Supporting Information

Distinct dual C-Cl isotope fractionation patterns during anaerobic biodegradation of 1,2-dichloroethane: potential for characterizing microbial degradation in the field

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MATERIALS AND METHODS

Chemicals and Medium. The purity and sources of chemicals used for the experiments with the *Dehalococcoides*-containing culture are: 1,2-DCA (99%, Mallinckrodt); VC (99.5%, Fluka); polymer grade ethene (99.9%, Airgas), and methane (99%, Matheson). Sodium lactate syrup was obtained from EM Science (58.8-61.2% sodium lactate; specific gravity = 1.31). All other chemicals were reagent grade. An enrichment culture capable of respiring 1,2-DCA was grown in a mineral salts medium,[1] modified to lower the chloride concentration from 13.2 to 2.3 mM, as follows: K_2HPO_4 was decreased to 41.4 g/L; $NaHCO_3$ was replaced by 15.1 g/L NH_4HCO_3; NH_4Cl was replaced by 3.2 g/L of $(NH_4)_2HPO_4$; $CaCl_2 \cdot 2H_2O$ was replaced by 5.5 g/L $CaHPO_4 \cdot 2H_2O$; HCl in the trace metal solution was replaced by 2.7 mL of H_3PO_4 per liter. The medium pH was adjusted with H_3PO_4 rather than sparging the headspace (HS) with 70% N_2/30% CO_2 gas.

The medium employed for the *Dehalogenimonas*-containing enrichment culture[2] was a sterilized anoxic synthetic medium previously used to grow *Dehalococcoides mccartyi* strain CBDB1.[3]

Enrichment cultures. The *Dehalococcoides*-containing anaerobic enrichment culture was gradually acclimated to a mineral salt medium with a lower chloride concentration (see above), in order to decrease the background level against which chloride from 1,2-DCA accumulated. Sodium lactate was used as the electron donor and sodium hydroxide was added periodically to keep the pH between 6.9 and 7.1.

For the *Dehalogenimonas*-containing enrichment culture, a defined bicarbonate-buffered mineral salts medium was prepared as previously described and was amended with either pyruvate or acetate at 5 mM each as substrates.[2] Hydrogen was only added to acetate-fed cultures and $Na_2S \cdot 9H_2O$ and L-cysteine (0.2 mM each) was used to chemically reduce the medium.

Batch experiments details. The *Dehalococcoides*-containing microcosms were incubated at room temperature (22-24 °C) in an inverted position on a shaker table, to keep the liquid phase completely mixed at all times. The serum bottles were monitored by headspace analysis and 3-4 bottles were sacrificed for isotope measurements after approximately 25, 50, 75, 88, 95, 97, and 99.5% of the 1,2-DCA was consumed. To stop biological activity, 950 μL of phosphoric acid was added (decreasing the pH to 3) and the bottles were placed in an ice bath for 30 min before storage at 4 °C. Preliminary

experiments demonstrated that this approach resulted in instantaneous cessation of dechlorination, with no significant loss of 1,2-DCA during storage.

The *Dehalogenimonas*-containing microcosms were incubated at 25 ºC in the dark. Incubation was performed in static conditions but the bottles were agitated for several minutes before headspace sampling. Similarly to the experiments with *Dehalococcoides*-containing culture, the serum bottles were monitored by analyzing 0.5 mL headspace samples and each was sacrificed at different stages of 1,2-DCA consumption. To stop biological transformations at different time points, the cultures were killed by adding 9 mL of a saturated Na_2SO_4 solution (pH 1) and bottles were preserved at 4 ºC until isotopic analysis.

At the completion of both experiments, the bottles were shipped on ice to Isotope Tracer Technologies Inc. and the Universities of Neuchâtel (UN) and Barcelona (UB) for the isotopic analysis.

Characterization of the *Dehalococcoides* enrichment cultures. Illumina 16S rRNA gene sequencing was performed to evaluate two types of *Dehalococcoides*-containing enrichment cultures. The first uses tetrachloroethene (PCE) and trichloroethene (TCE) as terminal electron acceptors and lactate as the electron donor, as described in Yu et al.[1] That culture has been continuously maintained for more than eight years on PCE, TCE, and lactate, and served as the starting point for the enrichment culture used in this study. The PCE/TCE enrichment stoichiometrically reduces these compounds to ethene and ethane.

The second enrichment culture sequenced was the inoculum used in the current study. As described in Peethambaram[4] and Yu et al.,[1] a subset of the PCE/TCE culture was transitioned to 1,2-DCA as the sole terminal electron acceptor.[5] While growing with 1,2-DCA, the yield for *Dehalococcoides* was similar to other halorespiring cultures. The 1,2-DCA enrichment was gradually transitioned to a lower chloride basal medium before being used in the current study. After being used as inoculum for this study, the enrichment culture was stored for nearly three years without being fed 1,2-DCA or lactate; consequently, endogenous decay of the biomass was expected. Nevertheless, it afforded an opportunity to determine which types of halorespiring microbes were present (see below).

