ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Molecular characterization of microbial communities in a peat-rich aquifer system contaminated with chlorinated aliphatic compounds

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:
Ghezzi D., Filippini M., Cappelletti M., Firrincieli A., Zannoni D., Gargini A., et al. (2021). Molecular characterization of microbial communities in a peat-rich aquifer system contaminated with chlorinated aliphatic compounds. ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH INTERNATIONAL, 28, 23017-23035 [10.1007/s11356-020-12236-3].

Availability:
This version is available at: https://hdl.handle.net/11585/804657 since: 2021-11-11

Published:
DOI: http://doi.org/10.1007/s11356-020-12236-3

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/).
When citing, please refer to the published version.

(Article begins on next page)

07 July 2024

Molecular characterization of microbial communities in a peat-rich aquifer system contaminated with chlorinated aliphatic compounds

Daniele Ghezzi[1,2] · Maria Filippini[3] · Martina Cappelletti[1] · Andrea Firrincieli[1] · Davide Zannoni[1] · Alessandro Gargini[3] · Stefano Fedi[1]

Abstract
In an aquifer-aquitard system in the subsoil of the city of Ferrara (Emilia-Romagna region, northern Italy) highly contaminated with chlorinated aliphatic toxic organics such as trichloroethylene (TCE) and tetrachloroethylene (PCE), a strong microbial-dependent dechlorination activity takes place during migration of contaminants through shallow organic-rich layers with peat intercalations. The in situ microbial degradation of chlorinated ethenes, formerly inferred by the utilization of contaminant concentration profiles and Compound-Specific Isotope Analysis (CSIA), was here assessed using Illumina sequencing of V4 hypervariable region of 16S rRNA gene and by clone library analysis of dehalogenase metabolic genes. Taxon-specific inves-tigation of the microbial communities catalyzing the chlorination process revealed the presence of not only dehalogenating genera such as *Dehalococcoides* and *Dehalobacter* but also of numerous other groups of non-dehalogenating bacteria and archaea thriving on diverse metabolisms such as hydrolysis and fermentation of complex organic matter, acidogenesis, acetogenesis, and methanogenesis, which can indirectly support the reductive dechlorination process. Besides, the diversity of genes encoding some reductive dehalogenases was also analyzed. Geochemical and 16S rRNA and RDH gene analyses, as a whole, provided insights into the microbial community complexity and the distribution of potential dechlorinators. Based on the data obtained, a possible network of metabolic interactions has been hypothesized to obtain an effective reductive dechlorination process.

Keywords Bioremediation · Reductive dechlorination · Chloroethylene · 16S rRNA · Microbial community

Introduction

Chlorinated ethylene contaminants such as perchloroethylene (PCE) and trichloroethylene (TCE) are commonly found in groundwater due to their frequent industrial use since the be-ginning of the last century (Balderacchi et al. 2014; McCarty 2010). Dichloroethylene isomers (mainly 1,2-cis dichloroethylene—cDCE) and vinyl chloride (VC) may be found associated to PCE and TCE in groundwater as by-products of anaerobic/reductive microbial dechlorination (Bradley and Chapelle 2010). Since VC is a known human carcinogen (IARC 2008) and is also highly soluble and vola-tile (Mackay et al. 2006), its possible occurrence in ground-water is of primary concern. In the past few years, several studies have used the Compound-Specific Isotope Analysis (CSIA) in combination with molecular biological analysis to evaluate the rate of reductive dechlorination (Chiu et al. 2013; Damgaard et al. 2013; Kotik et al. 2013). In particular, analy-ses applying isotope fractionation together with molecular bi-ology tools have been conducted to measure the dehalogenation efficiency in heterogeneous aquifers, where halogenated groundwater is mixed with open water bodies (Nijenhuis et al. 2007; Imfeld et al. 2011). In this respect, dechlorinating bacterial communities have been mostly inves-tigated through quantitative Polymerase Chain Reaction (qPCR) (Damgaard et al. 2013; Courbet et al. 2011).

However, as primers targeting specific microbes were used, the information obtained on the composition of the entire bacterial community was limited because microbial dehalogenase activity depends on metabolic interactions with other microorganisms (Damgaard et al. 2013; Hunkeler et al. 2011; Clark et al. 2018; Hermon et al. 2019). Therefore, to acquire a full overview of the entire microbial communities involved in putative mutualistic relationships between specific bacterial and/or archaeal groups is necessary to clarify the microbiology behind the dechlorination process (Lee et al. 2015). To this end, some studies have been done in the past but using low-resolution microbial community analysis methods such as clone libraries and Terminal Restriction Fragment Length Polymorphism (TRFLP) of 16S rRNA amplicons (Macbeth et al. 2004; Rahm et al. 2006). Therefore, a series of open questions on the microbiology related to the dehalogenation processes in aquifers contaminated by toxic PCE and TCE remains.

Different molecular methodologies have characterized functional genes for the dehalogenation of organic solvents in anaerobic and aerobic environments. Several reductive dehalogenase (RDH) genes have been examined using both microarray-based methods and PCR-based protocols, and different sets of primers proved useful for the identification of numerous RDH genes from a variety of samples, allowing their detection in some genera of anaerobic bacteria such as *Dehalococcoides*, *Dehalobacter*, and *Desulfitobacterium* (Futagami et al. 2009; Regeard et al. 2004). Alternatively, the aerobic degradation of halogenated organic solvents is performed through cometabolic transformation by aerobic bacteria containing oxygenase enzymes with broad substrate range which fortuitously degrade VC, cDCE, and 1,1,2,2-tetrachloroethane using phenol, toluene, ethene, methane, propane, or ammonia as the growth-supporting substrate (Mattes et al. 2010; Cappelletti et al. 2018).

The aquifers in the subsoil of the city of Ferrara (Emilia-Romagna region, northern Italy) are affected by chlorinated ethylene contamination at several field sites with concentrations up to 10 mg L^{-1} for single contaminants (Gargini et al. 2011). The contamination originated from the industrial spill of sol-vents or improper disposal of industrial wastes between the 1960s and the 1970s (Nijenhuis et al. 2013). Whereas dechlo-rination products cDCE and VC generally occur in groundwater associated with primary compounds PCE and TCE, at some of the sites of Ferrara VC, the composition of the contaminant plumes downgradient to the source dominates, suggesting the presence of exceptionally high dechlorination activity. Filippini et al. (2016) observed that dechlorination of PCE and TCE takes place during contaminant migration through shallow organic-rich layers with peat intercalations. Such layers are ubiquitous in the subsoil of Ferrara, and they were proposed to act as a "reactor" stimulating the degradation of contaminants with the accumulation of VC.

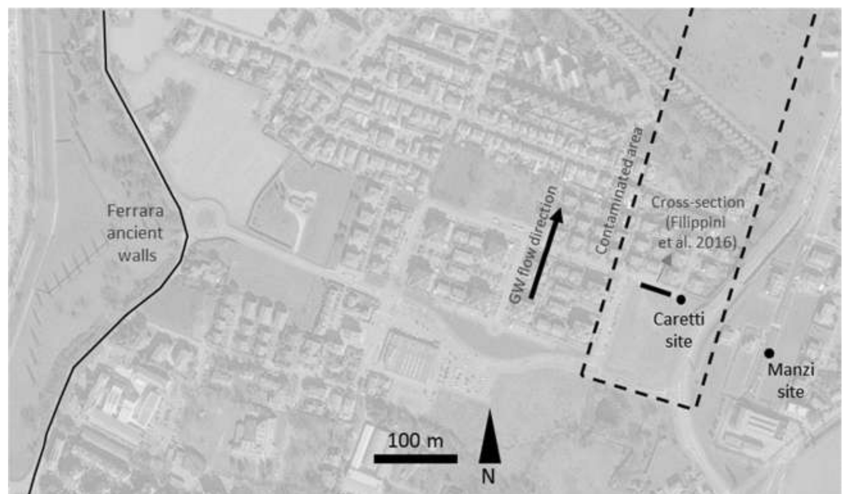
Here, Illumina sequencing of V4 hypervariable region of 16S rRNA gene in combination with clone library analysis of dehalogenase metabolic genes was applied to analyze the composition of the microbial community that drives the pecu-liar dehalogenation activity observed inside the organic-rich layers in the subsoil of Ferrara. The results were interpreted to show that the microbial communities catalyzing the chlorina-tion process are composed not only of dehalogenating bacte-rial genera such as *Dehalococcoides* and *Dehalobacter* but also of other groups of non-dehalogenating bacteria and ar-chaea involved in hydrolysis and fermentation of complex organic matter, acidogenesis, acetogenesis, and methanogenesis. Overall, both previous and present results allowed us to propose a working model in which syntrophic relationships within microbial communities are required to obtain an effective reductive dechlorination process.

Materials and methods

Description of sampling sites

Sediment samples were collected in June 2017 in a contaminated site in Ferrara, called "Caretti site" (hereinafter referred to as C), and in a second site 130 m southeastward, called "Manzi site" (hereinafter referred to as M) (Fig. 1). Filippini et al. (2016) thoroughly investigated the distribution of chloroethylene contaminants in the multilayered aquifer-aquitard system below the C site. Their research was focused along a cross section 60 m deep and 60 m wide adjacent to the local source of contamination (Fig. 1). The vertical distribu-tion of contaminant concentrations was reconstructed in detail along with the easternmost of three vertical profiles (labeled MC4–5 in Fig. 2) which was the most contaminated one. Sediment samples were collected from cores and analyzed for total contaminant concentration. Estimated pore water concentrations assuming equilibrium phase partitioning (Filippini et al. 2020) showed peaks of dechlorination prod-ucts cDCE and VC of 145 and 61 mg L^{-1}, respectively, within an organic-rich sub-layer between 5.9 and 7.5 m below sea level (bsl) (Fig. 2). A groundwater sample collected along the same profile showed PCE, TCE, and cDCE concentrations of 0.4, 0.8, and 63.6 mg L^{-1}, respectively, consistent with aver-aged pore water concentrations from sediments, whereas the VC was much higher in the groundwater sample (124.2 mg L^{-1}), suggesting that sediment sample analysis led to an underestimation of the actual VC concentration (Filippini et al. 2016). Ethylene was also detected in groundwater in a concentration of 169.1 mg L^{-1}, proving some complete dechlorination inside the organic-rich layer. A degree of dechlorination (DOD) of 0.8 was estimated for the groundwa-ter sample, where a DOD equal to 0 or 1 indicates no dichlorination or complete dichlorination, respectively

Fig. 1 Location of the study area. The black arrow indicates the main direction of groundwater flow. The dashed rectangle delimits the contaminated area



(Damgaard et al. 2013). The CSIA was performed by Filippini et al. (2016) showing isotopic fractionation within the organic-rich sub-layer that is consistent with the occurrence of reductive dechlorination. The same authors observed Eh values down to − 280 mV, oxygen concentrations lower than 0.4 mg L [1], and methane concentrations higher than 30 mg L [1] in the groundwater of the C site, corresponding to a methanogenic geochemical condition.

The M site is considered uncontaminated based on unpublished characterization efforts by the local authorities. The samples from this site were used to make a comparison between contaminated and uncontaminated sediments of the same aquifer-aquitard system. The new sampling locations were carefully selected based on previous characterization ef-forts. At the C site, which is well characterized in terms of aquifer-aquitard geometries, lithologies, hydrogeochemistry, and 3D contaminant distribution (Nijenhuis et al. 2013; Filippini et al. 2015, 2016, 2020), the new sampling was per-formed a few meters apart from the most contaminated hot spot in the source area, corresponding to the formerly

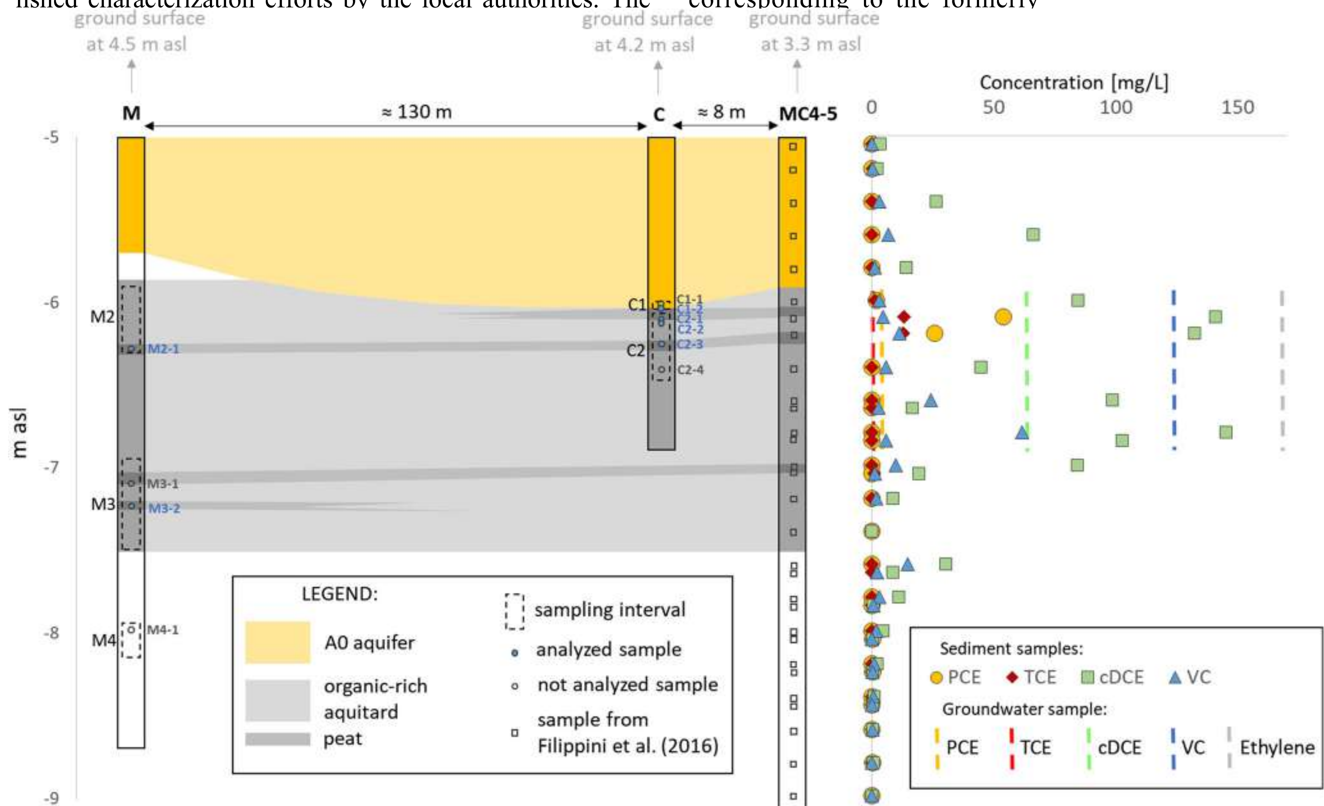


Fig. 2 Stratigraphic control points and sampling depths at the C and M sites. Profile MC4 5 from Filippini et al. (2016) is also depicted together with the vertical distribution of contaminant concentrations from sediment and water samples

investigated profile MC4–5. Such location was selected to ensure the collection of samples representative of strongly contaminated conditions since chloroethenes are known to assume complex distributions in the underground with abrupt changes in contaminant concentration over short distances (Parker et al. 2003). At site M, the new samples were collected close to an existing control point (piezometer) that allowed excluding the occurrence of chloroethene contamination in groundwater. Among existing piezometers at the M site, the selected one is the closest to the C site to maximize the correlability of geological layers between the two sites.

