

# Geochemical and microbial community determinants of reductive dechlorination at a site biostimulated with glycerol

Running title: Enhanced reductive dechlorination

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## Quality-Significance Statement

Efforts to enhance reductive dechlorination through biostimulation at contaminated sites are centered around the presence and activity of the key dechlorinating microbe, *Dehalococcoides mccartyi* (*Dcm*). Using an interdisciplinary analysis of geochemical parameters and microbial players, here we provide evidence indicating the importance of syntrophic interactions in sustaining robust dechlorination by *Dcm* at a contaminated site. The identified biogeochemical parameters that drive interlinked microbial networks should be assessed alongside the key *Dcm* for monitoring and steering of bioremediation.

## Summary

Biostimulation is widely used to enhance reductive dechlorination of chlorinated ethenes in contaminated aquifers. However, the knowledge on corresponding biogeochemical responses is limited. In this study glycerol was injected in an aquifer contaminated with *cis*-dichloroethene (cDCE), and geochemical and microbial shifts were followed for 265 days. Consistent with anoxic conditions and sulfate reduction after biostimulation, MiSeq 16S rRNA gene sequencing revealed temporarily increased relative abundance of Firmicutes, Bacteroidetes and sulfate reducing Deltaproteobacteria. In line with <sup>13</sup>C cDCE enrichment and increased *Dehalococcoides mccartyi* (*Dcm*) numbers, dechlorination was observed towards the end of the field experiment, albeit being incomplete with accumulation of vinyl chloride. This was concurrent with i) decreased concentrations of dissolved organic carbon (DOC), reduced relative abundances of fermenting and sulfate reducing bacteria that have been suggested to promote *Dcm* growth by providing electron donor (H<sub>2</sub>) and essential corrinoid cofactors, ii) increased sulfate concentration and increased relative abundance of Epsilonproteobacteria and Deferribacteres as putative oxidizers of reduced sulfur compounds. Strong correlations of DOC, relative abundance of fermenters and sulfate reducers, and dechlorination imply the importance of syntrophic interactions to sustain robust dechlorination. Tracking microbial and

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mediation strategies.

## Introduction

The widespread use of chlorinated ethenes (CEs) as solvents has resulted in severe groundwater contamination (Abelson, 1990). The incomplete transformation of CEs such as tetrachloroethene (PCE) and trichloroethene (TCE) in aquifers leads to the accumulation of *cis*-dichloroethene (cDCE) and vinyl chloride (VC) (Stroo et al., 2012), the latter known as a human carcinogen (Kielhorn et al., 2000). *In situ* bioremediation via enhanced reductive dechlorination (ERD) has become a widely applied remediation approach (Löffler and Edwards, 2006), and is achieved by biostimulation and in some cases bioaugmentation to activate microbial reductive dechlorination by organohalide-respiration (OHR) (Ellis et al., 2000; Major et al., 2002; Lendvay et al., 2003; Scheutz et al., 2010). Organohalide-respiring bacteria (OHRB) conserve energy by OHR (Leys et al., 2013) and belong to distinct bacterial genera distributed among the phyla Chloroflexi, Firmicutes, and Proteobacteria (Atashgahi et al., 2016). While many OHRB can only dechlorinate PCE and TCE to cDCE, *Dehalococcoides mccartyi* (*Dcm*) is capable of dechlorinating CEs to VC and nontoxic ethene. *Dcm* has been recognized as an essential member of the microbial community linked to biotransformation of a broad range of organohalides. This is due to its highly specialized metabolism restricted to OHR, rich repertoire of reductive dehalogenase genes (*rdhAB*) ranging from 10 to 36 copies in a single genome (Löffler et al., 2013) and widespread distribution in pristine and contaminated sites (Hendrickson et al., 2002). *Dcm* growth and dechlorination is more robust in mixed microbial communities as it depends on other organisms to provide hydrogen and acetate as electron donor and carbon source, respectively (Löffler et al., 2013), to produce organic cofactors (Schipp et al., 2013), to scavenge oxygen (Hug et al., 2012) and to remove the carbon monoxide that it produces but cannot tolerate (Zhuang et al., 2014).

Following addition of an electron donor in contaminated aquifers, OHRB will depend on

composition and activity of the resident microbial community, the local geochemical and This article has been accepted for publication and undergone full peer review but has not been through the copy editing, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article' only. doi: 10.1111/1462-2920.13531

biostimulation-induced subsurface feedback demands insight in microbial community dynamics and physiology and their interactions with geochemical and hydrological parameters across space and time. This knowledge is crucial to guide design and optimization of ERD efforts but remains limited. To date, ERD related microbial monitoring efforts have mainly focused on quantitative PCR (qPCR) based tracking of key OHRB and their *rdhA* genes (Lendvay et al., 2003; Lee et al., 2008; Scheutz et al., 2008; Ritalahti et al., 2010; Scheutz et al., 2010; Damgaard et al., 2013; Révész et al., 2014; Sutton et al., 2015b) or occasionally of selected non-dechlorinating microbial guilds (Pérez-De-Mora et al., 2014). While application of qPCR as a specific assay has highly refined diagnostic power and guided bioremediation efforts, it normally assays a limited number of targets and hence provides only fragmented information regarding microbial community composition, interactions between community members and community dynamics in response to ERD. Former studies on biogeochemical trajectories of ERD either applied low resolution microbial community analysis methods such as clone libraries and terminal restriction fragment length polymorphism of PCR-amplified 16S rRNA genes (Lendvay et al., 2003; Macbeth et al., 2004; Rahm et al., 2006) or were not complemented with detailed analysis of geochemical parameters and CE concentration dynamics (Lee et al., 2012). Knowledge of the interaction between geochemical parameters and the microbial communities involved in ERD will give insight into the 'black box' of processes underlying this accepted bioremediation solution. Some studies have addressed microbial community dynamics (Dojka et al., 1998; Bowman et al., 2006) and interactions with geochemical parameters (Hohnstock-Ashe et al., 2001; Lowe et al., 2002; Imfeld et al., 2008; Imfeld et al., 2011; Rossi et al., 2012) at CE contaminated sites undergoing natural attenuation without ERD. However, unlike the steady-state conditions under natural attenuation, biostimulation induces perturbations in the aquifer ecosystem that can reshape both living systems and the abiotic environment (Shade et al., 2012).

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site in an industrial area in Vilvoorde, Belgium (Schneidewind et al., 2014). Before and after injection groundwater geochemical parameters and bacterial community composition were analyzed over a period of 265 days. In parallel, the progress of ERD was followed by enumeration of *Dcm* and relevant *rdhA* genes using qPCR and monitoring of cDCE and VC isotopic signatures using compound specific isotope analysis (CSIA). This way, we aimed to gain an integrated geochemical and microbial insight that can be used to guide the design and optimization of ERD. Results showed how temporal succession of the predominant microbial guilds governed by their known/predicted physiology and aquifer geochemical feedback promoted and/or precluded ERD. Data presented here support the notion that microbial guilds known to sustain robust *Dcm* growth/dechlorination by syntrophic interactions seem pivotal for the success of ERD.

## Results

### *Geochemical characterisation*

Geochemical parameters were stable in the control well throughout the experiment. After biostimulation, DOC in the impacted filters was significantly ( $P < 0.05$ ) higher than before biostimulation over the experiment duration (Fig. S1, Table S1). The opposite pattern was observed for dissolved oxygen (DO) and oxidation-reduction potential (ORP) that declined and were significantly ( $P < 0.05$ ) lower throughout the experiment relative to pre-biostimulation values. ORP and DO slightly increased towards the end of the experiment. Nitrate was not detected. Fe(II) concentrations varied between day 0 and 72 in the down-gradient wells but showed a significant ( $P < 0.05$ ) reduction by day 195 and 265 relative to pre-biostimulation concentrations. This was accompanied by appearance of black precipitate indicative of ferrous sulfide formation. In most samples throughout the experiment, the Fe(II)/Fe ratio was close to 1 indicating lack of reducible Fe(III). Starting from a background concentration of  $463 \pm 133$  mg/l, sulfate steadily decreased to  $212 \pm 116$  mg/l by day 195 in down-gradient wells and rebounded in most wells by day 265. This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13531

### *Reductive dechlorination of CEs, CSIA and qPCR analysis*

The pre-stimulation carbon isotope values of cDCE (average  $\delta^{13}\text{C}$ ,  $-19.3\text{‰} \pm 0.6$ ) and the sum of carbon isotope values of all CEs weighted by their molar fractions (average  $\delta^{13}\text{C}_{\Sigma(\text{CE})}$ ,  $-21.4\text{‰} \pm 0.8$ ) were highly consistent in the seven filters later impacted by biostimulation. By day 195, there was clear onset of cDCE dechlorination with concomitant increase in its  $\delta^{13}\text{C}$  values and increased VC concentration in most filters.  $\delta^{13}\text{C}$  of VC remained more negative compared to cDCE in shallow filters of 2A, 3A and 7A and VC was not further transformed to ethene. Further degradation of cDCE to VC was observed at day 265 with progressive enrichment of cDCE in  $^{13}\text{C}$ . In addition, a significant increase (3‰) in the resultant  $\delta^{13}\text{C}_{\Sigma(\text{CE})}$  relative to the control well provided evidence for incipient dechlorination of VC in filters 2A, 2B and 7A between day 195 and 265 (Fig. 1, Table S2) (Hunkeler et al., 2008). The evidence from CE concentrations and isotopic signatures was further corroborated by over 1-2 orders of magnitude increased abundances of *Dcm* and *vcrA* and *bvcA* genes in the impacted wells relative to the control well. Among the *rdh* genes, *bvcA* became dominant in most filters, whereas *vcrA* was dominant in well 2 reaching above  $10^7$  copies/L by day 265. No ERD was noted in well 5 and the control well, in which cDCE concentration as well as isotopic and qPCR signatures were stable (Fig. 1, Table S2).

