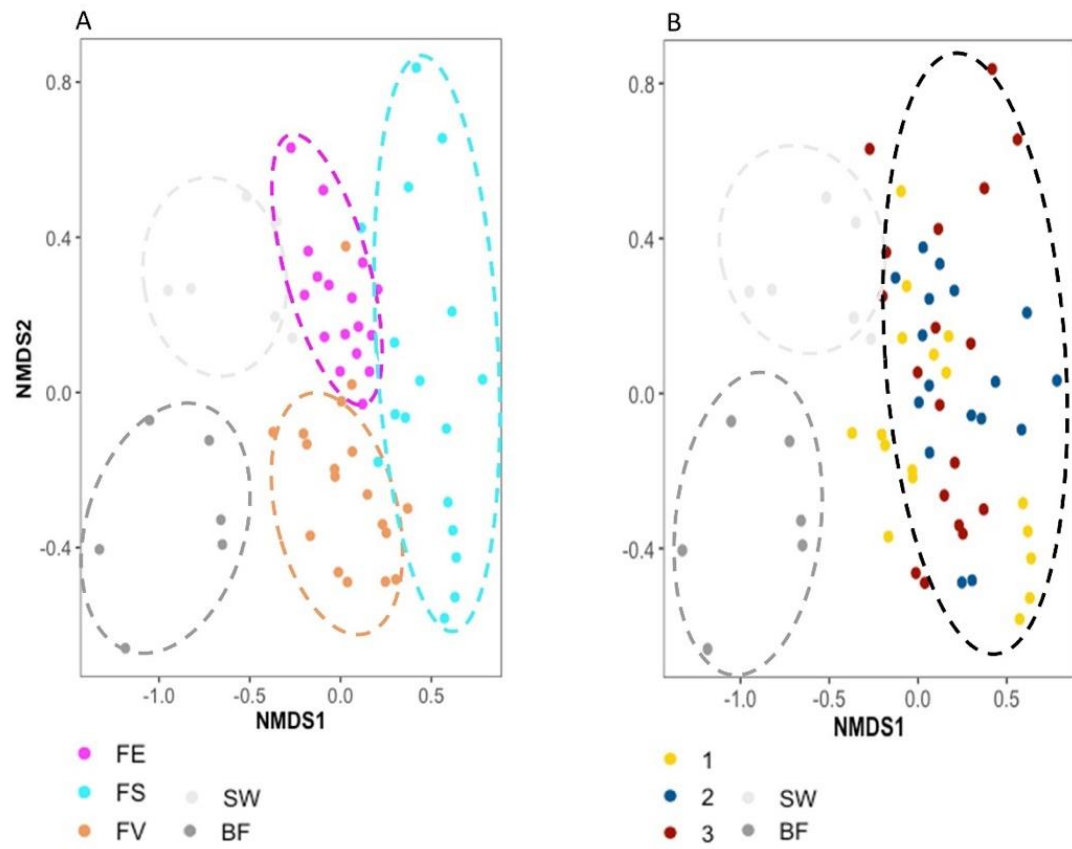


Figure S16. Beta diversity analysis of bacterial amplicon data based on Bray-Curtis distance calculation visualized by NMDS plots (A) according to sample origin (B) according to individual. FV: *Fucus vesiculosus*, FS: *F. serratus*, FE: *F. distichus* subsp. *evanescens*, BF: biofilm on stone, SW: seawater.

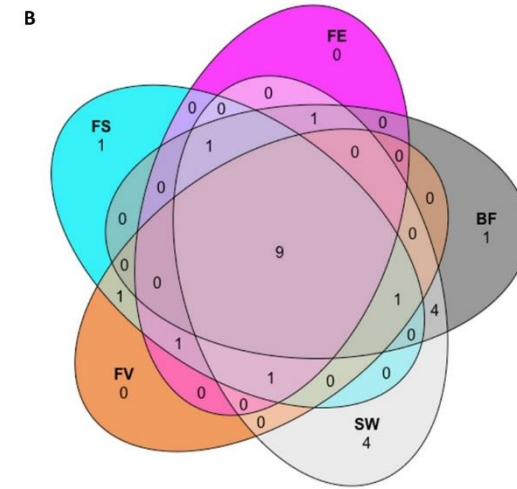
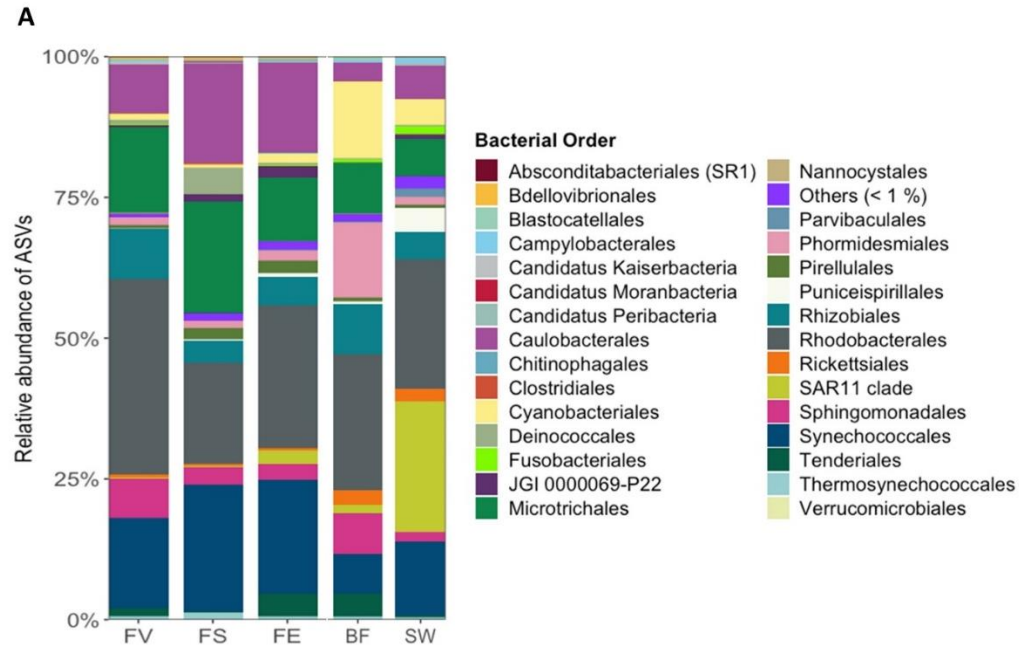


