

Supplementary Material for

Methane emissions offset atmospheric carbon dioxide uptake in coastal macroalgae, mixed vegetation and sediment ecosystems

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Supplementary Table 1. Daily net sea-air fluxes of CO₂, CH₄, CO₂-eq. of CH₄, and the net greenhouse gas (GHG) across four seasons in three coastal ecosystems. Values are means ± standard error. Positive fluxes refer to an efflux from the water to the atmosphere (source), while negative fluxes depict an uptake of atmospheric GHGs (sink). *CO₂-equivalent CH₄ fluxes were calculated using the sustained-flux global warming potential (SGWP) on a 100-year time horizon of 45 (21). †The net GHG balance is calculated based on net CO₂ and net CO₂-eq. CH₄ fluxes.

Habitat	Season	Net CO ₂ flux (mg CO ₂ m ⁻² d ⁻¹)	Net CH ₄ flux (mg CH ₄ m ⁻² d ⁻¹)	Net CO ₂ -eq. CH ₄ flux* (mg CO ₂ -eq. m ⁻² d ⁻¹)	Net GHG balance† (mg CO ₂ -eq. m ⁻² d ⁻¹)
Macroalgae	Spring	-148 ± 14	0.2 ± 0.0	9 ± 1	-139 ± 13
	Summer	-385 ± 54	1.8 ± 0.1	81 ± 6	-303 ± 51
	Fall	-159 ± 15	1.6 ± 0.1	70 ± 6	-89 ± 13
	Winter	117 ± 15	0.1 ± 0.0	1 ± 0	118 ± 15
Mixed vegetation	Spring	-126 ± 15	0.3 ± 0.0	16 ± 2	-111 ± 14
	Summer	-763 ± 99	2.7 ± 0.2	122 ± 9	-641 ± 92
	Fall	-275 ± 45	2.9 ± 0.3	129 ± 14	-146 ± 34
	Winter	390 ± 35	0.1 ± 0.0	4 ± 0	394 ± 35
Bare sediments	Spring	-21 ± 8	0.1 ± 0.1	7 ± 1	-15 ± 7
	Summer	157 ± 59	1.5 ± 0.1	66 ± 4	223 ± 59
	Fall	-132 ± 17	2.5 ± 0.2	112 ± 10	-21 ± 11
	Winter	326 ± 33	0.1 ± 0.0	2 ± 0	328 ± 33

Supplementary Table 2. Results of the Analysis of Variance of Aligned Rank Transformed Data of the daily net CH₄ fluxes across habitats and seasons. The ARTool R package was used to implement the Aligned Rank Transform for conducting nonparametric analyses of variance on the factorial model (CH₄ ~ Season*Habitat). The ART-C procedure was used to test for differences in pairwise combinations of levels between factors and interactions (i.e., “Contrasts”). P-values of the post hoc test are adjusted with the Tukey method.

Term	Df	Df.res	Sum Sq	Sum Sq.res	F value	Pr(>F)
Season	3	2098	301549223	4.13E+08	510.79	<0.0001
Habitat	2	2098	28390870	5.94E+08	50.13	<0.0001
Season:Habitat	6	2098	46288491	5.85E+08	27.66	<0.0001

Contrast	Estimate	SE	df	t ratio	p value
Fall, Macroalgae - Fall, Mixed vegetation	-162.92	36.45	2098	-4.47	0.0005
Fall, Macroalgae - Fall, Sediment	-138.84	37.81	2098	-3.67	0.0132
Fall, Macroalgae - Spring, Macroalgae	640.14	38.49	2098	16.63	<0.0001
Fall, Macroalgae - Summer, Macroalgae	-134.72	44.88	2098	-3.00	0.1086
Fall, Macroalgae - Winter, Macroalgae	1076.21	43.70	2098	24.63	<0.0001
Fall, Mixed vegetation - Fall, Sediment	24.08	38.50	2098	0.63	1.0000
Fall, Mixed vegetation - Spring, Mixed vegetation	610.34	38.26	2098	15.95	<0.0001
Fall, Mixed vegetation - Summer, Mixed vegetation	-211.61	52.81	2098	-4.01	0.0037
Fall, Mixed vegetation - Winter, Mixed vegetation	892.01	39.62	2098	22.52	<0.0001
Fall, Sediment - Spring, Sediment	884.90	40.33	2098	21.94	<0.0001
Fall, Sediment - Summer, Sediment	-32.22	45.42	2098	-0.71	0.9999
Fall, Sediment - Winter, Sediment	1042.39	47.05	2098	22.15	<0.0001
Spring, Macroalgae - Spring, Mixed vegetation	-192.72	40.21	2098	-4.79	0.0001
Spring, Macroalgae - Spring, Sediment	105.91	40.97	2098	2.59	0.2886
Spring, Macroalgae - Winter, Macroalgae	436.07	45.99	2098	9.48	<0.0001
Spring, Mixed vegetation - Spring, Sediment	298.64	40.11	2098	7.45	<0.0001
Spring, Mixed vegetation - Summer, Mixed vegetation	-821.95	53.59	2098	-15.34	<0.0001
Spring, Mixed vegetation - Winter, Mixed vegetation	281.67	40.65	2098	6.93	<0.0001
Spring, Sediment - Summer, Sediment	-917.12	45.90	2098	-19.98	<0.0001
Spring, Sediment - Winter, Sediment	157.50	47.51	2098	3.31	0.0437
Summer, Macroalgae - Summer, Mixed vegetation	-239.81	58.94	2098	-4.07	0.0029
Summer, Macroalgae - Winter, Macroalgae	1210.93	51.45	2098	23.54	<0.0001
Summer, Mixed vegetation - Summer, Sediment	203.47	58.05	2098	3.51	0.0235
Summer, Mixed vegetation - Winter, Mixed vegetation	1103.62	54.56	2098	20.23	<0.0001
Summer, Sediment - Winter, Sediment	1074.61	51.90	2098	20.70	<0.0001
Winter, Macroalgae - Winter, Mixed vegetation	-347.12	46.37	2098	-7.49	<0.0001
Winter, Macroalgae - Winter, Sediment	-172.66	51.90	2098	-3.33	0.0422
Winter, Mixed vegetation - Winter, Sediment	174.46	47.97	2098	3.64	0.0149

