Supplementary material

Compositional stability of sediment microbial communities during a seagrass meadow decline

Marsej Markovski¹, Mirjana Najdek¹, Gerhard J. Herndl^{2,3}, and Marino Korlević^{1*}

1. Center for Marine Research, Ruđer Bošković Institute, Croatia

2. Department of Functional and Evolutionary Ecology, University of Vienna, Austria

3. Department of Marine Microbiology and Biogeochemistry, Royal Netherlands Institute for Sea Research (NIOZ), Utrecht University, The Netherlands

*To whom correspondence should be addressed:

Marino Korlević

G. Paliaga 5, 52210 Rovini, Croatia

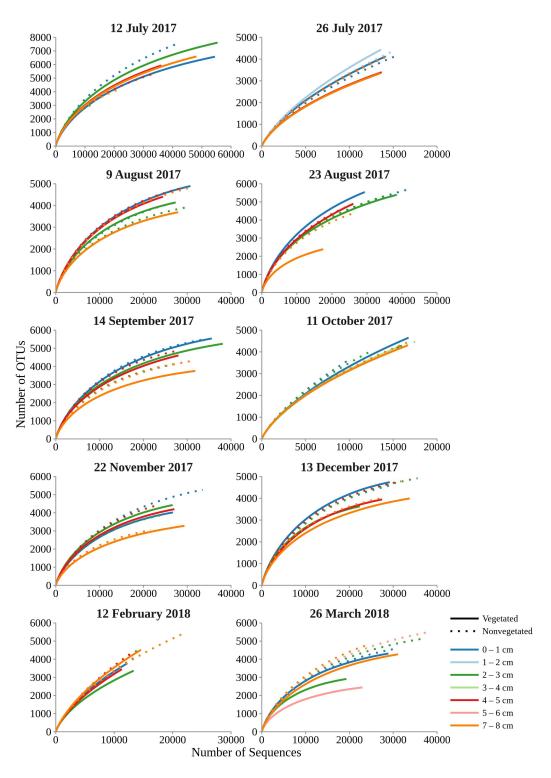
Tel.: +385 52 804 768