Figure S17. (A) Bacterial orders associated with surfaces of *Fucus* spp., and stone biofilm and seawater reference samples. Others (<1%) represents several orders with less than 1% relative abundance. (B) Venn diagram displaying bacterial orders with regard to different sample types FV: *Fucus vesiculosus*, FS: *F. serratus*, FE: *F. distichus* subsp. *evanescens*, BF: biofilm on stone, SW: seawater.

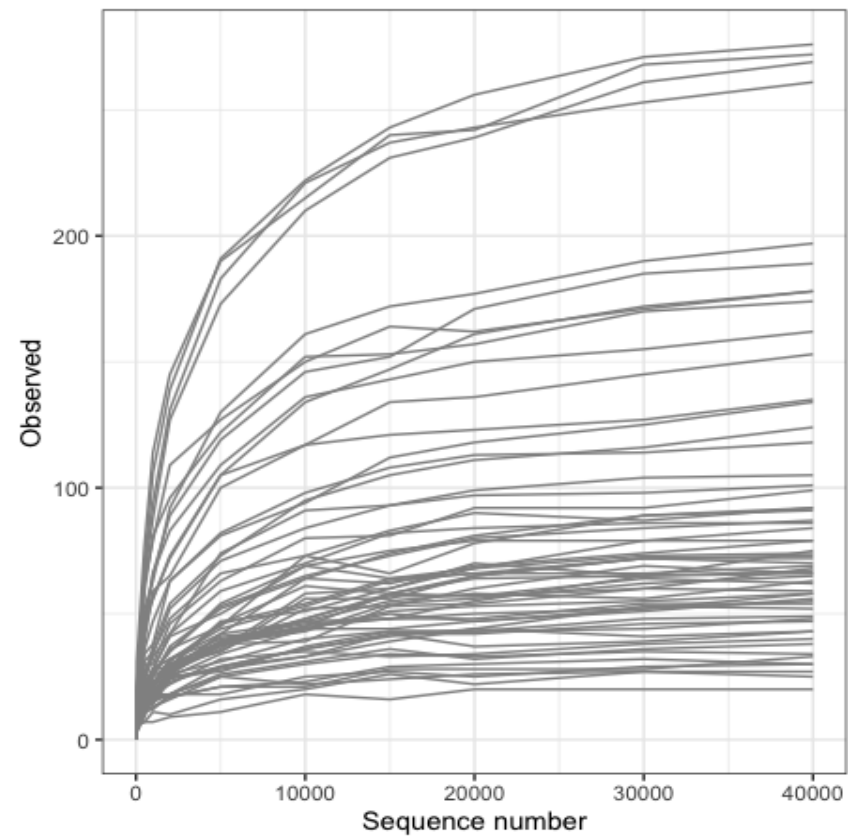


Figure S18. Rarefaction curves of eukaryotic ITS region amplicon sequences from all 60 samples.

