

**Supplementary Information for:**

**Methylotrophic methanogenesis: an ecological and genomic review**

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Table S2. Results of Kruskal-Wallis tests of the abundance of methanogenic genes in archaeal, bacterial, and other (including unclassified) metagenomic scaffolds across habitat types. Shown are the degrees of freedom (df), chi-square test statistic ( $\chi^2$ ), p-value with false discovery rate correction ( $P_{\text{fdr}}$ ), and significance (Sig., \*\*\* = < 0.001, \* = < 0.05, NSD = no significant difference).

| <b>Taxonomy</b> | <b>Gene</b> | <b>df</b> | <b><math>\chi^2</math></b> | <b>Pfdr</b> | <b>Sig.</b> |
|-----------------|-------------|-----------|----------------------------|-------------|-------------|
| Archaea         | <i>mcrA</i> | 9, 455    | 123.13                     | 1.22E-21    | ***         |
| Archaea         | <i>cdhD</i> | 9, 455    | 205.64                     | 1.74E-38    | ***         |
| Archaea         | <i>frhA</i> | 9, 455    | 88.08                      | 7.90E-15    | ***         |
| Archaea         | <i>mttC</i> | 9, 455    | 21.44                      | 1.08E-02    | *           |
| Archaea         | <i>mtbC</i> | 9, 455    | 76.35                      | 1.14E-12    | ***         |
| Archaea         | <i>mtmC</i> | 9, 455    | 71.76                      | 7.86E-12    | ***         |
| Archaea         | <i>mtaA</i> | 9, 455    | 77.18                      | 9.36E-13    | ***         |
| Archaea         | <i>mtsA</i> | 9, 455    | 95.34                      | 3.67E-16    | ***         |
| Bacteria        | <i>mcrA</i> | 9, 455    | 3.79                       | 9.25E-01    | NSD         |
| Bacteria        | <i>cdhD</i> | 9, 455    | 105.05                     | 1.19E-17    | ***         |
| Bacteria        | <i>frhA</i> | 9, 455    | 62.65                      | 8.26E-10    | ***         |
| Bacteria        | <i>mttC</i> | 9, 455    | 99.04                      | 9.85E-17    | ***         |
| Bacteria        | <i>mtbC</i> | 9, 455    | 51.56                      | 8.76E-08    | ***         |
| Bacteria        | <i>mtmC</i> | 9, 455    | 32.23                      | 2.43E-04    | ***         |
| Bacteria        | <i>mtaA</i> | 9, 455    | 94.79                      | 4.74E-16    | ***         |
| Bacteria        | <i>mtsA</i> | 9, 455    | 7.87                       | 6.26E-01    | NSD         |
| Other           | <i>mcrA</i> | 9, 455    | 39.66                      | 1.17E-05    | ***         |
| Other           | <i>cdhD</i> | 9, 455    | 109.18                     | 1.72E-18    | ***         |
| Other           | <i>frhA</i> | 9, 455    | 50.7                       | 1.27E-07    | ***         |
| Other           | <i>mttC</i> | 9, 455    | 56.4                       | 1.32E-08    | ***         |
| Other           | <i>mtbC</i> | 9, 455    | 38.27                      | 1.78E-05    | ***         |
| Other           | <i>mtmC</i> | 9, 455    | 28.38                      | 8.24E-04    | ***         |
| Other           | <i>mtaA</i> | 9, 455    | 81.75                      | 2.90E-13    | ***         |
| Other           | <i>mtsA</i> | 9, 455    | 58.56                      | 6.75E-09    | ***         |
| All             | <i>mcrA</i> | 9, 455    | 139.79                     | 4.55E-25    | ***         |
| All             | <i>cdhD</i> | 9, 455    | 147.71                     | 2.11E-26    | ***         |
| All             | <i>frhA</i> | 9, 455    | 97.24                      | 1.51E-16    | ***         |
| All             | <i>mttC</i> | 9, 455    | 72.54                      | 6.43E-12    | ***         |
| All             | <i>mtbC</i> | 9, 455    | 44.12                      | 1.34E-06    | ***         |
| All             | <i>mtmC</i> | 9, 455    | 63.61                      | 3.07E-10    | ***         |
| All             | <i>mtaA</i> | 9, 455    | 78.95                      | 4.18E-13    | ***         |
| All             | <i>mtsA</i> | 9, 455    | 84.74                      | 3.67E-14    | ***         |