Fax: +385 52 804 780

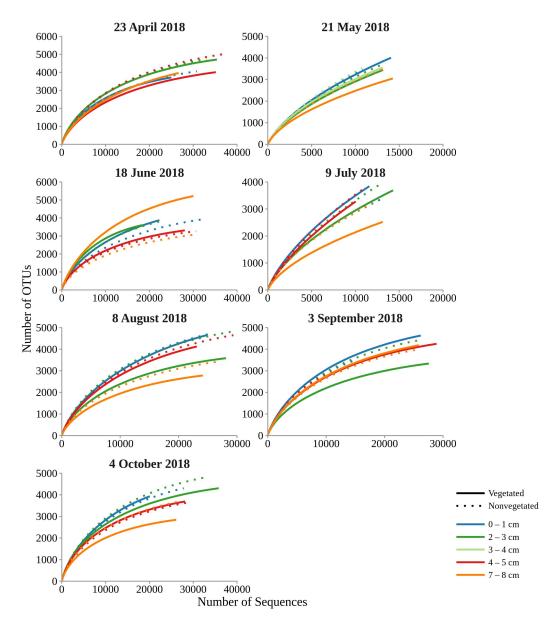
e-mail: marino.korlevic@irb.hr

Running title: Compositional stability of sediment communities

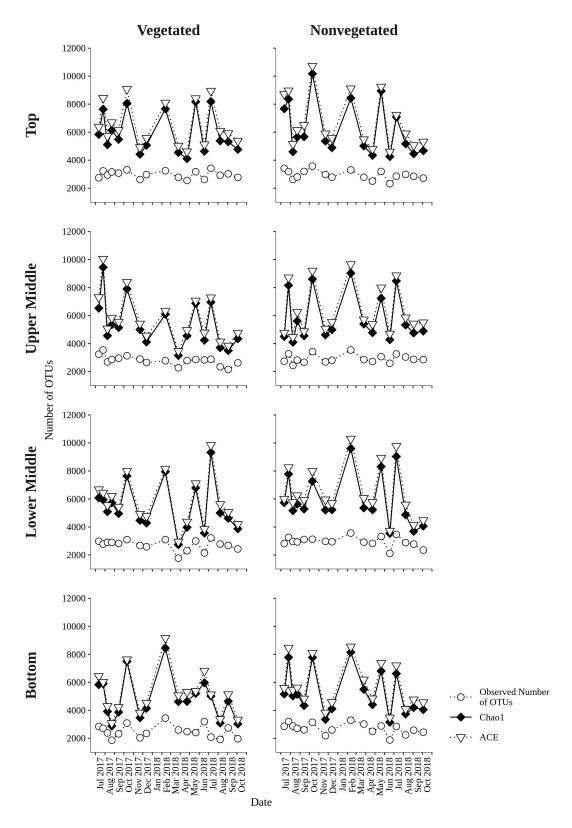
Supplementary figures



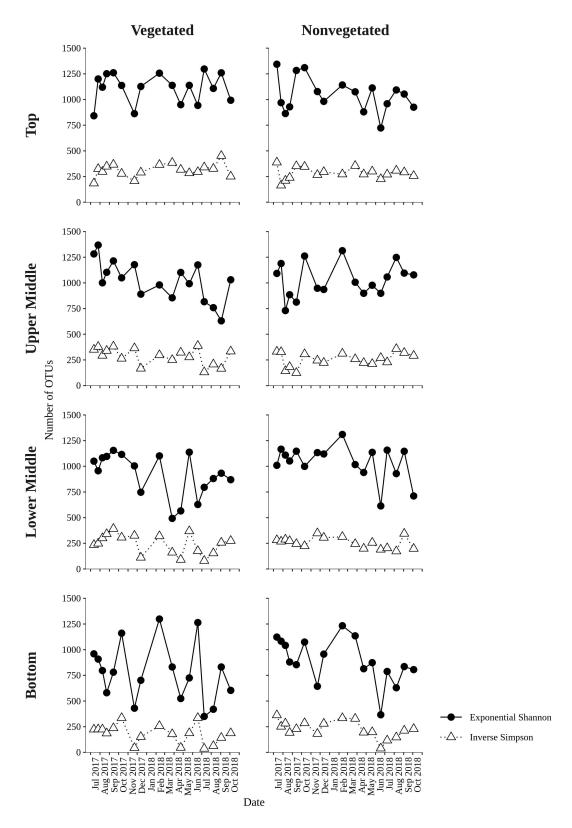
Supplementary Figure S1. Rarefaction curves of sediment microbial communities sampled at the vegetated and nonvegetated site in the Bay of Saline from 12 July 2017 to 26 March 2018.



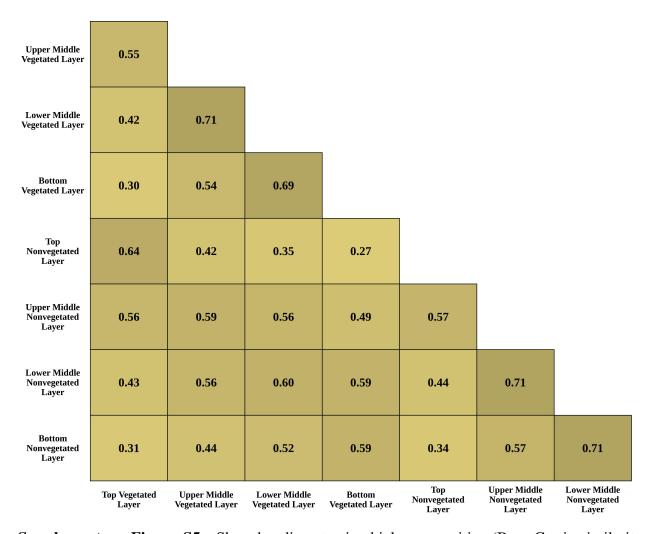
Supplementary Figure S2. Rarefaction curves of sediment microbial communities sampled at the vegetated and nonvegetated site in the Bay of Saline from 23 April 2018 to 4 October 2018.



