

Supplementary material

Assessing a Large-Scale Sequential In-Situ Chloroethene Bioremediation System Using Compound-Specific Isotope Analysis (CSIA) and Geochemical Modeling

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Enrichment factors - literature review

In the following tables (Table S1 and Table S2) enrichment factors obtained by [1] and [2] are reported.

Table S1 Enrichment factors for reductive dechlorination (ϵ_{RD}).

PCE				
Strain	ϵ (‰)		α	Reference
	lit	avg	avg	
Dehalococcoides ethenogenes strain 195	-6 ± 0.7	-6.00	0.994	Cichocka et al., 2008
Bacterial consortium (BDI) (dehalococcoides)	-7.12 ± 0.72	-7.12	0.9929	Liang et al., 2007
Dehalococcoides sp. strain CBDB1	-1.6 ± 0.3	-1.60	0.9984	Marco-Urrea et al., 2011
Dehalococcoides	-3.3 ± 1.2	-3.30	0.9967	Aeppli et al., 2010
	AVG	-4.51		
	MIN	-7.12		
	MAX	-1.60		
TCE				
Strain	ϵ (‰)		α	Reference
	lit	avg	avg	
Dehalococcoides mixed culture	-16.4 ± 0.4	-16.40	0.9836	Kuder et al., 2013
Dehalococcoides-containing enrichment	-16 ± 0.6	-16.00	0.9840	Lee et al., 2007
Dehalococcoides mixed culture	-15.3	-15.30	0.9847	Kuder et al., 2013
Bacterial consortium (BDI) (dehalococcoides)	-15.27 ± 0.79	-15.27	0.9847	Liang et al., 2007
Dehalococcoides ethenogenes strain 195	-13.7 ± 1.8	-13.70	0.9863	Cichocka et al., 2008
Dehalococcoides sp. strain CBDB1	-11.2 ± 2.6	-11.20	0.9888	Marco-Urrea et al., 2011
Dehalococcoides ethenogenes	-9.6 ± 0.4	-9.60	0.9904	Lee et al., 2007
Dehalococcoides pure culture FL2 (tceA)	-8.0 ± 0.4	-8.00	0.9920	Fletcher et al., 2011
Dehalobacter restrictus	-7.7 ± 0.4	-7.70	0.9923	Renpenning et al., 2015
Dehalobacter restrictus PER-K23	-3.3 ± 0.3	-3.30	0.9967	Lee et al., 2007
	AVG	-11.65		
	MIN	-16.40		
	MAX	-3.30		
cis-DCE				
Strain	ϵ (‰)		α	Reference
	lit	avg	avg	
Dehalococcoides BTF08	-30.5 ± 1.5	-30.50	0.9695	Schmidt et al., 2014b
Dehalococcoides-containing enrichment ANAS	-29.7 ± 1.6	-29.70	0.9703	Lee et al., 2007

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Dehalococcoides mixed culture	-26.8	-26.80	0.9732	Kuder et al., 2013
Dehalococcoides-containing consortia BDI	-25.3 ± 1.0	-25.30	0.9747	Fletcher et al., 2011
Dehalococcoides pure culture GT (vcrA)	-21.6 ± 1.3	-21.60	0.9784	Fletcher et al., 2011
Dehalococcoides ethenogenes	-21.1 ± 1.8	-21.10	0.9789	Lee et al., 2007
W. Dehalococcoides	-18.5 ± 1.8	-18.50	0.9815	Abe et al., 2009
W. Dehalococcoides	-18.5 ± 1.3	-18.50	0.9815	Abe et al., 2009
Dehalococcoides pure culture VS (vcrA)	-17.6 ± 2.7	-17.60	0.9824	Fletcher et al., 2011
Dehalococcoides sp. BAV1	-16.9 ± 1.4	-16.90	0.9831	Lee et al., 2007
Dehalococcoides pure culture FL2 (tceA)	-15.8 ± 1.1	-15.80	0.9842	Fletcher et al., 2011
Dehalococcoides pure culture BAV1 (bvcA)	-14.9 ± 0.5	-14.90	0.9851	Fletcher et al., 2011
	AVG	-21.43		
	MIN	-30.50		
	MAX	-14.90		

VC				
Strain	e (‰)		α	Reference
	lit	avg	avg	
W. Dehalococcoides	-25.0 ± 0.72	-25.00	0.9750	Abe et al., 2009
W. Dehalococcoides	-25.4 ± 1.1	-25.40	0.9746	Abe et al., 2009
Dehalococcoides pure culture BAV1 (bvcA)	-23.2 ± 1.8	-23.20	0.9768	Fletcher et al., 2011
Dehalococcoides pure culture GT (vcrA)	-23.8 ± 1.1	-23.80	0.9762	Fletcher et al., 2011
Dehalococcoides pure culture VS (vcrA)	-22.1 ± 1.2	-22.10	0.9779	Fletcher et al., 2011
Dehalococcoides-containing consortia BDI	-19.9 ± 1.6	-19.90	0.9801	Fletcher et al., 2011
Dehalococcoides mixed culture	-26.7 ± 1.9	-27.40	0.9726	Kuder et al., 2013
Dehalococcoides mixed culture	-28.1	-27.40	0.9726	Kuder et al., 2013
Dehalococcoides sp. BAV1	-24 ± 2.0	-24.00	0.9760	Lee et al., 2007
Dehalococcoides-containing enrichment ANAS	-22.7 ± 0.8	-22.70	0.9773	Lee et al., 2007
Dehalococcoides BTF08	-28.8 ± 1.5	-28.80	0.9712	Schmidt et al., 2014b
	AVG	-24.52		
	MIN	-28.80		
	MAX	-19.90		

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Table S2 – Enrichment factors for oxidation (ϵ_{ox}).