In both sites, the sediment samples were collected between 6 and 8 m bsl, i.e., the depth of the peat-rich layers showing the highest dechlorination activity at the C site. The depth of sampling was reached via percussion drilling. The technique allows advancing in the ground without the use of fluids that may disturb the sample. While advancing in the ground, a stainless-steel casing was put in place to avoid the collapse of the borehole or vertical cross-contamination. Cores 1.5 m long were recovered inside transparent plastic liners of 4 cm inner diameter. The use of transparent liners allowed for the selection of the sampling intervals of interest in the field by visual inspection, without the need of opening the liners. Three sampling intervals were selected at the M site corresponding to peat-rich layers (M2 to M4 in Fig. 2).

In contrast, two intervals were selected at the C site corresponding to a peat-rich layer and the bottom of a sandy aquifer layer overlying the peat (C1 and C2). The se-lected sampling intervals were separated from the rest of the liner using a hacksaw, plugged at both ends with plastic caps, and stored at − 20 °C. The remaining liners were opened in the field and used for stratigraphic de-scription. The liner sections selected for sampling were opened in the lab. The cores were scratched at the sur-face to remove disturbances caused by drilling, and a total of 6 samples were collected for DNA extraction and subsequent PCR amplification (Table 1).

DNA extraction, 16S rRNA gene amplification, and Illumina sequencing

The sediment samples were extracted for their total DNA using the DNeasy PowerSoil Kit (Qiagen) according to the manufacturer's protocol with slight modifications as previous-ly described (Cappelletti et al. 2016). The extracted DNA was used as a template for PCR amplification targeting the V4 hypervariable region of the 16S rRNA gene using the primer pair 515F (5′GTGCCAGCMGCCGCGGTAA3′) and 806R (5′GGACTACHVGGGTWTCTAAT3′) (Caporaso et al. 2010) modified with an Illumina adaptor sequence at the 5′end. PCR reactions were performed in a final volume of 50 μL containing 10 ng of total DNA, primers 500 nM, 1× Takara Ex Taq buffer with $MgCl_2$, dNTP mix 200 μM, and Takara Ex Taq Polymerase 0.5 U. The thermocycling program included 1 cycle at 95 °C for 10 s, 30 cycles at 95 °C for 10 s, 58 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 2 min. Amplicons were submitted to the library preparation and Illumina MiSeq sequencing platform for indexing and pair-end sequencing (2 × 250 bp; reagent kit, v2) at the sequencing service BMR Genomics SRL. The sequence analysis of 16S rRNA amplicons was performed by using QIIME2 version 2018.4 (Bolyen et al. 2019) and DADA2 package version 1.5.0 (Callahan et al. 2016). The demultiplexed and primer-clipped reads were trimmed based on quality and length. Trimmed sequences were dereplicated, denoised, and merged, and chimeras were removed. The taxonomic assignment of the resulting 16S rRNA gene amplicon sequence variants (ASVs) was performed by querying the ASVs against the SILVA SSU 132 reference database (Quast et al. 2013). The taxonomy nomenclature was further manually checked and modified according to the most recently published studies. Acco rdingly , *Bathyarchaeia* (former class of *Crenarchaeota*) and *Aminicentantia* (former class of *Acidobacteria*) were renamed as *Candidatus* (Ca.) Bathyarchaeota (Meng et al. 2014) and Ca. Aminicentantes (Rinke et al. 2013) phyla, respectively. One ASV belonging to C2.3 sample and affiliated to the *Escherichia*/*Shigella* genus

Table 1 List and description of samples analyzed in this work

Sample ID	Site	Depth (bsl)	Matrix	μg DNA per 1 g soil	Sequenced reads	ASVs	Shannon	Inverse Simpson's	Evenness
C1.2	Caretti	6.05 m	Peat	2.64	51,725	696	5.250	73.421	0.802
C2.1	Caretti	6.09 m	Interface between peat and organic rich aquitard	3.24	26,119	499	5.012	57.384	0.807
C2.2	Caretti	6.17 m	Organic rich aquitar	1.40	55,678	641	5.043	57.005	0.780
C2.3	Caretti	6.25 m	Peat	1.02	49,787	399	4.514	32.047	0.754
M2.1	Manzi	6.28 m	Peat	1.84	34,428	784	5.774	131.217	0.866
M3.2	Manzi	7.23 m	Interface between peat and organic rich aquitard	1.46	57,010	1075	5.741	86.631	0.822

was removed from further analysis being recognized as possible laboratory contaminant. Metabolic pathways contributing to PCE and TCE degradation were predicted from ASVs through the PICRUSt2 pipeline. Functional profiling was performed only for the most abundant ASVs (relative abundance > 1.0%). To further improve the reliability of the functional prediction, we focused only on ASVs having a Nearest-Sequenced Taxon Index (NSTI) value below 0.15. The NSTI score indicates to which extent a microorganism in a sample is related to a set of reference genomes in the PICRUSt database.

The Illumina sequencing raw data were deposited in the Sequence Read Archive of NCBI under accession number PRJNA635257. The 64 most abundant ASVs were submitted to the NCBI GenBank database under accession numbers MW136710-MW136773.

Clone library of dehalogenases metabolic genes

A set of primers selected to amplify from the total DNA different types of reductive dehalogenase homologous genes (*rdhA*) (Hug and Edwards 2013) was chosen accordingly to the most abundant microbial species retrieved from the 16S rRNA gene analysis. The list of *rdhA* primer used along with the expected product size, primer sequences, and melting temperature is summa-rized in Table S1. Around 10 ng of total DNA was added to a 50-μL (final volume) PCR reaction mixture containing Takara Ex Taq buffer with $MgCl_2$ (10×; Takara Bio Inc., Tokyo, Japan), primers 200 nM each, dNTP mix 200 μM, Takara Ex Taq Polymerase 1.25 U. PCR amplification conditions were applied according to Hug et al. reference (Hug and Edwards 2013) with some minor changes as shown in Table S2. The *rdhA* PCR products were purified using the Qiagen PCR pu-rification kit and eluted in 50 μL of Qiagen elution buffer. Aliquots (30 ng) of purified PCR products were cloned into the TOPO-TA vector (Thermo Fisher Scientific) and transformed into chemically competent *Escherichia coli* DH5α cells following the manufac-turer's instructions. White colonies isolated on LB agar plates containing 100 μg mL^{-1} ampicillin, 50 μg mL^{-1} kana-mycin, and 40 μg mL^{-1} X-gal were screened through colony PCR with the vector primers T7 and T3 (Invitrogen). After confirmation through electrophoresis, clones with the sup-posed correct size of the insert were sequenced in both for-ward and reverse directions using T7 and T3 primers (Invitrogen) using the sequencing service provided by Eurofins Genomics. Chimeras were identified and removed with Uchime2 v11 (Edgar et al. 2011). The clone library se-quences of dehalogenases were submitted to the NCBI GenBank database under accession numbers MT561367 and MT561376.

Phylogenetic and statistical analyses

Phylogenetic trees were constructed using, separately, (i) the ASVs resulting from the Illumina sequencing of V4 region of the 16S rRNA gene and (ii) the amino acid sequences of the *rdhA* genes obtained from in silico translation of the represen-tative clones obtained from the clone library analysis. For each sequence included in the tree, the most closely related se-quences retrieved from the NCBI database (Best Blast Hits) were downloaded. MEGAX (Kumar et al. 2018) was used to construct phylogenetic trees based on ClustalW sequence alignment and neighbor-joining clustering method with 1000 non-parametric bootstrap replicates for both the 16S rRNA gene (model: Jukes-Cantor; rates among site: uniform rates; gap/missing data treatment: pairwise deletion) and the amino acidic sequence of the *rdhA* genes (model: Poisson model; rates among site: uniform rates; gap/missing data treatment: pairwise deletion).

Diversity indices, richness estimations, and principal coor-dinate analysis (PCoA) were performed based on of ASVs using CALYPSO online software (Zakrzewski et al. 2017) and Primer-E v7 (Clarke and Gorley 2015).

Results

Stratigraphy at the C and M sites

The stratigraphy of boreholes M and C shows metric alterna-tion of sandy aquifer layers and silty-clayey aquitard layers locally enriched in peat and organic matter. Such configura-tion is consistent with previous observations in the region (Molinari et al. 2007). The present work focuses on the aquitard layer section enriched in organic matter, where Filippini et al. (2016) observed the dechlorination of chloroethylenes fostered by the organic-rich sediments. The aquitard section of interest is extended for a few meters below the bottom of the shallowest aquifer layer (regionally known as the "A0 aquifer"; Regione Emilia-Romagna and ENI-AGIP 1998) (Fig. 2). The bottom of the A0 aquifer is around 6 m bsl. Below the aquifer bottom, a fine-grained sub-layer enriched in organic matter was identified in M and C boreholes containing centimeter intercalations of peats. The peaty horizons ob-served inside the organic-rich sub-layer are expected to have a remarkable later extent (Bruno et al. 2019), and it was pos-sible to correlate some of them between boreholes M and C and with the previous borehole MC4–5.

Diversity of microbial communities

The sampling depth and sequencing coverage resulted in be-ing sufficient to describe the microbial diversity in each of the six samples under analysis (Fig. S1). As a result of the

processing of the demultiplexed fastq files with DADA2 package, a total of 284,110 total reads were obtained and clustered in 2781 sequence variants.

The microbial communities of the four contaminated C sediment samples (C1.2, C2.1, C2.2, and C2.3) showed lower richness, evenness, and diversity as compared to the two samples collected from the uncontaminated M site (M2.1 and M3.2) (Table 1). Among the C site samples, Shannon and Inverse Simpson's indices showed that the microbial communities' diversity decreased accordingly with the increment of the sampling depth. Moreover, the lean-peat samples (C2.1 and C2.3) showed less microbial richness than those located in the rich organic fraction of the semi-permeable layer and the aquifer fraction (C1.2 and C2.2), collected just above and below the peat horizon of the contaminated area (Table 1, see Fig. 2).

Principal coordinates analysis based on the taxonomy composition of the various sediments revealed that M samples grouped apart from C samples (Fig. 3). Among the four C samples, C1.2 and C2.1 shared higher taxonomic similarity. In contrast, the deepest sediment sample C2.3 was located separately from the other Caretti sediments on the plot (Fig. 3).

Bacteria were predominant in all analyzed samples representing at least 65% of each microbial community. Conversely, phylum-level taxonomic assignments of 16S rRNA gene sequences revealed that the Ca. Bathyarchaeota phylum was included in the top three microbial phyla of all six samples under analysis and represented the most abundant taxonomic group in the C samples C1.2 (24.5%), C2.1

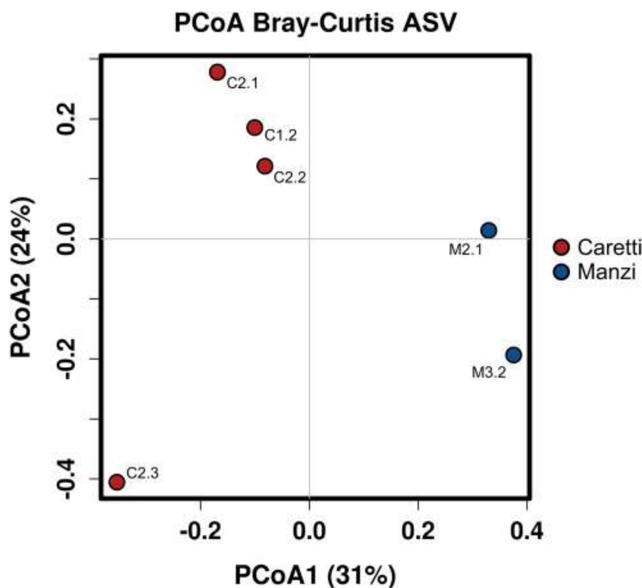


Fig. 3 Principal coordinates analysis (PCoA) based on ASV Bray Curtis similarity of Caretti (red dots) and Manzi (blue dots) sediments. Dots closer together in the plots indicate samples with similar community composition. Variance explained in PCoA axis 1 = 31% and PCoA axis 2 = 24%

(21.5%), and C2.2 (32.5%) (Fig. 4). These three samples were also abundant in *Chloroflexi* (7–17%) and *Elusimicrobia* (10–13%), whose relative abundances decreased accordingly to the sample deepness. Only in the C2.3 sample, *Firmicutes* represented the most abundant phylum (45.6%), followed by *Proteobacteria* (11.8%). Additional abundant phyla ranging between 5 and 10% of the total community in C sediments were *Spirochaetes*, Ca. Aminicenantes, and *Actinobacteria* (Fig. 4). The two samples M1.2 and M3.2, collected from the uncontaminated M site, were mainly constituted of *Chloroflexi* (35.3% and 23.9%, respectively), followed by Ca. Bathyarchaeota (12.7% and 19.6%, respectively). Other abundant phyla in the M sediments were *Elusimicrobia*, Ca. Aminicenantes, and *Spirochaetes* (Fig. 4).

