

**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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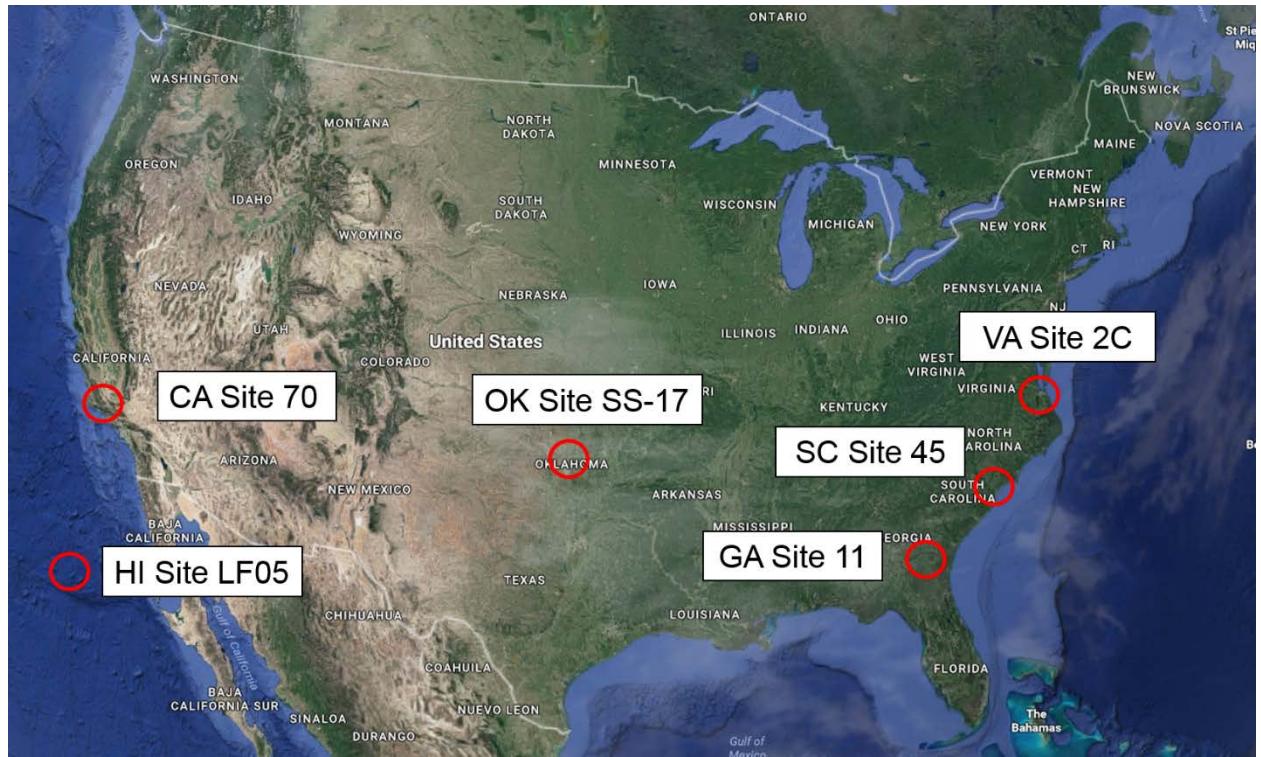


Figure S1. General location of the six VC-contaminated sites used in this study.

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 ground water surface)

Site	Ground water table (ft bgs)	Well screened interval (ft bgs)	pH		Temp (°C)		DO (mg/L)		ORP (mV)	
			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):

$$\frac{\text{genes}}{\text{L GW}} = \frac{\text{genes per qPCR}}{\text{ng DNA used for qPCR}} \times \frac{\text{ng DNA after extraction}}{\text{volume of groundwater}}$$

Equation used for transcript abundance calculation per liter of GW:

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

### 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:

$R$  = retardation factor

$\rho_b$  = bulk density

$K_{oc}$ = soil adsorption coefficient for soil organic carbon content

$f_{oc}$ = fraction soil organic carbon

$n$  = total porosity

$K_{oc}$  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 ( $\rho_b$ ,  $f_{oc}$ , 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.

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

Target gene	Primer name	Sequences (5'->3')	Expected product size	Ref.
16S rRNA	515f	GTCGCCAGCMGCCGCGGTAA	291 bp	5
	806r	GGACTACHVGGGTWTCTAAT		
luciferase mRNA	ref-STF	CCAGGGATTCAGTCGATGT	1014 bp	6
	ref-STR	TTTTCGTCATCGTCTTCC		
luciferase mRNA	ref-f	TACAAACACCCCCAACATCTTCGA	150 bp	7
	ref-r	GGAAGTTCACCGGCGTCAT		
<i>etnC</i>	JS614- <i>etnCF</i>	GCGATGGAGAATGAGAAGGA	1138 bp	1
	JS614- <i>etnCR</i>	TCCAGTCACAACCCTCACTG		
<i>etnC</i>	RTC-f	ACCCCTGGTCGGTGTKSTYTC	106 bp	1
	RTC-r	TCATGTAMGAGCCGACGAAGTC		
<i>etnE</i>	CoM-F1L	AACTACCCSAAYCCSCGCTGGTACGAC	891 bp	8
	CoM-R2E	GTCGGCAGTTCGGTGATCGTGTCTTGAC		
<i>etnE</i>	RTE-f	CAGAAYGGCTGYGACATYATCCA	151 bp	1
	RTE-r	CSGGYGTRCCCGAGTAGTTWCC		
<i>mmoX</i>	mmoX-std2f	AGGCAGTCAAGGACGAAAGG	1123 bp	9
	mmoX-std2r	ATCTGGCCGTTGTACTCGTG		
<i>mmoX</i>	mmoX536f	CGCTGTGGAAGGGCATGAAGCG	362 bp	10
	mmoX898r	GCTCGACCTTGAACTTGGAGCC		
<i>pmoA</i>	pmoA-std1f	TCGGTCCGTTCAACTCCG	703 bp	9
	pmoA-std1r	GAATACCAACGGCCCATGAA		
<i>pmoA</i>	pmoA472-A189f	GGNGACTGGGACTTCTGG	472 bp	11
	pmoA472-mb661r	CCGGMGCAACGTCYTTACC		
<i>bvcA</i>	Bvc925F	AAAAGCACTTGGCTATCAAGGAC	92 bp	2
	Bvc1017R	CCAAAAGCACCACCAAGGTC		
<i>vcrA</i>	Vcr1022F	CGGGCGGATGCACTATTTC	71 bp	2
	Vcr1093R	GAATAGTCCGTGCCCTTCCTC		