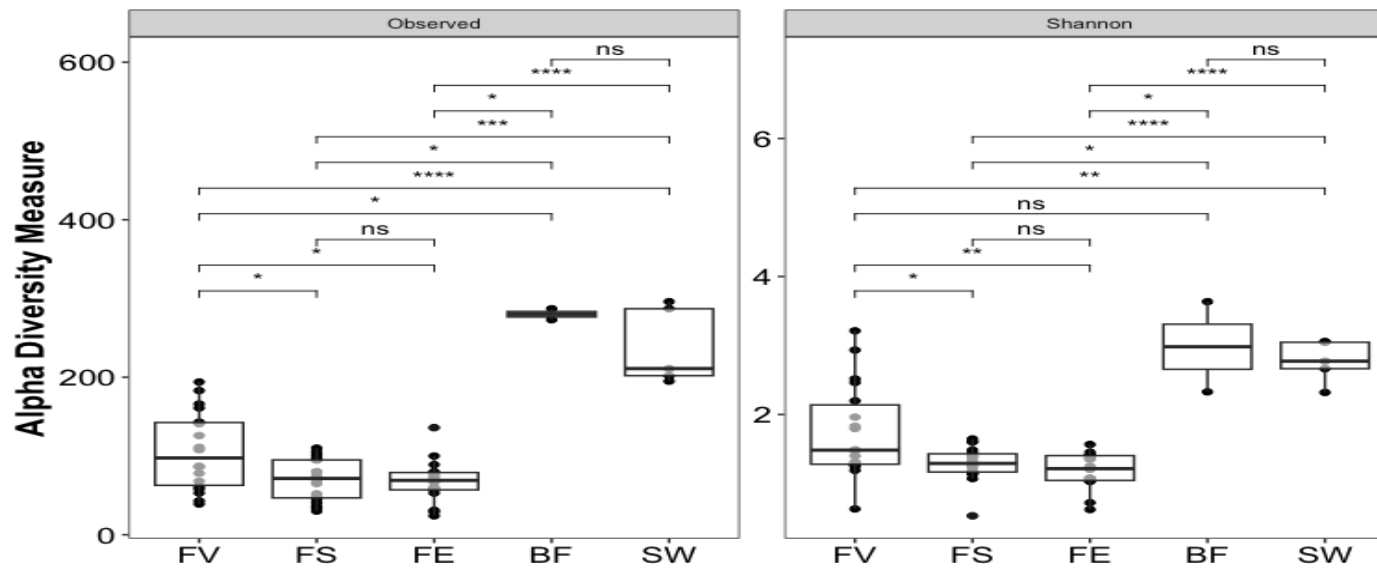


Figure S19. Alpha diversity (ASV-Observed vs. Shannon) of eukaryotic epiphytic community based on ITS fragment sequences with regard to sample source. FV: *F. vesiculosus*, FS: *F. serratus*, FE: *F. distichus* subsp. *evanescens*, BF: biofilm on stone, SW: seawater. Significance levels: >0:****, >0.0001: ***, >0.001: **, 0.01: *, >0.05: ns)

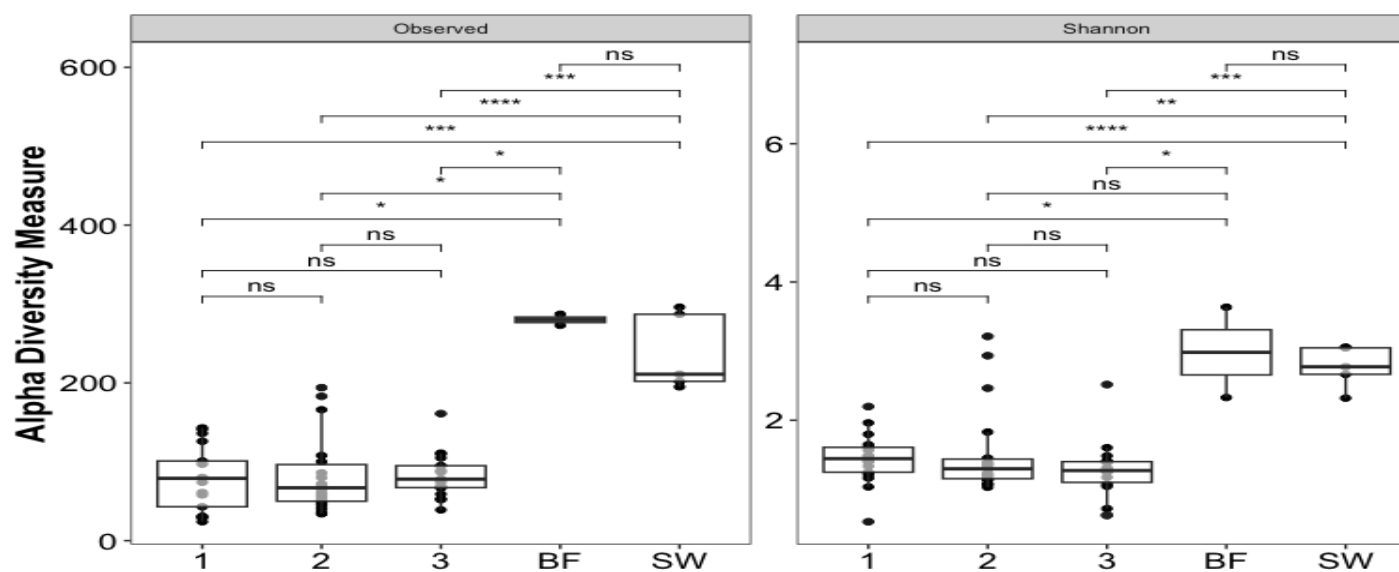


Figure S20. Alpha diversity (ASV-Observed vs. Shannon) of eukaryotic epiphytic community based on ITS fragment sequences with regard to individual. 1: individual 1, 2: individual 2, 3: individual 3, BF: biofilm on stone, SW: seawater. Significance levels: >0:****, >0.0001: ***, >0.001: **, 0.01: *, >0.05: ns)