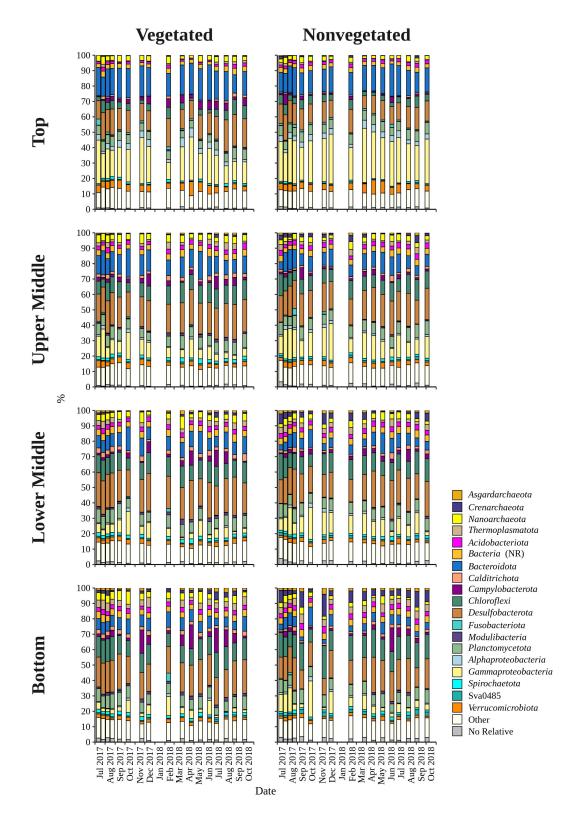
Supplementary Figure S3. Temporal dynamics of the observed number of OTUs, Chao1, and ACE of sediment microbial communities sampled in different sediment layers of the vegetated and nonvegetated site in the Bay of Saline.



Supplementary Figure S4. Temporal dynamics of the exponential Shannon diversity index and Inverse Simpson diversity index of sediment microbial communities sampled in different sediment layers of the vegetated and nonvegetated site in the Bay of Saline.



Supplementary Figure S5. Shared sediment microbial communities (Bray-Curtis similarity coefficient) between different sediment layers and sites in the Bay of Saline.



Supplementary Figure S6. Taxonomic classification and relative contribution of the most abundant bacterial and archaeal sequences ($\geq 3\%$) of each sample taken in different sediment layers from the vegetated and nonvegetated site in the Bay of Saline. No Relative (NR) – sequences without known relatives.

Supplementary tables

Supplementary Table S1. Sample ID, sampling date and site, sediment depth, no. of sequences and no. of OTUs of each sample. The number of sequences and OTUs was calculated after exclusion of sequences without known relatives (no relative sequences) and eukaryotic, chloroplast and mitochondrial sequences.

Sample ID	Date	Site	Sediment Depth	No. of Sequences	No. of OTUs
76	12 July 2017	Vegetated	0 – 1 cm	54,476	6,574
77	12 July 2017	Vegetated	2-3 cm	55,381	7,608
78	12 July 2017	Vegetated	4-5 cm	36,129	5,929
79	12 July 2017	Vegetated	7 – 8 cm	48,135	6,590
80	12 July 2017	Nonvegetated	0 - 1 cm	42,466	7,601
81	12 July 2017	Nonvegetated	2-3 cm	13,568	3,210
82	12 July 2017	Nonvegetated	4-5 cm	34,483	5,387
83	12 July 2017	Nonvegetated	7 – 8 cm	21,281	4,147
96	26 July 2017	Vegetated	0 - 1 cm	14,141	4,126
97	26 July 2017	Vegetated	1-2 cm	13,607	4,434
98	26 July 2017	Vegetated	4-5 cm	13,686	3,396
99	26 July 2017	Vegetated	7-8 cm	13,558	3,352
100	26 July 2017	Nonvegetated	0 - 1 cm	15,116	4,113
101	26 July 2017	Nonvegetated	1-2 cm	14,701	4,318
102	26 July 2017	Nonvegetated	4-5 cm	14,012	4,080
103	26 July 2017	Nonvegetated	7-8 cm	14,494	4,158
116	9 August 2017	Vegetated	0 - 1 cm	30,722	4,894
117	9 August 2017	Vegetated	2-3 cm	27,363	4,141
118	9 August 2017	Vegetated	4-5 cm	24,476	4,405
119	9 August 2017	Vegetated	7-8 cm	27,941	3,691
120	9 August 2017	Nonvegetated	0-1 cm	27,645	4,152
121	9 August 2017	Nonvegetated	2-3 cm	30,154	3,929
122	9 August 2017	Nonvegetated	4-5 cm	29,084	4,816
123	9 August 2017	Nonvegetated	7 – 8 cm	30,128	4,787
136	23 August 2017	Vegetated	0 - 1 cm	29,381	5,541
137	23 August 2017	Vegetated	2-3 cm	38,507	5,391
138	23 August 2017	Vegetated	4-5 cm	26,101	4,896
139	23 August 2017	Vegetated	7-8 cm	17,524	2,388

