Supplementary materials

Analysis of the Functional Gene Structure and Metabolic Potential of Microbial Community in High Arsenic Groundwater

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**Figure S1** Hierarchical cluster analysis of microbial functional genes of sample S1-19 from Inner Mongolia. Results were generated in CLUSTER and visualized using TREEVIEW. Blue indicates signal intensities below background, whereas red indicates signal intensities above background. Brighter red color indicates higher signal intensities. Sample S1-5 with lower arsenic concentrations clustered together and were well separated from other samples S6-19 with higher arsenic concentrations.



**Figure S2** Variation partitioning based on canonical correspondence analysis CCA for all functional gene signal intensities. CCA-based VIF shows the relative effects of the six environmental variables (As Tot, SO42-, NH4+, pH, ORP, and TOC) on the functional microbial community structure. The unexplained represents the effect that could not be explained by the six environmental variables.

**Table S1** Geochemistry of groundwater samples collected from Hangjinhouqi County, Inner Mongolia of China

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample Name | pH | EC | DO | ORP | TDS | AsTot | AsIII | CH4 |  | Sulfate | Sulfide | FeTot | FeII | Nitrate | Nitrite | NH4+ | TN | TC | TOC | Cl- |
| ms/cm | mg/L | mv | g/L | μg/L |  |  |  |  |  |  | mg/L |  |  |  |  |  |
| S1 | 7.51 | 1.89 | 2.38 | -15 | 1.37 | 38.93 | 0.00 | 7.60 |  | 323.81 | 0.007 | 0.021 | BD | 3.768 | 0.020 | 1.357 | 2.195 | 4.152 | 4.150 | 47.38 |
| S2 | 7.34 | 2.24 | 1.19 | -55 | 1.64 | 60.71 | 10.67 | 5.65 |  | 386.14 | 0.003 | 0.335 | 0.245 | 1.239 | 0.002 | 0.017 | 1.490 | 3.934 | 3.902 | 49.10 |
| S3 | 7.41 | 2.15 | 1.38 | -30 | 1.57 | 63.24 | 0.00 | 3.50 |  | 331.17 | 0.040 | 0.127 | 0.081 | 2.788 | 0.015 | 0.087 | 1.731 | 4.087 | 4.004 | 52.03 |
| S4 | 7.52 | 2.53 | 4.01 | -69 | 1.87 | 78.27 | 1.17 | 5.65 |  | 178.64 | 0.010 | 0.095 | 0.016 | 2.592 | 0.021 | 0.687 | 1.793 | 4.594 | 4.573 | 71.41 |
| S5 | 7.77 | 8.00 | 4.76 | -75 | 6.83 | 92.17 | 45.81 | 7.03 |  | 463.24 | BD | 0.060 | 0.043 | 2.392 | 0.002 | 0.967 | 1.919 | 5.414 | 5.404 | 155.50 |
