

Supplemental information for:

Epoxyalkane: Coenzyme M Transferase gene diversity in groundwater samples from chlorinated ethene contaminated sites

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Table S1. Field groundwater geochemical parameters collected from the monitoring wells in this study. These parameters and groundwater velocities were taken from the reports cited in Table 1.

Well	Sample Designation	pH	Temperature (°C)	ORP (millivolts)	Groundwater Flow Velocity (ft/yr)
Carver, MA RB46D* (09/29/2009)	CARV46-1	5.9	14.05	135	277.4
Carver, MA RB64I* (09/29/2009)	CARV64	5.28	12.04	152	
Carver, MA RB46D (09/15/2010)	CARV46	5.6	14.08	217	
Carver, MA RB63I (09/15/2010)	CARV63	5.91	13.36	91	
Oceana, VA MW18 (08/06/2009)	OCEA18	7.39	21.4	-53	10.0
Oceana, VA MW25 (11/21/2008)	OCEA25	6.37	20	-93	
Soldotna, AK MW6 (05/12/2009)	SOLD6	4.85	3.21	3.7	54.75
Soldotna, AK MW40 (09/22/2008)	SOLD40	6.11	6.16	-55	
Fairbanks, AK 4M (03/27/2014)	FAIR4	6.26	4.56	34.8	--
Fairbanks, AK 13M (03/27/2014)	FAIR13	6.68	3.8	-20.9	
Kotzebue, AK 10-01 (10/22/2013)	KOTZ01	7.2	0.4	-101.4	--
Kotzebue, AK 10-03 (10/22/2013)	KOTZ03	6.48 ⁺	1.9 ⁺	-125.1 ⁺	
Australia 039IJ-1,3,6,7,8 (Oct.2011)	AUS39-1,3,6,7,8	NA	NA	NA	123.03

NA: data not provided.

--: VC attenuation rates were not calculated for these sites as VC was not detected, thus groundwater flow velocities were not used in the analysis

*These samples were used in the correlation analysis of *etnE* abundance with geochemical parameters, ethene concentrations, VC concentrations and attenuation rates, but was not included in the T-RFLP and clone library analyses.

⁺Kotzebue, AK 10-03: The original data sheet marked as “Parameters measured in-situ - may not represent true groundwater conditions” therefore were excluded in correlation analysis of these parameters.

Table S2. Oligonucleotide primers used in this study.

Primer name	Sequence (5'-3')	Product size (bp)	Reference
COM-F1L	AACTACCCSAAYCCSCGCTGGTACGAC	834-891	(1)
COM-R2E	GTCGGCAGTTTCGGTGATCGTGCTCTTGAC		
RTE F	CAGAA YGGCTGYGACATYATCCA	151	(2)
RTE R	CSGGYGTRCCCGAGTAGTTWCC		
F131	GGAAGCGTTCGARGAYGCSGT	447-453	(3)
R562	TAGGGCCAGACRAACTCGTCGA		
M13F	GTAAAACGACGGCCAG	169-199	Invitrogen
M13R	CAGGAAACAGCTATGAC		

Table S3. Information of qPCR parameters in this study. All *etnE* genes were amplified with RTE primers. *Nocardioides* sp. strain JS614 (Genbank Accession No. CP000508) *etnE* PCR products amplified with CoM primers were used as standards. Standards were amplified from *Nocardioides* sp. strain JS614 genomic DNA. There are 1.025×10^9 genes per ng *etnE* template. Kotzebue and Fairbanks *etnC* and *etnE* were run on the sample plate.

Sample	Target Gene	Flourescence threshold	Slope(-)	PCR Efficiency (%)	R ²	Y-intercept
Australia (AUS)	<i>etnE</i>	0.089	3.31	100.43	0.993	38.72
Kotzebue (KOTZ)	<i>etnE</i>	0.123	3.28	101.6	0.996	36.79
Fairbanks (FAIR)	<i>etnE</i>	0.123	3.28	101.6	0.996	36.79

Table S4. EaCoMT clone sequences (139 in total) retrieved as a result of different PCR protocols attempted to amplify EaCoMT genes from environmental samples. -: negative result; NA: not attempted

Site	Well No.	Direct PCR	Direct PCR + second amplification	Direct touchdown PCR	Nested PCR	Nested PCR with touchdown	Total clones sequenced
Carver, MA (CARV)	RB46D	-	-	-	-	TD CARV46 Clones 2-6, 8-10	8
	RB63I	-	-	-	-	TD CARV63 Clones 1-3,5-10	9
Soldotna, AK (SOLD)	MW6	-	-	-	SOLD6 Clones 1-7,9,10	TD SOLD6 Clones 1,2,5,6,8	14
	MW40	-	-	-	SOLD40 Clones 1,2,5,7,9,10	NA	6
NAS Oceana, VA (OCEA)	MW18	-	-	-	OCEA18 Clones 1-3, 8	TD OCEA18 Clone 2-7, 9,10	12
	MW25	-	-	-	OCEA25 Clones 1-8	TD OCEA25 Clone 1,2,4,5,7,8	14
Melbourne, Australia (AUS)	39IJ-1	COM AUS39-1 Clone 1-8	NA	NA	AUS39-1 Clone 1-8	NA	16
	39IJ-3	COM AUS39-3 Clone 1-4, 7-10	NA	NA	NA	NA	8
	39IJ-6	COM AUS39-6 Clone 1,2, 4-10	NA	NA	AUS39-6 Clone 1,3,5-8	NA	15
	39IJ-7	COM AUS39-7 Clone 1-6, 8-10	NA	NA	NA	NA	9
	39IJ-8	COM AUS39-8 Clone 1-6, 9	NA	NA	NA	NA	7
Kotzebue, AK (KOTZ)	MW10-01	Faint Band	-	NA	KOTZ01 Clones 1-5	NA	5
	MW10-03	Faint Band	-	NA	KOTZ03 Clones 1-4	NA	4
Fairbanks, AK (FAIR)	MW-4M	-	-	NA	FAIR4 Clones 1-6	NA	6
	MW-13M	-	-	NA	FAIR13 Clones 1-6	NA	6

Table S5. Summary of REPK (4) predicted T-RF lengths from the products of F131/R562 primers (expected size 447-453bp) when subjected to in silico digestion with the AcoI (EaeI) restriction enzyme. Groups 1-8 were used to select the enzyme employed in T-RFLP analysis. The primary criterion for restriction enzyme selection was that it can distinguish EaCoMT genes from these different groups.