### *Impact on bacterial community alpha-diversity*

Biostimulation significantly affected bacterial community alpha-diversity. The post-biostimulation samples (seven samples at each sampling time point, excluding control well samples) showed significantly higher ( $P < 0.05$ ) predicted (Chao 1) and observed OTU richness, and phylogenetic diversity (PD) relative to the pre-biostimulation samples (Fig. 2). No significant difference was found between the post-biostimulation sampling dates, except for the PD at day 195 that was significantly higher ( $P < 0.05$ ) than at day 37 and

72 (Fig. 2). The control well showed progressively increasing alpha-diversity over time. This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13531

### *Bacterial community succession*

The pre-biostimulation bacterial community composition showed high between-sample similarity including the control samples (Fig. S3), and was significantly different from all post-biostimulation communities (Table S4). Remarkably, among the post-biostimulation communities, samples of day 265 were not significantly different from those taken at the control well (Fig. S3, Table S4). At day 0, the bacterial community was dominated by Campylobacterales (Epsilonproteobacteria) followed by Flavobacterales (Bacteroidetes), representing 75% and 18% of the average relative abundance (ARA), respectively (Fig. 3, Table S3). Recorded Campylobacterales comprised members of *Sulfuricurvum*, *Sulfurospirillum* and *Arcobacter* genera, while *Flavobacterium* was the main genus within Flavobacterales (Fig. S4, Table S3). Following biostimulation, clear shifts in the bacterial community structure were noted. Except in the 2B and 7A filters that did not show DOC change at day 37 (Fig. S1, Table S1), Campylobacterales and Flavobacterales were greatly reduced in ARA relative to day 0 and replaced by fermentative members of the Firmicutes (Clostridia and Bacilli orders) and Bacteroidetes (Bacteroidia order) and by sulfate reducing Deltaproteobacteria (Desulfobacterales order) (Fig. 3, Table S3). Within the Campylobacterales members of the genus *Sulfurospirillum* became predominant whereas *Sulfuricurvum*, *Arcobacter* and *Flavobacterium* showed drastic reduction in ARA. Members of the genus *Trichococcus* (Bacilli) within the Firmicutes increased substantially in relative abundance in 5A and 5B filters (Fig. S4, Table S3) that received the highest DOC input, whereas Clostridia genera of *Acetobacterium*, *Clostridium* and *Pelosinus* were noted in most filters. By day 72, Campylobacterales and Flavobacterales dropped to below 20 and 1% ARA, respectively in impacted filters. In contrast, the sulfate reducing Desulfobacterales dominated by *Desulfobulbus* genus flourished followed by Bacteroidia, Clostridia and Bacilli. Desulfobacterales continued to dominate communities observed in day 195 samples while Bacteroidia, Clostridia and Bacilli were fading. Interestingly, at day 195 and day 265, the Campylobacterales became predominant again in 5A and 5B filters. Dehalococcoidetes and *Geobacter* did

not surpass 1% of the community at any time point, and other OHRB including *Dehalobacter*, *Desulfitobacterium*, *Desulfomonile*, *Desulfuromonas* were not detected. Furthermore, there was notable emergence of sequences associated with Deferribacterales (Deferribacteres) and the candidate phylum OD1 by day 195 and 265, which were not observed in pre-biostimulation samples. The bacterial community showed a distinct succession pattern in the control well compared to stimulated wells. While Campylobacterales was the most predominant taxon at day 0 and 37, their relative abundance dropped to 41% by day 72 and below 2% by days 195 and 265. The community in the control well became more diverse over time (Fig. 2) with increased abundance of non-assigned reads and candidate phylum OD1 (Fig. 3, Table S3).

#### *Geochemical and microbial interactions*

The relative abundances of the orders Campylobacterales, Flavobacteriales, Burkholderiales and Pseudomonadales were positively correlated with DO, ORP and sulfate (Fig. 4). Putative fermenters belonging to Clostridiales, Lactobacillales, Bacteroidales and uncultured Bacterioidetes WCHB1-32 showed a positive correlation with DOC whereas DO was inversely correlated with the relative abundance of Clostridiales and uncultured Bacterioidetes WCHB1-32. A negative correlation was noted between Fe(II)/Fe and Dehalococcoidetes.

#### **Discussion**

There is growing interest in using the capacities of indigenous microbial communities to remediate CE contaminated aquifers by ERD. However, the ERD bioremediation efforts are mostly applied as a 'black box' without having fundamental insight into the underlying biogeochemical processes that govern the activity of key OHRB. In order to improve CE contaminated site bioremediation, the biogeochemical shifts induced by biostimulation must be understood, and the abundance and activity of key OHRB must be placed into the context of interlinked microbial networks, including the

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### *Geochemical and microbial dynamics: pre-biostimulation phase*

The almost complete removal of TCE and the dominance of cDCE before biostimulation indicated naturally occurring reductive dechlorination and hence TCE depletion (Table S4) formerly reported to be present at this site (Schneidewind et al., 2014). This could be an explanation for the presence of *Sulfurospirillum* as non-obligate dechlorinator of TCE to cDCE (Luijten et al., 2003) in the pre-biostimulation samples that was also reported from other TCE contaminated aquifers (Macbeth et al., 2004; Rahm et al., 2006; Dugat-Bony et al., 2012). By dechlorinating PCE and TCE to cDCE, *Sulfurospirillum* can facilitate CE dechlorination by *Dcm*. For example, synergistic interactions between *Sulfurospirillum* and *Dcm* members were reported for enrichment cultures where *Sulfurospirillum* dechlorinated PCE to cDCE and *Dcm* dechlorinated cDCE to ethene (Maillard et al., 2011).

These reports imply a potential role of *Sulfurospirillum* at CE contaminated sites. *Sulfurospirillum multivorans* has a distinct genomic region linked to OHR (Goris et al., 2014) that can be used as a target for design of molecular monitoring tools. Some members of this genus are capable of chemolithoautotrophic growth by coupling nitrate and oxygen reduction to the oxidation of sulfide, sulfur and thiosulfate (Eisenmann et al., 1995; Campbell et al., 2006) and therefore, their growth using sulfur released into groundwater from soils, rocks and minerals (Canfield, 2004) coupled to oxygen reduction cannot be excluded. *Sulfuricurvum* as another predominant genus currently contains only one characterised isolate, *Sulfuricurvum kujiense* strain YK-1, an obligate chemolithoautotroph (Kodama and Watanabe, 2004) that grows by oxidation of reduced sulfur compounds coupled to nitrate and oxygen (microaerophilic condition) respiration (Kodama and Watanabe, 2003). Although dechlorination or syntrophic interactions with OHRB by this strain are not reported, *Sulfuricurvum* was detected at TCE contaminated sites (Tsai et al., 2014; Kao et al., 2016) and in 1,2-dichloroethane dechlorinating enrichment cultures (Merlino et al., 2015). Another predominant genus in pre-biostimulation samples was *Flavobacterium* whose members play an important role in

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polysaccharides produced from cell debris in oligotrophic environments (Bernardet and Grimont, 1989; Bernardet et al., 1996).

#### *Geochemical and microbial dynamics: post-biostimulation phase*

Glycerol injection induced cDCE concentration reduction was accompanied by a shift of carbon isotope values. The most dynamic period was after day 72 during which the average  $\delta^{13}\text{C}$  cDCE values increased by +3.9 ‰ at day 195 and by +5.7‰ at day 265 (Fig. 5). This development was reflected by a successional change in groundwater bacterial community composition. There was a sharp increase in Firmicutes (Clostridiales and Lactobacillales) ARA followed by Bacteroidetes (Bacteroidales). These taxa that also showed positive correlation to DOC (Fig. 4) had low initial ARA and decreased again towards the end of the field experiment. Other studies at CE contaminated sites reported a similar transient peak of these microbes upon biostimulation (Macbeth et al., 2004; Lee et al., 2012; Pérez-De-Mora et al., 2014). The emergence of members of the genera *Trichococcus*, *Clostridium* and *Pelosinus* following biostimulation (Fig. S4, Table S3) is likely due to their glycerol fermentation capacity (Biebl, 2001; Moe et al., 2012; van Gelder et al., 2012; Wilkens et al., 2012). Moreover, *Pelosinus* strains were shown to sustain growth of *Dcm* by providing the necessary corrinoids (Men et al., 2014; Men et al., 2015) and were found to co-occur with OHRB in CE contaminated aquifers (Puigserver et al., 2016). The observed putative acetogenic bacteria belonging to *Acetobacterium*, *Clostridium* and Spirochaetaceae (Fig. S4, Table S3) have been proposed to stimulate *Dcm* growth by production of acetate and corrinoid cofactors (Macbeth et al., 2004; He et al., 2007; Pérez-De-Mora et al., 2014).

Sulfate reduction appeared to be the prevalent electron-accepting process at the site. Between day 37 and 195, Desulfobulbaceae flourished that can perform incomplete oxidation of propionate coupled to sulfate reduction (Rabus et al., 2006) yielding acetate and sulfide that can stimulate (Löffler et al., 2013) and inhibit (He et al., 2005) *Dcm* growth, respectively. On the other hand, both glycerol and its degradation product 1,3-

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of the genus *Desulfovibrio* (Qatibi et al., 1991; Qatibi et al., 1998) that were present during the same period. Further, *Desulfovibrio* strains are known to sustain *Dcm* growth by providing hydrogen, acetate and corrinoid cofactors (Men et al., 2012). However, sulfate at >500 mg/l as observed in 3B and 7A filters (Fig. S1, Table S1) might be inhibitory to *Dcm* due to electron donor exhaustion (Hoelen and Reinhard, 2004), though this seems unlikely on the basis of the observed dechlorination at these filters (Fig. 1). Methanogenesis as a sink of hydrogen and acetate (Smatlak et al., 1996) did not seem to be a major concern for OHR except at filters 5A and 5B that showed the highest DOC levels upon biostimulation and enhanced methanogenesis by day 265 (Fig. S1, Table S1) which can be an explanation for the lack of dechlorination at these filters.