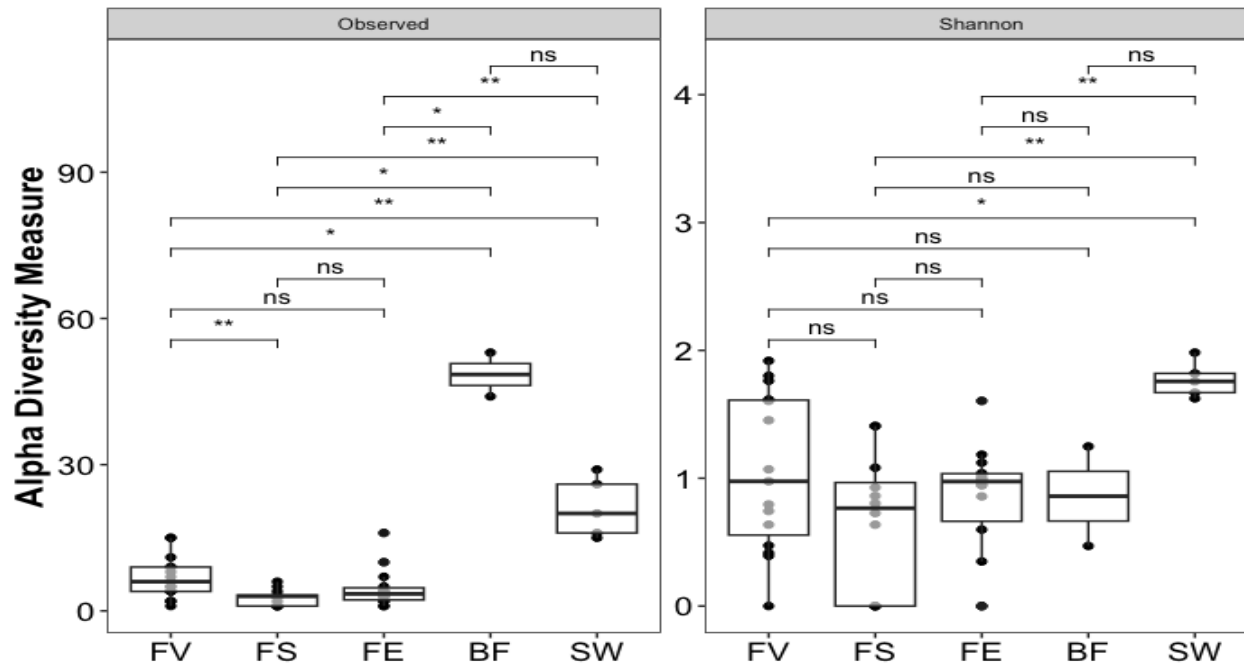


Figure S21. Alpha diversity (ASV-Observed vs. Shannon) of fungal epiphytic community based on ITS fragment sequences with regard to sample source. FV: *F. vesiculosus*, FS: *F. serratus*, FE: *F. distichus* subsp. *evanescens*, BF: biofilm on stone, SW: seawater. Significance levels: >0.***, >0.0001: ***, >0.001: **, 0.01: *, >0.05: ns)

