

Supplementary Material

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2 Supplementary Figures and Tables

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2.1 Supplementary Figures

Supplementary Table 1. Permanova analysis at univariate level on the environmental parameters measured at the experimental sites (Vent 1, Vent 2, REF 1, REF 2) at three times (Oct = October; Nov = November; Dec = December). Significant results are highlighted. Pair-wise tests were conducted after significant P(perm) results.

											pH [unit]				ORP [mV]				Salinity [psu]				
Temperature [°C]																							
Source	df	MS	Pseudo -F	P(perm)	Unique perms	df	MS	Pseudo -F	P(perm)	Unique perms	df	MS	Pseudo-F	P(perm)	Unique perms	df	MS	Pseudo-F	P(perm)	Unique perms			
Site	3	1,33E+02	4,67E+02	0,982	998	3	6,41E+01	2,08E+04	0,001	997	3	5,82E+04	1,21E+04	0,001	999	3	4,84E-01	4,08E-01	0,752	998			
Time	2	12.621	4,43E+04	0,001	998	2	50	1,62E+04	0,201	999	2	1,56E+04	3,26E+03	0,048	998	2	5,62E-01	4,74E-01	0,69	998			
Site x Time	6	2,57E+01	9,02E+00	1	997	6	1,71E+01	5,53E-01	0,77	999	6	2,50E-01	5,22E-01	0,799	998	6	4,70E-01	3,96E-01	0,834	999			
Residuals	20	0,28495				20	30,887				20	4,79E-01				20	1,19E+04						
Pair-wise tests	Groups		t	P(perm)	Unique perms	Groups		t	P(perm)	Unique perms	Groups		t	P(perm)	Unique perms								
	Oct, Nov		4,08E+04	0,003	996	Vent 1, Vent 2		3,89E+04	0,004	993	Vent 1, Vent 2		5,24E+04	0,001	998								
	Oct, Dec		1,93E+04	0,001	984	Vent 1, REF 1		4,95E+04	0,001	995	Vent 1, REF 1		5,65E+04	0,001	995								
	Nov, Dec		5,77E+04	0,001	999	Vent 1, REF 2		5,26E+04	0,001	999	Vent 1, REF 2		5,69E+04	0,001	997								
						Vent 2, REF 1		2,30E+04	0,043	998	Vent 2, REF 1		4,91E-01	0,635	999								
						Vent 2, REF 2		3,09E+04	0,007	998	Vent 2, REF 2		2,31E-01	0,824	997								
						REF 1, REF 2		1,69E+04	0,119	996	REF 1, REF 2		2,75E-01	0,754	996								

Supplementary Table 2. Permanova analysis at multivariate level on the community assemblages of biofilms. Pair-wise test showed comparison between sites (Vent 1, Vent 2, REF 1, REF 2) and times (t1 and t2). Significant results are highlighted. Pair-wise tests were conducted after significant P(perm) results.

Source	Phylum					Class					Genus				
	df	MS	Pseudo-F	P(perm)	Unique perms	df	MS	Pseudo-F	P(perm)	Unique perms	df	MS	Pseudo-F	P(perm)	Unique perms
Site	3	415,71	2,2684	0,007	997	3	1056,4	3,1224	0,001	999	3	1987,8	30,139	0,001	999
Time	1	301,28	1,644	0,17	999	1	806,17	2,3829	0,055	997	1	1423,3	21,579	0,049	997
Site x Time	3	230,17	1,2559	0,251	998	3	375,41	1,1097	0,366	998	3	769,23	11,663	0,29	998
Residuals	16					16					16				

Pair-wise tests Groups	t	P(perm)	Unique perms	t	P(perm)	Unique perms	t	P(perm)	Unique perms
Vent 1, Vent 2	2,1857	0,003	984	2,4654	0,004	987	25748	0,002	985
Vent 1, REF 1	3,0676	0,001	986	3,5595	0,001	979	32537	0,004	986
Vent 1, REF 2	1,2473	0,231	988	1,7276	0,02	982	17742	0,004	986
Vent 2, REF 1	1,5517	0,063	983	1,902	0,003	989	1763	0,006	988
Vent 2, REF 2	1,1193	0,313	984	1,2403	0,211	987	10777	0,351	992
REF 1, REF 2	1,1841	0,277	989	1,2193	0,25	981	10724	0,354	983

Supplementary Table 3. SIMPER analysis coupled with BLASTn (megablast) search against NCBI-NUCLEOTIDE DATABASE showing the relative contribution of 16S rRNA sequences to the dissimilarity among samples.