#### *Geochemical and microbial dynamics: the control well*

Despite stable geochemical parameters in the up-gradient control well, the bacterial community observed at days 195 and 265 highly diverged from the original state. This could be due to the high hydraulic conductivity of 1-3 m/day at this site (Hamonts et al., 2009) facilitating a rapid dispersion and community succession (Zhou et al., 2014). Accordingly, the pre-biostimulation bacterial community in the stimulated wells was significantly different from the control well (Table S4) whereas the geochemical parameters were similar between the pre-biostimulation and control well samples (Fig. S1, Table S1). On the other hand, the bacterial community in the stimulated wells at day 265 was significantly different from the pre-biostimulation community but not from the control well (Table S4). This indicates that the resident taxa in the impacted wells exploiting the stimulated conditions were also exposed to the arriving immigrant taxa brought by groundwater flow, in line with what has previously been suggested by Shade and co-workers (Shade et al., 2012). However, it was only towards the end of the experiment (and hence fading stimulation) that the immigrant taxa became established in stimulated wells which can be described by the niche occupation concept outlined in Fig. S5. The observed geochemical stability but compositional instability of the control

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relation to site geochemistry/hydrology as baseline prior to biostimulation to better guide intensity, duration and location of post-biostimulation sampling.

#### *Putative sulfur cycle following biostimulation*

After the dominance of sulfate reducers between day 37 and 195 and concurrent sulfate depletion, sulfate concentrations increased again in most wells at day 265. This was accompanied by a substantial reduction of Desulfobulbaceae ARA, re-establishment of initially abundant *Sulfuricurvum* and *Sulfurospirillum*, and appearance of putative sulfur oxidizers i.e. Deferribacteres and the candidate phylum OD1, which were barely detectable in pre-biostimulation samples (Fig. 3). The chemolithoautotrophic oxidation of the reduced sulfur compounds by *Sulfuricurvum* and *Sulfurospirillum* coupled to reduction of DO that was slightly increasing during the late phase may explain the increased sulfate levels. Accordingly, the epsilonproteobacterial Campylobacterales showed positive correlation with DO and ORP (Fig. 4). Enrichment of Epsilonproteobacteria as the successors of Deltaproteobacteria was previously reported during biostimulation of aquifers contaminated with uranium (Handley et al., 2012; Handley et al., 2013) and CEs (Lee et al., 2012). The resulting reduced sulfide levels can in turn support reductive dechlorination by decreasing the toxicity of sulfide to *Dcm* (Hoelen and Reinhard, 2004; He et al., 2005).

#### *Emergence of candidate phylum OD1*

Members of the candidate phylum OD1 (also known as Parcubacteria) (Brown et al., 2015) have mostly been connected with suboxic and anoxic pristine sulfur-rich environments (Elshahed et al., 2005; Briée et al., 2007; Peura et al., 2012). However, OD1 (Gihring et al., 2011; Kocur et al., 2016) and another candidate division, OP11 (Microgenomates), (Lee et al., 2012) also appeared at a later stage during aquifer biostimulation for uranium and CE bioremediation (Elshahed et al., 2005; Wrighton et al., 2012). Interestingly, the study of Kocur et al., (2016) showed dominance of OD1 where

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increased relative abundance at a later aquifer biostimulation stage when the redox potential increased and organic-rich condition faded. Owing to their fermentative lifestyle, the production of hydrogen and organic acids (e.g. acetate) from complex organic materials such as decaying biomass from microbial blooms by these candid phyla can fuel respiratory processes with nitrate, sulfate and Fe(III) (Wrighton et al., 2012; Wrighton et al., 2014) and perhaps CEs as terminal electron acceptors. Interestingly, OD1 has not been reported as member of CE dechlorinating communities in enrichment cultures (Richardson et al., 2002; Dennis et al., 2003; Duhamel et al., 2004; Gu et al., 2004; Freeborn et al., 2005; Yang et al., 2005; Duhamel and Edwards, 2006; Daprato et al., 2007; Rowe et al., 2008; Hug et al., 2012; Sutton et al., 2015a) that usually receive constant supply of easily accessible electron donors. As such, the OD1 is presumably outcompeted by fast-growing microbes and exterminated from the dechlorinating enrichment cultures, circumventing the services provided by this phylum. In line with this hypothesis, OD1 was recently shown as a persistent member of benzene-degrading methanogenic enrichment cultures where the slowly degradable benzene was used as the sole electron donor (Luo et al., 2016).

#### *Resilient and sensitive taxa*

Epsilonproteobacterial *Sulfuricurvum* and *Sulfurospirillum* recovered in ARA towards the end of the experiment, though remarkably only in stimulated wells. Resilience of the members of *Sulfurospirillum* spp. was previously reported in permanganate treated PCE dechlorinating enrichment cultures (Sutton et al., 2015a). This could be due to adaptation of some members like *S. multivorans* to oxic condition by hosting genes encoding key enzymes for detoxification of reactive oxygen species (Goris et al., 2014) and protection of PCE dechlorinating enzyme (PceA) by a peroxidase-like protein (Goris et al., 2015). In contrast, the initially predominant genera *Arcobacter* and *Flavobacterium* did not recover, which could be due to their high sensitivity to biostimulation-induced perturbation. However, they were also barely detectable from the up-gradient control

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well towards the end of the experiment indicating a role of other factors such as hydrology in controlling community succession.

### *Conclusions and perspectives*

Based on the observed geochemical, isotopic and bacterial patterns, a conceptual model of the metabolic interactions within the microbial foodweb during biostimulation was proposed (Fig. 5). cDCE dechlorination was only noted by day 195 and it was not fully converted to ethene likely due to the fading organic-rich condition towards the end of the experiment. Remarkably, during the same period, the relative abundance of non-dechlorinating fermenters and sulfate reducers decreased that are considered to stimulate robust *Dcm* dechlorination by providing organic cofactors such as the key vitamin B12 (Hug et al., 2012; Men et al., 2012). The increased relative abundance of Deferribacteres and Epsilonproteobacteria by the end of the field experiment likely supported the *Dcm* population by reducing sulfide level/toxicity. However, to our knowledge, these taxa are not known to provide the more important organic cofactors such as vitamin B12 needed by *Dcm*, hence they are not likely “sufficient” as companions to sustain *Dcm* growth. In line with this, other studies showed the importance of particular syntrophic partners belonging to Firmicutes and Deltaproteobacteria in providing the “right” cobamide lower base of vitamin B12 that is vital for *Dcm* dechlorination (Men et al., 2014; Men et al., 2015) but cannot be provided even by closely related microbes (Yan et al., 2012; Yan et al., 2013).

In case of incomplete dechlorination, biostimulation is performed together with bioaugmentation with dechlorinating enrichment cultures containing *Dcm* populations but also their non-dechlorinating partners (Rahm et al., 2006; Pérez-De-Mora et al., 2014). In fact, sustained presence of non-dechlorinating guilds that support *Dcm* growth might be the key to success of bioaugmentation as opposed to the initial biostimulation. Accordingly, a recent field biostimulation failed to induce complete dechlorination while dechlorination to ethene was achieved after bioaugmentation. This was concurrent with

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(Pérez-De-Mora et al., 2014). Therefore, in addition to monitoring dechlorinating guilds, future ERD monitoring efforts must consider composition and successional patterns of supportive non-dechlorination community members as well as geochemical factors controlling them to ensure robust *Dcm* growth and activity. This knowledge is pivotal to establish and maintain the required syntrophic relationships for OHR under challenging field conditions.

## **Experimental procedure**

### *Site description, treatment and sampling procedures*

At the contaminated site, a 1.4 km-wide groundwater plume mainly contaminated with cDCE flows towards the Zenne River in a northwesterly direction (Supplementary Fig. S6). Four injection wells were used to inject a single pulse of a glycerol:water mixture (1 : 3 v/v) by sonic drilling injection, at 20 L per vertical meter from 7 to 13 meters below surface (mbs) at each injection point. Eight monitoring wells were used for groundwater sampling and monitoring. Seven wells were located downstream of the injection points (wells 1-7) and one upstream (well 0, designated as the control well). All monitoring wells contained a shallow filter at 7-9 mbs (A-filters) and a deep filter at 11-13 mbs (B-filters), except the control well that only contained a shallow filter. Groundwater was sampled from the control well and the wells impacted by glycerol injection (wells 2, 3, 5, 7A; Supplementary Fig. S6) on day 0 (March 15<sup>th</sup> 2011) before glycerol injection, and 37, 72, 195 and 265 days after injection as outlined in supplementary information.

### *DNA extraction and qPCR*

For DNA extraction, 2 L groundwater samples were vacuum-filtered over a 0.22 µm membrane filter (Millipore, USA), and filters were stored at -80 °C. Filters were cut into small strips for DNA extraction by the FastDNA Spin Kit for Soil (MP Biomedicals). qPCRs were performed in triplicate in 25-µL reactions in an iQ5 iCycler using the iQ SYBR Green Supermix kit (Bio-Rad, Veenendaal, the Netherlands). A list of target genes, primers and

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curves were obtained using serial dilutions of a known concentration of plasmid DNA containing a suitable fragment of the target genes.