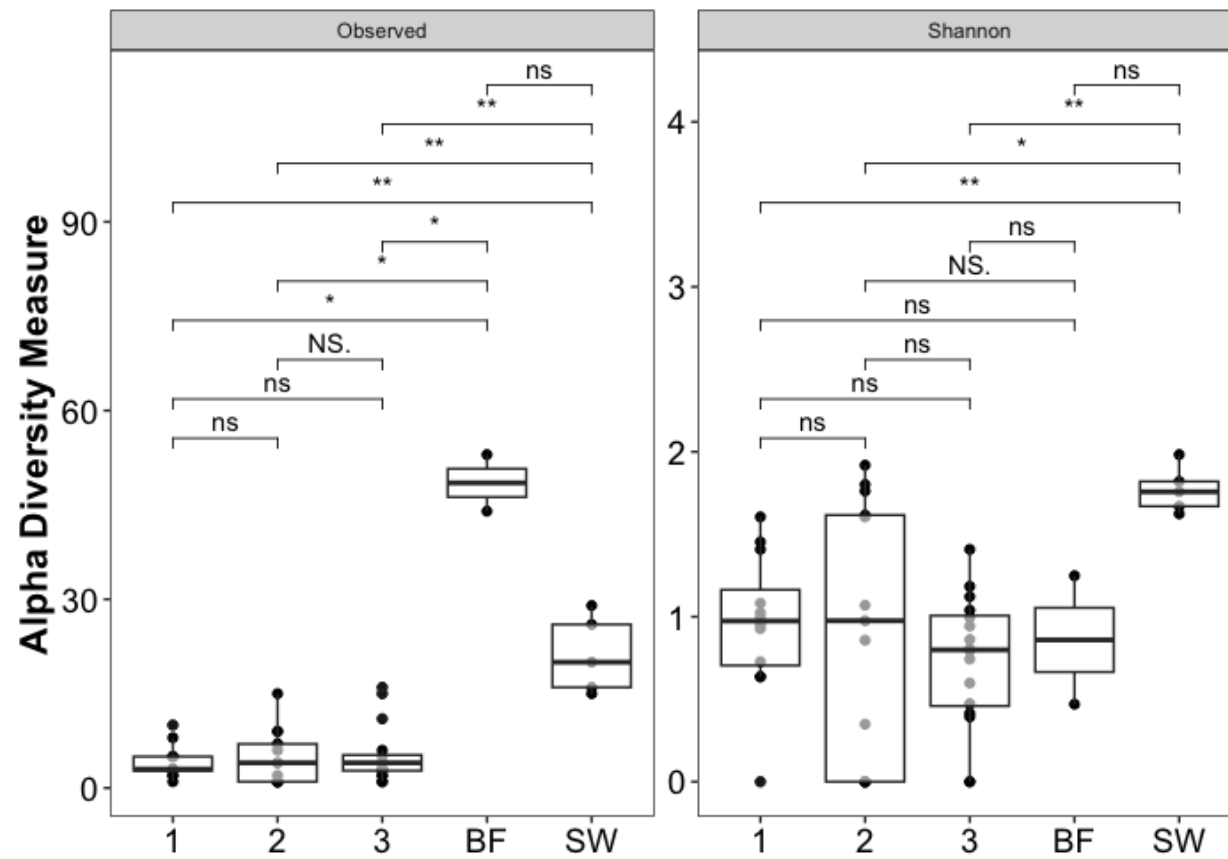


Figure S22. Alpha diversity (ASV-Observed vs. Shannon) of fungal epiphytic community based on ITS fragment sequences with regard to individual. 1: individual 1, 2: individual 2, 3: individual 3, BF: biofilm on stone, SW: seawater. Significance levels: >0:***, >0.0001: ***, >0.001: **, 0.01: *, >0.05: ns)

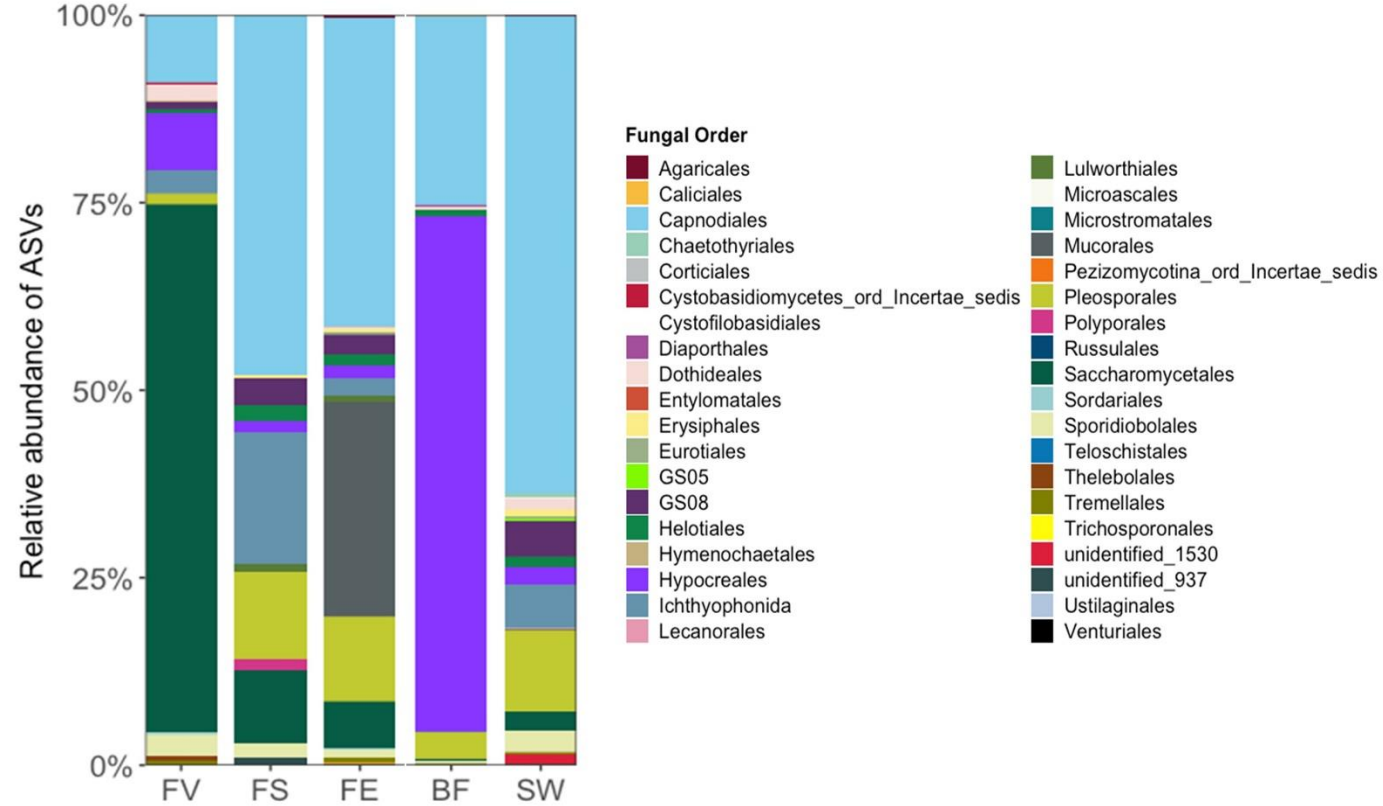


Figure S23. Fungal orders associated to surfaces of *Fucus* spp., and stone biofilm (BF) and seawater (SW) reference samples. FV: *Fucus vesiculosus*, FS: *F. serratus*, FE: *F. distichus* subsp. *evanescens*.

