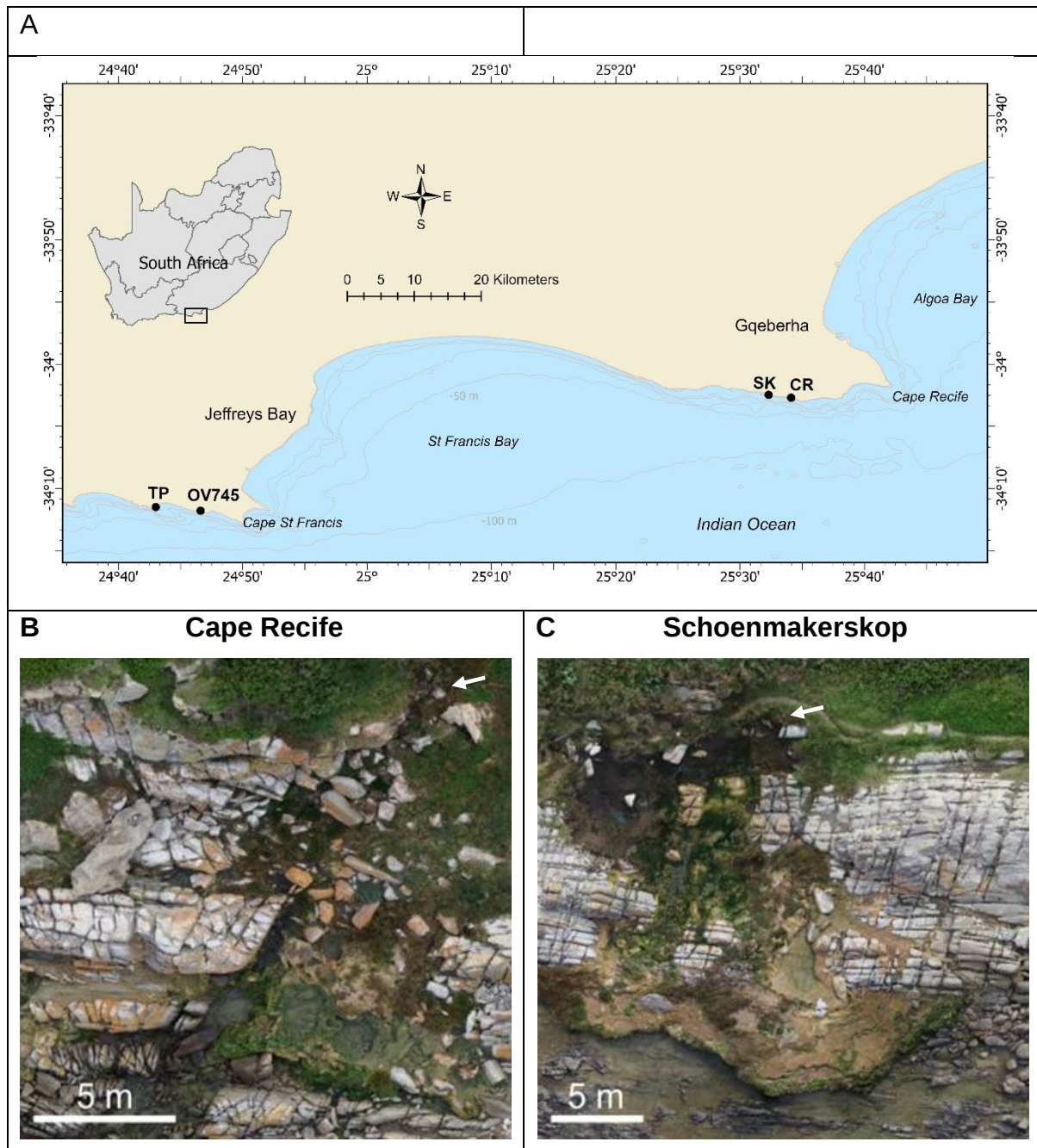
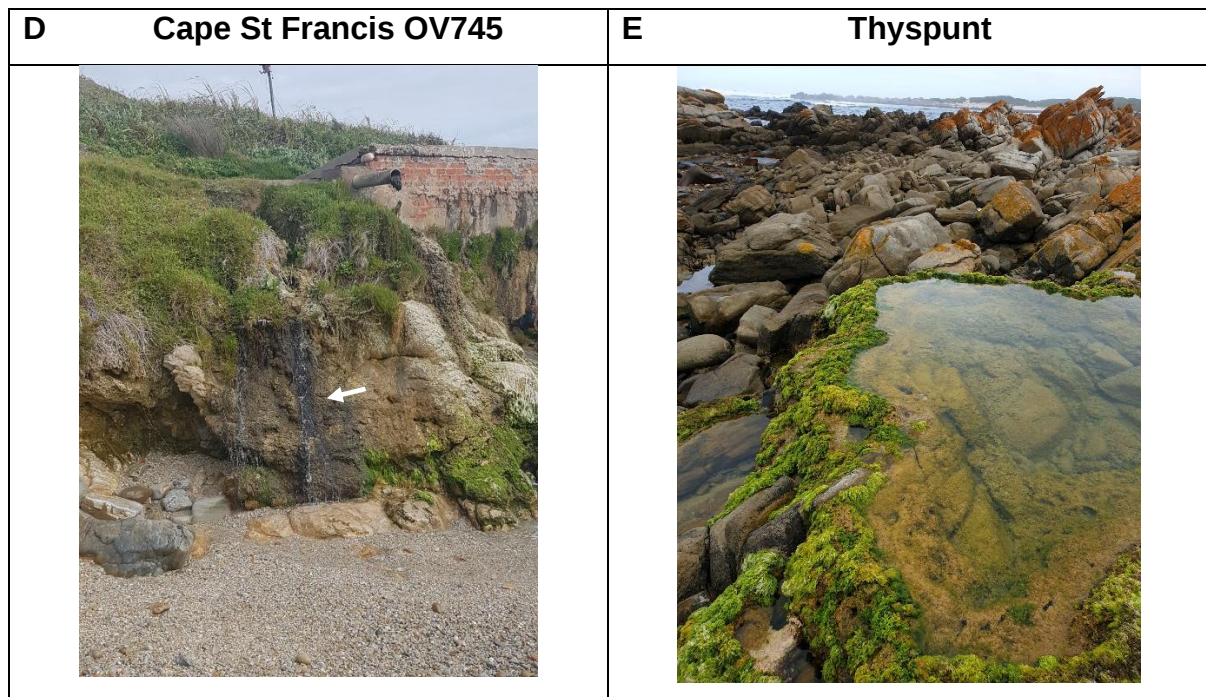


