Supplementary Data:

A.

B.

Supplementary Figure 1. A. Flow cytometric cell counts (SYBR green-stained cells) for sampled depths within Bundera sinkhole showing average of duplicate counts and standard error. B. representative images of flow cytometrically observed populations at different depths, showing that more than one discrete population were visualised at each depth and that the forward scatter characteristics of these populations differed at the examined depths.



Supplementary Figure 2. Stacked bar plots of taxonomic distribution by relative abundance at Family level showing the families that comprise over 1% of at least one of the sample replicates.

Query: ASV\_0000004 Query ID: lcl|Query\_28333 Length: 253

>ASV\_0000001

Sequence ID: Query\_28335 Length: 253

Range 1: 1 to 253

Score:324 bits(175), Expect:1e-93,

Identities:227/253(90%), Gaps:0/253(0%), Strand: Plus/Plus

Query 1 TACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGCGGCTAGA 60

 ||| |||||||||||||||||||||||||||||||||||||||||||||||| || ||||

Sbjct 1 TACTGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTAGA 60

Query 61 TCAGTCGGGTGTGAAAGCCCCGGGCTTAACCTGGGAACTGCATCCGATACTGTCTAGCTT 120

 ||||| |||||||||||||||||||| |||||||||||||||| | |||||||||||||

Sbjct 61 TCAGTTGGGTGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATTCAATACTGTCTAGCTA 120

Query 121 GAGTACAGGAGAGGAGAGTGGAATTCCACATGTAGCGGTGAAATGCGTAGATATGTGGAG 180

 ||||| | ||||| ||||||||||| ||||||||||||||||||||||| ||| ||||

Sbjct 121 GAGTATGGTAGAGGGAAGTGGAATTCCGCATGTAGCGGTGAAATGCGTAGAGATGCGGAG 180

Query 181 GAACACCAATGGCGAAGGCAGCTCTCTGGCCTGATACTGACGCTGAGGTGCGAAAGCGTG 240

 ||||| || |||||||||| || |||| | |||||||| ||||||||||||||||||

Sbjct 181 GAACATCAGTGGCGAAGGCGACTTCCTGGACCAATACTGACACTGAGGTGCGAAAGCGTG 240

Query 241 GGGAGCAAACAGG 253

 |||||||||||||

Sbjct 241 GGGAGCAAACAGG 253

Supplementary Figure 3. Nucleotide sequence alignment of ASV1 and 4 based on BLASTN.

Supplementary Table 1: Physicochemical data used to identify sampling depths of interest. Highlighting in yellow indicates depths that were selected for further analysis and microbiome sampling.



Supplementary Table 2: Physicochemical data for main sampling depths (data plotted in Figure 1). Measurements for all parameters apart from hydrogen sulphide were determined from water samples collected during the study and sent to analytical lab, while chemtet values taken on site are given for H2S.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Depth m**  | **DNA sample collection date** | **Sample collection details** | **DIC mg/L** | **DOC mg/L** | **Econd mS/m** | **Fe mg/L** | **N\_NH3 mg/L** | **N\_NO3 mg/L** | **P\_SR mg/L** | **SO4 mg/L** | **Si mg/L** | **methane mg/L** | **H2S Chemet** |
| -2 | 30/6/15 | via surface | 62 | 1.1 | 2190 | 0.032 | <0.01 | <0.02 | <0.01 | 1400 | 3.2 | <0.01 | 0.00 |
| -6 | 30/6/15 | via surface | 63 | 1.1 | 2930 | 0.044 | <0.01 | <0.02 | <0.01 | 1500 | 3.2 | <0.01 | 0.00 |
| -8 | 30/6/15 | via borehole 1 | 49 | <1.0 | 2800 | 0.09 | 0.02 | 0.05 | 0.01 | 2100 | 4.5 | <0.01 | 0.05 |
| -16 | 30/6/15 | via borehole 2 | 34 | <1.0 | 3430 | 0.15 | 0.09 | <0.02 | 0.02 | 2500 | 3.2 | 0.02 | 0.25 |
| -17 | 30/6/15 | via borehole 2 | 33 | <1.0 | 3450 | 0.059 | 0.02 | <0.02 | 0.01 | 2000 | 3 | <0.01 | 0.00 |
| -18 | 29/6/15 | via borehole 2 | 32 | <1.0 | 3250 | 0.075 | <0.01 | 0.05 | 0.01 | 1900 | 2.9 | <0.01 | 0.00 |
| -22 | 30/6/15 | via borehole 3 | 31 | <1.0 | 4740 | 0.049 | <0.01 | 0.03 | 0.01 | 2600 | 2.6 | <0.01 | 0.05 |
| -24 | 30/6/15 | via borehole 3 | 30 | <1.0 | 3770 | 0.057 | <0.01 | 0.06 | 0.01 | 2300 | 2.6 | <0.01 | 0.00 |
| -26 | 29/6/15 | via borehole 4 | 31 | <1.0 | 3340 | 0.081 | 0.02 | <0.02 | 0.02 | 2000 | 2.6 | <0.01 | 0.00 |
| -28 | 29/6/15 | via borehole 4 | 30 | 1 | 4130 | 0.076 | 0.03 | <0.02 | 0.02 | 2400 | 2.5 | <0.01 | 0.00 |

