Supplementary material

Shift in the metabolic profile of sediment microbial communities during seagrass decline

Marsej Markovski¹, Mirjana Najdek¹, Zihao Zhao², Gerhard J. Herndl^{2,3}, and Marino Korlević^{1*}

- 1. Centre 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

Marino Korlević

G. Paliaga 5, 52210 Rovinj, Croatia

Tel.: +385 52 804 768

e-mail: marino.korlevic@irb.hr

^{*}To whom correspondence should be addressed:

Supplementary tables

Supplementary Table S1 Sample ID, sampling date and site, and sediment depth and layer for each protein sample. Samples for which no MS/MS spectra were obtained are indicated with an asterisk.

Sample ID	Date	Site	Sediment Depth (cm)	Sediment Layer
1			0 – 1	Тор
MM_2	12 1.1. 2017	Nonviocatotad	2 - 3	Upper Middle
MM_3	12 July 2017	Nonvegetated	4 - 5	Lower Middle
MM_4			7 - 8	Bottom
MM_5			0 – 1	Тор
MM_6	12 July 2017	Vegetated	2 - 3	Upper Middle
MM_7	12 July 2017	vegetated	4 - 5	Lower Middle
MM_8			7 – 8	Bottom
MM_9			0 - 1	Тор
MM_10	9 August 2017	Nonvegetated	2 - 3	Upper Middle
MM_11	7 August 2017	Nonvegetated	4 - 5	Lower Middle
MM_12			7 – 8	Bottom
MM_13			0 - 1	Тор
MM_14	9 August 2017	Vegetated	2 - 3	Upper Middle
MM_15	9 August 2017	vegetated	4 - 5	Lower Middle
MM_16			7 – 8	Bottom
MM_17			0 - 1	Тор
MM_18	14 September 2017	Nonvegetated	2 - 3	Upper Middle
MM_19	14 September 2017		4 - 5	Lower Middle
MM_20			7 - 8	Bottom
MM_21			0 – 1	Тор
MM_22	14 Cantaurk au 2017	Vegetated	2 - 3	Upper Middle
MM_23	14 September 2017		4 - 5	Lower Middle
MM_24			7 - 8	Bottom
MM_25			0 – 1	Тор
MM_26	11 Ostala au 2017	Managaratatad	2 - 3	Upper Middle
MM_27	11 October 2017	Nonvegetated	3 - 4	Lower Middle
MM_28			7 - 8	Bottom
MM_29			0 – 1	Тор
MM_30	11 October 2017	Vagatatad	2 - 3	Upper Middle
MM_31	11 October 2017	Vegetated	3 - 4	Lower Middle
MM_32			7 - 8	Bottom
MM_33			0 – 1	Тор
MM_34	22 November 2017	Manuacatata 1	2 - 3	Upper Middle
MM_35		Nonvegetated	4 - 5	Lower Middle
MM_36			7 - 8	Bottom
MM_37			0 – 1	Тор
MM_38	22 November 2017	Vagatatad	2 - 3	Upper Middle
MM_39	22 November 2017	Vegetated	4 - 5	Lower Middle
MM_40			7 - 8	Bottom

Supplementary Table S1 Sample ID, sampling date and site, and sediment depth and layer for each protein sample. Samples for which no MS/MS spectra were obtained are indicated with an asterisk. *(continued)*

Sample ID	Date	Site	Sediment Depth (cm)	Sediment Layer
MM_41			0 – 1	Тор
MM_42	13 December 2017	Nonvegetated	2 - 3	Upper Middle
MM_43	15 December 2017	ronvegetated	4 - 5	Lower Middle
MM_44			7 – 8	Bottom
MM_45			0 - 1	Top
MM_46	13 December 2017	Vegetated	2 - 3	Upper Middle
MM_47	13 December 2017	vogetatea	4 - 5	Lower Middle
MM_48			7 – 8	Bottom
MM_49			0 - 1	Top
MM_50	12 February 2018	Nonvegetated	2 - 3	Upper Middle
MM_51	12 Teordary 2010	ronvegetatea	4 - 5	Lower Middle
MM_52			7 – 8	Bottom
MM_53			0 - 1	Top
MM_54	12 February 2018	Vegetated	2 - 3	Upper Middle
MM_55	12 Pediuary 2016	vegetateu	4 - 5	Lower Middle
MM_56			7 - 8	Bottom
MM_57*			0 – 1	Тор
MM_58	26 March 2019	Nonvogatatad	2 - 3	Upper Middle
MM_59	26 March 2018	Nonvegetated	5 – 6	Lower Middle
MM_60			7 - 8	Bottom
MM_61			0 – 1	Тор
MM_62	26 March 2019	Vacatatad	2 - 3	Upper Middle
MM_{63}^{*}	26 March 2018	Vegetated	5 – 6	Lower Middle
MM_64			7 - 8	Bottom
MM_65			0 – 1	Тор
MM_66	22 April 2019	Nonvogatatad	2 - 3	Upper Middle
MM_67	23 April 2018	Nonvegetated	4 - 5	Lower Middle
MM_68			7 - 8	Bottom
MM_69	<u> </u>		0 – 1	Тор
MM_70	23 April 2018	Vegetated	2 - 3	Upper Middle
MM_71	23 April 2016	vegetateu	4 - 5	Lower Middle
MM_72			7 - 8	Bottom
MM_73			0 – 1	Тор
MM_74	21 Mars 2010	Manua == 4=4 1	2 - 3	Upper Middle
MM_75	21 May 2018	Nonvegetated	3 - 4	Lower Middle
MM_76			7 - 8	Bottom
MM_77			0 – 1	Тор
MM_78	01 May 2010	Manager 1	2 - 3	Upper Middle
MM_79	21 May 2018	Vegetated	3 - 4	Lower Middle
MM_80			7 - 8	Bottom