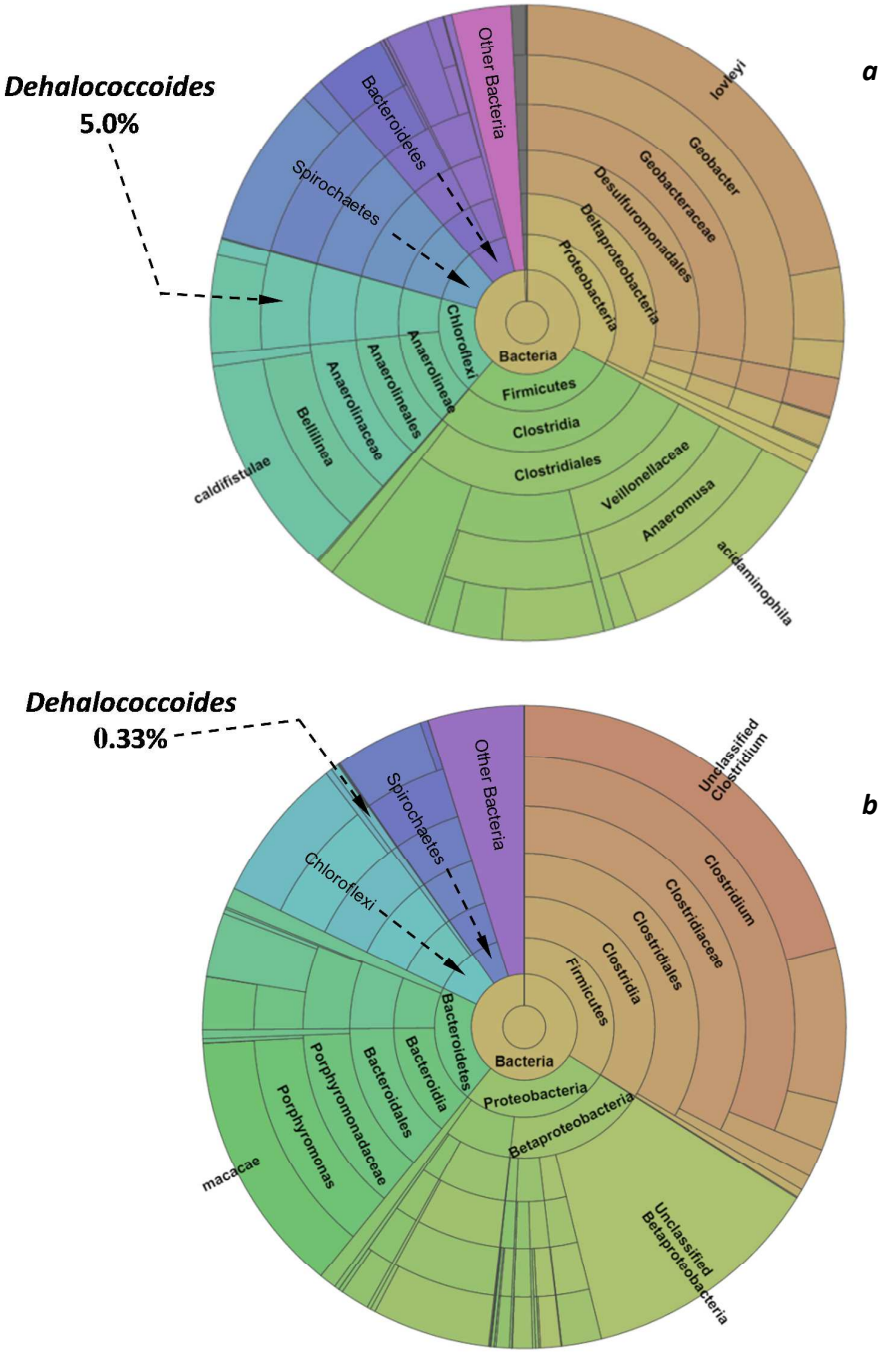

Fig. S1. Illumina 16S rRNA gene sequencing results for (a) PCE/TCE enrichment culture; and (b) 1,2-DCA enrichment culture.

Samples of both cultures were prepared for sequencing by centrifuging a 50 mL sample (4000x*g*, 10 min) and extracting DNA from the pellets using the MO BIO PowerSoil® DNA Isolation Kit. DNA was eluted in 75 μL buffer (10 mM Tris), quantified using Nanodrop, and sent to a commercial laboratory (SeqMatic, Fremont, CA) for Illumina 16S rRNA gene sequencing. Sequencing results (Table S1 and Fig. S1) indicated that i) the only strict organohalide-respiring microbe detected in the 1,2-DCA enrichment culture was *Dehalococcoides* and that ii) the presence of *Dehalococcoides* (0.33%) was much larger compared to *Desulfitobacterium* (0.001%). The relatively low level of *Dehalococcoides* reflects the fact that a high dose of lactate was used to provide hydrogen in excess, so that the enrichment was dominated by microbes consuming the lactate. In the PCE/TCE enrichment culture, *Dehalococcoides* was present at 5.0%, which was considerably higher than any other potential 1,2-DCA dechlorinator, i.e., *Dehalobacter* (0.39%), *Desulfitobacterium* (0.051%), and *Dehalogenimonas* (non-detectable). The lower level of *Dehalococcoides* in the 1,2-DCA culture in comparison to the PCE/TCE enrichment culture was consistent with the long storage time for the 1,2-DCA culture prior to DNA extraction.