Supplementary Table 3. Student’s T-test on flow cell counts

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 2 | 6 | 8 | 16 | 17 | 18 | 22 | 24 | 26 |
| 6 | 1 | NA | NA | NA | NA | NA | NA | NA | NA |
| 8 | 0.006 | 0.002 | NA | NA | NA | NA | NA | NA | NA |
| 16 | 0.019 | 0.005 | 1 | NA | NA | NA | NA | NA | NA |
| 17 | 0.019 | 0.005 | 1 | 1 | NA | NA | NA | NA | NA |
| 18 | 0.01 | 0.003 | 1 | 1 | 1 | NA | NA | NA | NA |
| 22 | 0.003 | 0.001 | 1 | 1 | 1 | 1 | NA | NA | NA |
| 24 | 0.003 | 0.001 | 1 | 1 | 1 | 1 | 1 | NA | NA |
| 26 | 0.009 | 0.003 | 1 | 1 | 1 | 1 | 1 | 1 | NA |
| 28 | 0.006 | 0.002 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

Supplementary Table 4A: Chao1, Shannon and Simpson diversity values for all replicates.

|  |  |  |  |
| --- | --- | --- | --- |
| Depth | Chao1 | Shannon | Simpson |
| 2A | 695 | 3.039 | 0.863 |
| 2B | 809 | 3.123 | 0.868 |
| 2C | 842 | 3.144 | 0.874 |
| 6A | 625 | 2.968 | 0.839 |
| 6B | 690 | 3.1 | 0.862 |
| 6C | 671 | 3.083 | 0.859 |
| 8A | 92 | 3.417 | 0.909 |
| 8B | 1438 | 3.476 | 0.86 |
| 8C | 1279 | 3.324 | 0.844 |
| 16A | 1188 | 3.529 | 0.875 |
| 16B | 1210 | 3.6 | 0.889 |
| 16C | 1167 | 3.693 | 0.902 |
| 17A | 716 | 3.633 | 0.919 |
| 17B | 617 | 3.506 | 0.917 |
| 17C | 739 | 3.433 | 0.898 |
| 18A | 760 | 3.477 | 0.904 |
| 18B | 644 | 3.409 | 0.899 |
| 18C | 778 | 3.361 | 0.89 |
| 22A | 1474 | 3.881 | 0.931 |
| 22B | 1528 | 3.757 | 0.926 |
| 22C | 1426 | 3.775 | 0.926 |
| 24A | 1890 | 3.988 | 0.923 |
| 24B | 1886 | 4.063 | 0.932 |
| 24C | 1945 | 4.125 | 0.934 |
| 26A | 610 | 2.144 | 0.633 |
| 26B | 765 | 2.268 | 0.668 |
| 26C | 892 | 2.219 | 0.654 |
| 28A | 956 | 2.126 | 0.599 |
| 28B | 1261 | 2.04 | 0.572 |
| 28C | 986 | 2.304 | 0.645 |
| Depth | Chao1 | Shannon | Simpson |
| 2A | 695 | 3.039 | 0.863 |
| 2B | 809 | 3.123 | 0.868 |
| 2C | 842 | 3.144 | 0.874 |
| 6A | 625 | 2.968 | 0.839 |
| 6B | 690 | 3.1 | 0.862 |
| 6C | 671 | 3.083 | 0.859 |
| 8A | 92 | 3.417 | 0.909 |
| 8B | 1438 | 3.476 | 0.86 |
| 8C | 1279 | 3.324 | 0.844 |
| 16A | 1188 | 3.529 | 0.875 |
| 16B | 1210 | 3.6 | 0.889 |
| 16C | 1167 | 3.693 | 0.902 |
| 17A | 716 | 3.633 | 0.919 |
| 17B | 617 | 3.506 | 0.917 |
| 17C | 739 | 3.433 | 0.898 |
| 18A | 760 | 3.477 | 0.904 |
| 18B | 644 | 3.409 | 0.899 |
| 18C | 778 | 3.361 | 0.89 |
| 22A | 1474 | 3.881 | 0.931 |
| 22B | 1528 | 3.757 | 0.926 |
| 22C | 1426 | 3.775 | 0.926 |
| 24A | 1890 | 3.988 | 0.923 |
| 24B | 1886 | 4.063 | 0.932 |
| 24C | 1945 | 4.125 | 0.934 |
| 26A | 610 | 2.144 | 0.633 |
| 26B | 765 | 2.268 | 0.668 |
| 26C | 892 | 2.219 | 0.654 |
| 28A | 956 | 2.126 | 0.599 |
| 28B | 1261 | 2.04 | 0.572 |
| 28C | 986 | 2.304 | 0.645 |