The most abundant taxa were unclassified at low taxonomy levels. In particular, sequences affiliated to Ca. Aminicenantes and *Elusimicrobia* did not provide any more in-depth classification. *Coriobacteriia* represented the only defined class of the *Actinobacteria* phylum with the highest relative abundance of 2.5% in C1.2. Most of *Chloroflexi* classified up to the class level was *Anaerolineae* and *Dehalococcoidia*, the latter being more abundant in M site (Fig. 4). Some of the less abundant taxa were classified at lower levels and mainly identified in C2.3 sample, including members of the *Gracilibacteraceae* family and *Dehalobacter* genus (both be-longing to *Firmicutes*) with relative abundances of 13% and 12%, respectively. Nevertheless, around 10% of *Firmicutes* in C2.3 remained unclassified at class level. *Deltaproteobacteria* and *Gammaproteobacteria* were the dominant classes of *Proteobacteria*, the first being more abundant in Manzi site (7% in M2.1 and 9% in M3.2) and the latter in Caretti site (1.5–8%). In Caretti, *Shewanella* and *Halomonas* represented the most abundant genera of *Gammaproteobacteria* showing the highest abundance in C2.3 (3.5% and 4.5%, respectively). Some bacterial members affiliated to the *Spirochaetes* phylum were classified up to the genus level, including *Spirochaeta* that represented 8% and 9% in C2.3 and M3.2, respectively (Fig. 4).

The dominance of Ca. Bathyarchaeota and *Elusimicrobia* observed in the phylum-level analysis among all samples was reflected in the ASV analysis (Table S3). Indeed, *Elusimicrobia*-affiliated ASV1 and ASV3 and Ca. Bathyarchaeota-affiliated ASV2 were among the dominant ASVs in C1.2, C2.1, and C2.2. The phylogenetic analysis of ASV1 and ASV3, along with other ASVs affiliated to the *Elusimicrobia* phylum, revealed sequence similarities with clones found in samples collected from sediments and groundwater, and also present in enriched archaeal methanogenic communities detected in subsurface freshwaters (Fig. 5). In contrast, ASV2 and other abundant ASVs belonging to the archaeal Ca. Bathyarchaeota phylum showed their affiliation with sequences retrieved from the transitional zone between rich organic river estuary water and coastal seawater (Fig. 6).

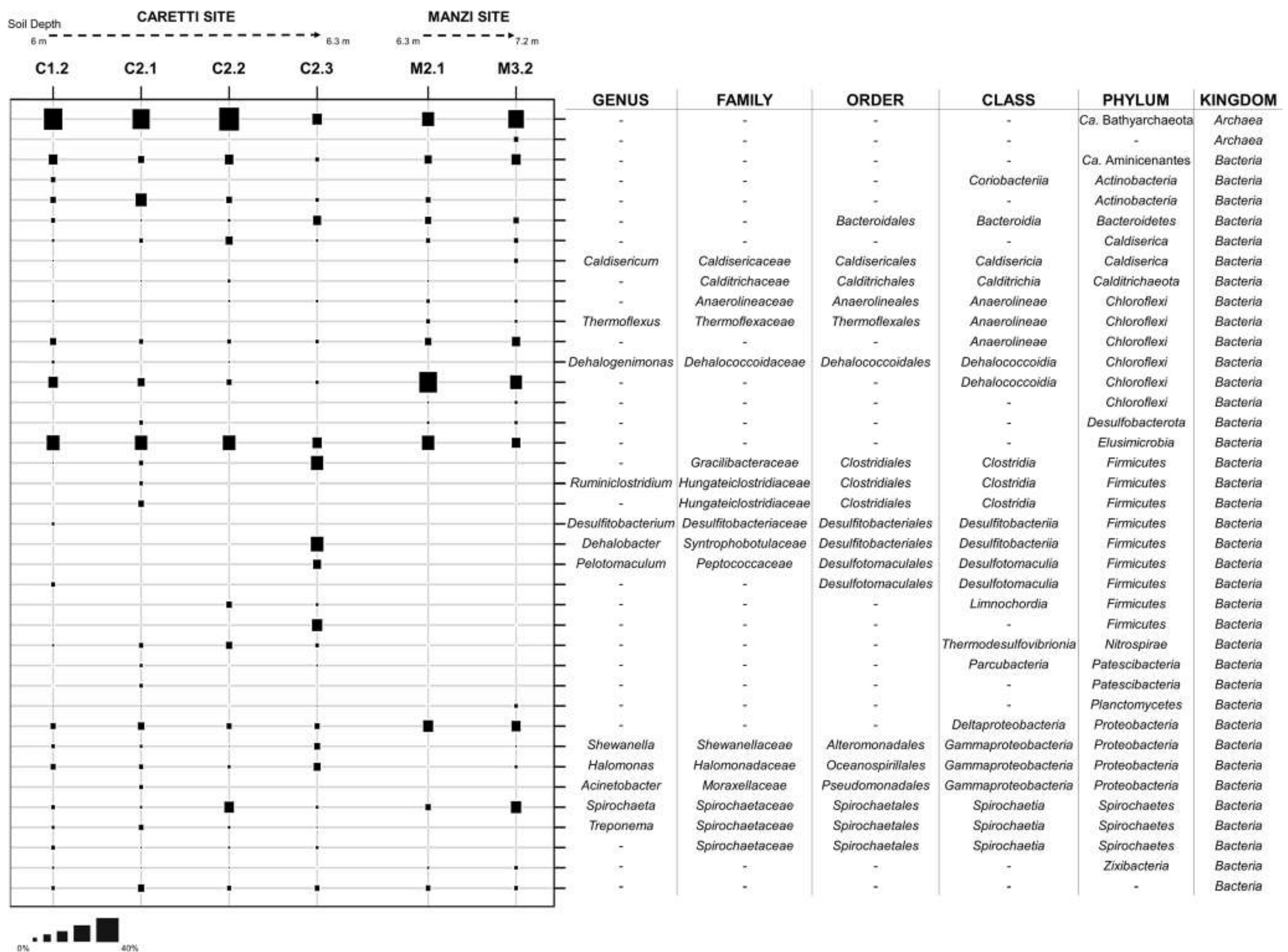


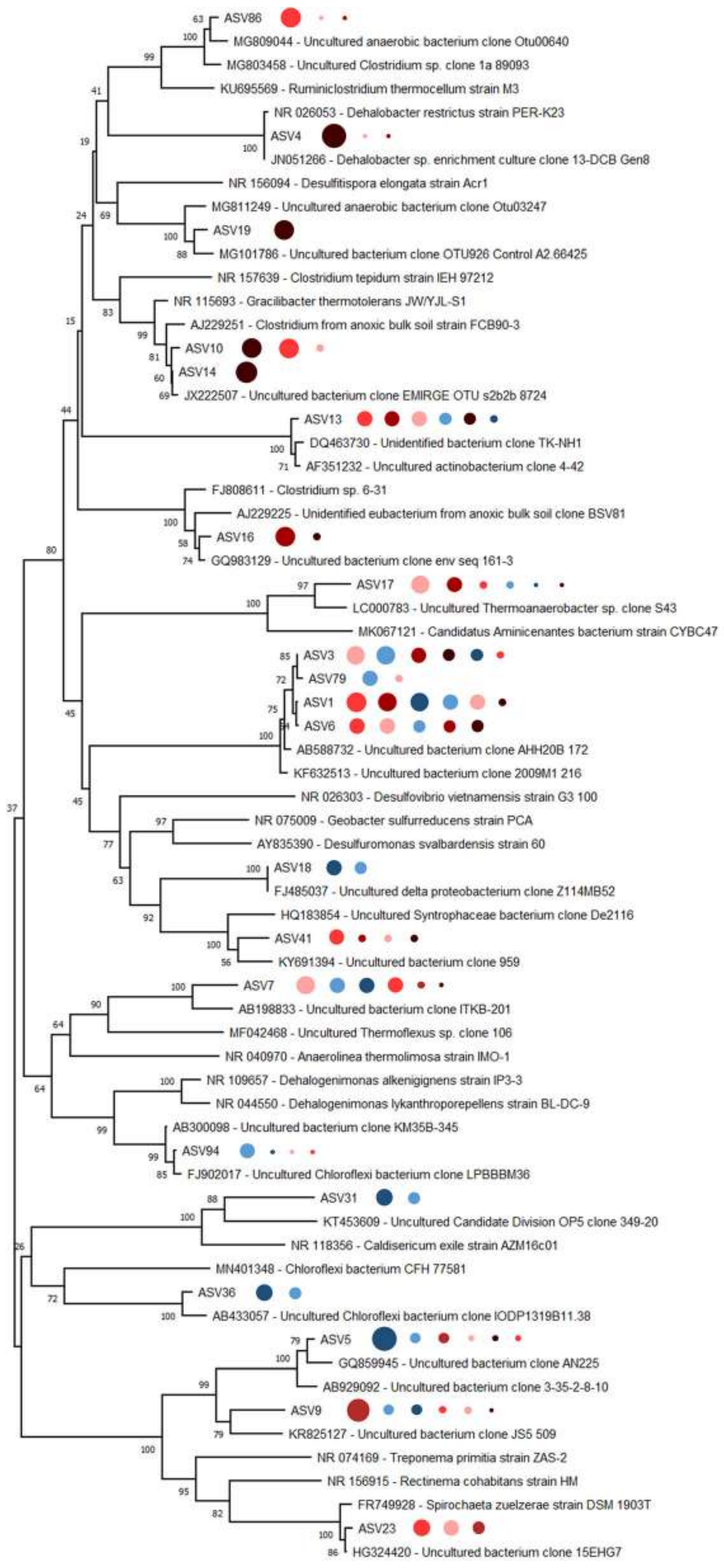
Fig. 4 Microbial community composition of samples collected from Caretti and Manzi sites. Microbial taxa with relative abundances > 1% in at least one sample are shown

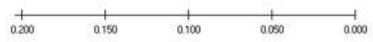
These ASVs were also abundant in M sediments in which, however, ASV5 was dominant in M3.2 and affiliated to the *Spirochaetes* phylum (Fig. 5).

Interestingly, the abundance of *Chloroflexi* observed in the taxonomy analysis of sequenced reads did not find correspon-dence with the analysis of the most abundant ASVs (Table S3). This evidence indicates that *Chloroflexi* (in partic-ular members of the *Dehaloccoidia* class) were highly diver-sified in the M and C sediments and that the *Chloroflexi* spe-cies were evenly spread among many different ASVs which, therefore, were detectable at low abundance. In the shallower sediments of the C site (C1.2 and C2.1), the most abundant ASV of *Chloroflexi* was ASV7, which belonged to the *Anaerolineae* class and was phylogenetically affiliated to se-quences recovered from subsurface sediments and groundwa-ter contaminated by arsenic compounds (Fig. 5). In the Caretti deepest sediment C2.3, nine abundant ASVs were affiliated to the *Clostridia* class of *Firmicutes* and were almost totally absent in the other C and M sediments. Among these, the most abundant ASV (ASV4) was affiliated to the genus *Dehalobacter* and shared high nucleotide similarity with sequences belonging to *Dehalobacter* spp. cultures that reduc-tively dehalogenate different dichlorobenzene isomers (Fig. 5). Other two abundant ASVs (ASV10 and ASV14) clustered in the phylogenetic tree with clones belonging to the *Gracilibacteraceae* family and retrieved from subsurface aquifer sediments (Fig. 5).

