# Supplementary Material

**for**

# Metagenomic Insights Into Microbial Controls of Carbon Cycling in Alpine Soils

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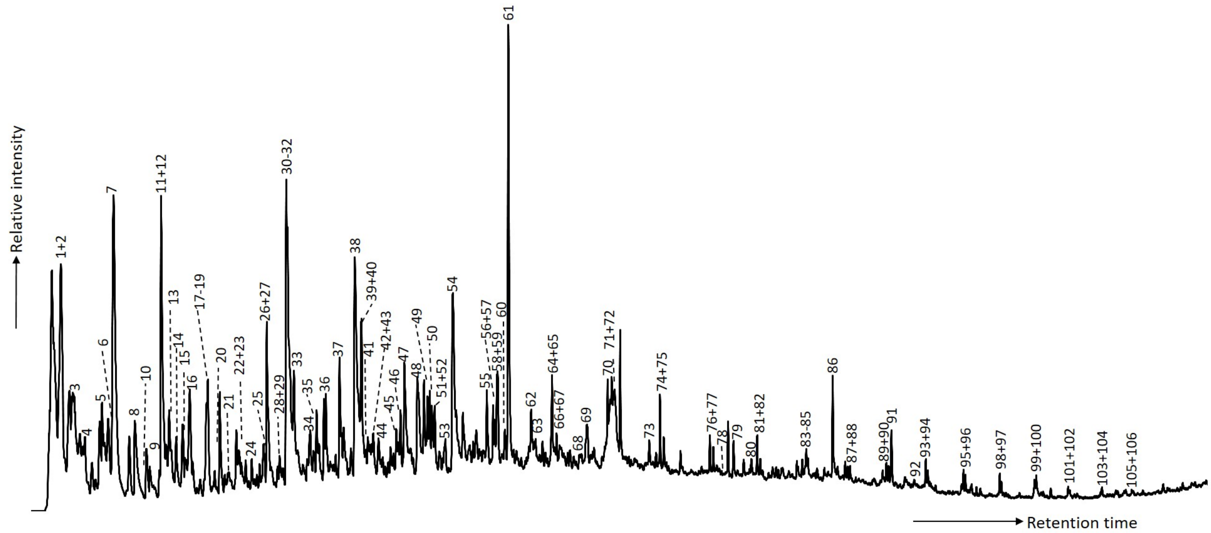


Figure S1: Example pyrogramm from pyrolysis gas chromatography–mass spectrometry analysis.

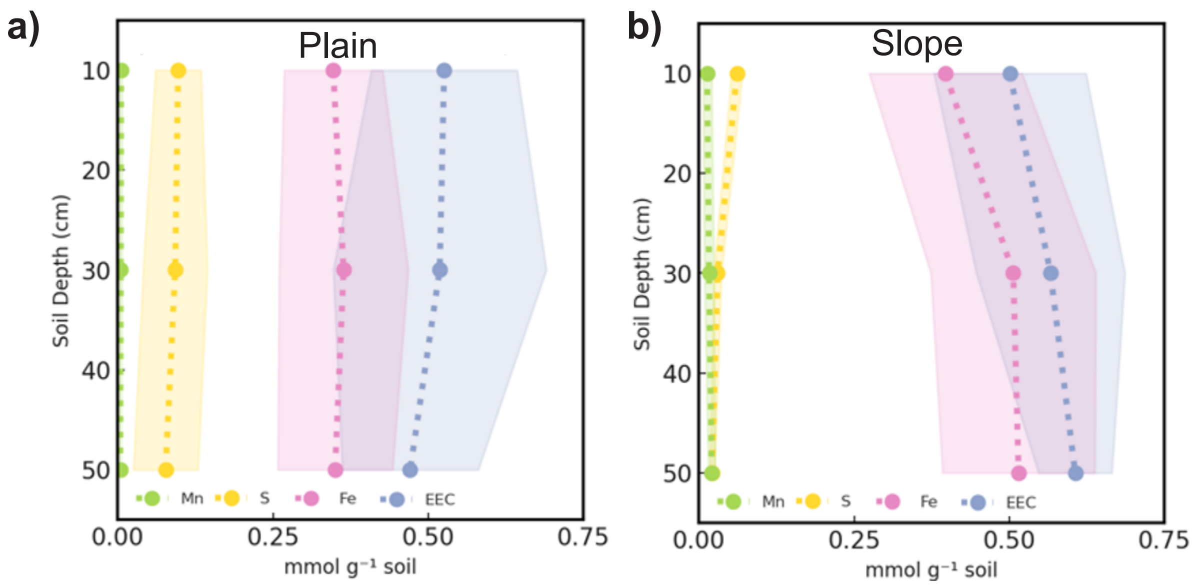


Figure S2: Electron exchanging capacity (EEC) and exchange capacities for iron, sulfur, and manganese. EEC values were determined from the sum of electron donating and accepting capacity. Element-specific exchange capacities were calculated from elemental concentrations, assuming one electron exchange per atom. Shaded areas represent the standard error of the mean. Each data point reflects the average of five soil samples.