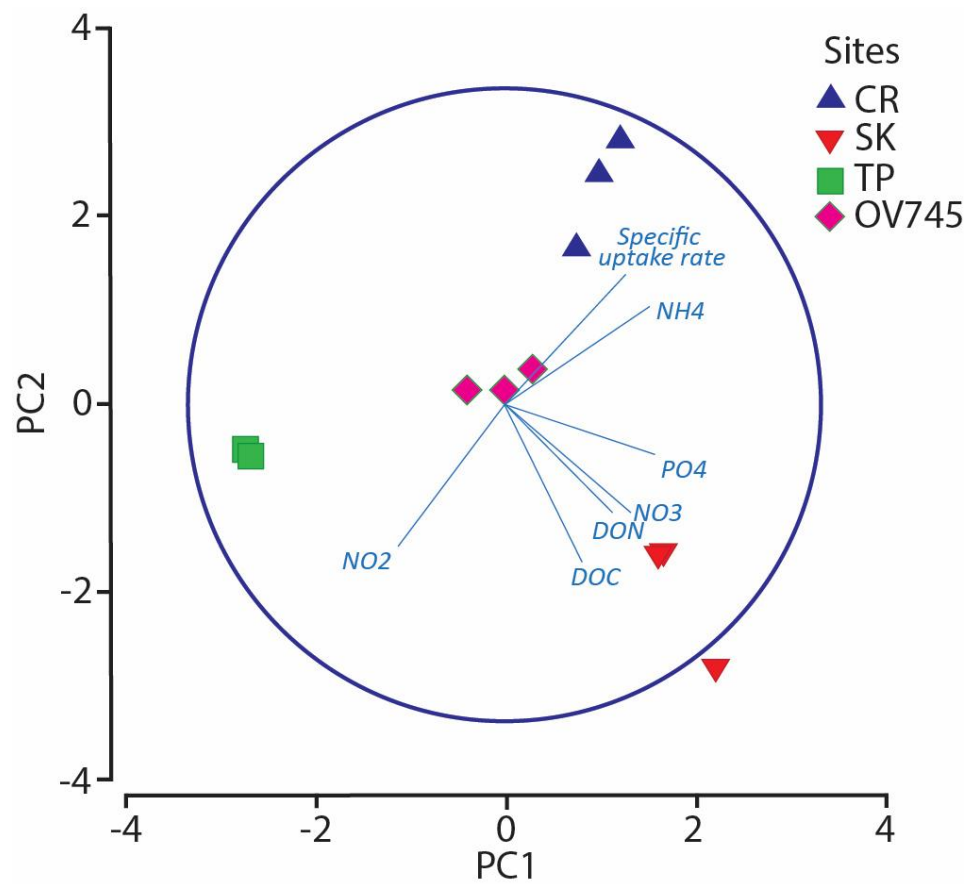
SUPPLEMENTARY FIGURES

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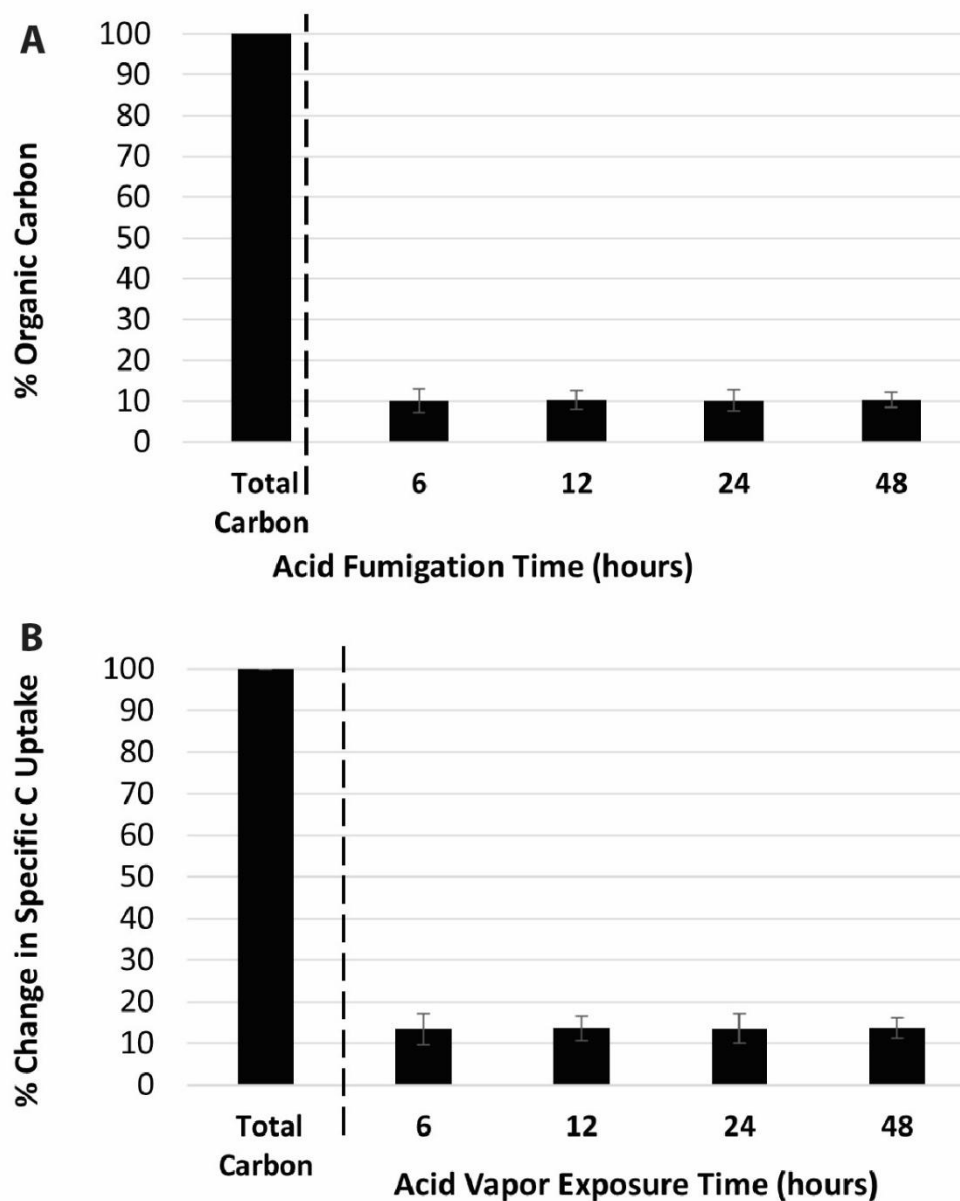