Functional inference of metabolic pathways involved in the synthesis of organic acids as reducing equivalents

We used PICRUSt2 to predict the functional capacity of the most abundant ASVs contributing to the reductive dechlori-nation of PCE and TCE in C sediments. We focused on func-tions involved in the degradation of organic matter and the synthesis of reducing equivalents used by dehalogenating mi-crobes in the reductive dechlorination. In this respect, for the most abundant ASVs in C samples with and NSTI < 0.15, functions were inferred from uncultured and environmental isolate-associated anaerobic niches such as hydrothermal vents, soil and aquatic sediments, and anaerobic digesters


ASV86
MG809044 - Uncultured anaerobic bacterium clone Otu00640
MG803458 - Uncultured Clostridium sp. clone 1a 89093
KU695569 - Ruminiclostridium thermocellum strain M3
NR 026053 - Dehalobacter restrictus strain PER-K23
ASV4
JN051266 - Dehalobacter sp. enrichment culture clone 13-DCB Gen8
NR 156094 - Desulfitispora elongata strain Acr1
MG811249 - Uncultured anaerobic bacterium clone Otu03247
ASV19
MG101786 - Uncultured bacterium clone OTU926 Control A2 66425
NR 157639 - Clostridium tepidum strain IEH 97212
NR 115693 - Gracilibacter thermotolerans JW/YJL-S1
AJ229251 - Clostridium from anoxic bulk soil strain FCB90-3
ASV10
ASV14
JX222507 - Uncultured bacterium clone EMIRGE OTU s2b2b 8724
ASV13
DQ463730 - Unidentified bacterium clone TK-NH1
AF351232 - Uncultured actinobacterium clone 4-42
FJ808611 - Clostridium sp. 6-31
AJ229225 - Unidentified eubacterium from anoxic bulk soil clone BSV81
ASV16
GQ983129 - Uncultured bacterium clone env seq 161-3
ASV17
LC000783 - Uncultured Thermoanaerobacter sp. clone S43
MK067121 - Candidatus Aminicenantes bacterium strain CYBC47
ASV3
ASV79
ASV1
ASV6
AB588732 - Uncultured bacterium clone AHH20B 172
KF632513 - Uncultured bacterium clone 2009M1 216
NR 026303 - Desulfovibrio vietnamensis strain G3 100
NR 075009 - Geobacter sulfurreducens strain PCA
AY835390 - Desulfuromonas svalbardensis strain 60
ASV18
FJ485037 - Uncultured delta proteobacterium clone Z114MB52
HQ183854 - Uncultured Syntrophaceae bacterium clone De2116
ASV41
KY691394 - Uncultured bacterium clone 959
ASV7
AB198833 - Uncultured bacterium clone ITKB-201
MF042468 - Uncultured Thermoflexus sp. clone 106
NR 040970 - Anaerolinea thermolimosa strain IMO-1
NR 109657 - Dehalogenimonas alkenigignens strain IP3-3
NR 044550 - Dehalogenimonas lykanthroporepellens strain BL-DC-9
AB300098 - Uncultured bacterium clone KM35B-345
ASV94
FJ902017 - Uncultured Chloroflexi bacterium clone LPBBBM36
ASV31
KT453609 - Uncultured Candidate Division OP5 clone 349-20
NR 118356 - Caldisericum exile strain AZM16c01
MN401348 - Chloroflexi bacterium CFH 77581
ASV36
AB433057 - Uncultured Chloroflexi bacterium clone IODP1319B11.38
ASV5
GQ859945 - Uncultured bacterium clone AN225
AB929092 - Uncultured bacterium clone 3-35-2-8-10
ASV9
KR825127 - Uncultured bacterium clone JS5 509
NR 074169 - Treponema primitia strain ZAS-2
NR 156915 - Rectinema cohabitans strain HM
FR749928 - Spirochaeta zuelzerae strain DSM 1903T
ASV23
HG324420 - Uncultured bacterium clone 15EHG7
Clostridia
(Firmicutes)
Actinobacteria
Limnochordia
(Firmicutes)
Candidatus
Aminicenantes
Elusimicrobia
Deltaproteobacteria
(Proteobacteria)
Anaerolinae
(Chloroflexi)
Dehalococcoidia
(Chloroflexi)
Calditrichaeota
Dehalococcoidia
(Chloroflexi)
Spirochaetia
(Spirochaetes)


0.200
0.150
0.100
0.050
0.000

0.5%
10%

C1.2
C2.1
C2.2
C2.3
Caretti
M2.1
M3.2
Manzi

◀ **Fig. 5** Phylogenetic tree of the dominant bacterial ASVs retrieved in the sediments under analysis. Colored dots represent the samples where each ASV is present. The dot size indicates the abundance of each ASV in the microbial community composition

(Table S4). Pathways involved in the synthesis of reducing substrates were the acetate synthesis via CO_2 fixation (Wood-Ljungdahl pathway, WLP) and anaerobic digestion of carbohydrates, and CO_2 synthesis via formate dehydrogenase (Fig. 7). Notably, the functional capacity of Ca. Bathyarchaeota ASVs to synthesize acetate was associated with enzymes linking the carbonyl and methyl-branch of the WLP with the synthesis of acetate from Acetyl-CoA (Fig. 7). Likewise, acetate synthesis from CO_2 fixation via the WLP methyl-branch of acetogenic bacteria was predicted from a *Desulfitobacterium* ASV (Fig. 7). In contrast, phosphate acetyltransferase and acetate kinase, which are also involved in the synthesis of acetate, were inferred from ASVs affiliated to Ca. Aminicenantes, *Spirochaetales*, *Halomonas*, and *Caldithrix* (Fig. 7). Therefore, we found that among the most abundant ASVs in C samples, these were affiliated to microbes having the metabolic capacity of synthetizing acetate as reducing equivalent for the reductive dechlorination. These findings do not necessarily exclude the presence of taxa capable of producing other organic acids such as butyrate or lactate as products of fermentative processes.

Reductive dehalogenases

Based on the results obtained from the sequencing of 16S rRNA, specific primers were used for the detection of the *vcrA*, *bvcA* and other *rdhA* genes (Table S2). VC-reductase genes *vcrA*-like were successfully amplified in samples C1.2 and C2.2 (Fig. 8), while the *bvcA* gene was not detected in any of the samples tested (Table S2). About the other reductive dehalogenases analyzed, only RDH22, i.e., the one containing the *crpA* cluster of the *Clostridiales* group, was found in sample C2.3. The finding of RDH22 in the only C2.3 sample agrees with the data obtained from the sequencing of the 16S rRNA, indicating an increase in the abundance of members of the *Clostridiales* order in that sample (Fig. 8).

Discussion

Among the different environmental remediation procedures, the use of indigenous microbial communities to bio-remediate chlorinated ethylene aquifers is gaining increasing interest (Adrian and Löffler 2016). However, previous attempts to increase the reductive dechlorination process have been conducted in the absence of a thorough knowledge of the fundamental biogeochemical cycles governing the activity of key organohalide-respiring bacteria. To fulfill this flaw, in the present work, we investigated the structure and composition of the whole microbial community present in an aquifer located in the subsoil of the city of Ferrara (northern Italy) contaminated with chlorinated ethylene compounds, along with the presence of different RDH genes.

The microbial diversity indices and estimated microbial population richness showed a partial reduction of both parameters in samples collected from the contaminated area C compared to those gathered from the non-contaminated area M (Table 1). This observation is not unusual as the influence of chlorinated solvents on the composition and diversity of microbial communities in enrichment procedures has previously been described (Atashgahi et al. 2017; Matturro et al. 2018). Furthermore, the microbial diversity was seen to decrease in parallel with the deepness of the different C sediments, and this phenomenon is likely due either to a higher concentration of VC at greater depths or a strong influence of depth on microbial diversity under anoxic conditions. Notably, the lean-peat samples (C2.1 and C2.3) showed a lower microbial richness of both the rich organic aquitard layer and the aquifer layer (C1.2 and C2.2). In recent years, several studies have examined the composition of peat microbial communities across different climate zones (Pankratov et al. 2008; Mishra et al. 2014). A common finding was that microbial communi-ties in the peat-ecosystem were composed of a few dominant taxa reflecting the specific features of the peat (Andersen et al. 2013; Gilbert and Mitchell 2006) and this observation might possibly explain the reduction of microbial diversity in peat samples C2.1 and C2.3.

The Illumina sequencing analysis of 16S rRNA gene revealed the presence of a complex microbial community that synergistically supports the activity of reductive dehalogenation. Although the *Bacteria* domain was dominant in all C and M sediments, archaeal sequences strongly affected each microbial community, as Ca. Bathyarchaeota consistently represented one of the most abundant phyla among the samples analyzed. Besides, it was evident that the contaminants represented one of the main factors that affected the microbial community structure present in the Caretti site. In line with our finding, a significant presence of *Archaea* in the microbial communities active in the dechlorination of halogenated hydrocarbons has also been reported in the past (Lee et al. 2012; Richardson et al. 2002). Most of them are related to different groups of methanogens belonging to both the *Euryarchaeota* and Ca. Bathyarchaeota phyla that may be co-localized together with the dehalogenating bacteria to form biofloccules (Rowe et al. 2008). It has been shown that some methanogens (e.g., *Methanosarcina* spp.) produce corrinoids that can be used as reductive dehalogenase cofactors by bacteria such as *Dehalococcoides* spp. (Mazumder et al. 1987). In the present work, the majority of the *Archaea* belonged to the Ca. Bathyarchaeota phylum. Members of Ca. Bathyarchaeota (formerly referred to as the "Miscellaneous Crenarchaeota

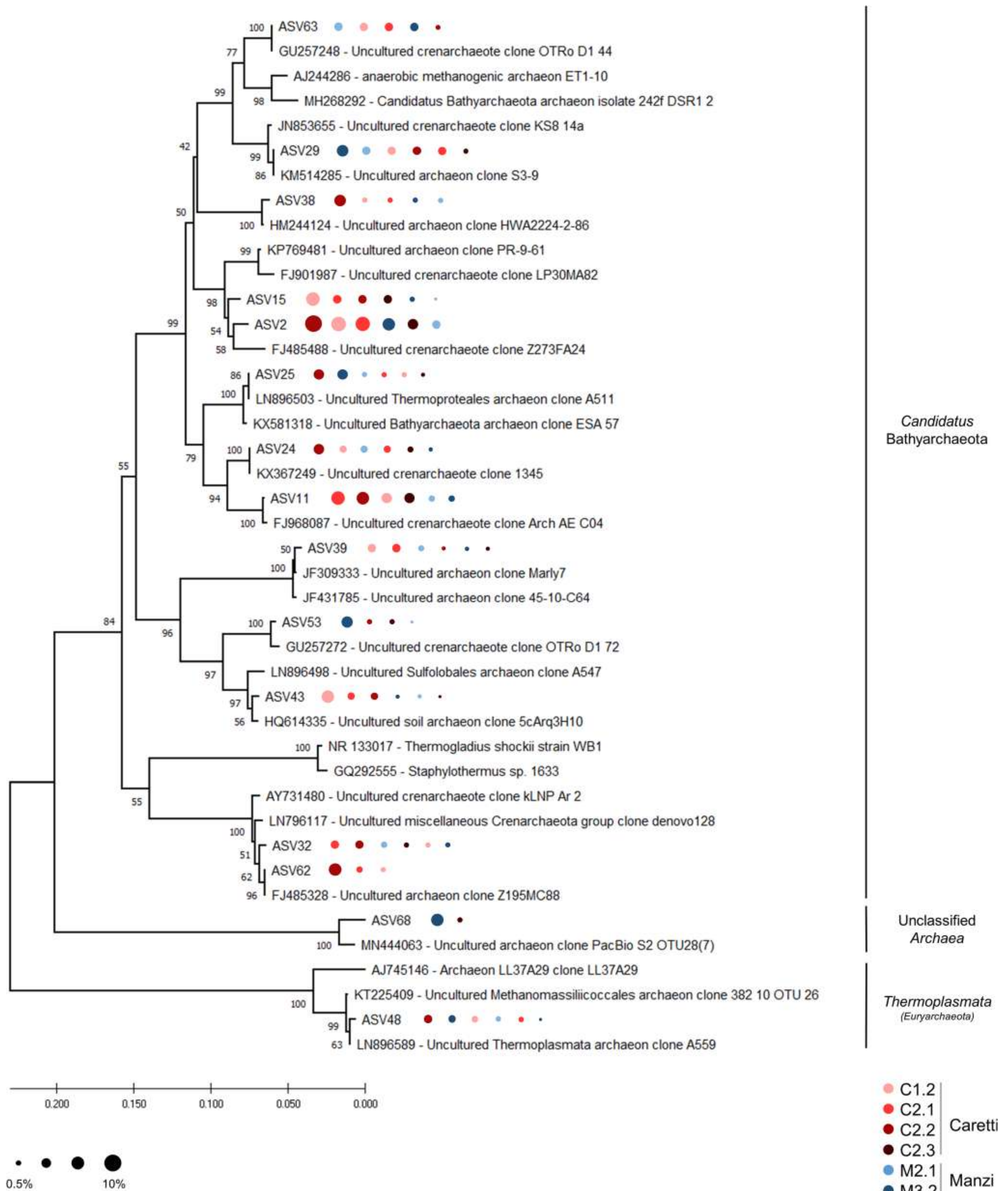


Fig. 6 Phylogenetic tree of the dominant archaeal ASVs retrieved in the sediments under analysis. Colored dots represent the samples where each ASV is present. The dot size indicates the abundance of each ASV in the microbial community composition

Group," Inagaki et al. 2003) are very frequently found in marine environments, particularly in the subsurface region and also in wetland and peat soils (Hawkins et al. 2014; Fillol et al. 2016).

Numerous reports have shown that *Bathyarchaeota* spp. can use a broad set of complex organic compounds as growth media, including proteins, cellulose, chitin, and aromatic com-pounds through fermentation (Lloyd et al. 2013), acetogenesis (He et al. 2016), and methanogenesis (Evans et al. 2015; Lloyd et al. 2013; Meng et al. 2014; Lazar et al. 2016). Recently, organoautotrophic growth of *Bathyarchaeota* with lignin as an energy source and bicarbonate as a carbon source has also been reported (Yu et al. 2018). Genetic and metabolic studies have also demonstrated that *Bathyarchaeota* spp. have the genetic potential for inorganic carbon fixation via the re-ductive acetyl-CoA Wood-Ljungdahl pathway (WLP) and some species also possess the carbon monoxide dehydrogenase/acetyl-CoA synthase complex (Codh/Acs) along with the capacity to produce acetate and H_2 (He et al. 2016). Functional features from the most abundant Ca. Bathyarchaeota ASVs found in PCE and TCE contaminated samples were predicted. According to the NSTI values, Ca. Bathyarchaeota ASV functional profiles were inferred from uncultured *Crenarchaeota* identified in geothermal systems and from a coalbed methane well (Table S2). According to the energy metabolism, all Ca. Bathyarchaeota ASVs shared functions involved in the utilization of methylated compounds for methanogenesis and associated with the carbonyl and methyl-branch of the WLP. Additionally, acetate biosynthetic pathways from CO_2 fixation and from glycolysis via pyruvate were also inferred from all Ca. Bathyarchaeota ASVs (Fig. 7 and Fig. 9).