Table S1: Soil physicochemical properties of the studied samples, including sampling depth (A = 0-10 cm, B = 10-30 cm, C = 30-50 cm), geographic coordinates, total C and N contents, texture fractions (clay, silt, sand), pH, and total Fe, Mn, S and Ca concentrations.

| **Name** | **Depth** | **X coordinate** | **Y coordinate** | **C** (%) | **N** (%) | **Clay** (%) | **Silt** (%) | **Sand** (%) | **pH** | **Fe** (µg/g) | **Mn** (µg/g) | **S** (µg/g) | **Ca** (µg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BF21 | A | 2664657 | 1136886 | 0.85 | 0.05 | 0.99 | 27.41 | 71.59 | 7.12 | 22681.88 | 453.64 | 447.31 | 52168.33 |
| BF21 | B | 2664657 | 1136886 | 1.97 | 0.12 | 1.50 | 41.86 | 56.64 | 7.28 | 26426.30 | 487.16 | 568.58 | 32066.74 |
| BF21 | C | 2664657 | 1136886 | 0.46 | 0.03 | 0.76 | 20.26 | 78.98 | 6.91 | 24476.06 | 609.95 | 350.95 | 45242.11 |
| BF26 | A | 2664636 | 1136854 | 1.05 | 0.05 | 1.41 | 38.95 | 59.63 | 7.54 | 26888.50 | 429.49 | 476.00 | 24799.30 |
| BF26 | B | 2664636 | 1136854 | 0.46 | 0.00 | 0.76 | 23.94 | 75.31 | 7.51 | 29581.46 | 536.06 | 353.19 | 13953.14 |
| BF26 | C | 2664636 | 1136854 | 0.18 | 0.00 | 0.99 | 24.72 | 74.30 | 7.79 | 23723.51 | 561.49 | 118.67 | 24174.14 |
| BS18 | A | 2664534 | 1137140 | 4.39 | 0.29 | 2.92 | 29.40 | 67.69 | 4.85 | 26674.56 | 522.83 | 1025.16 | 7360.88 |
| BS18 | B | 2664534 | 1137140 | 2.83 | 0.17 | 3.52 | 30.20 | 66.27 | 5.02 | 29746.87 | 919.81 | 724.24 | 7098.25 |
| BS18 | C | 2664534 | 1137140 | 1.68 | 0.10 | 4.95 | 36.56 | 58.49 | 5.22 | 31243.00 | 1293.76 | 512.53 | 6835.10 |
| BS21 | A | 2664590 | 1137072 | 3.81 | 0.27 | 1.96 | 26.52 | 71.53 | 5.21 | 20716.48 | 627.96 | 1088.37 | 7492.29 |
| BS21 | B | 2664590 | 1137072 | 2.70 | 0.21 | 3.39 | 34.58 | 62.03 | 5.02 | 25369.96 | 727.88 | 965.27 | 6729.88 |
| RF20 | A | 2605594 | 1116454 | 15.77 | 1.12 | 3.65 | 60.92 | 35.43 | 6.11 | 32182.22 | 2536.16 | 2882.33 | 10888.34 |
| RF20 | B | 2605594 | 1116454 | 3.38 | 0.26 | 3.14 | 76.22 | 20.64 | 6.52 | 30217.41 | 328.05 | 1541.43 | 5291.61 |
| RF20 | C | 2605594 | 1116454 | 1.13 | 0.09 | 3.97 | 69.80 | 26.22 | 6.63 | 41011.59 | 263.59 | 1675.46 | 4032.77 |
| RF24 | A | 2605579 | 1116699 | 7.33 | 0.57 | 3.71 | 80.98 | 15.32 | 5.59 | 42917.84 | 354.12 | 955.65 | 5006.22 |
| RF24 | B | 2605579 | 1116699 | 1.39 | 0.14 | 3.13 | 67.92 | 28.95 | 6.32 | 33530.86 | 426.83 | 606.04 | 3629.57 |
| RF24 | C | 2605579 | 1116699 | 0.53 | 0.04 | 3.85 | 56.51 | 39.64 | 7.01 | 46108.15 | 432.28 | 239.52 | 2336.01 |
| RS13 | A | 2605229 | 1116612 | 3.58 | 0.28 | 3.40 | 50.91 | 45.69 | 6.85 | 35590.51 | 299.27 | 1048.57 | 4279.37 |
| RS13 | B | 2605229 | 1116612 | 2.49 | 0.24 | 4.87 | 59.32 | 35.81 | 6.89 | 41163.72 | 2543.83 | 916.54 | 5306.71 |
| RS13 | C | 2605229 | 1116612 | 1.97 | 0.20 | 4.53 | 60.27 | 35.20 | 6.96 | 39876.05 | 992.94 | 1005.23 | 4996.69 |
| RS22 | A | 2605273 | 1116750 | 4.68 | 0.32 | 5.35 | 55.86 | 38.79 | 5.23 | 37034.96 | 848.14 | 994.31 | 2835.53 |
| RS22 | B | 2605273 | 1116750 | 1.49 | 0.12 | 3.88 | 47.78 | 48.34 | 6.47 | 34777.78 | 838.54 | 442.37 | 3028.28 |

