**Supplemental Information for:** 

Relationships between the Abundance and Expression of Functional Genes from Vinyl Chloride (VC)-Degrading Bacteria and Geochemical Parameters at VC-Contaminated Sites

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Figure S1. General location of the six VC-contaminated sites used in this study.

Table S1. Monitoring well information and field-measured geochemical parameters for each site. The geochemical parameter values listed are average values of all monitoring wells sampled over all sampling events. (NA: data not available; bgs: below groundwater surface)

Cite	Ground	Well screened	I	рН	Τe	emp (°C)	DO	(mg/L)		ORP (mV)
Site	(ft bgs)	interval (ft bgs)	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range
VA Site 2C	3-19	11-13	6.4	6.3-6.6	22.7	20.7-25.0	0.5	0.3-0.7	-71.0	(-123.0) – (-23.0)
GA Site 11	4	32-40	5.3	5.1-5.4	22.0	20.5-24.0	0.3	0.2-0.4	-97.5	(-107.1) – (-88.1)
OK Site SS-17	3	5-35	6.8	6.7-6.8	20.3	18.3-22.3	0.8	0.7-0.8	-133.3	(-185.0) – (-67.0)
CA Site 70	13	25-170	6.8	6.3-7.7	21.1	18.1-22.9	0.5	0.1-1.3	-237.1	(-351.2) – (-118.0)
SC Site 45	0-10	7-16	6.1	4.6-7.3	25.7	23.0-30.9	0.7	0.2-4.6	16.0	(-159.0) – (569.8)
HI Site LF05	2-28	1.5-20	7.5	7.1-8.2	25.5	24.8-26.2	0.5	0.2-1.8	-79.4	(-267.3) – (325.8)

#### **Supplemental Methods**

# qPCR primer specificity

Detailed qPCR primer specify checking has been accomplished in previous studies. The qPCR primer sets targeting *etnC* and *etnE* (i.e. RTC and RTE) were designed and tested for specificity with ethene- and VC-assimilating isolates and VC-contaminated environmental samples. Clone libraries were constructed with qPCR products and they were sequenced to test for possible non-specific amplification<sup>1</sup>. To date we have not observed any non-specific amplification products of the RTC and RTE primers.

The qPCR primer sets targeting *bvcA* (Bvc925f/1017r) and *vcrA* ((Vcr1022f/1093r) are based on genes from *Dehalococcoides mccartyi* strain BAV1, and strain GT and VS, respectively<sup>2</sup>. Primer sets targeting *mmoX* (mmoX536f/898r) and *pmoA* (pmoA472-A189f/ pmoA472-mb661r) have been widely used for the detection and quantification of methane monooxygenase genes in environmental samples <sup>3, 4</sup>.

## Calculation of gene and transcript abundances

Equation used for gene abundance calculation per liter of groundwater (GW):

genes _	genes per qPCR	, ng DNA after extraction
L GW	ng DNA used for qPCR	volume of groundwater

Equation used for transcript abundance calculation per liter of GW:

$$\frac{transcripts}{L \, GW} = \frac{transcripts \, per \, qPCR}{fraction \, RNA \, recovery} \times \frac{cDNA \, vol \, after \, R.T.}{cDNA \, volume \, used \, for \, qPCR} \\ \times \frac{RNA \, volume \, after \, RNA \, clean \, up}{RNA \, volume \, used \, for \, R.T.} \times \frac{2 \, (single \, strand \, correction)}{volume \, of \, groundwater}$$

### Estimation of VC travel time in groundwater

Values for VC retardation coefficient R and the groundwater seepage velocity (the average linear groundwater velocity parallel to groundwater flow direction) were estimated to develop VC travel times in groundwater. The apparent centerline of each VC plume at each site was determined using site maps showing estimated VC isocontour lines. Monitoring wells along the apparent centerline were picked for the bulk VC attenuation rate calculation. The groundwater seepage velocity, if not located in site reports, was estimated as follows:

$$v_x = \frac{K}{n_e} \frac{dH}{dL}$$

Where:  $v_x$  = average linear ground-water velocity parallel to ground-water flow direction (seepage velocity), K= hydraulic conductivity,  $n_e$  = effective porosity, and dH/dL=hydraulic gradient.

The Retardation factor, if not found in site reports, was calculated as follows:

$$R = 1 + \frac{\rho_b K_{oc} f_{oc}}{n}$$

Where:

 $\begin{array}{l} \mathsf{R} = \mbox{retardation factor} \\ \rho_b = \mbox{bulk density} \\ \mathsf{K}_{o\ c} = \mbox{soil adsorption coefficient for soil organic carbon content} \\ f_{oc} = \mbox{fraction soil organic carbon} \\ \mathsf{n} = \mbox{total porosity} \end{array}$ 

Koc was assumed to 29.6 L/kg for VC according to biochlor22 documentation (https://www.epa.gov/water-research/biochlor-natural-attenuation-decision-support-system).

Other parameters values ( $\rho_b$ , f<sub>oc</sub>, and n) were found in site reports or assumed if not available. Porosity was generally assumed to be 0.3. Calculated retardation factors ranged from 1.001-1.002. A report value for SC Site 45 (1.1) was used.

