Supplementary information for


**Arsenic behavior in groundwater in Hanoi (Vietnam) influenced by a complex biogeochemical network of iron, methane, and sulfur cycling**

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Table S1. List of primers, primer sequences and thermal programs used for quantification of bacterial and archaeal 16S rRNA genes, arsenate reductase genes (*arrA*), Geobacter specific 16S rRNA genes, particulate methane monooxygenase genes (*pmoA*) and methyl-coenzyme M reductase subunit alpha genes (*mcrA*).

| Specificity | Primer | Primer sequence (5´→ 3´) | Thermal program | References |
|---|---|---|---|---|
| 16S rRNA genes of *Bacteria* | 341f | CCTACGGGAGGCAGCAG | 98°C - 2´; (98°C - 5´´; 60°C - 12´´ | Muyzer et al., 1993 [1] |
| | 534r | ATTACCGCGGCTGCTGG | 95°C - 1´; 60°C - 1´) x 40; 60 – 95°C - 10´´ | |
| 16S rRNA genes of *Archaea* | Ar109f | ACK GCT GAG TAA CAC GT GTG CTC CCC CGC CAA TTC CT | 98°C - 3´; (98°C - 5´´; 52°C - 12´´ | Großkopf et al., 1998 [2] Stahl and Amann 1991 [3] |
| | Ar915r | | 72°C - 15´) x 40; 98°C - 1´; 52°C - 1´; 52 – 95°C - 10´´ | |
| *arrA* | arrA-F arrA-R | GGYSTGGGGCWSCGAYCC GGMASCCASTYGTGGGMC TT | 95°C - 2´; (95°C - 30´´; 62°C - 40´´) x 40; 95°C - 1´; 62°C - 1´; (62 – 95°C - 10´´) x 67 | Song et al. 2009 |
| 16S rRNA genes of *Geobacter* | Geo494F Geo825R | AGGAAGCACCGGCTAACTCC TACCCGCRACACCTAGT | 50°C - 2´; 95°C- 10´; (95°C - 15´´; 56°C - 60´´) x 30 | Hlmes et al. 2002 [4] Anderson et al. 1998 [5] |
| *pmoA* | A189f A682r | GGNGACTGGGACTTCTGG GAASGCNGAGAAGAASGC | 96°C - 5´; (94°C - 1´; 56°C - 1´; 72°C - 1´) x 38; 72°C - 5´ | Holmes et al. 1995 [6] |
| *mcrA* | ME1f ME1r | GCMATGCARATHGGWATG TC TCATKGCRTAGTTDGGRTA GT | 95°C - 5´; (95°C - 50´´; 54°C - 50´´ 72°C - 50´) x 34; 72°C - 10´ | Hales et al. 1996 [7] |

Table S2. Observed amplicon sequencing variants (ASVs) and alpha diversity indices in water samples from wells divided by geochemical zones as proposed in Stopelli et al. 2020 [8]. Pielou's index (a measure of community evenness), Faith's Phylogenetic Diversity (a qualitative measure of community richness that incorporates phylogenetic relationships between the features) and Shannon's diversity index (a quantitative measure of community richness).

| Zone | Well | Observed ASVs | Pielou e | Faith pd | Shannon |
|------|------|---------------|----------|----------|---------|
| **B** | **AMS12** | 2830 | 0.86 | 190 | 9.90 |
| | **AMS15** | 1906 | 0.79 | 130 | 8.64 |
| **C** | **AMS13** | 1860 | 0.77 | 109 | 8.35 |
| | **VPNS3** | 882 | 0.68 | 76 | 6.65 |
| | **VPMN5** | 738 | 0.64 | 54 | 6.08 |
| | **AMS5** | 1595 | 0.71 | 113 | 7.58 |
| **D** | **PC44** | 1905 | 0.80 | 124 | 8.69 |
| | **AMS11/32** | 1766 | 0.71 | 120 | 7.64 |
| | **AMS11/47** | 1897 | 0.86 | 122 | 9.32 |
| | **PC43** | 2134 | 0.80 | 136 | 8.90 |
| | **AMS32** | 950 | 0.78 | 73 | 7.67 |
| | **AMS31** | 1841 | 0.79 | 119 | 8.54 |
| | **AMS11/25** | 2260 | 0.79 | 139 | 8.81 |
| **E** | **VPML38** | 1744 | 0.71 | 108 | 7.67 |
| | **VPML54** | 1545 | 0.85 | 96 | 8.98 |
| | **AMS36** | 1646 | 0.83 | 122 | 8.89 |
| | **AMS4** | 2052 | 0.82 | 138 | 8.98 |
| | **VPML22** | 1097 | 0.69 | 81 | 6.96 |

Table S3. Quantitative PCR analysis of bacterial and archaeal 16S rRNA genes, arsenate reductase genes (*arrA*), *Geobacter* specific 16S rRNA genes, particulate methane monooxygenase genes (*pmoA*) and methyl-coenzyme M reductase subunit alpha genes (*mcrA*). The data is shown as gene copy numbers/mL in the groundwater samples from different geochemical zones of the Van Phuc aquifers [8].