OTU ID	SIMPER TEST		BLASTn (megablast) search against NCBI-NUCLEOTIDE DATABASE							
	Av. dissim	Contrib. %	Accession	Best BLAST hit	E value	Perc. Ident.	Reference	Isolation source	Phylogenetic ascription	Closest relative
1836083	2,696	3,792	AB611518.1	Uncultured gamma proteobacterium TKTMmva-B17	8E-134	98.90%	https://doi.org/10.1128/AEM.06491-11	Hydrothermal vents	Gammaproteobacteria	uncultured bacterium
264	1,729	2,432	JF261956.1	Uncultured bacterium clone Del10081A12	4E-132	98.53%	https://doi.org/10.1111/j.1574-6968.2011.02374.x	Marine biofilms from Great Barrier Reef	Bacteria; environmental samples	uncultured bacterium
428807	1,495	2,102	HM485054.1	Uncultured marine organism clone LON_240	4E-137	99.63%	10.1016/j.syapm.2011.01.003	Marine biofilms from Great Barrier Reef	Bacteria; environmental samples	uncultured marine organism
795504	1,109	1,56	HQ189720.1	Uncultured phototrophic eukaryote clone 40D06 chloroplast	4E-137	99.63%	10.3389/fmicb.2015.00818	Sea anemone Nematostella vectensis	Eukaryota; environmental samples	uncultured phototrophic eukaryote
			KY498709.1	<i>Angulodiscorbis quadrangularis</i> isolate AQ159-89 chloroplast	2E-135	99.27%	https://doi.org/10.1002/ece3.4358	Intertidal rocky shore	Eukaryota; Sar; Rhizaria; Retaria; Foraminifera; Rotaliida; Discorboidea; Glabratellidae; Angulodiscorbis	<i>Angulodiscorbis quadrangularis</i>
583758	1,017	1,43	MT229147.1	Uncultured bacterium clone 660336	7E-135	99.26%	10.1016/j.scitotenv.2020.141399	Hydrocarbon degrading bacteria	Bacteria; environmental samples	uncultured bacterium
			KP792487.1	Bacillariophyta sp. 867-32 plastid	8E-134	98.90%	doi.org/10.3389/fmars.2020.00585	Rocky-shore benthic foraminifera	Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta	Bacillariophyta sp. 867-32
			KP792475.1	Planoglabratella opercularis isolate GO_m22-06 plastid	8E-134	98.90%	doi.org/10.3389/fmars.2020.00586	Rocky-shore benthic foraminifera	Eukaryota; Sar; Rhizaria; Retaria; Foraminifera; Rotaliida; Discorboidea; Glabratellidae; Planoglabratella	<i>Planoglabratella opercularis</i>
517923	0,8387	1,18	FJ844231.1	Uncultured Firmicutes bacterium clone Fi07-21	4E-137	99.63%	10.1371/journal.pone.0027597	Salinity gradient	Bacteria; Firmicutes; environmental samples	uncultured bacterium

			LC164834.2	<i>Salinicoccus</i> sp. W19 gene	2E-135	99.27%	unpubl	Hypersalin environment	Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcaceae	<i>Salinicoccus</i> sp.
361839	0,8379	1,179	MH818895.1	Uncultured diatom clone H1716-16S_40 chloroplast	2E-135	99.27%	unpubl	Intertidal foraminifers	Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta	uncultured diatom
			EU182114.2	Uncultured bacterium clone D15_1_SW_H_48	2E-135	99.27%	10.1371/journal.pone.0066968	Bacterial communities from coastal areas	Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta	uncultured diatom
317794	0,7385	1,039	JF261956.1	Uncultured bacterium clone Del10081A12 16S	8E-134	98.90%	10.1111/j.1574-6968.2011.02374.x	Marine biofilms from the Great Barrier Reef	Bacteria; environmental samples	uncultured bacterium
New.ReferenceOTU145	0,6678	0,9394	MH818927.1	Uncultured diatom clone H1724-16S_26 chloroplast	9E-119	95.60%	unpubl	Intertidal foraminifers	Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta	uncultured diatom
			KC509521.1	<i>Coscinodiscus radiatus</i> chloroplast, complete genome	9E-119	95.60%	10.1093/gbe/evu039	Diatoms plastid genomes	Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Coscinodiscophycidae; Coscinodisciales; Coscinodiscaceae	<i>Coscinodiscus radiatus</i>
315090	0,5213	0,7333	GU118611.1	Uncultured bacterium clone Mfav_A15	9E-129	97.80%	10.1371/journal.pone.0009554	Caribbean Corals	Bacteria; environmental samples	uncultured bacterium
New.ReferenceOTU746	0,4682	0,6586	LC432074.1	Uncultured bacterium gene clone: GoM_AsphSed_Bac69	4E-122	96.34%	DOI:10.1007/978-3-030-34827-4_5	Asphaltic hydrocarbon emissions	Bacteria; environmental samples	hydrocarbon-degrading sulfate-reducing Deltaproteobacteria
New.ReferenceOTU772	0,4558	0,6412	KY276598.1	Uncultured bacterium clone CP_Otu1209	5E-126	99.60%	unpubl	Cochlodinium polykrikoides blooms (dinoflagellate)	Bacteria; environmental samples.	uncultured bacterium
			KC934138.1	Uncultured <i>Thiothrix</i> sp. clone PY10-10-194	4E-112	94.14%	10.1007/s11274-011-0812-5	Poyang Lake (China, freshwater)	Bacteria; Proteobacteria; Gammaproteobacteria; Thiotrichales; Thiotrichaceae; Thiothrix	uncultured <i>Thiothrix</i> sp.
			KF945141.1	Uncultured <i>Thiothrix</i> sp. clone DZ-8	4E-112	94.14%	unpubl	Activated sludge	Bacteria; Proteobacteria; Gammaproteobacteria; Thiotrichales; Thiotrichaceae; Thiothrix	uncultured <i>Thiothrix</i> sp.

Supplementary Table 4. Diversity Indexes of the biofilm samples across the four experimental sites (Vent 1, Vent 2, REF 1, REF 2) and times (t1 and t2). Values are reported as mean \pm standard deviation.