EaCoMT Group	EaCoMT gene source strain (Genbank accession no.)	Predicted T-RF (bp)	
1	<i>Mycobacterium rhodesiae</i> strain JS60 (AY243034) <i>Mycobacterium</i> sp. strain JS621 (AY243039) <i>Ochrobactrum</i> sp. TD LBB Enrichment (DQ370436) <i>Ochrobactrum</i> sp. TD VC Enrichment (AY858985) <i>Pseudomonas putida</i> strain AJ LBB-grown (DQ370435) <i>Pseudomonas putida</i> strain AJ VC-grown (AY858984)	363	
2	<i>Mycobacterium chubuense</i> strain NBB4 (GU174752)	453 (no cut)	
3	<i>Mycobacterium gadium</i> strain JS616 (AY243036) <i>Mycobacterium mageritense</i> strain JS625 (AY243043) <i>Mycobacterium moriokaense</i> strain JS619 (AY243038) <i>Mycobacterium</i> sp. strain JS624 (AY243042) <i>Mycobacterium tusciae</i> strain JS617 (AY243037)	24	
	<i>Mycobacterium aichiense</i> strain JS61 (AY243035)	48	
	<i>Mycobacterium rhodesiae</i> strain JS622 (AY243040) <i>Mycobacterium</i> sp. strain JS623 (AY243041)	312	
	4	<i>Nocardioides</i> sp. strain JS614 (AY243042)	240
	5	<i>Nocardioides</i> sp. strain JS614 <i>etnE1</i> (CP000508)	356
6	<i>Gordonia rubripertincta</i> B-276 (AF426826)	149	
7	<i>Xanthobacter</i> Py2 2-hydroxypropyl-CoM lyase (CP000782)	73	
8	<i>Haliea</i> sp. putative EaCoMT (AB691746)	151	
9	<i>Pseudomonas putida</i> MetE (AF363277)	450	

Table S6. Genbank accession numbers of existing EaCoMT sequences used in phylogenetic and diversity analyses in this study

Name	Accession Number
Carver MA Well RB63I-E01	GQ847819
Carver MA Well RB63I-E02	GQ847820
Carver MA Well RB63I-E03	GQ847821
Carver MA Well RB73-E05	GQ847813
Carver MA Well RB73-E06	GQ847814
Carver MA Well RB73-E10	GQ847817
Carver MA Well RB73-E11	GQ847818
Carver MA Well RB63I 75pc VC L4-1	KJ509931
Carver MA Well RB63I 75pc VC L4-4	KJ509932
Carver MA Well RB63I 75pc VC L4-10	KJ509933
Carver MA Well RB63I 75pc VC L9-1	KJ509934
Carver MA Well RB63I 75pc VC L9-4	KJ509935
Carver MA Well RB63I 75pc VC L9-8	KJ509936
<i>Mycobacterium chubuense</i> strain NBB4	GU174752
<i>Nocardioides</i> sp. strain JS614	AY772007
<i>Nocardioides</i> sp. strain JS614 <i>etnE1</i>	CP000508
<i>Mycobacterium rhodesiae</i> strain JS60	AY243034
<i>Mycobacterium aichiense</i> strain JS61	AY243035
<i>Mycobacterium gadium</i> strain JS616	AY243036
<i>Mycobacterium tusciae</i> strain JS617	AY243037
<i>Mycobacterium moriokaense</i> strain JS619	AY243038
<i>Mycobacterium</i> sp. strain JS621	AY243039
<i>Mycobacterium rhodesiae</i> strain JS622	AY243040
<i>Mycobacterium</i> sp. strain JS623	AY243041
<i>Mycobacterium</i> sp JS623 mutant 1	FJ602755
<i>Mycobacterium</i> sp. JS623 mutant 5	FJ602759
<i>Mycobacterium</i> sp. JS623 mutant 4	FJ602758
<i>Mycobacterium</i> sp. JS623 mutant 3	FJ602757
<i>Mycobacterium</i> sp. JS623 mutant 2	FJ602756
<i>Mycobacterium</i> sp. strain JS624	AY243042
<i>Mycobacterium mageritense</i> strain JS625	AY243043
<i>Pseudomonas putida</i> strain AJ VC Enrichment	AY858984
<i>Pseudomonas putida</i> strain AJ LBB Enrichment	DQ370435
<i>Ochrobactrum</i> sp. TD VC Enrichment	AY858985
<i>Ochrobactrum</i> sp. TD LBB Enrichment	DQ370436
<i>Gordonia rubripertincta</i> (<i>Rhodococcus rhodochrous</i>) B-276	AF426826
<i>Haliea</i> sp. ETY-M	AB691746
<i>Xanthobacter</i> Py2 2-hydroxypropyl-CoM lyase	CP000782

Table S7. Genbank accession numbers of MetE sequences used as outgroup sequences in phylogenetic tree construction.

Genus, Species and Strain	Genbank accession: genome location (Protein ID)	MetE Group
<i>Mycobacterium tusciae</i> JS617	NZ_KI912270.1:1983765-1984775 (WP_006245687.1)	1
<i>Mycobacterium smegmatis</i> JS623	CP003078.1:2183169-2184179 (AGB22566.1)	1
<i>Mycobacterium chubuense</i> NBB4	CP003053.1:1895804-1896820 (AFM16616.1)	1
<i>Mycobacterium smegmatis</i> str. mc ² -155	NC_018289.1:2441701-2442711 (WP_011728293.1)	1
<i>Gordonia</i> <i>polyisoprenivorans</i> VH2	CP003119.1:3444186-3445205 (AFA74057.1)	1
	CP003119.1:1951183-1952208 (AFA72765.1)	2
<i>Pseudomonas putida</i>	AF363277.1 (AAK29462.1)	2
<i>Pseudomonas putida</i> DOT- T1E	CP003734.1:2956795-2957880 (AFO48541.1)	2
<i>Nocardioides</i> sp. URHA0032	NZ_JIAV01000019.1:158602-159720 (WP_028637114.1)	2
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> PXO86	CP007166.1:476362-477390 (AJQ81599.1)	2
<i>Xanthomonas citri</i> subsp. <i>citri</i> strain UI7	CP008989.1:401372-402400 (AJY80461.1)	2

Table S8. Genbank accession number of representative clones in this study.