#### *Bacterial community analysis*

A 2-step PCR strategy was used to generate barcoded amplicons from the V1-V2 region of the 16S rRNA gene (primers and thermal cycling conditions shown in Table S6). The first PCR (50  $\mu$ l) contained 10  $\mu$ l 5 $\times$  HF buffer (Thermo Scientific<sup>TM</sup>, The Netherlands), 1  $\mu$ l dNTP Mix (10 mM; Promega, Leiden, The Netherlands), 1 U of Phusion<sup>®</sup> Hot Start II High-Fidelity DNA polymerase (Thermo Scientific<sup>TM</sup>), 500 nM of 27F-DegS forward primer, 500 nM of 338R I and II reverse primers, and 1  $\mu$ L template DNA (15-20 ng/ $\mu$ l). The forward and reverse primers were appended at the 5' end with 18 bp Universal Tag (Unitag) 1 and 2, respectively. The PCR product size was examined by gel electrophoresis. The second PCR (100  $\mu$ l) contained 20  $\mu$ L 5 $\times$  HF buffer, 2  $\mu$ l dNTP Mix, 2 U of Phusion<sup>®</sup> Hot Start II High-Fidelity DNA polymerase, 500 nM of a forward and reverse primer equivalent to the Unitag1 and Unitag2 sequences, respectively, that were each appended with an 8 nt sample specific barcode (Ramiro-Garcia J et al., 2016) at the 5' end, and 5  $\mu$ l PCR product of the first reaction. The PCR product size was examined by gel electrophoresis, purified with HighPrep<sup>TM</sup> (Magbio Genomics, Rockville, MD, USA) and quantified using a Qubit 2.0 Fluorometer (Life Technologies, Darmstadt, Germany) in combination with the dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA). Purified PCR products were pooled, underwent adaptor ligation and sequenced on a MiSeq platform (GATC-Biotech, Konstanz, Germany).

#### *Analysis of the MiSeq data*

NG-Tax, an in-house pipeline (Ramiro-Garcia J et al., 2016) was used for the analysis of the 16S rRNA gene sequencing data. In brief, paired-end libraries were filtered to contain only read pairs with perfectly matching barcodes, and those barcodes were used to separate reads by sample. Finally operational taxonomic units (OTUs) were assigned

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database (Quast et al., 2013). Bacterial composition plots were generated using a workflow based on Quantitative Insights Into Microbial Ecology (QIIME) v1.2 (Caporaso et al., 2010).

#### *Chemical and carbon isotope analysis:*

Concentrations of CEs, ethene, ethane, and methane in groundwater samples were determined on a Varian GC-FID (CP-3800) as described previously (Atashgahi et al., 2013). Sulfate concentrations were analyzed by ion chromatography using a Dionex DX-120 ion chromatograph equipped with a Dionex AS14A column (Dionex, Sunnyvale, CA). Concentrations of aqueous Fe(II), total Fe and NO<sub>3</sub>-N were determined using HACH kits (HACH, USA) according the manufacturer's instructions. Dissolved organic carbon (DOC) was determined from samples as the difference between total dissolved carbon and dissolved inorganic carbon, measured with a Shimadzu TOC-5000 analyzer equipped with an ASI-5000 auto-sampler. The stable carbon isotope composition of CEs in the groundwater samples were determined as described in supporting information. Concentration-weighted average values of the isotope signature for CEs ( $\Sigma(\text{EC})$ ) was calculated by weighting compound-specific values  $\delta^{13}\text{C}$  weighted by their molar fractions (x) (Aeppli et al., 2010):

$$\delta^{13}\text{C}_{\Sigma(\text{CE})} = \delta^{13}\text{C}_{\text{DCE}}x_{\text{DCE}} + \delta^{13}\text{C}_{\text{VC}}x_{\text{VC}}$$

#### *Statistical analysis*

In order to compare microbial communities observed at different times and locations, different statistical approaches were used based on Bray–Curtis distances. PERMANOVA (Permutational Multivariate Analysis of Variance) (Anderson, 2001) and ANOSIM (Analysis of Similarity) (Clarke, 1993) were performed using the Fathom Toolbox for Matlab (Jones, 2015). We used 10<sup>4</sup> permutations to assess significance of observed differences. MixOmics R package (Lê Cao et al., 2009; Dejean S, 2011) was used to

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regression analysis. Sparse partial least squares was used for simultaneous variable selection and integration to avoid selection of spurious associations. Geochemical and microbial data were set as dependent and independent variables, respectively. Interchanging both sets did not significantly impact the results. An additional threshold on correlation values was imposed by retaining only the 20% most relevant associations in the final network.

#### *Nucleotide sequences*

Nucleotide sequence data reported are available at the European Bioinformatics Institute under accession number PRJEB13312.

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#### **References**

Abelson, P.H. (1990) Inefficient remediation of ground-water pollution. *Science* **250**: 733.

Aeppli, C., Hofstetter, T.B., Amaral, H.I.F., Kipfer, R., Schwarzenbach, R.P., and Berg, M. (2010) Quantifying in situ transformation rates of chlorinated ethenes by combining compound-specific stable isotope analysis, groundwater dating, and carbon isotope mass balances. *Environ Sci Technol* **44**: 3705-3711.

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**: 32-46.

Atashgahi, S., Lu, Y., and Smidt, H. (2016) Overview of known organohalide-respiring bacteria - phylogenetic diversity and environmental distribution. In *Organohalide-Respiring Bacteria*, Adrian, L. and Löffler, F.E. (eds.). Springer Berlin Heidelberg, pp. 63-

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Atashgahi, S., Maphosa, F., Doğan, E., Smidt, H., Springael, D., and Dejonghe, W. (2013) Small-scale oxygen distribution determines the vinyl chloride biodegradation pathway in surficial sediments of riverbed hyporheic zones. *FEMS Microbiol Ecol* **84**: 133-142.

Bernardet, J.F., and Grimont, P.A.D. (1989) Deoxyribonucleic acid relatedness and phenotypic characterization of *Flexibacter columnaris* sp. nov., nom. rev., *Flexibacter psychrophilus* sp. nov., nom. rev., and *Flexibacter maritimus* Wakabayashi, Hikida, and Masumura 1986. *Int J Syst Bacteriol* **39**: 346-354.

Bernardet, J.F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K., and Vandamme, P. (1996) Cutting a gordian knot: Emended classification and description of the genus *Flavobacterium*, emended description of the family Flavobacteriaceae, and proposal of *Flavobacterium hydatis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* **46**: 128-148.

Biebl, H. (2001) Fermentation of glycerol by *Clostridium pasteurianum* - Batch and continuous culture studies. *J Ind Microbiol Biotechnol* **27**: 18-26.

Bowman, K.S., Moe, W.M., Rash, B.A., Bae, H.S., and Rainey, F.A. (2006) Bacterial diversity of an acidic Louisiana groundwater contaminated by dense nonaqueous-phase liquid containing chloroethanes and other solvents. *FEMS Microbiol Ecol* **58**: 120-133.

Bri e, C., Moreira, D., and L opez-Garc ıa, P. (2007) Archaeal and bacterial community composition of sediment and plankton from a suboxic freshwater pond. *Res Microbiol* **158**: 213-227.

Brown, C.T., Hug, L.A., Thomas, B.C., Sharon, I., Castelle, C.J., Singh, A. et al. (2015) Unusual biology across a group comprising more than 15% of domain Bacteria. *Nature* **523**: 208-211.

Campbell, B.J., Engel, A.S., Porter, M.L., and Takai, K. (2006) The versatile  $\epsilon$ -proteobacteria: Key players in sulphidic habitats. *Nat Rev Microbiol* **4**: 458-468.

Canfield, D.E. (2004) The evolution of the Earth surface sulfur reservoir. *Am J Sci* **304**: 839-861.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K. et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**: 335-336.

Clarke, K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Aust J Ecol* **18**: 117-143.

Damgaard, I., Bjerg, P.L., Jacobsen, C.S., Tsitonaki, A., Kern-Jespersen, H., and Broholm, M.M. (2013) Performance of full-scale enhanced reductive dechlorination in clay till. *Groundwater Monit Remediat* **33**: 48-61.

Daprato, R.C., L offler, F.E., and Hughes, J.B. (2007) Comparative analysis of three tetrachloroethene to ethene halo-respiring consortia suggests functional redundancy. *Environ Sci Technol* **41**: 2261-2269.

Dejean S, G.I., L e Cao K-A, Monget P. (2011) mixOmics: Omics data integration project. In: R Package version. 2-9.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

Dennis, P.C., Sleep, B.E., Fulthorpe, R.R., and Liss, S.N. (2003) Phylogenetic analysis of bacterial populations in an anaerobic microbial consortium capable of degrading saturation concentrations of tetrachloroethylene. *Can J Microbiol* **49**: 15-27.

Dojka, M.A., Hugenholtz, P., Haack, S.K., and Pace, N.R. (1998) Microbial diversity in a hydrocarbon- and chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation. *Appl Environ Microbiol* **64**: 3869-3877.

Dugat-Bony, E., Biderre-Petit, C., Jaziri, F., David, M.M., Denonfoux, J., Lyon, D.Y. et al. (2012) In situ TCE degradation mediated by complex dehalorespiring communities during biostimulation processes. *Microbial Biotechnol* **5**: 642-653.

Duhamel, M., and Edwards, E.A. (2006) Microbial composition of chlorinated ethene-degrading cultures dominated by *Dehalococcoides*. *FEMS Microbiol Ecol* **58**: 538-549.

Duhamel, M., Mo, K., and Edwards, E.A. (2004) Characterization of a highly enriched *Dehalococcoides*-containing culture that grows on vinyl chloride and trichloroethene. *Appl Environ Microbiol* **70**: 5538-5545.

Eisenmann, E., Beuerle, J., Sulger, K., Kroneck, P.M.H., and Schumacher, W. (1995) Lithotrophic growth of *Sulfurospirillum deleyianum* with sulfide as electron donor coupled to respiratory reduction of nitrate to ammonia. *Arch Microbiol* **164**: 180-185.

Ellis, D.E., Lutz, E.J., Odom, J.M., Buchanan Jr, R.J., Bartlett, C.L., Lee, M.D. et al. (2000) Bioaugmentation for accelerated in situ anaerobic bioremediation. *Environ Sci Technol* **34**: 2254-2260.

Elshahed, M.S., Najjar, F.Z., Aycock, M., Qu, C., Roe, B.A., and Krumholz, L.R. (2005) Metagenomic analysis of the microbial community at Zodletone Spring (Oklahoma): Insights into the genome of a member of the novel candidate division OD1. *Appl Environ Microbiol* **71**: 7598-7602.