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

Target gene	luciferase mRNA	<i>etnC</i>	<i>etnE</i>	<i>mmoX</i>	<i>pmoA</i>	<i>bvcA</i>	<i>vcrA</i>
primer conc, $\mu\text{M}$	0.1	0.8	0.8	0.3	0.3	0.3	0.3
template	2 $\mu\text{l}$ cDNA	10 ng DNA or 2 $\mu\text{l}$ cDNA	10 ng DNA or 2 $\mu\text{l}$ cDNA	10 ng DNA or 2 $\mu\text{l}$ cDNA	10 ng DNA or 2 $\mu\text{l}$ cDNA	10 ng DNA or 2 $\mu\text{l}$ cDNA	10 ng DNA or 2 $\mu\text{l}$ cDNA
linear range, gene copies/reaction	$30 - 30 \times 10^7$	$30 - 30 \times 10^7$	$30 - 30 \times 10^6$				
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
$R^2$	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

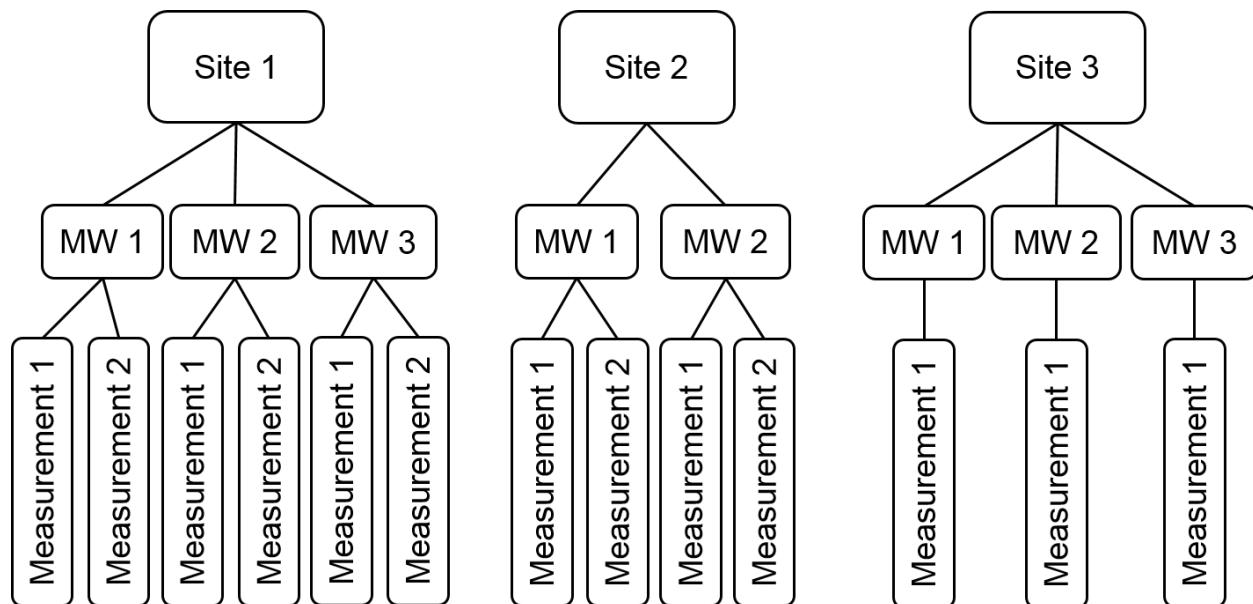


Figure S2. A general illustration of the hierarchical data structure in this study. Each site contains multiple monitoring wells (MW1, MW2, MW3...), and samples from each monitoring well could be analyzed at different sampling events (Measurement1, Measurement2...).

