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Supplementary Materials for

Effects of a deep-sea mining experiment on seafloor microbial communities and functions after 26 years

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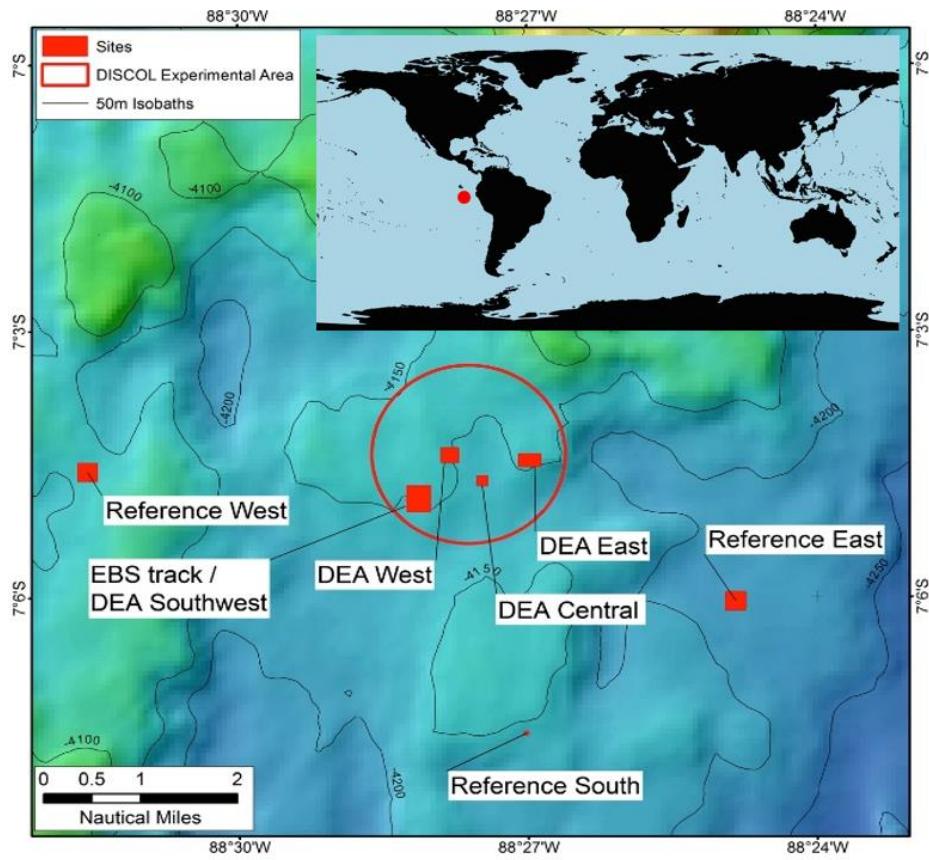


Fig S1. Sampling sites in the Peru basin (SO242) and location on the world map (upper right corner).

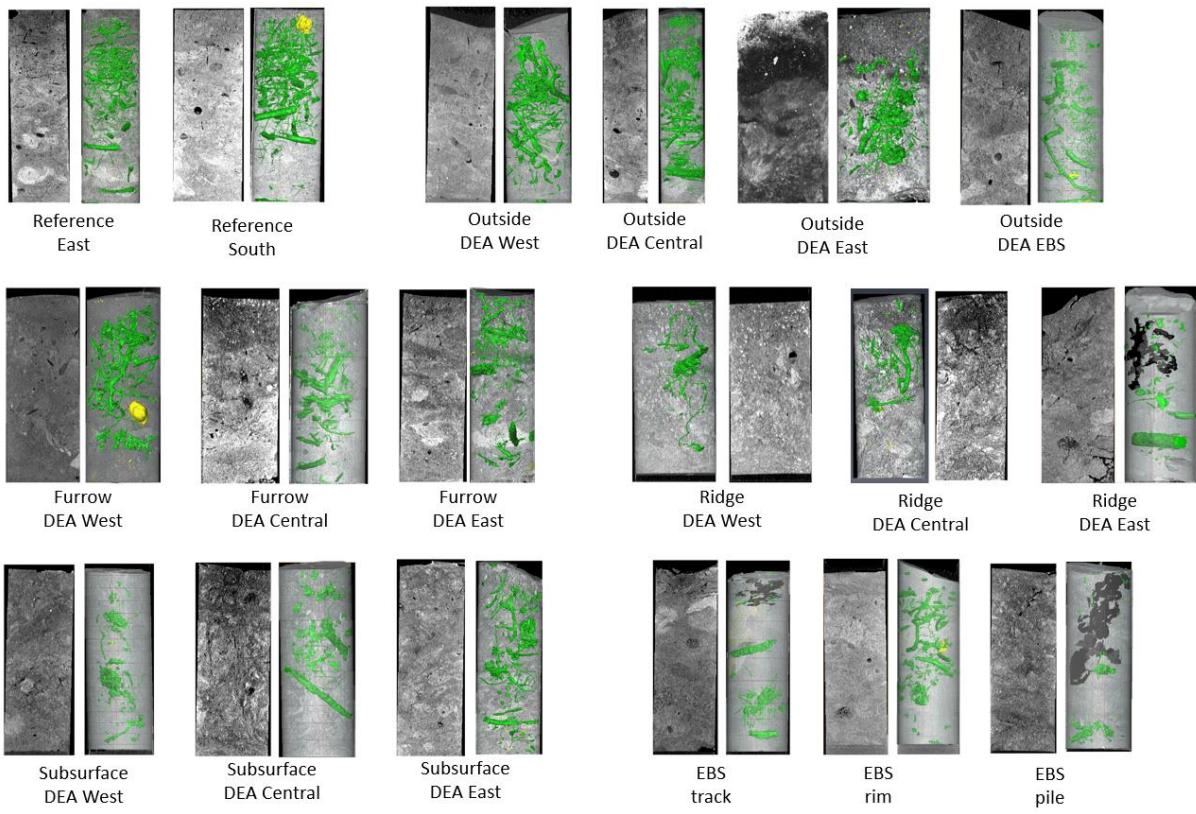


Fig S2. Middle section of the CT scans of all cores (left) and bioturbation outline (green), cracks (black) and nodule (yellow) outline of all sediment cores.