Table S3. Results of Kruskal-Wallis tests of the abundance of methanogenic families across habitat types. Shown are the degrees of freedom (df), chi-square test statistic ( $\chi^2$ ), and p-value with false discovery rate correction ( $P_{\text{fdr}}$ ).

| <b>Family</b>                   | <b>df</b> | <b><math>\chi^2</math></b> | <b><math>P_{\text{fdr}}</math></b> |
|---------------------------------|-----------|----------------------------|------------------------------------|
| Methanobacteriaceae             | 9, 455    | 92.13                      | < 0.001                            |
| Methanocaldococcaceae           | 9, 455    | 305.23                     | < 0.001                            |
| Methanocellaceae                | 9, 455    | 171.46                     | < 0.001                            |
| Methanococcaceae                | 9, 455    | 229.27                     | < 0.001                            |
| Methanocorpusculaceae           | 9, 455    | 85.6                       | < 0.001                            |
| Methanomassiliicoccaceae        | 9, 455    | 134.16                     | < 0.001                            |
| Methanomicrobiaceae             | 9, 455    | 114.36                     | < 0.001                            |
| Methanonatronarchaeaceae        | 9, 455    | 103.13                     | < 0.001                            |
| Methanopyraceae                 | 9, 455    | 63.08                      | < 0.001                            |
| Methanoregulaceae               | 9, 455    | 278.68                     | < 0.001                            |
| Methanosaetaceae                | 9, 455    | 142.3                      | < 0.001                            |
| Methanosarcinaceae              | 9, 455    | 177.35                     | < 0.001                            |
| Methanospirillaceae             | 9, 455    | 77.1                       | < 0.001                            |
| Methanothermaceae               | 9, 455    | 174.06                     | < 0.001                            |
| Methanotrichaceae               | 9, 455    | 215.67                     | < 0.001                            |
| unclassified Methanomicrobiales | 9, 455    | 48.56                      | < 0.001                            |
| unclassified Methanosarcinales  | 9, 455    | 33.4                       | < 0.001                            |

