



Unveiling complete natural reductive dechlorination mechanisms of chlorinated ethenes in groundwater: Insights from functional gene analysis

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Motivation

This study is aimed to highlight the significance of multi-lines of evidence with molecular biological markers, particularly CE-degrading bacteria and their association with RDase genes on the diverse mechanisms of CEs dechlorination under natural conditions, to provide valuable guidance for monitoring natural attenuation (MNA) strategies implementation for contaminated groundwater.

Materials and method

Two study areas were chosen from two sites: a pesticide manufacturing facility (PMF) and a fluorochemical plant (FCP). At each site, two sets of groundwater monitoring well clusters were installed, namely PMF-1/2 and FCP-1/2. Each well cluster consists of three monitoring wells, in which S/M/D represent monitoring wells screened in shallow/middle/deep aquifers, respectively (Fig. 1c and 1d).

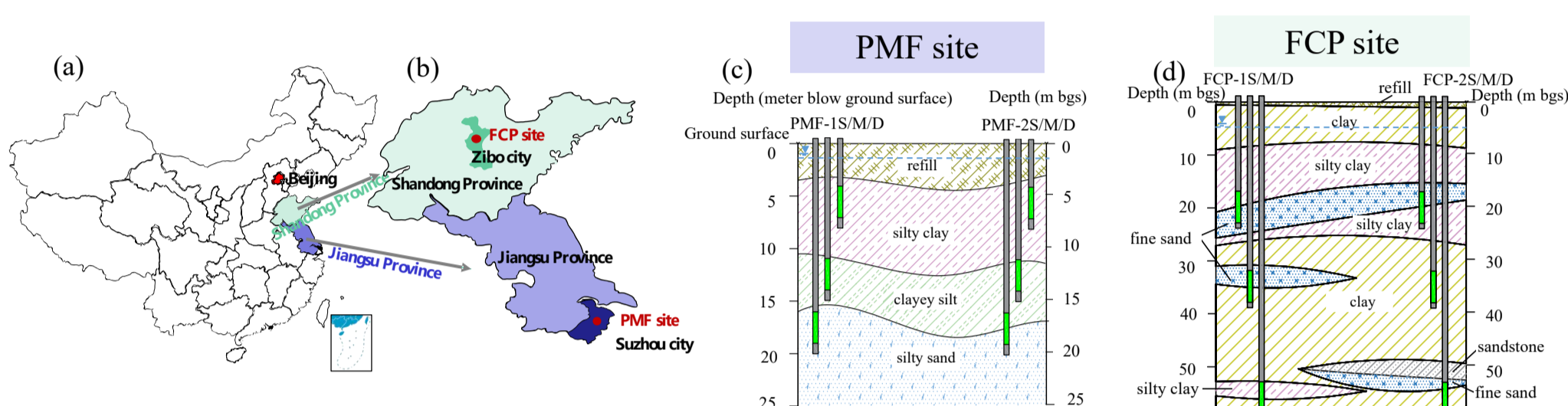


Fig. 1. Site locations (a, b), and layouts of groundwater monitoring wells with hydrogeological strata at the PMF (c) and FCP (d) sites.

Groundwater samples analysis:

- Groundwater measurement: pH, temperature, EC, ORP, DO
- Environmental parameters: TOC, Fe²⁺ - Fe³⁺, SO₄²⁻ - S²⁻, NO₃⁻ - NO₂⁻
- Chlorinated ethenes and ethene: PCE, TCE, DCE, VC, ethene
- Amplicon Sequencing: 16S rRNA sequencing
- qPCR: *Dhc* 16S rRNA, *tceA*, *vcrA*, *bvcA*