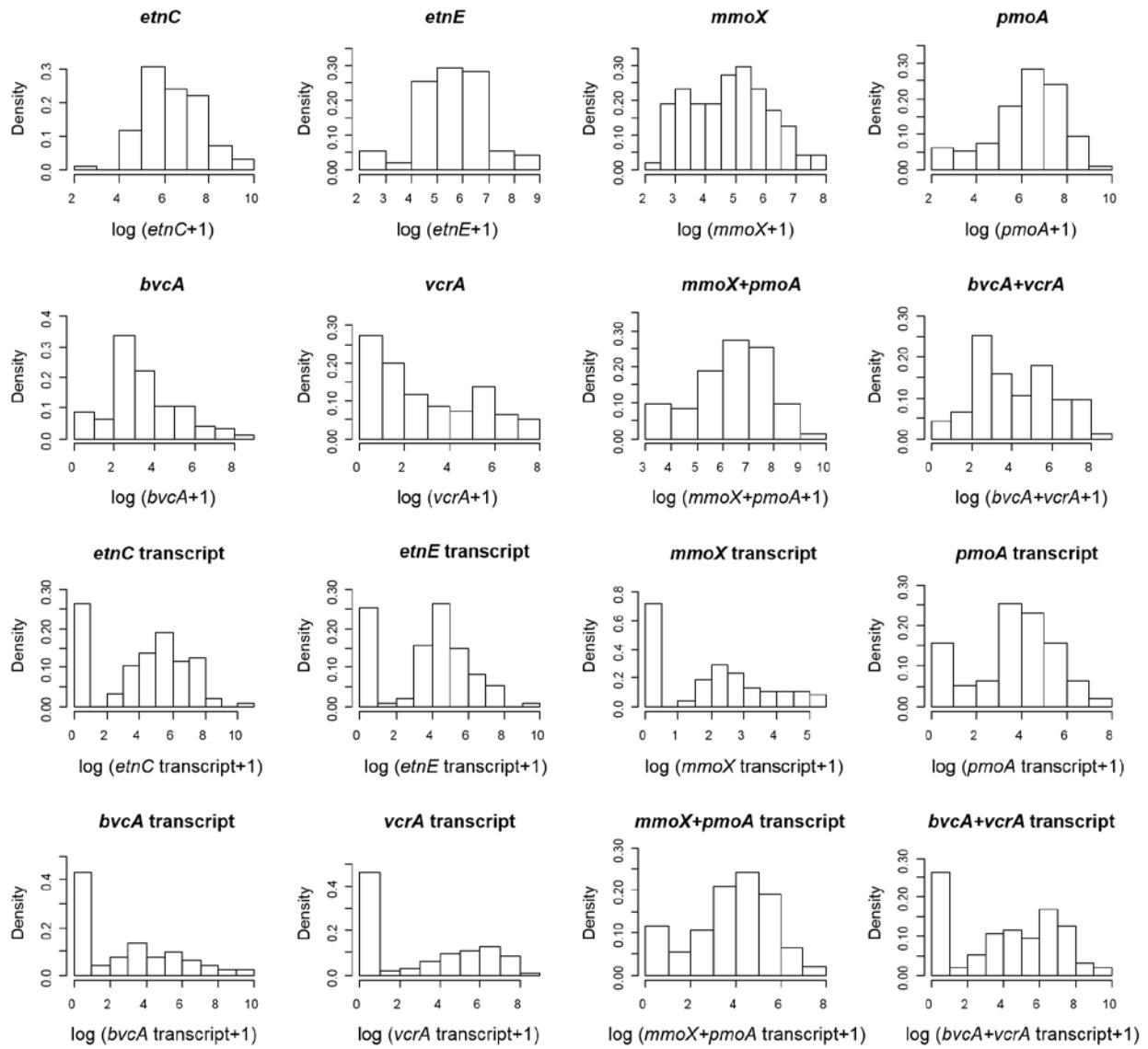


Figure S3. Histogram of gene and transcript abundance after log transformation.

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	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i> vs meth	95	0.41	<b>p&lt;0.001</b>	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	<b>p&lt;0.001</b>	0.20-0.39	263	0.32	<b>p&lt;0.001</b>	0.21-0.44	294	0.46	<b>p&lt;0.001</b>
meth vs dhc	95	0.20	<b>0.0015</b>	0.08-0.32	300	0.15	0.0544	0.00-0.30	341	0.22	0.0331

Transcript	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i> 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	<b>0.0249</b>	0.02-0.39	202	0.21	<b>0.0283</b>	0.02-0.40	197	0.29	<b>0.0320</b>
meth vs dhc	63	0.13	0.1084	-0.05-0.28	221	0.11	0.2264	-0.07-0.30	224	0.10	0.4196

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 others	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>cis</i> -DCE	95	0.48	<b>p&lt;0.001</b>	0.34-0.63	227	0.74	<b>p&lt;0.001</b>	0.59-0.89	287	0.77	<b>p&lt;0.001</b>
<i>trans</i> -DCE	95	0.45	<b>p&lt;0.001</b>	0.35-0.54	165	0.60	<b>p&lt;0.001</b>	0.50-0.70	215	0.72	<b>p&lt;0.001</b>
VC	95	0.13	0.070	-0.01-0.28	226	0.54	<b>p&lt;0.001</b>	0.37-0.71	309	0.59	<b>p&lt;0.001</b>
ethene	65	0.24	<b>p&lt;0.001</b>	0.10-0.37	94	0.31	<b>p&lt;0.001</b>	0.19-0.44	124	0.40	<b>p&lt;0.001</b>
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