| S6 | 7.99 | 1.42 | 3.23 | -122 | 1.00 | 157.71 | 76.30 | 19.22 |  | 89.80 | 0.010 | 0.320 | 0.259 | 0.239 | 0.008 | 0.713 | 2.251 | 4.208 | 4.185 | 58.09 |
| S7 | 7.91 | 4.49 | 3.35 | -124 | 3.42 | 189.10 | 101.37 | 17.77 |  | 34.24 | BD | 0.471 | 0.406 | 0.326 | 0.005 | 2.200 | 2.641 | 4.077 | 4.070 | 156.45 |
| S8 | 7.82 | 2.39 | 2.82 | -99 | 1.76 | 189.66 | 79.13 | 9.33 |  | 73.85 | 0.013 | 0.812 | 0.688 | 0.339 | 0.005 | 1.867 | 3.149 | 6.426 | 6.365 | 65.00 |
| S9 | 8.16 | 7.18 | 2.69 | -125 | 5.89 | 356.29 | 231.29 | 12.47 |  | 26.35 | 0.080 | 0.250 | 0.138 | BD | 0.012 | 3.533 | 3.677 | 4.636 | 4.520 | 124.65 |
| S10 | 8.22 | 2.16 | 1.77 | -126 | 1.58 | 270.57 | 185.66 | 24.93 |  | 25.19 | 0.030 | 0.210 | 0.049 | 0.406 | 0.026 | 3.400 | 4.339 | 8.003 | 7.966 | 73.68 |
| S11 | 7.95 | 7.61 | 3.69 | -131 | 0.52 | 380.91 | 216.35 | 15.23 |  | 78.81 | BD | 0.317 | 0.136 | BD | 0.008 | 2.300 | 3.867 | 8.042 | 7.951 | 32.47 |
| S12 | 8.25 | 2.57 | 2.68 | -88 | 1.91 | 389.83 | 252.86 | 9.82 |  | 32.73 | 0.007 | 0.361 | 0.328 | 0.321 | 0.034 | 3.867 | 4.704 | 5.825 | 5.647 | 86.40 |
| S13 | 8.03 | 2.33 | 2.57 | -132 | 1.71 | 398.83 | 95.31 | 42.16 |  | 25.71 | 0.010 | 0.224 | 0.091 | BD | 0.019 | 2.400 | 3.692 | 5.616 | 5.499 | 53.13 |
| S14 | 7.90 | 6.69 | 1.57 | -132 | 5.39 | 419.68 | 292.37 | 24.80 |  | 0.00 | BD | 1.056 | 0.965 | BD | 0.013 | 3.933 | 3.950 | 3.911 | 3.827 | 84.94 |
| S15 | 7.71 | 4.32 | 4.27 | -114 | 3.29 | 543.43 | 351.56 | 30.35 |  | 3.89 | 0.023 | 0.848 | 0.800 | BD | BD | 2.967 | 4.195 | 4.598 | 4.507 | 153.75 |
| S16 | 8.19 | 3.13 | 5.74 | -140 | 2.36 | 626.74 | 419.18 | 100.18 |  | 28.45 | 0.007 | 0.100 | 0.087 | 0.200 | 0.034 | 5.333 | 5.709 | 9.726 | 9.579 | 82.99 |
| S17 | 8.40 | 1.56 | 2.88 | -143 | 1.11 | 655.08 | 414.84 | 204.78 |  | 0.00 | BD | 0.174 | 0.129 | 0.339 | 0.018 | 4.500 | 6.527 | 9.067 | 8.924 | 80.32 |
| S18 | 8.31 | 1.30 | 1.16 | -120 | 0.92 | 766.86 | 524.01 | 93.44 |  | 12.82 | 0.010 | 0.607 | 0.513 | BD | 0.019 | 5.000 | 7.235 | 9.903 | 9.805 | 53.02 |
| S19 | 8.48 | 1.90 | 2.52 | -150 | 1.37 | 863.42 | 602.27 | 178.56 |  | 16.47 | 0.023 | 0.314 | 0.296 | BD | 0.022 | 5.633 | 7.901 | 11.452 | 11.285 | 43.29 |