cis-DCE				
Strain	e (‰)		α	Reference
	lit	avg	avg	
Beta-Proteobacterium strain JS666	-8.5 ± 0.1	-8.50	0.9915	Abe et al., 2009
Pseudomonas putida F1	-1.1 ± 0.77	-1.10	0.9989	Clingenpeel et al. 2012
Pseudomonas fluorescens CFS215	-1.23 ± 0.45	-1.20	0.9988	Clingenpeel et al. 2012
Pseudomonas mendocina KR1	-0.9 ± 0.51	-0.90	0.9991	Clingenpeel et al. 2012
Polaromonas s. strain JS666	-19.9 ± 2.5	-19.90	0.9801	Jennings et al., 2009
Enrichment culture	-15.2 ± 0.5	-15.20	0.9848	Schmidt et al., 2010
Enrichment culture	-9.8 ± 1.7	-9.80	0.9902	Tiehm et al., 2008
Enrichment culture	-8.8 ± 1.0	-8.80	0.9912	Tiehm et al., 2008
Enrichment culture	-7.1 ± 0.9	-7.10	0.9929	Tiehm et al., 2008
Enrichment culture	-8.2 ± 3.5	-8.20	0.9918	Tiehm et al., 2008
Mixed culture	-7.2	-7.20	0.9928	Pooley et al., 2009
	AVG	-7.99		
	MIN	-19.90		
	MAX	-0.90		
VC				
Strain	e (‰)		α	Reference
	lit	avg	avg	
Nocardioides strain JS614	-7.2 ± 0.16	-7.20	0.9928	Abe et al., 2009
Nocardioides strain JS614	-7.3 ± 0.07	-7.30	0.9927	Abe et al., 2009
Mycobacterium sp. JS60	-8.2	-8.20	0.9918	Chartrand et al., 2005
Mycobacterium sp. JS61	-7.1	-7.10	0.9929	Chartrand et al., 2005
Mycobacterium sp. JS62	-7	-7.00	0.9930	Chartrand et al., 2005
Mycobacterium sp. JS63	-7.6	-7.60	0.9924	Chartrand et al., 2005
Mycobacterium aurum L1	-5.7 ± 1.1	-5.70	0.9943	Chu et al., 2004
Methylosinus trichosporium OB3b	-3.2 ± 0.3	-3.20	0.9968	Chu et al., 2004
Mycobacterium vaccae JOB5	-4.8 ± 0.3	-4.80	0.9952	Chu et al., 2004
Enrichment culture (Alameda)	-4.5 ± 1.0	-4.50	0.9955	Chu et al., 2004
Enrichment culture (Travis)	-5.5 ± 0.8	-5.50	0.9945	Chu et al., 2004
Enrichment culture	-6.3/-6.5	-6.40	0.9936	Chu et al., 2004
Enrichment culture	-5.5/-5.4/-5.4	-5.40	0.9946	Chu et al., 2004
Enrichment culture	-6.3 ± 0.3	-6.30	0.9937	Tiehm et al., 2008
Enrichment culture	-6.5 ± 0.4	-6.50	0.9935	Tiehm et al., 2008
Enrichment culture	-5.5 ± 0.3	-5.50	0.9945	Tiehm et al., 2008
Enrichment culture	-5.4 ± 0.8	-5.40	0.9946	Tiehm et al., 2008
Enrichment culture	-5.4 ± 0.4	-5.40	0.9946	Tiehm et al., 2008
	AVG	-6.06		
	MIN	-8.20		
	MAX	-3.20		

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Monitoring data - complete time series

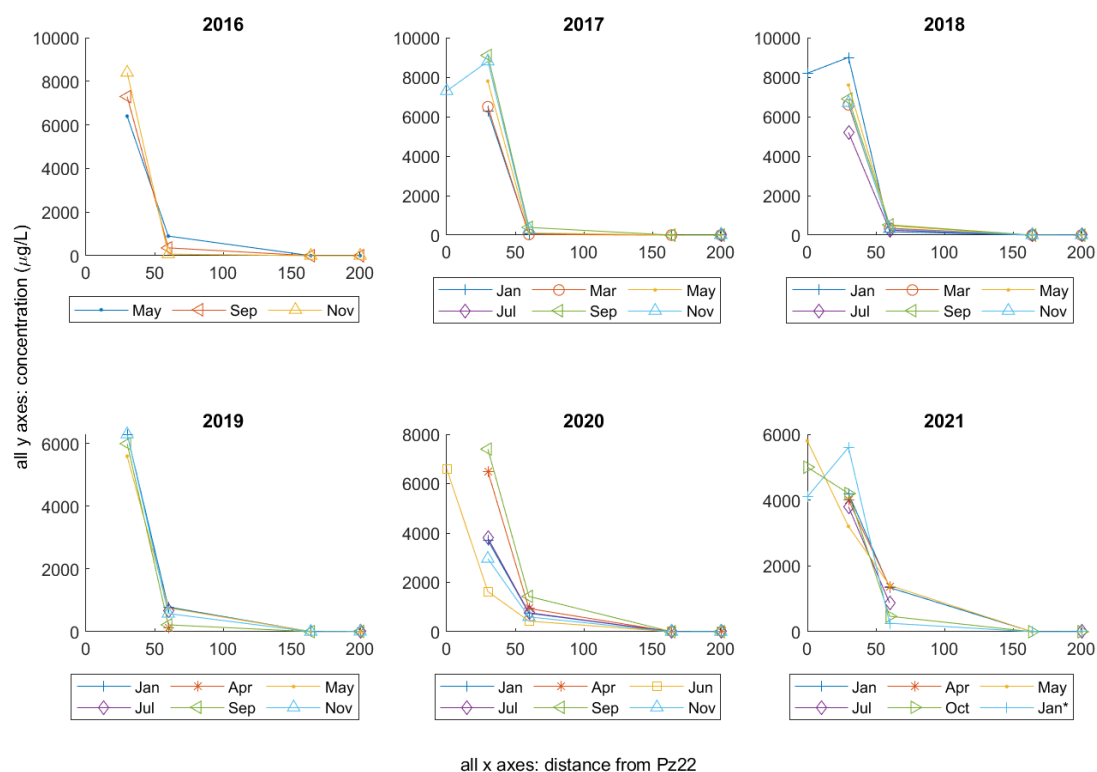


Figure S1 –Time series of PCE concentrations. In the 2021 subplot, the “Jan*” time series corresponds to measurements collected in January 2022.