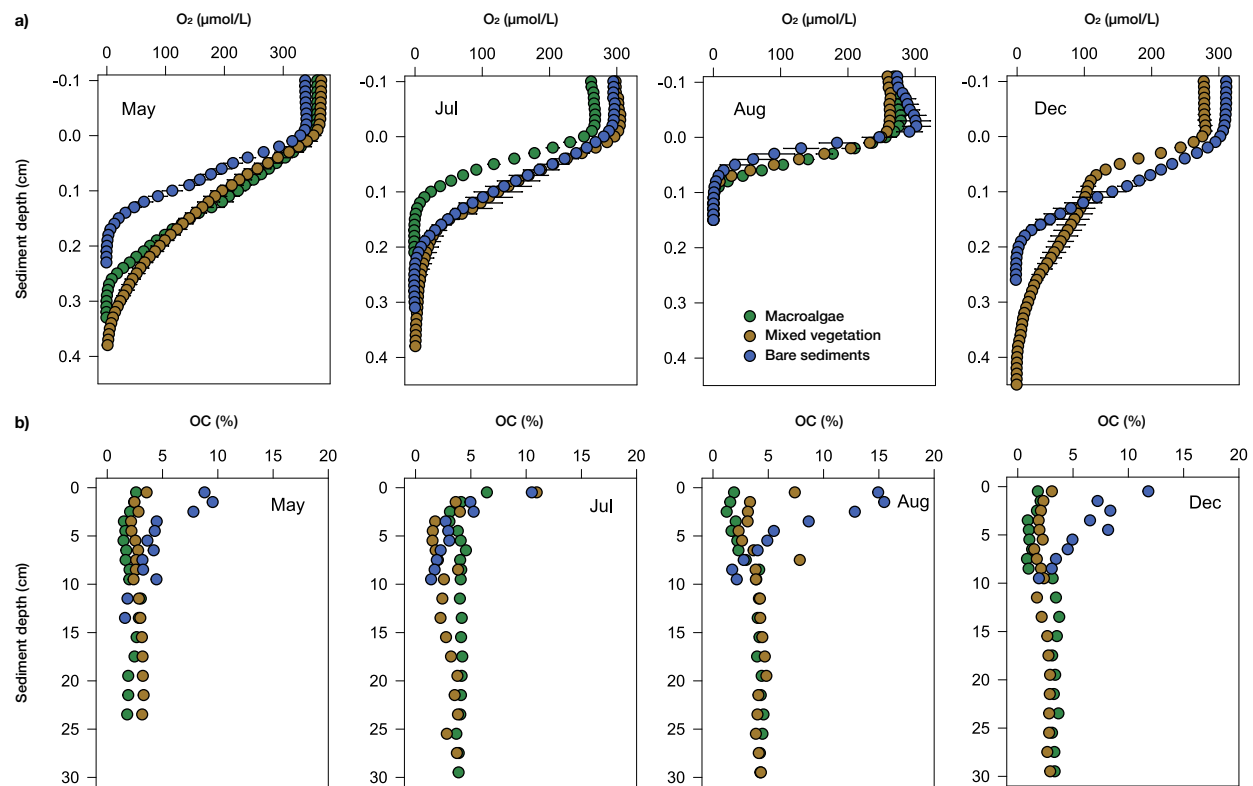
Supplementary Table 3. Results of the Analysis of Variance of Aligned Rank Transformed Data of the daily net CO₂ fluxes across habitats and seasons. The ARTool R package was used to implement the Aligned Rank Transform for conducting nonparametric analyses of variance on the factorial model (CO₂ ~ Season*Habitat). The ART-C procedure was used to test for differences in pairwise combinations of levels between factors and interactions (i.e., “Contrasts”). P-values of the post hoc test are adjusted with the Tukey method.