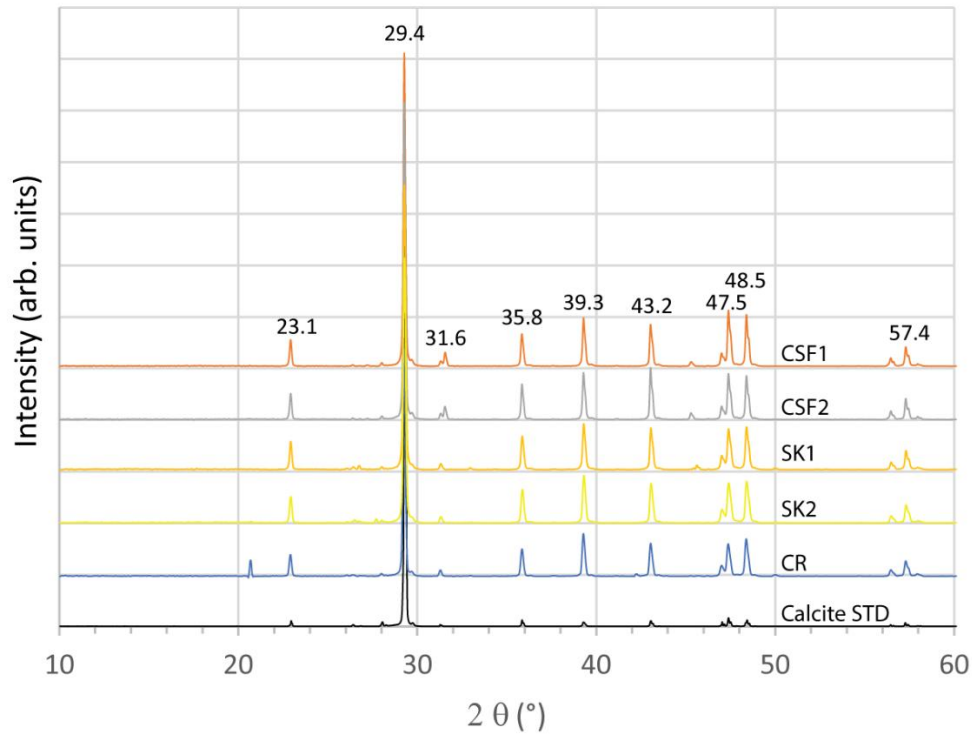
Supplementary Figure 1. Study sites. A Map of microbialite sampling locations. B Photogrammetric map of the Cape Recife (CR) and C Schoenmakerskop (SK) microbialite systems. The freshwater inflow point where samples were collected is indicated by a white arrow. D Microbialite face of the ov745 system. White arrows indicate freshwater flow path where samples were collected. E Thyspunt (TP) barrage pool formed by microbialite growth showing the flow of freshwater over the wall at in the subtidal zone. Site locations: TP (34°11'24"S 24°42'13"E), OV745 (34°11'47"S 24°46'37"E), CR (34°02'42"S 25°34'07"E), and SK (34°02'28"S 25°32'18"E).