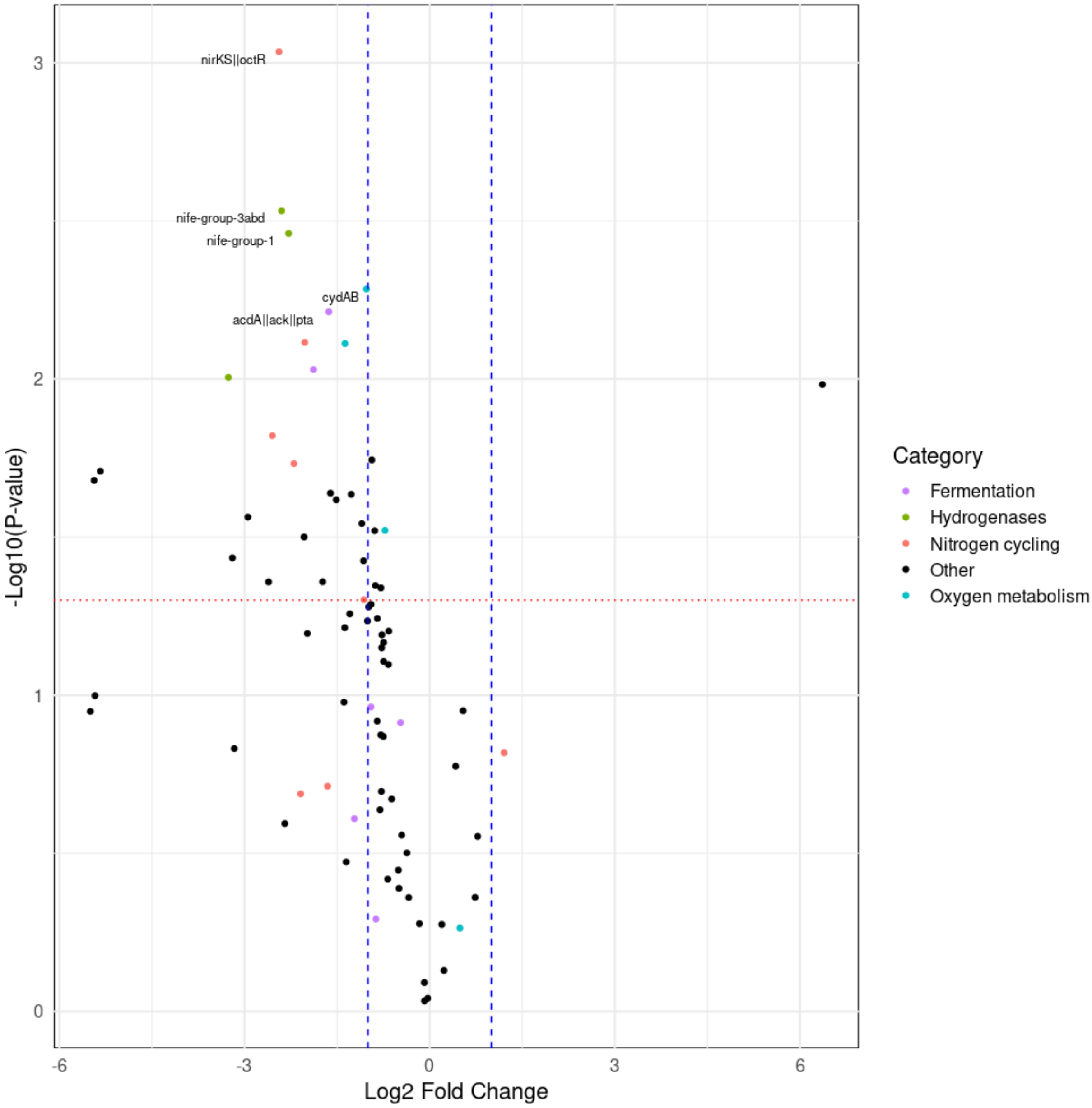


Figure S3: Volcano plot showing differential gene expression for metabolic pathways between plain (left) and slope soils (right).

Table S2: List of pyrolysis gas chromatography–mass spectrometry moieties found in the studied soils, containing peak number (as related to Figure S1), retention time, compound class, molecular weight and masses used quantification. [a] ncc = nitrogen containing compounds

| **Pyrolysis moiety** | **Peak #** | **Retention time (min)** | **Compound class[a]** | **Molecular weight** | **Masses** |
|---|---|---|---|---|---|
| 2-methylfurnan | 1 | 2.4 | polysaccharides | 82 | 53+82 |
| acetic acid | 2 | 2.4 | polysaccharides | 60 | 60 |
| benzene | 3 | 3.0 | aromatics | 78 | 77+78 |
| (1H)-pyrrole, dimethyl | 4 | 3.4 | ncc | 96 | 95+96 |
| Pyridine | 5 | 4.2 | ncc | 79 | 52+79 |
| Pyrrole | 6 | 4.5 | ncc | 67 | 67 |
| toluene | 7 | 4.7 | aromatics | 92 | 92+91 |
| (2H)-furan-3-one | 8 | 5.7 | polysaccharides | 84 | 54+84 |
| 3 furaldehyde | 9 | 6.2 | polysaccharides | 96 | 95+96 |
| methylpyridine | 10 | 6.4 | ncc | 93 | 66+93 |
| cyclopenten-1-one | 11 | 6.8 | polysaccharides | 82 | 82+54 |
| Furfural | 12 | 6.9 | polysaccharides | 96 | 95+96 |
| methyl-1H-pyrrole | 13 | 7.2 | ncc | 81 | 80+81 |
| methyl-1H-pyrrole | 14 | 7.5 | ncc | 81 | 80+81 |
| C2 bezene (xylene) | 15 | 7.8 | aromatics | 106 | 106+91 |
| C2 bezene (xylene) | 16 | 8.1 | aromatics | 106 | 106+91 |
| styrene | 17 | 8.9 | aromatics | 104 | 104+78 |
| C2 bezene (xylene) | 18 | 9.0 | aromatics | 106 | 106+91 |