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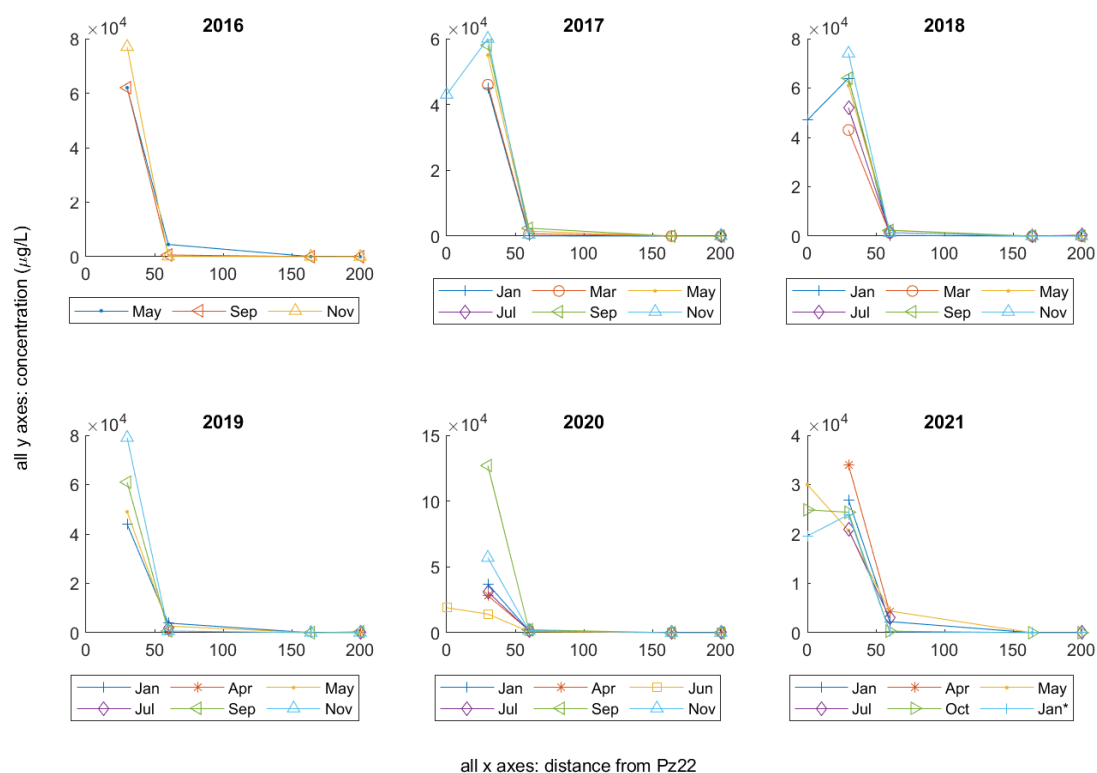


Figure S2 –Time series of TCE concentrations. In the 2021 subplot, the “Jan*” time series corresponds to measurements collected in January 2022.

Supplementary material

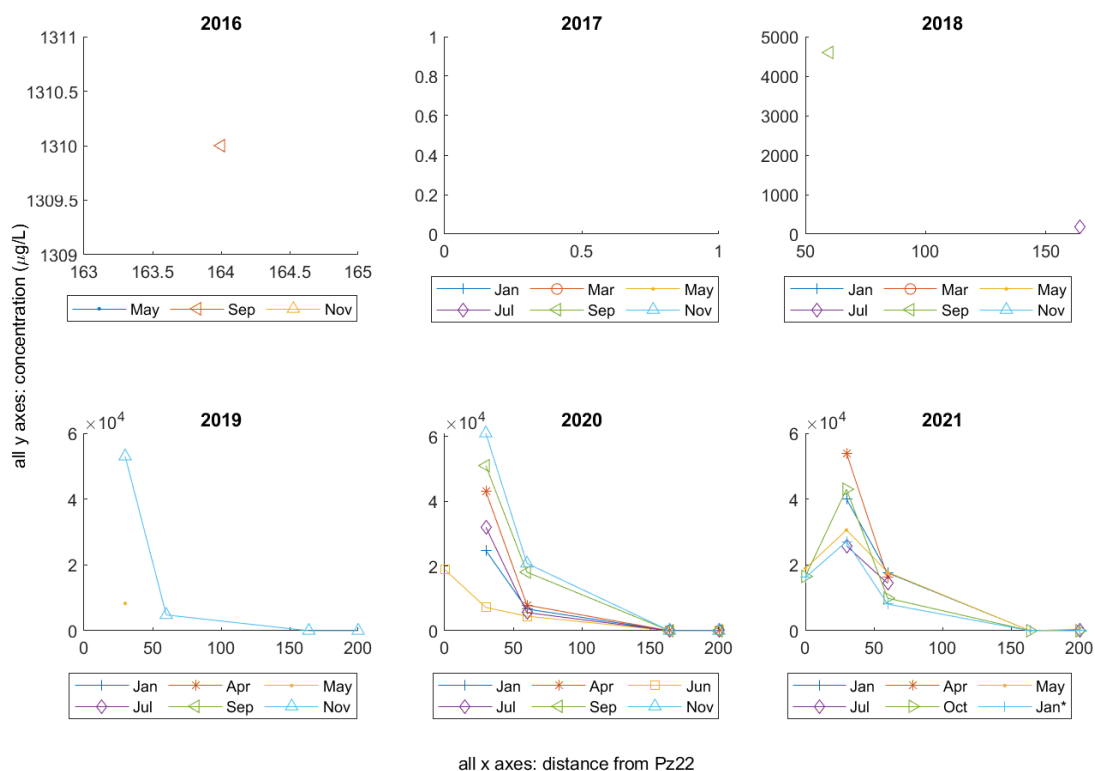


Figure S3 –Time series of *cis*-DCE concentrations. In the 2021 subplot, the “Jan*” time series corresponds to measurements collected in January 2022.

Supplementary material

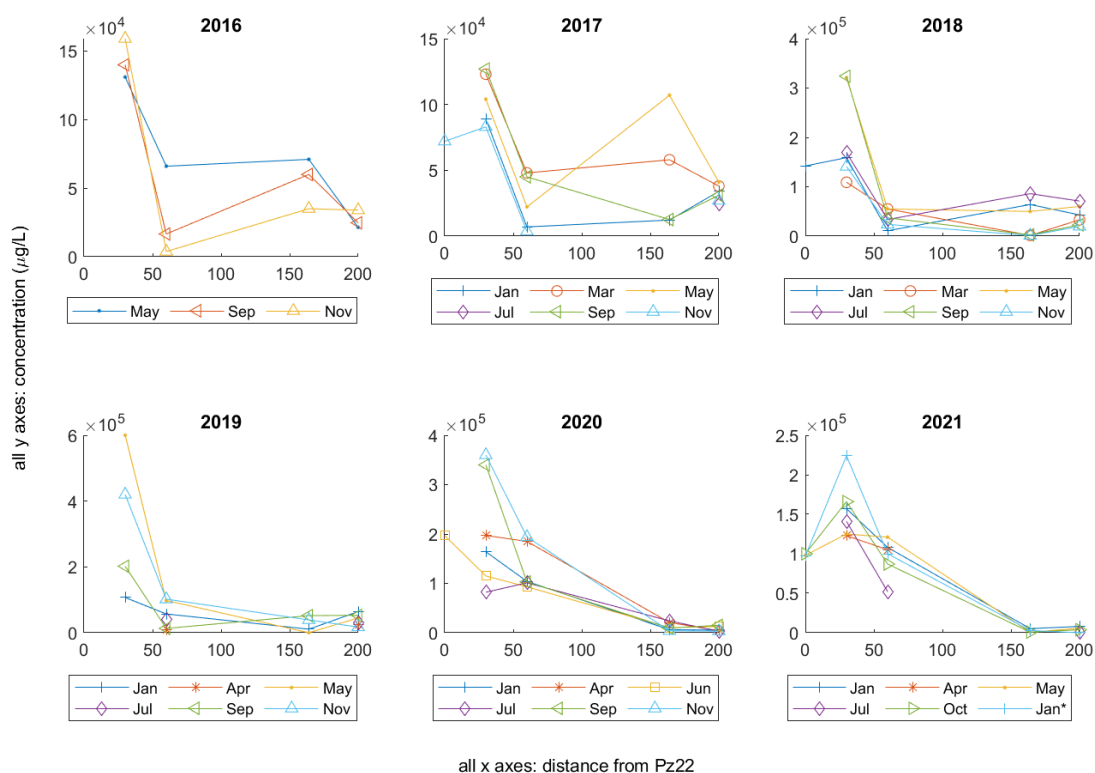


Figure S4 –Time series of VC concentrations. In the 2021 subplot, the “Jan*” time series corresponds to measurements collected in January 2022.