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

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

Gene vs <i>cis</i> -DCE	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i>	95	0.25	<b>0.002</b>	0.10-0.38	283	0.28	<b>0.001</b>	0.11-0.44	310	0.34	<b>0.001</b>
<i>etnE</i>	95	0.24	<b>0.002</b>	0.09-0.37	284	0.28	<b>0.001</b>	0.11-0.44	312	0.35	<b>p&lt;0.001</b>
<i>mmoX</i>	95	-0.13	0.146	-0.28-0.05	287	-0.19	<b>0.031</b>	-0.36--0.02	319	-0.23	<b>0.027</b>
<i>pmoA</i>	95	0.01	0.912	-0.15-0.22	323	-0.07	0.506	-0.28-0.14	357	-0.08	0.441
<i>bvcA</i>	95	0.36	<b>0.021</b>	0.07-0.63	367	0.34	<b>0.006</b>	0.10-0.58	382	0.25	<b>0.016</b>
<i>vcrA</i>	95	0.31	0.081	0.08-0.66	390	0.43	<b>0.005</b>	0.13-0.73	427	0.30	<b>0.003</b>
<i>mmoX+pmoA</i>	95	0.02	0.858	-0.16-0.18	310	-0.06	0.514	-0.26-0.13	345	-0.08	0.419
<i>bvcA+vcrA</i>	95	0.38	<b>0.024</b>	0.03-0.68	382	0.38	<b>0.003</b>	0.13-0.63	393	0.30	<b>0.003</b>
Transcript vs <i>cis</i> -DCE	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i>	70	0.38	<b>0.002</b>	0.16-0.62	259	0.40	<b>p&lt;0.001</b>	0.18-0.61	254	0.26	<b>0.010</b>
<i>etnE</i>	71	0.30	<b>0.003</b>	0.12-0.48	288	0.32	<b>0.001</b>	0.14-0.50	292	0.28	<b>0.006</b>
<i>mmoX</i>	61	-0.08	0.396	-0.27-0.10	181	-0.08	0.377	-0.27-0.10	189	-0.12	0.250
<i>pmoA</i>	80	0.05	0.616	-0.15-0.26	274	-0.03	0.791	-0.23-0.17	279	-0.09	0.378
<i>bvcA</i>	54	0.56	<b>0.003</b>	0.15-0.92	219	0.57	<b>0.001</b>	0.25-0.89	222	0.28	<b>0.007</b>
<i>vcrA</i>	51	-0.29	0.198	-0.77-0.15	192	-0.30	0.105	-0.66-0.06	200	0.24	<b>0.019</b>
<i>mmoX+pmoA</i>	84	0.04	0.707	-0.15-0.23	289	-0.05	0.648	-0.25-0.15	297	-0.09	0.367
<i>bvcA+vcrA</i>	70	0.35	0.054	-0.04-0.67	283	0.33	<b>0.027</b>	0.04-0.63	288	0.29	<b>0.004</b>

n: number of samples in the analysis

CI: confidence interval

Coef: regression coefficient

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

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

Gene vs <i>trans</i> -DCE	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i>	95	0.38	<b>0.01</b>	0.15-0.61	282	0.49	<b>p&lt;0.001</b>	0.29-0.70	300	0.43	<b>p&lt;0.001</b>
<i>etnE</i>	95	0.39	<b>0.001</b>	0.17-0.61	282	0.53	<b>p&lt;0.001</b>	0.33-0.74	298	0.47	<b>p&lt;0.001</b>
<i>mmoX</i>	95	-0.20	0.111	-0.46-0.06	285	-0.29	<b>0.012</b>	-0.52--0.07	317	-0.27	<b>0.009</b>
<i>pmoA</i>	95	0.03	0.828	-0.24-0.31	322	-0.29	<b>0.036</b>	-0.56--0.02	353	-0.20	<b>0.048</b>
<i>bvcA</i>	95	0.61	<b>0.004</b>	0.20-1.02	364	0.49	<b>0.003</b>	0.17-0.80	381	0.24	<b>0.021</b>
<i>vcrA</i>	95	0.64	<b>0.011</b>	0.14-1.15	387	0.87	<b>p&lt;0.001</b>	0.49-1.24	415	0.44	<b>p&lt;0.001</b>
<i>mmoX+pmoA</i>	95	0.03	0.829	-0.24-0.28	309	-0.28	<b>0.034</b>	-0.53--0.02	341	-0.21	<b>0.041</b>
<i>bvcA+vcrA</i>	95	0.71	<b>0.001</b>	0.34-1.08	375	0.69	<b>p&lt;0.001</b>	0.37-1.01	385	0.36	<b>p&lt;0.001</b>
Transcript vs <i>trans</i> -DCE	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i>	70	0.48	<b>0.005</b>	0.16-0.81	260	0.51	<b>0.001</b>	0.23-0.80	254	0.22	<b>0.033</b>
<i>etnE</i>	71	0.40	<b>0.009</b>	0.09-0.70	286	0.41	<b>0.002</b>	0.15-0.67	287	0.34	<b>0.001</b>
<i>mmoX</i>	61	-0.15	0.246	-0.43-0.11	179	-0.07	0.603	-0.32-0.19	190	-0.17	0.102
<i>pmoA</i>	80	0.00	0.980	-0.25-0.28	273	-0.17	0.211	-0.43-0.10	277	-0.25	<b>0.013</b>
<i>bvcA</i>	54	0.80	<b>0.003</b>	0.30-1.31	220	0.56	<b>0.012</b>	0.13-0.99	227	0.24	<b>0.017</b>
<i>vcrA</i>	51	0.06	0.835	-0.54-0.65	193	0.07	0.768	-0.44-0.57	203	0.38	<b>p&lt;0.001</b>
<i>mmoX+pmoA</i>	84	-0.04	0.806	-0.34-0.25	288	-0.21	0.118	-0.47-0.05	295	-0.25	<b>0.013</b>
<i>bvcA+vcrA</i>	70	0.44	0.061	0.00-0.91	283	0.48	<b>0.015</b>	0.10-0.87	287	0.37	<b>p&lt;0.001</b>