Clone ID	Genbank Accession Number
TD CARV 63 Clone 10	KR936138.1
TD CARV46 Clone 10	KR936139.1
TD CARV 63 Clone 1	KR936140.1
TD CARV46 Clone 3	KR936141.1
OCEA25 Clone 8	KR936142.1
OCEA18 Clone 2	KR936143.1
TD OCEA18 Clone 10	KR936144.1
OCEA18 Clone 1	KR936145.1
OCEA18 Clone 2	KR936146.1
OCEA18 Clone 3	KR936147.1
OCEA25 Clone 1	KR936148.1
OCEA25 Clone 2	KR936149.1
OCEA25 Clone 4	KR936150.1
SOLD6 Clone 2	KR936151.1
SOLD6 Clone 8	KR936152.1
SOLD6 Clone 1	KR936153.1
SOLD6 Clone 3	KR936154.1
SOLD6 Clone 6	KR936155.1
SOLD40 Clone 1	KR936156.1
KOTZ03 Clone 2	KR936157.1
KOTZ01 Clone 3	KR936158.1
KOTZ01 Clone 1	KR936159.1
FAIR4 Clone 2	KR936160.1
FAIR13 Clone 3	KR936161.1
FAIR4 Clone 1	KR936162.1
COM AUS39-1 Clone 1	KR936163.1
COM AUS39-1 Clone 2	KR936164.1
COM AUS39-1 Clone 3	KR936165.1
COM AUS39-1 Clone 4	KR936166.1
COM AUS39-1 Clone 7	KR936167.1

Table S9. Summary of EaCoMT sequences found in the MG-RAST metagenomics database by July 2015. MG-RAST metagenome numbers are provided, along with the sample source and location, the top BLAST hit, abundance of each EaCoMT gene type in the sample, % identity and average amino acid alignment length.

Metagenome	Sample Source	Location	Top Hit in BLAST	Abundance	% ID	Avg. Align Length (aa)
4508942.3	Agricultural soil	Richmond, Indiana, USA	epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	1	85.19	54
			epoxyalkane coenzyme M transferase, uncultured bacteria	1	74.14	58
4516651.3	Air	Beijing, China	epoxyalkane: coenzyme M transferase <i>Nocardioides</i> sp. JS614	124	84.47	26.483
			epoxyalkane:coenzyme M transferase <i>Rhodococcus rhodochrous</i>	51	86.245	27.72
			epoxyalkane:coenzyme M transferase <i>Mycobacterium chubuense</i> NBB4	14	83.062	22.462
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	10	84.458	23
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	9	71.18	24.625
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	8	83.664	27.231
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	5	77.66	23.556
			epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	7	84.549	23.714
			putative epoxyalkane:coenzyme M transferase <i>Taylorella equigenitalis</i> MCE9	75	78.742	25.232

			putative epoxyalkane:coenzyme M transferase <i>Corynebacterium aurimucosum</i> ATCC 700975	70	82.491	26.886
			putative epoxyalkane:coenzyme M transferase <i>Rhodobacterales</i> bacterium HTCC2654	40	74.761	25.043
			putative epoxyalkane:coenzyme M transferase <i>Nitrococcus mobilis</i> Nb-231	17	75.884	23.462
4497384.3	Bulk Soil	Anazonia, Brazil	2-hydroxypropyl-CoM lyase (EC 4.4.1.23) (Epoxyalkane:CoM transferase) (EaCoMT) (Aliphatic epoxide carboxylation component I), <i>Bacillus tusciae</i> DSM 2912	1	80	15
4537093.3	Marine Sediment	Santa Barbara, CA, USA	epoxyalkane coenzyme M transferase, uncultured bacteria (JS623 and JS614-like)	14	66.61	32.17
4520078.3	Livestock body fluid	Ellinbank, Victoria, Australia	2-hydroxypropyl-CoM lyase (EC 4.4.1.23) (Epoxyalkane:CoM transferase) (EaCoMT) (Aliphatic epoxide carboxylation component I), <i>Bacillus tusciae</i> DSM 2912	1	84.62	13
4470378.3	Permafrost Soil (DNA)	Active Layer	epoxyalkane coenzyme M transferase, uncultured bacteria (JS623 and JS614-like)	13	72.9	31.97
			epoxyalkane coenzyme M transferase mutant 3 , <i>Mycobacterium</i> sp. JS623	1	60	30
			epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	5	75.51	33.8
			epoxyalkane coenzyme M transferase mutant 5 , <i>Mycobacterium</i> sp. JS623	1	72.97	37
4491382.3	Permafrost Soil (RNA)	Active Layer	epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	4	73.29	37.25
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	2	69.4	42
			epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	1	78.26	46

4470381.3	Permafrost Soil	Thermokarst Bog	epoxyalkane coenzyme M transferase, <i>Mycobacterium</i> sp. JS623	2	66.115	32
			epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	1	63.89	39
4477875.3	Bulk Soil	Misiones, Argentina	epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	1	62.5	24
4524575.3	Rhizosphere Soil	Golm, Germany	epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	1	82.98	47
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	2	71.7	50.5
4480863.3	Anaerobic digester sludge	Ithaca, New York, USA	epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	2	66.03	20.5
4481985.3	River Sediment	Southeastern Montana, USA	epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	1	84.62	13
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	3	80.77	16.33
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	1	71.43	21
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	1	66.67	21
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	2	73.43	23
4541641.3	Bulk Soil	Cedar Creek, MN, USA	epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	9	74.577	41.334
			epoxyalkane coenzyme M transferase, <i>Mycobacterium</i> sp. JS623	8	76.636	42.875
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	3	78.367	42.667

			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	5	69.074	37.4
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	2	69.416	37.5
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	1	77.42	31
4480704.3	Dust	Chad Desert, Chad	epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	1	82.76	29
			epoxyalkane coenzyme M transferase, <i>Mycobacterium</i> sp. JS623	3	75.107	27.333
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	5	83.036	23.4
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	1	92.86	14
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	1	72.22	18
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	6	80.638	27.5
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	2	77.78	27
			epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	2	72.425	29
4523235.3	Paddy Field Soil	Changshu, China	epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	1	75.86	29
4477900.3	Bulk Soil	Garwood Valley, Antarctica	epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	1	84.62	13
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	3	84.843	22