Supplementary material

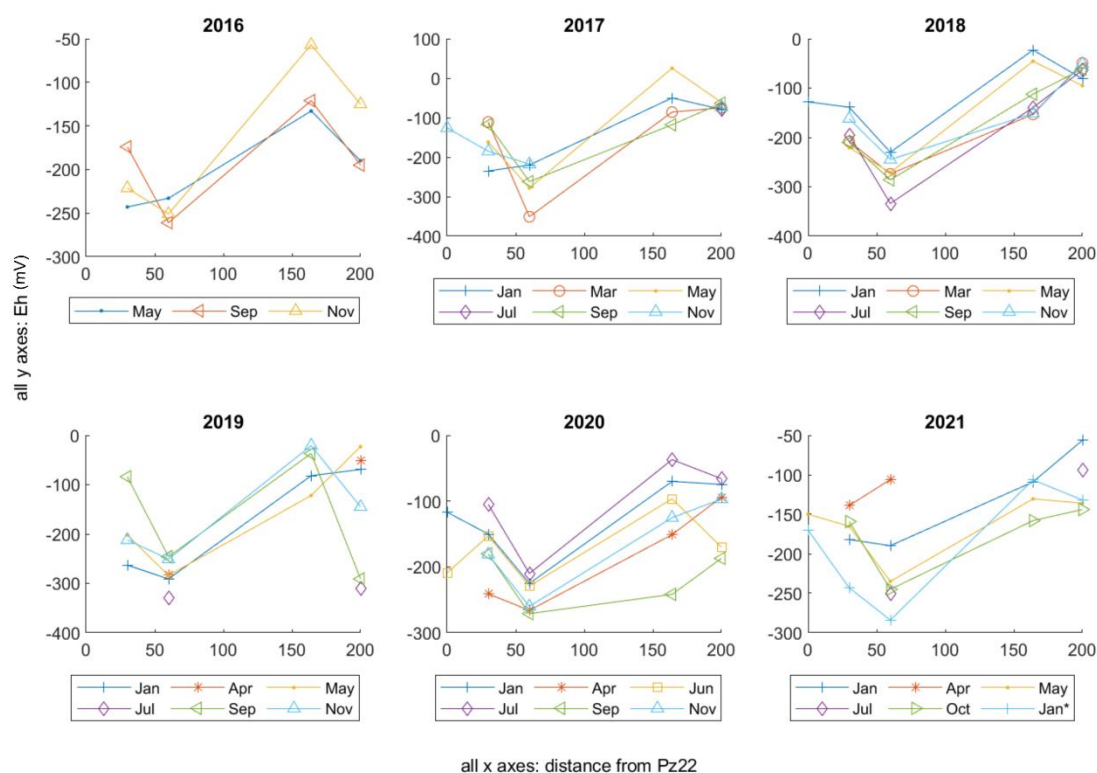


Figure S5 –Time series of Eh values. In the 2021 subplot, the “Jan*” time series corresponds to measurements collected in January 2022.

Supplementary material

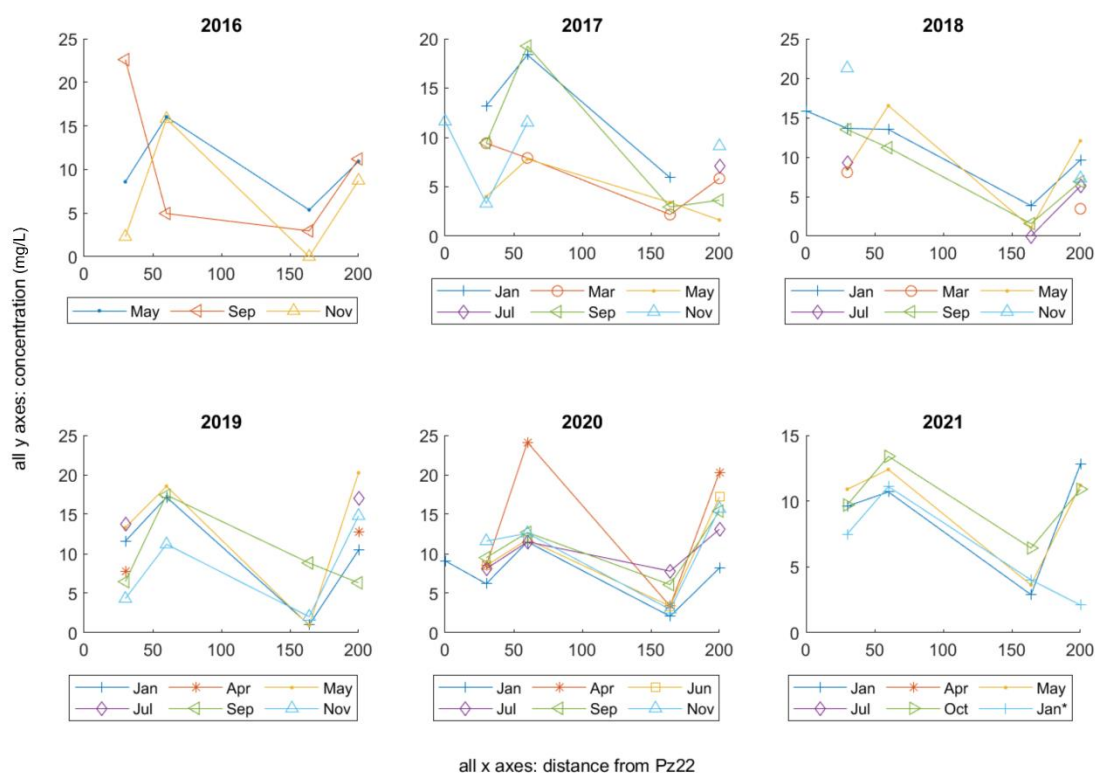


Figure S6 –Time series of ammonium concentrations. In the 2021 subplot, the “Jan*” time series corresponds to measurements collected in January 2022.