| C9 alkene | 19 | 9.0 | lipids | 126 | 55+69 |
| C9 alkane | 20 | 9.3 | lipids | 128 | 57+71 |
| acetylfuran | 21 | 9.7 | polysaccharides | 110 | 110+95 |

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Table S2: (cont.)

| **Pyrolysis moiety** | **Peak #** | **Retention time (min)** | **Compound class**[a] | **Molecular weight** | **Masses** |
|---|---|---|---|---|---|
| 2hydroxy-2-cyclopenten-1-one | 22 | 10.3 | polysaccharides | 98 | 98+55 |
| dimethylpyridine | 23 | 10.5 | ncc | 107 | 106+107 |
| propylbenzene | 24 | 11.2 | aromatics | 120 | 120+91 |
| C3 benzene | 25 | 11.5 | aromatics | 120 | 105+120 |
| 5 methyl fufural | 26 | 11.6 | polysaccharides | 110 | 110+109 |
| C3 benzene | 27 | 11.7 | aromatics | 120 | 105+120 |
| C3 benzene | 28 | 12.1 | aromatics | 120 | 105+120 |
| benzonitrile | 29 | 12.3 | ncc | 103 | 76+103 |
| Phenol | 30 | 12.5 | phenols | 94 | 94+66 |
| c10 alkene | 31 | 12.5 | lipids | 140 | 55+69 |
| C3 benzene | 32 | 12.6 | aromatics | 120 | 105+120 |
| C10 alkane | 33 | 12.9 | lipids | 142 | 57+71 |
| C3 benzene | 34 | 13.6 | aromatics | 120 | 105+120 |
| 3-hydroxy-2-methyl-2-cyclopenten-1-one | 35 | 13.9 | polysaccharides | 112 | 112 |
| Indene | 36 | 14.3 | aromatics | 116 | 116+115 |
| methylphenol | 37 | 14.9 | phenols | 108 | 107+108 |
| methylphenol | 38 | 15.6 | phenols | 108 | 107+108 |
| 4-methoxyphenol (guaicol) | 39 | 15.9 | lignins | 124 | 109+124 |
| c11 alkene | 40 | 15.9 | lipids | 154 | 55+69 |
| C11 alkane | 41 | 16.2 | lipids | 156 | 57+71 |
| methylbenzofuran | 42 | 16.3 | polysaccharides | 132 | 132+131 |