Target gene	Primer name	Sequences (5'->3')	Expected product size	Ref.
16S	515f	GTGCCAGCMGCCGCGGTAA	201 hr	5
rRNA	806r	GGACTACHVGGGTWTCTAAT	- 291 bp	
luciferase	ref-STF	CCAGGGATTTCAGTCGATGT	1011 hr	6
mRNA	ref-STR	TTTTCCGTCATCGTCTTTCC	- 1014 bp	
luciferase	ref-f	TACAACACCCCAACATCTTCGA	150 hr	7
mRNA	ref-r	GGAAGTTCACCGGCGTCAT	- 150 bp	
otaC	JS614-etnCF	GCGATGGAGAATGAGAAGGA	1120 hn	1
einc	JS614-etnCR	TCCAGTCACAACCCTCACTG	- 1130 bp	
otaC	RTC-f	ACCCTGGTCGGTGTKSTYTC	106 hr	1
einc	RTC-r	TCATGTAMGAGCCGACGAAGTC		
othE	CoM-F1L	AACTACCCSAAYCCSCGCTGGTACGAC	901 hn	8
eune	CoM-R2E	GTCGGCAGTTTCGGTGATCGTGCTCTTGAC	- 091 ph	
othE	RTE-f	CAGAAYGGCTGYGACATYATCCA	151 bp	1
eure	RTE-r	CSGGYGTRCCCGAGTAGTTWCC	- 151 bp	
mmoV	mmoX-std2f	AGGCAGTCAAGGACGAAAGG	1122 hn	9
mmox	mmoX-std2r	ATCTGGCCGTTGTACTCGTG	- 1123 bp	
mmoV	mmoX536f	CGCTGTGGAAGGGCATGAAGCG	262 hn	10
	mmoX898r	GCTCGACCTTGAACTTGGAGCC	- 302 bh	
nmo (	pmoA-std1f	TCGGTCCGTTCAACTCCG	702 hn	9
ρποΑ	pmoA-std1r	GAATACCAACGGCCCATGAA	- 703 bh	
nmoA -	pmoA472-A189f	GGNGACTGGGACTTCTGG	- 172 hn	11
ршод	pmoA472-mb661r	CCGGMGCAACGTCYTTACC	472.00	
byc A	Bvc925F	AAAAGCACTTGGCTATCAAGGAC	02 hn	2
DVCA -	Bvc1017R	CCAAAAGCACCACCAGGTC	- 92 bp	
vcrA	Vcr1022F	CGGGCGGATGCACTATTTT	71 hn	2
	Vcr1093R	GAATAGTCCGTGCCCTTCCTC		

Table S2. Primer sets used for PCR and qPCR analysis.

Target gene	luciferase mRNA	etnC	etnE	mmoX	pmoA	bvcA	vcrA
primer conc, μM	0.1	0.8	0.8	0.3	0.3	0.3	0.3
template	2 μl cDNA	10 ng DNA or 2 μl cDNA	10 ng DNA or 2 μl cDNA	10 ng DNA or 2 μl cDNA	10 ng DNA or 2 μl cDNA	10 ng DNA or 2 μl cDNA	10 ng DNA or 2 μl cDNA
linear range, gene copies/reaction	30 - 30 × 10 <sup>7</sup>	30 - 30 × 10 <sup>7</sup>	30 - 30 × 10 <sup>6</sup>				
qPCR efficiency	94.6 - 97.2%	94.4 - 103.1%	90.4 - 100.6%	87.3 - 93.5%	87.8 - 97.2%	96.0 - 104.0%	96.2 - 99.5%
Y-intercept	33.0-37.6	40.7 - 41.7	38.2 - 40.1	33.9 - 41.4	35.7 - 43.1	32.4 - 37.2	32.1 - 37.1
R <sup>2</sup>	0.999 - 1.000	0.995 - 0.999	0.998 - 0.999	0.995 - 1.000	0.993 - 0.999	0.996 - 1.000	0.997 - 1.000

Table S3. Pertinent qPCR parameters (primer concentration, template mass, linear range, PCR efficiency, and y-intercept of the qPCR standard curve.



Figure S2. A general illustration of the hierarchical data structure in this study. Each site contains multiple monitoring wells (MW1, MW2, MW3...), and samples from each monitoring well could be analyzed at different sampling events (Measurement1, Measurement2...).



Figure S3. Histogram of gene and transcript abundance after log transformation.

Table S4. Regression analysis comparing functional gene and transcript abundances from etheneotroph, methanotroph, and anaerobic VC-dechlorinating bacteria at six different VC-contaminated sites, p values < 0.05 are in bold.

Gene	n		multileve	el regression			simple line	ear regression		Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
etnC vs meth	95	0.41	p<0.001	0.24-0.57	274	-0.11	0.2364	-0.29-0.07	320	-0.08	0.4580
<i>etnC</i> vs dhc	95	0.30	p<0.001	0.20-0.39	263	0.32	p<0.001	0.21-0.44	294	0.46	p<0.001
meth vs dhc	95	0.20	0.0015	0.08-0.32	300	0.15	0.0544	0.00-0.30	341	0.22	0.0331
Transcript	n	_	multileve	el regression			simple line	ear regression		Spe cor	arman's relation
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
etnC vs meth	64	0.27	0.0630	0.01-0.57	243	-0.05	0.7407	-0.33-0.24	247	-0.02	0.8657
<i>etnC</i> vs dhc	55	0.22	0.0249	0.02-0.39	202	0.21	0.0283	0.02-0.40	197	0.29	0.0320

n: number of samples in the analysis

Coef: regression coefficient

CI: confidence interval

AIC: Akaike's Information Criterion. Lower AIC scores indicate improved fitting for the statistical model with the data.

meth: the sum of *mmoX* and *pmoA* (a proxy for total methanotroph abundance)

dhc: the sum of *bvcA* and *vcrA* (a proxy for total anaerobic VC-dechlorinating bacterial abundance)

Table S5. Regression analysis of TCE concentrations with *cis*-DCE, *trans*-DCE, VC, ethene, and methane concentrations. p values <0.05 are in bold.