Table S2

Relative abundances of bacterial genera (> 1%) associated to surfaces of *Fucus* spp., seawater and stone biofilm based on amplicon sequencing of the V3/V4 region of the 16S rRNA gene.

<i>F. vesiculosus</i> (FV)				<i>F. serratus</i> (FS)				<i>F. distichus</i> subsp. <i>evanescens</i> (FE)			
ASV No.	Genus	Phylum	% Abundance	ASV No.	Genus	Phylum	% Abundance	ASV No.	Genus	Phylum	% Abundance
ASV1	Schizothrix LEGE 07164	Cyanobacteria	24.41	ASV1	Schizothrix LEGE 07164	Cyanobacteria	29.61	ASV1	Schizothrix LEGE 07164	Cyanobacteria	26.02
ASV11	Sva0996 marine group	Actinobacteriota	11.78	ASV11	Sva0996 marine group	Actinobacteriota	13.97	ASV4	Robiginitomaculum	Proteobacteria	12.84
ASV12	Ilumatobacter	Actinobacteriota	9.85	ASV17	Litorimonas	Proteobacteria	13.65	ASV10	Yoonia-Loktanella	Proteobacteria	8.96
ASV10	Yoonia-Loktanella	Proteobacteria	8.68	ASV12	Ilumatobacter	Actinobacteriota	8.31	ASV11	Sva0996 marine group	Actinobacteriota	7.20
ASV20	Octadecabacter	Proteobacteria	6.85	ASV24	Truepera	Deinococcota	6.24	ASV12	Ilumatobacter	Actinobacteriota	7.12
ASV17	Litorimonas	Proteobacteria	5.08	ASV4	Robiginitomaculum	Proteobacteria	3.62	ASV13	Candidatus Tenderia	Proteobacteria	5.25
ASV14	Silicimonas	Proteobacteria	5.00	ASV21	Fretibacter	Proteobacteria	3.50	ASV20	Octadecabacter	Proteobacteria	3.96
ASV51	Erythrobacter	Proteobacteria	4.08	ASV10	Yoonia-Loktanella	Proteobacteria	2.99	ASV17	Litorimonas	Proteobacteria	3.86
ASV4	Robiginitomaculum	Proteobacteria	2.74	ASV68	Blastopirellula	Planctomycetota	2.67	ASV68	Blastopirellula	Planctomycetota	2.90
ASV39	Filomicrobium	Proteobacteria	2.48	ASV20	Octadecabacter	Proteobacteria	2.36	ASV21	Fretibacter	Proteobacteria	2.69
ASV13	Candidatus Tenderia	Proteobacteria	2.04	ASV88	Acaryochloris MBIC11017	Cyanobacteria	1.71	ASV19	Clade Ia	Proteobacteria	2.03
ASV73	Altererythrobacter	Proteobacteria	1.54	ASV14	Silicimonas	Proteobacteria	1.59	ASV14	Silicimonas	Proteobacteria	1.75
ASV21	Fretibacter	Proteobacteria	1.35	ASV61	Phormidesmis ANT.LACV5.1	Cyanobacteria	1.39	ASV61	Phormidesmis ANT.LACV5.1	Cyanobacteria	1.64
ASV61	Phormidesmis ANT.LACV5.1	Cyanobacteria	1.24	ASV39	Filomicrobium	Proteobacteria	1.05	ASV29	Planktomarina	Proteobacteria	1.31
ASV24	Truepera	Deinococcota	1.09								
seawater (SW)				stone biofilm (BF)							
ASV No.	Genus	Phylum	% Abundance	ASV No.	Genus	Phylum	% Abundance				
ASV19	Clade Ia	Proteobacteria	22.46	ASV61	Phormidesmis ANT.LACV5.1	Cyanobacteria	12.76				
ASV1	Schizothrix LEGE 07164	Cyanobacteria	14.56	ASV49	Rivularia PCC-7116	Cyanobacteria	11.30				
ASV29	Planktomarina	Proteobacteria	10.17	ASV12	Ilumatobacter	Actinobacteriota	9.39				
ASV79	Cand. Puniceispirillum	Proteobacteria	6.02	ASV1	Schizothrix LEGE 07164	Cyanobacteria	8.85				
ASV12	Ilumatobacter	Actinobacteriota	4.89	ASV13	Cand. Tenderia	Proteobacteria	5.80				
ASV11	Sva0996 marine group	Actinobacteriota	4.13	ASV10	Yoonia-Loktanella	Proteobacteria	5.07				
ASV4	Robiginitomaculum	Proteobacteria	4.09	ASV76	Sphingorhabdus	Proteobacteria	4.66				
ASV130	Aphanizomenon NIES81	Cyanobacteria	3.44	ASV116	Pleurocapsa PCC-7319	Cyanobacteria	4.37				
ASV158	Cyanobium PCC-6307	Cyanobacteria	3.42	ASV93	Phormidium MBIC10003	Cyanobacteria	4.05				
ASV10	Yoonia-Loktanella	Proteobacteria	3.38	ASV11	Sva0996 marine group	Actinobacteriota	3.06				
ASV171	Propionigenium	Fusobacteriota	2.09	ASV51	Erythrobacter	Proteobacteria	1.89				
ASV17	Litorimonas	Proteobacteria	1.88	ASV98	Ahrensia	Proteobacteria	1.61				
ASV20	Octadecabacter	Proteobacteria	1.73	ASV4	Robiginitomaculum	Proteobacteria	1.54				
ASV61	Phormidesmis ANT.LACV5.1	Cyanobacteria	1.52	ASV14	Silicimonas	Proteobacteria	1.34				
ASV14	Silicimonas	Proteobacteria	1.28	ASV19	Clade Ia	Proteobacteria	1.25				
				ASV158	Cyanobium PCC-6307	Cyanobacteria	1.22				
				ASV29	Planktomarina	Proteobacteria	1.12				
				ASV20	Octadecabacter	Proteobacteria	1.10				