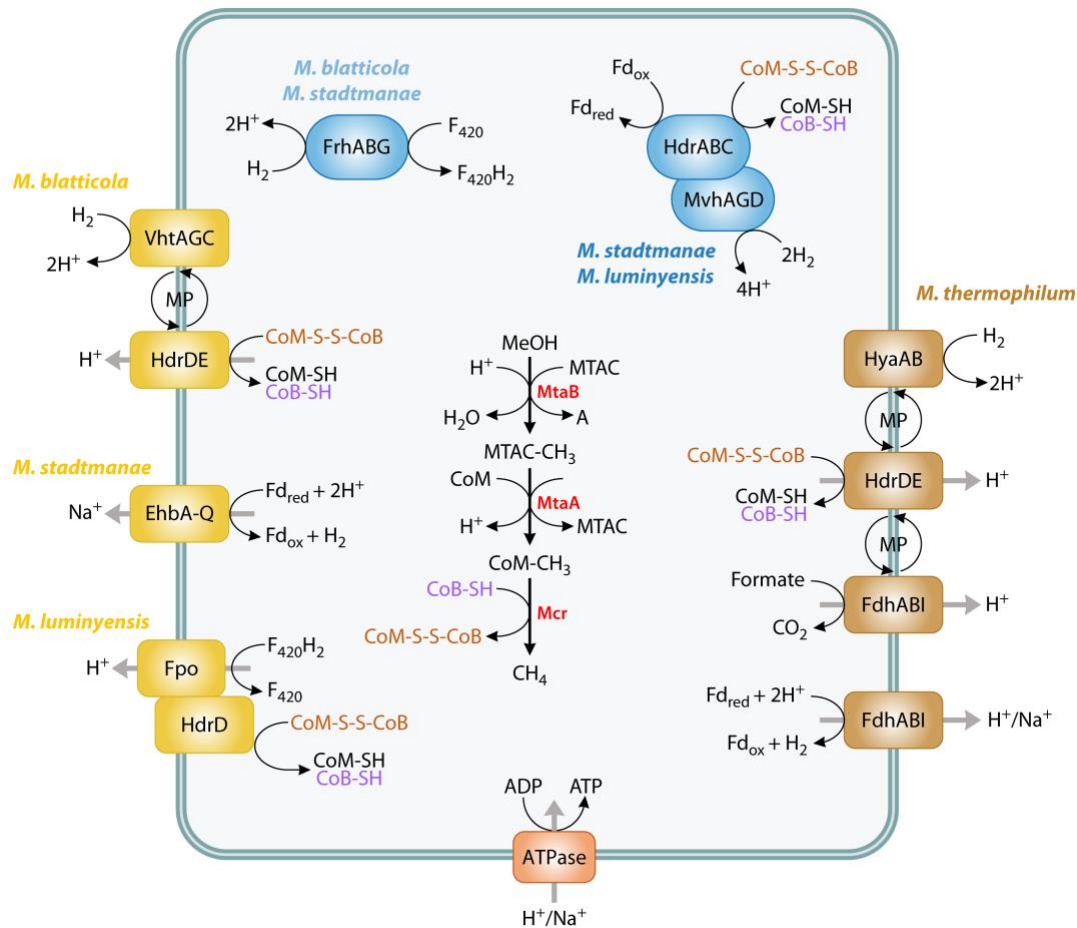


Figure S1. Diagram of methyl-reducing pathways from methanol from four different taxa (adapted from J. M. Kurth, H. J. M. Op den Camp, and Cornelia U. Welte, Appl Microbiol Biotechnol 104:6839-6854, 2020). The taxa are *Methanimicrococcus blatticola* (Methanosarcinaceae), *Methanosphaera stadtmanae* (Methanobacteriaceae), *Methanomassiliicoccus luminyensis* (Methanomassiliicoccaceae), and *Methanonatronarchaeum thermophilum* (Methanonatronarchaeaceae). Note that methyl-reducing taxa may or may not contain the genes for the methyl branch of the Wood-Ljungdahl pathway, and activity/growth experiments are recommended to confirm the methyl-reducing pathway. Mta = methyl-coenzyme M methyltransferase (methanol/glycine betaine-specific corrinoid protein, Mcr = methyl-coenzyme M reductase, Fpo =  $F_{420}H_2$  dehydrogenase, Hdr = membrane-bound

heterodisulfide reductase, Ech-H<sub>2</sub>ase = energy-conserving hydrogenase, Rnf = Na<sup>+</sup>-translocating ferredoxin:NAD<sup>+</sup> oxidoreductase complex, MP = methanophenazine, MTAC = CoI-Corrinoid-Fe-S-proteins, CoM = coenzyme M, CoB = coenzyme B, CoM-S-S-Cob = coenzyme B-coenzyme M heterodisulfide, F<sub>420</sub> = coenzyme F<sub>420</sub>, Fd = ferredoxin, a two electron carrier, red = reduced, ox = oxidized, ADP = adenosine diphosphate, ATP = adenosine triphosphate. Frh = coenzyme F<sub>420</sub>-reducing hydrogenase, Mvh = F<sub>420</sub>-non-reducing hydrogenase, Vht = [NiFed]-hydrogenase, Ehb = energy-conserving hydrogenase, Hya = H<sub>2</sub>-producing hydrogenase, 4 g Hyd = 4 g-type hydrogenase. Na<sup>+</sup>/H<sup>+</sup> translation stoichiometry is not represented in the figure.



Figure S2. Map of metagenome samples used in the IMG/M survey of methanogenic genes and families. 33 of the 465 samples did not have associated latitude and longitude coordinates and are missing from the map.