Supplementary Table S1. Sample ID, sampling date and site, sediment depth, no. of sequences and no. of OTUs of each sample. The number of sequences and OTUs was calculated after exclusion of sequences without known relatives (no relative sequences) and eukaryotic, chloroplast and mitochondrial sequences. *(continued)*

Sample ID	Date	Site	Sediment Depth	No. of Sequences	No. of OTUs
140	23 August 2017	Nonvegetated	0 – 1 cm	41,344	5,663
141	23 August 2017	Nonvegetated	2-3 cm	35,724	5,361
142	23 August 2017	Nonvegetated	4-5 cm	26,572	4,919
143	23 August 2017	Nonvegetated	7 – 8 cm	26,310	4,385
156	14 September 2017	Vegetated	0 - 1 cm	35,609	5,541
157	14 September 2017	Vegetated	2-3 cm	38,113	5,249
158	14 September 2017	Vegetated	4-5 cm	27,996	4,606
159	14 September 2017	Vegetated	7 – 8 cm	31,834	3,750
160	14 September 2017	Nonvegetated	0 - 1 cm	33,666	5,487
161	14 September 2017	Nonvegetated	2-3 cm	28,251	4,174
162	14 September 2017	Nonvegetated	4-5 cm	27,073	4,830
163	14 September 2017	Nonvegetated	7 – 8 cm	31,422	4,316
176	11 October 2017	Vegetated	0 - 1 cm	16,751	4,653
177	11 October 2017	Vegetated	2-3 cm	15,991	4,292
178	11 October 2017	Vegetated	3-4 cm	15,385	4,191
179	11 October 2017	Vegetated	7 – 8 cm	16,641	4,300
180	11 October 2017	Nonvegetated	0 - 1 cm	9,722	3,576
181	11 October 2017	Nonvegetated	2-3 cm	12,470	4,038
182	11 October 2017	Nonvegetated	3-4 cm	17,612	4,486
183	11 October 2017	Nonvegetated	7 – 8 cm	17,080	4,514
196	22 November 2017	Vegetated	0 - 1 cm	26,747	4,023
197	22 November 2017	Vegetated	2-3 cm	26,663	4,429
198	22 November 2017	Vegetated	4-5 cm	27,062	4,200
199	22 November 2017	Vegetated	7-8 cm	29,368	3,280
200	22 November 2017	Nonvegetated	0-1 cm	33,555	5,271
201	22 November 2017	Nonvegetated	2-3 cm	24,602	3,898
202	22 November 2017	Nonvegetated	4-5 cm	22,820	4,398
203	22 November 2017	Nonvegetated	7 – 8 cm	21,685	3,046
216	13 December 2017	Vegetated	0 - 1 cm	29,217	4,742
217	13 December 2017	Vegetated	2-3 cm	22,379	3,630

Supplementary Table S1. Sample ID, sampling date and site, sediment depth, no. of sequences and no. of OTUs of each sample. The number of sequences and OTUs was calculated after exclusion of sequences without known relatives (no relative sequences) and eukaryotic, chloroplast and mitochondrial sequences. *(continued)*