Term	Df	Df.res	Sum Sq	Sum Sq.res	F value	Pr(>F)
Season	3	2098	235125157	521997662	315.00	<0.0001
Habitat	2	2098	51513911	657353277	82.21	<0.0001
Season:Habitat	6	2098	54204608	671646215	28.22	<0.0001

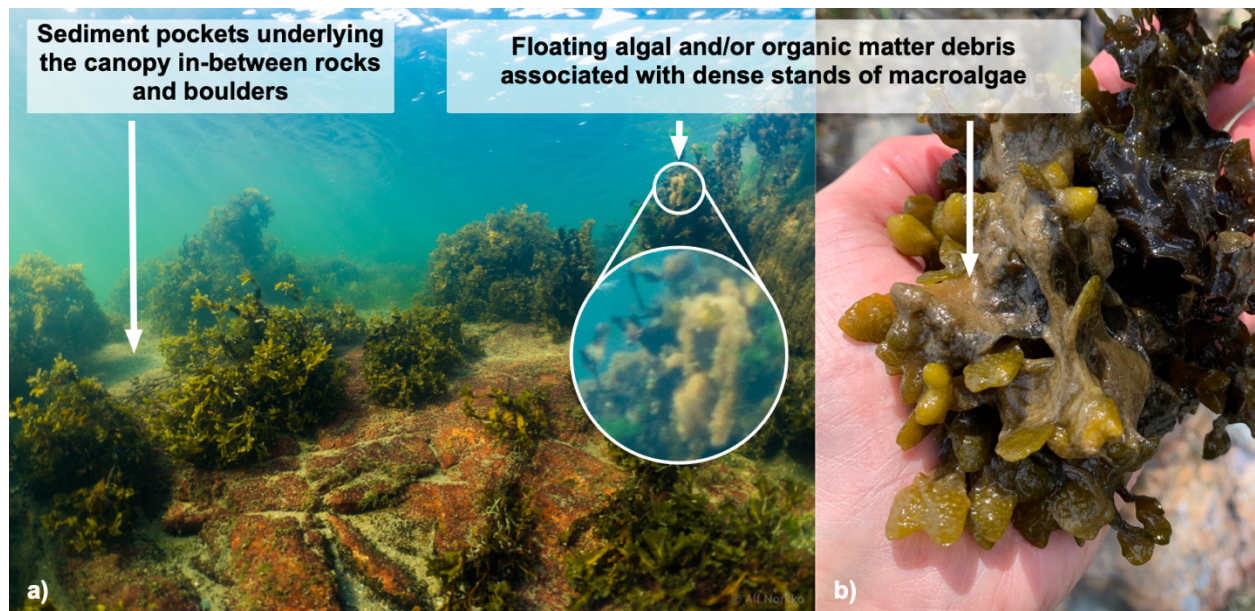
Contrast	Estimate	SE	df	t ratio	p value
Fall, Macroalgae - Fall, Mixed vegetation	-24.04	40.07	2098	-0.60	1.0000
Fall, Macroalgae - Fall, Sediment	-126.07	41.57	2098	-3.03	0.0999
Fall, Macroalgae - Spring, Macroalgae	16.22	42.31	2098	0.38	1.0000
Fall, Macroalgae - Summer, Macroalgae	107.61	49.33	2098	2.18	0.5636
Fall, Macroalgae - Winter, Macroalgae	-847.39	48.04	2098	-17.64	<0.0001
Fall, Mixed vegetation - Fall, Sediment	-102.03	42.33	2098	-2.41	0.3990
Fall, Mixed vegetation - Spring, Mixed vegetation	-79.69	42.06	2098	-1.89	0.7629
Fall, Mixed vegetation - Summer, Mixed vegetation	323.44	58.05	2098	5.57	<0.0001
Fall, Mixed vegetation - Winter, Mixed vegetation	-1022.44	43.55	2098	-23.48	<0.0001
Fall, Sediment - Spring, Sediment	-318.49	44.34	2098	-7.18	<0.0001
Fall, Sediment - Summer, Sediment	-468.00	49.93	2098	-9.37	<0.0001
Fall, Sediment - Winter, Sediment	-943.61	51.73	2098	-18.24	<0.0001
Spring, Macroalgae - Spring, Mixed vegetation	-119.94	44.20	2098	-2.71	0.2202
Spring, Macroalgae - Spring, Sediment	-460.78	45.04	2098	-10.23	<0.0001
Spring, Macroalgae - Summer, Macroalgae	91.39	51.79	2098	1.76	0.8370
Spring, Macroalgae - Winter, Macroalgae	-863.61	50.56	2098	-17.08	<0.0001
Spring, Mixed vegetation - Spring, Sediment	-340.84	44.09	2098	-7.73	<0.0001
Spring, Mixed vegetation - Summer, Mixed vegetation	403.12	58.91	2098	6.84	<0.0001
Spring, Mixed vegetation - Winter, Mixed vegetation	-942.76	44.68	2098	-21.10	<0.0001
Spring, Sediment - Summer, Sediment	-149.51	50.45	2098	-2.96	0.1202
Spring, Sediment - Winter, Sediment	-625.12	52.23	2098	-11.97	<0.0001
Summer, Macroalgae - Summer, Mixed vegetation	191.79	64.79	2098	2.96	0.1212
Summer, Macroalgae - Summer, Sediment	-701.68	56.56	2098	-12.41	<0.0001
Summer, Macroalgae - Winter, Macroalgae	-955.00	56.56	2098	-16.88	<0.0001
Summer, Mixed vegetation - Summer, Sediment	-893.48	63.81	2098	-14.00	<0.0001
Summer, Mixed vegetation - Winter, Mixed vegetation	-1345.88	59.98	2098	-22.44	<0.0001
Summer, Sediment - Winter, Sediment	-475.60	57.06	2098	-8.34	<0.0001
Winter, Macroalgae - Winter, Mixed vegetation	-199.09	50.98	2098	-3.91	0.0055

Supplementary Table 4. Measured environmental variables in the three habitats across four seasons. Values include all measurements over 24h cycles for multiple days within the seasonal sampling campaigns (i.e., spring = 18 – 29 May; summer = 06 – 11 July; fall = 22 October – 2 November; and winter = 30 November – 08 December 2020). Accordingly, values are not representative for the “meteorological” seasons at the study locations. Note: Daily-integrated sea-air fluxes of CO₂ and CH₄ are not based on the mean values presented here, but result from continuous measurements averaged to 15 min intervals.