Results

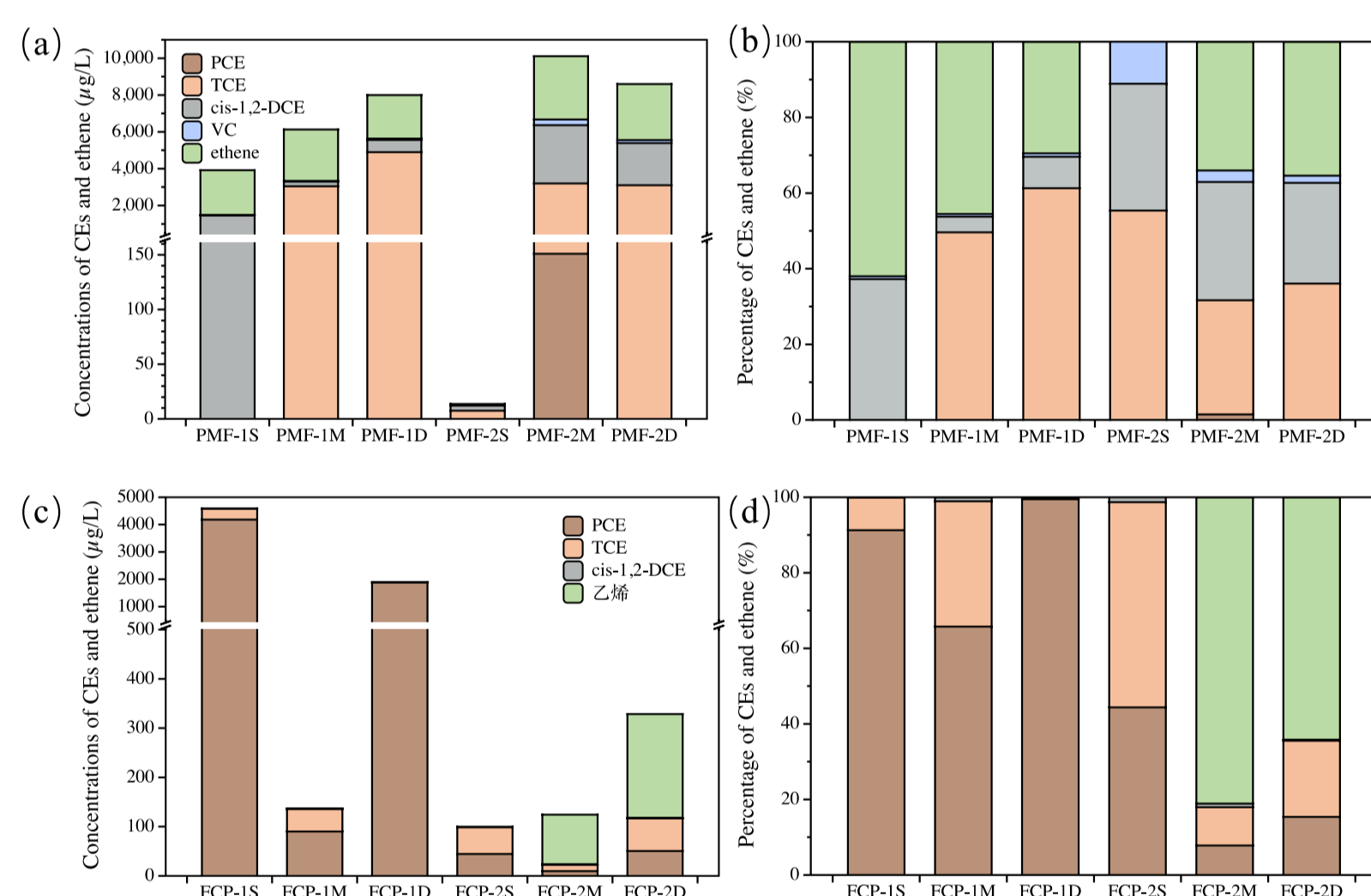


Fig. 2. Concentration and percentage of CEs and ethene in groundwater collected at the PMF(a, b) and FCP(c, d) sites.

Significant amounts of *cis*-1,2-DCE, VC, and ethene were detected at both well clusters in both sites, indicating the typical reductive dechlorination pathway and complete reductive dechlorination under natural conditions. Furthermore, significant amount of ethene, found at FCP-2M and FCP-2D (57-60 m bgs), provides new insights for the treatment of CEs in deep bedrock aquifers.