			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	1	62.5	24
4564114.3	Sea Sediment	Elba, Mediteranian, Italy	putative epoxyalkane:coenzyme M transferase, <i>Nitrococcus mobilis</i> Nb-231	3	67.65	73.667
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium chubuense</i> NBB4	1	52.46	61
4512589.3	Oil Contaminated Soil	Varenes, QC, Canada	epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	2	77.27	22
4511145.3	Soil	Loma Ridge, CA, USA	putative epoxyalkane:coenzyme M transferase, <i>Nitrococcus mobilis</i> Nb-231	1	73.33	30
			putative epoxyalkane:coenzyme M transferase, <i>Taylorella equigenitalis</i> MCE9	1	78.26	23
4529836.3	Soil	Koeln, Germany	epoxyalkane: coenzyme M transferase, <i>Nocardioides</i> sp. JS614	26	74.377	33.122
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium tusciae</i> JS617	1	82.5	40
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium chubuense</i> NBB4	6	63.101	36.636
			epoxyalkane:coenzyme M transferase, <i>Rhodococcus rhodochrous</i>	15	72.168	36.379
4554870.3	Aquatic microbial mat	Kowary, Poland	putative epoxyalkane:coenzyme M transferase, <i>Nitrococcus mobilis</i> Nb-231	1	84.38	32
			putative epoxyalkane:coenzyme M transferase, <i>Rhodobacterales</i> bacterium HTCC2654	1	61.11	54
4484670.3	Chyme	Salt Lake City, UT, USA	putative epoxyalkane:coenzyme M transferase, <i>Taylorella equigenitalis</i> MCE9	3	80.888	23.2
			putative epoxyalkane:coenzyme M transferase, <i>Corynebacterium aurimucosum</i> ATCC 700975	1	84	25

4530091.3	Forest organic material	Johnsbach, Austria	epoxyalkane: coenzyme M transferase, <i>Nocardioides</i> sp. JS614	8	72.674	32.732
			epoxyalkane: coenzyme M transferase, <i>Mycobacterium rhodesiae</i> JS60	1	61.54	39
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	1	89.29	28
			putative epoxyalkane:coenzyme M transferase, <i>Rhodobacterales</i> bacterium HTCC2654	35	69.874	37.47
			putative epoxyalkane:coenzyme M transferase, <i>Nitrococcus mobilis</i> Nb-231	3	74.857	34.333
4482593.3	Tundra Soil	Daring Lake, NWT, Canada	epoxyalkane: coenzyme M transferase, <i>Nocardioides</i> sp. JS614	142	80.226	29.636
			epoxyalkane:coenzyme M transferase, <i>Rhodococcus rhodochrous</i>	41	79.474	28.875
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium chubuense</i> NBB4	40	79.054	27.405
			epoxyalkane: coenzyme M transferase, <i>Mycobacterium rhodesiae</i> JS60	20	76.425	29.2
			epoxyalkane: coenzyme M transferase, <i>Mycobacterium rhodesiae</i>	10	76.455	27
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium tusciae</i> JS617	6	71.198	31.833
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium</i> sp. JS621	5	79.76	27.2
			epoxyalkane:coenzyme M transferase, <i>Ochrobactrum</i> sp. TD	4	74.803	24.75
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium mageritense</i>	4	76.825	25
epoxyalkane:coenzyme M transferase, <i>Mycobacterium gadium</i>	2	72.857	27			

			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	23	75.773	28.739
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	12	71.595	30.304
			epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	17	77.505	30.273
			epoxyalkane coenzyme M transferase mutant 5 <i>Mycobacterium</i> sp. JS623	5	81.377	25.444
			putative epoxyalkane:coenzyme M transferase, <i>Nitrococcus mobilis</i> Nb-231	55	76.949	22.399
			putative epoxyalkane:coenzyme M transferase, <i>Rhodobacteriales</i> bacterium HTCC2654	52	71.176	25.935
			putative epoxyalkane:coenzyme M transferase, <i>Taylorella equigenitalis</i> MCE9	45	73.13	23.891
			putative epoxyalkane:coenzyme M transferase, <i>Corynebacterium aurimucosum</i> ATCC 700975	37	74.491	22.331
4494863.3	Activated Sludge	Xiangcheng, Henan, China	epoxyalkane: coenzyme M transferase, <i>Nocardioides</i> sp. JS614	1	84.62	13
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium rhodesiae</i> JS60	1	66.67	21
4508039.3	Marine Sediment	Gulf of Mexico, USA	putative epoxyalkane:coenzyme M transferase, <i>Corynebacterium aurimucosum</i> ATCC 700975	1	84.62	13
4502924.3	Arable Soil	Auburn, IL, USA	epoxyalkane:coenzyme M transferase, <i>Mycobacterium chubuense</i> NBB4	33	67.86	23.365
			epoxyalkane:coenzyme M transferase, <i>Rhodococcus rhodochrous</i>	21	75.322	23.256
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium tusciae</i> JS617	3	62.145	26.5
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium rhodesiae</i> JS60	3	68.965	24.75

			epoxyalkane: coenzyme M transferase, <i>Nocardioides</i> sp. JS614	59	71.111	23.065
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	4	68.733	29
			epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	2	79.15	16
4502926.3	Arable Soil	Mansfield, IL, USA	epoxyalkane: coenzyme M transferase, <i>Nocardioides</i> sp. JS614	97	75.81	21.961
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium chubuense</i> NBB4	34	70.866	22.079
			epoxyalkane:coenzyme M transferase, <i>Rhodococcus rhodochrous</i>	21	73.414	23.897
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	5	76.389	22
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	5	83.666	24.8
			epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	7	82.629	23.429
			epoxyalkane coenzyme M transferase mutant 4 <i>Mycobacterium</i> sp. JS623	3	75.657	30.667
4502927.3	Arable Soil	Urbana, IL, USA	epoxyalkane:coenzyme M transferase, <i>Mycobacterium chubuense</i> NBB4	46	72.736	22.416
			epoxyalkane:coenzyme M transferase, <i>Rhodococcus rhodochrous</i>	44	70.166	23.228
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium tusciae</i> JS617	3	84.207	29
			epoxyalkane: coenzyme M transferase <i>Nocardioides</i> sp. JS614	99	71.358	22.421
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	5	78.918	22