Site	Time	Taxa S		Individuals		Dominance D		Simpson 1-D		Shannon H		Evenness e ^{H/S}		Margalef		Chao-1	
		mean	sd(\pm)	mean	sd(\pm)	mean	sd(\pm)	mean	sd(\pm)	mean	sd(\pm)	mean	sd(\pm)	mean	sd(\pm)	mean	sd(\pm)
Vent 1		6333,83	3073,78	99139,50	49401,81	0,03	0,01	0,97	0,01	5,62	0,38	0,06	0,05	546,08	248,73	10655,17	4925,05
	t1	6959,00	2980,93	101243,67	53269,88	0,04	0,01	0,96	0,01	5,44	0,09	0,04	0,03	601,50	233,33	12154,67	4913,71
	t2	5708,67	3682,64	97035,33	57012,15	0,02	0,01	0,98	0,01	5,81	0,50	0,09	0,06	490,67	301,68	9155,67	5454,37
Vent 2		7222,00	3740,03	86342,67	49272,45	0,01	0,00	0,99	0,00	6,79	0,44	0,15	0,07	632,92	306,61	11837,00	6084,06
	t1	8446,67	5360,48	93084,33	47681,10	0,01	0,00	0,99	0,00	6,61	0,56	0,12	0,05	729,07	446,63	13988,33	8611,27
	t2	5997,33	1317,37	79601,00	60494,61	0,00	0,00	1,00	0,00	6,98	0,27	0,19	0,07	536,77	88,35	9685,67	2121,51
REF 1		8479,17	4422,69	102029,50	47877,12	0,01	0,00	0,99	0,00	6,49	0,50	0,10	0,05	731,43	358,48	13831,00	6870,98
	t1	10359,33	4885,00	103621,67	63772,89	0,01	0,00	0,99	0,00	6,87	0,27	0,12	0,07	894,30	379,20	16447,33	7811,83
	t2	6599,00	3798,95	100437,33	40693,50	0,02	0,00	0,98	0,00	6,12	0,33	0,08	0,03	568,57	312,90	11214,67	6038,69
REF 2		6186,00	3965,22	69518,00	32925,01	0,02	0,02	0,98	0,02	6,14	1,33	0,11	0,05	546,66	337,76	9909,97	6536,39
	t1	7928,00	4024,89	82573,00	31716,27	0,01	0,01	0,99	0,01	6,64	0,60	0,11	0,04	695,20	336,49	13038,00	6493,51
	t2	4444,00	3742,19	56463,00	34538,66	0,03	0,03	0,97	0,03	5,63	1,81	0,11	0,07	398,12	325,26	6781,93	5940,68

Supplementary Table 5. Permanova analysis on Dominance and Evenness diversity indexes calculated for the biofilm community. Significant results are highlighted. Pair-wise tests were conducted after significant P(perm) results.

Source	Dominance					Evenness			
	df	MS	Pseudo-F	P(perm)	Unique perms	MS	Pseudo-F	P(perm)	Unique perms
Site	3	918,35	5,3794	0,009	998	600,77	3,8492	0,024	999
Time	1	103,16	0,60426	0,5	999	138,77	0,88916	0,345	999
Site x Time	3	482,71	2,8276	0,053	999	194,38	1,2454	0,325	999
Residuals	16					156,07			

Pair-wise tests Groups	t	P(perm)	Unique perms	t	P(perm)	Unique perms
Vent 1, Vent 2	6,8914	0,003	985	3,0848	0,029	985
Vent 1, REF 1	3,3849	0,015	994	1,6732	0,127	987
Vent 1, REF 2	1,3987	0,169	987	2,1255	0,066	978
Vent 2, REF 1	3,7637	0,011	984	1,7293	0,115	985
Vent 2, REF 2	1,6714	0,114	977	1,1046	0,308	986
REF 1, REF 2	0,46971	0,769	986	0,57976	0,573	987

Supplementary Table 6. Permanova analysis on diversity indexes calculated for the biofilm community.

Taxa S					Individuals					Simpson			
Source	df	MS	Pseudo-F	P(perm)	Unique perms	MS	Pseudo-F	P(perm)	Unique perms	MS	Pseudo-F	P(perm)	Unique perms
Site	3	131,23	0,36	0,85	999	90,38	0,33	0,81	998	0,33	2,72	0,05	999
Time	1	583,39	1,61	0,22	999	90,54	0,34	0,56	997	0,01	0,05	0,85	998
Site x Time	3	110,89	0,31	0,89	998	53,20	0,20	0,91	999	0,29	2,39	0,08	999
Residuals	16	363,14				269,92				0,12			

Shannon					Margalef				Chao-1				
Source	df	MS	Pseudo-F	P(perm)	Unique perms	MS	Pseudo-F	P(perm)	Unique perms	MS	Pseudo-F	P(perm)	Unique perms
Site	3	26,31	2,09	0,12	998	124,43	0,39	0,84	998	164,88	0,46	0,78	999
Time	1	9,12	0,72	0,45	998	567,93	1,77	0,20	999	662,15	1,86	0,17	999
Site x Time	3	16,26	1,29	0,31	998	104,34	0,33	0,87	995	138,50	0,39	0,87	999
Residuals	16	12,59				320,75				356,44			

Supplementary Table 7. Predicted gene abundance of selected metabolic pathways from biofilms collected at Levante Bay. Values are reported as mean \pm standard deviation for each site (Vent 1, Vent 2, REF 1, REF 2) and time (t1, t2).