Supplementary material

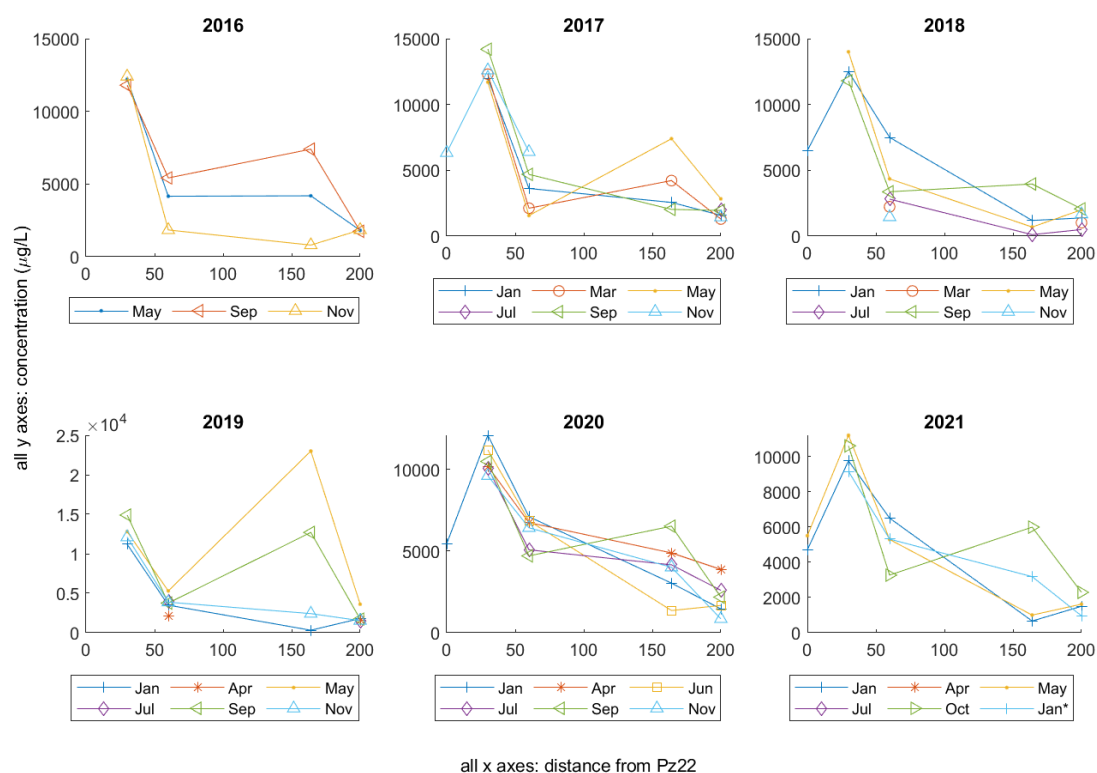


Figure S7 –Time series of iron concentrations. In the 2021 subplot, the “Jan*” time series corresponds to measurements collected in January 2022.

Supplementary material

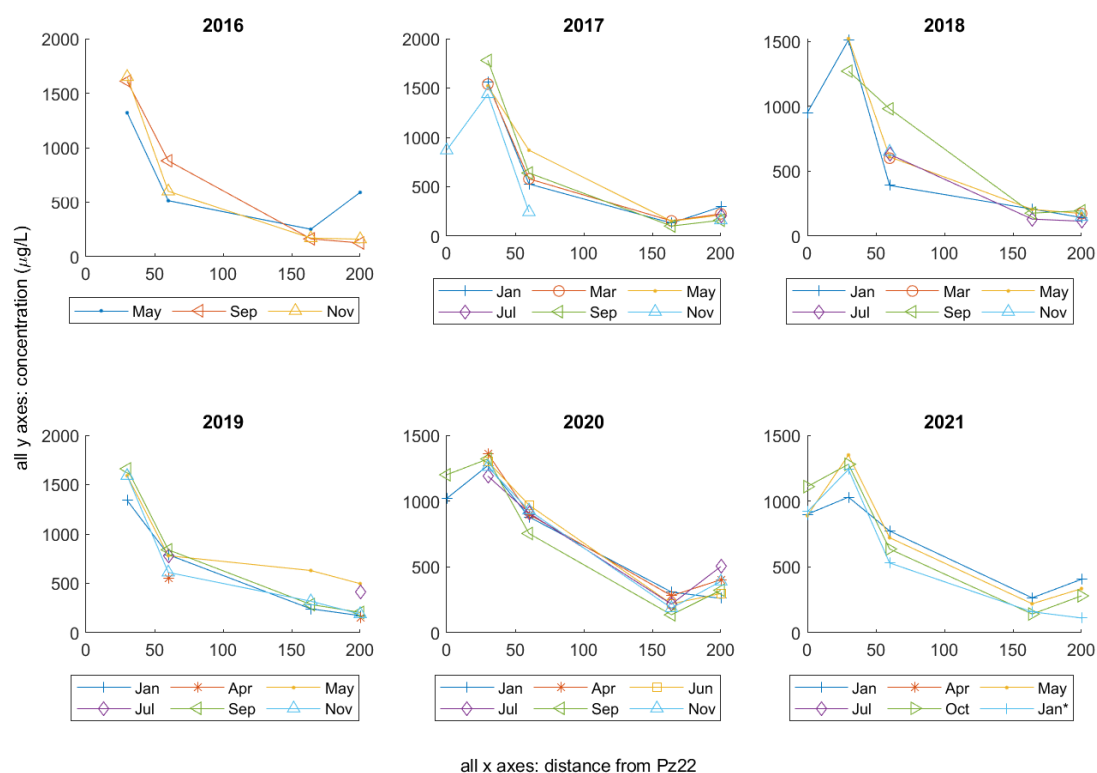


Figure S8 –Time series of manganese concentrations. In the 2021 subplot, the “Jan*” time series corresponds to measurements collected in January 2022.