			epoxyalkane coenzyme M transferase mutant 5 <i>Mycobacterium</i> sp. JS623	3	85.12	15.333
			putative epoxyalkane:coenzyme M transferase, <i>Nitrococcus mobilis</i> Nb-231	252	74.985	23.502
			putative epoxyalkane:coenzyme M transferase, <i>Rhodobacteriales</i> bacterium HTCC2654	174	74.938	22.701
			putative epoxyalkane:coenzyme M transferase, <i>Taylorella equigenitalis</i> MCE9	167	73.661	21.975
			putative epoxyalkane:coenzyme M transferase, <i>Corynebacterium aurimucosum</i> ATCC 700975	109	72.434	23.424
4469540.3	Permafrost Soil	USA	epoxyalkane:coenzyme M transferase, <i>Mycobacterium chubuense</i> NBB4	4	77.81	27
			epoxyalkane:coenzyme M transferase, <i>Rhodococcus rhodochrous</i>	4	77.235	27.5
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium rhodesiae</i> JS60	2	75.313	29.667
			epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	1	76.47	17
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	3	81.96	24
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	3	86.49	37
			epoxyalkane coenzyme M transferase mutant 5 <i>Mycobacterium</i> sp. JS623	1	80.65	31
			putative epoxyalkane:coenzyme M transferase, <i>Rhodobacteriales</i> bacterium HTCC2654	44	75.44	20.278
			putative epoxyalkane:coenzyme M transferase, <i>Nitrococcus mobilis</i> Nb-231	23	73.278	25.726

			putative epoxyalkane:coenzyme M transferase, <i>Taylorella equigenitalis</i> MCE9	16	71.455	22.304
4520035.3	Intertidal Sediment	Plum Island, USA	epoxyalkane: coenzyme M transferase <i>Nocardioides</i> sp. JS614	1	84.54	17.778
			epoxyalkane:coenzyme M transferase, <i>Mycobacterium chubuense</i> NBB4	1	64	25
			putative epoxyalkane:coenzyme M transferase, <i>Rhodobacteriales</i> bacterium HTCC2654	5	77.142	41.5
4508984.3	Sea water	Kalvhagefjorden, Sweden	2-hydroxypropyl-CoM lyase (EC 4.4.1.23) (Epoxyalkane:CoM transferase) (EaCoMT) (Aliphatic epoxide carboxylation component I) <i>Bacillus tusciae</i> DSM 2912	1	100	10
4472705.3	Mucus, Human Microbe	USA	2-hydroxypropyl-CoM lyase (EC 4.4.1.23) (Epoxyalkane:CoM transferase) (EaCoMT) (Aliphatic epoxide carboxylation component I) <i>Bacillus tusciae</i> DSM 2912	1	84.62	13
4519770.3	Oil contaminated soil	Houston, TX, USA	2-hydroxypropyl-CoM lyase (EC 4.4.1.23) (Epoxyalkane:CoM transferase) (EaCoMT) (Aliphatic epoxide carboxylation component I) <i>Bacillus tusciae</i> DSM 2912	1	84.62	13
4460182.3	Estuarine bulk water	Puget Sound, WA, USA	putative epoxyalkane:coenzyme M transferase, <i>Kocuria rhizophila</i> DC2201	1	55	80
4582806.3	Soil	Dumpas West, Malaysia	epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	8	75.597	81.875
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS614-like)	4	78.925	38.5
			epoxyalkane coenzyme M transferase, uncultured bacteria (JS623-like)	3	78.52	44
			epoxyalkane: coenzyme M transferase <i>Nocardioides</i> sp. JS614	30	81.419	54.1

			epoxyalkane:coenzyme M transferase <i>Rhodococcus rhodochrous</i>	10	78.844	53
			epoxyalkane:coenzyme M transferase <i>Mycobacterium tusciae</i> JS617	1	78.57	42
			epoxyalkane coenzyme M transferase mutant 1 <i>Mycobacterium</i> sp. JS623	1	57.89	57
			epoxyalkane coenzyme M transferase mutant 2 <i>Mycobacterium</i> sp. JS623	2	57.451	47
			epoxyalkane coenzyme M transferase mutant 3 <i>Mycobacterium</i> sp. JS623	2	71.155	32.5
			epoxyalkane coenzyme M transferase mutant 5 <i>Mycobacterium</i> sp. JS623	1	51.791	56
			putative epoxyalkane:coenzyme M transferase, <i>Nitrococcus mobilis</i> Nb-231	24	65.98	44.417
			putative epoxyalkane:coenzyme M transferase, <i>Rhodobacteriales</i> bacterium HTCC2654	17	68.826	43.177
			putative epoxyalkane:coenzyme M transferase, <i>Taylorella equigenitalis</i> MCE9	5	66.18	40.4
4554871.3	Gold Mine Aquatic Microbial Mat	Zolty Stok, Poland	epoxyalkane:coenzyme M transferase, <i>Mycobacterium chubuense</i> NBB4	7	65.253	37.429
			epoxyalkane:coenzyme M transferase <i>Rhodococcus rhodochrous</i>	2	59.795	42.5

Table S10. Examples of EaCoMT sequences in VC-, ethene- and propene-assimilating bacteria that are incorrectly annotated as MetE sequences in Genbank.

Genus, Species and Strain	Genbank accession: genome location (Protein ID)	Genbank annotation	Uniprot annotation (accession no.; % identity)	% identity (ID) with top EaCoMT BLAST hit
<i>Mycobacterium</i> sp. JS617	NZ_KI912270.1: 4898170-4899279 (WP_006247398.1)	methionine synthase (cobalamin-independent)	Epoxyalkane coenzyme M transferase (B9VTP0; 97.6% to <i>Mycobacterium</i> sp. JS623)*	99% ID with JS617 EaCoMT gene (AY243037.1)
<i>Mycobacterium</i> sp. JS623	CP003078.1: 1868236-1869345 (AGB22266.1)		Epoxyalkane coenzyme M transferase (B9VTP0; 100%)*	100% ID with JS623 EaCoMT gene (AY243041)
<i>Mycobacterium chubuense</i> strain NBB4	YP_006442905 (WP_014805821.1)		Epoxyalkane:coenzyme M transferase (D2K2D7; 100%)	94% ID with JS623 EaCoMT gene (ACM61851)
<i>Nocardioides</i> sp. JS614	CP000508.1: 134268-135380 (ABL79394.1)	methionine synthase (B12-independent) (plasmid)	Epoxyalkane:coenzyme M transferase (Q5U9J8; 100%)	100% ID with JS614 EaCoMT gene (AY772007.1)
	NC_008697.1: 134268-135380 (WP_011751519.1)	methionine synthase	Epoxyalkane:coenzyme M transferase (Q5U9J8;100%)	100% ID with JS614 EaCoMT gene (AY772007.1)
<i>Xanthobacter autotrophicus</i> Py2 plasmid pXAUT01	CP000782.1: 81145-82275 (ABS70076.1)	methionine synthase (vitamin-B12 independent)	2-hydroxypropyl-CoM lyase (Q56837; 100%)	72% ID with putative <i>Haliea</i> EaCoMT gene (AB691746.2)
	CP000782.1: 266692-267822 (ABS70245.1)		2-hydroxypropyl-CoM lyase (Q56837l; 100%)	Apparent duplication of CP000782.1:81145-82275