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Table S2: (cont.)

| **Pyrolysis moiety** | **Peak #** | **Retention time (min)** | **Compound class**[a] | **Molecular weight** | **Masses** |
|---|---|---|---|---|---|
| methylbenzofuran | 43 | 16.4 | polysaccharides | 132 | 132+131 |
| maltol | 44 | 16.8 | polysaccharides | 126 | 126 |
| benzylnitrile | 45 | 17.5 | ncc | 117 | 90+117 |
| 3methyl 1H-indene | 46 | 17.6 | aromatics | 130 | 130+115 |
| dimethyl/ethylphenol | 47 | 17.8 | phenols | 122 | 107+122 |
| dimethyl/ethylphenol | 48 | 18.4 | phenols | 122 | 107+122 |
| naphthalene | 49 | 18.7 | aromatics | 128 | 128 |
| c12 alkene | 50 | 19.0 | lipids | 168 | 55+69 |
| 4-methylguiaacol (creosol) | 51 | 19.1 | lignins | 138 | 123+138 |
| C12 alkane | 52 | 19.2 | lipids | 170 | 57+71 |
| 4,7-dimethylbenzofuran | 53 | 19.7 | polysaccharides | 146 | 145+146 |
| 4-vinylphenol | 54 | 20.1 | phenols | 120 | 120+91 |
| 4-ethylguaiacol | 55 | 21.5 | lignins | 152 | 137+152 |
| c13 alkene | 56 | 21.8 | lipids | 182 | 55+69 |
| methyl naphthalene | 57 | 21.9 | aromatics | 142 | 142+141 |
| Indole | 58 | 22.0 | ncc | 117 | 90+117 |
| C13 alkane | 59 | 22.0 | lipids | 184 | 57+71 |
| methyl naphthalene | 60 | 22.4 | aromatics | 142 | 142+141 |
| 4-vinylguaiacol | 61 | 22.5 | lignins | 150 | 135+150 |
| 2,6-dimethoxyphenol (syringol) | 62 | 23.6 | lignins | 154 | 139+154 |
| 4-Propenylguaiacol | 63 | 23.7 | lignins | 164 | 164+149 |

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Table S2: (cont.)

| **Pyrolysis moiety** | **Peak #** | **Retention time (min)** | **Compound class**[a] | **Molecular weight** | **Masses** |
|---|---|---|---|---|---|
| methyl indole | 64 | 24.5 | ncc | 131 | 131+130 |
| c14 alkene | 65 | 24.5 | lipids | 196 | 55+69 |
| C14 alkane | 66 | 24.7 | lipids | 198 | 57+71 |
| 4-formylguaicol (Vanillin) | 67 | 24.9 | lignins | 152 | 151+152 |
| 4-methylsyringol | 68 | 26.0 | lignins | 168 | 153+168 |
| trans-4-(2-propenyl)guaiacol (eugenol) | 69 | 26.0 | lignins | 164 | 164+149 |
| C15 alkene | 70 | 27.0 | lipids | 210 | 55+69 |
| levoglucosan | 71 | 27.3 | polysaccharides | 162 | 73+60 |
| C15 alkane | 72 | 27.2 | lipids | 212 | 57+71 |
| 4-vinylsyringol | 73 | 28.9 | lignins | 180 | 165+180 |
| C16 alkene | 74 | 29.3 | lipids | 224 | 55+69 |
| C16 alkane | 75 | 29.5 | lipids | 226 | 57+71 |
| C17 alkene | 76 | 31.6 | lipids | 238 | 55+69 |
| C17 alkane | 77 | 31.8 | lipids | 240 | 57+71 |
| diketodipyrrole | 78 | 32.0 | NCC | 186 | 93+186 |
| 4-acetylsyringol | 79 | 32.7 | lignins | 196 | 181+196 |
| phenanthrene | 80 | 33.5 | aromatics | 178 | 178 |
| C18 alkene | 81 | 33.7 | lipids | 252 | 55+69 |
| C18 alkane | 82 | 33.9 | lipids | 254 | 57+71 |
| C19 alkene | 83 | 35.8 | lipids | 266 | 55+69 |
| C19 alkane | 84 | 35.9 | lipids | 268 | 57+71 |