Freeborn, R.A., West, K.A., Bhupathiraju, V.K., Chauhan, S., Rahm, B.G., Richardson, R.E., and Alvarez-Cohen, L. (2005) Phylogenetic analysis of TCE-dechlorinating consortia enriched on a variety of electron donors. *Environ Sci Technol* **39**: 8358-8368.

Gihring, T.M., Zhang, G., Brandt, C.C., Brooks, S.C., Campbell, J.H., Carroll, S. et al. (2011) A limited microbial consortium is responsible for extended bioreduction of uranium in a contaminated aquifer. *Appl Environ Microbiol* **77**: 5955-5965.

Goris, T., Schubert, T., Gadkari, J., Wubet, T., Tarkka, M., Buscot, F. et al. (2014) Insights into organohalide respiration and the versatile catabolism of *Sulfurospirillum multivorans* gained from comparative genomics and physiological studies. *Environ Microbiol* **16**: 3562-3580.

Goris, T., Schiffmann, C.L., Gadkari, J., Schubert, T., Seifert, J., Jehmlich, N. et al. (2015) Proteomics of the organohalide-respiring Epsilonproteobacterium *Sulfurospirillum multivorans* adapted to tetrachloroethene and other energy substrates. *Sci Rep* **5**: 13794.

Gu, A.Z., Hedlund, B.P., Staley, J.T., Strand, S.E., and Stensel, H.D. (2004) Analysis and comparison of the microbial community structures of two enrichment cultures capable of reductively dechlorinating TCE and cis-DCE. *Environ Microbiol* **6**: 45-54.

Hamonts, K., Kuhn, T., Maesen, M., Bronders, J., Lookman, R., Kalka, H. et al. (2009) Factors determining the attenuation of chlorinated aliphatic hydrocarbons in eutrophic

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

river sediment impacted by discharging polluted groundwater. *Environ Sci Technol* **43**: 5270-5275.

Handley, K.M., Wrighton, K.C., Piceno, Y.M., Andersen, G.L., DeSantis, T.Z., Williams, K.H. et al. (2012) High-density PhyloChip profiling of stimulated aquifer microbial communities reveals a complex response to acetate amendment. *FEMS Microbiol Ecol* **81**: 188-204.

Handley, K.M., VerBerkmoes, N.C., Steefel, C.I., Williams, K.H., Sharon, I., Miller, C.S. et al. (2013) Biostimulation induces syntrophic interactions that impact C, S and N cycling in a sediment microbial community. *ISME J* **7**: 800-816.

He, J., Holmes, V.F., Lee, P.K.H., and Alvarez-Cohen, L. (2007) Influence of vitamin B12 and cocultures on the growth of *Dehalococcoides* isolates in defined medium. *Appl Environ Microbiol* **73**: 2847-2853.

He, J., Sung, Y., Krajmalnik-Brown, R., Ritalahti, K.M., and Löffler, F.E. (2005) Isolation and characterization of *Dehalococcoides* sp. strain FL2, a trichloroethene (TCE)- and 1,2-dichloroethene-respiring anaerobe. *Environ Microbiol* **7**: 1442-1450.

Hendrickson, E.R., Payne, J.A., Young, R.M., Starr, M.G., Perry, M.P., Fahnestock, S. et al. (2002) Molecular analysis of *Dehalococcoides* 16S ribosomal DNA from chloroethene-contaminated sites throughout North America and Europe. *Appl Environ Microbiol* **68**: 485-495.

Hoelen, T.P., and Reinhard, M. (2004) Complete biological dehalogenation of chlorinated ethylenes in sulfate containing groundwater. *Biodegradation* **15**: 395-403.

Hohnstock-Ashe, A.M., Plummer, S.M., Yager, R.M., Baveye, P., and Madsen, E.L. (2001) Further biogeochemical characterization of a trichloroethene-contaminated fractured dolomite aquifer: Electron source and microbial communities involved in reductive dechlorination. *Environ Sci Technol* **35**: 4449-4456.

Hug, L.A., Beiko, R.G., Rowe, A.R., Richardson, R.E., and Edwards, E.A. (2012) Comparative metagenomics of three *Dehalococcoides*-containing enrichment cultures: the role of the non-dechlorinating community. *BMC Genomics* **13**: 327.

Hunkeler, D., Meckenstock, R.U., Sherwood Lollar, B., Schmidt, T.C., and Wilson, J. (2008) *A guide for assessing biodegradation and source identification of organic ground water contaminants using compound specific isotope analysis (CSIA)*. EPA 600/R-08/148: Office of Research and Development, National Risk Management Research Laboratory, US Environmental Protection Agency.

Imfeld, G., Nijenhuis, I., Nikolausz, M., Zeiger, S., Paschke, H., Drangmeister, J. et al. (2008) Assessment of in situ degradation of chlorinated ethenes and bacterial community structure in a complex contaminated groundwater system. *Water Res* **42**: 871-882.

Imfeld, G., Pieper, H., Shani, N., Rossi, P., Nikolausz, M., Nijenhuis, I. et al. (2011) Characterization of groundwater microbial communities, dechlorinating bacteria, and in situ biodegradation of chloroethenes along a vertical gradient. *Water Air Soil Pollut* **221**: 107-122.

Jones, D. (2015) The Fathom Toolbox for MATLAB: software for multivariate ecological and oceanographic data analysis. College of Marine Science, University of South Florida, Tampa, FL, USA. Available from: <http://www.marine.usf.edu/user/djones/>.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

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Kao, C.M., Liao, H.Y., Chien, C.C., Tseng, Y.K., Tang, P., Lin, C.E., and Chen, S.C. (2016) The change of microbial community from chlorinated solvent-contaminated groundwater after biostimulation using the metagenome analysis. *J Haz Mat* **302**: 144-150.

Kielhorn, J., Melber, C., Wahnschaffe, U., Aitio, A., and Mangelsdorf, I. (2000) Vinyl chloride: Still a cause for concern. *Environ Health Perspect* **108**: 579-588.

Kocur, C.M.D., Lomheim, L., Molenda, O., Weber, K.P., Austrins, L.M., Sleep, B.E. et al. (2016) Long-Term field study of microbial community and dechlorinating activity following carboxymethyl cellulose-stabilized nanoscale zero-valent iron injection. *Environ Sci Technol* **50**: 7658-7670.

Kodama, Y., and Watanabe, K. (2004) *Sulfuricurvum kujiense* gen. nov., sp. nov., a facultatively anaerobic, chemolithoautotrophic, sulfur-oxidizing bacterium isolated from an underground crude-oil storage cavity. *Int J Syst Evol Microbiol* **54**: 2297-2300.

Lê Cao, K.A., González, I., and Déjean, S. (2009) IntegrOmics: An R package to unravel relationships between two omics datasets. *Bioinformatics* **25**: 2855-2856.

Lee, P.K.H., Macbeth, T.W., Sorenson Jr, K.S., Deeb, R.A., and Alvarez-Cohen, L. (2008) Quantifying genes and transcripts to assess the in situ physiology of "*Dehalococcoides*" spp. in a trichloroethene-contaminated groundwater site. *Appl Environ Microbiol* **74**: 2728-2739.

Lee, P.K.H., Warnecke, F., Brodie, E.L., MacBeth, T.W., Conrad, M.E., Andersen, G.L., and Alvarez-Cohen, L. (2012) Phylogenetic microarray analysis of a microbial community performing reductive dechlorination at a TCE-contaminated site. *Environ Sci Technol* **46**: 1044-1054.

Lendvay, J.M., Löffler, F.E., Dollhopf, M., Aiello, M.R., Daniels, G., Fathepure, B.Z. et al. (2003) Bioreactive barriers: A comparison of bioaugmentation and biostimulation for chlorinated solvent remediation. *Environ Sci Technol* **37**: 1422-1431.

Leys, D., Adrian, L., and Smidt, H. (2013) Organohalide respiration: Microbes breathing chlorinated molecules. *Phil Trans R Soc B: Biol Sci* **368**: 20120316.

Löffler, F.E., and Edwards, E.A. (2006) Harnessing microbial activities for environmental cleanup. *Curr Opin Biotechnol* **17**: 274-284.

Löffler, F.E., Yan, J., Ritalahti, K.M., Adrian, L., Edwards, E.A., Konstantinidis, K.T. et al. (2013) *Dehalococcoides mccartyi* gen. nov., sp. nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation, belong to a novel bacterial class, *Dehalococcoidia classis nov.*, order *Dehalococcoidales ord. nov.* and family *Dehalococcoidaceae fam. nov.*, within the phylum *Chloroflexi*. *Int J Syst Evol Microbiol* **63**: 625-635.

Lowe, M., Madsen, E.L., Schindler, K., Smith, C., Emrich, S., Robb, F., and Halden, R.U. (2002) Geochemistry and microbial diversity of a trichloroethene-contaminated Superfund site undergoing intrinsic in situ reductive dechlorination. *FEMS Microbiol Ecol* **40**: 123-134.

Luijten, M.L.G.C., de Weert, J., Smidt, H., Boschker, H.T.S., de Vos, W.M., Schraa, G., and Stams, A.J.M. (2003) Description of *Sulfurospirillum halorespirans* sp. nov., an anaerobic, tetrachloroethene-respiring bacterium, and transfer of *Dehalospirillum multivorans* to the genus *Sulfurospirillum* a *Sulfurospirillum multivorans* comb. nov. *Int J Syst Evol Microbiol* **53**: 787-793.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Wiley-Blackwell and Society for Applied Microbiology

Luo, F., Devine, C.E., and Edwards, E.A. (2016) Cultivating microbial dark matter in benzene-degrading methanogenic consortia. *Environ Microbiol.* doi:10.1111/1462-2920.13121

Macbeth, T.W., Cummings, D.E., Spring, S., Petzke, L.M., and Sorenson Jr, K.S. (2004) Molecular characterization of a dechlorinating community resulting from in situ biostimulation in a trichloroethene-contaminated deep, fractured basalt aquifer and comparison to a derivative laboratory culture. *Appl Environ Microbiol* **70**: 7329-7341.