TCE vs n			multileve	l regression			simple line	ar regression		Spearman's correlation		
others		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value	
cis-DCE	95	0.48	p<0.001	0.34-0.63	227	0.74	p<0.001	0.59-0.89	287	0.77	p<0.001	
trans-DCE	95	0.45	p<0.001	0.35-0.54	165	0.60	p<0.001	0.50-0.70	215	0.72	p<0.001	
VC	95	0.13	0.070	-0.01-0.28	226	0.54	p<0.001	0.37-0.71	309	0.59	p<0.001	
ethene	65	0.24	p<0.001	0.10-0.37	94	0.31	p<0.001	0.19-0.44	124	0.40	p<0.001	
methane	74	0.08	0.336	-0.09-0.26	138	0.17	0.086	-0.22-0.36	201	0.15	0.207	

n: number of samples in the analysis

Coef: regression coefficient

CI: confidence interval

Gene vs <i>cis</i> -	n	mu	ltilevel regr	ession		simp	ole linear re	gression		Spear corre	rman's elation
DCE	-	Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
etnC	95	0.25	0.002	0.10-0.38	283	0.28	0.001	0.11-0.44	310	0.34	0.001
etnE	95	0.24	0.002	0.09-0.37	284	0.28	0.001	0.11-0.44	312	0.35	p<0.001
ттоХ	95	-0.13	0.146	-0.28-0.05	287	-0.19	0.031	-0.360.02	319	-0.23	0.027
ртоА	95	0.01	0.912	-0.15-0.22	323	-0.07	0.506	-0.28-0.14	357	-0.08	0.441
bvcA	95	0.36	0.021	0.07-0.63	367	0.34	0.006	0.10-0.58	382	0.25	0.016
vcrA	95	0.31	0.081	0.08-0.66	390	0.43	0.005	0.13-0.73	427	0.30	0.003
ттоХ+ртоА	95	0.02	0.858	-0.16-0.18	310	-0.06	0.514	-0.26-0.13	345	-0.08	0.419
bvcA+vcrA	95	0.38	0.024	0.03-0.68	382	0.38	0.003	0.13-0.63	393	0.30	0.003
Transcript vs	n	mu	ltilevel regr	ession		simp	ole linear re	gression		Speai corre	rman's lation
Transcript vs <i>cis</i> -DCE	n _	mu Coef	ltilevel regr p value	ession 95% Cl	AIC	simp Coef	ple linear re	gression 95% Cl	AIC	Spear corre Rho	rman's lation p value
Transcript vs <i>cis</i> -DCE <i>etnC</i>	n _ 70	mu Coef 0.38	ltilevel regr p value <b>0.002</b>	ession 95% Cl 0.16-0.62	AIC 259	simp Coef 0.40	ple linear re p value <b>p&lt;0.001</b>	gression 95% Cl 0.18-0.61	AIC 254	Spear corre Rho 0.26	rman's elation p value 0.010
Transcript vs cis-DCE etnC etnE	n 70 71	mu Coef 0.38 0.30	ltilevel regr p value 0.002 0.003	ession 95% Cl 0.16-0.62 0.12-0.48	AIC 259 288	simp Coef 0.40 0.32	ble linear re p value p<0.001 0.001	gression 95% Cl 0.18-0.61 0.14-0.50	AIC 254 292	Spear corre Rho 0.26 0.28	rman's elation p value 0.010 0.006
Transcript vs <i>cis</i> -DCE <i>etnC</i> <i>etnE</i> <i>mmoX</i>	n 70 71 61	mu Coef 0.38 0.30 -0.08	ltilevel regr p value 0.002 0.003 0.396	95% Cl 0.16-0.62 0.12-0.48 -0.27-0.10	AIC 259 288 181	simp Coef 0.40 0.32 -0.08	ble linear re p value p<0.001 0.001 0.377	gression 95% Cl 0.18-0.61 0.14-0.50 -0.27-0.10	AIC 254 292 189	Spear corre Rho 0.26 0.28 -0.12	rman's 2lation p value 0.010 0.006 0.250
Transcript vs cis-DCE etnC etnE mmoX pmoA	n 70 71 61 80	mu Coef 0.38 0.30 -0.08 0.05	ltilevel regr p value 0.002 0.003 0.396 0.616	ession 95% Cl 0.16-0.62 0.12-0.48 -0.27-0.10 -0.15-0.26	AIC 259 288 181 274	simp Coef 0.40 0.32 -0.08 -0.03	ble linear re p value p<0.001 0.001 0.377 0.791	gression 95% Cl 0.18-0.61 0.14-0.50 -0.27-0.10 -0.23-0.17	AIC 254 292 189 279	Spear corre Rho 0.26 0.28 -0.12 -0.09	rman's 2lation p value 0.010 0.006 0.250 0.378
Transcript vs cis-DCE etnC etnE mmoX pmoA bvcA	n 70 71 61 80 54	mu Coef 0.38 0.30 -0.08 0.05 0.56	ltilevel regr p value 0.002 0.003 0.396 0.616 0.003	95% Cl 0.16-0.62 0.12-0.48 -0.27-0.10 -0.15-0.26 0.15-0.92	AIC 259 288 181 274 219	simp Coef 0.40 0.32 -0.08 -0.03 0.57	De linear re p value p<0.001 0.001 0.377 0.791 0.001	gression 95% Cl 0.18-0.61 0.14-0.50 -0.27-0.10 -0.23-0.17 0.25-0.89	AIC 254 292 189 279 222	Spear corre Rho 0.26 0.28 -0.12 -0.09 0.28	rman's elation p value 0.010 0.006 0.250 0.378 0.007
Transcript vs cis-DCE etnC etnE mmoX pmoA bvcA vcrA	n 70 71 61 80 54 51	mu Coef 0.38 0.30 -0.08 0.05 0.56 -0.29	ltilevel regr p value 0.002 0.396 0.616 0.003 0.198	ession 95% Cl 0.16-0.62 0.12-0.48 -0.27-0.10 -0.15-0.26 0.15-0.92 -0.77-0.15	AIC 259 288 181 274 219 192	simp Coef 0.40 0.32 -0.08 -0.03 0.57 -0.30	ble linear re p value p<0.001 0.001 0.377 0.791 0.001 0.105	gression 95% Cl 0.18-0.61 0.14-0.50 -0.27-0.10 -0.23-0.17 0.25-0.89 -0.66-0.06	AIC 254 292 189 279 222 200	Spear corre Rho 0.26 0.28 -0.12 -0.09 0.28 0.24	rman's 2lation p value 0.010 0.006 0.250 0.378 0.007 0.019
Transcript vs cis-DCE etnC etnE mmoX pmoA bvcA vcrA mmoX+pmoA	n 70 71 61 80 54 51 84	mu Coef 0.38 0.30 -0.08 0.05 0.56 -0.29 0.04	ltilevel regr p value 0.002 0.396 0.616 0.003 0.198 0.707	ession 95% Cl 0.16-0.62 0.12-0.48 -0.27-0.10 -0.15-0.26 0.15-0.92 -0.77-0.15 -0.15-0.23	AIC 259 288 181 274 219 192 289	simp Coef 0.40 0.32 -0.08 -0.03 0.57 -0.30 -0.05	p value p value p<0.001 0.377 0.791 0.001 0.105 0.648	gression 95% Cl 0.18-0.61 0.14-0.50 -0.27-0.10 -0.23-0.17 0.25-0.89 -0.66-0.06 -0.25-0.15	AIC 254 292 189 279 222 200 297	Spear corre Rho 0.26 0.28 -0.12 -0.09 0.28 0.24 -0.09	rman's elation p value 0.010 0.006 0.250 0.378 0.007 0.019 0.367