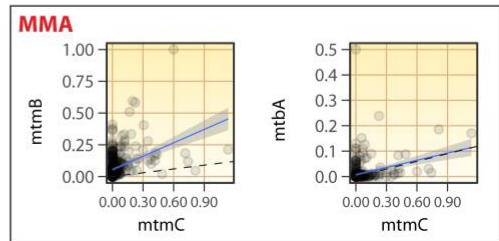
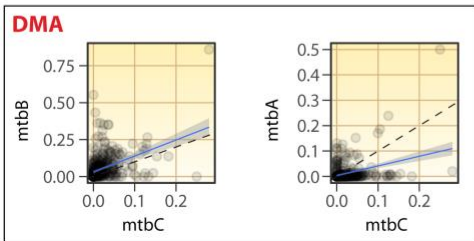
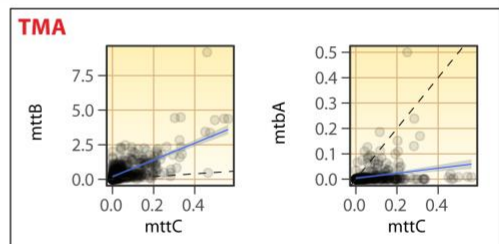
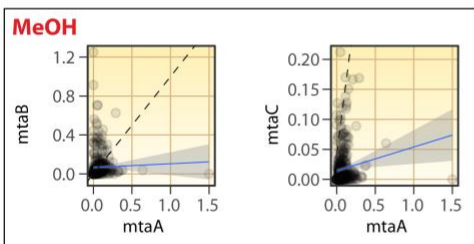
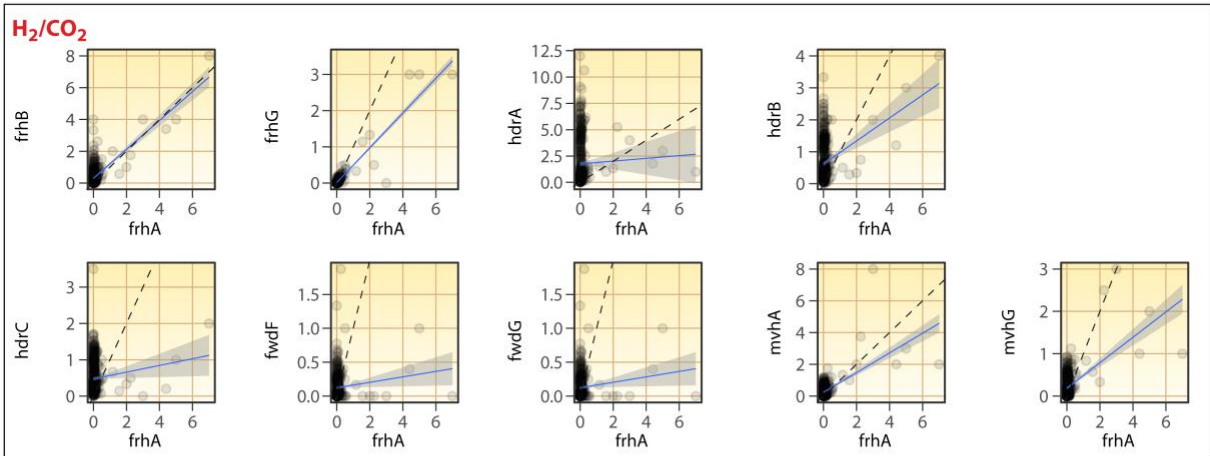
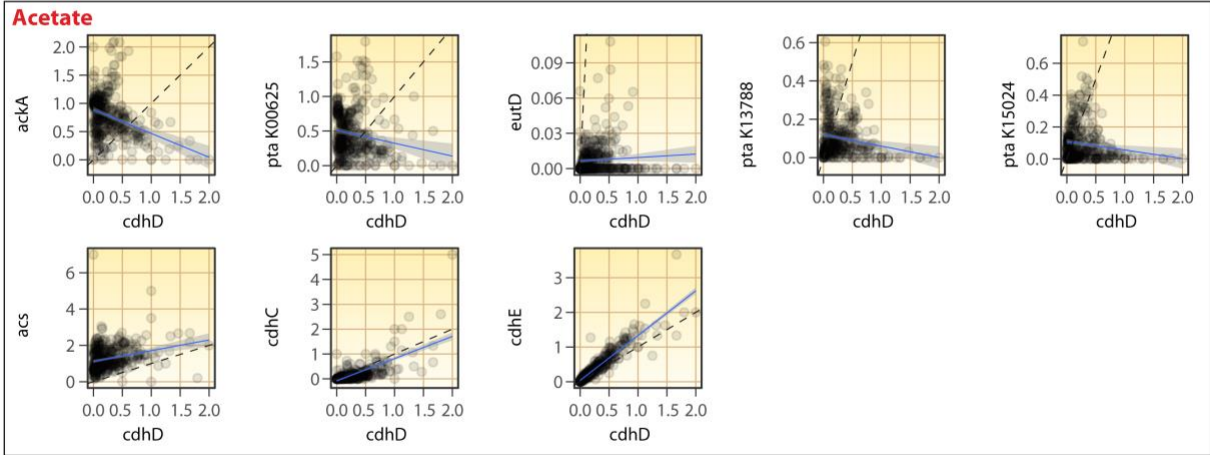
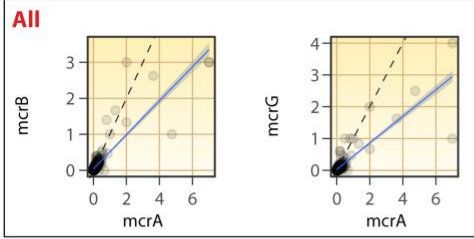