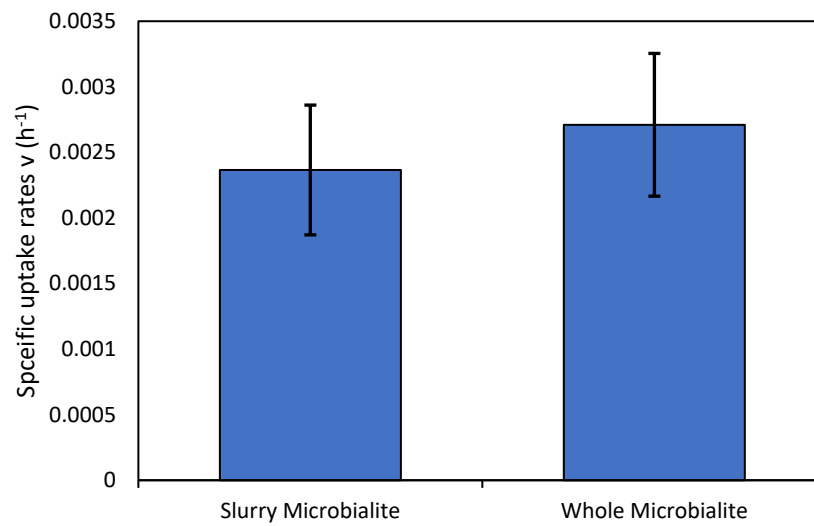
Supplementary Figure 2. PCA plot comparing 2018 microbialite samples based on nutrient factors and specific $\text{H}^{13}\text{CO}_3^-$ uptake rates, using Euclidean distance.



Supplementary Figure 3. Bar graph showing (A) the change in percent organic carbon over a 6, 12, 24, and 48-hour period, with acid fumigation to remove inorganic carbon and (B) the corresponding change in bicarbonate uptake rates associated with the removal of inorganic carbon over the same 48-hour time period. Changes in the proportion of inorganic carbon closely replicate the decrease in uptake rate indicating that the bicarbonate taken up during the initial 24-hour incubation was stored within the inorganic and not the organic carbon fraction. Error bars: n=3.



Supplementary Figure 4. CaCO_3 XRD spectra from Cape St Francis (CSF1, 2; sample OV745), Schoenmakerskop (SK1, 2) and Cape Recife (CR). Characteristic calcite peaks include the main peak at 2θ of 29.4° and several secondary peaks between 35.8° and 48.5° . A pure hydrothermal calcite standard (Calcite STD) shows the same peak distribution. Analyses of CSF and SK samples were repeated to confirm reproducibility. Prominent peaks indicative of aragonite at 26.1° , 27.2° and 45.9° or of vaterite at 24.8° , 27.0° , 32.7° and 49.9° are absent (peak positions from Luo et al., 2020; Hossein and Ahmed, 2023).



Supplementary Figure 5. Comparison of specific uptake rates using a slurry versus whole microbialite pieces. No significant difference ($p=0.4$) was observed between the two preparation methods. Error bars: $n=3$

SUPPLEMENTARY TABLES

Supplementary Table 1. ANOSIM test of bacterial 16S rRNA gene sequence OTU diversity and abundance at the four study sites.

Pairwise test			
Groups	R-value	Significance %	Possible permutations
CR-SK	0.619	0.2	462
CR-TP	0.969	3.6	28
CR-OV745	1	1.2	84
SK-TP	0.958	3.6	28
SK-OV745	0.958	0.1	3003
TP-OV745	0.948	2.2	45

Supplementary Table 2. Tukey Honest Significance Difference (HSD) test for specific uptake rates (v) for the four study sites.

Tukey Honest Significance Difference (HSD) Test	
groups	p-value
CR-SK	0.0010
CR-TP	0.0000
CR-OV745	0.0442
SK-TP	0.0051
SK-OV745	0.0619
TP-OV745	0.0002

Supplementary Table 3. Mass of carbon assessed ($\mu\text{g C}$) and specific uptake rate (v; h^{-1}). CR: Cape Recife; SK: Schoenmakerskop; TP: Thyspunt.