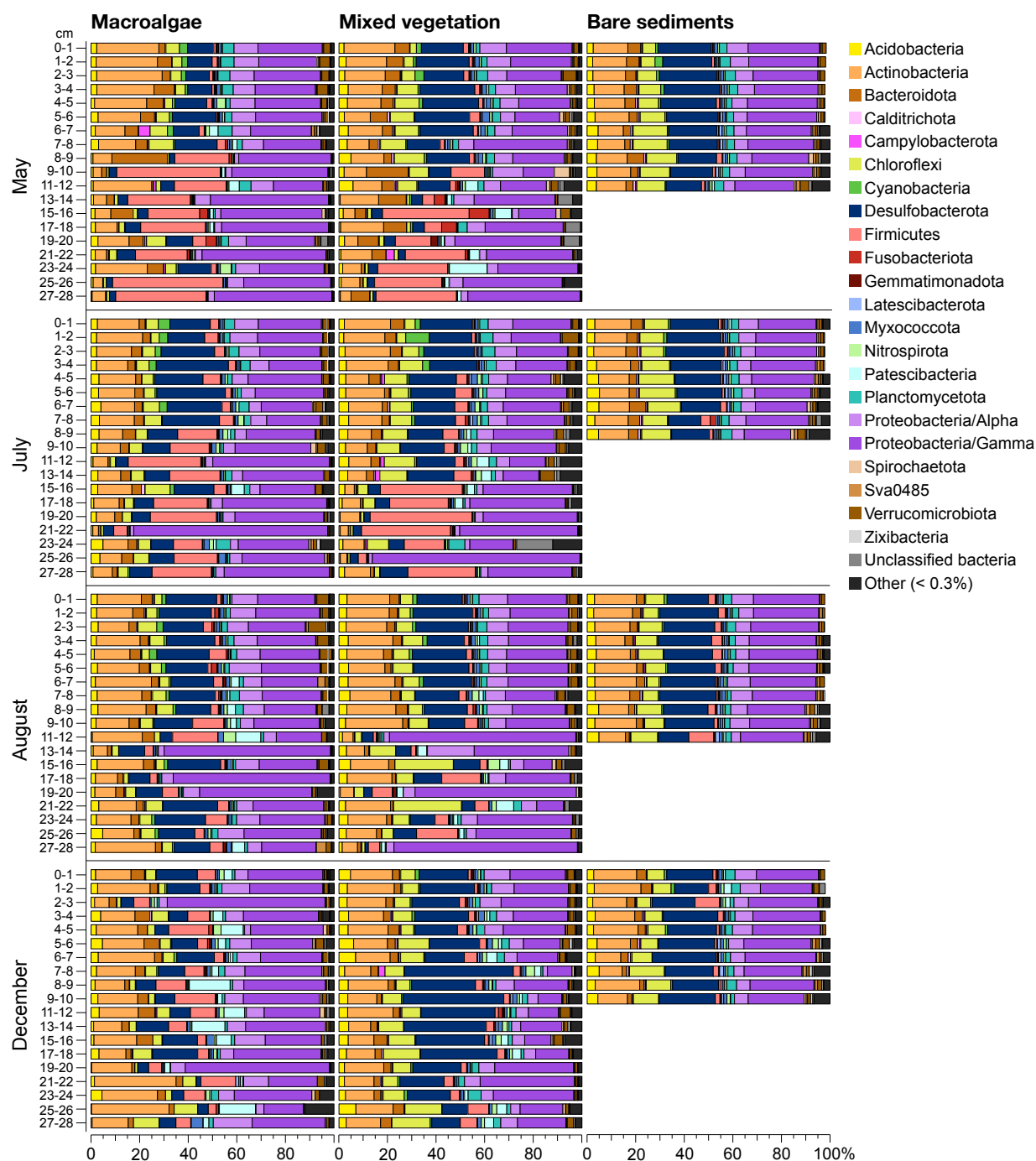
	Spring		Summer		Fall		Winter	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
<u>Macroalgae</u>								
Water temperature (°C)	8.55 – 11.58	9.77 $\pm$ 0.76	17.08 – 22.17	19.80 $\pm$ 1.53	10.82 – 12.59	11.56 $\pm$ 0.43	5.87 – 6.62	6.26 $\pm$ 0.16
Salinity	6.17 – 6.66	6.62 $\pm$ 0.08	6.47 – 6.61	6.52 $\pm$ 0.04	6.34 – 6.50	6.43 $\pm$ 0.06	6.84 – 7.03	6.99 $\pm$ 0.04
pCO ₂ water (μ atm)	134 – 352	235 $\pm$ 53	62 – 635	245 $\pm$ 137	73 – 537	264 $\pm$ 119	349 – 710	620 $\pm$ 64
CH ₄ water (nmol/L)	17 – 101	41 $\pm$ 15	41 – 260	144 $\pm$ 53	45 – 169	80 $\pm$ 24	9 – 23	13 $\pm$ 3
Windspeed (m/s)	0.1 – 9.1	2.1 $\pm$ 1.6	0.1 – 9.4	2.7 $\pm$ 1.6	0.3 – 11.9	4.9 $\pm$ 2.7	0.1 – 7.2	1.7 $\pm$ 1.3
<u>Mixed vegetation</u>								
Water temperature (°C)	8.43 – 11.32	9.53 $\pm$ 0.85	16.53 – 22.04	19.93 $\pm$ 1.80	10.21 – 12.81	11.46 $\pm$ 0.75	5.69 – 6.90	6.54 $\pm$ 0.28
Salinity	6.39 – 6.66	6.61 $\pm$ 0.05	6.46 – 6.58	6.51 $\pm$ 0.04	6.36 – 6.49	6.41 $\pm$ 0.02	6.78 – 7.05	6.96 $\pm$ 0.09
pCO ₂ water (μ atm)	131 – 765	317 $\pm$ 112	55 – 1628	313 $\pm$ 286	78 – 617	206 $\pm$ 138	505 – 813	669 $\pm$ 73
CH ₄ water (nmol/L)	17 – 103	56 $\pm$ 17	53 – 460	186 $\pm$ 71	58 – 204	119 $\pm$ 33	6 – 37	18 $\pm$ 7
Windspeed (m/s)	0.1 – 9.5	2.4 $\pm$ 1.8	0.1 – 12.5	2.9 $\pm$ 1.8	0.6 – 11.3	5.1 $\pm$ 2.1	0.1 – 14.1	3.2 $\pm$ 2.6
<u>Bare sediments</u>								
Water temperature (°C)	8.39 – 11.39	9.51 $\pm$ 0.71	16.59 – 21.75	20.12 $\pm$ 1.64	10.34 – 12.86	11.85 $\pm$ 0.40	5.73 – 6.45	6.13 $\pm$ 0.19
Salinity	6.21 – 6.67	6.61 $\pm$ 0.07	6.47 – 6.57	6.51 $\pm$ 0.03	6.01 – 6.49	6.38 $\pm$ 0.07	6.78 – 7.01	6.92 $\pm$ 0.11
pCO ₂ water (μ atm)	238 – 735	414 $\pm$ 85	122 – 1245	379 $\pm$ 155	110 – 759	465 $\pm$ 201	552 – 756	604 $\pm$ 35
CH ₄ water (nmol/L)	20 – 69	40 $\pm$ 8	41 – 324	160 $\pm$ 52	34 – 115	69 $\pm$ 17	6 – 17	9 $\pm$ 2
Windspeed (m/s)	0.1 – 7.4	1.7 $\pm$ 1.5	0.1 – 9.8	3.3 $\pm$ 2.0	0.9 – 9.6	4.8 $\pm$ 1.6	0.2 – 13.4	3.4 $\pm$ 2.0