Figure S3. Correlations between the total abundance of the selected gene to represent each pathway used in the comparative analysis, and other genes in that pathway. The dashed line is the 1:1 line and the blue line with shaded 95% confidence interval is the linear regression line. If the regression line is to the right of the 1:1 line, the selected gene is more abundant than the other gene. If the regression line is to the left of the 1:1 line, the selected gene is less abundant than the other gene. The large discrepancy between *cdhD* and *ackA* and *pta* is likely because *ackA* and *pta* are involved in acetyl-CoA production, which is used in many other reactions that are not involved in methanogenesis and is thus not expected to track methanogenesis gene abundances.



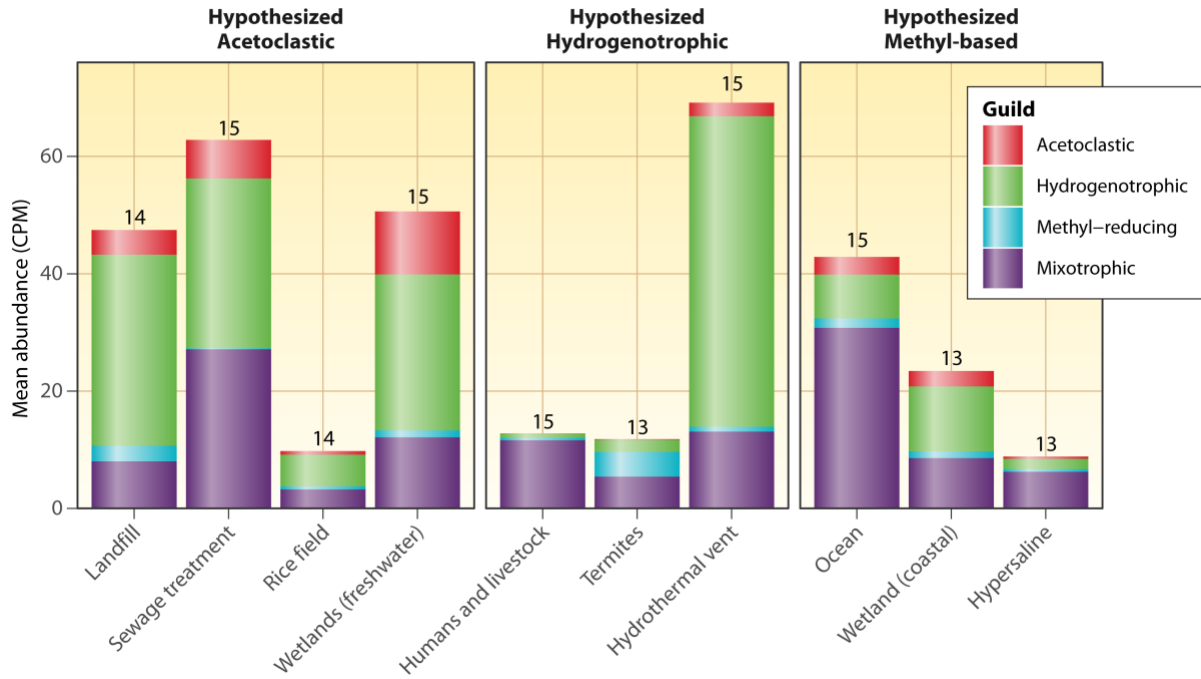


Figure S4. Methanogen family abundances from Figure 5 aggregated by guild. The mixotrophic guild includes Methanosarcinaceae, which can perform the acetoclastic, hydrogenotrophic, methyl-dismutation, and methyl-reduction pathways, and Methanobacteriaceae, which can perform the hydrogenotrophic and methyl-reducing pathways. For families included in the other guilds, see Figure 5. Numbers above the bars represent the number of methanogenic families present.