Supplementary Table 3. $\text{H}^{13}\text{CO}_3^-$ uptake data		
Sample Replicate	Carbon Mass (μg)	Specific Uptake Rate (h^{-1})
2018 carbon uptake assessment		
CR_2018_REP1	1091.20	0.0148
CR_2018_REP2	1062.97	0.0152
CR_2018_REP3	1308.06	0.0116
SK_2018_REP1	1121.92	0.0080
SK_2018_REP2	1583.42	0.0055
SK_2018_REP3	2069.09	0.0068
TP 2018_REP1	1799.59	0.0014
TP 2018_REP2	1551.48	0.0013
TP 2018_REP3	3072.94	0.0012
OV-745_2018_REP1	189.78	0.0107
OV-745_2018_REP2	232.25	0.0112
OV-745_2018_REP3	195.41	0.0086
2019 carbon uptake assessment		
OV745-1 Daytime_REP1	179.61	0.0148
OV745-1 Daytime_REP2	236.30	0.0145
OV745-1 Nighttime_REP1	113.67	0.0041
OV745-1 Nighttime_REP2	97.48	0.0150
OV745-2 Daytime_REP1	221.82	0.0136
OV745-2 Daytime_REP2	326.92	0.0079
OV745-2 Daytime_REP3	302.79	0.0131
OV745-2 Nighttime_REP1	318.96	0.0124
OV745-2 Nighttime_REP2	437.20	0.0079
OV745-2 Nighttime_REP3	306.36	0.0118
OV745-3 Daytime_REP1	225.23	0.0131
OV745-3 Daytime_REP2	397.51	0.0069
OV745-3 Daytime_REP3	500.46	0.0094
OV745-3 Nighttime_REP1	261.48	0.0065
OV745-3 Nighttime_REP2	198.54	0.0080
OV745-1 Daytime Control_REP1	130.83	-0.0004
OV745-1 Daytime Control_REP2	196.84	-0.0002
OV745-1 Nighttime Control_REP1	300.13	-0.0001
OV745-1 Nighttime Control_REP2	202.97	-0.0002
OV745-2 Daytime Control_REP1	628.26	-0.0001
OV745-2 Daytime Control_REP2	570.18	-0.0001
OV745-2 Nighttime Control_REP1	235.41	-0.0001
OV745-3 Daytime Control_REP1	232.40	-0.0001
OV745-3 Daytime Control_REP2	331.89	-0.0001
OV745-3 Nighttime Control_REP1	309.27	0.0000
OV745-3 Nighttime Control_REP2	127.21	-0.0002
OV745-1 - HgCl control	182.57	0.002
OV745-2 - HgCl control	276.66	0.001
OV745-3 - HgCl control	342.08	0.001

Supplementary Table 3 (cont.).

Acidification test to assess precipitation as inorganic carbon		
OV-745 No Acid_REP1	235.30	0.0013
OV-745 No Acid_REP2	385.55	0.0004
OV-745 No Acid_REP3	144.65	0.0015
OV-745 Acidified for 6 hours_REP1	70.85	0.0001
OV-745 Acidified for 6 hours_REP2	48.95	0.0001
OV-745 Acidified for 6 hours_REP3	49.35	0.0002
OV-745 Acidified for 12 hours_REP1	68.95	0.0001
OV-745 Acidified for 12 hours_REP2	57.75	0.0001
OV-745 Acidified for 12 hours_REP3	42.70	0.0002
OV-745 Acidified for 24 hours_REP1	67.90	0.0001
OV-745 Acidified for 24 hours_REP2	102.95	0.0001
OV-745 Acidified for 24 hours_REP3	52.75	0.0002
OV-745 Acidified for 48 hours_REP1	83.90	0.0001
OV-745 Acidified for 48 hours_REP2	115.30	0.0001
OV-745 Acidified for 48 hours_REP3	73.20	0.0002
Crushed vs whole microbialite comparison		
OV-12_day_crushed_REP1	556.89	0.0030
OV-12_day_crushed_REP2	544.26	0.0024
OV-12_day_crushed_REP3	533.71	0.0020
OV-12_day_whole_REP1	1635.20	0.0025
OV-12_day_whole_REP2	1389.40	0.0025
OV-12_day_whole_REP3	846.10	0.0035

Supplementary Table 4. Principle coordinate analysis of C uptake vs inflow nutrient analysis