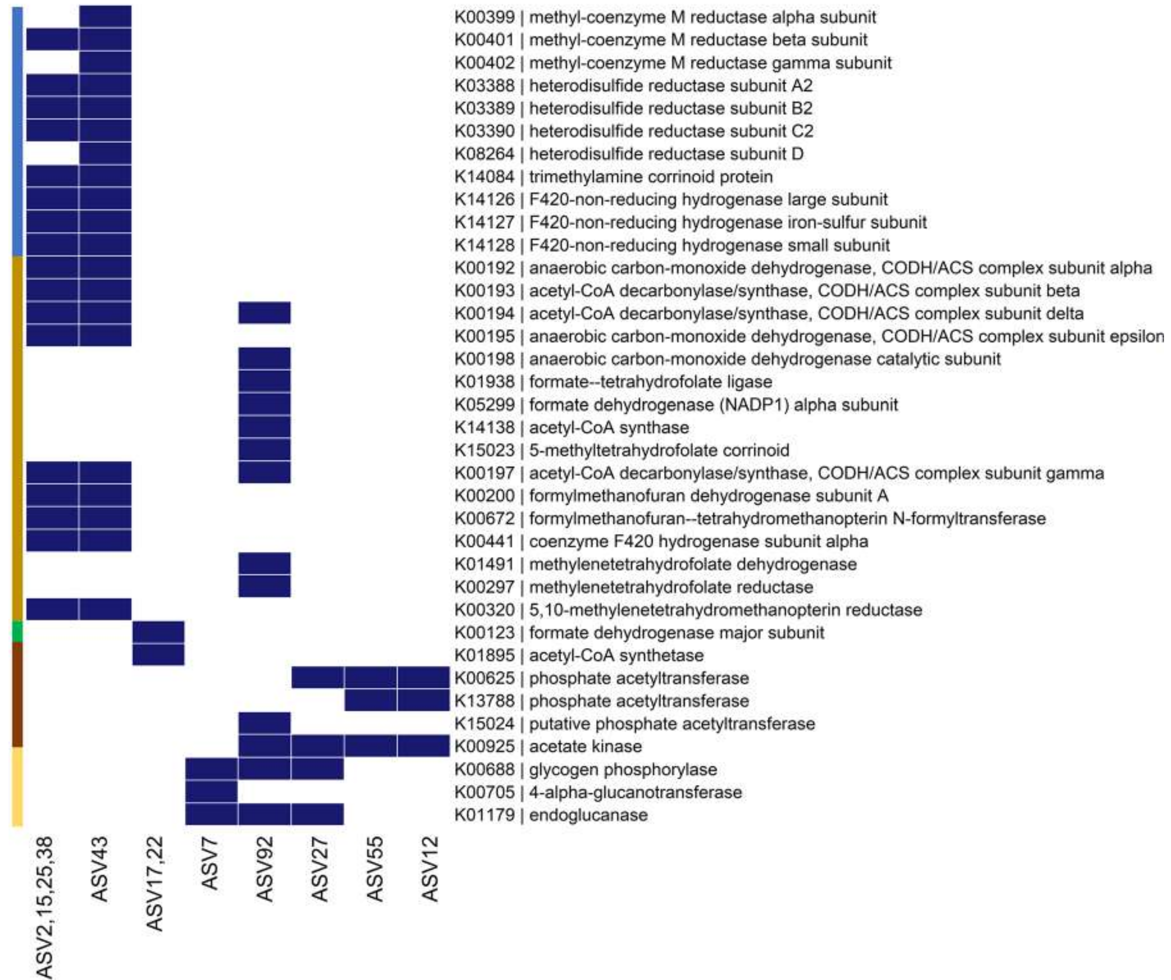
Sequences affiliated to the *Anaerolineae* class of the *Chloroflexi* phylum were abundant in all analyzed samples. These bacteria are considered typical fermenting microorgan-isms catalyzing the anaerobic digestion of organic matter (Rivière et al. 2009). Recently, the capacity of *Anaerolineae* spp. to grow on starch (Yamada et al. 2006) and cellulose (Podosokorskaya et al. 2013) has been demonstrated. Furthermore, analysis of fully sequenced genomes in combi-nation with meta-transcriptomics data demonstrated that ace-tate and lactate are likely the main products of glycolytic met-abolic pathway of this bacterial class (Xia et al. 2016). In respect to the *Anaerolineae* ASVs, the functional prediction was inferred from uncultured bacteria identified in deep oce-anic hydrothermal fluid (Table S2). While the functional pro-file associated to *Anaerolineae* ASVs indicated the presence of pathways involved in the degradation of starch and cellu-lose, the lack of genes linking the glycolytic pathways to the synthesis of acetate and lactate indicated that this microbial group does not contribute to the degradation of chlorinated solvents. The presence of [Ni-Fe]-hydrogenase was also re-ported in several *Chloroflexi* confirming the metabolic ability to produce hydrogen during fermentation (Nunoura et al. 2013; Imachi et al. 2014). In this respect, members of the *Anaerolineae* lineage of *Chloroflexi* are capable of autotrophy with molecular hydrogen and carbon dioxide as substrates since they encode both the WLP and a sodium-motive ferredoxin:NAD oxidoreductase complex used for energy conservation (Schuchmann and Müller 2016). However, in the functional profiling of *Anaerolineae* ASV, both [Ni-Fe]-hydrogenase and enzymes of the WLP were missing (Fig. 7). Core functions involved in the synthesis of fermentation prod-ucts such as acetate and CO_2 were inferred for ASVs affiliated to Ca. Aminicenantes (Fig. 7). Specifically, CO_2 synthesis via formate dehydrogenase and functions involved in the conver-sion of acetyl-CoA to acetate were predicted from an uncul-tured *Aminicenantes* isolated from Sakinaw Lake.

Elusimicrobia spp. were also found in abundance in all samples tested. Until recently, bacteria belonging to the *Elusimicrobia* class have only been associated with the intes-tinal tract of termites, but phylogenetic analyses of environ-mental sequences have also revealed their presence in differ-ent habitats such as groundwater, soil, and sediments (Herlemann et al. 2007). Groundwater-associated *Elusimicrobia* bacteria have considerable metabolic versatili-ty, and most of their genomes have the potential for expressing genes involved in the fermentation of sugars to acetate, buty-rate, and ethanol with the generation of ATP by substrate phosphorylation. Despite being identified among the most abundant ASVs, NSTI values associated with *Elusimicrobia* were far above the confidence threshold chosen as an accurate prediction of the functional profile.

Methanogenic bacteria were also detected in the C sedi-ments but in small amounts. Most of them were found in sample C1.2 and belonged to the *Methanocellaceae* family and the genus *Methanocella*. Isolated strains of this latter ge-nus were shown to utilize H_2/CO_2 and formate for growth and methane production (Sakai et al. 2008).

Based on our observation, it is therefore likely that part of H_2, CO_2, and acetate generated by acetogenic bacteria are converted into CH_4 through methanogenesis. On the other hand, methanogens may be necessary, together with other *Firmicutes* and *Deltaproteobacteria* syntrophic partners, to provide the appropriate cobamide lower base of vitamin B_{12} which is vital for *Dehalococcoides* dechlorination (Men et al. 2014).

Sequences identified as uncultured *Deltaproteobacteria* Sva0485 were found in all samples analyzed and represented between 5.3 and 7.2% of all sequences classified at the order level. Recently, uncultured *Deltaproteobacteria* Sva0485 ge-nomes were retrieved from a metagenomics study of an arti-ficial acid mine drainage system and analyzed in-depth to characterize the metabolism of this clade (Tan et al. 2019). Reconstructed metabolic pathways and gene expression pro-files showed that they were likely facultative anaerobic auto-trophs capable of both nitrogen fixation and fermentation of simple sugars into ethanol and lactate. The reconstruction of metabolic pathways and gene expression profiles also showed that these microorganisms are able to perform dissimilatory



ASV	C1.2	C2.1	C2.2	C2.3	Taxonomy
ASV2	5.78	5.43	7.29	1.67	p_*Ca.* Bathyarchaeota
ASV7	2.74	1.26	0.51	0.06	p_*Chloroflexi*; c_*Anaerolineae*
ASV12	1.70	0.91	0.70	1.93	p_*Proteobacteria*; g_*Halomonas*
ASV15	1.65	0.88	0.71	0.81	p_*Ca.* Bathyarchaeota
ASV17	2.09	0.32	1.24	0.03	p_*Ca.* Aminicenantes
ASV22	1.10	0.08	0.75	0.11	p_*Ca.* Aminicenantes
ASV25	0.23	0.31	1.03	0.21	p_*Ca.* Bathyarchaeota
ASV27	1.16	0.28	0.67	0.86	p_*Spirochaetes*; o_*Spirochaetales*
ASV38	0.30	0.27	1.30	0	p_*Ca.* Bathyarchaeota
ASV43	1.23	0.41	0.38	0.03	p_*Ca.* Bathyarchaeota
ASV55	0.14	0.52	1.06	0	p_*Calditrichaeota*
ASV92	1.16	0	0.01	0	p_*Firmicutes*; g_*Desulfitobacterium*

◀ **Fig. 7** Heatmap displaying the predicted functional profile of ASVs involved in the synthesis of reducing equivalents and reducing equivalent and carbohydrates digestion. Upper panel, presence/ absence of functional orthologues is encoded in midnight blue and white, respec tively. Lower panel, ASVs relative abundance in C samples

sulfate reduction and/or oxidize sulfide depending on the oxygen concentration present.

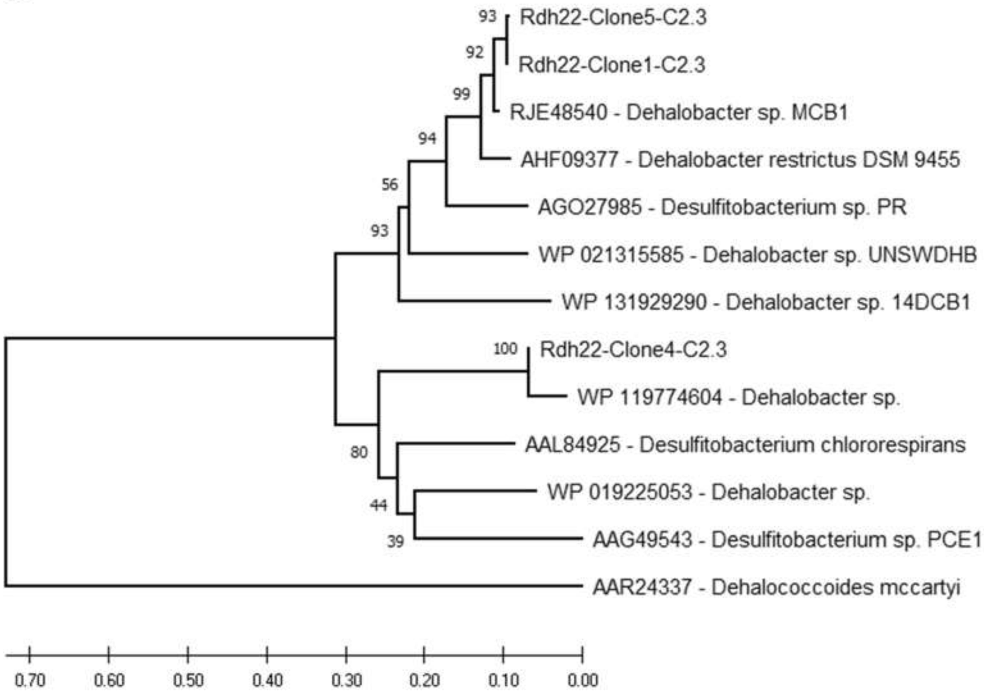
Dehalococcoidia spp. were found in all characterized samples. It should be noted that in contaminated samples, their percentage decreased from the shallowest C1.2 sample (12%) to the deepest C2.3 (2.3%) collected in the peaty zone. Members of the *Dehalococcoidia* class have the peculiar ability to use halogenated organic compounds (PCE and TCE) as terminal electron acceptors in the respiratory chain coupled to H_2 as the sole electron donor and acetate as carbon source. Furthermore, some species of *Dehalococcoidia*, such as *Dehalococcoides mccartyi*, are known to metabolically respire PCE to the non-toxic ethylene (Maymó-Gatell et al. 1997) using reductive dehalogenase enzymes BvcA and VcrA (encoded by genes *bvcA* and *vcrA*) (Krajmalnik-Brown et al. 2004; Müller et al. 2004; Sung et al. 2006). On the other hand, the consumption of H_2 by *Dehalogenomonas* spp. can create a thermodynamically favorable environment for fermenting microorganisms that could be inhibited by high concentrations of hydrogen (Lee et al. 2012).