Table S1. 1,2-DCA dechlorinators identified by Illumina 16S rRNA gene sequencing

Known 1,2-DCA dechlorinators	Occurrence in the PCE/TCE enrichment	Occurrence in the 1,2-DCA enrichment
Dehalococcoides	5.0%	0.33%
Dehalobacter	0.39%	non-detectable
Desulfitobacterium	0.051%	0.001%
Dehalogenimonas	non-detectable	non-detectable

Groundwater sampling and redox conditions. In site A, groundwater samples for concentration and isotopic analysis of 1,2-DCA were collected from 6 long screen monitoring wells (MW-1 to 6) and 1 multilevel well (MW-7, Fig. S2). The long screen wells were sampled using a 2 inch submersible pump (Grundfos, MP 1), which was placed in the middle of the screened interval. Physicochemical parameters including temperature, pH, redox potential (Eh), electrical conductivity (EC) and dissolved oxygen (DO) were measured using a flow-through cell. Prior to samples collection,

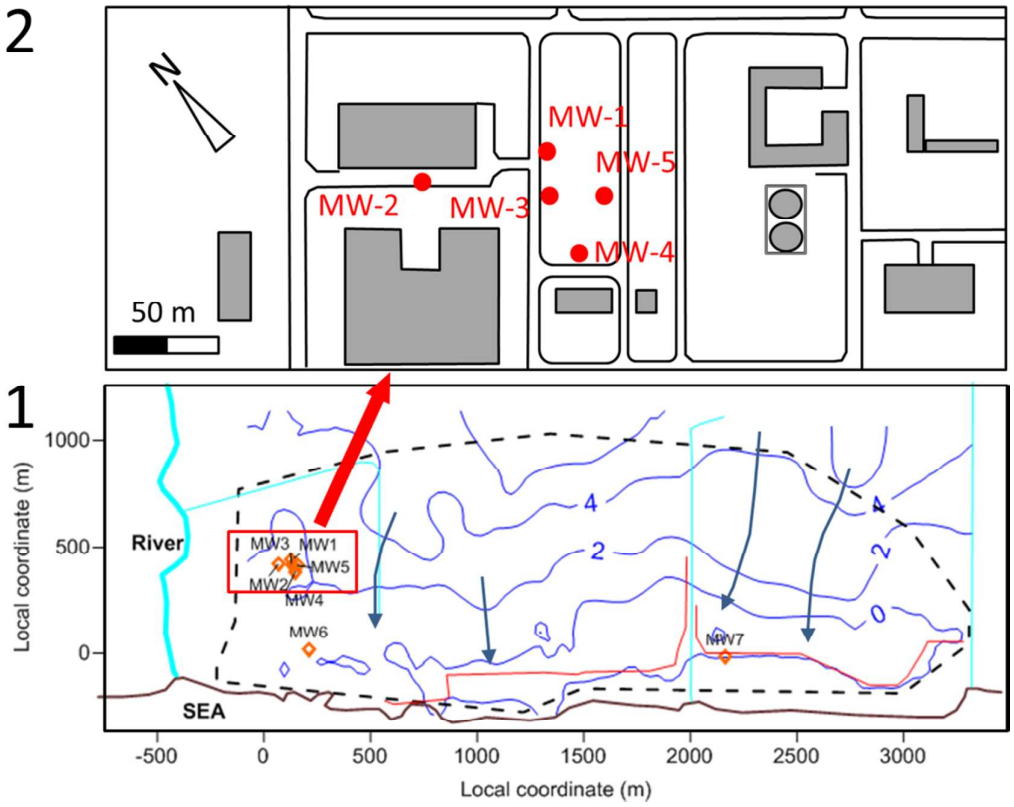

Fig. S2. (1) Site A map showing the piezometric surface (blue lines in m above sea level), the groundwater flow direction (blue arrows) and the monitoring wells. The industrial site limits (black dashed line) and a horizontal flow barrier (red line) are also indicated. The flow barrier consists of a vertical bentonite wall and it was constructed to contain the migration of light non-aqueous phase liquids towards the sea. (2) Zoom-in on the area indicated by the red box in panel 1 showing the monitoring wells and the industrial facilities (grey boxes).