Eigenvalues					
PC	Eigenvalues	%Variation	Cum.%Variation		
1	3.24	46.3	46.3		
2	2.76	39.5	85.8		
3	0.559	8.0	93.8		
4	0.333	4.8	98.5		
5	0.0622	0.9	99.4		
Eigenvectors					
(Coefficients in the linear combinations of variables making up PC's)					
Variable	PC1	PC2	PC3	PC4	PC5
DOC	0.239	-0.505	0.352	-0.224	0.573
NH4	0.453	0.307	-0.191	-0.357	0.008
NO3	0.393	-0.355	-0.293	-0.539	-0.243
NO2	-0.344	-0.455	0.205	-0.051	0.060
DON	0.328	-0.350	-0.558	0.639	0.188
PO4	0.464	-0.163	0.559	0.318	-0.580
Specific rate (13C)	0.377	0.410	0.297	0.139	0.487
Principal Component Scores					
Sample	SCORE1	SCORE2	SCORE3	SCORE4	SCORE5
CR	0.969	2.44	-0.446	-0.293	0.196
CR	1.20	2.80	-0.36	-0.236	0.024
CR	0.735	1.65	-0.685	0.703	-0.28
SK	1.65	-1.58	0.455	-1.2	0.006
SK	1.59	-1.59	-0.651	-0.54	-0.219
SK	2.20	-2.80	-0.570	1.01	0.148
TP	-2.75	-0.524	-0.268	0.003	-0.123
TP	-2.69	-0.557	-0.307	-0.0462	-0.0377
TP	-2.75	-0.490	-0.414	-0.186	0.0532
OV745	0.292	0.359	1.6	0.325	-0.476
OV745	-0.0386	0.133	1.09	0.328	0.358
OV745	-0.403	0.148	0.556	0.128	0.350