Table S3

Bacterial beta diversity statistics based on Bray-Curtis dissimilarity, left: PERMANOVA results, right: pairwise permutation test for homogeneity of multivariate dispersions

PERMANOVA		Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1								pairwise permutation for homogeneity of multivariate dispersion										
	Df	Sum Squares	R2	F	Pr(>F)	Signifi- cance	Resi- duals df	Residuals Sum Squares	Residuals R2		Df	Sum Squares	Mean Squares	F	N permut	Pr(>F)	Signifi- cance	Resi- duals df	Residuals sum squares	Residuals mean squares
full dataset										full dataset										
origin	4	4.8181	0.39588	9.6655	0.001	***	59	7.3572	0.60412	4	0.13976	0.034941	8.1559	1000	0.000999	***	59	0.25276	0.004284	
individual no.	4	3.1344	0.25753	5.1162	0.001	***	59	9.0364	0.74247	4	0.13753	0.034383	7.4047	1000	0.000999	***	59	0.27396	0.004643	
FV										FV										
individual no.	2	0.50452	0.26094	2.6481	0.015	*	15	1.42895	0.73906	2	0.00199	0.000966	0.2325	1000	0.7972	ns	15	0.064251	0.004283	
tissue age	1	0.58970	0.30499	7.0214	0.001	***	16	1.3438	0.69501	1	0.00586	0.005862	1.361	1000	0.2677	ns	16	0.068909	0.004307	
FS										FS										
individual no.	2	1.16040	0.42611	5.1974	0.001	***	14	1.5629	0.57389	2	0.03316	0.01658	2.1023	1000	0.1638	ns	14	0.110414	0.007887	
FE										FE										
individual no.	2	0.39425	0.23931	2.2022	0.013	*	14	1.25319	0.76069	2	0.00709	0.003544	1.0939	1000	0.3716	ns	14	0.045354	0.00324	

Table S4

Relative abundances of eukaryote genera (> 1%) associated to surfaces of *Fucus* spp., seawater and stone biofilm based on amplicon sequencing of the ITS fragment.

<i>F. vesiculosus</i> (FV)				<i>F. serratus</i> (FS)				<i>F. distichus</i> subsp. <i>evanescens</i> (FE)			
ASV No.	Genus	Phylum	% Abundance	ASV No.	Genus	Phylum	% Abundance	ASV No.	Genus	Phylum	% Abundance
ASV52	Zoothamnium	Ciliophora	39.26	ASV45	unidentified_91524	Ciliophora	33.17	ASV28	Sarsia	Cnidaria	27.22
ASV45	unidentified_91524	Ciliophora	28.84	ASV87	Ulva	Chlorophyta	17.18	ASV19	unidentified_12446	Ciliophora	25.46
ASV64	Candida	Ascomycota	16.26	ASV19	unidentified_12446	Ciliophora	11.57	ASV221	Mucor	Mucoromycota	11.60
ASV106	Pseudovorticella	Ciliophora	3.83	ASV106	Pseudovorticella	Ciliophora	9.69	ASV87	Ulva	Chlorophyta	8.74
ASV178	unidentified_15	unidentified	2.32	ASV28	Sarsia	Cnidaria	7.14	ASV45	unidentified_91524	Ciliophora	6.17
ASV210	Myoschiston	Ciliophora	1.92	ASV280	Uronema_91786	Ciliophora	5.94	ASV108	unidentified_5383	unidentified_6967	3.81
ASV10	Haptocillium	Ascomycota	1.82	ASV108	unidentified_5383	unidentified_6967	4.17	ASV97	Alternaria	Ascomycota	2.64
ASV216	unidentified_4835	unidentified_6334	1.62	ASV366	Haliclystus	Cnidaria	2.07	ASV127	Pseudendoconium	Chlorophyta	2.22
				ASV52	Zoothamnium	Ciliophora	1.25	ASV106	Pseudovorticella	Ciliophora	2.08
								ASV166	unidentified_33279	Chlorophyta	1.23
								ASV244	unidentified_2488	Rozellomycota	1.02
seawater (SW)				stone biofilm (BF)							
ASV No.	Genus	Phylum	% Abundance	ASV No.	Genus	Phylum	% Abundance				
ASV28	Sarsia	Cnidaria	39.62	ASV10	Haptocillium	Ascomycota	76.14				
ASV19	unidentified_12446	Ciliophora	35.05	ASV87	Ulva	Chlorophyta	5.84				
ASV108	unidentified_5383	unidentified_6967	5.81	ASV75	Cladosporium	Ascomycota	3.94				
ASV45	unidentified_91524	Ciliophora	4.89	ASV127	Pseudendoconium	Chlorophyta	3.47				
ASV87	Ulva	Chlorophyta	3.30	ASV97	Alternaria	Ascomycota	2.80				
ASV106	Pseudovorticella	Ciliophora	1.94	ASV45	unidentified_91524	Ciliophora	2.34				
				ASV166	unidentified_33279	Chlorophyta	1.17				