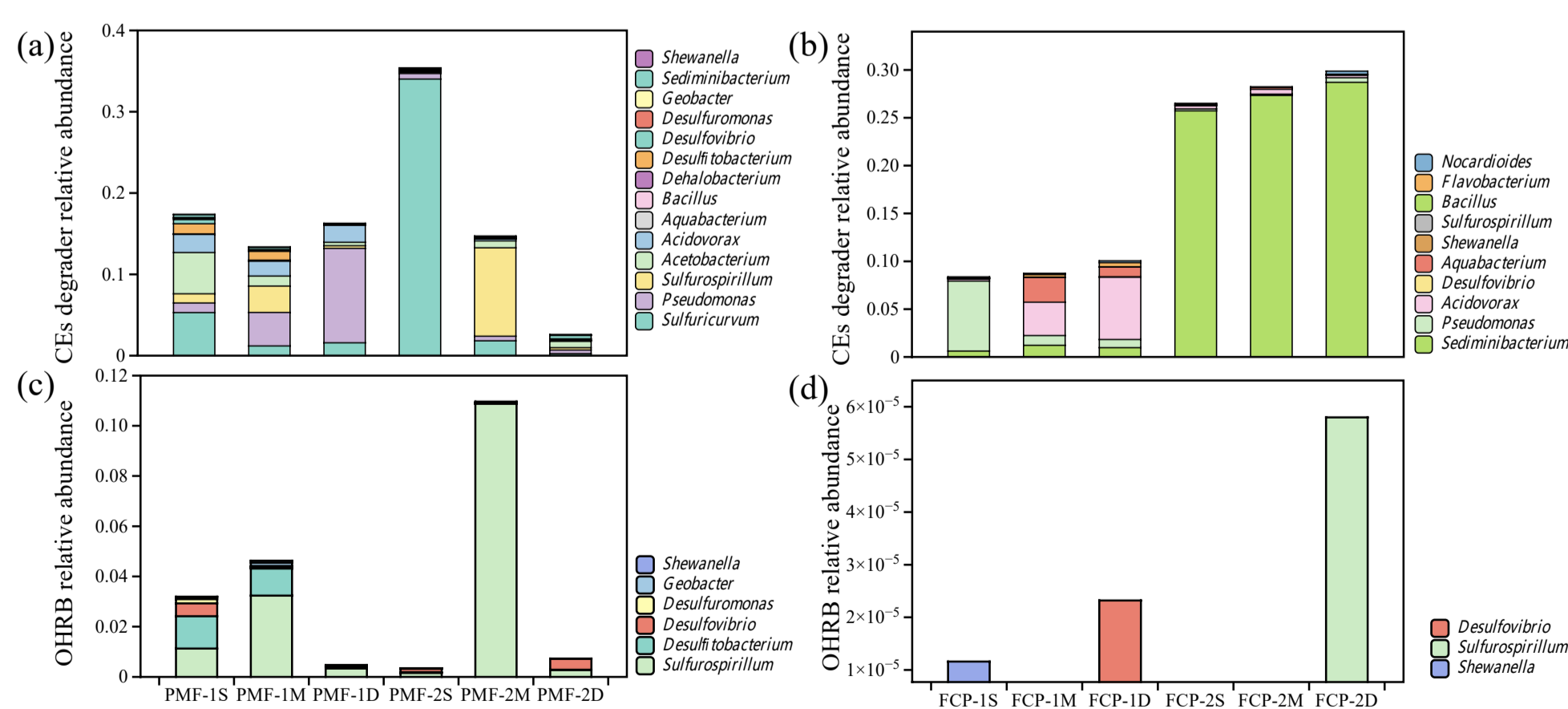


Fig. 3. Relative abundance of CEs degrader and OHRB at PMF (a,c) and FCP(c,d) sites

A higher abundance of CE-degrader and OHRB was identified at the PMF site compared to the FCP site, suggesting heightened dechlorination activity at the PMF site. The higher abundance of *Sediminibacterium* with larger amount of ethene in FCP-2 wells, indicating the potential key role of *Sediminibacterium* for dechlorination, which need further investigation.

