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Ammonium-oxidizing archaea drive in groundwater contaminated with chlorinated compounds – novel implications of dark oxygen production for contaminated aquifers?

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Microbial dark-oxygen production has been observed recently in marine and groundwater environments carried out by ammonium-oxidizing archaea (AOA), that oxidize ammonium to nitrite as part of their energy requirement and are abundant in environments with high, very low, or depleted oxygen concentrations. Recent investigations have shown that the ammonium-oxidizer *Nitrosopumilus maritimus* can produce oxygen and dinitrogen in the dark (Kraft *et al.,* Science, 2022), and oxygen anomalies in deep aquifers might be explained by microbial dark-oxygen production (Strous *et al.*, Nature Communications, 2023). Here, we investigated the microbiology, hydrology, and geochemistry of five monitoring wells characterized by different contamination levels with the chlorinated compounds perchloroethylene (PCE), trichloroethylene (TCE), cis-dichloroethylene (c-DCE) and vinyl chloride (VC). Electrical Resistivity Imaging (ERI) indicated low bioactivity in the contaminated groundwater plume, which was consistent with total cell numbers ranging from $1.1x10^6$ up to $9.1x10^7$ cells/mL. The range was similar to the background control well ($3.7x10^7$ cells/mL). Plume groundwater geochemistry was distinct from the control well, showing no methane, lower sodium and manganese, but higher sulfate concentrations. PCE and TCE were detected in all contaminated groundwater wells, whereas small concentrations of daughter products (cis-DCE and VC) were only observed in one contaminated well. No trans-DCE or ethene was detected. 16S rRNA sequencing and quantitative (q)PCR of bacterial, archaeal, and dehalogenase genes revealed that dehalogenating bacteria belonging to the phyla Chloroflexi and Deltaproteonacteria are present in very low abundance (below 1% relative abundance). They are likely mostly inactive due to high oxygen concentrations and groundwater redox potential. Surprisingly, we detected an aerobic or facultative anaerobic microbial community consisting of bacteria and archaea, but mostly ammonium-oxidizing archaea (AOA). The microbial communities were dominated by the 2 AOA representatives, *Nitrosopumilus* and *Nitrososphaera* species. The species had a relative abundance of up to 65% and 21% of the overall community, consistent with high total abundances of ammonium-oxidizing genes (*amo*A). Metagenomic analyses linked to culture-based physiological studies are currently in progress to resolve the challenging current observation of the predominance of AOA communities in contaminated groundwater for bioremediation.