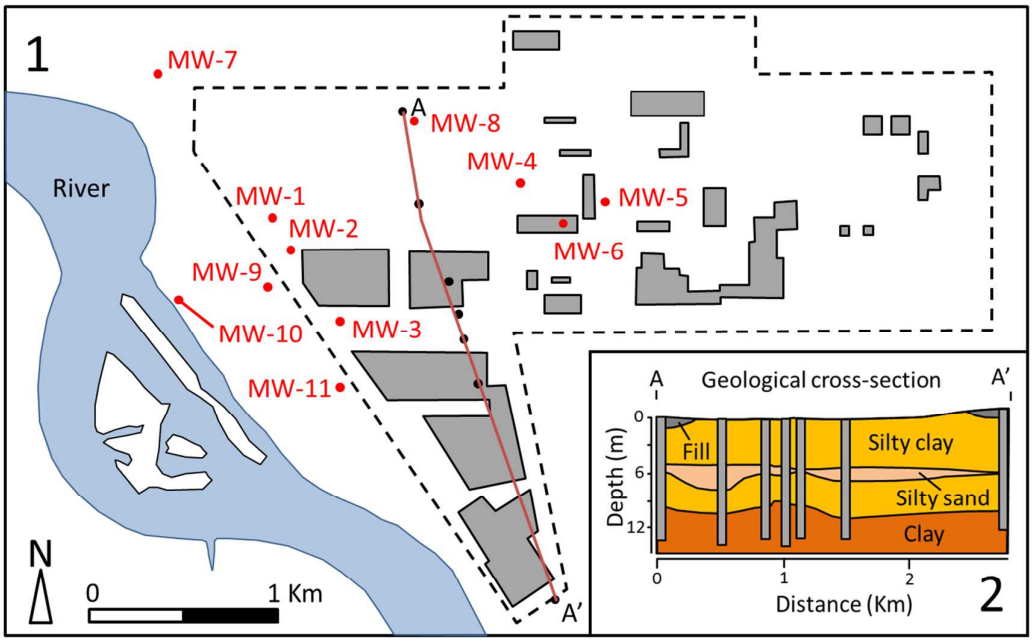

Fig. S3. (1) Site B map showing the monitoring wells, the industrial site limits (black dashed line) and the industrial facilities (grey boxes). A–A′ refers to the cross-section in panel 2. (2) Geological cross-section.

Table S2. Carbon and chlorine isotope ratios of 1,2-DCA in sites A and B. Concentrations of 1,2-DCA in each monitoring well are also indicated (LS: Long screen, n.d.: not detected).

Site	Well	Sampling depth (m a.s.l.)	1,2-DCA		
			Conc. (mg/L)	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{37}Cl_{SMOC}$ (‰)
A	MW-1	LS	2410	-21.3±0.5	+1.1±0.2
A	MW-2	LS	14	-21.7±0.5	+2.3±0.2
A	MW-3	LS	86	-18.6±0.5	+2.2±0.2
A	MW-4	LS	0.003	- - -	- - -
A	MW-5	LS	9.9	-12.2±0.5	+7.1±0.2
A	MW-6	LS	n.d.	- - -	- - -
A	MW-7	-9	0.03	- - -	+34.1±0.2
A	MW-7	-14	0.06	+51.5±0.5	+35.6±0.2
A	MW-7	-19	0.04	- - -	+33.1±0.2
A	MW-7	-24	0.1	+33.5±0.5	+28.4±0.2
A	MW-7	-29	1.0	-19.0±0.5	+3.6±0.2
B	MW-1	LS	5.6	-13.6±0.5	+5.6±0.2
B	MW-2	LS	1710	-11.7±0.5	+8.4±0.2
B	MW-3	LS	0.006	- - -	+5.2±0.3
B	MW-4	LS	0.1	-5.5±0.5	+9.3±0.2
B	MW-5	LS	0.004	- - -	+28.9±0.4
B	MW-6	LS	1810	-18.5±0.5	+4.6±0.2
B	MW-7	LS	0.009	- - -	- - -
B	MW-8	LS	n.d.	- - -	- - -
B	MW-9	LS	n.d.	- - -	- - -
B	MW-10	LS	n.d.	- - -	- - -
B	MW-11	LS	n.d.	- - -	- - -

monitoring wells were purged (a minimum of 4 well volumes) until physicochemical parameters stabilized. The multilevel sampling in MW-7 was performed using a Straddle Packer system (Solinst) and 5 groundwater samples were collected at different depths (Table S2). The packer assemblage consisted of a 0.9 m long sampling port situated between two inflatable packers. Depth discrete samples were collected after the multilevel sampling system was purged (a minimum of 3 volumes) and physicochemical parameters stabilized. In site B, groundwater samples were collected from 11 long screen monitoring wells (Fig. S3 and Table S2) using a sampling approach similar to site A.

Samples for volatile organic compounds concentration and isotope analysis of 1,2-DCA were collected in glass vials closed without headspace using screwcaps with Teflon coated septa, preserved at pH ~2 with HNO_3 (10%). The sample vials were shipped on ice to the University of Waterloo (UW) and stored at 4 ºC in the dark until analysis.

In site A, hydrochemical data from multilevel wells showed very low DO levels (< 0.2 mg/L for most of the sampling depths), suggesting anoxic conditions in the aquifer. Low Eh values decreasing in depth, from -50 to -100 mV at around -4 and -29 m a.s.l., respectively, confirm that groundwater was under reducing conditions. In addition, methane was also detected in the aquifer. In site B, the presence of dissolved iron (up to 56 mg/L) and methane (up to 1.3 mg/L) indicated the groundwater is also under reducing conditions.

ANALYTICAL METHODS

Concentration analysis. The amounts of 1,2-DCA, VC and ethene in the *Dehalococcoides*-containing microcosms were measured at Clemson University (CU) laboratories using a GC-FID (Hewlett Packard 5890 Series II). Further information is available in a previous study.[1] Briefly, aqueous phase detection limits were 13, 5.2, and 0.7 µg/L, respectively. The GC-FID response to a headspace sample (0.5 mL) was calibrated to give the total mass of the compound in that bottle,[6] which was then converted to an aqueous-phase concentration using Henry's Law constants.[7] Indicated products concentrations are net amounts, i.e., adjusted for the amounts that carrier over from the enrichment culture (~0.21±0.05 mg/L ethene and 0.11±0.05 mg/L VC).