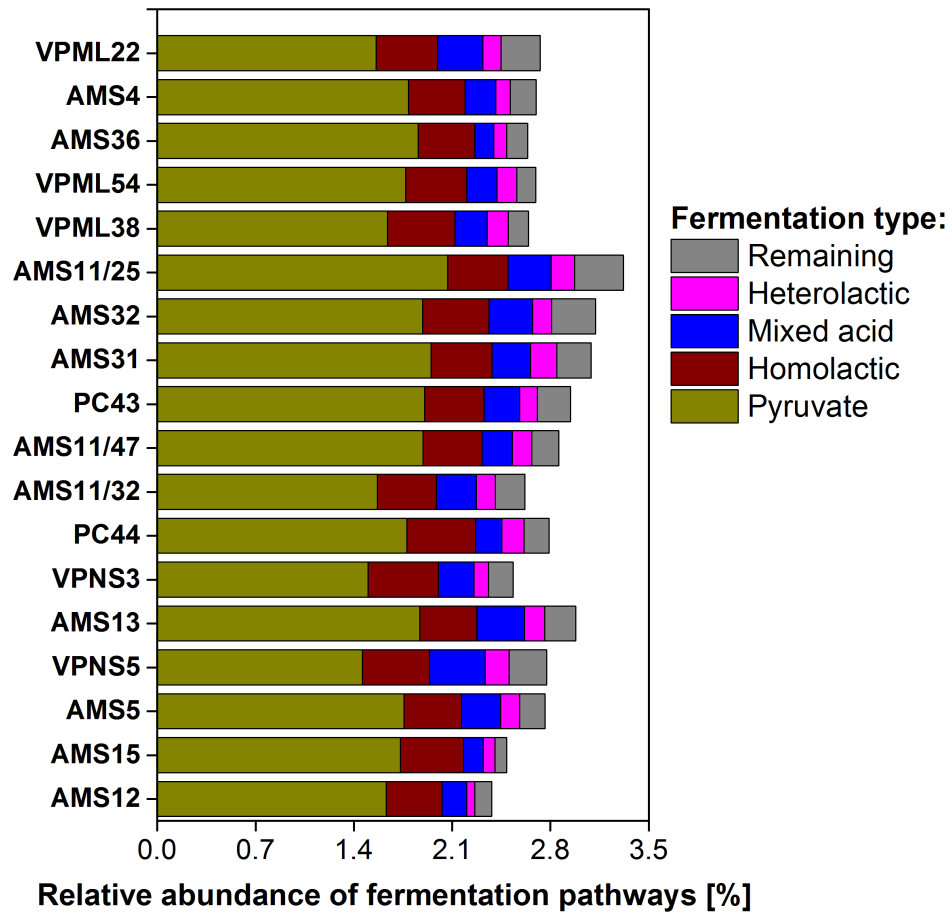| Zone | Well | *Bacteria* | *Archaea* | *arrA* | *Geobacter* | *mcrA* | *pmoA* |
|------|------|-----------|-----------|--------|-------------|--------|--------|
| B | AMS12 | 1256 ± 120 | 3727 ± 346 | 146 ± 21 | 47.1 ± 0.8 | 1 ± 0 | 82 ± 6 |
| | AMS15 | 50610 ± 5553 | 542 ± 16 | 949 ± 95 | 522.4 ± 34 | 71 ± 8 | 1736 ± 214 |
| C | AMS13 | 1132444 ± 197401 | 42118 ± 7799 | 9765 ± 1300 | 9808 ± 770 | 7390 ± 395 | 1444555 ± 248776 |
| | VPN3 | 870 ± 74 | 59 ± 2 | 6 ± 1 | 6.3 ± 0.2 | 2 ± 1 | 31 ± 5 |
| | VPMN5 | 159500 ± 14110 | 15 ± 5 | 3127 ± 83 | 1136 ± 36 | 122 ± 9 | 18243 ± 2288 |
| | AMS5 | 44786 ± 2398 | 1111 ± 84 | 2625 ±107 | 2955 ± 148 | 369 ± 48 | 174733 ± 5774 |
| D | PC44 | 45676 ± 9053 | 468 ± 29 | 7092 ± 168 | 713 ± 13 | 87 ± 5 | 4481 ± 144 |
| | AMS 11/32 | 37296 ± 2750 | 131 ± 15 | 252 ± 15 | 201 ± 18 | 14 ± 2 | 1087 ± 91 |
| | AMS 11/47 | 114633 ± 8528 | 207 ± 8 | 3051 ± 324 | 4271 ± 235 | 133 ± 7 | 6070 ± 3195 |
| | PC43 | 16916 ± 2282 | 185 ± 4 | 328 ± 16 | 278 ± 17 | 91 ± 7 | 1267 ± 54 |
| | AMS32 | 111100 ± 4258 | 8 ± 1 | 1477 ± 42 | 1839 ± 88 | 290 ± 15 | 6879 ± 782 |
| | AMS31 | 32923 ± 5563 | 67 ± 2 | 346 ± 26 | 204 ± 14 | 72 ± 5 | 2991 ± 229 |
| | AMS 11/25 | 9467 ± 453 | 182 ± 16 | 290 ± 12 | 250 ± 16 | 171 ± 15 | 3954 ± 356 |
| E | VPML38 | 31283 ± 585 | 347 ± 14 | 404 ± 22 | 1180 ± 30 | 18 ± 1 | 6274 ± 1927 |
| | VPML54 | 126733 ± 3190 | 6657 ± 174 | 3220 ± 102 | 1451 ± 41 | 15 ± 2 | 1613 ± 421 |
| | AMS36 | 170866 ± 10651 | 1862 ± 74 | 1417 ± 240 | 1903 ± 64 | 1104 ± 191 | 6266 ± 172 |
| | AMS4 | 217300 ± 9945 | 2044 ± 88 | 2154 ± 54 | 1793 ± 116 | 996 ± 36 | 2690 ± 522 |
| | VPML22 | 19283 ± 42 | 91 ± 17 | 142 ± 15 | 100 ± 4 | 3 ± 1 | 1466 ± 36 |