* These genes also had Uniprot hits to Methionine synthase II (Cobalamin independent) from *Mycobacterium* sp. strain JS623 (L0IWC5)

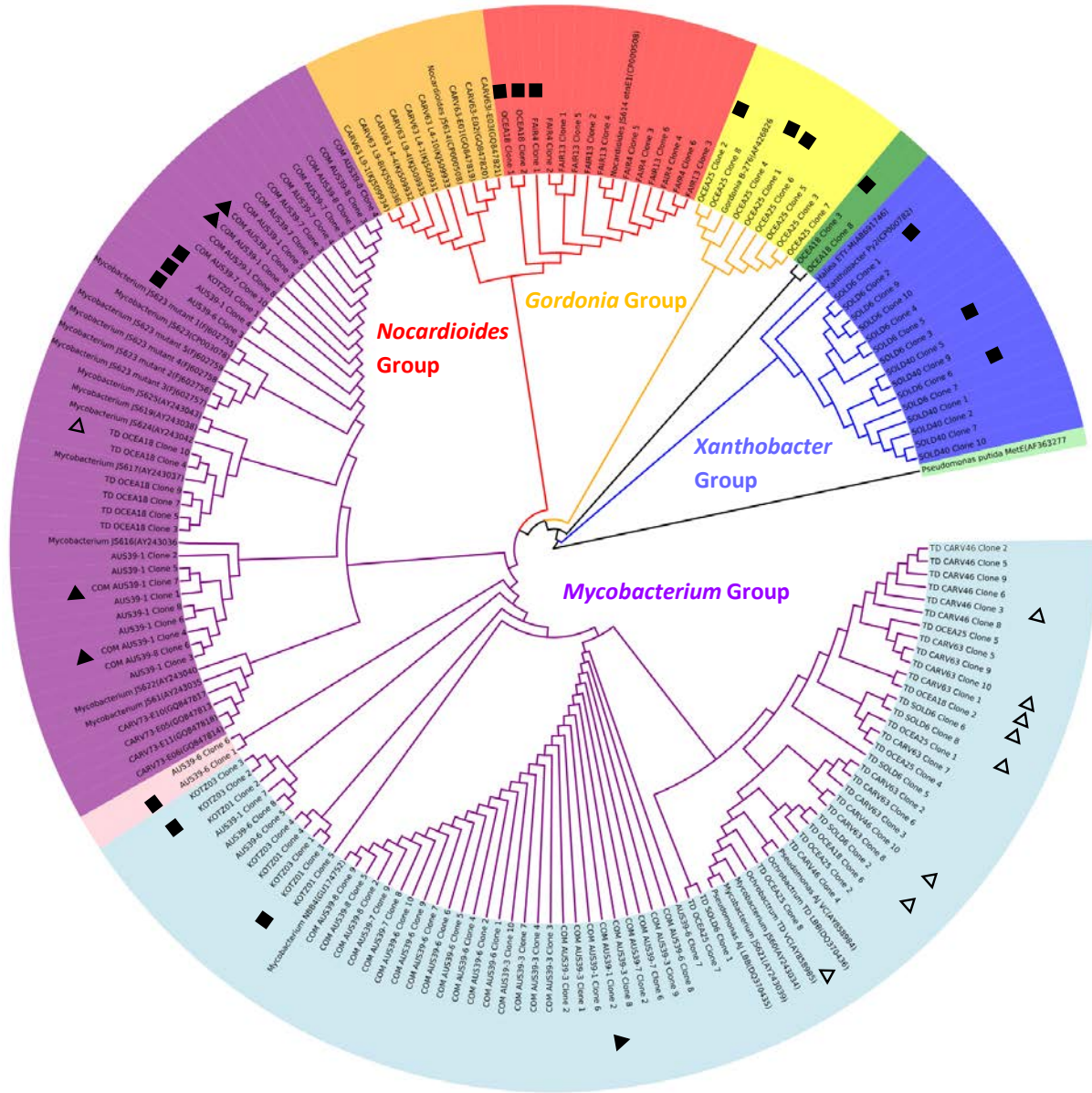


Figure S1. A phylogenetic tree depicting the relationship of deduced EaCoMT sequences from environmental samples in this study (Table S2), enrichment cultures and isolates from Genbank (Table S5). Genes are colored by group. The clones used for amino acid tree (Fig. 2) were marked with symbols. The symbols refer to the PCR amplification method used: ▲ Direct PCR; ■ Nested PCR; △ Nested PCR with a touchdown modification. A total of 178 sequences were aligned with ClustalW (5) and trimmed to 464 bp (including gaps) in MEGA5 (6, 7). The circle tree was constructed using the maximum likelihood (7) method with *Pseudomonas putida MetE* gene (8) as the outgroup. The circular tree was visualized using EvolView (9).