EC: electrical conductivity; DO: dissolved oxygen, ORP: oxidation reduction potentials; TDS: total dissolved solids, AsTot: total arsenic; AsIII: arsenite; FeTot: total dissolved iron, FeII: ferrous iron; TOC: total organic carbon, TC: total carbon, TN: total nitrogen; BD: below detection limit.

Table S2 Statistical correlations (r values) between geochemical variables and functional microbial community richness and diversity indexes of groundwater samples collected from Hangjinhouqi County, Inner Mongolia of China

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 　 | pH | EC | DO | ORP | TDS | AsTot | AsIII | CH4 | Sulfate | Sulfide | FeTot | FeII | Fe(II/III) | Nitrate | Nitrite | NH4+ | TN | TC | TOC | Cl- | *H’* | EI | Genes |
| pH | 1.0000 | -0.0809 | 0.0585 | -0.8055 | -0.0871 | 0.7972 | 0.7870 | 0.6845 | -0.7343 | 0.0865 | 0.0483 | 0.0444 | 0.2977 | -0.6972 | 0.4855 | 0.8635 | 0.8594 | 0.7523 | 0.7468 | -0.0084 | -0.3040 | -0.0816 | -0.3224 |
| EC | -0.0809 | 1.0000 | 0.3422 | -0.1510 | 0.7497 | -0.1097 | -0.0710 | -0.3383 | 0.0933 | 0.1135 | 0.1613 | 0.1306 | -0.0074 | -0.1005 | -0.4108 | -0.0358 | -0.2133 | -0.2342 | -0.2351 | 0.5124 | -0.3120 | -0.5097 | -0.0613 |
| DO | 0.0585 | 0.3422 | 1.0000 | -0.2125 | 0.2507 | 0.0543 | 0.0441 | 0.0429 | -0.0344 | -0.1860 | -0.2316 | -0.1822 | 0.1141 | -0.0158 | -0.0017 | 0.0658 | -0.0303 | 0.1055 | 0.1065 | 0.4618 | -0.3261 | -0.1446 | -0.2886 |
| ORP | **-0.8055** | -0.1510 | -0.2125 | 1.0000 | -0.0333 | -0.7337 | -0.6930 | -0.5526 | 0.8269 | -0.0031 | -0.3216 | -0.2826 | -0.3684 | 0.8968 | -0.1208 | -0.7198 | -0.6797 | -0.5638 | -0.5596 | -0.1213 | 0.2744 | 0.0841 | 0.2705 |
| TDS | -0.0871 | **0.7497** | 0.2507 | -0.0333 | 1.0000 | -0.1484 | -0.0907 | -0.2764 | 0.1643 | 0.2520 | 0.1744 | 0.2098 | 0.1218 | 0.0265 | -0.3223 | -0.0075 | -0.2327 | -0.3787 | -0.3805 | 0.7618 | -0.4831 | -0.5453 | -0.2394 |
| AsTot | **0.7972** | -0.1097 | 0.0543 | **-0.7337** | -0.1484 | 1.0000 | 0.9777 | 0.8068 | -0.6991 | 0.0178 | 0.2613 | 0.2861 | 0.5824 | -0.6686 | 0.3603 | 0.9172 | 0.9667 | 0.7924 | 0.7848 | -0.0671 | -0.1136 | -0.0098 | -0.1287 |
| AsIII | **0.7870** | -0.0710 | 0.0441 | **-0.6930** | -0.0907 | **0.9777** | 1.0000 | 0.7857 | -0.6572 | 0.0416 | 0.2830 | 0.3196 | 0.6277 | -0.6293 | 0.3517 | 0.9290 | 0.9618 | 0.7869 | 0.7803 | -0.0174 | -0.1002 | -0.0370 | -0.0985 |
| CH4 | **0.6845** | -0.3383 | 0.0429 | **-0.5526** | -0.2764 | **0.8068** | **0.7857** | 1.0000 | -0.4257 | -0.1169 | -0.0798 | -0.0284 | 0.3318 | -0.3557 | 0.3413 | 0.7110 | 0.8341 | 0.7867 | 0.7833 | -0.1705 | -0.1455 | -0.1128 | -0.1164 |
| Sulfate | **-0.7343** | 0.0933 | -0.0344 | **0.8269** | 0.1643 | **-0.6991** | **-0.6572** | -0.4257 | 1.0000 | -0.1190 | -0.4561 | -0.4185 | -0.4237 | 0.8280 | -0.3241 | -0.7422 | -0.6762 | -0.4281 | -0.4205 | -0.0283 | 0.0068 | -0.2592 | 0.1572 |
| Sulfide | 0.0865 | 0.1135 | -0.1860 | -0.0031 | 0.2520 | 0.0178 | 0.0416 | -0.1169 | -0.1190 | 1.0000 | -0.1076 | -0.1369 | -0.0704 | -0.0601 | 0.0385 | 0.0694 | 0.0102 | -0.0839 | -0.0896 | 0.1293 | -0.1764 | -0.1753 | -0.1149 |