Concentration measurements of chlorinated volatile compounds and ethene in the *Dehalogenimonas*-containing microcosms were performed at the Universitat

Autònoma de Barcelona (UAB) laboratories as previously described.[2] Briefly, samples of the headspace (0.5 mL) were analyzed using a GC model 6890N (Agilent Technologies; Santa Clara, CA, USA) equipped with a FID detector. The target compounds were identified using retention times of chemical standards. Calibration was based on aqueous standards, with the same liquid and headspace volumes as in the microcosms.

Concentration analysis of volatile organic compounds in field samples was performed using a GC-MS (Thermo Electron, MAT 95 XP).

Chlorine isotope analysis. Samples from laboratory batch experiments were measured at Isotope Tracer Technologies Inc., Canada. Pure 1,2-DCA isotopic working standards were used for instrument monitoring and external calibration of sample raw $\delta^{37}Cl$ values to the international Standard Mean Ocean Chloride (SMOC) scale: IT2-3001 and IT2-3002 ($\delta^{37}Cl_{SMOC}$ = +0.83 ± 0.09 and −0.19 ± 0.12‰, respectively) and CHYN1 and CHYN2 ($\delta^{37}Cl_{SMOC}$ = +6.30 ± 0.06 and +0.84 ± 0.14‰, respectively). Further information about working standards characterization is available in a previous study.[8] Chlorine compound-specific isotope analysis (CSIA) was performed using a 6890 GC (Agilent, Santa Clara, CA, U.S.) coupled to a MAT 253 IRMS (Thermo Finnigan, Bremen, Germany). This IRMS, equipped with nine collectors, is a continuous flow IRMS with a dual-inlet (DI) mode option. The DI bellows are used as the monitoring gas reservoir and reference peaks were introduced at the beginning of each analysis run.

Samples and standards were prepared in 20 mL vials and sealed with crimped septa caps (PTFE/Silicone). These vials contain 16 mL of solution and 4 mL of headspace. The 1,2-DCA in solution was extracted by headspace solid phase micro extraction (SPME) fiber (75 μm Carboxen-PDMS for Merlin Microseal™, 23 gauge needle holder from Supelco, Bellefonte, PA, US) using a CombiPAL SPME autosampler (CTC Analytics, Zwingen, Switzerland). The SPME fiber was desorbed into the GC inlet at 270 °C. A SPME injection sleeve (0.75 mm ID) (Supelco, Bellefonte, PA, U.S.) was used as an inlet liner. The chromatographic separation was performed with a DB-5 MS column (60 m x 0.32 mm x 1 μm) (Agilent, Santa Clara, CA, U.S.). The carrier gas (He) flow rate of the GC was set at 1.8 mL/min and the oven temperature was programmed as follows: 40 °C (9 min), ramp at 10 °C/min to 100 °C and ramp at 46 °C/min to 250 °C (6.75 min). A 4-way VALCO valve (Valco Intruments, Houston, TX, US) with two positions is installed between the GC and the

IRMS. This setup is important as it allows a non-stop constant flow of He into the IRMS. The end part of the GC column is connected to one of the two in-ports of the valve, while the other in-port is connected with ultra-pure He gas. The two out-ports are connected to the IRMS and to a FID detector mounted on the GC. A 100 μm deactivated capillary column was used to connect the valve port with the IRMS.

The analysis scheme for the samples calibration with respect to SMOC scale consists of a sequence of samples bracketed by two sets of standards, one set at the beginning of the run and another set at the end of the analysis. Each set of standards comprise six replicates for each of the two standards in varying concentrations, and each run is composed of 12 to 20 samples. Blanks are included at the beginning of each run, however, there is no carry-over observed along many years of analyzing these compounds by this method.

Field samples were analyzed at the UW laboratories using the same analytical method described above. Chlorine CSIA was performed by direct injection of the GC-separated 1,2-DCA into the ion source of a Micromass Isoprime IRMS (Cheadle Hume, UK).

Carbon isotope analysis. Samples from *Dehalococcoides*-containing microcosms were measured for carbon isotope ratios at the UN laboratories. An isotopic working standard of 1,2-DCA (Fluka, ≥99.5% pure) was used to ensure stability of the measurements during the course of samples analysis. The isotopic signature of the working standard ($\delta^{13}C_{VPDB}$ = −29.47 ± 0.05‰, ±1σ, n = 5) was determined beforehand using an elemental analyzer coupled to an IRMS system (EA-IRMS). Carbon CSIA of 1,2-DCA was performed using a 7890A GC (Agilent, Santa Clara, CA, U.S.) coupled to an Isoprime^{TM} 100 IRMS via an Isoprime^{TM} GC5 combustion interface set to 970°C (Isoprime Ltd., Manchester, U.K.). Aqueous samples and standards were prepared without HS in 40 mL (nominal volume) VOC vials. These were subsequently preconcentrated with a Stratum purge & trap system (Teledyne Tekmar Dohrmann, Mason, OH, U.S.) connected to a cryogenic trap (Teledyne Tekmar Dohrmann). A 25 mL sample volume was purged with N_2 for 10 min, at a rate of 40 mL/min, and the volatile compounds were retained on a VOCARB 3000 trap (Supelco, Bellefonte, PA, U.S.) at room temperature. After desorption from the trap at 250°C, the compounds were (i) condensed in the cryogenic unit at -80°C, (ii) released by heating to 180°C and (iii) injected splitless into the GC column. The GC was equipped with a DB-VRX