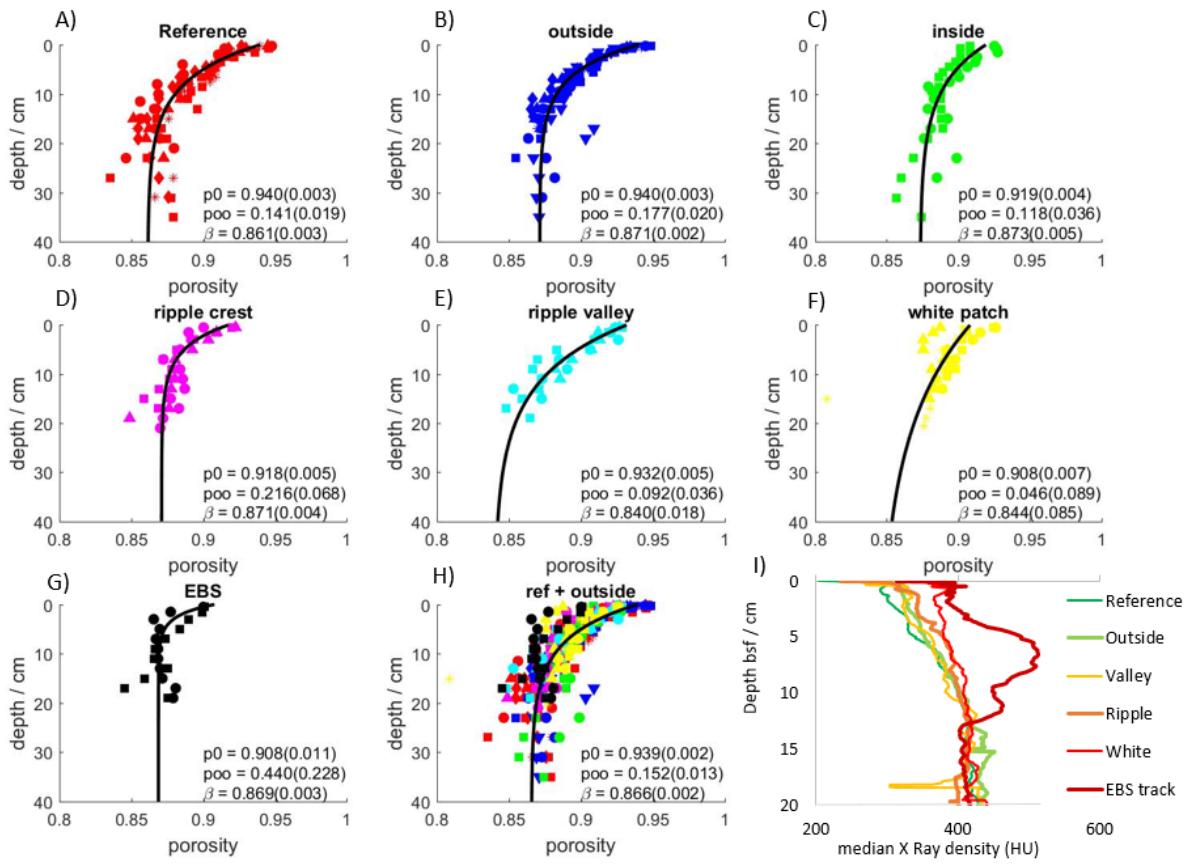


Fig S3. Density profiles of all cores **A-H)** Porosity profiles and fitted curves for parameter estimations used in the biogeochemical model for estimating dissolved oxygen uptake. **I)** Median X-ray densities over depth in houndsfield units.

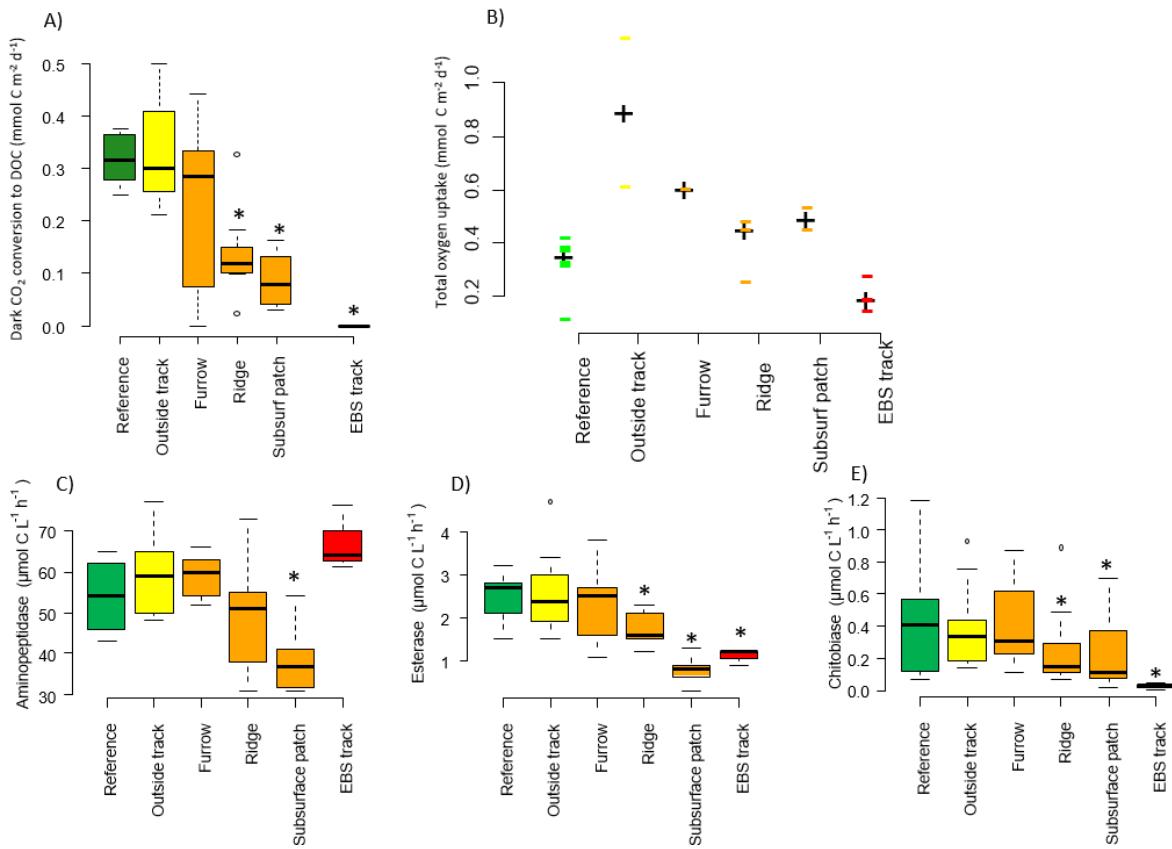


Fig S4. Microbial activities in the different microhabitats **A)** Dark carbon fixation as DOC. **B)** Total oxygen uptake based on benthic flux chambers. Each – represents a single measurement, while + indicates the median. **C-E)** Extracellular enzymatic activities of beta Glucosidase, Chitobiase, Aminopeptidase, and Esterase at the surface cm. Boxplots show the median (horizontal line), interquartile ranges (box limits), range (whiskers), and outliers (circles). The colors represent the different sampling sites: reference station (green), outside the DEA tracks (yellow), DEA track furrows, ridges, and subsurface patches (orange), fresh EBS track (red), and subsurface, i.e., at 14-16 cm bsf (grey). Significant differences to the Reference (Kruskall wallis test, $p < 0.05$, TableS1) are indicated with an asterix.