Table S6. Regression analysis of functional gene and transcript abundances with *cis*-DCE. p values < 0.05 are in bold

CI: confidence interval

Coef: regression coefficient

Gene vs <i>trans</i> -	n		multileve	l regression			simple linea	ar regression		Spea corre	rman's elation
DCE		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
etnC	95	0.38	0.01	0.15-0.61	282	0.49	p<0.001	0.29-0.70	300	0.43	p<0.001
etnE	95	0.39	0.001	0.17-0.61	282	0.53	p<0.001	0.33-0.74	298	0.47	p<0.001
ттоХ	95	-0.20	0.111	-0.46-0.06	285	-0.29	0.012	-0.520.07	317	-0.27	0.009
ртоА	95	0.03	0.828	-0.24-0.31	322	-0.29	0.036	-0.560.02	353	-0.20	0.048
bvcA	95	0.61	0.004	0.20-1.02	364	0.49	0.003	0.17-0.80	381	0.24	0.021
vcrA	95	0.64	0.011	0.14-1.15	387	0.87	p<0.001	0.49-1.24	415	0.44	p<0.001
ттоХ+ртоА	95	0.03	0.829	-0.24-0.28	309	-0.28	0.034	-0.530.02	341	-0.21	0.041
bvcA+vcrA	95	0.71	0.001	0.34-1.08	375	0.69	p<0.001	0.37-1.01	385	0.36	p<0.001
Transcript vs	n		multileve	l regression			simple linea	ar regression		Spea corre	rman's elation
Transcript vs trans-DCE	n _	Coef	multileve p value	l regression 95% Cl	AIC	Coef	simple linea	ar regression 95% Cl	AIC	Spea corro Rho	rman's elation p value
Transcript vs trans-DCE etnC	n _ 70	Coef 0.48	multileve p value <b>0.005</b>	l regression 95% Cl 0.16-0.81	AIC 260	Coef 0.51	simple linea p value <b>0.001</b>	ar regression 95% Cl 0.23-0.80	AIC 254	Spea corro Rho 0.22	rman's elation p value <b>0.033</b>
Transcript vs trans-DCE etnC etnE	n 70 71	Coef 0.48 0.40	multileve p value 0.005 0.009	l regression 95% Cl 0.16-0.81 0.09-0.70	AIC 260 286	Coef 0.51 0.41	simple linea p value 0.001 0.002	95% CI 0.23-0.80 0.15-0.67	AIC 254 287	Spea corre Rho 0.22 0.34	rman's elation p value 0.033 0.001
Transcript vs trans-DCE etnC etnE mmoX	n 70 71 61	Coef 0.48 0.40 -0.15	multileve p value 0.005 0.009 0.246	l regression 95% Cl 0.16-0.81 0.09-0.70 -0.43-0.11	AIC 260 286 179	Coef 0.51 0.41 -0.07	simple linea p value 0.001 0.002 0.603	95% Cl 0.23-0.80 0.15-0.67 -0.32-0.19	AIC 254 287 190	Spea corre Rho 0.22 0.34 -0.17	rman's elation p value 0.033 0.001 0.102
Transcript vs trans-DCE etnC etnE mmoX pmoA	n 70 71 61 80	Coef 0.48 0.40 -0.15 0.00	multileve p value 0.005 0.009 0.246 0.980	l regression 95% Cl 0.16-0.81 0.09-0.70 -0.43-0.11 -0.25-0.28	AIC 260 286 179 273	Coef 0.51 0.41 -0.07 -0.17	simple linea p value 0.001 0.603 0.211	95% Cl 0.23-0.80 0.15-0.67 -0.32-0.19 -0.43-0.10	AIC 254 287 190 277	Spea corre Rho 0.22 0.34 -0.17 -0.25	rman's elation p value 0.033 0.001 0.102 0.013
Transcript vs trans-DCE etnC etnE mmoX pmoA bvcA	n 70 71 61 80 54	Coef 0.48 0.40 -0.15 0.00 0.80	multileve p value 0.005 0.246 0.980 0.003	l regression 95% Cl 0.16-0.81 0.09-0.70 -0.43-0.11 -0.25-0.28 0.30-1.31	AIC 260 286 179 273 220	Coef 0.51 0.41 -0.07 -0.17 0.56	simple linea p value 0.001 0.002 0.603 0.211 0.012	95% Cl 0.23-0.80 0.15-0.67 -0.32-0.19 -0.43-0.10 0.13-0.99	AIC 254 287 190 277 227	Spea corre Rho 0.22 0.34 -0.17 -0.25 0.24	rman's elation p value 0.033 0.001 0.102 0.013 0.017
Transcript vs trans-DCE etnC etnE mmoX pmoA bvcA vcrA	n 70 71 61 80 54 51	Coef 0.48 0.40 -0.15 0.00 0.80 0.06	multileve p value 0.005 0.009 0.246 0.980 0.003 0.835	l regression 95% Cl 0.16-0.81 0.09-0.70 -0.43-0.11 -0.25-0.28 0.30-1.31 -0.54-0.65	AIC 260 286 179 273 220 193	Coef 0.51 0.41 -0.07 -0.17 0.56 0.07	simple lines p value 0.001 0.603 0.211 0.012 0.768	95% Cl 0.23-0.80 0.15-0.67 -0.32-0.19 -0.43-0.10 0.13-0.99 -0.44-0.57	AIC 254 287 190 277 227 203	Spea corro Rho 0.22 0.34 -0.17 -0.25 0.24 0.38	rman's elation p value 0.033 0.001 0.102 0.013 0.017 p<0.001
Transcript vs trans-DCE etnC etnE mmoX pmoA bvcA vcrA mmoX+pmoA	n 70 71 61 80 54 51 84	Coef 0.48 0.40 -0.15 0.00 0.80 0.06 -0.04	multileve p value 0.005 0.009 0.246 0.980 0.003 0.835 0.806	95% Cl 0.16-0.81 0.09-0.70 -0.43-0.11 -0.25-0.28 0.30-1.31 -0.54-0.65 -0.34-0.25	AIC 260 286 179 273 220 193 288	Coef 0.51 0.41 -0.07 -0.17 0.56 0.07 -0.21	simple linea p value 0.001 0.603 0.211 0.012 0.768 0.118	95% Cl 0.23-0.80 0.15-0.67 -0.32-0.19 -0.43-0.10 0.13-0.99 -0.44-0.57 -0.47-0.05	AIC 254 287 190 277 227 203 295	Spea corro Rho 0.22 0.34 -0.17 -0.25 0.24 0.38 -0.25	rman's elation p value 0.033 0.001 0.102 0.013 0.017 p<0.001 0.013