Supplementary Table 5. Metagenomic carbon pathway KEGG database hits

genetic group	Pathway	KO numbers	2018				2019		
			CR	SK	TP	OV745	OV745-1	OV745-2	OV745-3
PS1	Photosynthesis	K02689-K02700, K08905, K14332	234	251	158	97	111	160	93
PS2	Photosynthesis	K02703-K02724, K03541-K03542, K08901-K08904	216	211	218	61	118	121	97
B6F	Photosynthesis	K02634-K02637, K02640, K03689, K02642-K02643	233	205	188	66	89	123	84
PET	Photosynthesis	K02638-K02639, K02641, K08906	301	280	145	107	124	143	98
APC	Photosynthesis	K02092-K02097	291	303	177	127	57	133	130
Phycocyanin	phorosynthetic pigments	K02284-K02290	6	11	8	1	15	16	8
Phycocerythrocyanin	phorosynthetic pigments	K02628-K02632	397	363	329	115	197	198	169
Phycocerythrin	phorosynthetic pigments	K05376-K05386	675	625	538	192	292	347	246
CODH-ACS complex (<i>acs/cdh</i>)	Wood-Ljungdahl (carbonyl)	K00192-K00197	0	0	12	45	45	146	69
CODH (<i>acsAB</i>)	Wood-Ljungdahl (carbonyl)	K00198, K14138	0	0	8	39	28	73	79
Ni-CODH (<i>cooFS</i>)	Wood-Ljungdahl (carbonyl)	K00196, K00198	0	0	8	38	53	134	78
MTHFR	Wood-Ljungdahl (methyl)	K00297	71	118	159	113	251	269	139
MTHFD	Wood-Ljungdahl (methyl)	K01491	113	180	177	153	377	334	196
FTHFS	Wood-Ljungdahl (methyl)	K01938	127	139	146	124	292	345	236
5,10-methylene-THMPT hydrogenase	Proton generating	K13492	0	0	0	0	0	0	0
Coenzyme M biosynthesis [<i>comA-E</i>]	CoB-CoM complex	K08097, K05979, K05884, K13039, K06034	54	63	89	51	92	106	68
Coenzyme M methyltransferase	CoB-CoM complex	K14080, K14082	0	1	0	9	2	9	4
F420-dependent N5,N10-methenylTHMPT reductase	CO ₂ -methanogenesis	No KO assigned, Tsp 14558	7	19	23	10	41	26	12
Formyldehyde dehydrogenase	Oxidation of methane	K00148	5	13	4	3	30	9	10
Formylmethanofuran dehydrogenase	CO ₂ -methanogenesis	K00200-K00203, K00205, K11260-K11261	47	37	61	33	87	106	38
Formylmethanofuran-THMPT-N-formyltransferase	CO ₂ -methanogenesis	K00672	12	11	29	7	12	5	10
Hetrodisulfide reductase	CoB-CoM complex	K03388-K03390, K08264, K08265	37	36	153	287	345	494	285
Methanol corrinoid protein	CH ₃ OH-methanogenesis	K01480	0	0	0	0	1	2	0
Methanol-5-hydroxybenzimidazolylcobamide Co-methyltransferase	CH ₃ OH-methanogenesis	K04480	1	0	1	5	12	14	4
MethenylTHMPT cyclohydrolase	CO ₂ -methanogenesis	K00288	8	12	23	6	20	13	8
Methyl coenzyme M reductase	CoB-CoM complex	K00400	0	0	0	2	0	1	0
Methylene-THMPT dehydrogenase	CO ₂ -methanogenesis	K00319	11	5	25	5	8	5	4