Maillard, J., Charnay, M.P., Regeard, C., Rohrbach-Brandt, E., Rouzeau-Szynalski, K., Rossi, P., and Holliger, C. (2011) Reductive dechlorination of tetrachloroethene by a stepwise catalysis of different organohalide-respiring bacteria and reductive dehalogenases. *Biodegradation* **22**: 949-960.

Major, D.W., McMaster, M.L., Cox, E.E., Edwards, E.A., Dworatzek, S.M., Hendrickson, E.R. et al. (2002) Field demonstration of successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene. *Environ Sci Technol* **36**: 5106-5116.

Men, Y., Seth, E.C., Yi, S., Allen, R.H., Taga, M.E., and Alvarez-Cohen, L. (2014) Sustainable growth of *Dehalococcoides mccartyi* 195 by corrinoid salvaging and remodeling in defined lactate-fermenting consortia. *Appl Environ Microbiol* **80**: 2133-2141.

Men, Y., Seth, E.C., Yi, S., Crofts, T.S., Allen, R.H., Taga, M.E., and Alvarez-Cohen, L. (2015) Identification of specific corrinoids reveals corrinoid modification in dechlorinating microbial communities. *Environ Microbiol* **17**: 4873-4884.

Men, Y., Feil, H., Verberkmoes, N.C., Shah, M.B., Johnson, D.R., Lee, P.K.H. et al. (2012) Sustainable syntrophic growth of *Dehalococcoides ethenogenes* strain 195 with *Desulfovibrio vulgaris* hildenborough and *Methanobacterium congolense*: Global transcriptomic and proteomic analyses. *ISME J* **6**: 410-421.

Merlino, G., Balloi, A., Marzorati, M., Mapelli, F., Rizzi, A., Lavazza, D. et al. (2015) Diverse reductive dehalogenases are associated with clostridiales-enriched microcosms dechlorinating 1,2-dichloroethane. *BioMed Res Int* **2015**: 242856.

Moe, W.M., Stebbing, R.E., Rao, J.U., Bowman, K.S., Fernanda Nobre, M., da Costa, M.S., and Rainey, F.A. (2012) *Pelosinus defluvii* sp. nov., isolated from chlorinated solvent-contaminated groundwater, emended description of the genus *Pelosinus* and transfer of *Sporotalea propionica* to *Pelosinus propionicus* comb. nov. *Int J Syst Evol Microbiol* **62**: 1369-1376.

Pérez-De-Mora, A., Zila, A., McMaster, M.L., and Edwards, E.A. (2014) Bioremediation of chlorinated ethenes in fractured bedrock and associated changes in dechlorinating and nondechlorinating microbial populations. *Environ Sci Technol* **48**: 5770-5779.

Peura, S., Eiler, A., Bertilsson, S., Nykänen, H., Tirola, M., and Jones, R.I. (2012) Distinct and diverse anaerobic bacterial communities in boreal lakes dominated by candidate division OD1. *ISME J* **6**: 1640-1652.

Puigserver, D., Herrero, J., Torres, M., Cortés, A., Nijenhuis, I., Kuntze, K. et al. (2016) Reductive dechlorination in recalcitrant sources of chloroethenes in the transition zone between aquifers and aquitards. *Environ Sci Pollut Res.* DOI 10.1007/s11356-016-7068-4.

Qatibi, A.I., Bories, A., and Garcia, J.L. (1991) Sulfate reduction and anaerobic glycerol fermentation by *Desulfohalobium* sp. nov. *Environ Microbiol* **22**: 117-122.

This article has been accepted for publication and underwent peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

Qatibi, A.I., Bennisse, R., Jana, M., and Garcia, J.L. (1998) Anaerobic degradation of glycerol by *Desulfovibrio fructosovorans* and *D. carbinolicus* and evidence for glycerol-dependent utilization of 1,2-propanediol. *Curr Microbiol* **36**: 283-290.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2013) The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res* **41**: D590-D596.

Rabus, R., Hansen, T.A., and Widdel, F. (2006) Dissimilatory sulfate- and sulfur-reducing prokaryotes. In *The Prokaryotes*, Rosenberg, E., DeLong, E.F., Stackenbrandt, E., Lory, S., Thompson, F. (eds.). New York: Springer-Verlag, pp. 659-768.

Rahm, B.G., Chauhan, S., Holmes, V.F., Macbeth, T.W., Sorenson Jr, K.S., and Alvarez-Cohen, L. (2006) Molecular characterization of microbial populations at two sites with differing reductive dechlorination abilities. *Biodegradation* **17**: 523-534.

Ramiro-Garcia J, Hermes G, Giatsis C, Sipkema D, Zoetendal EG, PJ, S., and H, S. (2016) A highly accurate and validated pipeline for analysis of 16S rRNA amplicons from complex biomes. [version 1 referees: awaiting peer review]. *F1000Research* **5**: 1791. doi: 10.12688/f1000research.9227.1

Révész, K.M., Lollar, B.S., Kirshtein, J.D., Tiedeman, C.R., Imbrigiotta, T.E., Goode, D.J. et al. (2014) Integration of stable carbon isotope, microbial community, dissolved hydrogen gas, and  $^2\text{H}_{\text{H}_2\text{O}}$  tracer data to assess bioaugmentation for chlorinated ethene degradation in fractured rocks. *J Contam Hydrol* **156**: 62-77.

Richardson, R.E., Bhupathiraju, V.K., Song, D.L., Goulet, T.A., and Alvarez-Cohen, L. (2002) Phylogenetic characterization of microbial communities that reductively dechlorinate TCE based upon a combination of molecular techniques. *Environ Sci Technol* **36**: 2652-2662.

Ritalahti, K.M., Hatt, J.K., Lugmayr, V., Henn, K., Petrovskis, E.A., Ogles, D.M. et al. (2010) Comparing on-site to off-site biomass collection for *Dehalococcoides* biomarker gene quantification to predict in situ chlorinated ethene detoxification potential. *Environ Sci Technol* **44**: 5127-5133.

Rossi, P., Shani, N., Kohler, F., Imfeld, G., and Holliger, C. (2012) Ecology and biogeography of bacterial communities associated with chloroethene-contaminated aquifers. *Front Microbiol* **3**: 260.

Rowe, A.R., Lazar, B.J., Morris, R.M., and Richardson, R.E. (2008) Characterization of the community structure of a dechlorinating mixed culture and comparisons of gene expression in planktonic and biofloc-associated "*Dehalococcoides*" and *Methanospirillum* species. *Appl Environ Microbiol* **74**: 6709-6719.

Scheutz, C., Durant, N.D., Dennis, P., Hansen, M.H., Jørgensen, T., Jakobsen, R. et al. (2008) Concurrent ethene generation and growth of *Dehalococcoides* containing vinyl chloride reductive dehalogenase genes during an enhanced reductive dechlorination field demonstration. *Environ Sci Technol* **42**: 9302-9309.

Scheutz, C., Broholm, M.M., Durant, N.D., Weeth, E.B., Jørgensen, T.H., Dennis, P. et al. (2010) Field evaluation of biological enhanced reductive dechlorination of chloroethenes in clayey till. *Environ Sci Technol* **44**: 5134-5141.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

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Schipp, C.J., Marco-Urrea, E., Kublik, A., Seifert, J., and Adrian, L. (2013) Organic cofactors in the metabolism of *Dehalococcoides mccartyi* strains. *Phil Trans R Soci B: Biol Sci* **368**: 20120321.

Schneidewind, U., Haest, P.J., Atashgahi, S., Maphosa, F., Hamonts, K., Maesen, M. et al. (2014) Kinetics of dechlorination by *Dehalococcoides mccartyi* using different carbon sources. *J Contam Hydrol* **157**: 25-36.

Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Buergermann, H. et al. (2012) Fundamentals of microbial community resistance and resilience. *Front Microbiol* **3**: 417.

Smatlak, C.R., Gossett, J.M., and Zinder, S.H. (1996) Comparative kinetics of hydrogen utilization for reductive dechlorination of tetrachloroethene and methanogenesis in an anaerobic enrichment culture. *Environ Sci Technol* **30**: 2850-2858.

Stroo, H.F., Leeson, A., Marqusee, J.A., Johnson, P.C., Ward, C.H., Kavanaugh, M.C. et al. (2012) Chlorinated ethene source remediation: Lessons learned. *Environ Sci Technol* **46**: 6438-6447.

Sutton, N.B., Atashgahi, S., Saccenti, E., Grotenhuis, T., Smidt, H., and Rijnaarts, H.H.M. (2015a) Microbial community response of an organohalide respiring enrichment culture to permanganate oxidation. *PLoS ONE* **10**: e0134615.

Sutton, N.B., Atashgahi, S., van der Wal, J., Wijn, G., Grotenhuis, T., Smidt, H., and Rijnaarts, H.H.M. (2015b) Microbial dynamics during and after in situ chemical oxidation of chlorinated solvents. *Groundwater* **53**: 261-270.

Tsai, T.T., Liu, J.K., Chang, Y.M., Chen, K.F., and Kao, C.M. (2014) Application of polycolloid-releasing substrate to remediate trichloroethylene-contaminated groundwater: A pilot-scale study. *J Haz Mat* **268**: 92-101.

van Gelder, A.H., Aydin, R., Alves, M.M., and Stams, A.J.M. (2012) 1,3-Propanediol production from glycerol by a newly isolated *Trichococcus* strain. *Microbial Biotechnol* **5**: 573-578.

Wilkins, E., Ringel, A.K., Hortig, D., Willke, T., and Vorlop, K.D. (2012) High-level production of 1,3-propanediol from crude glycerol by *Clostridium butyricum* AKR102a. *Appl Microbiol Biotechnol* **93**: 1057-1063.