| FeTot | 0.0483 | 0.1613 | -0.2316 | -0.3216 | 0.1744 | 0.2613 | 0.2830 | -0.0798 | **-0.4561** | -0.1076 | 1.0000 | 0.9850 | 0.6104 | -0.5336 | -0.3699 | 0.2287 | 0.1752 | -0.1121 | -0.1156 | 0.1768 | 0.1903 | 0.2225 | 0.1128 |
| FeII | 0.0444 | 0.1306 | -0.1822 | -0.2826 | 0.2098 | 0.2861 | 0.3196 | -0.0284 | -0.4185 | -0.1369 | **0.9850** | 1.0000 | 0.6984 | -0.4819 | -0.3472 | 0.2519 | 0.1964 | -0.1138 | -0.1179 | 0.2309 | 0.1432 | 0.1959 | 0.0728 |
| Fe(II/III) | 0.2977 | -0.0074 | 0.1141 | -0.3684 | 0.1218 | **0.5824** | **0.6277** | 0.3318 | -0.4237 | -0.0704 | **0.6104** | **0.6984** | 1.0000 | -0.4561 | -0.0205 | 0.5085 | 0.4950 | 0.2219 | 0.2142 | 0.2668 | -0.1660 | 0.1797 | -0.2820 |
| Nitrate | **-0.6972** | -0.1005 | -0.0158 | **0.8968** | 0.0265 | **-0.6686** | **-0.6293** | -0.3557 | **0.8280** | -0.0601 | **-0.5336** | **-0.4819** | **-0.4561** | 1.0000 | -0.0194 | -0.6345 | -0.6005 | -0.4143 | -0.4069 | -0.0829 | 0.1734 | -0.0215 | 0.2116 |
| NitrIte | **0.4855** | -0.4108 | -0.0017 | **-0.1208** | -0.3223 | 0.3603 | 0.3517 | 0.3413 | -0.3241 | 0.0385 | -0.3699 | -0.3472 | -0.0205 | -0.0194 | 1.0000 | 0.5353 | 0.4787 | 0.4760 | 0.4700 | -0.3523 | 0.2350 | 0.2912 | 0.0871 |
| NH4+ | **0.8635** | -0.0358 | 0.0658 | **-0.7198** | -0.0075 | **0.9172** | **0.9290** | **0.7110** | **-0.7422** | 0.0694 | 0.2287 | 0.2519 | **0.5085** | **-0.6345** | **0.5353** | 1.0000 | 0.9398 | 0.7535 | 0.7463 | 0.0288 | -0.1031 | 0.0067 | -0.1327 |
| TN | **0.8594** | -0.2133 | -0.0303 | **-0.6797** | -0.2327 | **0.9667** | **0.9618** | **0.8341** | **-0.6762** | 0.0102 | 0.1752 | 0.1964 | **0.4950** | **-0.6005** | **0.4787** | **0.9398** | 1.0000 | 0.8709 | 0.8649 | -0.1479 | -0.0585 | 0.0566 | -0.1091 |
| TC | **0.7523** | -0.2342 | 0.1055 | **-0.5638** | -0.3787 | **0.7924** | **0.7869** | **0.7867** | -0.4281 | -0.0839 | -0.1121 | -0.1138 | 0.2219 | -0.4143 | 0.4760 | **0.7535** | **0.8709** | 1.0000 | 0.9998 | -0.3154 | 0.0528 | 0.0864 | -0.0006 |
| TOC | **0.7468** | -0.2351 | 0.1065 | **-0.5596** | -0.3805 | **0.7848** | **0.7803** | **0.7833** | -0.4205 | -0.0896 | -0.1156 | -0.1179 | 0.2142 | -0.4069 | **0.4700** | **0.7463** | **0.8649** | **0.9998** | 1.0000 | -0.3158 | 0.0546 | 0.0874 | 0.0012 |
| Cl- | -0.0084 | **0.5124** | **0.4618** | -0.1213 | **0.7618** | -0.0671 | -0.0174 | -0.1705 | -0.0283 | 0.1293 | 0.1768 | 0.2309 | 0.2668 | -0.0829 | **-0.3523** | 0.0288 | -0.1479 | -0.3154 | -0.3158 | 1.0000 | -0.5949 | -0.2229 | -0.5463 |
| *H’* | -0.3040 | -0.3120 | -0.3261 | 0.2744 | **-0.4831** | -0.1136 | -0.1002 | -0.1455 | 0.0068 | -0.1764 | 0.1903 | 0.1432 | -0.1660 | 0.1734 | 0.2350 | -0.1031 | -0.0585 | 0.0528 | 0.0546 | -0.5949 | 1.0000 | 0.5025 | 0.8752 |
| EI | -0.0816 | **-0.5097** | -0.1446 | 0.0841 | **-0.5453** | -0.0098 | -0.0370 | -0.1128 | -0.2592 | -0.1753 | 0.2225 | 0.1959 | 0.1797 | -0.0215 | 0.2912 | 0.0067 | 0.0566 | 0.0864 | 0.0874 | -0.2229 | **0.5025** | 1.0000 | 0.0231 |
| Genes | -0.3224 | -0.0613 | -0.2886 | 0.2705 | -0.2394 | -0.1287 | -0.0985 | -0.1164 | 0.1572 | -0.1149 | 0.1128 | 0.0728 | -0.2820 | 0.2116 | 0.0871 | -0.1327 | -0.1091 | -0.0006 | 0.0012 | **-0.5463** | **0.8752** | 0.0231 | 1.0000 |