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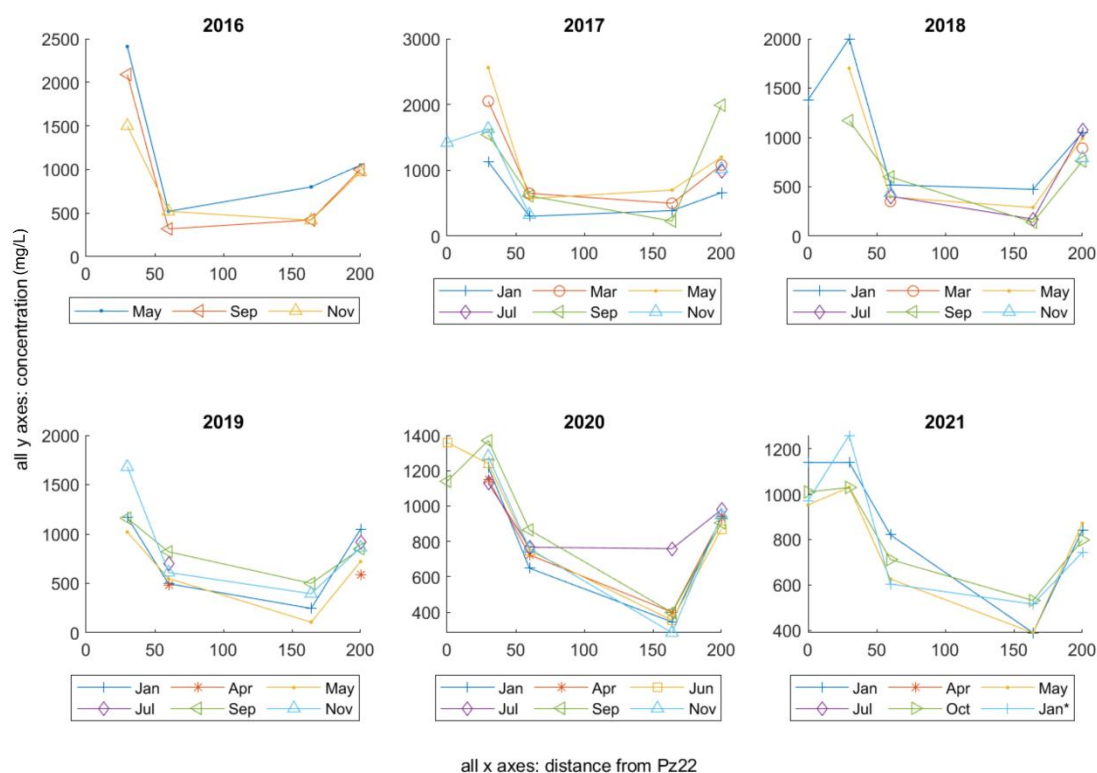


Figure S9 –Time series of sulfate concentrations. In the 2021 subplot, the “Jan*” time series corresponds to measurements collected in January 2022.

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Sensitivity analysis – key results

In “Approach 2”, k and ϵ values were fitted considering two further values of longitudinal dispersion ($\alpha_L=5$ and 10m) in addition to $\alpha_L=22.4$ m determined following [3]. The results are reported in Table S1. Overall lower wRMSE were obtained using $\alpha_L=22.4$ m. Slightly different calibrated values for k and ϵ were obtained in the three cases, with ϵ values generally more similar to literature mean values for $\alpha_L=22.4$ m. For lower α_L ($\alpha_L=5$ m, in particular) calibrated ϵ_{RD} values almost coincided with the upper limit of the literature range for TCE and DCE.

Table S3 - Calibrated k and ϵ values for the tested scenarios with May 2021 data.

	PCE	TCE	cis-DCE	VC
$\alpha_L = 22.4$m				
<i>Approach 2 - Reductive Dechlorination (RD)</i>				
k_{RD1} (y^{-1})	0.3	0.2	0	0
k_{RD2} (y^{-1})	6.5	2.9	0.6	0
ϵ_{RD} (‰)	-5.6	-5.7	-16.0	0
<i>Approach 2 – Oxidation (OX) using the average ϵ_{OX}</i>				
k_{OX} (y^{-1})	-	-	4.7	2.9
ϵ_{OX} (‰)	-	-	-7.99	-6.06
<i>wRMSE (average ϵ_{OX})</i>				
concentrations	1.10	13.14	24.08	157.76
CSIA	1.88	0.13	1.12	0.80
$\alpha_L = 10$m				
<i>Approach 2 - Reductive Dechlorination (RD)</i>				
k_{RD1} (y^{-1})	0.38	0.25	0	0
k_{RD2} (y^{-1})	5.5	2.8	0.53	0
ϵ_{RD} (‰)	-4.3	-4.4	-15.2	-
<i>Approach 2 – Oxidation (OX) using the average ϵ_{OX}</i>				
k_{OX} (y^{-1})	-	-	2.3	2.1
ϵ_{OX} (‰)	-	-	-7.99	-6.06
<i>wRMSE</i>				
concentrations	1.15	14.42	28.26	193.77
CSIA	1.89	0.10	1.19	0.73
$\alpha_L = 5$m				
<i>Approach 2 - Reductive Dechlorination (RD)</i>				
k_{RD1} (y^{-1})	0.4	0.3	0	0
k_{RD2} (y^{-1})	4.8	2.7	0.5	0
ϵ_{RD} (‰)	-3.4	-3.5	-14.9	-
<i>Approach 2 – Oxidation (OX) using the average ϵ_{OX}</i>				
k_{OX} (y^{-1})	-	-	1.3	1.8
ϵ_{OX} (‰)	-	-	-7.99	-6.06
<i>wRMSE</i>				
concentrations	1.09	13.82	30.81	212.49
CSIA	2.02	0.14	1.25	0.69

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A higher α_L value was also tested ($\alpha_L=32.4\text{m}$), but it resulted in an ε_{RD} for PCE equal to the lower limit of the literature range ($\varepsilon_{RD}=-7.2\text{‰}$), so the calibration of k and ε for the other daughter compounds (TCE, cis-DCE and VC) was not carried out and data not reported for this case. Due to this reasons $\alpha_L=22.4\text{m}$ was chosen.

The second step of the sensitivity analysis was carried out by varying the best-fitted ε_{RD} values obtained for PCE, TCE and cis-DCE by ± 0.1 and ± 1.0 . The variation of k_{RD2} by ± 0.1 did not produce noteworthy modifications of isotopic compositions and concentrations for PCE and TCE. Thus, only variations of $\pm 1.0\text{y}^{-1}$ were further considered for these compounds. As for cis-DCE only a k_{RD2} variation of ± 0.1 was evaluated due to the low degradation rates of this compound.

For PCE, variations of ± 0.1 of both parameters did not produce noteworthy modifications to simulated concentrations. While enrichment factors changed, they remained within standard deviations for PCE $\delta^{13}\text{C}$ ($\pm 0.25\text{‰}$ for k_{RD2} variation and $\pm 0.55\text{‰}$ for ε_{RD} variation) and daughter products ($<0.02\text{‰}$ for TCE and even smaller for cis-DCE and VC). For PCE, a change by $\pm 1.0\text{‰}$ in ε_{RD} (Figure S10) generated an isotopic shift of $\Delta^{13}\text{C}=\pm 5.3\text{‰}$. For TCE, the shift remained below $<0.2\text{‰}$. No shift was observed for cis-DCE and VC.