Supplementary Table 4B. T-test support for differences in Shannon alpha diversity showing p-values adjusted by the Holm-Bonferroni correction for intergroup comparisons (3 replicates per depth). All values are rounded at three decimal places. “NA’ indicates unity where the samples are the same, or that the values are depicted in the left bottom triangle of the matrix where they are not.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Depth | 2 | 6 | 8 | 16 | 17 | 18 | 22 | 24 | 26 |
| 6 | 1 | NA | NA | NA | NA | NA | NA | NA | NA |
| 8 | 0.002 | 0 | NA | NA | NA | NA | NA | NA | NA |
| 16 | 0 | 0 | 0.057 | NA | NA | NA | NA | NA | NA |
| 17 | 0 | 0 | 0.534 | 0.889 | NA | NA | NA | NA | NA |
| 18 | 0.002 | 0 | 1 | 0.062 | 0.584 | NA | NA | NA | NA |
| 22 | 0 | 0 | 0 | 0.06 | 0.004 | 0 | NA | NA | NA |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | NA | NA |
| 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

Supplementary Table 4C. T-test support for differences in Simpson alpha diversity showing p-values adjusted by the Holm-Bonferroni correction for intergroup comparisons (3 replicates per depth). All values are rounded at three decimal places. “NA’ indicates unity where the samples are the same, or that the values are depicted in the left bottom triangle of the matrix where they are not.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 2 | 6 | 8 | 16 | 17 | 18 | 22 | 24 | 26 |
| 6 | 1 | NA | NA | NA | NA | NA | NA | NA | NA |
| 8 | 1 | 1 | NA | NA | NA | NA | NA | NA | NA |
| 16 | 1 | 0.471 | 1 | NA | NA | NA | NA | NA | NA |
| 17 | 0.203 | 0.023 | 0.263 | 1 | NA | NA | NA | NA | NA |
| 18 | 0.901 | 0.168 | 1 | 1 | 1 | NA | NA | NA | NA |
| 22 | 0.021 | 0.002 | 0.028 | 0.282 | 1 | 0.804 | NA | NA | NA |
| 24 | 0.017 | 0.002 | 0.023 | 0.247 | 1 | 0.69 | 1 | NA | NA |
| 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.135 |
| Depth | 2 | 6 | 8 | 16 | 17 | 18 | 22 | 24 | 26 |
| 6 | 1 | NA | NA | NA | NA | NA | NA | NA | NA |
| 8 | 1 | 1 | NA | NA | NA | NA | NA | NA | NA |
| 16 | 1 | 0.471 | 1 | NA | NA | NA | NA | NA | NA |
| 17 | 0.203 | 0.023 | 0.263 | 1 | NA | NA | NA | NA | NA |
| 18 | 0.901 | 0.168 | 1 | 1 | 1 | NA | NA | NA | NA |
| 22 | 0.021 | 0.002 | 0.028 | 0.282 | 1 | 0.804 | NA | NA | NA |
| 24 | 0.017 | 0.002 | 0.023 | 0.247 | 1 | 0.69 | 1 | NA | NA |
| 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.135 |