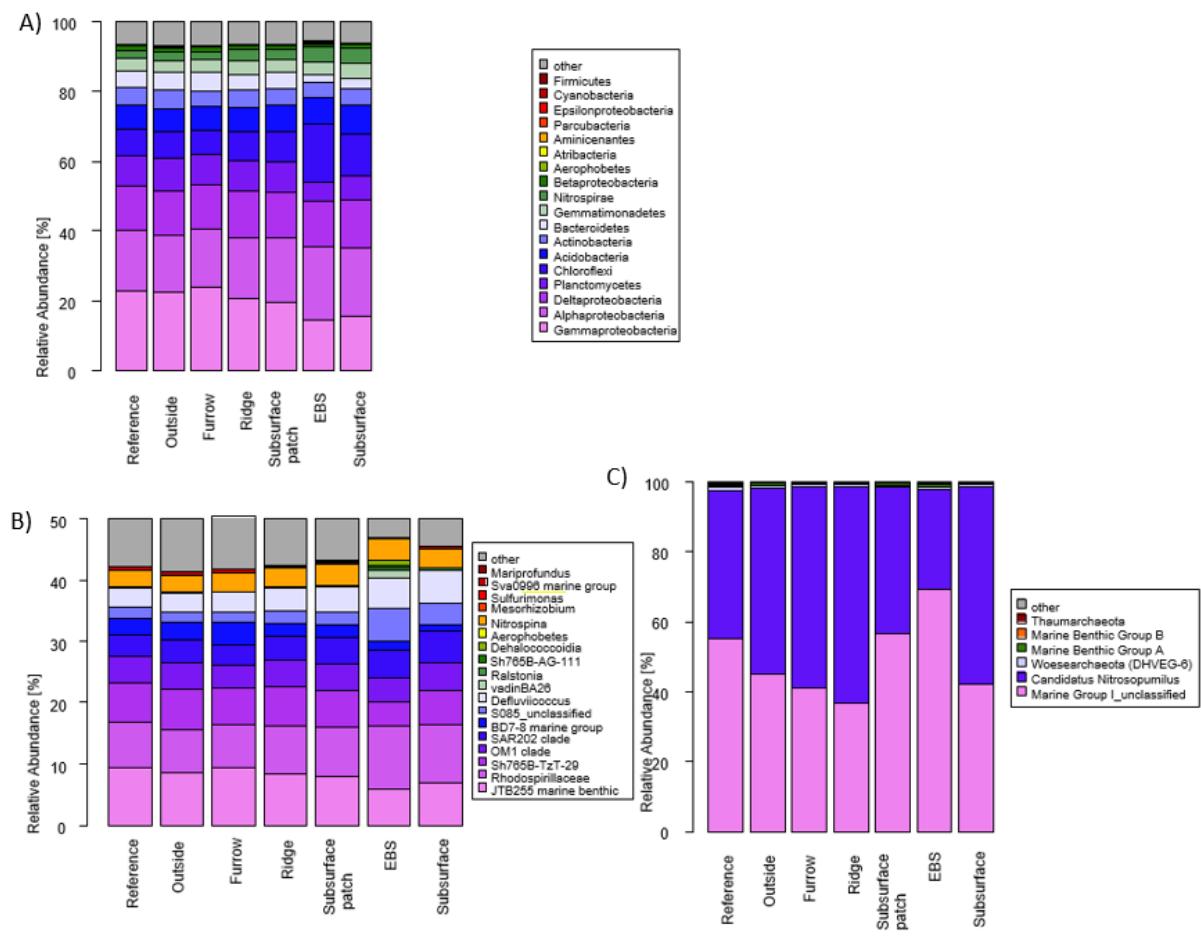


Fig S5. Microbial community structures on **A)** phylum level of bacteria with higher resolution for Proteobacteria, **B)** genus level of bacteria (or lowest taxonomic level), **C)** phylum level of archaea (or lowest taxonomic level).

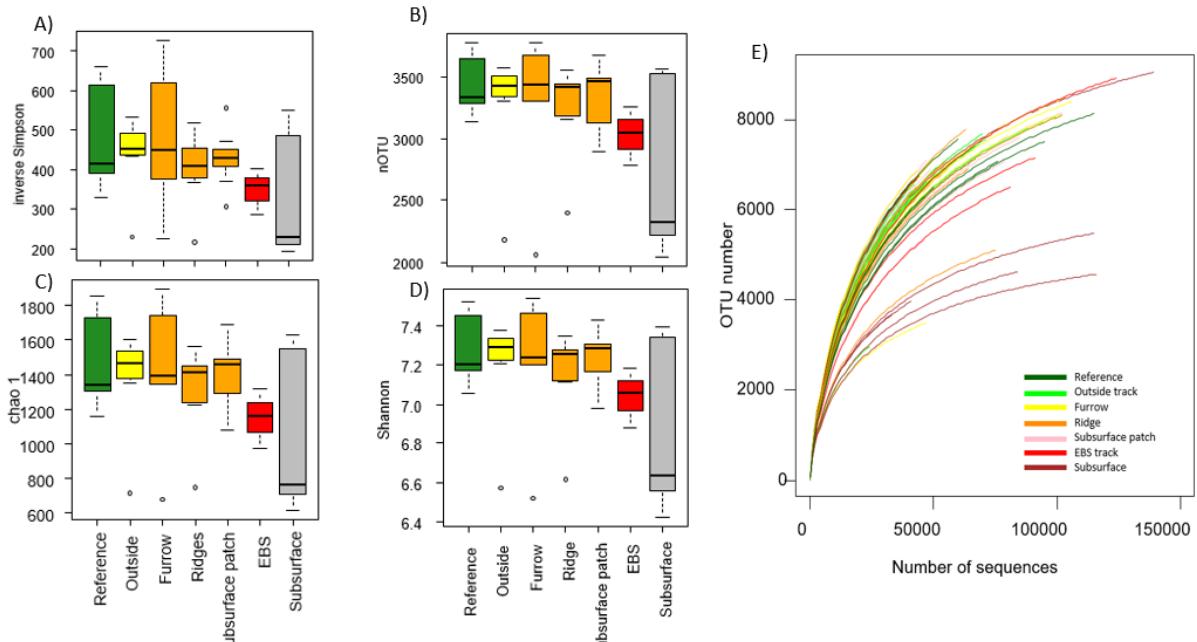


Fig S6. Diversity estimates of bacteria A-D) Diversity indices of bacteria OTUs. Boxplots show the median (horizontal line), interquartile ranges (box limits), range (whiskers), and outliers (circles). The colors represent the different sampling sites: reference station (green), outside the DEA tracks (yellow), DEA track furrows, ridges, and subsurface patches (orange), fresh EBS track (red), and subsurface, i.e., at 14-16 cm bsf (grey). **E)** Rarefaction curve of bacteria OTUs.

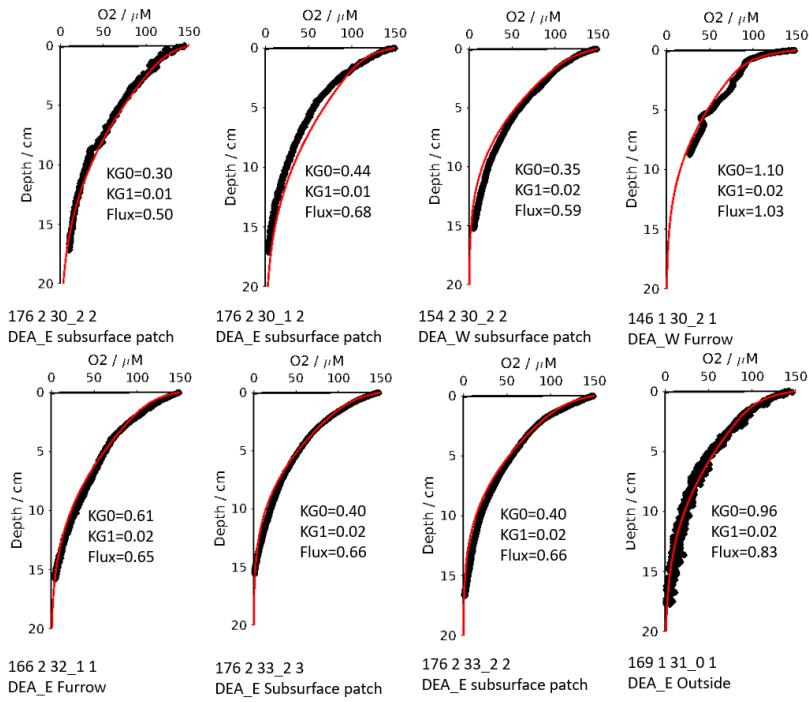


Fig S7. Validation plots for the diagenetic models described in 4.3.1. Modelled O_2 profiles (red line) vs calibrated O_2 data from the microsensors (black dots) for estimates of diffused oxygen uptake. Station, sensor ID, and microhabitat are given below each individual plot. Modelled rates of diffusive oxygen uptake of example profiles (RO2). RO2 KG1 is the rate caused by reactive TOC degradation and RO2 KG0 the rate caused by refractory TOC degradation. The highest rates are observed at the surface 3cm, indicating that the model should have the best fit in this depth for a robust estimation of total diffusive oxygen uptake. The kinetic constants for use of labile (KG0) and reactive (KG1) carbon (in year^{-1}) as well as the resulting flux (in $\text{mmol m}^{-2} \text{d}^{-1}$) used in the manuscript are given in the plot.