		Vent 1				Vent 2				REF 1				REF 2			
		t1		t2		t1		t2		t1		t2		t1		t2	
PATHWAY	GENE	mean	sd (\pm)	mean	sd (\pm)	mean	sd (\pm)	mean	sd (\pm)	mean	sd (\pm)	mean	sd (\pm)	mean	sd (\pm)	mean	sd (\pm)
CARBON (r-TCA)	ATP-citrate lyase [EC:2.3.3.8]	3,47E-06	2,31E-11	9,46E-07	2,51E-12	4,62E-07	2,79E-13	6,81E-08	1,55E-14	1,08E-07	2,28E-14	3,36E-07	1,24E-13	9,99E-08	1,52E-14	1,41E-07	9,50E-14
CARBON (CBB)	ribulose- biphosphate carboxylase [EC:4.1.1.39]	1,74E-04	2,07E-09	1,32E-04	7,75E-09	1,11E-04	8,52E-10	1,02E-04	3,28E-10	1,31E-04	3,09E-10	6,42E-05	7,63E-11	1,02E-04	1,28E-09	9,44E-05	1,36E-09
NITROGEN	nitrate reductase / nitrite oxidoreductase [EC:1.7.5.1 1.7.99.-]	1,85E-04	1,33E-09	2,04E-04	4,03E-09	1,54E-04	1,41E-09	1,72E-04	3,28E-09	1,52E-04	1,53E-10	1,22E-04	2,23E-10	1,46E-04	4,81E-10	3,03E-04	3,73E-08
	nitric oxide reductase [EC:1.7.2.5]	1,65E-04	1,08E-10	1,46E-04	4,59E-09	1,27E-04	3,29E-09	1,41E-04	5,61E-10	1,67E-04	2,12E-10	1,29E-04	9,70E-10	1,44E-04	3,84E-09	1,09E-04	9,32E-09
	nitrite reductase (cytochrome c- 552) [EC:1.7.2.2]	1,51E-06	2,36E-13	4,90E-07	5,54E-13	3,25E-06	1,24E-12	3,26E-06	5,61E-12	4,02E-06	3,91E-13	2,17E-06	6,88E-13	2,29E-06	2,02E-12	3,84E-06	2,28E-11
	nitrite reductase (NADH) [EC:1.7.1.15]	4,69E-04	1,69E-09	4,68E-04	3,34E-10	4,83E-04	1,02E-09	5,03E-04	1,98E-10	4,26E-04	1,68E-09	4,82E-04	6,76E-10	4,84E-04	4,00E-09	5,38E-04	3,55E-09
	nitrite reductase (NO-forming) / hydroxylamine reductase [EC:1.7.2.1 1.7.99.1]	3,05E-05	6,10E-11	1,21E-05	1,03E-11	2,30E-05	1,53E-10	3,13E-05	2,69E-10	2,00E-05	2,07E-11	9,63E-06	6,72E-11	2,37E-05	4,11E-11	1,33E-05	1,62E-10
	nitrite reductase (NO-forming) [EC:1.7.2.1]	7,42E-05	4,29E-10	9,92E-05	1,55E-10	8,59E-05	1,91E-10	9,60E-05	1,82E-10	1,04E-04	7,25E-12	9,68E-05	7,29E-10	8,87E-05	1,83E-09	1,09E-04	8,74E-09
	nitrogen fixation protein NifBQTUXZ	2,14E-04	2,01E-09	2,62E-04	2,31E-08	2,61E-04	5,60E-09	2,52E-04	2,68E-10	3,65E-04	4,93E-09	1,94E-04	7,08E-10	2,78E-04	1,64E-08	2,64E-04	8,07E-09
	nitrogenase protein Nif [EC:1.18.6.1]	2,67E-04	2,04E-09	2,58E-04	7,98E-09	2,56E-04	1,58E-08	2,66E-04	3,33E-09	3,74E-04	8,73E-10	2,45E-04	5,36E-10	2,98E-04	2,73E-08	2,00E-04	3,75E-08
	nitroreductase	5,62E-05	1,34E-11	3,54E-05	4,43E-10	4,21E-05	4,70E-10	4,91E-05	1,88E-10	3,91E-05	6,47E-10	3,14E-05	1,67E-10	4,40E-05	8,28E-10	5,17E-05	2,35E-09
	nitrous-oxide reductase [EC:1.7.2.4]	2,45E-05	7,51E-11	1,30E-05	3,33E-11	2,87E-05	1,33E-10	2,20E-05	3,36E-11	1,91E-05	1,42E-10	1,13E-05	1,62E-12	1,47E-05	1,33E-14	1,26E-05	7,41E-12
SULFUR	adenylsulfate reductase (aprAB) [EC:1.8.99.2]	3,80E-05	2,05E-10	1,67E-05	2,64E-11	1,12E-05	1,88E-10	2,20E-05	2,81E-12	1,14E-05	2,29E-11	3,63E-06	2,45E-11	1,03E-05	1,04E-11	9,29E-06	1,31E-10
	polysulfide reductase (psrBC)	4,14E-07	8,47E-14	2,28E-07	1,41E-13	6,11E-07	1,36E-13	1,21E-06	1,09E-12	1,77E-06	2,59E-14	9,64E-07	8,03E-15	1,41E-06	4,89E-13	1,89E-06	8,52E-12