Supplementary Table 4D. T-test support for differences in Chao1 alpha diversity showing p-values adjusted by the Holm-Bonferroni correction for intergroup comparisons (3 replicates per depth). All values are rounded at three decimal places. “NA’ indicates unity where the samples are the same, or that the values are depicted in the left bottom triangle of the matrix where they are not.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Depth | 2 | 6 | 8 | 16 | 17 | 18 | 22 | 24 | 26 |
| 6 | 1 | NA | NA | NA | NA | NA | NA | NA | NA |
| 8 | 1 | 1 | NA | NA | NA | NA | NA | NA | NA |
| 16 | 1 | 0.519 | 1 | NA | NA | NA | NA | NA | NA |
| 17 | 1 | 1 | 1 | 0.682 | NA | NA | NA | NA | NA |
| 18 | 1 | 1 | 1 | 0.965 | 1 | NA | NA | NA | NA |
| 22 | 0.085 | 0.025 | 0.464 | 1 | 0.033 | 0.05 | NA | NA | NA |
| 24 | 0.001 | 0 | 0.004 | 0.067 | 0 | 0 | 1 | NA | NA |
| 26 | 1 | 1 | 1 | 1 | 1 | 1 | 0.067 | 0.001 | NA |
| 28 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0.019 | 1 |

Supplementary Table 5: PERMANOVA on groups inferred from beta diversity visualisation utilising Bray Curtis distance with the pairwise.adonis function using the vegan function adonis to calculate inter-group differences in a pairwise fashion. P.adjusted p-values used Bonferroni correction.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Pairs | Df | SumsOfSqs | F.Model | R2 | p.value | p.adjusted | sig |
| 8\_vs\_2\_6 | 1 | 1.7154286 | 359.42576 | 0.9808965 | 0.004 | 0.0241 | . |
| 8\_vs\_26\_28 | 1 | 1.8391562 | 186.40602 | 0.9638067 | 0.018 | 0.1082 |  |
| 8\_vs\_16\_17\_18\_22\_24 | 1 | 1.8849839 | 33.69994 | 0.6780681 | 0.002 | 0.0123 | . |
| 2\_6\_vs\_26\_28 | 1 | 2.9323664 | 717.63227 | 0.9862568 | 0.003 | 0.0184 | . |
| 2\_6\_vs\_16\_17\_18\_22\_24 | 1 | 3.9535490 | 86.66601 | 0.8201882 | 0.001 | 0.0065 | \* |
| 26\_28\_vs\_16\_17\_18\_22\_24 | 1 | 0.9479592 | 19.95920 | 0.5123103 | 0.001 | 0.0066 | \* |

Supplementary Table 6A. Relative abundance (%) of Archaea and Bacteria



Supplementary Table 6B. Relative abundance (%) at class level for Proteobacteria, phyla level for all others (> 0.01 %)



Supplementary Table 6C. Family relative abundances. Sample names comprise depth sample was taken and replicate. ASV number represents the most abundant ASV classified at that taxonomic level, all other ASVs contributions are aggregated to this family designation. File called Supplementary\_Table\_6C.tsv (attached).

Supplementary Table 6D. ASV relative abundances. Sample names comprise depth sample was taken and replicate. File called Supplementary\_Table\_6D.tsv (attached).