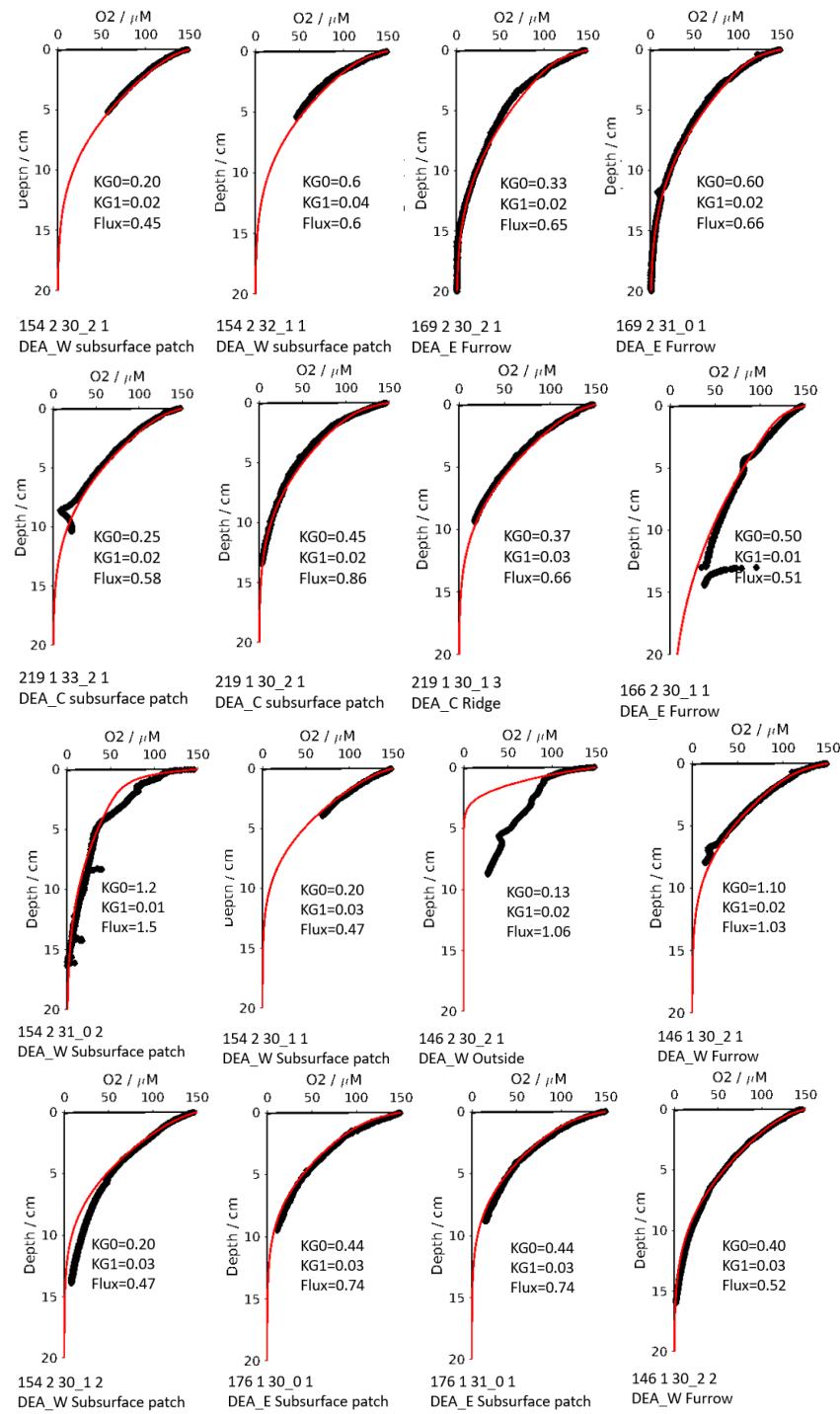


Fig S8 (continued)

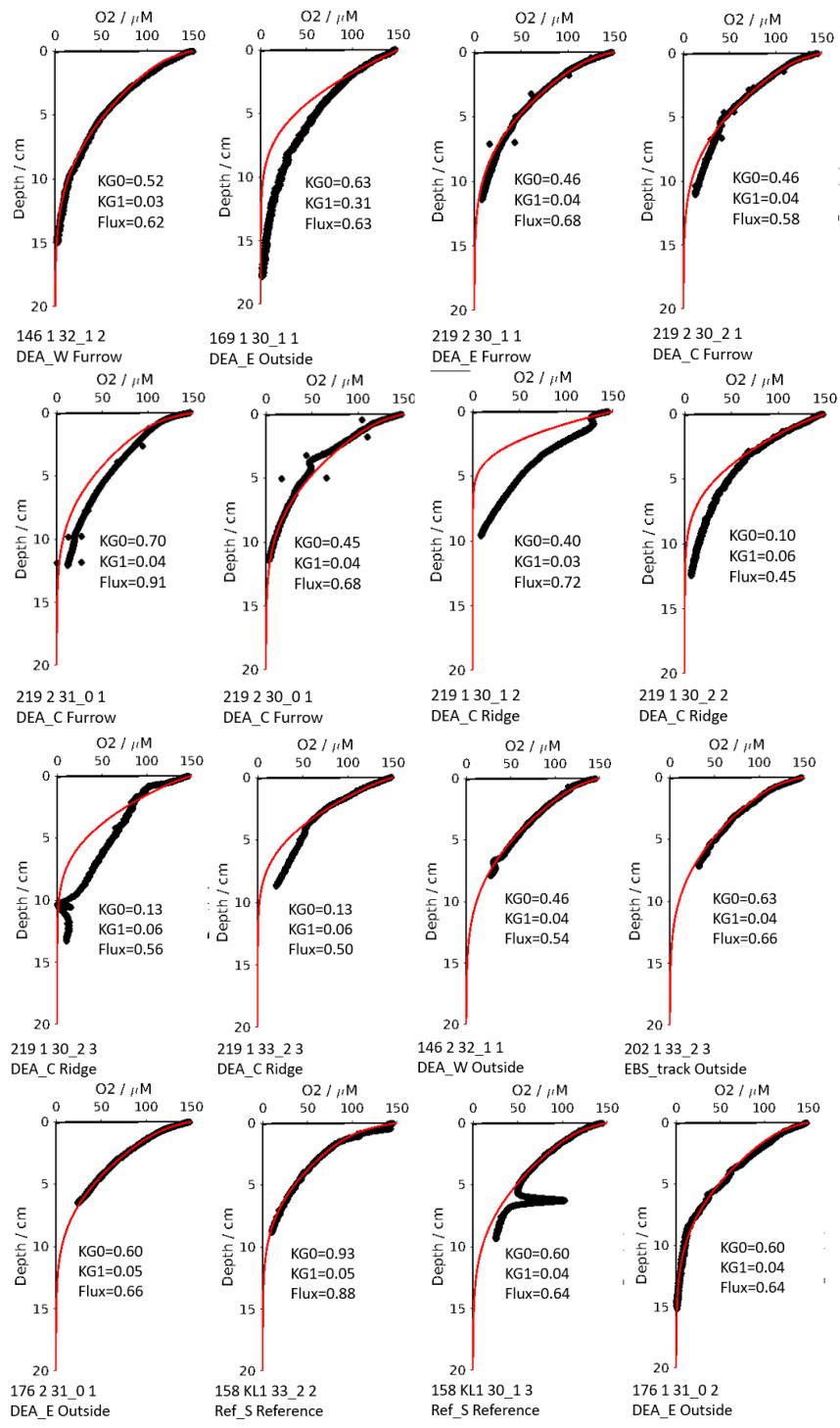


Fig S8 (continued)