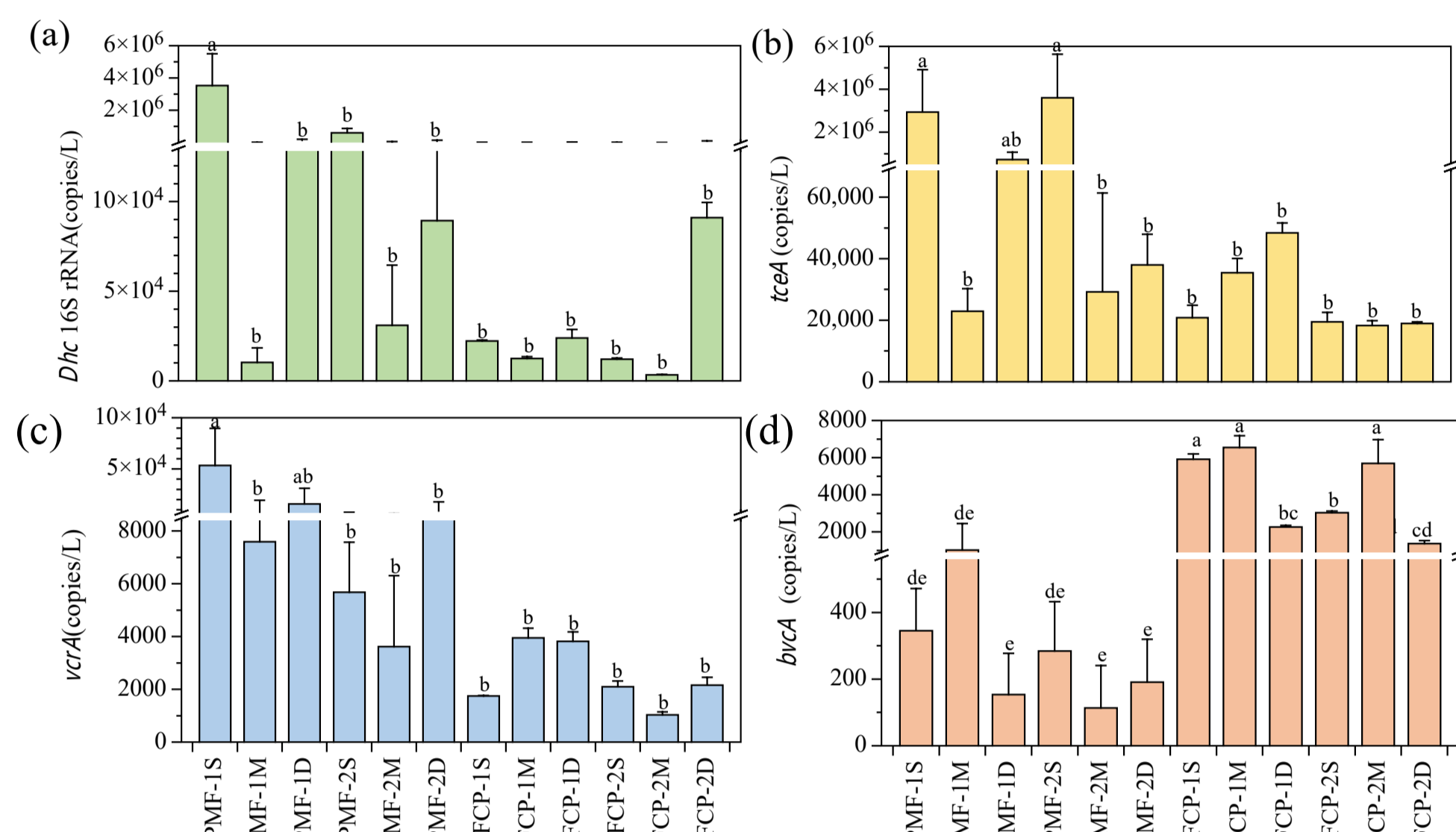


Fig. 4. *Dhc* and RDase genes abundance at the PMF and FCP sites.

Dhc 16S rRNA, *tceA* and *vcrA* are widely abundant at PMF than FCP site, similar to the relative abundance of CE-degrader and OHRB, implying the active dechlorination. However, the higher abundance of *bvcA* was observed at FCP site, indicating its key role for ethene formation at FCP site.