Table S7. Regression analysis of functional gene and transcript abundances with trans-DCE. p values < 0.05 are in bold

CI: confidence interval

Coef: regression coefficient

Gene vs TCE	n		multilevel re	egression			simple line	ar regression		Spea corre	rman's elation
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
etnC	95	0.21	0.009	0.07-0.38	287	0.33	p<0.001	0.16-0.49	307	0.34	0.001
etnE	95	0.19	0.017	0.04-0.33	288	0.30	0.001	0.13-0.47	311	0.35	0.001
ттоХ	95	-0.07	0.179	-0.24-0.11	288	-0.22	0.015	-0.400.04	318	-0.19	0.067
ртоА	95	0.03	0.725	-0.18-0.22	323	-0.18	0.109	-0.39-0.04	355	-0.11	0.279
bvcA	95	0.41	0.005	0.13-0.67	365	0.37	0.004	0.12-0.62	381	0.30	0.003
vcrA	95	0.35	0.029	0.03-0.67	389	0.54	0.001	0.23-0.85	423	0.36	p<0.001
ттоХ+ртоА	95	0.04	0.669	-0.14-0.21	310	-0.16	0.119	-0.36-0.04	343	-0.12	0.261
bvcA+vcrA	95	0.45	0.004	0.15-0.79	379	0.50	p<0.001	0.24-0.76	388	0.38	p<0.001
Transcript vs	n		multilevel re	egression			simple line	ar regression		Spea corre	rman's elation
Transcript vs TCE	n _	Coef	multilevel ro	egression 95% Cl	AIC	Coef	simple line p value	ar regression 95% Cl	AIC	Spea corre Rho	rman's elation p value
Transcript vs TCE etnC	n	Coef 0.38	multilevel ro p value 0.003	egression 95% Cl 0.16-0.62	AIC 261	Coef 0.39	simple line p value 0.001	ar regression 95% Cl 0.18-0.61	AIC 254	Spea corre Rho 0.30	rman's elation p value <b>0.003</b>
Transcript vs TCE etnC etnE	n	Coef 0.38 0.28	multilevel ro p value 0.003 0.011	egression 95% Cl 0.16-0.62 0.05-0.49	AIC 261 288	Coef 0.39 0.33	simple line p value 0.001 0.001	ar regression 95% Cl 0.18-0.61 0.14-0.52	AIC 254 292	Spea corre Rho 0.30 0.36	rman's elation p value 0.003 p<0.001
Transcript vs TCE <i>etnC</i> <i>etnE</i> <i>mmoX</i>	n _ 70 71 61	Coef 0.38 0.28 -0.14	multilevel re p value 0.003 0.011 0.179	egression 95% Cl 0.16-0.62 0.05-0.49 -0.35-0.05	AIC 261 288 179	Coef 0.39 0.33 -0.10	simple line p value 0.001 0.001 0.312	ar regression 95% Cl 0.18-0.61 0.14-0.52 -0.30-0.10	AIC 254 292 189	Spea corre Rho 0.30 0.36 -0.15	rman's elation p value 0.003 p<0.001 0.155
Transcript vs TCE <i>etnC</i> <i>etnE</i> <i>mmoX</i> <i>pmoA</i>	n	Coef 0.38 0.28 -0.14 0.04	multilevel ro p value 0.003 0.011 0.179 0.725	egression 95% Cl 0.16-0.62 0.05-0.49 -0.35-0.05 -0.17-0.26	AIC 261 288 179 273	Coef 0.39 0.33 -0.10 -0.06	simple line p value 0.001 0.312 0.613	ar regression 95% Cl 0.18-0.61 0.14-0.52 -0.30-0.10 -0.29-0.17	AIC 254 292 189 278	Spea corre Rho 0.30 0.36 -0.15 -0.11	rman's elation p value 0.003 p<0.001 0.155 0.307
Transcript vs TCE <i>etnC</i> <i>etnE</i> <i>mmoX</i> <i>pmoA</i> <i>bvcA</i>	n 70 71 61 80 54	Coef 0.38 0.28 -0.14 0.04 0.76	multilevel ro p value 0.003 0.011 0.179 0.725 p<0.001	egression 95% Cl 0.16-0.62 0.05-0.49 -0.35-0.05 -0.17-0.26 0.36-1.11	AIC 261 288 179 273 216	Coef 0.39 0.33 -0.10 -0.06 0.45	simple line p value 0.001 0.312 0.613 0.009	ar regression 95% Cl 0.18-0.61 0.14-0.52 -0.30-0.10 -0.29-0.17 0.12-0.78	AIC 254 292 189 278 226	Spea corre Rho 0.30 0.36 -0.15 -0.11 0.25	rman's elation p value 0.003 p<0.001 0.155 0.307 0.016
Transcript vs TCE etnC etnE mmoX pmoA bvcA vcrA	n	Coef 0.38 0.28 -0.14 0.04 0.76 0.02	multilevel ro p value 0.003 0.011 0.179 0.725 p<0.001 0.921	egression 95% Cl 0.16-0.62 0.05-0.49 -0.35-0.05 -0.17-0.26 0.36-1.11 -0.35-0.38	AIC 261 288 179 273 216 195	Coef 0.39 0.33 -0.10 -0.06 0.45 0.05	simple line p value 0.001 0.312 0.613 0.009 0.743	ar regression 95% Cl 0.18-0.61 0.14-0.52 -0.30-0.10 -0.29-0.17 0.12-0.78 -0.24-0.34	AIC 254 292 189 278 226 203	Spea corre Rho 0.30 0.36 -0.15 -0.11 0.25 0.28	rman's elation p value 0.003 p<0.001 0.155 0.307 0.016 0.006
Transcript vs TCE <i>etnC</i> <i>etnE</i> <i>mmoX</i> <i>pmoA</i> <i>bvcA</i> <i>vcrA</i> <i>mmoX+pmoA</i>	n	Coef 0.38 0.28 -0.14 0.04 0.76 0.02 -0.02	multilevel ro p value 0.003 0.011 0.179 0.725 p<0.001 0.921 0.861	egression 95% Cl 0.16-0.62 0.05-0.49 -0.35-0.05 -0.17-0.26 0.36-1.11 -0.35-0.38 -0.23-0.21	AIC 261 288 179 273 216 195 289	Coef 0.39 0.33 -0.10 -0.06 0.45 0.05 -0.14	simple line p value 0.001 0.312 0.613 0.009 0.743 0.175	ar regression 95% Cl 0.18-0.61 0.14-0.52 -0.30-0.10 -0.29-0.17 0.12-0.78 -0.24-0.34 -0.35-0.07	AIC 254 292 189 278 226 203 296	Spea corre Rho 0.30 0.36 -0.15 -0.11 0.25 0.28 -0.10	rman's elation p value 0.003 p<0.001 0.155 0.307 0.016 0.006 0.341