column (60m x 0.32 mm ID x 1.8 μm) (Agilent, Santa Clara, CA, U.S.). The He carrier gas was set at 1.7 mL/min and the oven temperature program was 40 ºC (6 min) followed by a ramp of 10 ºC/min to 130 and 20 ºC/min to 220 ºC (1 min). Aqueous samples and isotopic standards interspersed along the sequence were diluted to a similar concentration and analyzed by duplicate as a quality control.

For the experiments with *Dehalogenimonas*-containing cultures, samples from the microcosms were analyzed for carbon isotope ratios at the UB laboratories. Liquid aliquots were diluted to a similar 1,2-DCA concentration in 20 mL vials containing a 30 mm PTFE-coated stir bar. The vials were immediately sealed with PTFE/Silicone septa and aluminum crimp caps. Then, the sample solution was stirred at room temperature and 1,2-DCA was extracted during 20 min by headspace SPME using a manual sampler holder equipped with a 75 μm Carboxen-PDMS fiber (Supelco, Bellefonte, PA).[9] The GC was equipped with a Supelco SPB-624 column (60 m × 0.32 mm, 1.8 μm film thickness; Bellefonte, PA). The injector was set at 220 ºC in split mode (1:10) and the oven temperature program was kept at 60 ºC for 2 min, heated to 220 ºC at a rate of 8 ºC/min and finally held at 220 ºC for 5 min. Helium was used as a carrier gas with a gas flow rate of 1.8 mL/min. An isotopic working standard of 1,2-DCA (99%, Sigma-Aldrich) was used to ensure accuracy of the isotopic measurements during the course of samples analysis. The isotopic signature of the working standard ($\delta^{13}C_{VPDB}$ = -28.74 ± 0.04‰, ±1σ, n = 10) was determined beforehand by EA-IRMS. Several 1,2-DCA aqueous standards were prepared daily from the same pure 1,2-DCA (stock solutions were prepared first in HPLC grade methanol) and analyzed on the same days as the samples. Standards and samples and were analyzed by duplicate as a quality control.

Field samples were analyzed for carbon CSIA at the UW laboratories using a Trace GC (Thermo) coupled to a Delta plus XP IRMS (Thermo Finnigan) via a GC-CIII combustion interface set at 940 ºC (Thermo Scientific, Waltham, MA, USA).

RAYLEIGH ISOTOPE PLOTS

Bulk carbon and chlorine ε values were obtained from the slopes of Rayleigh plots according to eq. 2 in the main text (Fig. S4).

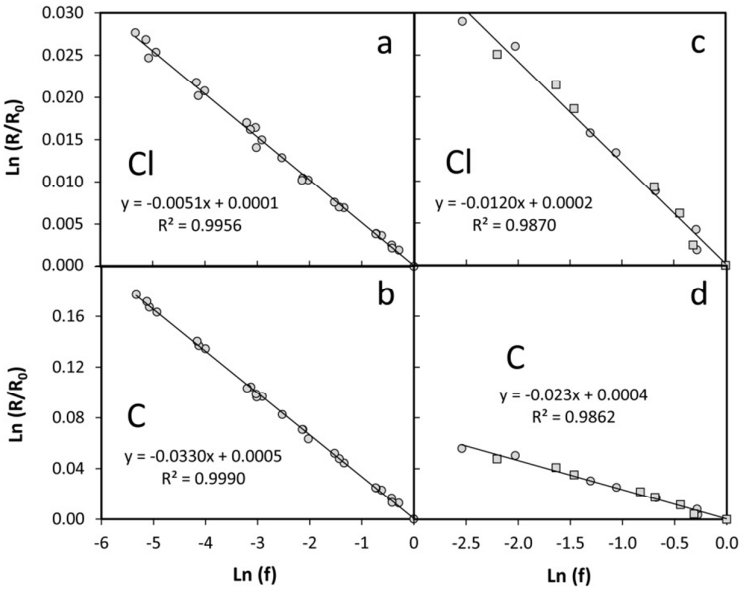

Fig. S4. Chlorine and carbon isotopes regression during 1,2-DCA transformation by *Dehalococcoides* (a, b) and *Dehalogenimonas*-containing cultures (c, d). For the experiments with *Dehalogenimonas*-containing culture, data from the microcosms with acetate (circles) or pyruvate (squares) are combined.