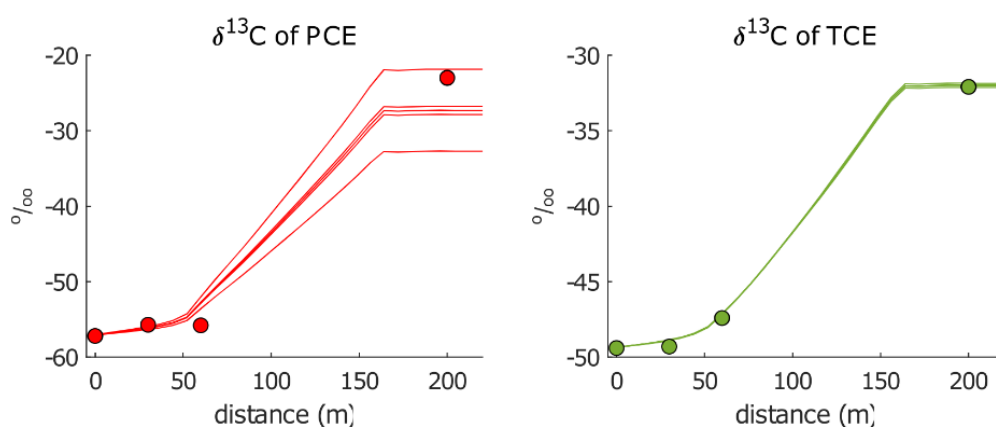


Figure S10 – Variation of isotopic composition of PCE and TCE after varying ε_{RD} by $\pm 1.0\text{‰}$ and 0.1‰ .

Considering the calibrated value of ε_{RD} for PCE (-5.6‰), a change in k_{RD2} of $\pm 1.0\text{y}^{-1}$ produces a $\Delta^{13}\text{C}=\pm 2.5\text{‰}$ in PCE, while only a maximum variation of 0.2‰ for TCE (Figure S11). Cis-DCE and

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VC isotopic variations are negligible, as well as concentration variations for all compounds, even for PCE. This means that PCE degradation does not affect significantly the evolution of concentrations and isotopic compositions of daughter chloroethenes due to its much lower concentration.

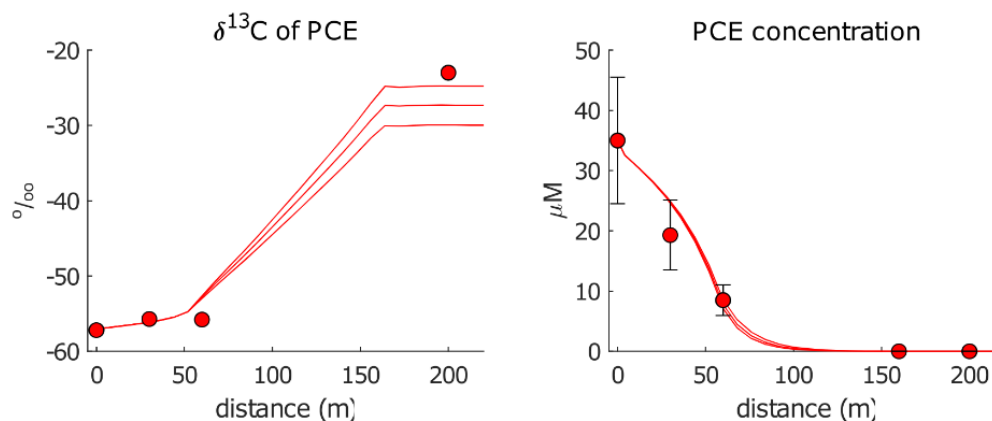


Figure S11 – k_{RD2} variation of PCE: $\pm 1.0\%$.

For TCE the variation of $\pm 0.1\%$ in ϵ_{RD} produces a negligible variation of the isotopic value ($\pm 0.3\%$), while for a variation of $\pm 1.0\%$ the isotopic variation is ten times higher ($\pm 3.2\%$; Figure S12). No influence on the isotopic composition of DCE was observed.

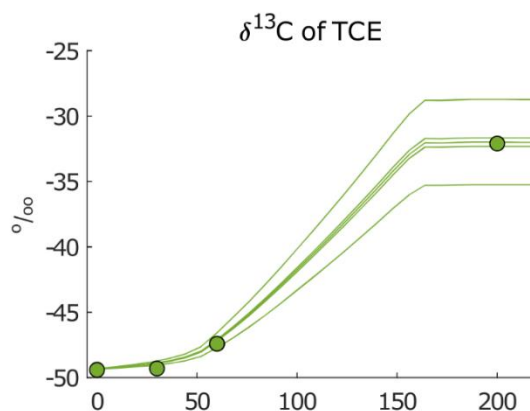


Figure S12 – ϵ_{RD} variation of TCE: $\pm 1.0\%$ and 0.1% .

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The variation of k_{RD2} of TCE by ± 1.0 , instead, caused an enrichment of 2.9‰ and a decrease of 3.7‰ in the isotopic composition of TCE as well as a variation of concentrations after the AN barrier. Maximum variations were observed at $x=100\text{m}$ where the initial value of $20.1\mu\text{M}$ was almost doubled ($35.7\mu\text{M}$) for increased k_{RD2} and almost halved ($12.5\mu\text{M}$) for decreased k_{RD2} .

Moreover, the variation of k_{RD2} caused a slight enrichment of 0.5‰ and a decrease of 0.9‰ in the isotopic composition of cis-DCE as well (measured in 206S), and a small variation in the accumulation of cis-DCE at the AN barrier was induced (Figure S13). Maximum variations were observed in Pz13: from $256.5\mu\text{M}$ to $266.3\mu\text{M}$ for decreased k_{RD2} and from $256.5\mu\text{M}$ to $242.8\mu\text{M}$ for increased k_{RD2} . No noteworthy variations for VC isotopic composition or concentration were observed.