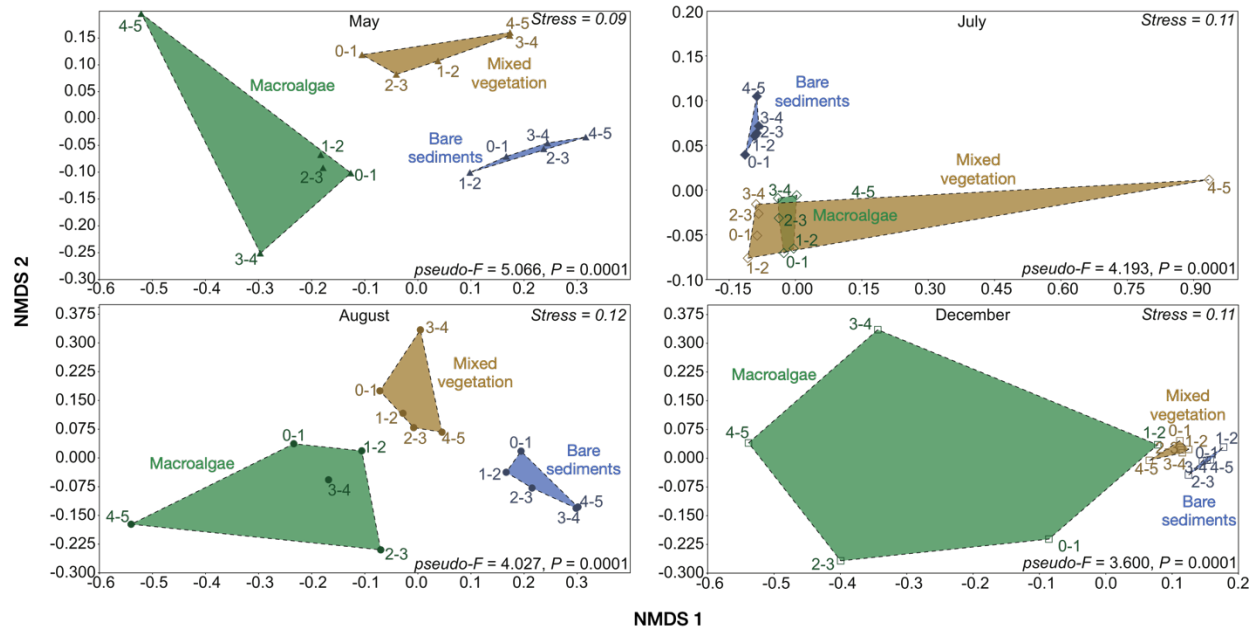
Supplementary Figure 1. Seasonal sediment microprofiles for O_2 (a) and organic carbon (OC) contents (b) in the three habitats.