Supplementary Table 7. Relative abundance percentages from Tax4Fun2 used to generate Figure 7, with averages and standard deviation.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Pathway | 2m | 6m | 8m | 16m | 17m | 18m | 22m | 24m | 26m | 28m | Average | St.Dev |
| Pentose phosphate pathway | 5.29 | 5.38 | 4.22 | 3.36 | 3.54 | 4.23 | 4.51 | 4.51 | 4.05 | 4.3 | 4.34 | 0.61 |
| Entner-Doudoroff pathway | 1.76 | 1.73 | 0 | 0.7 | 0 | 0 | 0 | 0.75 | 1.38 | 1.85 | 0.94 | 0.63 |
| Ascorbate degradation | 0.56 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.14 | 0.22 |
| Ethylmalonyl pathway | 10.54 | 9.56 | 2.6 | 6.84 | 4.85 | 5.6 | 5.23 | 5.69 | 8.56 | 9.63 | 6.91 | 2.43 |
| Methylaspartate cycle | 3.01 | 3.2 | 3.67 | 3.65 | 3.85 | 5.39 | 5.63 | 4.79 | 3.44 | 3.29 | 3.99 | 0.89 |
| Reductive pentose phosphate cycle  | 5.61 | 5.98 | 7.85 | 4.59 | 4.27 | 5.2 | 5.67 | 6.02 | 4.69 | 4.94 | 5.48 | 0.97 |
| Reductive citrate cycle | 11.34 | 11.91 | 13.88 | 12.22 | 13.47 | 14.02 | 15.02 | 14.43 | 11.08 | 10.66 | 12.8 | 1.47 |
| 3-Hydroxypropionate bi-cycle | 7.72 | 7.82 | 7.53 | 7.07 | 6.04 | 7.74 | 8 | 8.13 | 7.54 | 8.11 | 7.57 | 0.59 |
| Hydroxypropionate-hydroxybutylate cycle | 5.74 | 5.09 | 3.13 | 5.33 | 5.28 | 7.5 | 7.27 | 5.61 | 5.64 | 5.51 | 5.61 | 1.14 |
| Dicarboxylate-hydroxybutyrate cycle | 9.59 | 9.29 | 10 | 8.24 | 8.43 | 9.85 | 10.13 | 9.59 | 8.52 | 8.41 | 9.21 | 0.69 |
| Reductive acetyl-CoA pathway | 0 | 1.62 | 1.51 | 1.87 | 2.34 | 3.53 | 1.18 | 1.39 | 1.59 | 2.46 | 1.75 | 0.87 |
| Phosphate acetyltransferase-acetate kinase pathway | 0.56 | 0.57 | 0 | 0 | 0 | 0 | 0 | 0.54 | 0 | 0.5 | 0.41 | 0.15 |
| Methanogenesis (CO2) | 0.82 | 0.8 | 0 | 3.29 | 4.8 | 0.95 | 0.85 | 0.75 | 2.07 | 1.17 | 1.58 | 1.34 |
| Methanogenesis (acetate) | 3.09 | 3.05 | 2.58 | 5.39 | 7.03 | 3.42 | 3.44 | 3.2 | 4.1 | 3.09 | 3.84 | 1.29 |
| Methanogenesis (methanol) | 0 | 0 | 0 | 2.34 | 3.88 | 0 | 0 | 0 | 1.52 | 0.77 | 0.97 | 1.2 |
| Methanogenesis (methylamine) | 0 | 0 | 0 | 2.71 | 4.18 | 0 | 0 | 0 | 1.64 | 0.87 | 1.1 | 1.29 |
| Methane oxidation | 0 | 0 | 0.52 | 1.12 | 1.15 | 2.06 | 2.03 | 1.27 | 0.62 | 0 | 0.93 | 0.68 |
| Serine pathway | 3.89 | 4.12 | 4.4 | 3.71 | 3.54 | 4.78 | 4.91 | 4.42 | 3.41 | 3.21 | 4.04 | 0.55 |
| Ribulose monophosphate pathway | 0.83 | 0.83 | 0.78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.49 | 0.23 |
| Xylulose monophosphate pathway | 0.51 | 0.53 | 0.72 | 0 | 0 | 0 | 0 | 0.54 | 0.56 | 0.76 | 0.47 | 0.19 |
| Assimilatory nitrate reduction | 0 | 0 | 1.4 | 0.68 | 0.57 | 0 | 0 | 0.57 | 0.9 | 0.99 | 0.64 | 0.35 |
| Dissimilatory nitrate reduction | 0.52 | 0.54 | 4.82 | 1.53 | 1.11 | 0.57 | 0.66 | 1.7 | 1.53 | 1.67 | 1.47 | 1.21 |
| Denitrification | 0.53 | 0.6 | 6.23 | 1.79 | 2.02 | 3.16 | 3.23 | 3.06 | 1.63 | 1.41 | 2.37 | 1.59 |
| Nitrification | 0 | 0 | 0.51 | 0.72 | 1.11 | 2 | 1.98 | 1.14 | 0 | 0 | 0.8 | 0.71 |
| Complete nitrification | 0 | 0 | 1.87 | 1.11 | 1.34 | 2.15 | 2.15 | 1.58 | 0.79 | 0.55 | 1.17 | 0.75 |
| Assimilatory sulfate reduction | 2.44 | 2.46 | 2.66 | 3.57 | 3.23 | 4.08 | 4.3 | 4.05 | 3.32 | 3.51 | 3.36 | 0.64 |
| Dissimilatory sulfate reduction | 0.61 | 0.66 | 3.85 | 1.36 | 1.54 | 1.72 | 1.85 | 1.99 | 1.26 | 1.14 | 1.6 | 0.87 |
| Thiosulfate oxidation | 0.69 | 0.76 | 7.59 | 1.07 | 0 | 0.56 | 0.72 | 1.89 | 1.08 | 1.23 | 1.6 | 2.03 |
| Ectoine biosynthesis | 1.9 | 2.1 | 2.69 | 2.66 | 2.9 | 4.37 | 4.49 | 3.44 | 2.11 | 1.73 | 2.84 | 0.93 |
| Homoprotocatechuate degradation | 2.09 | 2.03 | 0 | 1 | 0 | 0 | 0 | 0.66 | 1.49 | 1.95 | 1.05 | 0.73 |
| Trans-cinnamate degradation | 5.93 | 5.12 | 1.17 | 2.21 | 1.83 | 2.53 | 2.38 | 2.16 | 2.97 | 3 | 2.93 | 1.4 |
| Nicotinate degradation | 1.15 | 1.34 | 0 | 1.03 | 0 | 0 | 0 | 0 | 0.79 | 0.78 | 0.65 | 0.4 |
| Toluene degradation | 0.86 | 0.74 | 0 | 1.85 | 1.88 | 0 | 0 | 0.57 | 1.51 | 1.43 | 0.95 | 0.63 |
| Xylene degradation | 0.61 | 0.54 | 0 | 0 | 0 | 0 | 0 | 0 | 0.72 | 0.89 | 0.38 | 0.27 |
| Benzene degradation | 0.87 | 0.77 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.3 | 0.28 |
| Benzoate degradation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.91 | 1.09 | 0.41 | 0.32 |
| Catechol ortho-cleavage | 1.75 | 1.57 | 0 | 0.63 | 0 | 0 | 0 | 0.54 | 1.44 | 1.69 | 0.87 | 0.62 |
| Catechol meta-cleavage | 3.82 | 3.43 | 0.69 | 2.72 | 1.29 | 1.24 | 1.14 | 1.75 | 2.93 | 3.42 | 2.24 | 1.09 |
| Biphenyl degradation | 0.72 | 0.69 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.21 | 0.26 |
| Benzoate degradation | 1.57 | 1.33 | 0 | 0 | 0 | 0 | 0 | 0 | 0.95 | 1.11 | 0.65 | 0.51 |
| Naphthalene degradation | 0.5 | 0.52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.21 | 0.16 |
| Phthalate degradation | 0.62 | 0.71 | 0 | 0 | 0 | 0 | 0 | 0 | 0.53 | 0.56 | 0.3 | 0.26 |