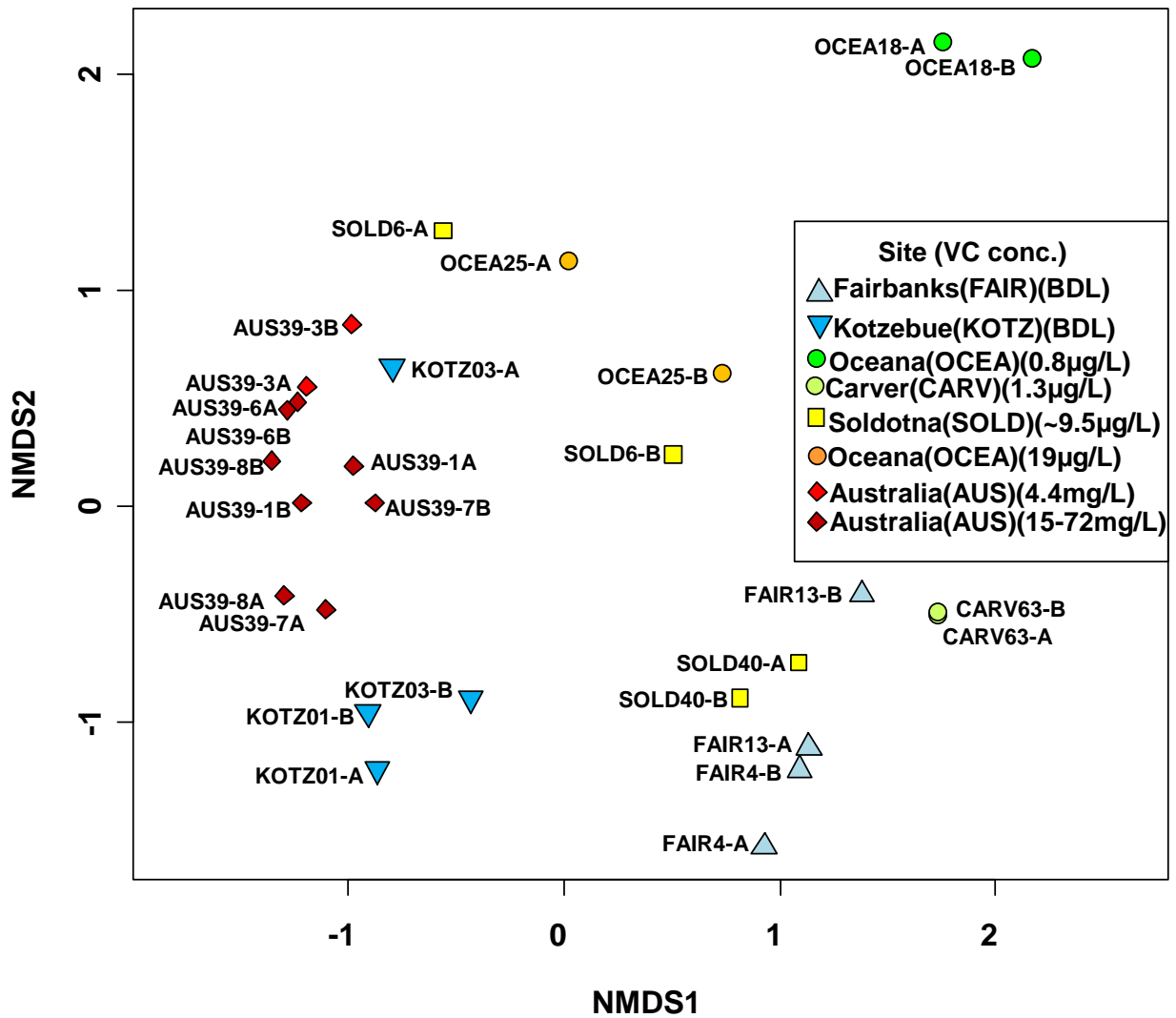


Figure S2. NMDS ordination of EaCoMT gene T-RFLP profiles from different groundwater samples. Sample identifiers are formatted by site, well number, and replicate (A or B). VC concentrations estimated in each of the wells is provided for reference. The final stress was 0.1402.

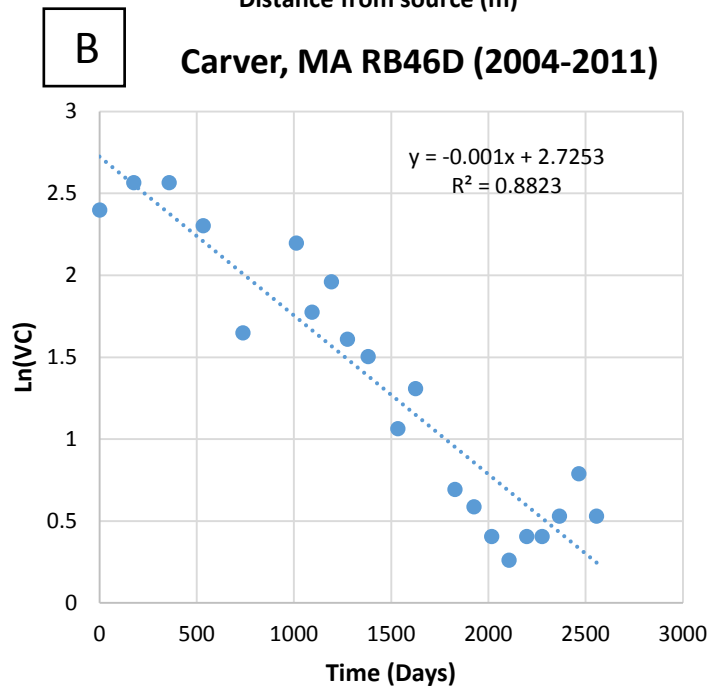
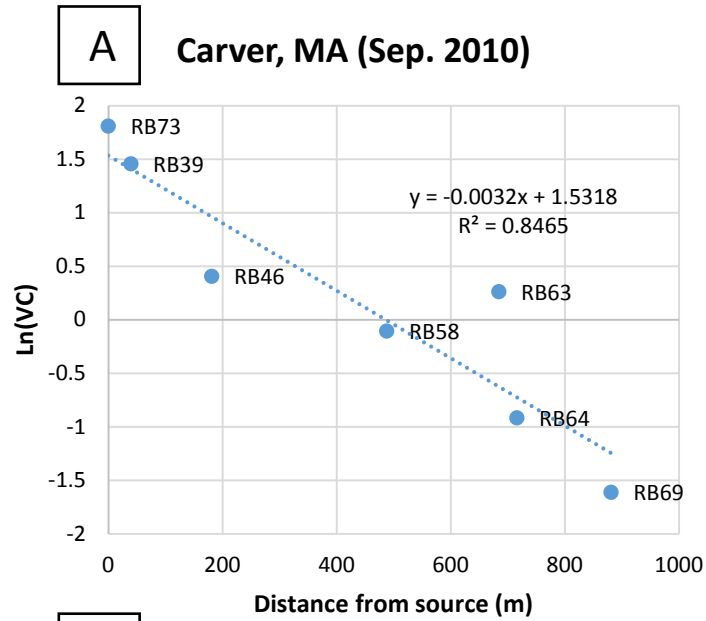


Figure S3. Examples of bulk VC attenuation rate (k_{bulk}) (A) and point VC decay rate (k_{point}) (B) calculations. To estimate the k_{bulk} , a transect was developed across source zone and selected wells on the map, then the natural log of VC concentration from each well was plotted against the distance between each well and the source zone. Linear regression was performed and the k_{bulk} is equal to slope times groundwater flow rate (Table S2). To estimate the k_{point} , the \ln VC concentration was plotted against time and a linear regression was performed. The k_{point} is equal to the slope (unit conversions should be made if necessary). The retardation factor for VC was assumed to be 1.7.