Sample ID	Date	Site	Sediment Depth	No. of Sequences	No. of OTUs
218	13 December 2017	Vegetated	4 – 5 cm	27,460	3,948
219	13 December 2017	Vegetated	7 – 8 cm	33,757	3,994
220	13 December 2017	Nonvegetated	0 - 1 cm	30,110	4,634
221	13 December 2017	Nonvegetated	2-3 cm	35,557	4,921
222	13 December 2017	Nonvegetated	4-5 cm	31,732	4,784
223	13 December 2017	Nonvegetated	7 – 8 cm	26,860	3,988
236	12 February 2018	Vegetated	0 - 1 cm	12,249	3,778
237	12 February 2018	Vegetated	2-3 cm	13,328	3,364
238	12 February 2018	Vegetated	4-5 cm	11,317	3,449
239	12 February 2018	Vegetated	7 – 8 cm	14,574	4,518
240	12 February 2018	Nonvegetated	0 - 1 cm	13,730	4,146
241	12 February 2018	Nonvegetated	2-3 cm	14,416	4,590
242	12 February 2018	Nonvegetated	4-5 cm	13,317	4,423
243	12 February 2018	Nonvegetated	7 – 8 cm	21,480	5,363
256	26 March 2018	Vegetated	0 - 1 cm	28,906	4,313
257	26 March 2018	Vegetated	2-3 cm	19,307	2,911
258	26 March 2018	Vegetated	5-6 cm	22,957	2,444
259	26 March 2018	Vegetated	7 – 8 cm	31,090	4,270
260	26 March 2018	Nonvegetated	0 - 1 cm	30,528	4,579
261	26 March 2018	Nonvegetated	2-3 cm	36,972	5,142
262	26 March 2018	Nonvegetated	5-6 cm	38,650	5,522
263	26 March 2018	Nonvegetated	7 – 8 cm	24,660	4,834
276	23 April 2018	Vegetated	0-1 cm	25,010	3,714
277	23 April 2018	Vegetated	2-3 cm	35,406	4,712
278	23 April 2018	Vegetated	4-5 cm	35,154	4,008
279	23 April 2018	Vegetated	7-8 cm	26,658	3,965
280	23 April 2018	Nonvegetated	0-1 cm	30,854	4,055
281	23 April 2018	Nonvegetated	2-3 cm	33,005	4,742
282	23 April 2018	Nonvegetated	4-5 cm	37,048	5,017
283	23 April 2018	Nonvegetated	7 – 8 cm	16,686	3,271

Supplementary Table S1. Sample ID, sampling date and site, sediment depth, no. of sequences and no. of OTUs of each sample. The number of sequences and OTUs was calculated after exclusion of sequences without known relatives (no relative sequences) and eukaryotic, chloroplast and mitochondrial sequences. *(continued)*

Sample ID	Date	Site	Sediment Depth	No. of Sequences	No. of OTUs
296	21 May 2018	Vegetated	0 – 1 cm	14,063	4,009
297	21 May 2018	Vegetated	2-3 cm	13,148	3,441
298	21 May 2018	Vegetated	3-4 cm	13,120	3,553
299	21 May 2018	Vegetated	7 – 8 cm	14,266	3,050
300	21 May 2018	Nonvegetated	0-1 cm	10,825	3,462
301	21 May 2018	Nonvegetated	2-3 cm	13,392	3,750
302	21 May 2018	Nonvegetated	3-4 cm	10,768	3,541
303	21 May 2018	Nonvegetated	7 – 8 cm	13,103	3,471
316	18 June 2018	Vegetated	0 - 1 cm	22,280	3,874
317	18 June 2018	Vegetated	2-3 cm	18,356	3,602
318	18 June 2018	Vegetated	4-5 cm	28,066	3,309
319	18 June 2018	Vegetated	7 – 8 cm	30,028	5,217
320	18 June 2018	Nonvegetated	0 - 1 cm	32,190	3,929
321	18 June 2018	Nonvegetated	2-3 cm	22,167	3,797
322	18 June 2018	Nonvegetated	4-5 cm	30,626	3,264
323	18 June 2018	Nonvegetated	7 – 8 cm	30,259	3,071
336	9 July 2018	Vegetated	0-1 cm	11,589	3,844
337	9 July 2018	Vegetated	2-3 cm	14,299	3,690
338	9 July 2018	Vegetated	4-5 cm	10,031	3,276
339	9 July 2018	Vegetated	7 – 8 cm	13,117	2,521
340	9 July 2018	Nonvegetated	0 - 1 cm	13,328	3,425
341	9 July 2018	Nonvegetated	2-3 cm	12,897	3,926
342	9 July 2018	Nonvegetated	4-5 cm	11,252	3,822
343	9 July 2018	Nonvegetated	7-8 cm	11,902	3,211
356	8 August 2018	Vegetated	0-1 cm	24,862	4,654
357	8 August 2018	Vegetated	2-3 cm	28,104	3,584
358	8 August 2018	Vegetated	4-5 cm	23,108	4,125
359	8 August 2018	Vegetated	7-8 cm	24,151	2,782
360	8 August 2018	Nonvegetated	0 - 1 cm	25,781	4,741
361	8 August 2018	Nonvegetated	2-3 cm	29,308	4,822