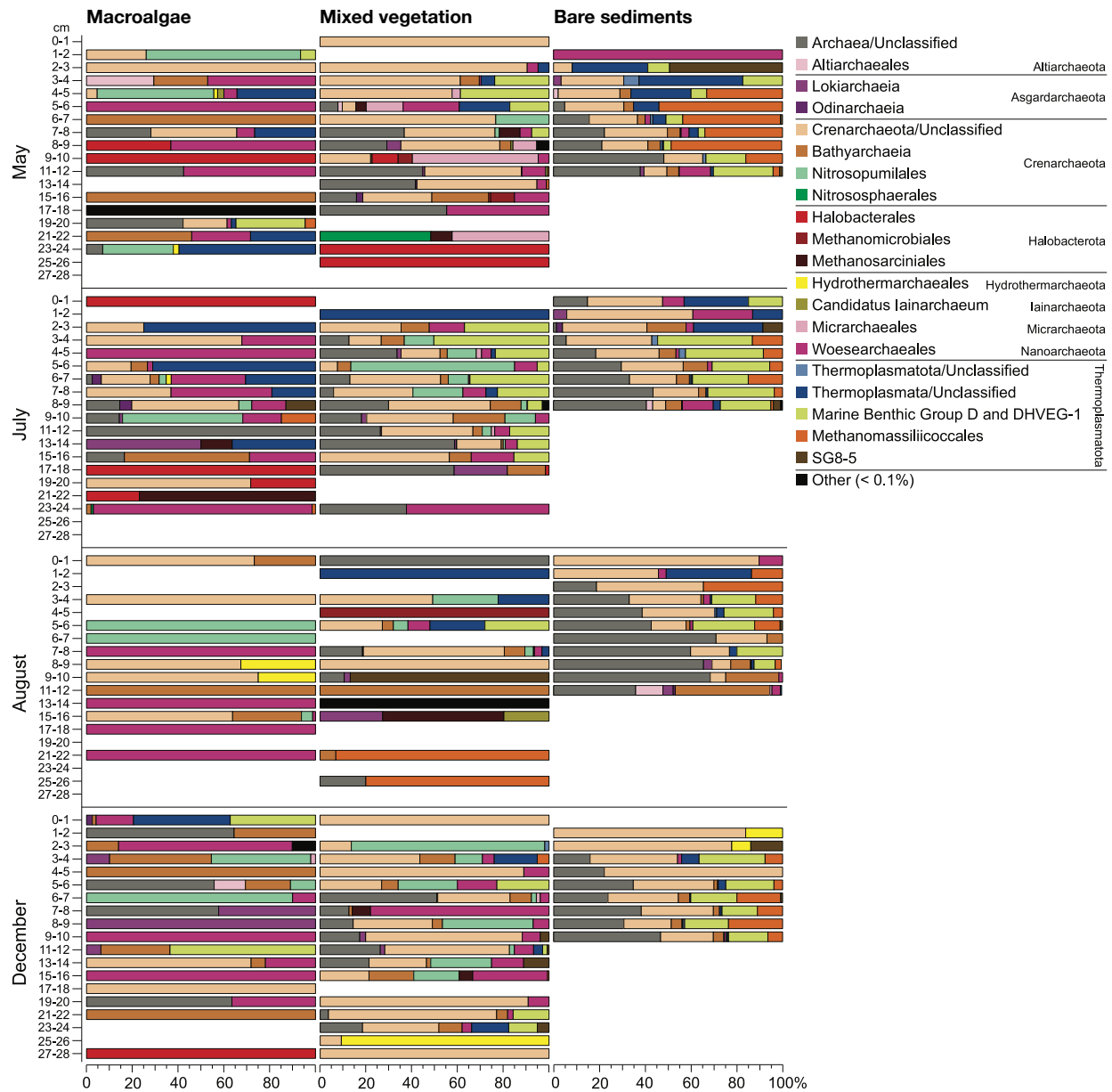
Supplementary Figure 2. Exemplary picture from the macroalgae habitat dominated by *Fucus vesiculosus* (A) and ex situ close-up of *Fucus vesiculosus* with associated floating algal and/or organic matter debris. Depicted in the pictures are exemplary locations from which additional samples for 16S rRNA gene amplicon sequencing were taken: i.e., sediment pockets underlying the canopy in-between rocks and boulders, and floating algal and/or organic matter debris associated with dense stands of *Fucus vesiculosus*. Picture in a) taken by Alf Norkko; picture in b) taken by Florian Roth.