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Table S2: (cont.)

| **Pyrolysis moiety** | **Peak #** | **Retention time (min)** | **Compound class**[a] | **Molecular weight** | **Masses** |
|---|---|---|---|---|---|
| C17 methylketone | 85 | 36.0 | lipids | 254 | 58+59 |
| C16 fatty acid | 86 | 37.1 | lipids | 256 | 60+73 |
| C20 alkene | 87 | 37.7 | lipids | 280 | 55+69 |
| C20 alkane | 88 | 37.8 | lipids | 282 | 57+71 |
| C21 alkene | 89 | 39.5 | lipids | 294 | 55+69 |
| C21 alkane | 90 | 39.7 | lipids | 296 | 57+71 |
| C19 methylketone | 91 | 39.8 | lipids | 282 | 58+59 |
| C18 fatty acid | 92 | 40.8 | lipids | 284 | 60+73 |
| C22 alkene | 93 | 41.3 | lipids | 308 | 55+69 |
| C22 alkane | 94 | 41.4 | lipids | 310 | 57+71 |
| C23 alkene | 95 | 43.0 | lipids | 322 | 55+69 |
| C23 alkane | 96 | 43.1 | lipids | 324 | 57+71 |
| C24 alkene | 97 | 44.7 | lipids | 336 | 55+69 |
| C24 alkane | 98 | 44.8 | lipids | 338 | 57+71 |
| C25 alkene | 99 | 46.2 | lipids | 350 | 55+69 |
| C25 alkane | 100 | 46.3 | lipids | 352 | 57+71 |
| C26 alkene | 101 | 47.8 | lipids | 364 | 55+69 |
| C26 alkane | 102 | 47.8 | lipids | 366 | 57+71 |
| C27 alkene | 103 | 49.2 | lipids | 378 | 55+69 |
| C27 alkane | 104 | 49.3 | lipids | 380 | 57+71 |
| C28 alkane | 105 | 50.7 | lipids | 394 | 57+71 |
| C29 alkane | 106 | 52.0 | lipids | 408 | 57+71 |

Table S3: Spearman correlations between functional gene categories and taxonomic lineages identified in metagenomic data. Lineages are grouped by taxonomic rank: p = phylum, c = class, o = order. Only statistically significant associations ($p < 0.05$) are shown.

| **Taxon Lineage** | **Potential Function** | **Correlation Estimate** | **P-Value** |
|---|---|---|---|
| p__Chloroflexota<br>c__Anaerolineae<br>o__Anaerolineales | Nitrate reduction | 0.598 | 0.00535 |
| p__Chloroflexota<br>c__Dehalococcoidia<br>o__DSTF029 | Nitrate reduction | 0.560 | 0.01025 |
| p__Chloroflexota<br>c__Dehalococcoidia<br>o__SM23-31 | Nitrate reduction | 0.372 | 0.01802 |
| p__Acidobacteriota<br>c__Thermoanaerobaculia<br>o__Thermoanaerobaculales | Fe/Mn reduction | 0.786 | 9.83e-14 |
| p__Acidobacteriota<br>c__Acidobacteriae<br>o__Acidobacterales | Fe/Mn reduction | 0.738 | 0.000202 |
| p__Acidobacteriota<br>c__Blastocatellia<br>o__Pyrinomonadales | Fe/Mn reduction | 0.734 | 0.000228 |
| p__Desulfobacterota<br>c__Desulfuromonadia<br>o__Geobacterales | Sulfate reduction | 0.400 | 0.01051 |
| p__Desulfobacterota<br>c__BSN033<br>o__BSN033 | Sulfate reduction | 0.466 | 0.03830 |
| p__Desulfobacterota<br>c__DSM-4660<br>o__Desulfatiales | Sulfate reduction | 0.457 | 0.04254 |