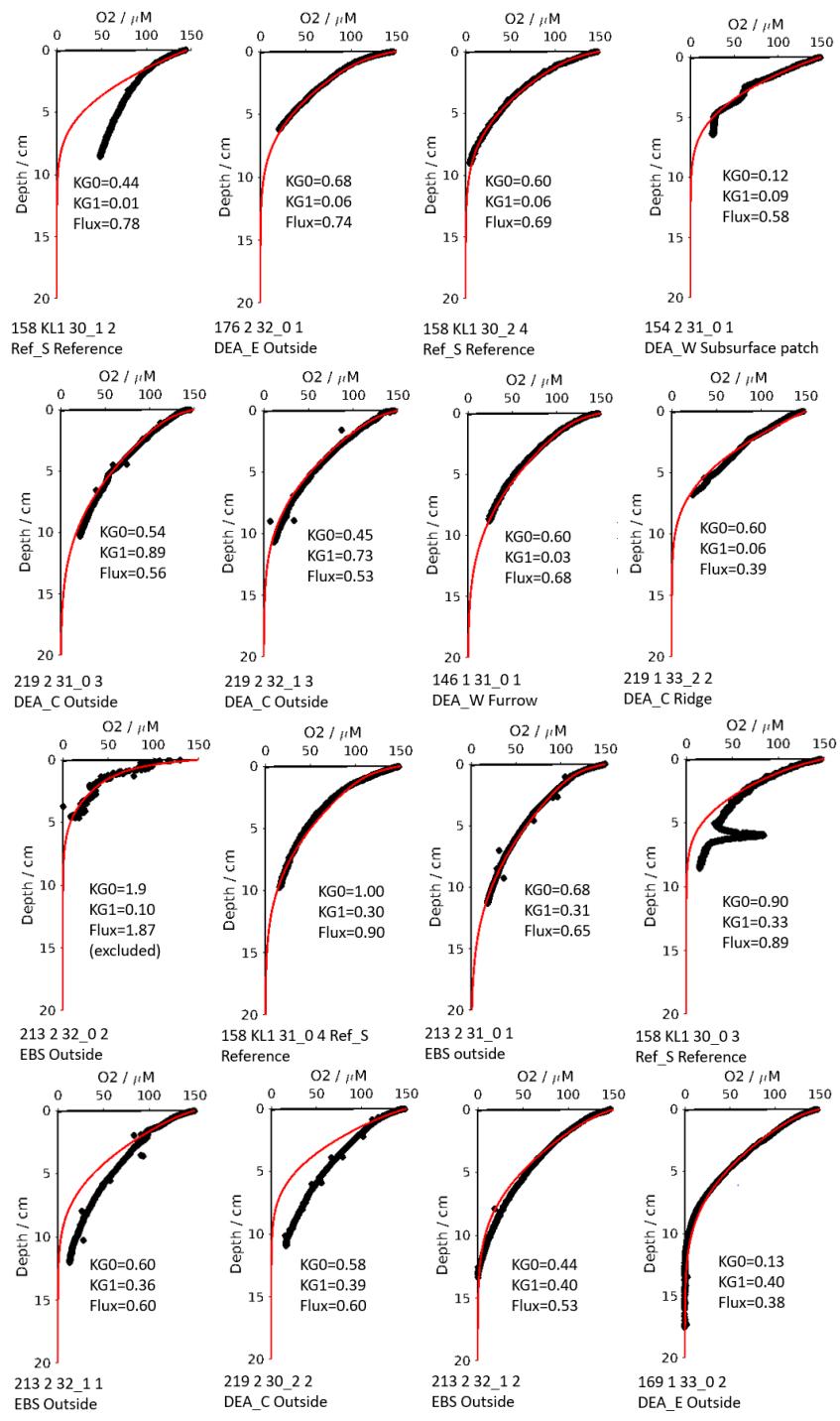


Fig S8 (continued)

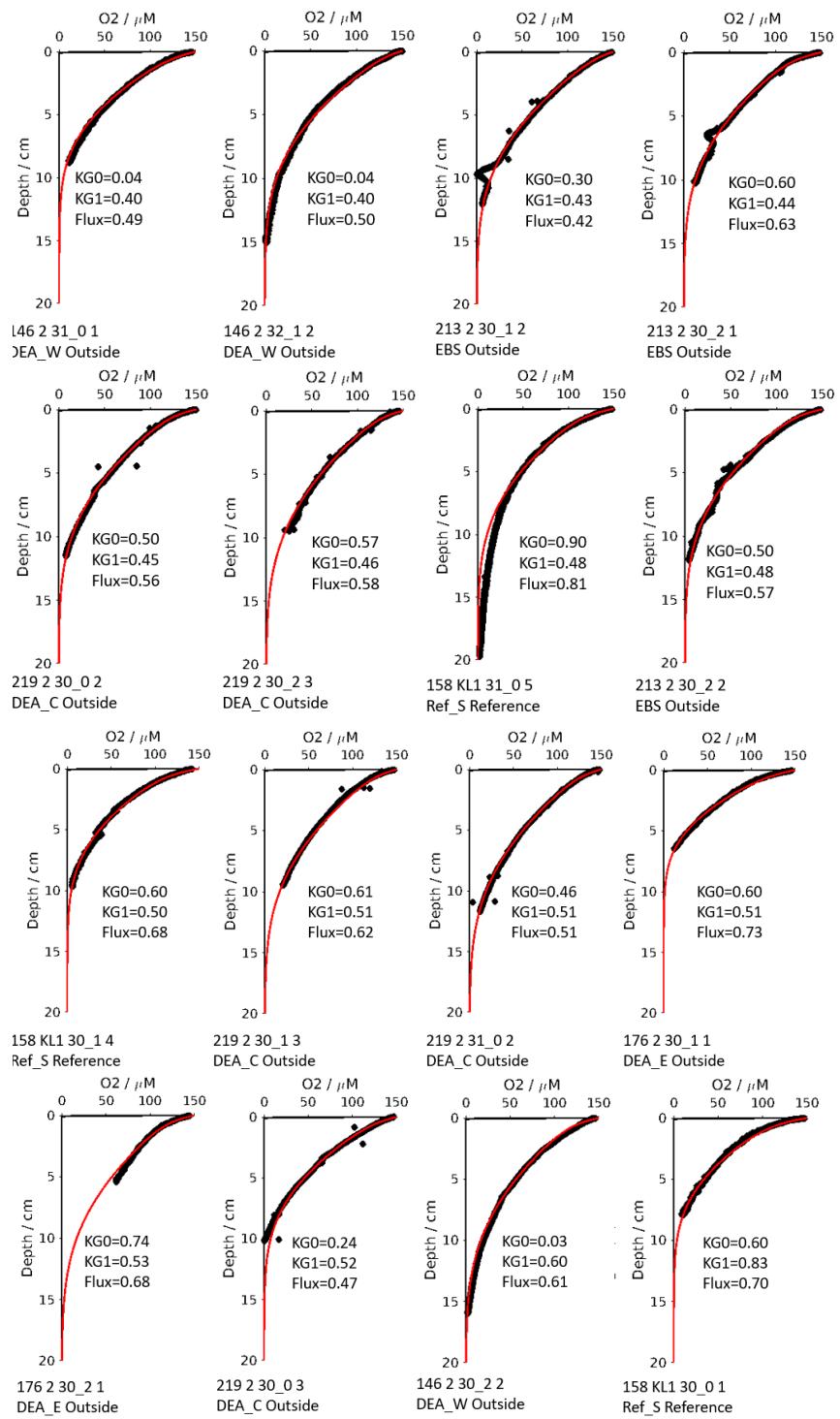


Fig S8 (continued)