n: number of samples in the analysis

CI: confidence interval

Coef: regression coefficient

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

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

Gene vs TCE	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i>	95	0.21	<b>0.009</b>	0.07-0.38	287	0.33	<b>p&lt;0.001</b>	0.16-0.49	307	0.34	<b>0.001</b>
<i>etnE</i>	95	0.19	<b>0.017</b>	0.04-0.33	288	0.30	<b>0.001</b>	0.13-0.47	311	0.35	<b>0.001</b>
<i>mmoX</i>	95	-0.07	0.179	-0.24-0.11	288	-0.22	<b>0.015</b>	-0.40--0.04	318	-0.19	0.067
<i>pmoA</i>	95	0.03	0.725	-0.18-0.22	323	-0.18	0.109	-0.39-0.04	355	-0.11	0.279
<i>bvcA</i>	95	0.41	<b>0.005</b>	0.13-0.67	365	0.37	<b>0.004</b>	0.12-0.62	381	0.30	<b>0.003</b>
<i>vcrA</i>	95	0.35	<b>0.029</b>	0.03-0.67	389	0.54	<b>0.001</b>	0.23-0.85	423	0.36	<b>p&lt;0.001</b>
<i>mmoX+pmoA</i>	95	0.04	0.669	-0.14-0.21	310	-0.16	0.119	-0.36-0.04	343	-0.12	0.261
<i>bvcA+vcrA</i>	95	0.45	<b>0.004</b>	0.15-0.79	379	0.50	<b>p&lt;0.001</b>	0.24-0.76	388	0.38	<b>p&lt;0.001</b>
Transcript vs TCE	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i>	70	0.38	<b>0.003</b>	0.16-0.62	261	0.39	<b>0.001</b>	0.18-0.61	254	0.30	<b>0.003</b>
<i>etnE</i>	71	0.28	<b>0.011</b>	0.05-0.49	288	0.33	<b>0.001</b>	0.14-0.52	292	0.36	<b>p&lt;0.001</b>
<i>mmoX</i>	61	-0.14	0.179	-0.35-0.05	179	-0.10	0.312	-0.30-0.10	189	-0.15	0.155
<i>pmoA</i>	80	0.04	0.725	-0.17-0.26	273	-0.06	0.613	-0.29-0.17	278	-0.11	0.307
<i>bvcA</i>	54	0.76	<b>p&lt;0.001</b>	0.36-1.11	216	0.45	<b>0.009</b>	0.12-0.78	226	0.25	<b>0.016</b>
<i>vcrA</i>	51	0.02	0.921	-0.35-0.38	195	0.05	0.743	-0.24-0.34	203	0.28	<b>0.006</b>
<i>mmoX+pmoA</i>	84	-0.02	0.861	-0.23-0.21	289	-0.14	0.175	-0.35-0.07	296	-0.10	0.341
<i>bvcA+vcrA</i>	70	0.32	0.050	-0.02-0.66	284	0.35	<b>0.015</b>	0.07-0.63	287	0.34	<b>0.001</b>

n: number of samples in the analysis

CI: confidence interval

Coef: regression coefficient

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

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

Gene vs ethene	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i>	65	0.53	<b>0.009</b>	0.13-0.92	190	0.59	<b>0.001</b>	0.24-0.94	188	0.35	<b>0.004</b>
<i>etnE</i>	65	0.46	<b>0.028</b>	0.04-0.85	194	0.53	<b>0.004</b>	0.17-0.88	190	0.28	<b>0.024</b>
<i>mmoX</i>	65	-0.05	0.813	-0.50-0.39	198	0.02	0.935	-0.40-0.43	209	-0.01	0.963
<i>pmoA</i>	65	-0.13	0.590	-0.61-0.35	205	-0.08	0.705	-0.47-0.32	204	0.02	0.871
<i>bvcA</i>	65	0.87	<b>0.014</b>	0.21-1.55	240	1.19	<b>p&lt;0.001</b>	0.62-1.75	250	0.37	<b>0.003</b>
<i>vcrA</i>	65	0.83	<b>0.020</b>	0.10-1.58	239	0.93	<b>0.010</b>	0.23-1.62	278	0.17	0.171
<i>mmoX+pmoA</i>	65	-0.14	0.512	-0.60-0.30	198	-0.09	0.620	-0.47-0.28	196	0.01	0.912
<i>bvcA+vcrA</i>	65	0.96	<b>0.007</b>	0.34-1.65	241	1.19	<b>p&lt;0.001</b>	0.62-1.76	252	0.39	<b>0.002</b>
Transcript vs ethene	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i>	45	0.85	<b>0.008</b>	0.30-1.37	173	0.86	<b>0.005</b>	0.28-1.44	167	0.32	<b>0.010</b>
<i>etnE</i>	51	0.63	<b>0.017</b>	0.13-1.11	178	0.63	<b>0.012</b>	0.15-1.11	172	0.24	0.055
<i>mmoX</i>	51	0.16	0.433	-0.26-0.57	148	0.25	0.236	-0.17-0.67	146	0.18	0.163
<i>pmoA</i>	59	-0.09	0.711	-0.58-0.39	191	-0.09	0.712	-0.56-0.38	189	0.05	0.691
<i>bvcA</i>	42	1.45	<b>p&lt;0.001</b>	0.77-2.10	165	1.53	<b>p&lt;0.001</b>	0.92-2.13	160	0.31	<b>0.012</b>
<i>vcrA</i>	36	0.44	0.163	-0.12-1.05	121	0.49	0.115	-0.13-1.10	135	0.22	0.077
<i>mmoX+pmoA</i>	61	-0.04	0.864	-0.56-0.41	199	-0.01	0.970	-0.49-0.47	199	0.04	0.756
<i>bvcA+vcrA</i>	51	1.39	<b>p&lt;0.001</b>	0.83-1.98	184	1.49	<b>p&lt;0.001</b>	0.97-2.00	181	0.38	<b>0.002</b>