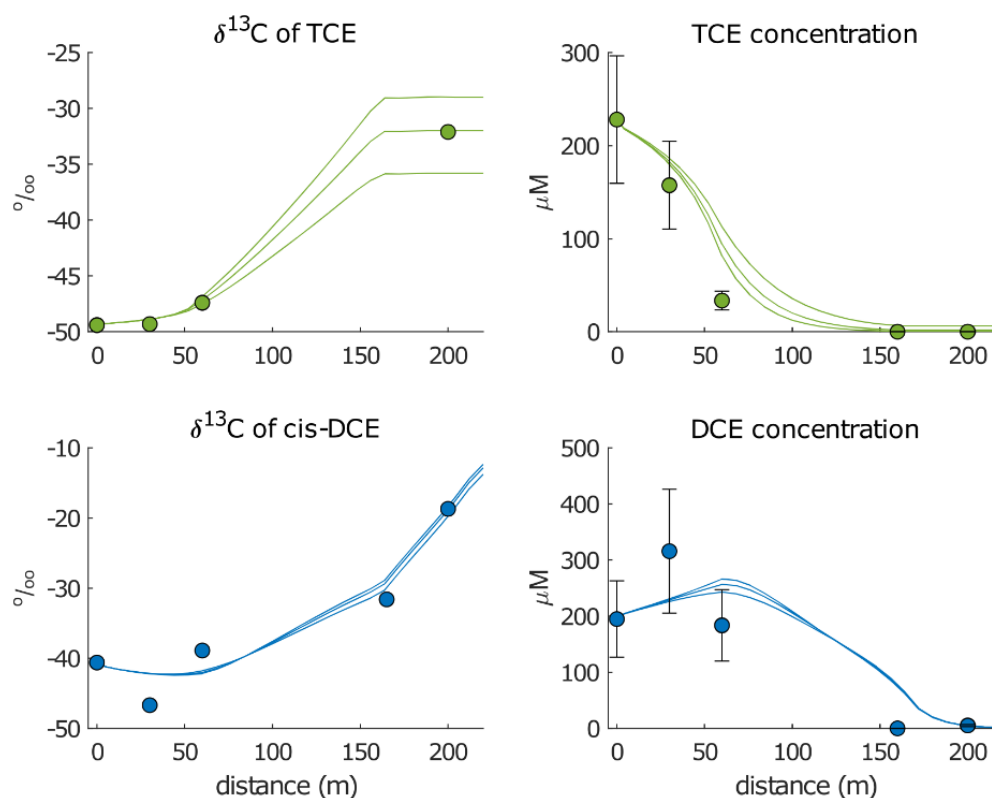


Figure S13 – k_{RD2} variation of TCE: $\pm 1.0\%$.

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For cis-DCE an ϵ_{RD} variation of $\pm 0.1\text{‰}$ did not produce noteworthy changes in isotopic values, while a bit higher variation was induced for $\Delta\epsilon=\pm 1.0\text{‰}$ ($\Delta^{13}\text{C}=\pm 1.0\text{‰}$; Figure S14). No influence on the isotopic composition of VC was observed.

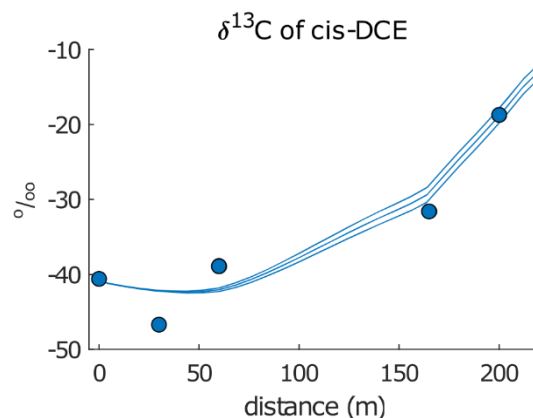


Figure S14 -- ϵ variation of cis-DCE: $\pm 1.0\text{‰}$

The variation of k_{RD2} for cis-DCE by ± 0.1 produced in 206S an enrichment of 1.7‰ and an impoverishment of 2.2‰ . As for cis-DCE concentrations, the highest variations were observed between Pz13 and 206S were negative and positive variations of $20\mu\text{M}$ compared to the initial value took place. No significant variations were observed for VC isotopic composition and concentrations.

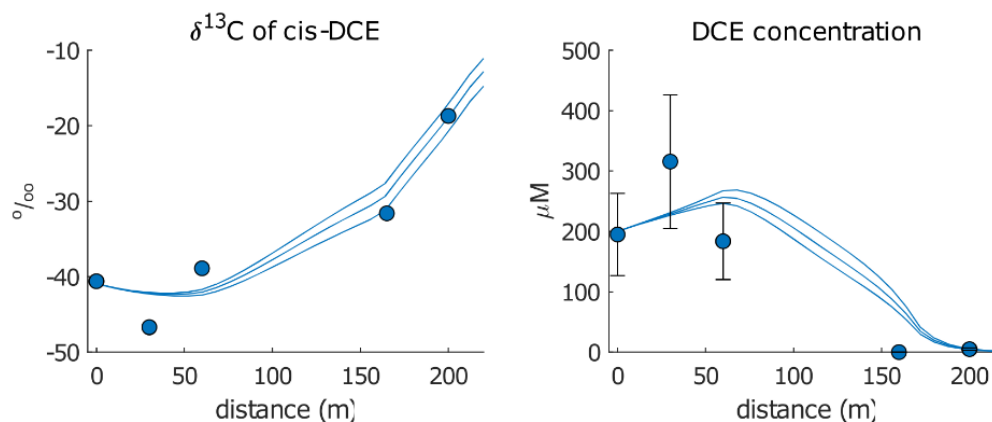


Figure S15 – k_{RD2} variation of cis-DCE: $\pm 0.1\text{‰}$.

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Isotopic shifts

Isotopic shifts ($\Delta^{13}\text{C}$) calculated for each compound as the difference between $\delta^{13}\text{C}$ in the downgradient piezometer and $\delta^{13}\text{C}$ in the respective upgradient piezometer.

Table S4 Isotopic shifts ($\Delta^{13}\text{C}$) along the transect for January and May 2021 data.

$\Delta^{13}\text{C}$ (‰)	ANAEROBIC		AEROBIC	
January 2021				
	Pz22→Pz10		Pz10→206S	206S→AEext4
PCE	3.0		12.9 (Pz10→AEext4)	
TCE	2.4		8.5 (Pz10→AEext4)	
DCE	2.5		11.4	14.9
VC	-0.2		8.5	1.2
May 2021				
	Pz22→Pz13	Pz13→Pz10	Pz10→206S	206S→AEext4
PCE	1.43	-0.06	32.8 (Pz10→AEext4)	
TCE	0.12	1.87	15.3 (Pz10→AEext4)	
DCE	-6.16	7.79	7.4	12.9
VC	-0.64	-0.18	-9.3	14.5

Casiraghi *et al* (2022), Assessing a large-scale sequential in-situ chloroethene bioremediation system using compound-specific isotope analysis (CSIA) and geochemical modeling. *Pollutants*.

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1. Antelmi, M.; Mazzon, P.; Höhener, P.; Marchesi, M.; Alberti, L. Evaluation of MNA in A Chlorinated Solvents-Contaminated Aquifer Using Reactive Transport Modeling Coupled with Isotopic Fractionation Analysis. *Water* **2021**, *13*, 2945, doi:10.3390/w13212945.
2. Kuder, T.; Philp, P.; Van Breukelen, B.M.; Thouement, H.A.A.; Vanderford, M.; Newell, C.J. Integrated Stable Isotope – Reactive Transport Model Approach for Assessment of Chlorinated Solvent Degradation 2014.
3. Gelhar, L.W.; Welty, C.; Rehfeldt, K.R. A Critical Review of Data on Field-Scale Dispersion in Aquifers. *Water Resour. Res.* **1992**, *28*, 1955–1974, doi:10.1029/92WR00607.