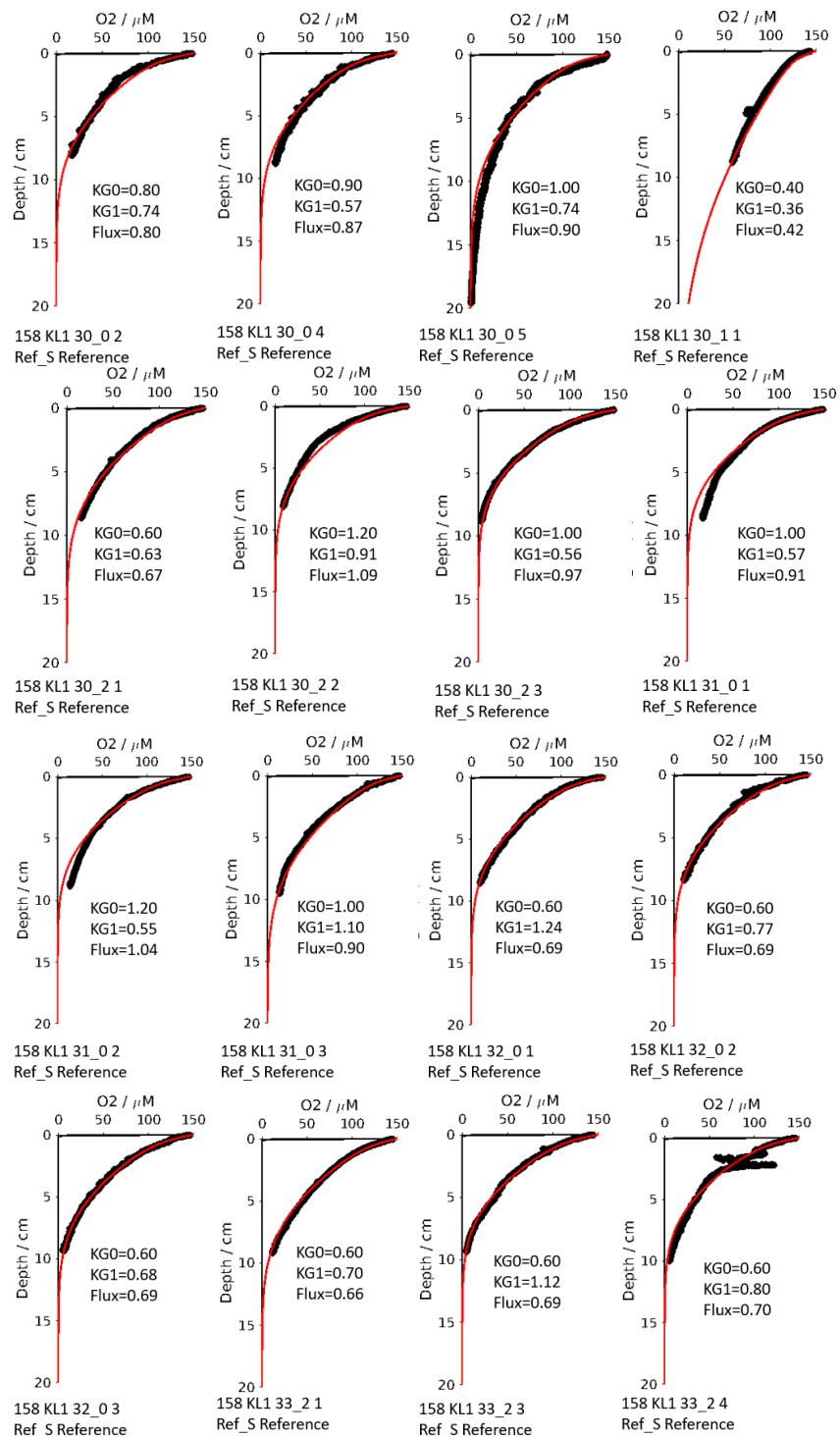


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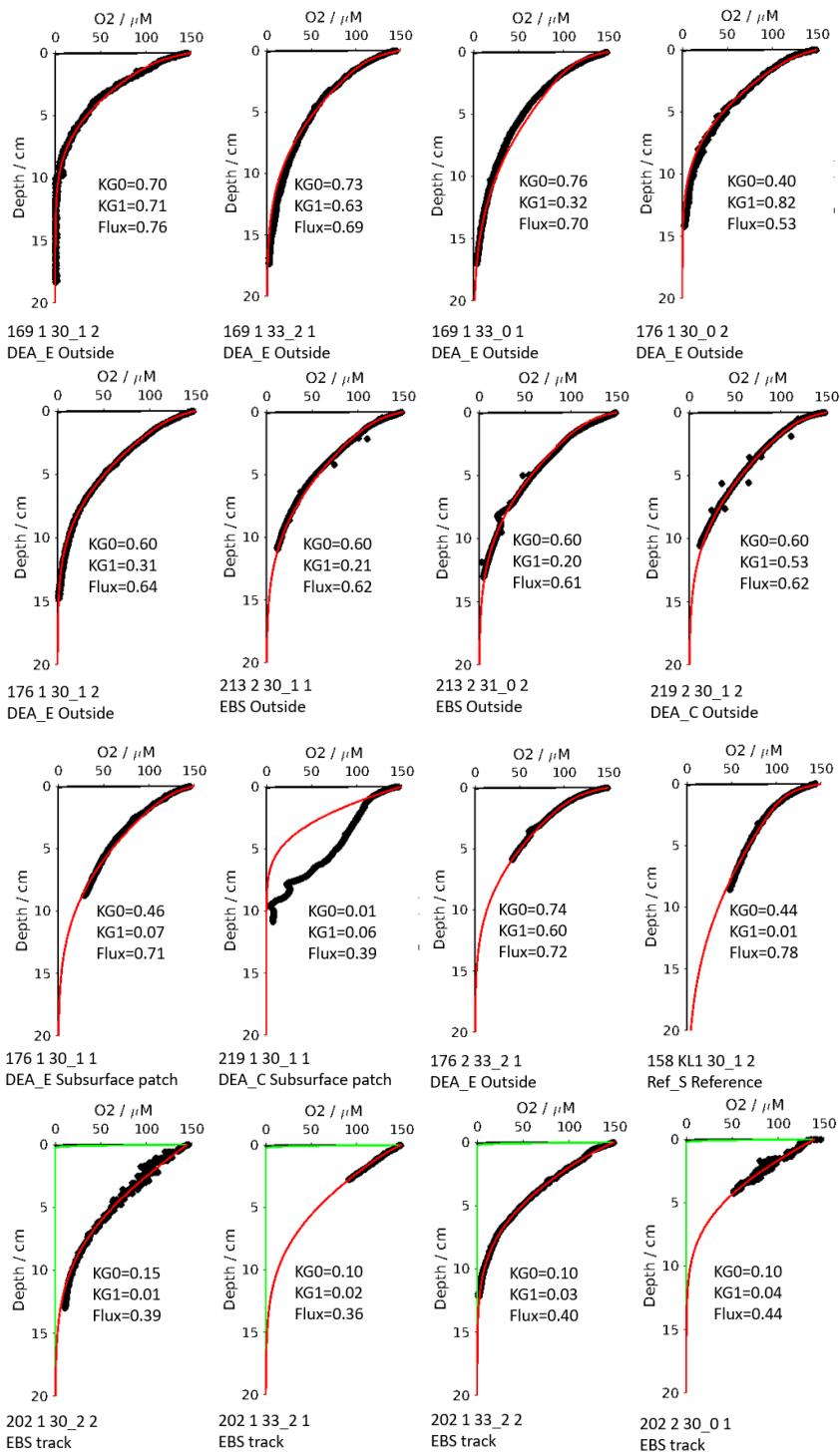


Fig S8 (continued)

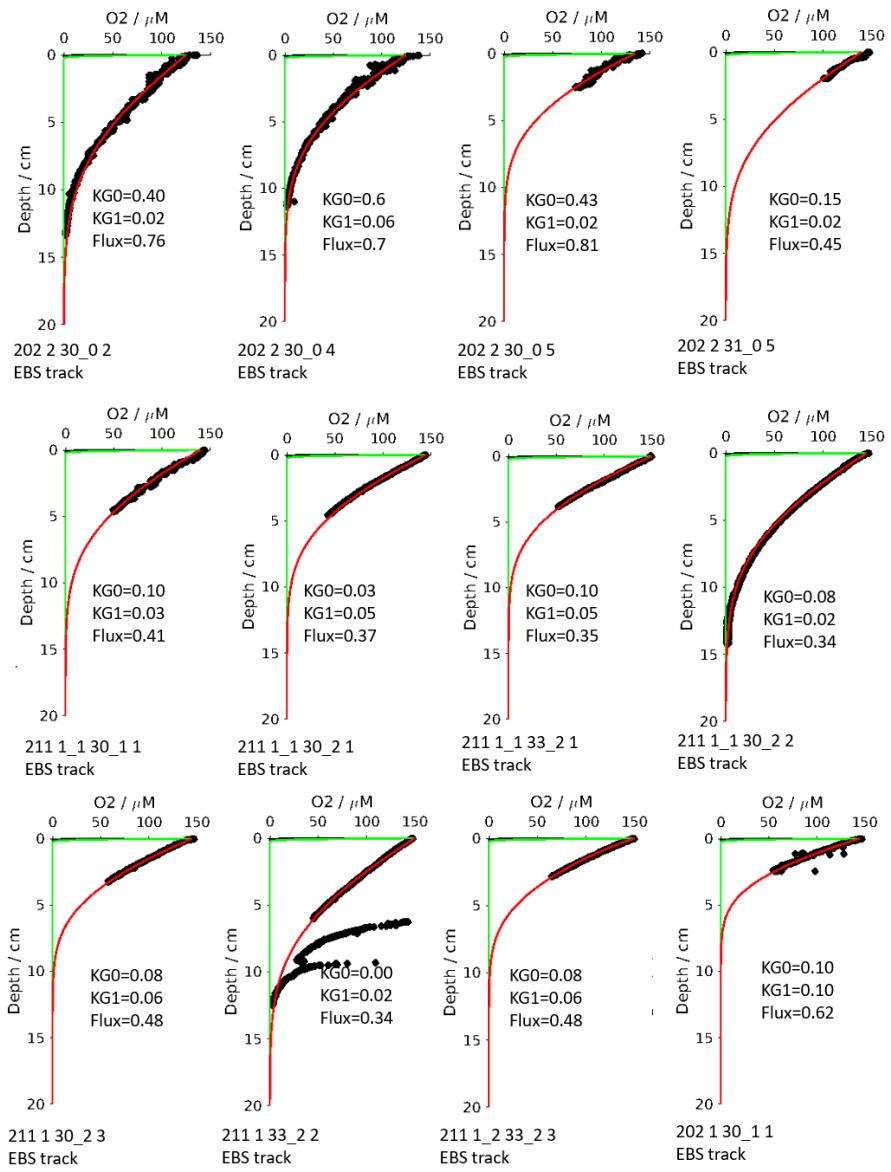


Fig S8 (continued)