CALCULATION OF APPARENT KINETIC CARBON ISOTOPE EFFECTS (AKIEs) AND REPORTED VALUES FOR ABIOTIC REACTIONS OF CHLORINATED ETHANES IN PREVIOUS LABORATORY STUDIES

Intrinsic KIEs are position specific whereas ε_{bulk} values are calculated from compound-average isotope data (eq. 2 in the main text). Therefore, observable ε_{bulk} values have to be converted into AKIEs in order to obtain information about the underlying reaction mechanisms.[10] For the calculation and interpretation of AKIEs a hypothesis about the reaction mechanism, or assumed reaction mechanism, is necessary. The effects of non-reacting positions within the molecule, as well as of intramolecular competition, are then taken into account using eqs. S1 and S2, respectively,[10]

$$\varepsilon_{rp} \approx \frac{n}{x} \cdot \varepsilon_{bulk} \qquad \text{(S1)}$$

$$AKIE_C = \frac{1}{z \cdot \varepsilon_{rp} + 1} \qquad \text{(S2)}$$

where ε_{rp} is the isotopic fractionation at the reactive position, n is the number of atoms of the element considered, x is the number of reactive sites and z the number of identical reactive sites undergoing intramolecular competition. These equations assume the absence of secondary isotope effects. Secondary carbon isotope effects are usually insignificant.[10] In symmetric molecules such as 1,2-DCA, all atoms are in equivalent reactive positions (n = x), and therefore, ε_{rp} is directly obtained from the slopes of the Rayleigh plots (Fig. S4). If the two C-Cl bonds are broken in sequence (i.e. *stepwise* dihaloelimination), assuming that the first bond cleavage is the rate determining step, then z = 2 in eq. S2 as both C-Cl bonds compete for reaction. In contrast, if the two C-Cl bonds are broken simultaneously (i.e. *concerted* dihaloelimination), then z = 1 since there is no intramolecular competition between them.

Abiotic reductive dechlorination of three polychlorinated ethanes in a previous study, 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA), pentachloroethane (PCA) and hexachloroethane (HCA) by Cr(II), an inner sphere one-electron reductant, in aqueous homogeneous solution via sequential β-elimination of two chlorine atoms showed $AKIE^{C}_{stepwise}$ values from 1.0212 ± 0.0005 to 1.0303 ± 0.0006.[11] A similar $AKIE^{C}_{stepwise}$ of 1.037 ± 0.001 was also obtained by Elsner et al.[12] for abiotic dihaloelimination of 1,1,2,2-TeCA by Cr(II).

KINETICS OF 1,2-DCA BIODEGRADTION AND PRODUCT FORMATION

Substrate degradation and product formation rates were estimated using first-order kinetic equations S3-S5. Lag times of approximately 31 and 137 hours were considered for the experiments with *Dehalococcoides* and *Dehalogenimonas*-containing cultures, respectively (~15% of the overall duration of each experiment).

$$[1,2-\mathrm{DCA}] = [1,2-\mathrm{DCA}]_0 \cdot e^{-k_{obs} \cdot t} \quad \text{(S3)}$$

$$[\mathrm{Ethene}] = \frac{k_{Ethene} \cdot [1,2-\mathrm{DCA}]_0}{k_{obs}} \cdot (1 - e^{-k_{obs} \cdot t}) \quad \text{(S4)}$$

$$[\mathrm{VC}] = \frac{k_{VC} \cdot [1,2-\mathrm{DCA}]_0}{k_{obs}} \cdot (1 - e^{-k_{obs} \cdot t}) \quad \text{(S5)}$$

Substrate degradation and product formation rate constants were calculated by non-linear regression using SigmaPlot™, as follows: k_{obs} = 0.023±0.002 h^{-1} (± 95% C.I., r^2 = 0.91), k_{Ethene} = 0.0222±0.0009 h^{-1} (± 95% C.I., r^2 = 0.90) and k_{VC} = 0.002±0.001 h^{-1} (± 95% C.I., r^2 = 0.58) for the microcosms containing *Dehalococcoides* and k_{obs} = 0.0024±0.0002 h^{-1} (± 95% C.I., r^2 = 0.92) and k_{Ethene} = 0.004±0.001 h^{-1} (± 95% C.I., r^2 = 0.82) for those containing *Dehalogenimonas*. The results indicated that the substrate decay can be fairly well described by a first-order kinetic equation (half-lives ($t_{1/2}$) of 30 and 289 h for the experiments with *Dehalococcoides* and *Dehalogenimonas*-containing cultures, respectively). For VC in *Dehalococcoides*-containing microcosms, only concentration data measured at times <100 hours were used in the correlation calculations. The reason is that VC concentrations at later times are probably affected by further transformation of VC to ethene, which agrees with the observed values being lower than the model prediction (Fig. 1).

The *Dehalococcoides*-containing enrichment culture used in this study was derived from the one described by Yu et al.[1] Monod parameters determined by Yu et al.[1] were also used to fit the 1,2-DCA concentration data (Fig. S5), based on equations S6 and S7.

$$\frac{dS}{dt} = \frac{\hat{\mu}}{Y} \cdot \frac{X(S - S_t)}{K_s + (S - S_t)} \quad \text{(S6)}$$

$$\frac{dX}{dt} = -\frac{dS}{dt} Y - bX \quad \text{(S7)}$$

where S = 1,2-DCA concentration, $\hat{\mu}$ = maximum specific growth rate (0.020 h^{-1}), X = biomass concentration, S_t = transition concentration for 1,2-DCA (2.14×10^{-2} mg/L), Y = yield (4.6×10^{7} gene copies/μmol Cl^-), K_S = half saturation coefficient (21.7 mg/L), and b = endogenous decay coefficient (0.002 h^{-1}). The initial biomass concentration (X_o) was determined by fitting to be 5.52×10^{10} gene copies/L. The 1,2-DCA concentration data were fit to equations S6 and S7 using AQUASIM 2.0.