n: number of samples in the analysis

CI: confidence interval

Coef: regression coefficient

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

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

Gene vs methane	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i>	74	0.30	0.087	-0.07-0.63	222	-0.01	0.966	-0.29-0.28	234	-0.09	0.449
<i>etnE</i>	74	0.30	0.09	-0.05-0.62	224	0.07	0.638	-0.22-0.35	231	-0.07	0.581
<i>mmoX</i>	74	0.52	<b>0.004</b>	-0.15-0.85	219	0.62	<b>p&lt;0.001</b>	-0.35-0.90	228	0.49	<b>p&lt;0.001</b>
<i>pmoA</i>	74	0.51	<b>0.007</b>	-0.12-0.86	232	0.46	<b>0.002</b>	-0.18-0.74	230	0.31	<b>0.007</b>
<i>bvcA</i>	74	0.43	0.118	-0.11-1.04	269	0.50	<b>0.020</b>	-0.08-0.93	291	0.33	<b>0.004</b>
<i>vcrA</i>	74	1.00	<b>0.001</b>	0.37-1.57	286	1.13	<b>p&lt;0.001</b>	0.64-1.61	311	0.48	<b>p&lt;0.001</b>
<i>mmoX+pmoA</i>	74	0.48	<b>0.006</b>	-0.14-0.79	224	0.44	<b>0.002</b>	-0.17-0.70	222	0.33	<b>0.005</b>
<i>bvcA+vcrA</i>	74	0.76	<b>0.005</b>	-0.23-1.31	283	0.79	<b>p&lt;0.001</b>	-0.37-1.20	290	0.46	<b>p&lt;0.001</b>
Transcript vs methane	n	multilevel regression				simple linear regression				Spearman's correlation	
		Coef	p value	95% CI	AIC	Coef	p value	95% CI	AIC	Rho	p value
<i>etnC</i>	54	0.36	0.204	-0.19-0.98	213	0.18	0.480	-0.32-0.67	213	-0.06	0.606
<i>etnE</i>	59	0.16	0.470	-0.31-0.66	211	-0.07	0.708	-0.47-0.32	214	-0.10	0.385
<i>mmoX</i>	57	0.36	<b>0.021</b>	-0.07-0.63	160	0.54	<b>p&lt;0.001</b>	-0.27-0.82	163	0.37	<b>0.001</b>
<i>pmoA</i>	68	0.51	<b>0.011</b>	-0.15-0.88	219	0.51	<b>0.003</b>	-0.17-0.85	226	0.30	<b>0.008</b>
<i>bvcA</i>	46	0.75	0.068	-0.08-1.53	192	0.52	0.126	-0.15-1.20	198	0.22	0.065
<i>vcrA</i>	38	1.37	<b>0.001</b>	-0.63-2.11	132	1.17	<b>p&lt;0.001</b>	-0.57-1.77	132	0.57	<b>p&lt;0.001</b>
<i>mmoX+pmoA</i>	70	0.58	<b>0.002</b>	-0.17-0.92	224	0.60	<b>p&lt;0.001</b>	-0.30-0.91	230	0.34	<b>0.004</b>
<i>bvcA+vcrA</i>	57	1.06	<b>0.011</b>	-0.25-1.93	229	1.17	<b>0.001</b>	-0.50-1.85	228	0.43	<b>p&lt;0.001</b>

n: number of samples in the analysis

CI: confidence interval

Coef: regression coefficient

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

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.

Site	Remediation approach	Time	Bulk VC attenuation rate, yr <sup>-1</sup>	R <sup>2</sup>	p Value	Rate Category
VA Site 2C	Oxygen releasing compound injection	2013.11	0.0006	0.972	0.518	Low
		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 bioremediation with biobarriers	2015.07	0.0849	0.909	0.012	High
		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
		2015.10	0.4350	0.932	0.008	High
SC Site 45* (north plume lower aquifer)	Enhanced anaerobic bioremediation – emulsified vegetable oil injection	2016.07	0.3752	0.879	0.019	High
		2016.07	1.0858	0.344	0.299	High
SC Site 45* (north plume upper aquifer)		2015.10	1.0409	0.23	0.414	High
		2015.10	0.5182	0.764	0.126	High
SC Site 45* (south plume lower aquifer)	In situ chemical oxidation	2016.07	0.2836	0.840	0.028	High

\*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.