SULFUR	sulfate adenyltransferase (sat) [EC:2.7.7.4]	4,59E-04	1,63E-09	4,31E-04	6,43E-09	5,01E-04	1,00E-08	5,14E-04	7,36E-10	4,25E-04	1,36E-09	5,54E-04	6,47E-09	5,27E-04	3,27E-08	4,74E-04	1,63E-08
	sulfide dehydrogenase [flavocytochrome c] flavoprotein chain [EC:1.8.2.3]	1,57E-04	3,40E-10	1,79E-04	1,74E-10	1,45E-04	6,18E-10	1,78E-04	6,76E-10	1,24E-04	5,69E-10	1,63E-04	7,77E-11	1,37E-04	4,65E-11	1,41E-04	2,76E-09
	sulfide dehydrogenase [EC:1.8.1.19]	2,37E-07	2,50E-13	4,13E-09	1,03E-16	3,88E-08	3,95E-15	2,38E-08	3,40E-15	7,05E-08	8,34E-16	2,41E-08	3,48E-15	3,63E-08	4,17E-15	1,42E-08	1,20E-15
	sulfide:quinone oxidoreductase [EC:1.8.5.4]	4,07E-04	1,73E-10	3,42E-04	1,35E-09	3,44E-04	3,74E-09	3,96E-04	2,43E-09	3,56E-04	2,59E-09	3,83E-04	1,68E-09	3,59E-04	1,72E-09	3,18E-04	2,38E-08
	sulfite reductase (NADPH) (dsrAB) [EC:1.8.1.2]	4,05E-04	8,68E-11	4,22E-04	2,56E-10	4,76E-04	7,22E-09	4,53E-04	1,48E-09	4,09E-04	8,42E-10	4,77E-04	2,23E-09	4,24E-04	5,22E-11	4,34E-04	2,06E-09
	sulfur oxygenase/reductase [EC:1.13.11.55]	1,75E-06	2,17E-13	3,68E-07	2,12E-13	2,59E-08	4,03E-15	2,00E-07	6,65E-14	5,47E-09	1,79E-16	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0
PHOTOSYNTHESIS	sulfur-oxidizing protein Sox system (ABXYZ)	8,53E-04	1,46E-09	9,21E-04	5,41E-09	8,25E-04	3,76E-09	8,45E-04	1,18E-10	7,44E-04	9,92E-09	8,70E-04	4,02E-10	7,42E-04	2,43E-08	7,89E-04	1,94E-08
	photosynthetic reaction center	3,62E-04	9,41E-09	2,87E-04	3,18E-09	2,50E-04	2,30E-08	3,23E-04	1,08E-08	2,91E-04	1,09E-09	3,91E-04	6,11E-10	2,48E-04	2,15E-08	2,14E-04	6,97E-08
	photosystem I	3,76E-04	1,83E-08	1,49E-05	3,33E-10	1,61E-04	7,92E-09	8,26E-05	5,78E-09	3,42E-04	6,35E-09	9,48E-05	6,79E-09	2,44E-04	3,67E-09	3,10E-05	2,11E-09
	photosystem II photosystem P840 reaction center	7,54E-04	6,50E-08	2,99E-05	1,35E-09	3,36E-04	3,42E-08	1,70E-04	2,45E-08	7,05E-04	2,96E-08	2,04E-04	3,20E-08	5,09E-04	1,65E-08	6,50E-05	9,26E-09
OXYGEN REDUCTION		4,26E-06	1,09E-10	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0	0,00E+0 0
	cytochrome aa3 [EC:1.10.3.12]	1,07E-04	1,66E-10	1,19E-04	1,41E-09	1,30E-04	1,15E-08	7,42E-05	2,05E-11	8,18E-05	4,59E-11	7,74E-05	4,72E-10	1,07E-04	4,43E-09	2,20E-04	8,69E-08
	cytochrome bd [EC:1.10.3.14]	6,13E-04	1,16E-11	5,48E-04	5,77E-09	5,98E-04	4,42E-08	5,66E-04	8,29E-09	5,92E-04	5,24E-10	5,31E-04	3,39E-09	5,77E-04	3,87E-09	6,15E-04	4,98E-08
HEAVY METAL DETOXIFICATION	cytochrome cbb3 [EC:1.9.3.1]	1,48E-03	3,78E-09	1,54E-03	2,49E-08	1,44E-03	1,54E-07	1,67E-03	3,55E-09	1,45E-03	6,60E-09	1,61E-03	1,62E-08	1,43E-03	4,08E-08	1,22E-03	5,99E-07
	arsenate reductase [EC:1.20.4.1]	7,42E-04	1,63E-09	6,47E-04	4,59E-10	7,63E-04	3,12E-09	7,60E-04	1,37E-10	7,64E-04	1,30E-09	7,97E-04	6,87E-10	7,67E-04	4,54E-10	7,70E-04	5,14E-08
	mercuric reductase [EC:1.16.1.1]	1,34E-04	3,16E-11	1,23E-04	8,01E-10	1,43E-04	1,72E-09	1,65E-04	1,74E-09	1,58E-04	4,28E-10	1,06E-04	8,71E-10	1,64E-04	1,45E-09	1,51E-04	2,72E-09
CCMs	carbon dioxide concentrating mechanism protein CcmKLMNO	1,90E-05	1,55E-10	1,08E-05	3,53E-10	4,37E-05	2,03E-09	2,61E-05	8,58E-12	8,44E-05	2,62E-10	3,04E-05	2,52E-10	7,74E-05	3,11E-10	3,11E-05	1,82E-10
	carbonic anhydrase [EC:4.2.1.1]	2,87E-04	5,19E-10	2,92E-04	4,13E-09	3,26E-04	2,10E-09	2,95E-04	8,82E-10	3,30E-04	5,55E-09	2,78E-04	9,14E-10	3,07E-04	7,62E-11	3,24E-04	6,10E-09

Supplementary Table 8. Permanova analysis at univariate level of key metabolic pathways. Pair-wise test showed comparison between sites (Vent 1, Vent 2, REF 1, REF 2) and times (t1 and t2). Significant results are highlighted. Pair-wise tests were conducted after significant P(permanova) results.