Table S8. Regression analysis of functional gene and transcript abundances with TCE. p values < 0.05 are in bold

CI: confidence interval

Coef: regression coefficient

Gene vs	n		multilevel	regression		:	simple linea	r regression		Spea corre	rman's elation
ethene		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
etnC	65	0.53	0.009	0.13-0.92	190	0.59	0.001	0.24-0.94	188	0.35	0.004
etnE	65	0.46	0.028	0.04-0.85	194	0.53	0.004	0.17-0.88	190	0.28	0.024
ттоХ	65	-0.05	0.813	-0.50-0.39	198	0.02	0.935	-0.40-0.43	209	-0.01	0.963
ртоА	65	-0.13	0.590	-0.61-0.35	205	-0.08	0.705	-0.47-0.32	204	0.02	0.871
bvcA	65	0.87	0.014	0.21-1.55	240	1.19	p<0.001	0.62-1.75	250	0.37	0.003
vcrA	65	0.83	0.020	0.10-1.58	239	0.93	0.010	0.23-1.62	278	0.17	0.171
ттоХ+ртоА	65	-0.14	0.512	-0.60-0.30	198	-0.09	0.620	-0.47-0.28	196	0.01	0.912
bvcA+vcrA	65	0.96	0.007	0.34-1.65	241	1.19	p<0.001	0.62-1.76	252	0.39	0.002
Transcript vs	n		multilevel	regression		:	simple linea	r regression		Spea corre	rman's elation
Transcript vs ethene	n	Coef	multilevel p value	regression 95% Cl	AIC	Coef	simple linea	r regression 95% Cl	AIC	Spea corre Rho	rman's elation p value
Transcript vs ethene <i>etnC</i>	n 45	Coef 0.85	multilevel p value <b>0.008</b>	regression 95% Cl 0.30-1.37	AIC 173	Coef 0.86	simple linea p value <b>0.005</b>	95% Cl 0.28-1.44	AIC 167	Spear corre Rho 0.32	rman's elation p value <b>0.010</b>
Transcript vs ethene <i>etnC</i> <i>etnE</i>	n 45 51	Coef 0.85 0.63	multilevel p value 0.008 0.017	regression 95% Cl 0.30-1.37 0.13-1.11	AIC 173 178	Coef 0.86 0.63	simple linea p value 0.005 0.012	95% Cl 0.28-1.44 0.15-1.11	AIC 167 172	Spea corre Rho 0.32 0.24	rman's elation p value <b>0.010</b> 0.055
Transcript vs ethene <i>etnC</i> <i>etnE</i> <i>mmoX</i>	n 45 51 51	Coef 0.85 0.63 0.16	multilevel p value 0.008 0.017 0.433	regression 95% Cl 0.30-1.37 0.13-1.11 -0.26-0.57	AIC 173 178 148	Coef 0.86 0.63 0.25	simple linea p value 0.005 0.012 0.236	95% Cl 0.28-1.44 0.15-1.11 -0.17-0.67	AIC 167 172 146	Spea corre Rho 0.32 0.24 0.18	rman's elation p value 0.010 0.055 0.163
Transcript vs ethene etnC etnE mmoX pmoA	n 45 51 51 59	Coef 0.85 0.63 0.16 -0.09	multilevel p value 0.008 0.017 0.433 0.711	regression 95% Cl 0.30-1.37 0.13-1.11 -0.26-0.57 -0.58-0.39	AIC 173 178 148 191	Coef 0.86 0.63 0.25 -0.09	simple linea p value 0.005 0.012 0.236 0.712	95% Cl 0.28-1.44 0.15-1.11 -0.17-0.67 -0.56-0.38	AIC 167 172 146 189	Spear corre Rho 0.32 0.24 0.18 0.05	rman's elation p value 0.010 0.055 0.163 0.691
Transcript vs ethene etnC etnE mmoX pmoA bvcA	n 45 51 51 59 42	Coef 0.85 0.63 0.16 -0.09 1.45	multilevel p value 0.008 0.017 0.433 0.711 p<0.001	regression 95% Cl 0.30-1.37 0.13-1.11 -0.26-0.57 -0.58-0.39 0.77-2.10	AIC 173 178 148 191 165	Coef 0.86 0.63 0.25 -0.09 1.53	simple linea p value 0.005 0.012 0.236 0.712 p<0.001	95% Cl 0.28-1.44 0.15-1.11 -0.17-0.67 -0.56-0.38 0.92-2.13	AIC 167 172 146 189 160	Spear corre Rho 0.32 0.24 0.18 0.05 0.31	rman's elation p value 0.010 0.055 0.163 0.691 0.012
Transcript vs ethene etnC etnE mmoX pmoA bvcA vcrA	n 45 51 51 59 42 36	Coef 0.85 0.63 0.16 -0.09 1.45 0.44	multilevel p value 0.008 0.017 0.433 0.711 p<0.001 0.163	regression 95% Cl 0.30-1.37 0.13-1.11 -0.26-0.57 -0.58-0.39 0.77-2.10 -0.12-1.05	AIC 173 178 148 191 165 121	Coef 0.86 0.63 0.25 -0.09 1.53 0.49	simple linea p value 0.005 0.012 0.236 0.712 p<0.001 0.115	95% Cl 0.28-1.44 0.15-1.11 -0.17-0.67 -0.56-0.38 0.92-2.13 -0.13-1.10	AIC 167 172 146 189 160 135	Spear corre Rho 0.32 0.24 0.18 0.05 0.31 0.22	rman's elation p value 0.010 0.055 0.163 0.691 0.012 0.077
Transcript vs ethene etnC etnE mmoX pmoA bvcA vcrA mmoX+pmoA	n 51 51 59 42 36 61	Coef 0.85 0.63 0.16 -0.09 1.45 0.44 -0.04	multilevel p value 0.008 0.017 0.433 0.711 p<0.001 0.163 0.864	regression 95% Cl 0.30-1.37 0.13-1.11 -0.26-0.57 -0.58-0.39 0.77-2.10 -0.12-1.05 -0.56-0.41	AIC 173 178 148 191 165 121 199	Coef 0.86 0.63 0.25 -0.09 1.53 0.49 -0.01	simple linea p value 0.005 0.236 0.712 p<0.001 0.115 0.970	95% Cl 0.28-1.44 0.15-1.11 -0.17-0.67 -0.56-0.38 0.92-2.13 -0.13-1.10 -0.49-0.47	AIC 167 172 146 189 160 135 199	Spear corre Rho 0.32 0.24 0.18 0.05 0.31 0.22 0.04	rman's elation p value 0.055 0.163 0.691 0.012 0.077 0.756