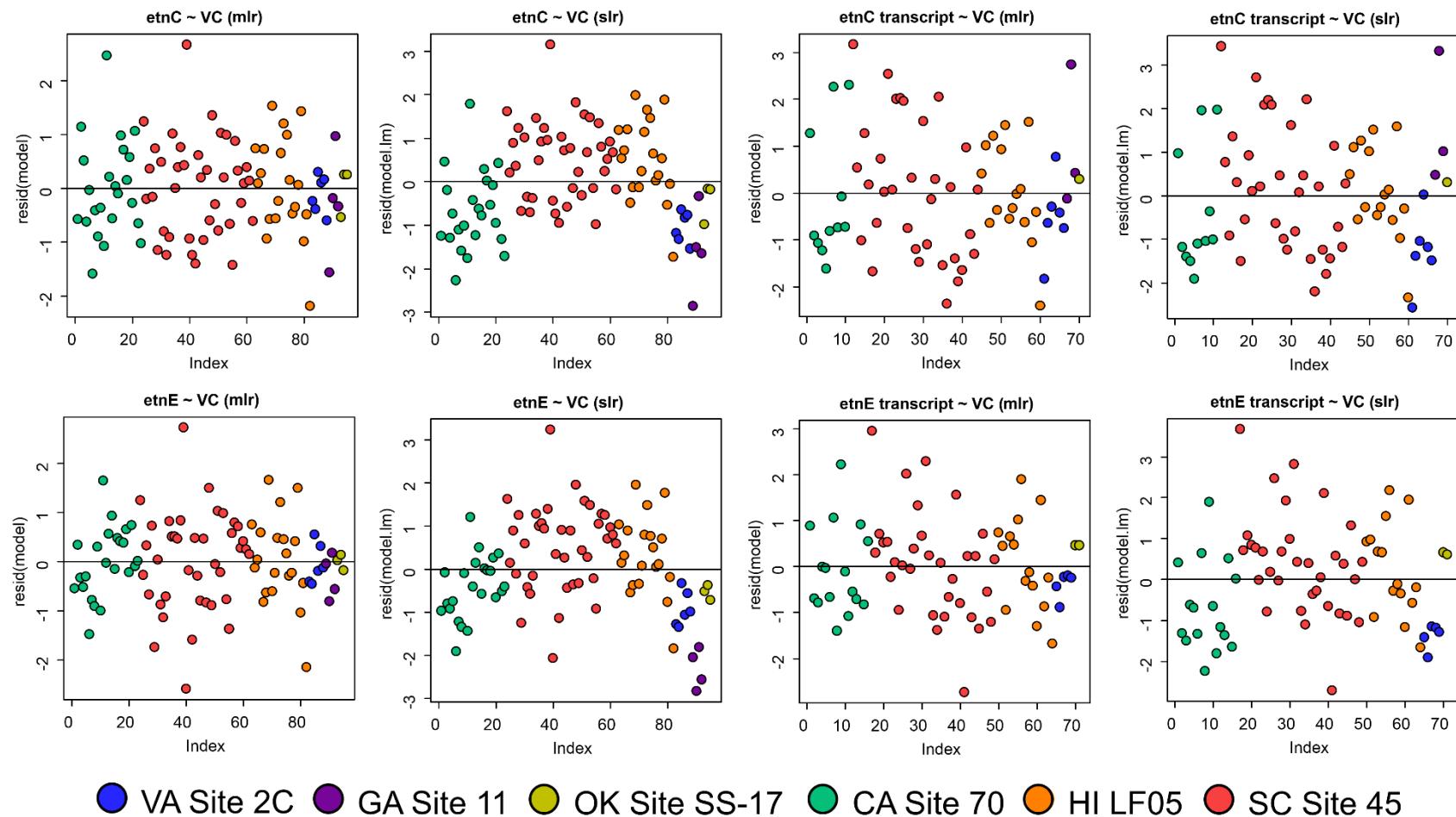


Figure S4. Residuals of multilevel regression (mlr) and simple linear regression (slr) analyses of VC concentration vs. *etnC* and *etnE* gene and transcript abundance.