		Nitrogen				Oxygen Reduction				Heavy Metals Detoxification							
Source	df	MS	Pseudo-F	P(perm)	Unique perms	MS	Pseudo-F	P(perm)	Unique perms	MS	Pseudo-F	P(perm)	Unique perms				
Site	3	37,968	1,0802	0,38	997	27,183	0,97456	0,459	999	15,885	2,5704	0,063	999				
Time	1	86,025	2,4475	0,06	999	2,7863	0,099897	0,859	998	7,2415	1,1718	0,318	999				
Site x Time	3	53,202	1,5137	0,158	999	25,671	0,92036	0,494	999	10,014	1,6203	0,205	997				
Residuals	16	35,148				27,892				6,1801							
		Carbon (r-TCA)				Carbon (CBB)				CCMs				Photosynthesis			
Source	df	MS	Pseudo-F	P(perm)	Unique perms	MS	Pseudo-F	P(perm)	Unique perms	MS	Pseudo-F	P(perm)	Unique perms	MS	Pseudo-F	P(perm)	Unique perms
Site	3	831,1	7,0621	0,001	998	1765,5	4,6063	0,012	999	117,51	4,7023	0,009	998	626,06	1,2221	0,238	998
Time	1	313,28	2,3102	0,136	998	3075	8,0227	0,016	996	226,68	9,0709	0,003	998	4334,3	8,4606	0,001	999
Site x Time	3	441,23	2,1358	0,109	998	712,56	1,8591	0,158	999	21,08	0,84357	0,514	999	783,22	1,5288	0,079	998
Residuals	16	315,04				383,29				24,989				512,3			
Pair-wise tests Groups		t	P(perm)	Unique perms		t	P(perm)	Unique perms		t	P(perm)	Unique perms					
Vent 1, Vent 2		2,1597	0,011	991		2,0957	0,076	990		1,5864	0,126	986					
Vent 1, REF 1		1,9704	0,016	992		2,8384	0,026	994		3,2948	0,003	991					
Vent 1, REF 2		2,1109	0,006	988		2,3601	0,046	985		3,2401	0,008	988					
Vent 2, REF 1		0,78339	0,918	973		1,4608	0,166	979		1,4032	0,17	987					
Vent 2, REF 2		0,70692	0,403	988		0,72076	0,462	984		1,1181	0,298	991					
REF 1, REF 2		1,0354	0,434	987		0,19254	0,81	991		Negative							
t1, t2						2,8324	0,015	999		3,0118	0,006	998		2,9087	0,001	999	

Supplementary Table 9. Permanova analysis at univariate level of sulfur metabolism. Pair-wise test showed comparison between sites (Vent 1, Vent 2, REF 1, REF 2) and times (t1 and t2). Significant results are highlighted. Pair-wise tests were conducted after significant P(permanova) results.

Sulfur					
Source	df	MS	Pseudo-F	P(permanova)	Unique perms
Site	3	21,902	2,3766	0,01	999
Time	1	21,484	2,3313	0,062	999
Site x Time	3	20,465	2,2207	0,019	999
Residuals	16	9,2157			

Pair-wise tests									
Within level 't1' of factor 'Time'					Within level 't2' of factor 'Time'				
Groups	t	P(permanova)	Unique perms	P(MC)	Groups	t	P(permanova)	Unique perms	P(MC)
Vent1, Vent2	1,9413	0,076	10	0,066	Vent1, Vent2	2,1119	0,092	10	0,04
Vent1, REF1	3,0333	0,106	10	0,021	Vent1, REF1	2,6727	0,105	10	0,023
Vent1, REF2	2,6062	0,113	10	0,045	Vent1, REF2	1,0167	0,486	10	0,399
Vent2, REF1	1,3825	0,094	10	0,204	Vent2, REF1	2,0992	0,105	10	0,05
Vent2, REF2	1,0094	0,624	10	0,387	Vent2, REF2	1,1652	0,216	10	0,274
REF1, REF2	0,93756	0,491	10	0,463	REF1, REF2	0,91698	0,605	10	0,475

Within level 'Vent1' of factor 'Site'					Within level 'Vent2' of factor 'Site'				
Groups	t	P(permanova)	Unique perms	P(MC)	Groups	t	P(permanova)	Unique perms	P(MC)
t1, t2	2,414	0,101	10	0,027	Groups				
					t1, t2	1,3243	0,103	10	0,206

Within level 'REF1' of factor 'Site'					Within level 'REF2' of factor 'Site'				
Groups	t	P(permanova)	Unique perms	P(MC)	Groups	t	P(permanova)	Unique perms	P(MC)
t1, t2	3,012	0,103	10	0,02	t1, t2	0,61077	0,889	10	0,705

Supplementary Table 10. SIMPER analysis on predicted abundance of genes for Sulfur metabolism across groups of sites (Vent 1, Vent 2, REF 1, REF 2) and times (t1 and t2).

Sulfur												
Groups Vent1 & Vent2							Groups Vent1 & REF1					
Average dissimilarity = 4,30							Average dissimilarity = 4,99					
	Group Vent1	Group Vent2						Group Vent1	Group REF1			
Enzyme	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%	Enzyme	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
adenylylsulfate reductase (aprAB) [EC:1.8.99.2]	0,01	0	0,92	0,95	21,38	21,38	adenylylsulfate reductase (aprAB) [EC:1.8.99.2]	0,01	0	1,22	2,57	24,54
sulfate adenylyltransferase (sat) [EC:2.7.7.4]	0,02	0,02	0,72	1,28	16,62	38	sulfate adenylyltransferase (sat) [EC:2.7.7.4]	0,02	0,02	0,85	1,21	17,12
sulfide:quinone oxidoreductase [EC:1.8.5.4]	0,02	0,02	0,71	1,61	16,46	54,45	sulfur-oxidizing protein Sox system (ABXYZ)	0,03	0,03	0,68	1,37	13,61
sulfite reductase (NADPH) (dsrAB) [EC:1.8.1.2]	0,02	0,02	0,56	1,21	13	67,46	sulfide:quinone oxidoreductase [EC:1.8.5.4]	0,02	0,02	0,57	1,49	11,38
sulfur-oxidizing protein Sox system (ABXYZ)	0,03	0,03	0,46	1,49	10,61	78,06	sulfide dehydrogenase [flavocytochrome c] flavoprotein chain [EC:1.8.2.3]	0,01	0,01	0,47	1,35	9,38
sulfur oxygenase/reductase [EC:1.13.11.55]	0	0	0,37	1,6	8,48	86,55	sulfur oxygenase/reductase [EC:1.13.11.55]	0	0	0,41	1,81	8,23
sulfide dehydrogenase [flavocytochrome c] flavoprotein chain [EC:1.8.2.3]	0,01	0,01	0,28	1,31	6,51	93,06	sulfite reductase (NADPH) (dsrAB) [EC:1.8.1.2]	0,02	0,02	0,39	1,29	7,89