Quinohemoprotein amine dehydrogenase	Oxidation of methane	K08685	1	4	9	3	16	9	4
S-(hydroxymethyl)glutathione synthase	Oxidation of methane	K003396	11	5	14	8	30	3	5
S-formylglutathione hydrolase	Oxidation of methane	K01070	67	72	60	38	79	72	48
THMPT hydro-lyase	CO ₂ -methanogenesis	K10713	15	16	31	10	23	21	7
THMPT S-methyltransferase	CO ₂ -methanogenesis	K00577-K00584	0	1	1	0	1	0	0
Trimethylamine corrinoid protein	(CH ₃) ₃ NH-methanogenesis	K14084	0	0	1	1	4	10	5
Trimethylamine dehydrogenase	(CH ₃) ₃ NH-methanogenesis	K00317	5	6	8	5	13	9	9
Trimethylamine-corrinoid protein Co-methyltransferase	(CH ₃) ₃ NH-methanogenesis	K14083	83	91	133	67	170	114	106
(S)-malate CoA-transferase	3-hydroxypropionate bicycle	K14471	2	8	70	10	42	26	22
2-methylfumaryl-CoA isomerase	3-hydroxypropionate bicycle	K14470	6	17	63	16	38	32	21
3-hydroxyacyl-CoA dehydrogenase	3-hydroxypropionate bicycle	K07516	128	212	217	166	421	308	220
3-hydroxypropionate dehydrogenase (NADP+)	3-hydroxypropionate bicycle	K14468	8	27	31	22	64	54	53
3-hydroxypropionyl CoA complex	3-hydroxypropionate bicycle	K14469	17	33	51	36	98	74	56
3-methylfumaryl-CoA hydratase	3-hydroxypropionate bicycle	K09709	9	8	47	5	39	26	19
Succinate semialdehyde reductase	3-hydroxypropionate bicycle	K14465	0	0	0	1	0	0	0
Carbonic anhydrase	Carbonic anhydrases	K01673-K01674, K01743	235	231	313	177	474	352	268
Coenzyme F420 hydrogenase	Proton generating	K00440-K00443	81	74	59	42	62	75	56
ECH hydrogenase	Proton generating	K14086-K14091	0	0	0	15	25	39	11
Energy converting hydrogenase	Proton generating	K06862	0	6	10	0	13	9	3
F420 non-reducing hydrogenase	Proton generating	K14126-K14128	0	0	12	47	57	117	63
Ferredoxin hydrogenase	Proton generating	K00532-K00534, K06441	2	1	3	6	19	20	13
H2:NAD+ oxidoreductase (bidirectional hydrogenase)	Proton generating	K00336, K018005-K018007	14	13	65	79	140	157	111
MoCu-CODH	Carboxidotrophy	K03518-K03520	245	282	675	179	630	386	287
NA+:H+ antiporters (<i>nhaA-C</i>)	Proton generating	K03313-K03315	98	124	139	134	300	243	173

For full list of all KEGG gene annotations please see [Google Sheet here](#)

Supplementary Table 6. Metagenomic shotgun sequence library information			
Year		Reads	Average read length
2018	CR	39,138,286	184 bp
	SK	37,724,541	190 bp
	OV 745	42,973,598	187 bp
	TP	41,813,029	192 bp
2019	OV 745-1	97,196,393	187 bp
	OV 745-2	102,861,899	184 bp
	OV 745-3	85,074,306	181 bp

References

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