Table S9. Regression analysis of functional gene and transcript abundances with ethene. p values < 0.05 are in bold

CI: confidence interval

Coef: regression coefficient

Gene vs	n		multileve	regression			simple linea	r regression		Spea corre	rman's elation
methane		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
etnC	74	0.30	0.087	-0.07-0.63	222	-0.01	0.966	-0.29-0.28	234	-0.09	0.449
etnE	74	0.30	0.09	-0.05-0.62	224	0.07	0.638	-0.22-0.35	231	-0.07	0.581
ттоХ	74	0.52	0.004	-0.15-0.85	219	0.62	p<0.001	-0.35-0.90	228	0.49	p<0.001
ртоА	74	0.51	0.007	-0.12-0.86	232	0.46	0.002	-0.18-0.74	230	0.31	0.007
bvcA	74	0.43	0.118	-0.11-1.04	269	0.50	0.020	-0.08-0.93	291	0.33	0.004
vcrA	74	1.00	0.001	0.37-1.57	286	1.13	p<0.001	0.64-1.61	311	0.48	p<0.001
ттоХ+ртоА	74	0.48	0.006	-0.14-0.79	224	0.44	0.002	-0.17-0.70	222	0.33	0.005
bvcA+vcrA	74	0.76	0.005	-0.23-1.31	283	0.79	p<0.001	-0.37-1.20	290	0.46	p<0.001
Transcript vs	n		multileve	regression			simple linea	r regression		Spea corre	rman's elation
Transcript vs methane	n	Coef	multileve p value	regression 95% Cl	AIC	Coef	simple linea p value	r regression 95% Cl	AIC	Spea corre Rho	rman's elation p value
Transcript vs methane <i>etnC</i>	n 54	Coef 0.36	multileve p value 0.204	l regression 95% Cl -0.19-0.98	AIC 213	Coef 0.18	simple linea p value 0.480	95% Cl -0.32-0.67	AIC 213	Spea corre Rho -0.06	rman's elation p value 0.606
Transcript vs methane <i>etnC</i> <i>etnE</i>	n 54 59	Coef 0.36 0.16	multileve p value 0.204 0.470	l regression 95% Cl -0.19-0.98 -0.31-0.66	AIC 213 211	Coef 0.18 -0.07	simple linea p value 0.480 0.708	95% Cl -0.32-0.67 -0.47-0.32	AIC 213 214	Spea corre Rho -0.06 -0.10	rman's elation p value 0.606 0.385
Transcript vs methane <i>etnC</i> <i>etnE</i> <i>mmoX</i>	n 54 59 57	Coef 0.36 0.16 0.36	multilevel p value 0.204 0.470 <b>0.021</b>	95% Cl -0.19-0.98 -0.31-0.66 -0.07-0.63	AIC 213 211 160	Coef 0.18 -0.07 0.54	simple linea p value 0.480 0.708 p<0.001	95% Cl -0.32-0.67 -0.47-0.32 -0.27-0.82	AIC 213 214 163	Spea corre Rho -0.06 -0.10 0.37	rman's elation p value 0.606 0.385 <b>0.001</b>
Transcript vs methane <i>etnC</i> <i>etnE</i> <i>mmoX</i> <i>pmoA</i>	n 54 59 57 68	Coef 0.36 0.16 0.36 0.51	multilevel p value 0.204 0.470 0.021 0.011	95% Cl -0.19-0.98 -0.31-0.66 -0.07-0.63 -0.15-0.88	AIC 213 211 160 219	Coef 0.18 -0.07 0.54 0.51	simple linea p value 0.480 0.708 p<0.001 0.003	95% Cl -0.32-0.67 -0.47-0.32 -0.27-0.82 -0.17-0.85	AIC 213 214 163 226	Spea corre Rho -0.06 -0.10 0.37 0.30	rman's elation p value 0.606 0.385 0.001 0.008
Transcript vs methane <i>etnC</i> <i>etnE</i> <i>mmoX</i> <i>pmoA</i> <i>bvcA</i>	n 54 59 57 68 46	Coef 0.36 0.16 0.36 0.51 0.75	multilevel p value 0.204 0.470 <b>0.021</b> 0.011 0.068	95% Cl -0.19-0.98 -0.31-0.66 -0.07-0.63 -0.15-0.88 -0.08-1.53	AIC 213 211 160 219 192	Coef 0.18 -0.07 0.54 0.51 0.52	simple linea p value 0.480 0.708 p<0.001 0.003 0.126	95% Cl -0.32-0.67 -0.47-0.32 -0.27-0.82 -0.17-0.85 -0.15-1.20	AIC 213 214 163 226 198	Spea corre Rho -0.06 -0.10 0.37 0.30 0.22	rman's elation p value 0.606 0.385 0.001 0.008 0.065