Supplementary Table S1. Sample ID, sampling date and site, sediment depth, no. of sequences and no. of OTUs of each sample. The number of sequences and OTUs was calculated after exclusion of sequences without known relatives (no relative sequences) and eukaryotic, chloroplast and mitochondrial sequences. *(continued)*

Sample ID	Date	Site	Sediment Depth	No. of Sequences	No. of OTUs
362	8 August 2018	Nonvegetated	4-5 cm	29,364	4,649
363	8 August 2018	Nonvegetated	7 – 8 cm	26,580	3,422
376	3 September 2018	Vegetated	0 - 1 cm	26,186	4,632
377	3 September 2018	Vegetated	2-3 cm	27,575	3,337
378	3 September 2018	Vegetated	4-5 cm	28,887	4,249
379	3 September 2018	Vegetated	7 – 8 cm	25,855	4,184
380	3 September 2018	Nonvegetated	0 - 1 cm	14,868	3,481
381	3 September 2018	Nonvegetated	2-3 cm	26,415	4,443
382	3 September 2018	Nonvegetated	4-5 cm	11,945	3,010
383	3 September 2018	Nonvegetated	7 – 8 cm	25,845	3,993
396	4 October 2018	Vegetated	0 - 1 cm	20,085	3,933
397	4 October 2018	Vegetated	2-3 cm	35,809	4,306
398	4 October 2018	Vegetated	4-5 cm	28,130	3,692
399	4 October 2018	Vegetated	7 – 8 cm	26,114	2,845
400	4 October 2018	Nonvegetated	0 - 1 cm	27,823	4,307
401	4 October 2018	Nonvegetated	2-3 cm	32,430	4,796
402	4 October 2018	Nonvegetated	4-5 cm	28,865	3,645
403	4 October 2018	Nonvegetated	7 – 8 cm	26,764	3,695

Supplementary Table S2. Statistic of richness estimator and diversity index parameters of sediment microbial communities sampled in different sediment layers of the vegetated site in the Bay of Saline. Parameters were tested by applying the Kruskal-Wallis H test followed by a pairwise comparison using the Mann-Whitney U test. Bonferroni correction was used to address the problem of multiple comparisons.

	Krus	kal-Wallis	H test	Mann-Whitney U test		
Parameter	H df		p	Comparisons Between Sediment Layers	p	
				Top Layer – Upper Middle Layer	0.78	
				Top Layer – Lower Middle Layer	0.38	
Observed No. of		2		Top Layer – Bottom Layer	< 0.05	
OTUs	11.5	3	< 0.01	Upper Middle Layer – Lower Middle Layer	1.00	
				Upper Middle Layer – Bottom Layer	0.21	
				Lower Middle Layer – Bottom Layer	0.63	
				Top Layer – Upper Middle Layer	1.00	
				Top Layer – Lower Middle Layer	1.00	
			0.00	Top Layer – Bottom Layer	0.23	
Chao1	4.7	3	3 0.20	Upper Middle Layer – Lower Middle Layer	1.00	
				Upper Middle Layer – Bottom Layer	1.00	
				Lower Middle Layer – Bottom Layer	1.00	
				Top Layer – Upper Middle Layer	0.78	
				Top Layer – Lower Middle Layer	1.00	
				Top Layer – Bottom Layer	0.15	
ACE	5.7	3	0.13	Upper Middle Layer – Lower Middle Layer	1.00	
				Upper Middle Layer – Bottom Layer	1.00	
				Lower Middle Layer – Bottom Layer	1.00	
				Top Layer – Upper Middle Layer	0.95	
				Top Layer – Lower Middle Layer	< 0.05	
	16.4	2	0.001	Lower Middle Layer – Bottom Layer Top Layer – Upper Middle Layer Top Layer – Lower Middle Layer Top Layer – Bottom Layer <	< 0.01	
Exponential Shannon	16.4	3	< 0.001	Upper Middle Layer – Lower Middle Layer	1.00	
				Upper Middle Layer – Bottom Layer	< 0.05	
				Lower Middle Layer – Bottom Layer	0.63	