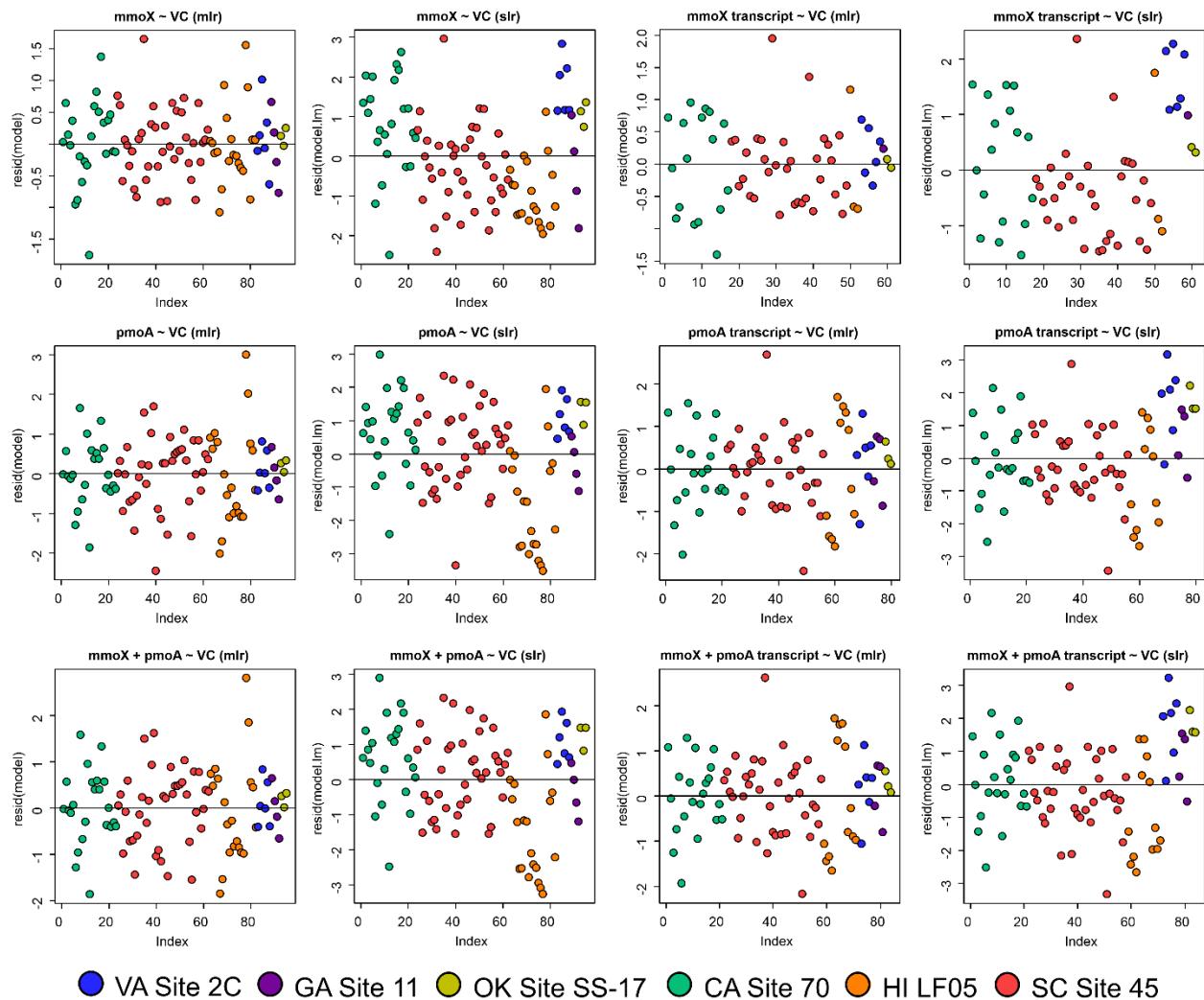


Figure S5. Residuals of multilevel regression (mlr) and simple linear regression (slr) analyses of VC concentration vs. *mmoX* and *pmoA* gene and transcript abundance.

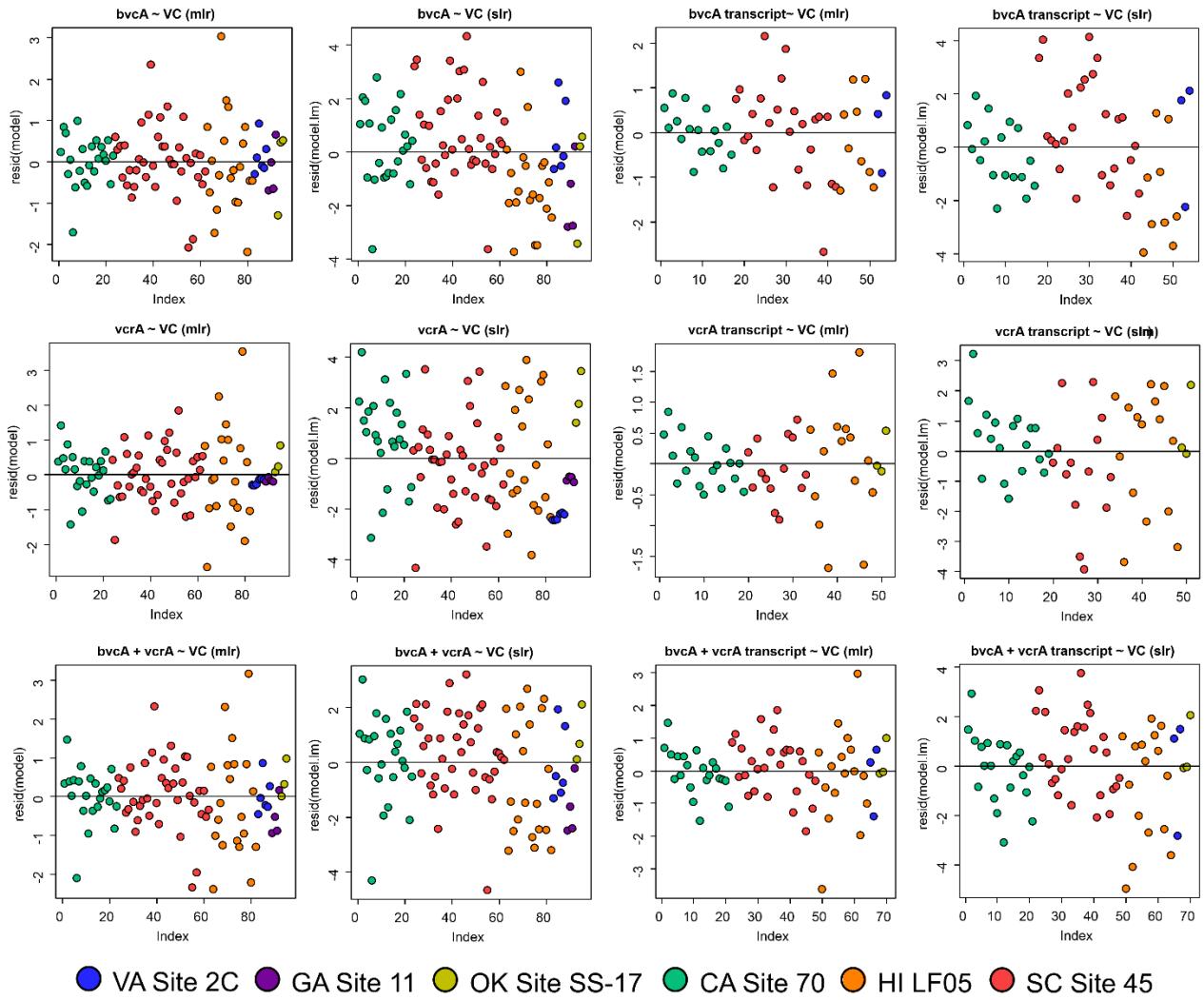


Figure S6. Residuals of multilevel regression (mlr) and simple linear regression (slr) analyses of VC concentration vs. *bvcA* and *vcrA* gene and transcript abundance.

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