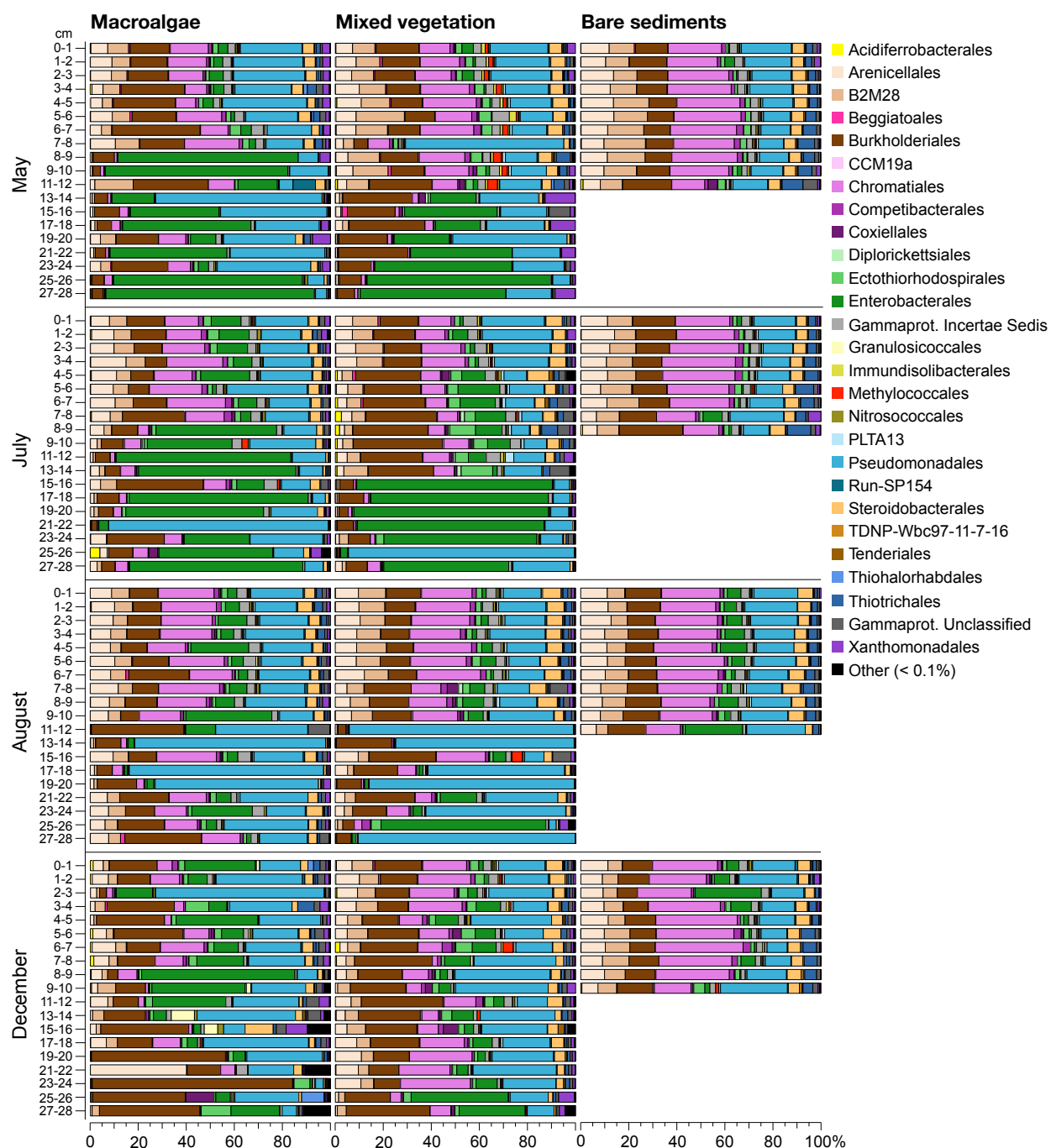
Supplementary Figure 3. Stacked bars showing the microbial community on the phyla level for the three habitats during sampling in May, July, August, and December. The y-axes show the different sediment layers, while the x-axes show the relative abundance (% of all phyla). “Other” denote groups < 0.3% average of all samples. Note that for the station “Sediments” samples could only be collected down to 12 cm (May), 9 cm (July), 12 cm (August), and 10 cm (December).



Supplementary Figure 4. NMDS plots of the Bray-Curtis dissimilarity index between the three habitats during sampling in May, July, August, and December. Each plot shows data from the first 5 cm layers in the sediment. The $pseudo-F$ and P values show the statistical results from PERMANOVA (9999 permutations) based on testing all groups together.

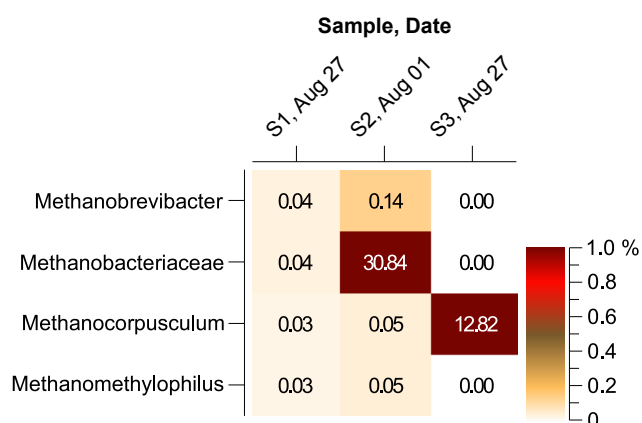


Supplementary Figure 5. Stacked bars showing the archaeal community on the lowest taxonomic classified level for the three habitats during sampling in May, July, August, and December. The y-axes show the different sediment layers, while the x-axes show the relative abundance (% of all archaea). "Other" denote groups < 0.1% average of all samples. Note that for the station "Sediments" samples could only be collected down to 12 cm (May), 9 cm (July), 12 cm (August), and 10 cm (December). Empty cells denote that no archaea were detected in the dataset.