Supplementary Table S2. Statistic of richness estimator and diversity index parameters of sediment microbial communities sampled in different sediment layers of the vegetated site in the Bay of Saline. Parameters were tested by applying the Kruskal-Wallis H test followed by a pairwise comparison using the Mann-Whitney U test. Bonferroni correction was used to address the problem of multiple comparisons. (continued)

	Krus	skal-Wallis	H test	Mann-Whitney U test		
Parameter	Н	df	p	Comparisons Between Sediment Layers	p	
				Top Layer – Upper Middle Layer	1.00	
				Top Layer – Lower Middle Layer	0.32	
	16.4	2	0.001	Top Layer – Bottom Layer	< 0.0	
Inverse Simpson	16.4	3	< 0.001	Upper Middle Layer – Lower Middle Layer	1.00	
				Upper Middle Layer – Bottom Layer	< 0.03	
				Lower Middle Layer – Bottom Layer	0.44	

Supplementary Table S3. Statistic of richness estimator and diversity index parameters of sediment microbial communities sampled in different sediment layers of the nonvegetated site in the Bay of Saline. Parameters were tested by applying the Kruskal-Wallis H test followed by a pairwise comparison using the Mann-Whitney U test. Bonferroni correction was used to address the problem of multiple comparisons.

	Krusl	kal-Wallis	H test	${\bf Mann\text{-}Whitney}\ U\ {\bf test}$	
Parameter	Н	df	p	Comparisons Between Sediment Layers	
				Top Layer – Upper Middle Layer	1.00
				Top Layer – Lower Middle Layer	1.00
OL IN COTTU	. ·	2	0.16	Top Layer – Bottom Layer	0.59
Observed No. of OTUs	5.1	3	0.16	Upper Middle Layer – Lower Middle Layer	1.00
				Upper Middle Layer – Bottom Layer	0.89
				Lower Middle Layer – Bottom Layer	0.2
				Top Layer – Upper Middle Layer	1.00
				Top Layer – Lower Middle Layer	1.00
Cl. 1	2.7	2	0.20	Top Layer – Bottom Layer	0.5
Chao1	3.7	3	0.30	Upper Middle Layer – Lower Middle Layer	1.00
				Upper Middle Layer – Bottom Layer	1.0
				Lower Middle Layer – Bottom Layer	0.9
				Top Layer – Upper Middle Layer	1.00
				Top Layer – Lower Middle Layer	1.0
A CIT	4.1			Top Layer – Bottom Layer	0.4
ACE	4.1	3	0.26	Upper Middle Layer – Lower Middle Layer	1.0
				Upper Middle Layer – Bottom Layer	1.0
				Lower Middle Layer – Bottom Layer	0.9
				Top Layer – Upper Middle Layer	1.0
				Top Layer – Lower Middle Layer	1.0
E	7.1	2	0.07	Top Layer – Bottom Layer	0.2
Exponential Shannon	7.1	3	0.07	Upper Middle Layer – Lower Middle Layer	1.0
				Upper Middle Layer – Bottom Layer	0.3
				Lower Middle Layer – Bottom Layer	0.1

Supplementary Table S3. Statistic of richness estimator and diversity index parameters of sediment microbial communities sampled in different sediment layers of the nonvegetated site in the Bay of Saline. Parameters were tested by applying the Kruskal-Wallis H test followed by a pairwise comparison using the Mann-Whitney U test. Bonferroni correction was used to address the problem of multiple comparisons. (continued)

	Krusl	kal-Wallis	H test	Mann-Whitney U test		
Parameter	Н	df	p	Comparisons Between Sediment Layers	p	
				Top Layer – Upper Middle Layer	1.00	
				Top Layer – Lower Middle Layer	1.00	
I 0:	4.7	2	0.20	Top Layer – Bottom Layer	0.2	
Inverse Simpson	4.7	3	0.20	Upper Middle Layer – Lower Middle Layer	1.00	
				Upper Middle Layer – Bottom Layer	1.00	
				Lower Middle Layer – Bottom Layer	1.00	