Dehalobacter members were highly present in sample C2.3. The genus *Dehalobacter* is phylogenetically closely related to the *Desulfitobacterium* genus (Villemur et al. 2006). However, compared to the latter, most strains of *Dehalobacter* are specialized for organohalide respiration, a lifestyle similar to *Dehalococcoides* or *Dehalogenimonas* in the *Choroflexi* phylum (Siddaramappa et al. 2012). Furthermore, sample C2.3 was also rich in other bacteria of the *Clostridia* class which can ferment numerous compounds, including glucose, arabinose, fructose, pectin, glycerol, starch, fumarate with ethanol, acetate, and hydrogen production (Blouzard et al. 2010; Giallo et al. 1985; Liu et al. 2014; Ravachol et al. 2016). Their presence might favor the biodegradation of chlorinated organics by *Dehalobacter* spp. (Shan et al. 2010). Indeed, the mixed acid fermentation activity is likely to lower the ambient redox potential, thereby creating conditions for a better reductive dechlorination activity by *Dehalobacter* (Shan et al. 2010).

The *vrcA* metabolic genes for reductive VC dechlorination were amplified in samples C1.2 and C2.2, while the Rdh22 genes were found only in sample C2.3. The *vcrA* genes encode for the PCE, DCE, and VC, to ethylene-reducing dehalogenase and are mainly found in *Dehalococcoides* spp. Their presence in C1.2 and C2.2 samples was correlated with the abundance of 16S rRNA amplicons belonging to *Dehalococcoides*, along with the concentration of VC detected in samples C2.2. In this respect, a positive correlation between the abundance of *Dehalococcoides* spp., *vcrA* gene amplification, and VC transformation to ethylene has previ-ously been reported in an anoxic dechlorinating bioreactor, anaerobic microcosms containing superficial river sediments, and sediment samples collected from sites contaminated ini-tially with PCE (Maphosa et al. 2010; Yoshikawa et al. 2017; Atashgahi et al. 2013). However, as reported by Takeuchi et al. (2011), numerous reductive dehalogenase genes in sed-iment might also be affected by multiple chemical properties, such as natural organic matter, and not only VC concentration. This latter observation may explain why, in our study, we failed in amplifying complementary Rdhase genes such as *tceA* and *bvcA* from all the contaminated samples we tested.

The genes encoding reductive dehalogenases that were am-plified using the Rdhase group 22 primers were related to those of the *Dehalobacter* genus. They were amplified only from the C2.3 sample according to the prevalence of 16S rRNA sequences affiliated to the genus *Dehalobacter* found exclusively in this contaminated sample. *Dehalobacter* spp. are known for respiring both aliphatic, e.g., PCE (Holliger et al. 1998), and aromatic-like dichlorobenzoate, and they can dechlorinate PCE and TCE to cDCE (Grostern and Edwards 2006; Tang and Edwards 2013). Notably, no further reductive dechlorination of cDCE to VC and ethylene has been found in species of this genus (Regeard et al. 2004). These results, combined with data on the distribution of 16S rRNA sequences of *Dehalococcoides* and *Dehalobacter* in contaminated samples, allowed us to conclude that PCE and TCE were mainly dehalogenated in sample C2.3. Concurrently, VC dehalogenation is likely to occur in samples closest to the surface where *Dehalococcoides* are more numer-ous as compared to sample C2.3, and where *vcrA* genes have been detected (Fig. 8).

Taken all together, based on the present and past observa-tions describing the contaminated samples of the Caretti site, we propose a working model of the possible metabolic inter-actions within the microbial communities involved in the re-ductive dehalogenation process analyzed here (Fig. 9). Such model also takes in consideration the functional profiling of ASVs affiliated to taxa involved in the synthesis of fermenta-tion products that supports the dechlorination process. In our tentative scheme, several groups of *Anaerolineae* and Ca. Bathyarchaeota can transform some of the main organic com-ponents of peat, such as cellulose, hemicellulose, lignin, hu-mic acids, fulvic acids, and mixed carbohydrates, into low molecular weight metabolites that can be further fermented by *Elusimicrobia*, Ca. Aminicenantes, and *Clostridia*, with organic acids, CO_2, and hydrogen as final products. These compounds are essential to stimulate the reductive dechlori-nation of TCE and PCE to ethylene by *Dehalococcoides* that have been found predominant in C1.2, C2.1, and C2.2 sam-ples, together with the presence of *vcrA* genes which code for the VcrA enzyme that dechlorinates vinyl chloride to



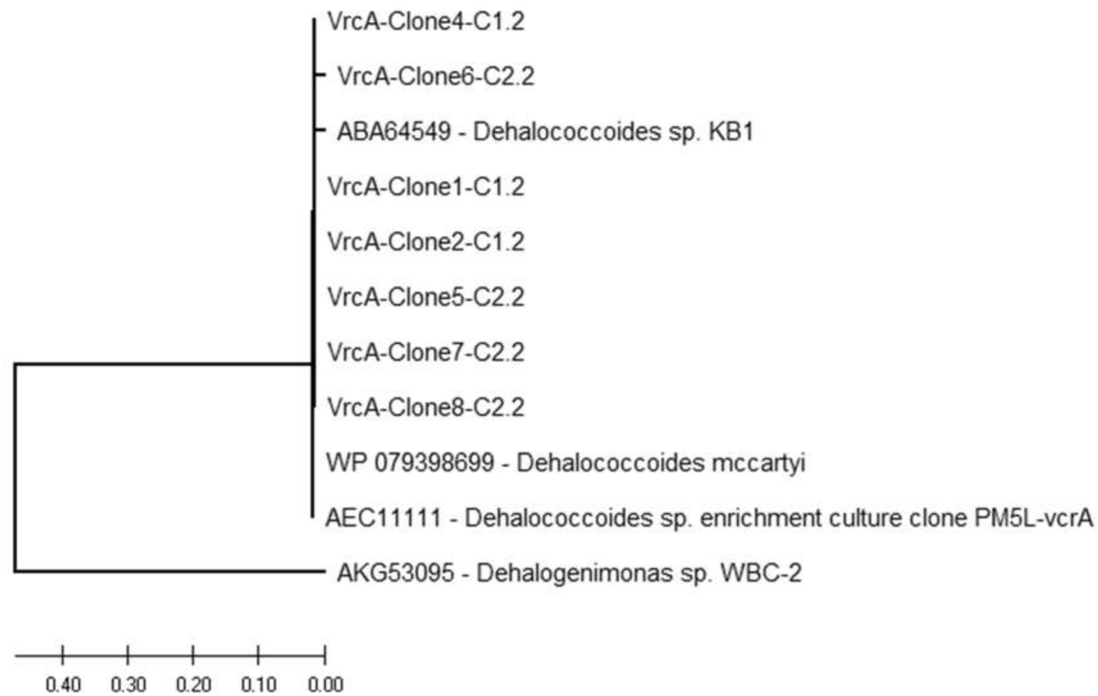


Fig. 8 Phylogenetic trees of the amino acidic sequences retrieved from clone library analysis of **a** Rdh22 and **b** VrcA

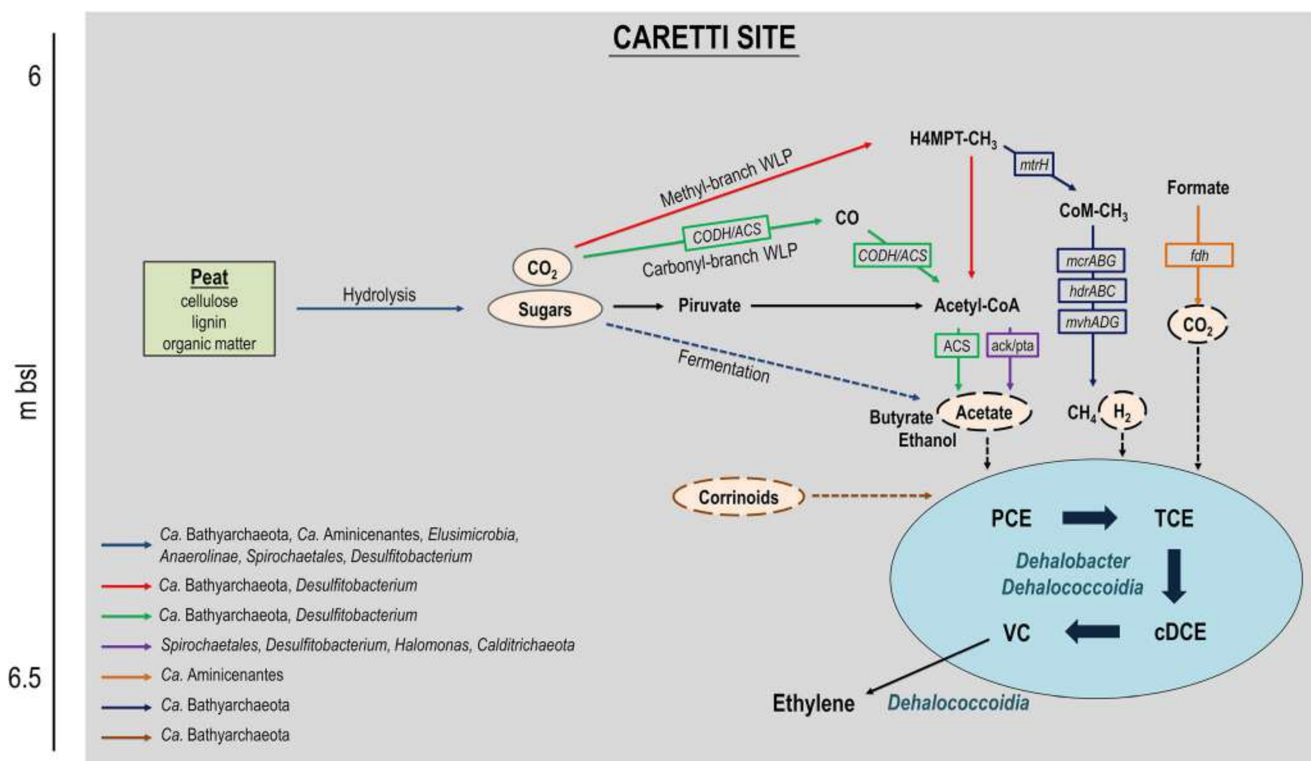


Fig. 9 Schematic overview of the metabolic processes which are proposed to take place within the dechlorinating microbial consortia at the Caretti site aquifer. Metabolic pathways are indicated with different colors and the microorganism contributing to each metabolic pathway are reported in the figure legend. Dashed lines indicate pathways non predicted from the functional profiling of ASVs. Gene names are *mcr* (methyl coenzyme M reductase); *hdr* (heterodisulfide reductase subunit); *mvh* (F420 non reducing hydrogenase); CODH/ACS (carbon monoxide dehydrogenase/acetyl CoA synthase); *ack*/*pta* (acetate kinase/phosphate acetyltransferase); *fdh* (formate dehydrogenase)

ethylene, thus avoiding its accumulation. In the deepest sample C2.3, members of the *Dehalobacter* genus contribute to the reductive dechlorination of TCE to cDCE and VC, together with the syntrophic fermentation activity of other microorganisms of the *Firmicutes* phylum.

Numerous reports describe the enrichment and isolation of anaerobic microbial consortia in microcosms or bioreactors amended with organic acids or alcohols and in the presence of halogenated aliphatic compounds (Yohda et al. 2015; Hug et al. 2012). It is interesting to note that these consortia's microbial composition resembles the natural microbial population structure present in the Caretti site. The peat layers provide the organic material for fermentation and methanogenesis metabolisms assisting the reductive dechlorination process carried out by *Dehalococcoidia* and *Dehalobacter* and naturally created an involved microbial selection community capable of dehalogenating organic halogenated compounds. The implementation of metagenomic analyses with the monitoring of the expression of reductive dehalogenases and quantification of the main actors involved in the dechlorination process by qPCR along with the comparison with adjacent uncontaminated sites, where dechlorination fails, will provide indications to understand and optimize the natural microbial consortium activity. This information will be critical to promote and stimulate the processes of reductive dechlorination in natural environments.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356 020 12236 3.

Acknowledgments We acknowledge the Environmental Service of the Municipality of Ferrara for their support during field investigation. We are also grateful to the UNIBO student Alessia De Matteis for the pre cious contribution in the experimental work.

Authors' contributions Conceptualization: Stefano Fedi, Daniele Ghezzi, Maria Filippini, Martina Cappelletti; methodology: Stefano Fedi, Daniele Ghezzi, Martina Cappelletti; formal analysis and investigation: Stefano Fedi, Daniele Ghezzi, Maria Filippini, Andrea Firrincieli; writing original draft preparation: Stefano Fedi, Daniele Ghezzi, Maria Filippini; Andrea Firrincieli; writing review and editing: Stefano Fedi, Daniele Ghezzi, Maria Filippini, Davide Zannoni; funding acquisition: Davide Zannoni, Alessandro Gargini; supervision: Stefano Fedi, Alessandro Gargini, Davide Zannoni

Funding This study was funded by the University of Bologna (RFO grant). AF is financed by the ELECTRA project that has received funding from the European Union's Horizon 2020 research and innovation pro gram under grant agreement no. 826244.

Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

References

Adrian L, Löffler FE (2016) Organohalide respiring bacteria. Springer Verlag Berlin Heidelberg https:/ /doi.org/10.1007/978 3 662 49875 0_2

Andersen R, Chapman SJ, Artz RRE (2013) Microbial communities in natural and disturbed peatlands: a review. Soil Biol Biochem 57: 979 994. : https: //doi.org/10.1016/j.soilbio.2012.10.003

Atashgahi S, Maphosa F, Doğan E, Smidt H, Springael D, Dejonghe W (2013) Small scale oxygen distribution determines the vinyl chlo ride biodegradation pathway in surficial sediments of riverbed hyporheic zones. FEMS Microbiol Ecol 84:133 142. https :// doi. org/10.1111/1574 6941.12044

Atashgahi S, Lu Y, Zheng Y, Saccenti E, Suarez Diez M, Ramiro Garcia J, Eisenmann H, Elsner M, JM Stams A, Springael D, Dejonghe W, Smidt H (2017) Geochemical and microbial community determi nants of reductive dechlorination at a site biostimulated with glyc erol. Environ Microbiol 19:968 981. : https :// doi.org/10.1111/ 1462 2920.13531

Balderacchi M, Filippini M, Gemitzi A, Klöve B, Petitta M, Trevisan M, Wachniew P, Witczak S, Gargini A (2014) Does groundwater pro tection in Europe require new EU wide environmental quality stan dards? Front Chem 2:32. .org/https ://doi.org/10.3389/fchem 2014. 00032

Blouzard JC, Coutinho PM, Fierobe HP, Henrissat B, Lignon S, Tardif C, Pagès S, de Philip P (2010) Modulation of cellulosome composition in *Clostridium cellulolyticum*: adaptation to the polysaccharide en vironment revealed by proteomic and carbohydrate active enzyme analyses. Proteomics 10:541 554. : https ://doi.org/10.1002/pmic. 200900311

Bolyen E, Rideout JR, Dillon M.R., Dillon MR, Bokulich NA, Abnet CC, al Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo Rodríguez AM, Chase J, Cope EK, da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson II MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul Hasan S, van der Hooft JJJ, Vargas F, Vázquez Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG (2019) Reproducible, interactive, scalable and exten sible microbiome data science using QIIME 2. Nat Biotechnol 37: 852 857. https ://doi.org/10.1038/s41587 019 0209 9

Bradley PM, Chapelle FH (2010) Biodegradation of chlorinated ethenes, In Stroo HF, Ward CH (Eds) In: In situ remediation of chlorinated solvent plumes, Springer, New York, pp. 39 67. https://doi.org/10. 1007/978 1 4419 1401 9_1

Bruno L, Campo B, Di Martino A, Hong W, Amorosi A (2019) Peat layer accumulation and post burial deformation during the mid late Holocene in the Po coastal plain (Northern Italy). Basin Res 31: 621 639. https://doi.org/10.1111/bre. 12339

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP (2016) DADA2: high resolution sample inference from Illumina amplicon data. Nat Methods 13:581 583. https:// doi.org/10.1038/nmeth. 3869

Caporaso JG, Lauber CL, Walters WA, Berg Lyons D, Lozupone CA Turnbaugh PJ, Fierer N, Knight R, (2010) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A 108 Suppl 1:4516 4522. : https:// doi.org/10. 1073/ pnas.1000080107

Cappelletti M, Ghezzi D, Zannoni D, Capaccioni B, Fedi S (2016) Diversity of methane oxidizing bacteria in soils from "Hot Lands of Medolla" (Italy) featured by anomalous high temperatures and biogenic CO_2 emission. Microbes Environ 31:369 377. : https://doi.org/10.1264/jsme2.ME1 6087

Cappelletti M, Pinelli D, Fedi S, Zannoni D, Frascari D (2018) Aerobic co metabolism of 1,1,2,2 tetrachloroethane by *Rhodococcus aetherivorans* TPA grown on propane: kinetic study and bioreactor configuration analysis. J Chem Technol Biotechnol 93:155 165. https ://doi.org/10.1002/jctb.5335

Chiu HY, Liu KK, Chien HY, Surampalli RY, Kao CM (2013) Evaluation of enhanced reductive dechlorination of trichloroethy lene using gene analysis: pilot scale study. J Environ Eng 139: 428 437. : https ://doi.org/10.1061/(ASCE)EE.1943 7870.0000654

Clark K, Taggart DM, Baldwin BR, Ritalahti KM, Murdoch RW, Hatt JK, Löffler FE (2018) Normalized quantitative PCR measurements as predictors for ethene formation at sites impacted with chlorinated ethenes. Environ Sci Technol 52:13410 13420. https ://doi.org/10. 1021/acs.est.8b04373

Clarke KR, Gorley RN (2015) Getting started with PRIMER V7 "User Manual/Tutorial" PRIMER E, Plymouth Marine Laboratory

Courbet C, Riviere A, Jeannottat S, Rinaldi S, Hunkeler D, Bendjoudi H, de Marsily G (2011) Complementing approaches to demonstrate chlorinated solvent biodegradation in a complex pollution plume: mass balance, PCR and compound specific stable isotope analysis. J Contam Hydrol 126:315 329. https :// doi.org/10.1016/j.jconhyd. 2011.08.009

Damgaard I, Bjerg PL, Baelum J, Scheutz C, Hunkeler D, Jacobsen CS, Tuxen N, Broholm MM (2013) Identification of chlorinated sol vents degradation zones in clay till by high resolution chemical, microbial and compound specific isotope analysis. J Contam Hydrol 146:37 50. : https ://doi.org/10.1016/j.jconhyd.2012.11.010

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194 2200. https ://doi.org/10.1093/bioinformatics/btr381

Evans PN, Parks DH, Chadwick GL, Robbins SJ, Orphan VJ, Golding SD, Tyson GW (2015) Methane metabolism in the archaeal phylum *Bathyarchaeota* revealed by genome centric metagenomics. Science. 350:434 438. :https ://doi.org/10.1126/science.aac7745

Filippini M, Stumpp C, Nijenhius I, Richnow HH, Gargini A (2015) Evaluation of aquifer recharge and vulnerability in an alluvial low land using environmental tracers. J Hydrol 529:1657 1668. https :// doi.org/10.1016/j.jhydrol.2015.07.055

Filippini M, Amorosi A, Campo B, Herrero Martin S, Nijenhuis I, Parker BL, Gargini A, (2016) Origin of VC only plumes from naturally enhanced dechlorination in a peat rich hydrogeologic setting. J Contam Hydrol 192:129 139. :https ://doi.org/10.1016/ j.jconhyd. 2016.07.003

Filippini M, Parker BL, Dinelli E, Wanner P, Chapman SW, Gargini A (2020) Assessing aquitard integrity in a complex aquifer aquitard system contaminated by chlorinated hydrocarbons. Water Res 171: 115388. : https ://doi.org/10.1016/j.watres.2019.115388

Fillol M, Auguet JC, Casamayor EO, Borrego CM (2016) Insights in the ecology and evolutionary history of the Miscellaneous Crenarchaeotic Group lineage. ISME J 10:665 677. https ://doi. org/10.1038/ismej.2015.143

Futagami T, Morono Y, Terada T, Kaksonen AH, Inagaki F (2009) Dehalogenation activities and distribution of reductive dehalogenase homologous genes in marine subsurface sediments. Appl Environ Microbiol 75:6905 6909. https ://doi.org/10.1128/AEM.01124 09

Gargini A, Pasini M, Picone S, Rijnaarts H. Van Gaans P (2011) Chlorinated hydrocarbons plumes in a residential area. Site investi gation to assess indoor vapor intrusion and human health risks. In: Saponaro S, Sezenna E, Bonomo L, (Eds) Vapor emission to out door air and enclosed spaces for human health risk assessment: site characterization, monitoring and modelling. Nova Science Publishers, Milan, pp. 211 233

Giallo J, Gaudin C, Belaich JP (1985) Metabolism and solubilization of cellulose by *Clostridium cellulolyticum* H10. Appl Environ Microbiol 49:1216 1221. https ://doi.org/10.1128/ AEM.49.5.1216 1221.1985

Gilbert D, Mitchell EAD (2006) Microbial diversity in *Sphagnum peatlands* In: Peatlands: evolution and records of environmental and climate changes. Martini IP, Cortizas AM, Chesworth W (eds) Elsevier, Amsterdam, pp 287 318

Grostern A, Edwards EA (2006) A 1,1,1 trichloroethane degrading an aerobic mixed microbial culture enhances biotransformation of mix tures of chlorinated ethenes and ethanes. Appl Environ Microbiol 72:7849 7856. : https ://doi.org/10.1128/AEM.01269 06

Hawkins AN, Johnson KW, Brauer SL (2014) Southern Appalachian Peatlands support high Archaeal diversity. Microb Ecol 67:587 602. https ://doi.org/10.1007/s00248 013 0352 7

He Y, Li M, Perumal V, Feng X, Fang J, Xie J, Sievert S.M, Wang F (2016) Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum *Bathyarchaeota* wide spread in marine sediments. Nat Microbiol 1:16035. https ://doi. org/10.1038/nmicrobiol.2016 35

Herlemann DP, Geissinger O, Brune A (2007) The termite group I phy lum is highly diverse and widespread in the environment. Appl Environ Microbiol 73:6682 6685. https ://doi.org/10.1128/ AEM. 00712 07

Hermon L, Hellal J, Denonfoux J, Vuilleumier S, Imfeld G, Urien C, Ferreira S, Joulian C (2019) Functional genes and bacterial commu nities during organohalide respiration of chloroethenes in micro cosms of multi contaminated groundwater. Front Microbiol 10:89. https ://doi.org/10.3389/fmicb.2019.00089

Holliger C, Hahn D, Harmsen H, Ludwig W, Schumacher W, Tindall B, Vazquez F, Weiss N, Zehnder AJ (1998) *Dehalobacter restrictus* gen. nov. and sp. nov , a strictly anaerobic bacterium that reductively dechlorinates tetra and trichloroethene in an anaerobic respiration. Arch Microbiol 169:313 321. http s://doi.org/10.1007/s002030050577

Hug LA, Edwards EA (2013) Diversity of reductive dehalogenase genes from environmental samples and enrichment cultures identified with degenerate primer PCR screens. Front Microbiol 4:341. https ://doi. org/10.3389/fmicb.2013.00341. eCollection 2013. Erratum in: Front Microbiol (2015) 6:30

Hug LA, Beiko RG, Rowe AR, Richardson RE, Edwards EA (2012) Comparative metagenomics of three Dehalococcoides containing en richment cultures: the role of the non dechlorinating community. BMC Genomics 13:1 19. https: //doi.org/10.1186/1471 2164 13 327

Hunkeler D, Abe Y, Broholm MM, Jeannottat S, Westergaard C, Jacobsen CS, Aravena R, Bjerg PL (2011) Assessing chlorinated ethene degradation in a large scale contaminant plume by dual carbon chlorine isotope analysis and quantitative PCR. J Contam Hydrol 119:69 79. https ://doi.org/10.1016/j.jconhyd.2010.09.009

IARC (2008) IARC monographs on the evaluation of carcinogenic risks to humans. 1, 3 butadiene, ethylene oxide and vinyl halides (vinyl fluoride, vinyl chloride and vinyl bromide). IARC Monogr Eval Carcinog Risks Hum 97:3 471

Imachi H, Sakai S, Lipp JS, Miyazaki M, Saito Y, Yamanaka Y, Hinrichs KU, Inagaki F, Takai K (2014) *Pelolinea submarina gen*. nov , sp. nov., an anaerobic, filamentous bacterium of the phylum *Chloroflexi* isolated from subseafloor sediment. Int J Syst Evol Microbiol 64: 812 818. https ://doi.org/10.1099/ijs.0.057547 0

Imfeld G, Pieper H, Shani N, Rossi P, Nikolausz M, Nijenhuis I, Paschke H, Weiss H, Richnow HH (2011) Characterization of groundwater microbial communities, dechlorinating bacteria, and in situ biodeg radation of chloroethenes along a vertical gradient. Water Air Soil Pollut 211:107 122. https ://doi.org/10.1007/s11270 011 0774 0

Inagaki F, Suzuki M, Takai K, Oida H, Sakamoto T, Aoki K, Nealson KH, Horikoshi K (2003) Microbial communities associated with geological horizons in coastal subseafloor sediments from the sea of okhotsk. Appl Environ Microbiol 69:7224 7235. https ://doi.org/ 10.1128/aem.69.12.7224 7235.2003

Kotik M, Davidová A, Voříšková J, Baldrian P (2013) Bacterial commu nities in tetrachloroethene polluted groundwaters: a case study. Sci Total Environ 454 455:517 527. https :// doi.org/10.1016/j. scitotenv 2013.02.082

Krajmalnik Brown R, Hölscher T, Thomson IN, Saunders FM, Ritalahti KM, Löffler FE (2004) Genetic identification of a putative vinyl chloride reductase in *Dehalococcoides* sp. strain BAV1. Appl Environ Microbiol 70:6347 6351. https :// doi.org/10.1128/AEM. 70.10.6347 6351 2004

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing plat forms. Mol Biol Evol 35:1547 1549. https :// doi.org/10.1093/molbev/msy096

Lazar CS, Baker BJ, Seitz K, Hyde AS, Dick GJ, Hinrichs KU, Teske AP (2016) Genomic evidence for distinct carbon substrate preferences and ecological niches of *Bathyarchaeota* in estuarine sediments. Environ Microbiol 18:1200 1211. https ://doi.org/10.1111/1462 2920.13142

Lee PK, Warnecke F, Brodie EL, Macbeth TW, Conrad ME, Andersen GL, 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. https ://doi.org/10.1021/es203005k

Lee SS, Kaown D, Lee KK (2015) Evaluation of the fate and transport of chlorinated ethenes in a complex groundwater system discharging to a stream in Wonju, Korea. J Contam Hydrol 182:231 243. https ://doi.org/10.1016/j.jconhyd.2015.09.005

Liu Y, Qiao JT, Yuan XZ, Guo RB, Qiu YL (2014) Hydrogenispora ethanolica gen. nov., sp. nov., an anaerobic carbohydrate fermenting bacterium from anaerobic sludge. Int J Syst Evol Microbiol 64:1756 1762. https ://doi.org/10.1099/ijs.0.060186 0