EC: electrical conductivity; DO: dissolved oxygen, ORP: oxidation reduction potentials; TDS: total dissolved solids, AsTot: total arsenic; AsIII: arsenite; FeTol: total dissolved iron, FeII: ferrous iron; Fe(II/III): the ratio of Fe(II/III), TOC: total organic carbon, TC: total carbon, TN: total nitrogen; BD: below detection limit. H’: Shannon-Weiner diversity, EI: Evenness index. Genes: number of genes.

Red color represents P<0.001, blue color represents P<0.01, green color represents P<0.05.

Table S3Richness and diversity indexes and the functional gene numbers detected by geochip 5.0.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sample No. | Shannon | Pielou's evenness | No. of functional genes | Sample No. | Shannon | Pielou's evenness | No. of functional genes |
| S1 | 9.5225 | 0.886 | 46510 | S11 | 9.5911 | 0.8856 | 50538 |
| S2 | 9.5821 | 0.8831 | 51572 | S12 | 9.4926 | 0.8912 | 42252 |
| S3 | 9.6267 | 0.891 | 49258 | S13 | 9.4088 | 0.8881 | 39912 |
| S4 | 9.6938 | 0.8898 | 53844 | S14 | 9.6026 | 0.8842 | 52064 |
| S5 | 9.2622 | 0.8763 | 38949 | S15 | 9.3868 | 0.8916 | 37350 |
| S6 | 9.3983 | 0.8828 | 42037 | S16 | 9.5565 | 0.8864 | 48114 |
| S7 | 9.4211 | 0.8889 | 40101 | S17 | 9.4346 | 0.8823 | 44081 |
| S8 | 9.6514 | 0.8924 | 49788 | S18 | 9.6248 | 0.887 | 51573 |
| S9 | 9.3554 | 0.8738 | 44669 | S19 | 9.423 | 0.8858 | 41675 |
| S10 | 9.5122 | 0.8955 | 41015 |  |  |  |  |