Supplementary Table S1 Sample ID, sampling date and site, and sediment depth and layer for each protein sample. Samples for which no MS/MS spectra were obtained are indicated with an asterisk. *(continued)*

Sample ID	Date	Site	Sediment Depth (cm)	Sediment Layer
MM_81 MM_82	10.1		0-1 $2-3$	Top Upper Middle
MM_83	18 June 2018	Nonvegetated	4 - 5	Lower Middle
MM_84			7 - 8	Bottom
MM_85			0 – 1	Тор
MM_86	18 June 2018	Vegetated	2 - 3	Upper Middle
MM_87	10 June 2010	vegetated	4 - 5	Lower Middle
MM_88			7 – 8	Bottom
MM_89			0 - 1	Top
MM_90	9 July 2018	Nonvegetated	2 - 3	Upper Middle
MM_91) July 2010	ronvegetated	4 - 5	Lower Middle
MM_92			7 – 8	Bottom
MM_93			0 – 1	Тор
MM_94	9 July 2018	Vegetated	2 - 3	Upper Middle
MM_95	9 July 2016	vegetated	4 - 5	Lower Middle
MM_96			7 – 8	Bottom
MM_97			0 – 1	Тор
MM_98	8 August 2018	Managaratata d	2 - 3	Upper Middle
MM_99	o August 2010	Nonvegetated	4 - 5	Lower Middle
MM_100			7 - 8	Bottom
MM_101			0 – 1	Тор
MM_102	9 August 2019	Vacatatad	2 - 3	Upper Middle
MM_103	8 August 2018	Vegetated	4 - 5	Lower Middle
MM_104			7 - 8	Bottom
MM_105			0 – 1	Тор
MM_106	2 Cantanal an 2010	Managara da da d	2 - 3	Upper Middle
MM_107	3 September 2018	Nonvegetated	4 - 5	Lower Middle
MM_108			7 - 8	Bottom
MM_109			0 – 1	Тор
MM_110	2.0010	Manadada 1	2 - 3	Upper Middle
MM_111	3 September 2018	Vegetated	4 - 5	Lower Middle
MM_112			7 - 8	Bottom
MM_113			0 – 1	Тор
MM_114	4.0-4-12010	Manage 1	2 - 3	Upper Middle
MM_115	4 October 2018	Nonvegetated	4 - 5	Lower Middle
MM_116			7 - 8	Bottom
MM_117			0 – 1	Тор
MM_118	4.0-4-12010	Manadada 1	2 - 3	Upper Middle
MM_119	4 October 2018	Vegetated	4 - 5	Lower Middle
MM_120			7 - 8	Bottom

Supplementary Table S2 Sample ID, sampling site, sediment layer and depth, sampling date, number of raw sequence pairs, number of assembled contigs by MEGAHIT, N50 and L50 assembly statistics, number of predicted CDSs by Prodigal, and number of eggNOG-mapper annotated CDSs.

Sample ID	Site	Layer (Depth)	Date	No. of Raw Sequence Pairs	No. of Contigs	N50*	L50 (bp)*	No. of Predicted CDSs	No. of Annotated CDSs
356 358	Vegetated	Top $(0 - 1 \text{ cm})$ Lower Middle $(4 - 5 \text{ cm})$	8 August 2018	205,085,833 209,632,803	32,026,408 33,248,196	8,760,379 9,111,820	601 590	40,693,178 42,249,295	29,364,186 29,892,039
360 362	Nonvegetated	Top $(0-1 \text{ cm})$ Lower Middle $(4-5 \text{ cm})$	8 August 2018	213,766,540 216,556,629	21,634,340 27,534,653	6,073,512 8,174,204	595 592	27,526,969 34,788,216	19,599,377 24,307,842

^{*} The notation was preserved from the original output of BBTools statswrapper.sh.