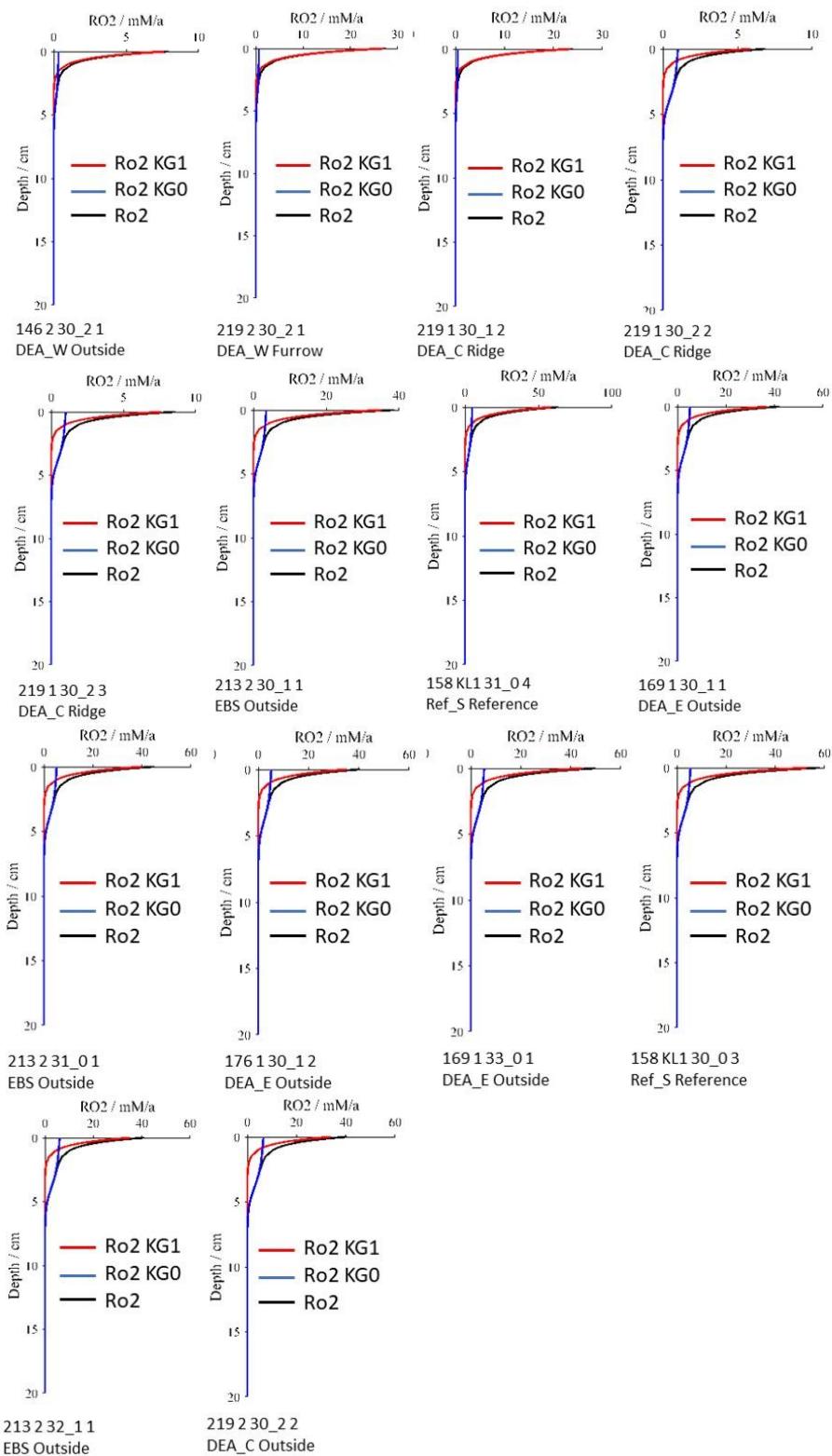


Fig S8 (continued)

Table S1 Kruskall wallis test statistics and power analyses for all tests performed.

Adjusted p values using the false discovery rate. Chi square values and number of distinct replicates. Median (Q2) and Interquartile ranges (25%: Q1, 75%: Q3) of the data used for statistical tests and not given in the main manuscript, including units. Power analyses based on the student's t test based on measured distributions estimating needed sample sizes for the differences observed between the Reference and subsurface patch, and potential differences of 25% and 50%.

p adjust	Outside	Furrow	Ridge	Subsurf patch	EBS	Subsurf
TOC/TN	0.679	0.93	0.679	0.679	0.075	0.726
cells	0.659	0.576	0.576	0.025	0.025	0.016
TIC	0.038	0.014	0.002	0.001	0.015	0
TOC	0.781	0.495	0.006	0.001	0.781	0.001
Chlorophyll a	0.452	0.275	0.001	0.001	0.275	0.001
Phaeophytin	0.926	0.926	0.305	0.656	0.926	0.279
Beta-Glucosidase	0.93	0.93	0.349	0.104	0.038	0.005
Beta-Glucosidase per cell	0.895	0.849	0.849	0.849	0.201	0.887
Chitobiase	0.825	0.825	0.602	0.245	0.038	0.038
Chitobiase per cell	0.825	0.825	0.825	0.825	0.474	0.825
Aminopeptidase	0.255	0.255	0.626	0.012	0.255	0.378
Aminopeptidase per cell	0.301	0.29	0.895	0.301	0.038	0.016
Esterase	0.825	0.643	0.022	0.001	0.022	0
Esterase per cell	0.965	0.965	0.502	0.146	0.776	0.502
DCF as DOC	0.886	0.554	0.014	0.01	0.018	NA
DCF as POC	0.352	0.056	0.012	0.01	0.033	NA
DOU	0	0.006	0.005	0.001	0	NA
brown layer	0.934	0.014	0.082	0.016	0	NA
Oxygen penetration	0.311	0.199	0.043	0.199	0.091	NA

χ^2	Outside	Furrow	Ridge	Subsurf patch	EBS	Subsurf
TOC/TN	0.56	0.01	1.22	0.56	6.23	0.27
cells	0.2	0.56	0.5	6.79	6.23	9
TIC	0.2	0.95	8.77	12.84	0.08	15.23
TOC	0.07	0.13	2.68	0.96	0.01	3.96
Chlorophyll a	0.57	1.53	12.17	12.79	1.44	12.79
Phaeophytin	0.07	0.01	1.42	3.78	6.23	11.33
Beta-Glucosidase	0.02	0.33	0.56	0.86	4.52	0.11
Beta-Glucosidase per cell	0.1	0.05	0.7	2.39	6.23	7.47
Chitobiase	0.16	0.16	0.7	0.05	3.09	0.22
Chitobiase per cell	1.88	2.01	0.24	9.61	2.79	1.01
Aminopeptidase	1.32	2.12	0.02	1.64	6.23	9
Aminopeptidase per cell	0.05	0.38	5.95	12.83	6.27	16.13
Esterase	0	0.02	1.32	5.07	0.42	1.6
Esterase per cell	8.31	0.01	0.42	2.61	1.68	0
DCF as DOC	0.02	0.59	7.68	9.60	5.59	NA
DCF as POC	0.87	4.02	8.01	9.64	5.44	NA
DOU	21.25	7.54	8.31	12.63	19.04	NA