Table S4 Arsenic related genes percentages and organisms in high arsenic groundwater samples collected from Hangjinhouqi County, Inner Mongolia of China.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene | Percentage | Organism | Gene | Percentage | Organism |
| *arsC* | 4.39  | *Rhodanobacter spathiphylli* B39 | *arsB* | 13.64  | *Methylobacterium* sp. 4-46 |
|  | 3.98  | *Rhodococcus erythropolis* PR4 |  | 7.98  | *Micromonospora* sp. ATCC 39149 |
|  | 3.64  | *Stenotrophomonas maltophilia* K279a |  | 6.38  | *Ethanoligenens harbinense* YUAN-3 |
|  | 3.59  | *Aurantimonas manganoxydans* SI85-9A1 |  | 4.96  | *Acidovorax* sp. JS42 |
|  | 3.36  | *Nitrobacter winogradskyi* Nb-255 |  | 2.86  | *Methylobacterium extorquens* DM4 |
|  | 3.28  | *Xanthobacter autotrophicus* Py2 |  | 2.43  | *Oceanimonas doudoroffii* |
|  | 3.14  | *Agrobacterium sp.* GW4 |  | 1.66  | *Afipia sp.* 1NLS2 |
|  | 2.61  | *Rhodococcus sp.* RHA1 |  | 1.65  | *Conexibacter woesei* DSM 14684 |
|  | 2.54  | *Acinetobacter lwoffii* SH145 |  | 1.52  | *Achromobacter xylosoxidans* A8 |
|  | 2.19  | *Thioalkalivibrio* sp. K90mix |  | 1.31  | *Tsukamurella paurometabola* DSM 20162 |
|  | 2.11  | *Mycobacterium* sp. JLS |  | 1.22  | *Ectothiorhodospira* sp. PHS-1 |
|  | 1.87  | *Burkholderia multivorans* ATCC 17616 |  | 1.20  | *Desulfovibrio alaskensis* G20 |
|  | 1.84  | *Propionibacterium freudenreichii subsp. shermanii* CIRM-BIA1 |  | 1.20  | *Caldalkalibacillus thermarum* TA2.A1 |
|  | 1.79  | *Desulfovibrio sp.* FW1012B |  | 1.19  | *Achromobacter arsenitoxydans* SY8 |
|  | 1.69  | *Enterobacter cloacae* ATCC 13047 |  |  |  |
|  | 1.54  | *Polaromonas naphthalenivorans* CJ2 | *aoxB* | 26.62  | *Vibrio* sp. MED222 |
|  | 1.41  | *Roseobacter* sp. AzwK-3b |  | 7.68  | *Acidiphilium multivorum* AIU301 |
|  | 1.34  | *Gluconacetobacter xylinus* NBRC 3288 |  | 5.95  | *Halomonas boliviensis* LC1 |
|  | 1.21  | *Azoarcus* sp. BH72 |  | 5.90  | *Variovorax* sp. RM1 |
|  |  |  |  | 2.14  | *Burkholderia multivorans* ATCC 17616 |
| *arsM* | 29.96  | *Conexibacter woesei* DSM 14684 |  | 1.75  | *uncultured alpha proteobacterium* |
|  | 20.63  | *Desulfohalobium retbaense* DSM 5692 |  | 1.46  | *Acidiphilium* sp. PM |
|  | 8.09  | *Thiocapsa marina* 5811 |  | 1.17  | *Polymorphum gilvum* SL003B-26A1 |
|  | 7.73  | *Nitrosomonas* sp. Is79A3 |  | 1.16  | *Chloroflexus aggregans* DSM 9485 |
|  | 4.79  | *Cupriavidus metallidurans* CH34 |  | 1.15  | *Aeropyrum pernix* K1 |
|  | 3.79  | *Leptonema illini* DSM 21528 |  | 1.07  | *Roseobacter litoralis* Och 149 |
|  | 3.57  | *Halomonas* sp. HAL1 |  |  |  |
|  | 2.57  | *Pelotomaculum thermopropionicum* SI | *arrA* | 24.08  | *Azoarcus* sp. KH32C |
|  | 2.36  | *Blastococcus saxobsidens* DD2 |  | 20.21  | *Aromatoleum aromaticum* EbN1 |
|  | 2.07  | *Serinicoccus profundi* MCCC 1A05965 |  | 18.39  | *Chlorobium luteolum* DSM 273 |
|  | 1.99  | *Symbiobacterium thermophilum* IAM 14863 |  | 3.37  | *Thauera sp.* MZ1T |
|  | 1.85  | *Sphaerobacter thermophilus* DSM 20745 |  | 1.36  | *Geobacter uraniireducens* |
|  | 1.39  | *Candidatus Nitrospira defluvii* |  | 1.16  | *Alkaliphilus oremlandii* OhILAs |
|  | 1.32  | *Desulfurivibrio alkaliphilus* AHT2 |  | 1.11  | *Desulfonatronospira thiodismutans* ASO3-1 |
|  |  |  |  |  |  |