Transcript vs methane <i>etnC</i> <i>etnE</i> <i>mmoX</i> <i>pmoA</i> <i>bvcA</i> <i>vcrA</i>	n 54 59 57 68 46 38	Coef 0.36 0.16 0.36 0.51 0.75 1.37	multilevel p value 0.204 0.470 0.021 0.011 0.068 0.001	95% Cl -0.19-0.98 -0.31-0.66 -0.07-0.63 -0.15-0.88 -0.08-1.53 -0.63-2.11	AIC 213 211 160 219 192 132	Coef 0.18 -0.07 0.54 0.51 0.52 1.17	simple linea p value 0.480 0.708 p<0.001 0.003 0.126 p<0.001	95% Cl -0.32-0.67 -0.47-0.32 -0.27-0.82 -0.17-0.85 -0.15-1.20 -0.57-1.77	AIC 213 214 163 226 198 132	Spea corre Rho -0.06 -0.10 0.37 0.30 0.22 0.57	rman's elation p value 0.606 0.385 0.001 0.008 0.065 p<0.001
Transcript vs methane <i>etnC</i> <i>etnE</i> <i>mmoX</i> <i>pmoA</i> <i>bvcA</i> <i>vcrA</i> <i>mmoX+pmoA</i>	n 54 59 57 68 46 38 70	Coef 0.36 0.16 0.36 0.51 0.75 1.37 0.58	multilevel p value 0.204 0.470 0.021 0.011 0.068 0.001 0.002	95% Cl -0.19-0.98 -0.31-0.66 -0.07-0.63 -0.15-0.88 -0.08-1.53 -0.63-2.11 -0.17-0.92	AIC 213 211 160 219 192 132 224	Coef 0.18 -0.07 0.54 0.51 0.52 1.17 0.60	simple linea p value 0.480 0.708 p<0.001 0.003 0.126 p<0.001 p<0.001	95% Cl -0.32-0.67 -0.47-0.32 -0.27-0.82 -0.17-0.85 -0.15-1.20 -0.57-1.77 -0.30-0.91	AIC 213 214 163 226 198 132 230	Spea corre Rho -0.06 -0.10 0.37 0.30 0.22 0.57 0.34	rman's elation p value 0.606 0.385 0.001 0.008 0.065 p<0.001 0.004

Table S10. Regression analysis of functional gene and transcript abundances with methane. p values < 0.05 are in bold

CI: confidence interval

Coef: regression coefficient

Site	Remediation approach	Time	Bulk VC attenuation rate, yr <sup>-1</sup>	R <sup>2</sup>	p Value	Rate Category
	Oxygen releasing	2013.11	0.0006	0.972	0.518	Low
VA Sile 2C	injection	2014.07	-0.0009	0.472	0.108	Low
GA Site 11	Pump and treat; in situ chemical oxidation	2015.01	0.0037	0.003	0.950	Low
CA Site 70	Enhanced anaerobic	2015.07	0.0849	0.909	0.012	High
	bioremediation with biobarriers	2015.12	0.0958	0.891	0.006	High
OK Site SS-17	Mulch biowalls	2015.05	0.1210	0.942	0.030	High
HI Site LF05	Enhanced anaerobic bioremediation - bioreactor	2015.04	0.1057	0.969	0.016	High
SC Site 45*	Enhanced	2015.10	0.4350	0.932	0.008	High
aquifer)	anaerobic bioremediation –	2016.07	0.3752	0.879	0.019	High
SC Site 45* (north plume upper	vegetable oil injection	2016.07	1.0858	0.344	0.299	High
aquifer)		2015.10	1.0409	0.23	0.414	High
SC Site 45*	In situ chemical	2015.10	0.5182	0.764	0.126	High
aquifer)	oxidation	2016.07	0.2836	0.840	0.028	High

Table S11. Bulk VC attenuation rate estimate and rate categories. p values < 0.05 are in bold. Low rate: bulk VC attenuation rates < 0.005 per year. High rates: bulk VC attenuation rate >0.08 per year.

\*At SC site 45, a northern and southern VC plume exist within a surficial aquifer which has upper and lower hydrogeologic units. Bulk VC attenuation rates were estimated in each of these cases.



Figure S4. Residuals of multilevel regression (mlr) and simple linear regression (slr) analyses of VC concentration vs. *etnC* and *etnE* gene and transcript abundance.



Figure S5. Residuals of multilevel regression (mlr) and simple linear regression (slr) analyses of VC concentration vs. *mmoX* and *pmoA* gene and transcript abundance.



Figure S6. Residuals of multilevel regression (mlr) and simple linear regression (slr) analyses of VC concentration vs. *bvcA* and *vcrA* gene and transcript abundance.

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