Supplementary Table S3 The proportion of each COG functional category (NAAF) and the number of proteins assigned to each category. The proportion and the number of proteins assigned to category C (energy production and conversion) are highlighted.

COG Category	NAAF (%)	Number of Proteins
C – Energy production and conversion	15.18	8,224
S – Function unknown	12.62	6,299
G – Carbohydrate transport and metabolism	11.45	6,823
E – Amino acid transport and metabolism	9.25	6,893
M – Cell wall/membrane/envelope biogenesis	8.89	2,999
P – Inorganic ion transport and metabolism	8.43	4,441
Multiple functional categories	7.65	2,603
O – Posttranslational modification, protein turnover, chaperones	7.02	2,901
J – Translation, ribosomal structure and biogenesis	5.28	1,507
H – Coenzyme transport and metabolism	2.29	1,494
Q – Secondary metabolites biosynthesis, transport and catabolism	2.26	1,356
F – Nucleotide transport and metabolism	1.78	1,475
I – Lipid transport and metabolism	1.72	1,421
N – Cell motility	1.38	599
K – Transcription	1.35	1,169
U – Intracellular trafficking, secretion, and vesicular transport	1.27	731
L – Replication, recombination and repair	0.98	397
T – Signal transduction mechanisms	0.79	574
V – Defense mechanisms	0.23	217
D – Cell cycle control, cell division, chromosome partitioning	0.18	147
Total	100.00	52,270

Supplementary Table S4 Overview of selected enzymes, their enzymatic products, and corresponding KO entries used to evaluate mediation processes of various fermentation products.

Name	Product	KO Entry	
		K00169	
		K00170	
Pyruvate:ferredoxin oxidoreductase	Acetyl-CoA, carbon dioxide	K00171	
		K00172	
		K03737	
Pyruvate formate-lyase	Acetyl-CoA, formate	K00656	
Acetyl-CoA hydrolase	Acetate	K01067	
Acetate kinase	Acetate	K00925	
Acetoacetate decarboxylase	Acetone, carbon dioxide	K01574	
		K00001	
		K04022	
		K13951	
		K13952	
Alcohol dehydrogenase	Ethanol	K13954	
Alcohol denydrogenase	Ethanoi	K13980	
		K18857	
		K00002	
		K13979	
		K00114	
		K00122	
		K00123	
		K00124	
		K00126	
Ermente deberderenses	Carbon dioxide	K00127	
Formate dehydrogenase	Carbon dioxide	K22515	
		K05299	
		K15022	
		K00125	
		K22516	
Lactate dehydrogenase	Lactate	K00016	
Acetolactate decarboxylase	Acetoin, carbon dioxide	K01575	
Methylmalonyl-CoA decarboxylase	Propionyl-CoA, carbon dioxide	K11264	
wichiyinalonyi-coa decalboxyiase	1 ropionys-coa, carbon dioxide	K01604	
Lactoyl-CoA dehydratase	Acryloyl-CoA	K20626	
Zactoji Cori deliyaratuse	710171071 0011	K20627	
Propionaldehyde dehydrogenase	Propionyl-CoA	K13922	
Butyrate kinase	Butyrate	K00929	
Butyryl-CoA:acetate CoA transferase	Butyrate, acetyl-CoA	K01034	
Daty 1 y 1 Cor 1. acctate Cor 1 transferase	Daily rate, accept Cort	K01035	

 $\textbf{Supplementary Table S5} \ \ \textbf{Overview of KEGG modules used for assessing various types of microbial metabolism}.$

Type of Metabolism	KEGG Module
	M00567
Mathanaganasis	M00357
Methanogenesis	M00356
	M00563
Methane oxidation	M00174
Nitrogen fixation	M00175
Assimilatory nitrate reduction	M00531
Dissimilatory nitrate reduction	M00530
Denitrification	M00529
Nitrification	M00528
Complete nitrification, comammox	M00804
Anammox	M00973
Assimilatory sulphate reduction	M00176
Dissimilatory sulphate reduction	M00596
Thiosulfate oxidation by SOX complex	M00595

Supplementary Table S6 Enzymes involved in dissimilatory sulphate reduction and their KO entries.

Name	KO Entry
Sulphate adenylyltransferase	K00958
Adenylylsulphate reductase	K00394 K00395
Dissimilatory sulphite reductase	K11180 K11181 K27196