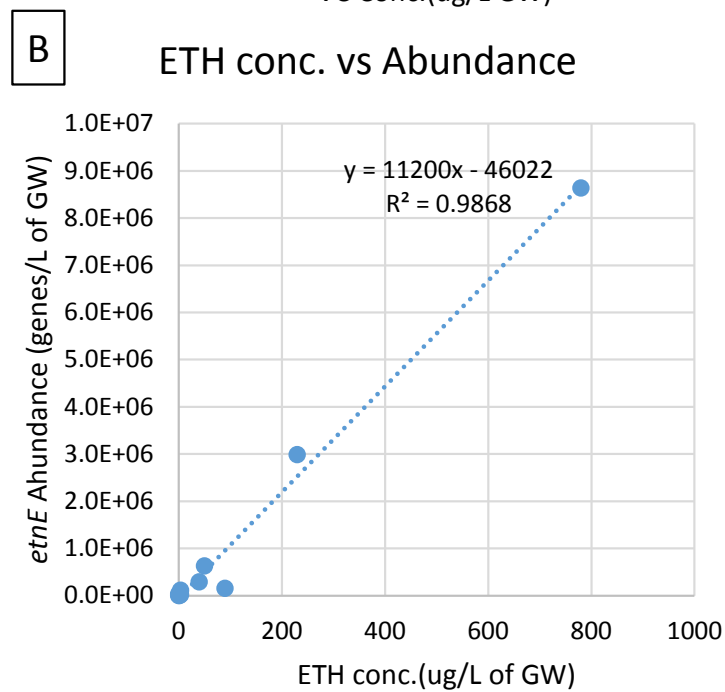
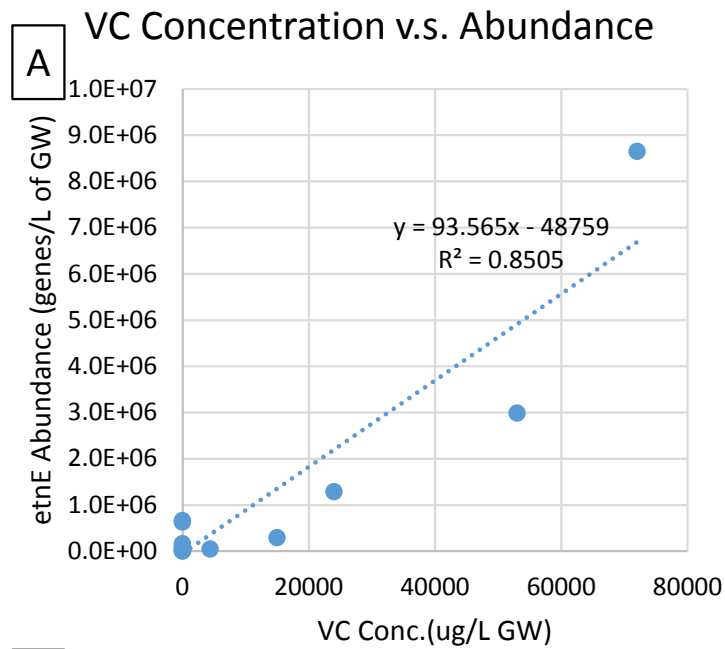


Figure S4. Linear regression of EaCoMT gene (*etnE*) abundance with vinyl chloride (VC) (A) and ethene (B) concentration. Each data point represents a sampling well from the sites surveyed in this study (Table 1).

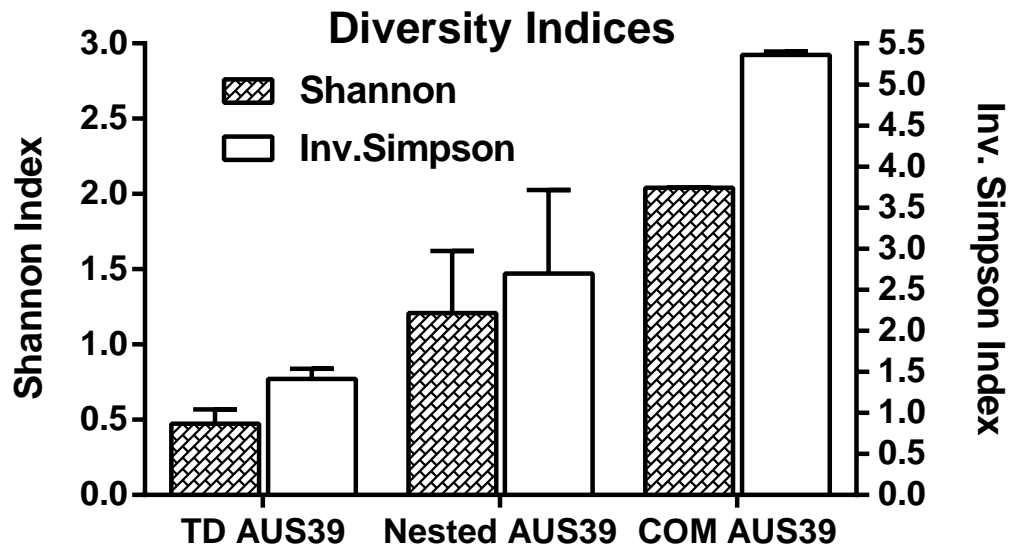


Figure S5. Analysis of potential bias introduced by touch down and nested PCR. A composite DNA sample from Australia (AUS39) was used for T-RFLP analysis of EaCoMT genes. Shannon-Wiener and inverse Simpson diversity indices (48) were calculated based on the T-RFLP profiles. Bar heights represent the average of duplicate T-RFLP analyses and the error bars represent the range. All the T-RFs <48 bp and >453 bp were excluded. The COM, Nested and TD designation indicates where the COM primer set, nested PCR and touchdown-nested PCR methods were used for T-RFLP.

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