brown layer	0.01	7.74	3.39	6.74	22.78	NA		
Oxygen penetration	1.02	2.36	6.9	1.98	4.37	NA		
n	Outside	Furrow	Ridge	Subsurf patch	EBS	Subsurf		
TOC/TN	9	9	9	9	3	9		
cells	9	9	9	9	4	5		
TIC	9	9	9	9	3	9		
TOC	9	9	9	9	3	9		
Chlorophyll a	9	9	9	9	3	9		
Phaeophytin	9	9	9	9	3	9		
Beta-Glucosidase	9	9	9	9	3	9		
Beta-Glucosidase per cell	9	9	9	9	3	9		
Chitobiase	9	9	9	9	3	9		
Chitobiase per cell	9	9	9	9	3	9		
Aminopeptidase	9	9	9	9	3	9		
Aminopeptidase per cell	9	9	9	9	3	9		
Esterase	9	9	9	9	3	9		
Esterase per cell	9	9	9	9	3	9		
DCF as DOC	7	9	9	8	3	NA		
DCF as POC	7	9	9	8	3	NA		
DOU	37	13	6	18	15	NA		
brown layer	28	20	17	18	10	NA		
Oxygen penetration	28	10	9	21	4	NA		
parameter	Reference	Outside track	Furrow	Ridge	Subsurface patches	Subsurface		
oxpen (mm bsf)	Q2 Q1 Q3	99 93 113	128 118 143	136 124 144	106 95 114	133 110 150	110 106 122	NA NA NA
brown (mm bsf)	Q2 Q1 Q3	10 12 13	9 10 11	4 5 6	3 5 6	0 0 4	0 0 0	NA NA NA
DOU (mmol m ⁻² d ⁻¹)	Q2 Q1 Q3	0.78 0.69 0.90	0.62 0.54 0.66	0.66 0.62 0.68	0.53 0.46 0.64	0.65 0.50 0.68	0.41 0.37 0.48	NA NA NA
DCF as DOC (mmol m ⁻² d ⁻¹)	Q2 Q1 Q3	0.31 0.28 0.35	0.30 0.26 0.41	0.29 0.07 0.33	0.12 0.10 0.15	0.08 0.04 0.13	0.00 0.00 0.00	NA NA NA
DCF as POC (mmol m ⁻² d ⁻¹)	Q2 Q1 Q3	0.12 0.10 0.15	0.08 0.03 0.13	0.09 0.05 0.10	0.05 0.04 0.07	0.03 0.01 0.04	0.01 0.01 0.01	NA NA NA
Beta Glucosidase (μmol L ⁻¹ h ⁻¹)	Q2 Q1 Q3	0.08 0.04 0.17	0.07 0.04 0.16	0.07 0.03 0.22	0.04 0.02 0.15	0.02 0.00 0.17	0.01 0.01 0.01	0.02 0.01 0.05
Chitobiase (μmol L ⁻¹ h ⁻¹)	Q2 Q1 Q3	0.41 0.12 1.19	0.34 0.19 0.93	0.31 0.23 0.87	0.15 0.11 0.89	0.11 0.07 0.70	0.03 0.02 0.04	0.10 0.03 0.18

Esterase ($\mu\text{mol L}^{-1} \text{ h}^{-1}$)	Q2	2.7	2.4	2.5	1.6	0.8	1.2	1.2
Aminopeptidase ($\mu\text{mol L}^{-1} \text{ h}^{-1}$)	Q1	2.1	1.9	1.6	1.5	0.6	1.05	0.8
	Q3	3.2	4.7	3.8	2.3	1.3	1.2	2.7
cell number (cells ml^{-1} wet sed.)	Q2	54	59	60	51	37	64	56
	Q1	46	50	54	38	32	62	52
	Q3	65	77	66	73	54	76	63
	Q2	3.8E+08	3.3E+08	3.2E+08	3.2E+08	2.2E+08	1.3E+08	1.1E+08
	Q1	3.0E+08	3.0E+08	2.9E+08	2.2E+08	1.7E+08	1.3E+08	1.0E+08
	Q3	4.2E+08	3.9E+08	3.9E+08	4.1E+08	2.5E+08	1.4E+08	1.2E+08

Parameter	difference	Power for n=9	n for Power=0.8	n for Power=0.9	n for Power=0.99
Chlorophyll	48 %	0.88	7	10	16
	25 %	0.37	24	32	56
	50 %	0.9	7	9	15
TOC	11 %	1	4	5	7
	25 %	1	1	2	3
	50 %	1	1	1	1
Cell numbers	41 %	0.63	13	17	29
	25 %	0.29	33	44	76
	50 %	0.79	9	12	20
Beta Glucosidase	49 %	0.25	39	52	90
	25 %	0.1	145	194	338
	50 %	0.26	37	49	85
DCF	77 %	1	4	4	7
	25 %	0.39	23	30	52
	50 %	0.92	7	8	14
DOU	22 %	0.52	16	22	37
	25 %	0.62	13	17	29
	50 %	0.99	4	5	8
oxygen penetration	20 %	0.34	27	36	63
	25 %	0.49	18	23	40
	50 %	0.97	5	7	11
Brown layer depth	84 %	1	3	3	4
	25 %	0.57	15	19	33
	50 %	0.99	5	6	9

Table S2. Test statistics for community structure analyses. Bacterial OTU turnover between different microhabitats. ANOSIM test statistics for Bray curtis dissimilarity matrices (top) and Jaccard (presence-absence) dissimilarities (bottom) between the sites, showing R values and adjusted p values (false discovery rate)

	Reference	Outside	Ridge	Subsurface	Furrow	Subsurf patch
Outside	9 %					
Ridge	9 %	10 %				
Subsurface	12 %	11 %	12 %			
Furrow	10 %	9 %	10 %	11 %		
Subsurf patch	11 %	11 %	12 %	12 %	11 %	
EBS	30 %	29 %	29 %	28 %	28 %	26 %

R (Bray curtis)						
	Outside	Reference	Ridge	Subsurface	Furrow	Subsurf patch
Outside	NA	NA	NA	NA	NA	NA
Reference	0.01	NA	NA	NA	NA	NA
Ridge	0.16	0.12	NA	NA	NA	NA
Subsurface	0.25	0.4	0.36	NA	NA	NA
Furrow	-0.01	0.06	0.21	0.17	NA	NA
Subsurf patch	0.02	0.08	0.05	0.22	0.06	NA
Subsurf patch_scratch	0.58	1	0.82	0.1	0.52	0.53

P (adjusted via false discovery rate)						
	Outside	Reference	Ridge	Subsurface	Furrow	Subsurf patch
Outside	NA	NA	NA	NA	NA	NA
Reference	0.36	NA	NA	NA	NA	NA
Ridge	0.04	0.1	NA	NA	NA	NA
Subsurface	0.08	0.03	0.03	NA	NA	NA
Furrow	0.51	0.19	0.03	0.15	NA	NA
Subsurf patch	0.26	0.19	0.23	0.09	0.21	NA
Subsurf patch_scratch	0.04	0.03	0.04	0.22	0.05	0.03

R (Jaccard)						
	outside	Reference	Ridge	Subsurface	Furrow	Subsurf patch
Outside	NA	NA	NA	NA	NA	NA
Reference	0.03	NA	NA	NA	NA	NA
Ridge	0.16	0.16	NA	NA	NA	NA
Subsurface	0.2	0.41	0.31	NA	NA	NA
Furrow	-0.04	0.06	0.24	0.18	NA	NA
Subsurf patch	-0.01	0.13	0.04	0.18	0.04	NA
Subsurf patch_scratch	0.49	0.99	0.56	0.06	0.49	0.35

P (adjusted via false discovery rate)

	outside	Reference	Ridge	Subsurface	Furrow	Subsurf patch
Outside	NA	NA	NA	NA	NA	NA
Reference	0.26	NA	NA	NA	NA	NA
Ridge	0.07	0.07	NA	NA	NA	NA
Subsurface	0.09	0.05	0.07	NA	NA	NA
Furrow	0.67	0.26	0.07	0.14	NA	NA
Subsurf patch	0.46	0.13	0.26	0.11	0.26	NA
Subsurf patch_scratch	0.07	0.05	0.07	0.27	0.07	0.07