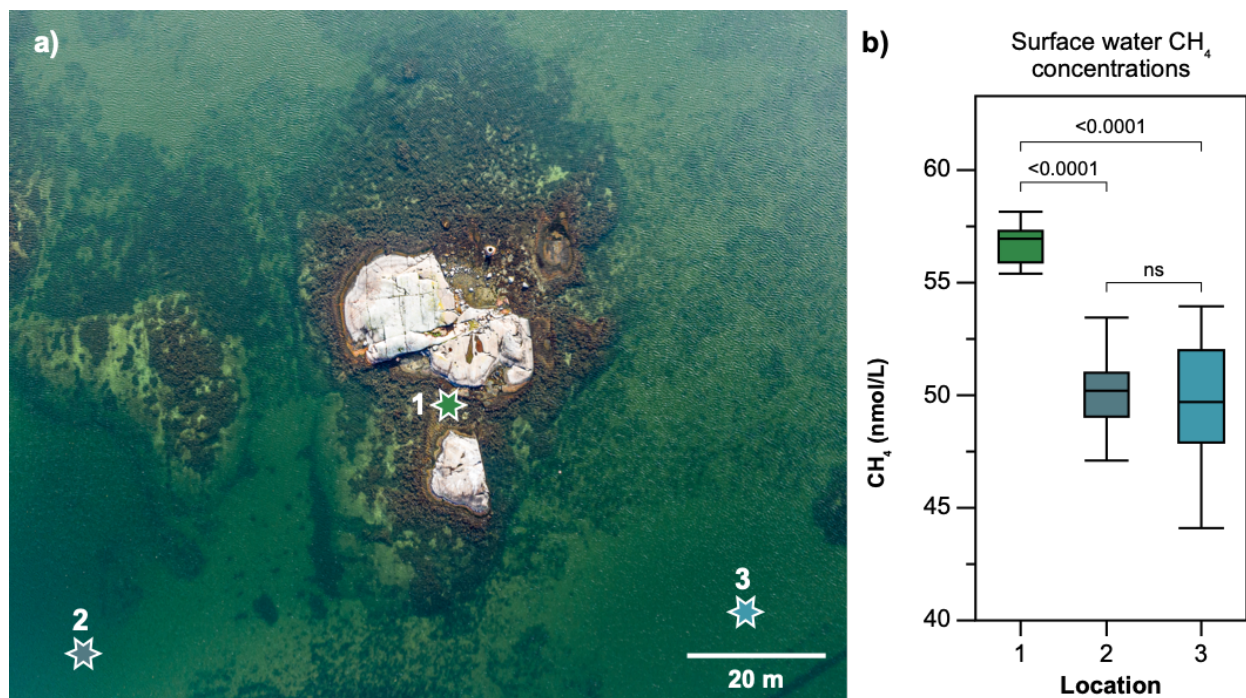


Supplementary Figure 6. Stacked bars showing the gammaproteobacterial community to the lowest taxonomic classified level for the three habitats during sampling in May, July, August, and December. The y-axes show the different sediment layers, while the x-axes show the relative abundance. “Other” denote groups < 0.1% average of all samples. Note that for the station “Sediments” samples could only be collected down to 12 cm (May), 9 cm (July), 12 cm (August), and 10 cm (December).

Methanogenic archaea (% of archaeal community)



Supplementary Figure 7. Sequencing results from organic matter aggregates (i.e., floating filamentous algal and/or organic matter debris) associated with dense stands of *Fucus vesiculosus*. Floating filamentous algal and/or organic matter debris (exemplary pictures in Fig. S2A and S2B) were collected from within dense stands of *Fucus vesiculosus* (i.e., the macroalgae habitat) in August 2020. Shown is the relative abundance (in % of all archaea) of methanogenic archaea. The figure shows the results from sequencing of the archaeal 16S rRNA gene V4–V5 region and the lowest level of classified taxonomy.



Supplementary Figure 8. (a) Aerial image of the sampling sites around a rocky island in the Finnish archipelago (59°50'30.8"N 23°15'01.0"E) surrounded by dense stands of *Fucus vesiculosus* (brown patches) and (b) results of surface water CH₄ concentration measurements at the different locations indicated in (a). Location “1” refers to within the dense macroalgae stands directly surrounding the island, while “2” and “3” are open water control sites. The concentration was recorded for approximately 1h continuously at each location between 09:00 – 12:00 on October 13, 2021. During this period, the following environmental conditions were recorded across the three stations (mean ± SD): water temperature = 11.34 ± 0.03 °C; salinity = 5.98 ± 0.00; windspeed = 3.4 ± 0.6 m/s. A one-way ANOVA was performed to compare the effect of the three different locations on surface water CH₄ concentrations. There was a statistically significant difference in mean surface water CH₄ concentrations between at least two groups ($F(2, 291) = [348.2133]$, $p < 0.0001$). Tukey’s HSD Test for multiple comparisons found that the mean value of surface water CH₄ concentrations was significantly different between location 1 (mean = 56.69, SD = 0.76, n = 70) and 2 (mean = 50.41, SD = 1.48, n = 105) ($p < 0.0001$, 95% C.I. = [-6.95, -5.61]) and location 1 and 3 (mean = 49.73, SD = 2.48, n = 119) ($p < 0.0001$, 95% C.I. = [6.31, 7.62]). There was no statistically significant difference in mean surface water CH₄ concentrations between location 2 and 3 ($p = 0.167$). Abbreviations: ns = not significant. Picture in (a) taken by Alf Norkko.