Wrighton, K.C., Thomas, B.C., Sharon, I., Miller, C.S., Castelle, C.J., VerBerkmoes, N.C. et al. (2012) Fermentation, hydrogen, and sulfur metabolism in multiple uncultivated bacterial phyla. *Science* **337**: 1661-1665.

Wrighton, K.C., Castelle, C.J., Wilkins, M.J., Hug, L.A., Sharon, I., Thomas, B.C. et al. (2014) Metabolic interdependencies between phylogenetically novel fermenters and respiratory organisms in an unconfined aquifer. *ISME J* **8**: 1452-1463.

Yan, J., Ritalahti, K.M., Wagner, D.D., and Löffler, F.E. (2012) Unexpected specificity of interspecies cobamide transfer from *Geobacter* spp. to organohalide-respiring *Dehalococcoides mccartyi* strains. *Appl Environ Microbiol* **78**: 6630-6636.

Yan, J., Im, J., Yang, Y., and Löffler, F.E. (2013) Guided cobalamin biosynthesis supports *Dehalococcoides mccartyi* reductive dechlorination activity. *Phil Trans R Soci B: Biol Sci* **368**: 20120320.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

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Yang, Y., Pesaro, M., Sigler, W., and Zeyer, J. (2005) Identification of microorganisms involved in reductive dehalogenation of chlorinated ethenes in an anaerobic microbial community. *Water Res* **39**: 3954-3966.

Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J.D. et al. (2014) Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. *Proc Natl Acad Sci USA* **111**: E836-E845.

Zhuang, W.Q., Yi, S., Bill, M., Brisson, V.L., Feng, X., Men, Y. et al. (2014) Incomplete Wood-Ljungdahl pathway facilitates one-carbon metabolism in organohalide-respiring *Dehalococcoides mccartyi*. *Proc Natl Acad Sci USA* **111**: 6419-6424.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

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## Figure legends

**Fig. 1.** Progress of reductive dechlorination over time. Left: concentrations of cDCE, VC, ethene,  $\delta^{13}\text{C}$  of cDCE and VC and concentration-weighted average of CE  $\delta^{13}\text{C}$  values ( $\delta^{13}\text{C}_{\Sigma(\text{CE})}$ ). Right: qPCR quantification of 16S rRNA gene copy numbers of *Dcm* and reductive dehalogenase encoding *vcrA*, *bvcA* and *tceA* genes. Each qPCR value represents the average of triplicate reactions. ETH: ethene.

**Fig. 2.** Predicted (Chao 1) and observed OTU richness, and phylogenetic diversity in the control (five samples) and impacted filters at each sampling campaign (seven samples). D: day; Cont: control well.

**Fig. 3.** Distribution of the most abundant phyla (> 1% average relative abundance across all samples, left panel) and orders (> 0.1% average relative abundance across all samples, right panel) in the control and impacted filters. D: day; Cont: control well.

**Fig. 4.** Correlation between bacterial orders and geochemical parameters. A color gradient from dark blue to dark red depicting the strength and direction of the correlation is given.

**Fig. 5.** Summary of major geochemical and microbial evolution before and after biostimulation (left), and a conceptual model of glycerol degradation based of the observed taxa from MiSeq analysis and their known/putative physiology (right). The proposed sulfur cycle is shown in red dotted square and the putative corrinoid supply to *Dcm* is shown by dashed gray arrows. All values are averages of the seven impacted filters. Error bars are not shown for clarity. PDO: 1,3-propanediol, SCFA: short chain fatty acids.

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## Supporting information

**Fig. S1.** Groundwater geochemical parameters. Scales on Y-axes are: dissolved organic carbon (DOC) (mg/L), electrical conductivity (EC) ( $\mu\text{S}/\text{cm}$ ), Fe (mg/l), Fe(II) (mg/l), methane ( $\mu\text{g}/\text{l}$ ), oxidation-reduction potential (ORP) (mV), oxygen (mg/l), sulfate (mg/l).

Cont: control well.

**Fig. S2.** Predicted (Chao 1) and observed OTU richness, and phylogenetic diversity at each filter over time (five samples per filter). D: day; Cont: control well.

**Fig. S3.** Ordination of community composition by nonmetric multidimensional scaling (NMDS) based on Bray–Curtis distances. D: day; Cont: control well.

**Fig. S4.** Distribution of the most abundant phyla (> 1% average relative abundance across all samples, left panel) and genera (> 0.7% average relative abundance across all samples, right panel) in the control (Cont) and impacted filters. D: day.

**Fig. S5.** Niche occupation concept. According to phylogenetic instability in the control well (well 0 in left figure), groundwater brings in immigrant microbes (the arrow from the control well towards the stimulated zone or active growth zone). The resident taxa in the stimulated wells exploit the stimulated conditions and occupy the available niches in the active growth zone. However, they were exposed to the newly arriving taxa brought by natural groundwater flow but it is only towards the end of the experiment (and hence fading stimulation) that the immigrants pass the threshold after day 195 (dashed line in right figure) and become established in the stimulated wells by day 265.

**Fig. S6.** Schematic presentation of the study site close to the Zenne River. Monitoring

wells (black circles) and injection wells used for glycerol delivery (red stars) at the site. This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/1462-2920.13361

are indicated. Well 0, located upstream of the injection wells, is taken as control. Samples were taken from the monitoring wells impacted by glycerol injection (shown in the dashed oval) i.e. well 2, 3, 5 and 7 (only shallow filter, 7A) as well as for the control well throughout the study.

**Table S1.** Groundwater geochemical parameters. Cont: control well.

**Table S2.** Concentrations of TCE, cDCE, VC, ethene and  $\delta^{13}\text{C}$  of cDCE and VC and concentration-weighted average values of the isotope signature ( $\delta^{13}\text{C}_{\text{TCE}}$ ). Right: qPCR quantification of 16S rRNA gene copy numbers of *Dcm*, and *vcrA*, *bvcA* and *tceA* genes. Each qPCR value represents the average value obtained from triplicate reactions. NA: not analyzed; ETH: ethene.

**Table S3.** Distribution of the most abundant phyla, orders and genera in the control and impacted filters. D: day; Cont: control well.

**Table S4.** Significance tests of the differences of microbial communities at order level. The groups with no significant differences ( $P>0.05$ ) are shown in bold.

**Table S5.** Overview of primer sequences and thermal cycling conditions used for qPCR in this study.

**Table S6.** Overview of MiSeq primers and Unitags and thermal cycling conditions.

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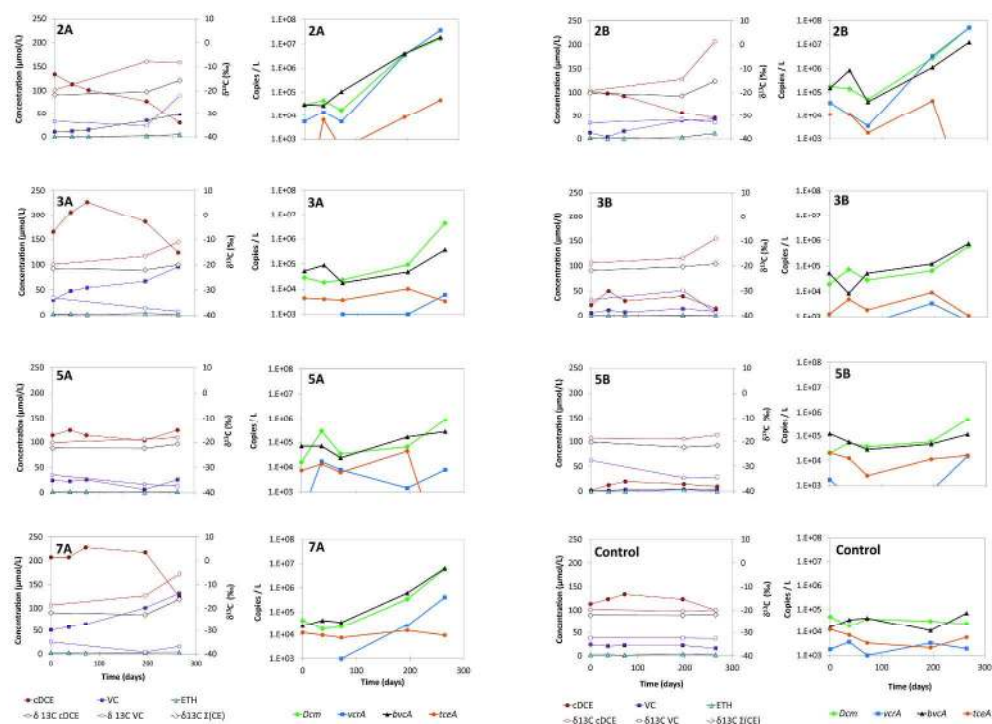


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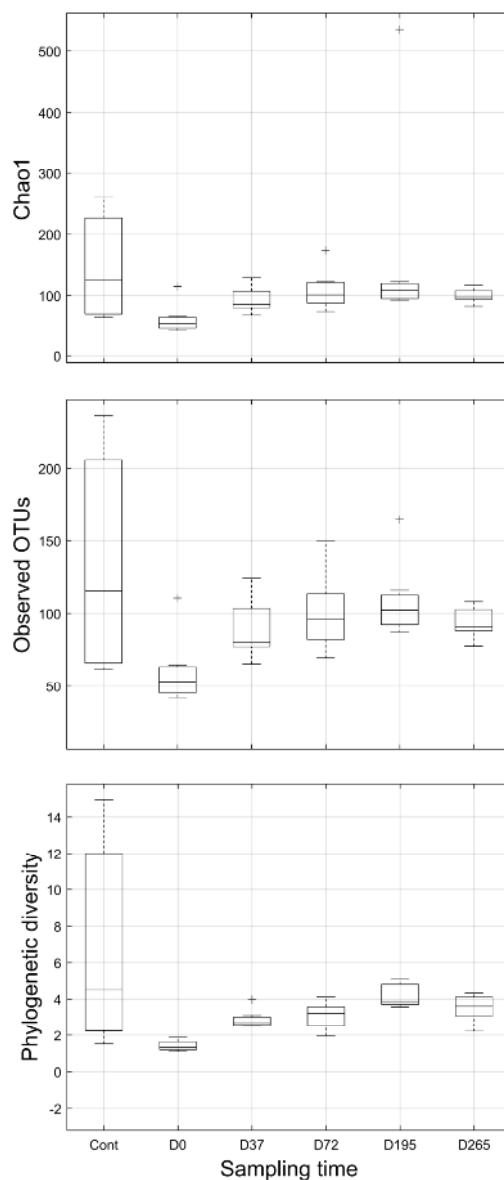


Fig. 2. Predicted (Chao 1) and observed OTU richness, and phylogenetic diversity in the control (five samples) and impacted filters at each sampling campaign (seven samples). D: day; Cont: control well.