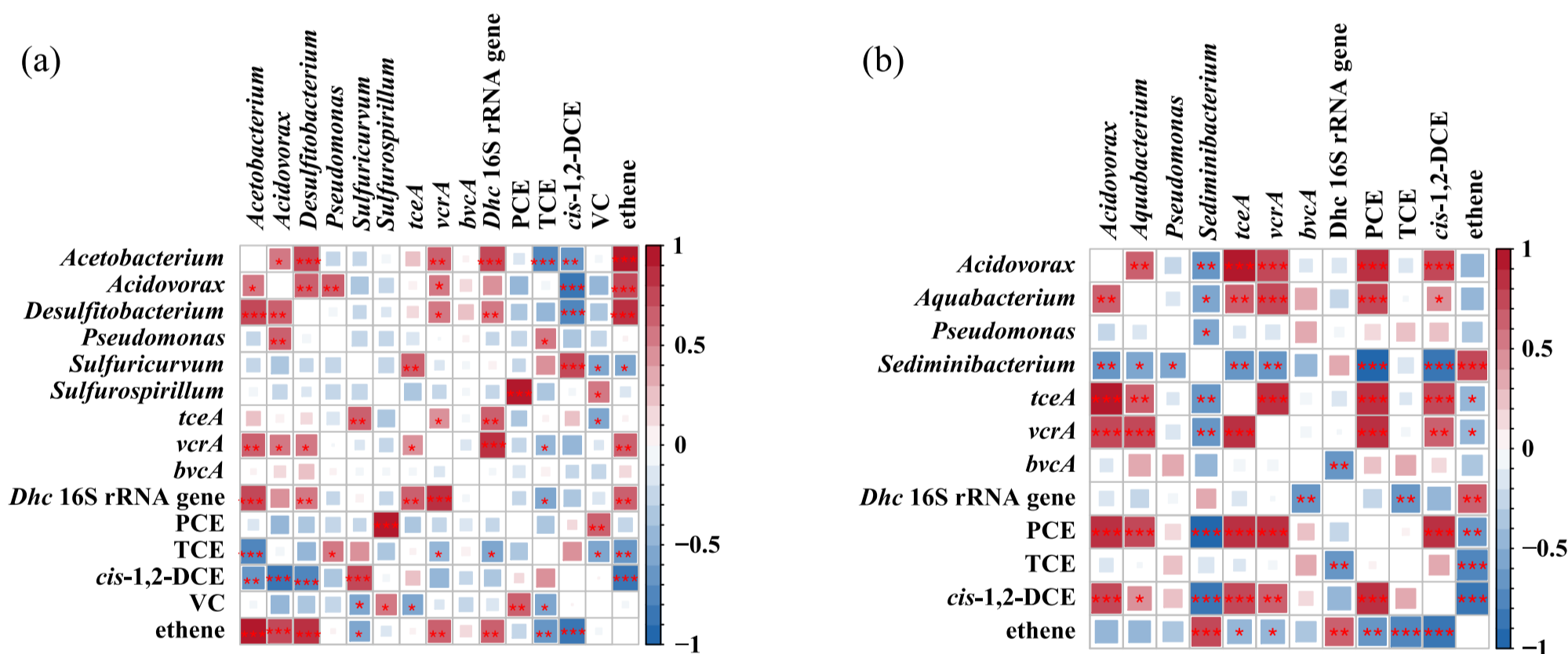


Fig. 5. Relationships between degrading bacteria, genes and CEs with ethene at sites PMF (a) and FCP (b). *** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$.

It was found that *vcrA* contributed to the complete dechlorination of CEs and formation of ethene at the PMF. However, *Sediminibacterium* and *bvcA* played a role for ethene formation at FCP site. Notably, *Sediminibacterium* was firstly reported to have the potential to achieve the complete dechlorination to form ethene at FCP site.

Conclusions

The research presents key insights into natural reductive dechlorination of CEs within groundwater, shedding light on distinct microbial compositions and gene profiles at varied contamination sites. It notably identifies the potential for complete CE dechlorination at great depths in fully weathered bedrock aquifers. Moreover, the study highlights the significance of molecular biological markers, particularly CE-degrading bacteria and their association with RDase genes, serving as effective indicators for evaluating MNA in groundwater remediation. Overall, these findings offer a more comprehensive understanding of reductive dechlorination processes, potentially guiding the development of targeted remediation strategies for contaminated sites.

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