Supplementary Table 8. Bioenv results for comparisons of physicochemical parameters. Best model is in bold. Also shown are PERMANOVA for the physicochemical parameters versus community structure.

 size correlation

DIC 1 0.8697

DIC P\_SR 2 0.8823

DIC ECond P\_SR 3 0.8775

DIC DOC P\_SR Si 4 0.8882

DIC DOC ECond P\_SR Si 5 0.8912

**DIC DOC ECond N\_NO3 P\_SR Si 6 0.9086**

DIC DOC ECond N\_NO3 P\_SR SO4 Si 7 0.9017

DIC DOC ECond Fe N\_NO3 P\_SR SO4 Si 8 0.8624

DIC DOC ECond Fe N\_NH3 N\_NO3 P\_SR SO4 Si 9 0.7582

DIC DOC ECond Fe N\_NH3 N\_NO3 P\_SR SO4 Si H2S 10 0.6157

DIC DOC ECond Fe N\_NH3 N\_NO3 P\_SR SO4 Si H2S methane 11 0.5518

PERMANOVA results from pairwise.adonis2 function for comparisons of physicochemical parameters by depth, p-values adjusted by Bonferroni method.

 Df SumOfSqs R2 F Pr(>F)

DIC 1 4.2919 0.55471 1078.368 0.001 \*\*\*

DOC 1 0.8455 0.10928 212.442 0.001 \*\*\*

ECond 1 0.3681 0.04758 92.494 0.001 \*\*\*

Fe 1 0.2954 0.03818 74.226 0.001 \*\*\*

N\_NH3 1 0.3276 0.04234 82.302 0.001 \*\*\*

N\_NO3 1 0.4002 0.05172 100.545 0.001 \*\*\*

P\_SR 1 0.6903 0.08922 173.449 0.001 \*\*\*

SO4 1 0.1028 0.01329 25.833 0.001 \*\*\*

Si 1 0.3358 0.04340 84.365 0.001 \*\*\*

H2S 1 0.3526 0.04557 88.585 0.001 \*\*\*

methane 1 0.5776 0.07465 145.126 0.001 \*\*\*

Residual 20 0.0796 0.01029

Total 29 7.7372 1.00000

---

Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1