Groups Vent2 & REF1

Average dissimilarity = 3,97

	Group Vent2	Group REF1				
Enzyme	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
adenylylsulfate reductase (aprAB) [EC:1.8.99.2]	0	0	1,1	1,61	27,64	27,64
sulfate adenylyltransferase (sat) [EC:2.7.7.4]	0,02	0,02	0,67	1,24	16,86	44,5
sulfite reductase (NADPH) (dsrAB) [EC:1.8.1.2]	0,02	0,02	0,58	1,2	14,52	59,02
sulfur-oxidizing protein Sox system (ABXYZ)	0,03	0,03	0,45	0,86	11,28	70,3
sulfide:quinone oxidoreductase [EC:1.8.5.4]	0,02	0,02	0,42	1,2	10,59	80,89
sulfide dehydrogenase [flavocytochrome c] flavoprotein chain [EC:1.8.2.3]	0,01	0,01	0,41	1,36	10,36	91,25

Groups Vent2 & REF2

Average dissimilarity = 4,82

	Group Vent2	Group REF2				
Enzyme	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	
adenylylsulfate reductase (aprAB) [EC:1.8.99.2]	0	0	0,92	1,21	19,13	
sulfide:quinone oxidoreductase [EC:1.8.5.4]	0,02	0,02	0,84	0,83	17,4	
sulfate adenylyltransferase (sat) [EC:2.7.7.4]	0,02	0,02	0,79	0,99	16,45	
sulfur-oxidizing protein Sox system (ABXYZ)	0,03	0,03	0,78	1,28	16,14	
sulfide dehydrogenase [flavocytochrome c] flavoprotein chain [EC:1.8.2.3]	0,01	0,01	0,55	1,08	11,34	
sulfite reductase (NADPH) (dsrAB) [EC:1.8.1.2]	0,02	0,02	0,5	1,25	10,37	

Groups Vent1 & REF2

Average dissimilarity = 5,54

Enzyme	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Group Vent1		Group REF2				
adenylylsulfate reductase (aprAB) [EC:1.8.99.2]	0,01	0	1,07	1,44	19,29	19,29
sulfur-oxidizing protein Sox system (ABXYZ)	0,03	0,03	1,04	1,39	18,86	38,15
sulfate adenylyltransferase (sat) [EC:2.7.7.4]	0,02	0,02	0,89	1,16	16,05	54,2
sulfide:quinone oxidoreductase [EC:1.8.5.4]	0,02	0,02	0,83	1,14	14,9	69,1
sulfide dehydrogenase [flavocytochrome c] flavoprotein chain [EC:1.8.2.3]	0,01	0,01	0,59	1,17	10,59	79,69
sulfur oxygenase/reductase [EC:1.13.11.55]	0	0	0,42	1,83	7,58	87,27
polysulfide reductase (psrBC)	0	0	0,35	1,47	6,36	93,62

Groups REF1 & REF2

Average dissimilarity = 4,31

Enzyme	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%
Group REF1		Group REF2			
sulfate adenylyltransferase (sat) [EC:2.7.7.4]	0,02	0,02	1,06	1,06	24,55
sulfur-oxidizing protein Sox system (ABXYZ)	0,03	0,03	0,78	1,37	18,12
sulfide:quinone oxidoreductase [EC:1.8.5.4]	0,02	0,02	0,76	0,79	17,7
adenylylsulfate reductase (aprAB) [EC:1.8.99.2]	0	0	0,57	1,05	13,22
sulfide dehydrogenase [flavocytochrome c] flavoprotein chain [EC:1.8.2.3]	0,01	0,01	0,49	1,2	11,33
sulfite reductase (NADPH) (dsrAB) [EC:1.8.1.2]	0,02	0,02	0,35	1,02	8,18

Groups t1 & t2

Average dissimilarity = 4,69

Enzyme	Group t1	Group t2				
	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
sulfate adenylyltransferase (sat) [EC:2.7.7.4]	0,02	0,02	0,93	1,4	19,91	19,91
adenylylsulfate reductase (aprAB) [EC:1.8.99.2]	0	0	0,85	1,28	18,09	38,01
sulfide:quinone oxidoreductase [EC:1.8.5.4]	0,02	0,02	0,74	1,1	15,86	53,87
sulfur-oxidizing protein Sox system (ABXYZ)	0,03	0,03	0,71	1,31	15,1	68,97
sulfide dehydrogenase [flavocytochrome c] flavoprotein chain [EC:1.8.2.3]	0,01	0,01	0,58	1,69	12,31	81,28
sulfite reductase (NADPH) (dsrAB) [EC:1.8.1.2]	0,02	0,02	0,44	1,3	9,49	90,76

Supplementary Table 11. SIMPER analysis on predicted genes of Photosynthesis and CCMs metabolic pathways across groups of sites (Vent 1, Vent 2, REF 1, REF 2) and times (t1 and t2).