Table S5

ITS beta diversity statistics based on Bray-Curtis dissimilarity, left: PERMANOVA results, right: pairwise permutation test for homogeneity of multivariate dispersions

PERMANOVA		Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1								pairwise permutation for homogeneity of multivariate dispersion										
	Df	Sum Squares	R2	F	Pr(>F)	Signifi- cance	Resi- duals df	Residuals Sum Squares	Residuals R2		Df	Sum Squares	Mean Squares	F	N permut	Pr(>F)	Signifi- cance	Resi- duals df	Residuals sum squares	Residuals mean squares
full dataset										full dataset										
origin	4	4.0572	0.29338	5.7089	0.001	***	55	9.7718	0.70662	4	0.13976	0.034941	8.1559	1000	0.000999	***	59	0.25276	0.004284	
individual no.	4	3.7628	0.2721	5.1398	0.001	***	55	10.0662	0.7279	4	0.13753	0.034383	7.4047	1000	0.000999	***	59	0.27396	0.004643	
FV										FV										
individual no.	2	0.7918	0.2223	2.1438	0.054	.	15	2.77	0.7777	2	0.00199	0.000966	0.2325	1000	0.7972	ns	15	0.064251	0.004283	
FS										FS										
individual no.	2	0.37763	0.18583	1.7118	0.076	.	15	1.65453	0.81417	2	0.03316	0.01658	2.1023	1000	0.1638	ns	14	0.110414	0.007887	
FE										FE										
individual no.	2	0.27035	0.09155	0.7054	0.493	ns	14	2.68281	0.90845	2	0.00709	0.003544	1.0939	1000	0.3716	ns	14	0.045354	0.00324	

Relative abundances of fungal genera (>1%) associated to the surfaces of *Fucus* spp., seawater and stone biofilm based on amplicon sequencing of the ITS fragment.

<i>F. vesiculosus</i> (FV)				<i>F. serratus</i> (FS)				<i>F. distichus</i> subsp. <i>evanescens</i> (FE)			
ASV No.	Genus	Phylum	% Abundance	ASV No.	Genus	Phylum	% Abundance	ASV No.	Genus	Phylum	% Abundance
ASV64	Candida	Ascomycota	82.30	ASV186	Sphaeroforma	Ichthyosporia_phy_Incertae_sedis	38.04	ASV221	Mucor	Mucoromycota	65.41
ASV10	Haptocillium	Ascomycota	9.20	ASV97	Alternaria	Ascomycota	25.00	ASV97	Alternaria	Ascomycota	14.91
ASV186	Sphaeroforma	Ichthyosporia_phy_Incertae_sedis	3.68	ASV342	Metschnikowia	Ascomycota	17.39	ASV244	unidentified_2488	Rozellomycota	5.77
ASV97	Alternaria	Ascomycota	1.61	ASV244	unidentified_2488	Rozellomycota	7.61	ASV186	Sphaeroforma	Ichthyosporia_phy_Incertae_sedis	5.37
ASV244	unidentified_2488	Rozellomycota	1.18	ASV1291	Wickerhamomyces	Ascomycota	3.26	ASV871	unidentified_99109	Ascomycota	2.19
				ASV451	Claviceps	Ascomycota	3.26				
				ASV1208	unidentified_786	Ascomycota	2.17				
				ASV871	unidentified_99109	Ascomycota	2.17				
				ASV1107	Blumeria	Ascomycota	1.09				
seawater (SW)				stone biofilm (BF)							
ASV No.	Genus	Phylum	% Abundance	ASV No.	Genus	Phylum	% Abundance				
ASV97	Alternaria	Ascomycota	26.17	ASV10	Haptocillium	Ascomycota	90.82				
ASV186	Sphaeroforma	Ichthyosporia_phy_Incertae_sedis	17.73	ASV75	Cladosporium	Ascomycota	4.70				
ASV244	unidentified_2488	Rozellomycota	14.69	ASV97	Alternaria	Ascomycota	3.35				
ASV75	Cladosporium	Ascomycota	13.98								
ASV342	Metschnikowia	Ascomycota	7.11								
ASV451	Claviceps	Ascomycota	4.30								
ASV440	unidentified_1163	Chytridiomycota	3.67								
ASV573	Pyrenophora	Ascomycota	2.89								
ASV568	Knufia	Ascomycota	1.64								