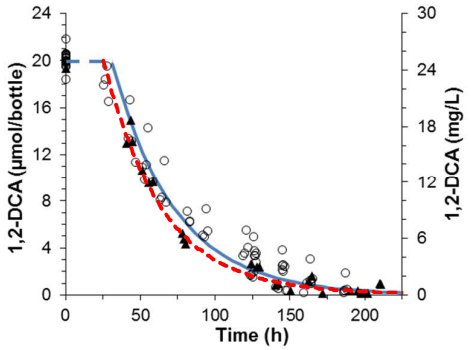

Fig. S5. Blue line is the first-order fit as shown in Figure 1. The red line is the Monod fit according to equations S6 and S7 and the parameter values from Yu et al.[1]

As shown in Figure S5, the Monod model follows the first-order fit very closely. The maximum specific growth rate (0.020 h^{-1}) is very close to k_{obs} (0.023 ± 0.002 h^{-1}). This outcome was expected, since the majority of the 1,2-DCA degradation occurred at concentrations below the half-saturation constant (21.7 mg/L), where Monod kinetics resemble first-order kinetics.

References

1. Yu, R.; Peethambaram, H. S.; Falta, R. W.; Verce, M. F.; Henderson, J. K.; Bagwell, C. E.; Brigmon, R. L.; Freedman, D. L., Kinetics of 1,2-Dichloroethane and 1,2-Dibromoethane Biodegradation in Anaerobic Enrichment Cultures. *Appl Environ Microbiol* **2013,** *79*, (4), 1359-1367.

2. Martin-Gonzalez, L.; Hatijah Mortan, S.; Rosell, M.; Parlade, E.; Martinez-Alonso, M.; Gaju, N.; Caminal, G.; Adrian, L.; Marco-Urrea, E., Stable Carbon Isotope Fractionation During 1,2-Dichloropropane-to-Propene Transformation by an Enrichment Culture Containing Dehalogenimonas Strains and a dcpA Gene. *Environ Sci Technol* **2015,** *49*, (14), 8666-74.

3. Adrian, L.; Szewzyk, U.; Wecke, J.; Gorisch, H., Bacterial dehalorespiration with chlorinated benzenes. *Nature* **2000,** *408*, (6812), 580-3.

4. Peethambaram, H. S. Molecular Characterization of Enrichment Cultures That Grow on Tetrachloroethene, 1,2 Dichloroethane and Ethylene Dibromide. Clemson University, 2010.

5. Eaddy, A. Scale-up and Characterization of an Enrichment Culture for Bioaugmentation of the P-Area Chlorinated Ethene Plume at the Savannah River Site. Clemson University, 2008.

6. Verce, M. F.; Ulrich, R. L.; Freedman, D. L., Characterization of an isolate that uses vinyl chloride as a growth substrate under aerobic conditions. *Appl Environ Microbiol* **2000,** *66*, (8), 3535-42.

7. Gossett, J. M., Measurement of Henry's law constants for C1 and C2 chlorinated hydrocarbons. *Environ Sci Technol* **1987,** *21*, (2), 202-208.

8. Palau, J.; Cretnik, S.; Shouakar-Stash, O.; Hoche, M.; Elsner, M.; Hunkeler, D., C and Cl Isotope Fractionation of 1,2-Dichloroethane Displays Unique delta(13)C/delta(37)Cl Patterns for Pathway Identification and Reveals Surprising C-Cl Bond Involvement in Microbial Oxidation. *Environ Sci Technol* **2014,** *48*, (16), 9430-7.

9. Palau, J.; Soler, A.; Teixidor, P.; Aravena, R., Compound-specific carbon isotope analysis of volatile organic compounds in water using solid-phase microextraction. *Journal of Chromatography A* **2007,** *1163*, (1-2), 260-268.

10. Elsner, M.; Zwank, L.; Hunkeler, D.; Schwarzenbach, R. P., A new concept linking observable stable isotope fractionation to transformation pathways of organic pollutants. *Environ Sci Technol* **2005,** *39*, (18), 6896-6916.

11. Hofstetter, T. B.; Reddy, C. M.; Heraty, L. J.; Berg, M.; Sturchio, N. C., Carbon and chlorine isotope effects during abiotic reductive dechlorination of polychlorinated ethanes. *Environ Sci Technol* **2007,** *41*, (13), 4662-4668.

12. Elsner, M.; Cwiertny, D. M.; Roberts, A. L.; Lollar, B. S., 1,1,2,2-tetrachloroethane reactions with OH-, Cr(II), granular iron, and a copper-iron bimetal: Insights from product formation and associated carbon isotope fractionation. *Environ Sci Technol* **2007,** *41*, (11), 4111-4117.