Lloyd KG, Schreiber L, Petersen DG, Kjeldsen KU, Lever MA, Steen AD, Stepanauskas R, Richter M, Kleindienst S, Lenk S, Schramm A, Jørgensen BB (2013) Predominant archaea in marine sediments degrade detrital proteins. Nature 496:215 218. https ://doi.org/10. 1038/nature12033

Macbeth TW, Cummings DE, Spring S, Petzke LM, Sorenson KS Jr (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. https ://doi.org/10.1128/AEM.70.12.7329 7341.2004

Mackay D, Shiu W Y, Ma K C, Lee SC (2006) Handbook of physical chemical properties and environmental fate for organic chemicals. CRC press

Maphosa F, Smidt H, de Vos WM, Röling WF (2010) Microbial community and metabolite dynamics of an anoxic dechlorinating bioreactor. Environ Sci Technol 44:4884 4890. https ://doi.org/10. 1021/es903721s

Mattes TE; Alexander AK, Coleman NV (2010) Aerobic biodegradation of the chloroethenes: pathways, enzymes, ecology, and evolution. FEMS Microbiol Rev 34:445 475. https :// doi.org/10.1111/j.1574 6976.2010.00210.x

Matturro B, Pierro L, Frascadore E, Petrangeli Papini M, Rossetti S (2018) Microbial community changes in a chlorinated solvents pol luted aquifer over the field scale treatment with poly 3 hydroxybutyrate as amendment. Front Microbiol 9:1664. https ://doi.org/10.3389/fmicb.2018.01664

Maymó Gatell X, Chien Y, Gossett JM, Zinder SH (1997) Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. Science 276:1568 1571. https ://doi.org/10.1126/ science.276 5318. 1568

Mazumder TK, Nishio N, Fukuzaki S, Nagai S (1987) Production of extracellular vitamin B 12 compounds from methanol by *Methanosarcina barkeri*. Appl Microbiol Biotechnol 26:511 516. https ://doi.org/10.1007/BF00253023

McCarty PL (2010) Groundwater contamination by chlorinated solvents: history, remediation technologies and strategies. In: Stroo H, Ward C (eds) In situ remediation of chlorinated solvent plumes. SERDP/ ESTCP Environmental Remediation Technology. Springer, New York, pp 1 28. https ://doi.org/10.1007/978 1 4419 1401 9_1

Men Y, Seth EC, Yi S, Allen RH, Taga ME, 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. https :// doi.org/10.1128/AEM.03477 13

Meng J, Xu J, Qin D, He Y, Xiao X, Wang F (2014) Genetic and func tional properties of uncultivated MCG archaea assessed by metagenome and gene expression analyses. ISME J 8:650 659. https ://doi.org/10.1038/ismej.2013.174

Mishra S, Lee WA, Hooijer A, Reuben S, Sudiana IM, Idris A, Swarup S (2014) Microbial and metabolic profiling reveal strong influence of water table and land use patterns on classification of degraded trop ical peatlands. Biogeosciences 11:14009 14042. https ://doi.org/10. 5194/bg 11 1727 2014

Molinari FC, Severi P, Boldrini G, Dugoni G, Caputo DR, Martinelli G, Colombani N, Gargini A, Mastrocicco M, Messina A (2007) Risorse idriche sotterranee della Provincia di Ferrara (in italian; transl. Groundwater resources of the Ferrara Province), Risorse idriche sotterranee della Provincia di Ferrara (in italian; transl. Groundwater resources of the Ferrara Province). DB MAP printer, Florence, Italy, pp. 1 62

Müller JA, Rosner BM, Von Abendroth G, Meshulam Simon G, McCarty PL, Spormann AM (2004) Molecular identification of the catabolic vinyl chloride reductase from *Dehalococcoides* sp. strain VS and its environmental distribution. Appl Environ Microbiol 70:4880 4888. https ://doi.org/10.1128/ AEM.70.8.4880 4888.2004

Nijenhuis I, Nikolausz M, Köth A, Felföldi T, Weiss H, Drangmeister J, Groβmann J, Kästner M, Richnow HH (2007) Assessment of the natural attenuation of chlorinated ethenes in an anaerobic contami nated aquifer in the bitterfeld/wolfen area using stable isotope tech niques, microcosm studies and molecular biomarkers. Chemosphere 67:300 311. https ://doi.org/10.1016/j.chemosphere.2006.09.084

Nijenhuis I, Schmidt M, Pellegatti E, Paramatti E, Richnow HH, Gargini A (2013) A stable isotope approach for source apportionment of chlorinated ethene plumes at a complex multi contamination events urban site. J Contam Hydrol 153:92 105. https ://doi.org/10.1016/j. jconhyd.2013.06.004

Nunoura T, Hirai M, Miyazaki M, Kazama H, MakitaH HH, Furushima Y, Yamamoto H, Imachi H, Takai K (2013) Isolation and characterization of a thermophilic, obligately anaerobic and hetero trophic marine *Chloroflexi* bacterium from a *Chloroflexi* dominated microbial community associated with a Japanese shallow hydrother mal system, and proposal for *Thermomarinilinea lacunofontalis* gen. nov., sp. nov. Microbes Environ 28:228 235. https :// doi.org/ 10.1264/jsme2.me12193

Pankratov TA, Serkebaeva YM, Kulichevskaya IS, Liesack W, Dedysh SN (2008) Substrate induced growth and isolation of *Acidobacteria* from acidic Sphagnum peat. ISME J 2:551 560. https ://doi.org/10. 1038/ismej.2008.7

Parker BL, Cherry JA, Chapman SW, Guilbeault MA (2003) Review and analysis of chlorinated solvent DNAPL distributions in five sandy aquifers. Vadose Zone J 2:116 137. https :// doi.org/10.2136/vzj2003.1160

Podosokorskaya OA, Bonch Osmolovskaya EA, Novikov AA, Kolganova TV, Kublanov IV (2013) *Ornatilinea apprima* gen. nov., sp. nov., a cellulolytic representative of the class Anaerolineae. Int J Syst Evol Microbiol 63:86 92. https :// doi.org/ 10.1099/ijs.0.041012 0

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web based tools. Nucleic Acids Res 41:D590 D596. https ://doi.org/10.1093/nar/gks1219

Rahm BG, Chauhan S, Holmes VF, Macbeth TW, Sorenson KS Jr, Alvarez Cohen L (2006) Molecular characterization of microbial populations at two sites with differing reductive dechlorination abil ities. Biodegradation 17:523 534. https :// doi.org/10.1007/s10532 005 9023 9

Ravachol J, de Philip P, Borne R, Mansuelle P, Maté MJ, Perret S, Fierobe HP (2016) Mechanisms involved in xyloglucan catabolism by the cellulosome producing bacterium *Ruminiclostridium cellulolyticum*. Sci Rep 6:22770. https ://doi.org/10.1038/srep22770

Regeard C, Maillard J, Holliger C (2004) Development of degenerate and specific PCR primers for the detection and isolation of known and putative chloroethene reductive dehalogenase genes. J Microbiol Methods 56:107 118. https ://doi.org/10.1016/j.mimet.2003.09.019

Regione Emilia Romagna and ENI AGIP, 1998. Riserve idriche sotterranee della Regione Emilia Romagna (In italian; transl.: groundwater resources of the Emilia Romagna Region). S.EL.CA. printer, Florence

Richardson RE, Bhupathiraju VK, Song DL, Goulet TA, 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. https ://doi.org/10.1021/es0157797

Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng J F, Darling A, Malfatti S, Swan BK, Gies EA, Dodsworth JA, Hedlund BP, Tsiamis G, Sievert SM, Liu W T, Eisen JA, Hallam SJ, Kyrpides NC, Stepanauskas R, Rubin EM, Hugenholtz P, Woyke T (2013) Insights into the phylogeny and coding potential of microbial dark matter. Nature 499:431 437. https :// doi.org/10. 1038/nature12352

Rivière D, Desvignes V, Pelletier E, Chaussonnerie S, Guermazi S, Weissenbach J, Li T, Camacho P, Sghir A (2009) Towards the definition of a core of microorganisms involved in anaerobic diges tion of sludge. ISME J 3:700 714. https ://doi.org/10.1038/ ismej. 2009.2

Rowe AR, Lazar BJ, Morris RM, Richardson RE (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. https :// doi.org/10.1128/AEM. 00445 08

Sakai S, Imachi H, Hanada S, Ohashi A, Harada H, Kamagata Y (2008) *Methanocella paludicola* gen. nov., sp. nov., a methane producing archaeon, the first isolate of the lineage ‘Rice Cluster I’, and

proposal of the new archaeal order *Methanocellales* ord. nov. Int J Syst Evol Microbiol 58:929 936. https: //doi.org/10.1099/ ijs.0. 65571 0

Schuchmann K, Müller V (2016) Energetics and application of hetero trophy in acetogenic bacteria. Appl Environ Microbiol 82:4056 4069. https ://doi.org/10.1128/AEM.00882 16

Shan H, Kurtz HD, Mykytczuk N, Trevors JT, Freedman DL (2010) Anaerobic biotransformation of high concentrations of chloroform by an enrichment culture and two bacterial isolates. Appl Environ Microbiol 76:6463 6469. https ://doi.org/10.1128/AEM.01191 10

Siddaramappa S, Challacombe JF, Delano SF, Green LD, Daligault H, Bruce D, Detter C, Tapia R, Han S, Goodwin L, Han J, Woyke T, Pitluck S, Pennacchio L, Nolan M, Land M, Chang YJ, Kyrpides NC, Ovchinnikova G, Hauser L, Lapidus A, Yan J, Bowman KS, da Costa MS, Rainey FA, Moe WM (2012) Complete genome se quence of *Dehalogenimonas lykanthroporepellens* type strain (BL DC 9(T)) and comparison to "*Dehalococcoides*" strains. Stand. Genomic Sci 6:251 264. https ://doi.org/10.4056/sigs.2806097

Sung Y, Ritalahti KM, Apkarian RP, Löffler FE (2006) Quantitative PCR confirms purity of strain GT, a novel trichloroethene to ethene respiring *Dehalococcoides* isolate. Appl. Environ Microbiol 72: 1980 1987. https ://doi.org/10.1128/AEM.72.3.1980 1987.2006

Takeuchi M, Kawabe Y, Watanabe E, Oiwa T, Takahashi M, Nanba K, Kamagata Y, Hanada S, Ohko Y, Komai T (2011) Comparative study of microbial dechlorination of chlorinated ethenes in an aqui fer and a clayey aquitard. J Contam Hydrol 124:14 24. https: //doi. org/10.1016/j.jconhyd.2011.01.003

Tan S, Liu J, Fang Y, Hedlund BP, Lian ZH, Huang LY, Li JT, Huang LN, Li WJ, Jiang HC, Dong HL, Shu WS (2019) Insights into ecological role of a new deltaproteobacterial order *Candidatus Acidulodesulfobacterales* by metag eno mics and metatranscriptomics. ISME J 13:2044 2057. https :// doi.org/10. 1038/s41396 019 0415 y

Tang S, Edwards EA (2013) Identification of *Dehalobacter* reductive dehalogenases that catalyse dechlorination of chloroform, 1,1,1 trichloroethane and 1,1 dichloroethane. Philos Trans R Soc Lond Ser B Biol Sci 368:20120318. https :// doi.org/10.1098/rstb.2012. 0318

Villemur R, Lanthier M, Beaudet R, Lépine F (2006) The *Desulfitobacterium* genus. FEMS Microbiol Rev 30:706 733. https ://doi.org/10.1111/j.1574 6976.2006.00029.x

Xia Y, Wang Y, Wang Y, Chin FY, Zhang T (2016) Cellular adhesive ness and cellulolytic capacity in *Anaerolineae* revealed by omics based genome interpretation. Biotechnol Biofuels 9:111. https :// doi. org/10.1186/s13068 016 0524 z

Yamada T, Sekiguchi Y, Hanada S, Imachi H, Ohashi A, Harada H, Kamagata Y (2006) *Anaerolinea thermolimosa* sp. nov., *Levilinea saccharolytica* gen. nov., sp. nov.and *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes,and description of the new classes *Anaerolineae* classis nov. and *Caldilineae* classis nov. in the bacterial phylum *Chloroflexi*. Int J Syst Evol Microbiol 56: 1331 1340. https ://doi.org/10.1099/ijs.0.64169 0

Yohda M, Yagi O, Takechi A, Kitajima M, Matsuda H, Miyamura N, Aizawa T, Nakajima M, Sunairi M, Daiba A, Miyajima T, Teruya M, Teruya K, Shiroma A, Shimoji M, Tamotsu H, Juan A, Nakano K, Aoyama M, Terabayashi Y, Satou K, Hirano T (2015) Genome sequence determination and metagenomic characterization of a *Dehalococcoides* mixed culture grown on cis 1,2 dichloroethene. J Biosci Bioeng 120:69 77. https ://doi.org/10.1016/ j.jbiosc.2014.12. 001

Yoshikawa M, Takeuchi M, Zhang M (2017) Distribution of *Dehalococcoides* 16S rRNA and dehalogenase genes in contaminat ed sites. Environ Nat Res Research 7:37. https :// doi.org/10.5539/enrr.v7n2p37

Yu T, Wu W, Liang W, Lever MA, Hinrichs KU, Wang F (2018) Growth of sedimentary *Bathyarchaeota* on lignin as an energy source. Proc Natl Acad Sci U S A 115:6022 6027. https ://doi.org/10.1073/ pnas. 1718854115

Zakrzewski M, Proietti C, Ellis JJ, Hasan S, Brion MJ, Berger B, Krause L (2017) Calypso: a user friendly web server for mining and visu alizing microbiome environment interactions. Bioinformatics 33: 782 783. https ://doi.org/10.1093/bioinformatics/btw725