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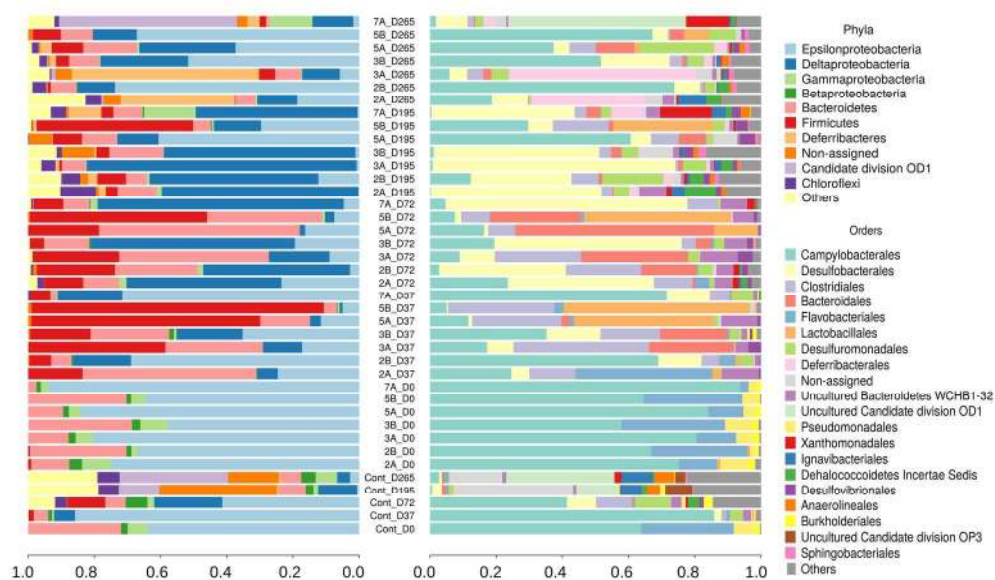


Fig. 3. Distribution of the most abundant phyla (> 1% average relative abundance across all samples, left panel) and orders (> 0.1% average relative abundance across all samples, right panel) in the control and impacted filters. D: day; Cont: control well.

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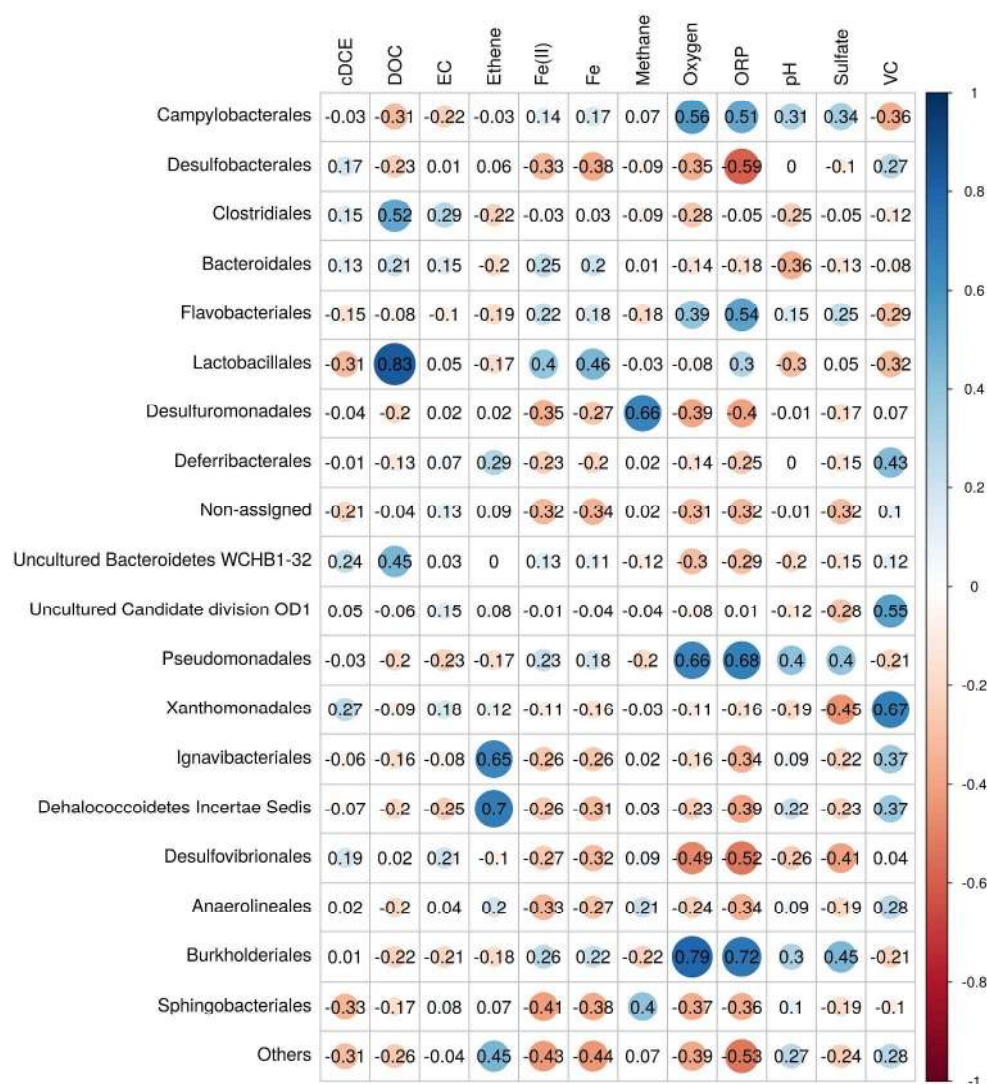


Fig. 4. Correlation between bacterial orders and geochemical parameters. A color gradient from dark blue to dark red depicting the strength and direction of the correlation is given.

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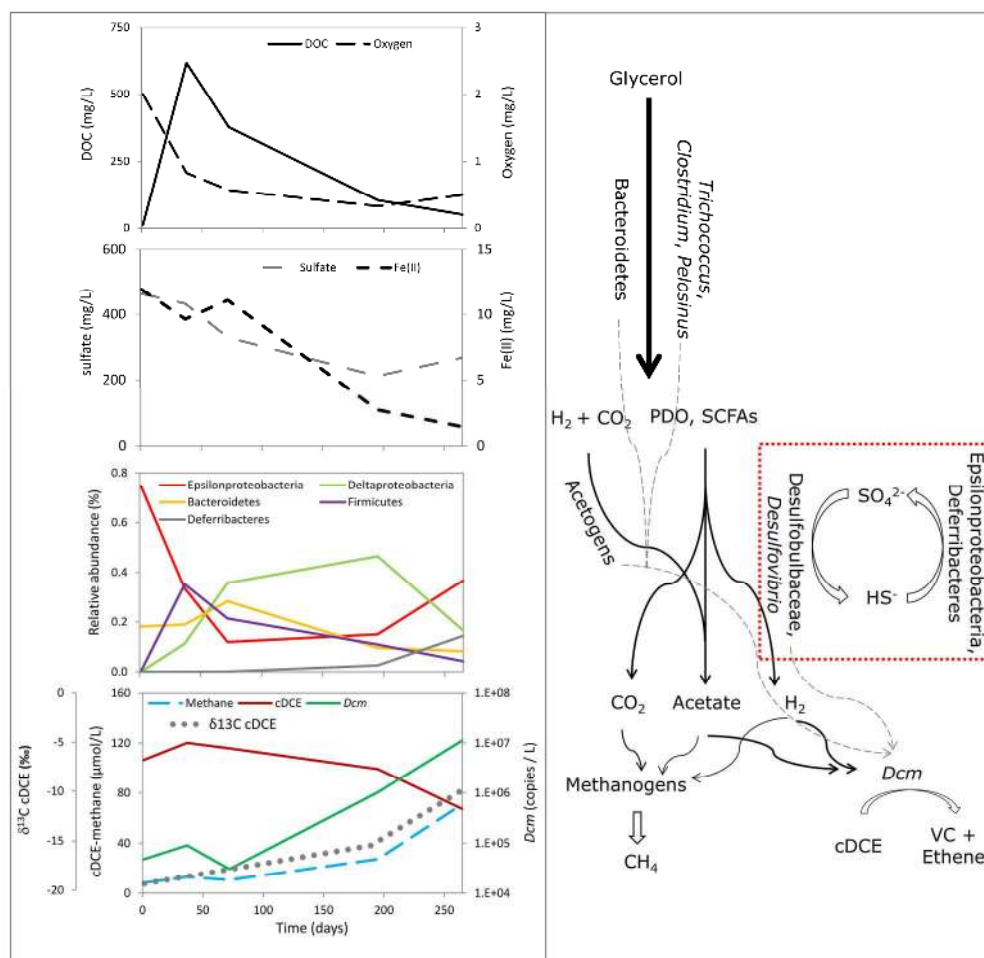


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