Photosynthesis						
Groups t1 & t2						
Average dissimilarity = 34,73						
	Group t1	Group t2				
Enzyme	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
photosystem II	0,02	0,01	17,26	1,65	49,69	49,69
photosystem I	0,02	0,01	12,07	1,65	34,74	84,44
photosynthetic reaction center	0,02	0,02	5,12	0,64	14,74	99,17
CCMs						
Groups Vent1 & Vent2						
Average dissimilarity = 8,08						
	Group Vent1	Group Vent2				
Enzyme	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
carbon dioxide concentrating mechanism protein CcmKLMNO	0	0,01	5,67	1,3	70,22	70,22
carbonic anhydrase [EC:4.2.1.1]	0,02	0,02	2,41	1,33	29,78	100

Groups Vent1 & REF1						
Average dissimilarity = 10,92						
	Group Vent1	Group REF1				
Enzyme	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
carbon dioxide concentrating mechanism protein CcmKLMNO	0	0,01	8,47	1,68	77,52	77,52
carbonic anhydrase [EC:4.2.1.1]	0,02	0,02	2,46	1,23	22,48	100

Groups Vent2 & REF1

Average dissimilarity = 7,77

	Group Vent2	Group REF1				
Enzyme	Av.Ab und	Av.Ab und	Av.D iss	Diss/ SD	Contri b%	Cum. %
carbon dioxide concentrating mechanism protein CcmKLMNO	0,01	0,01	5,48	1,49	70,55	70,55
carbonic anhydrase [EC:4.2.1.1]	0,02	0,02	2,29	1,2	29,45	100

Groups Vent2 & REF2

Average dissimilarity = 6,95

	Group Vent2	Group REF2				
Enzyme	Av.Ab und	Av.Ab und	Av.D iss	Diss/ SD	Contri b%	Cum. %
carbon dioxide concentrating mechanism protein CcmKLMNO	0,01	0,01	5,03	1,4	72,37	72,37
carbonic anhydrase [EC:4.2.1.1]	0,02	0,02	1,92	1,15	27,63	100

Groups t1 & t2

Average dissimilarity = 9,24

	Group t1	Group t2				
Species	Av.Ab und	Av.Ab und	Av.D iss	Diss/ SD	Contri b%	Cum. %
carbon dioxide concentrating mechanism protein CcmKLMNO	0,01	0	6,84	1,7	74	74
carbonic anhydrase [EC:4.2.1.1]	0,02	0,02	2,4	1,32	26	100

Groups Vent1 & REF2

Average dissimilarity = 10,62

	Group Vent1	Group REF2				
Enzyme	Av.Ab und	Av.Ab und	Av.D iss	Diss/ SD	Contri b%	Cum. %
carbon dioxide concentrating mechanism protein CcmKLMNO	0	0,01	8,07	1,66	76,04	76,04
carbonic anhydrase [EC:4.2.1.1]	0,02	0,02	2,54	1,33	23,96	100

Groups REF1 & REF2

Average dissimilarity = 6,74

	Group REF1	Group REF2				
Enzyme	Av.Ab und	Av.Ab und	Av.D iss	Diss/ SD	Contri b%	Cum. %
carbon dioxide concentrating mechanism protein CcmKLMNO	0,01	0,01	4,35	1,31	64,6	64,6
carbonic anhydrase [EC:4.2.1.1]	0,02	0,02	2,39	1,22	35,4	100

Supplementary Fig. 1. Taxonomic affiliation of the 16S rRNA transcript sequences. Taxa representing genera that account for at least 1% (on average) of the overall abundance in all samples are shown. A) Phylum-level. B) Class-level.

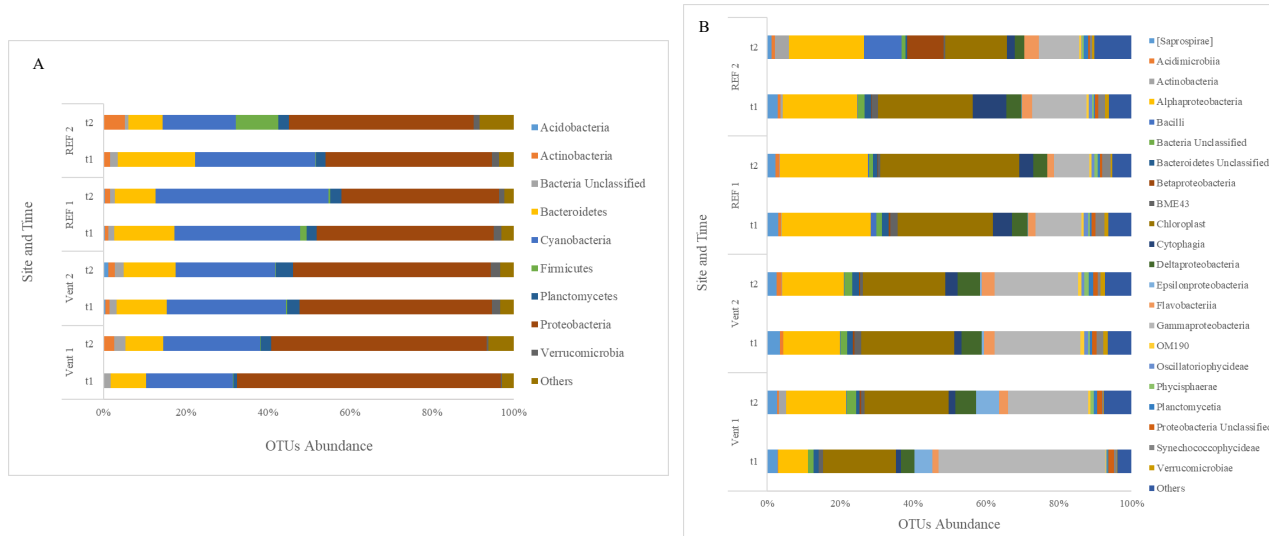


Fig. S1