Fig. S1. Relative abundance of predicted fermentation pathways in groundwater samples as inferred from 16S rRNA amplicon sequences based on MetaCyc pathways.
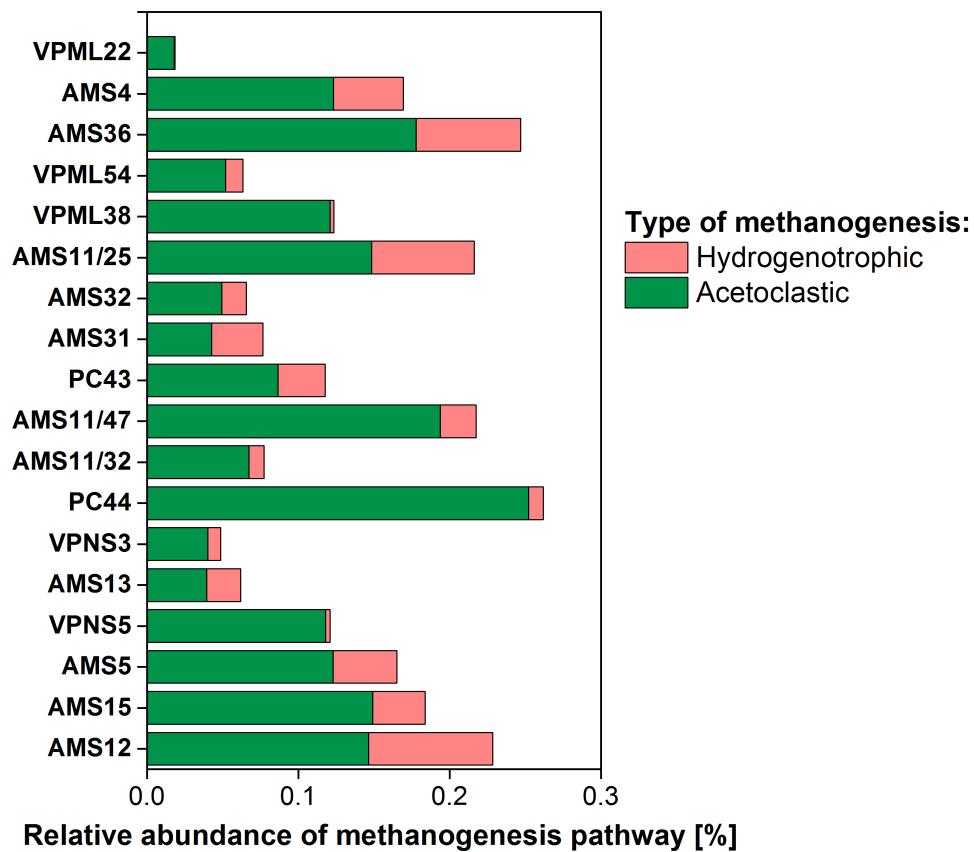
Fig. S2. Relative abundance of predicted methanogenic pathways in groundwater samples as inferred from 16S rRNA amplicon sequences based on MetaCyc pathways.

**Supplementary references**

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