

# Isotopic and Geochemical Assessment of in Situ Biodegradation of Chlorinated Hydrocarbons

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Currently there is no in situ method to detect and quantify complete mineralization of chlorinated hydrocarbons (CHCs) to CO<sub>2</sub>. Combined isotopic measurements in conjunction with traditional chemical techniques were used to assess in situ biodegradation of trichloroethylene (TCE) and carbon tetrachloride (CT). Vadose zone CHC, ethene, ethane, methane, O<sub>2</sub>, and CO<sub>2</sub> concentrations were analyzed using gas chromatography over 114 days at the Savannah River Site.  $\delta^{13}\text{C}$  of CHC and  $\delta^{13}\text{C}$  and  $^{14}\text{C}$  of vadose zone CO<sub>2</sub>, sediment organic matter, and groundwater dissolved inorganic carbon (DIC) were measured. Intermediate metabolites of TCE and CT accounted for  $\leq 10\%$  of total CHCs.  $\delta^{13}\text{C}$  of *cis*-1,2-dichloroethylene (DCE) was always heavier than TCE indicating substantial DCE biodegradation.  $^{14}\text{C}$ -CO<sub>2</sub> values ranged from 84 to 128 percent modern carbon (pMC), suggesting that plant root-respired CO<sub>2</sub> was dominant.  $^{14}\text{C}$ -CO<sub>2</sub> values decreased over time (up to 12 pMC), and contaminated groundwater  $^{14}\text{C}$ -DIC (76 pMC) was substantially depleted relative to the control (121 pMC).  $^{14}\text{C}$  provided a direct measure of complete CHC mineralization in vadose zone and groundwater in situ and may improve remediation strategies.

## Introduction

Field monitoring of chlorinated hydrocarbon (CHC) biodegradation is an essential component of any monitored natural attenuation program. Monitoring chlorinated solvent biodegradation is particularly difficult due to low transformation rates and complex microbial pathways that can potentially form daughter products that are more toxic than the parent compound. For instance, sequential reductive dechlorination of perchloroethylene (PCE) produces trichloroethylene (TCE), *cis*-1,2-dichloroethylene (DCE), vinyl chloride (VC), and eventually ethene and ethane in anaerobic environments (1). Laboratory radioisotope studies have shown that CO<sub>2</sub> is an important end product of chlorinated ethene anaerobic oxidation under varying redox conditions (2–4). In aerobic environments, microbes may quickly utilize DCE (5) or VC (6) as a sole carbon source or utilize methane and ethene as primary substrates for VC mineralization (7). Reductive dechlorination of carbon tetrachloride (CT) sequentially forms chloroform (CF), methylene chloride (MC), and eventually CO<sub>2</sub> (8, 9). It is difficult to demonstrate that

contaminants in situ are being biodegraded instead of attenuated by physical processes such as sorption to sediment, volatilization, or dilution. Biogeochemical indicators in groundwater (e.g., oxidation–reduction potential, terminal electron acceptors) and in vadose zone sediments (O<sub>2</sub> and CO<sub>2</sub>) are capable of detecting potential microbial processes but cannot differentiate between the degradation of natural carbon or contaminant carbon.

Recently,  $\delta^{13}\text{C}$  measurements of individual chlorinated ethenes have been shown to be a powerful tool for detecting CHC biodegradation in groundwater. Studies have shown that  $\delta^{13}\text{C}$  ratios are largely conserved when compounds undergo physical processes such as sorption, volatilization, or dissolution (10, 11). However, biological reductive dechlorination may invoke large reproducible fractionation generally following a Rayleigh type model. Laboratory studies have shown that the initial dechlorination product will be depleted in  $^{13}\text{C}$  relative to the corresponding precursor due to the microbial discrimination against  $^{13}\text{C}$  molecules (12–15). This isotopic fractionation increases with decreasing chlorination and can provide evidence of the occurrence and relative extent of microbial reductive dechlorination of chlorinated ethenes (12–15).

Currently there is no method to detect and quantify complete mineralization of CHCs in the field.  $\delta^{13}\text{C}$ -CO<sub>2</sub> (16–21) or  $^{14}\text{C}$ -CO<sub>2</sub> (22–24) measurements in the vadose zone or groundwater have been used to detect the complete aerobic and anaerobic biodegradation of petroleum hydrocarbons to CO<sub>2</sub> and may be equally effective in monitoring in situ CHC mineralization. Noncontaminant sources of CO<sub>2</sub> in the subsurface have a distinctive range of  $\delta^{13}\text{C}$  values and include atmospheric CO<sub>2</sub> (–7.4 to –12‰ (25)), root-respired CO<sub>2</sub> from C<sub>3</sub> (–22 to –33‰ (26)) or C<sub>4</sub> plants (–5.6 to –18.6‰ (27)), or microbial degradation of plant material. Theoretically,  $\delta^{13}\text{C}$ -CO<sub>2</sub> of CHC metabolites will be depleted in  $^{13}\text{C}$  relative to the precursor, but the  $\delta^{13}\text{C}$  of the precursor can vary widely ( $\pm 60\%$ ) (12–15). The effectiveness of  $\delta^{13}\text{C}$ -CO<sub>2</sub> to detect biodegradation is dependent on the (1)  $\delta^{13}\text{C}$  of the parent compound, (2) fractionation factor associated with biochemical metabolism, and (3) proportion of contaminant-derived CO<sub>2</sub> versus natural CO<sub>2</sub>.  $^{14}\text{C}$ -CO<sub>2</sub> may differentiate contaminant and natural carbon biodegradation because of vast differences in  $^{14}\text{C}$  activity between the two sources. All  $^{14}\text{C}$  has radioactively decayed (0 percent modern carbon (pMC)) in the CO<sub>2</sub> and methane produced from CHCs (synthesized from petroleum) and is vastly dissimilar to plant-derived or atmospheric inputs which are modern in  $^{14}\text{C}$  activity (>100 pMC).

The objectives of this field study were to assess biodegradation processes in (1) vadose zone sediments using traditional biogeochemical measurements (CHCs, O<sub>2</sub>, CO<sub>2</sub>) and isotopic measurements ( $\delta^{13}\text{C}$  of individual CHCs,  $\delta^{13}\text{C}$  and  $^{14}\text{C}$  of vadose zone CO<sub>2</sub>) and (2) in groundwater by measuring geochemical parameters, CHCs, and  $\delta^{13}\text{C}$  and  $^{14}\text{C}$  of DIC. We believe this field study is the first to report the use of combined isotopic methods ( $^{14}\text{C}$  and  $\delta^{13}\text{C}$  of CO<sub>2</sub> and

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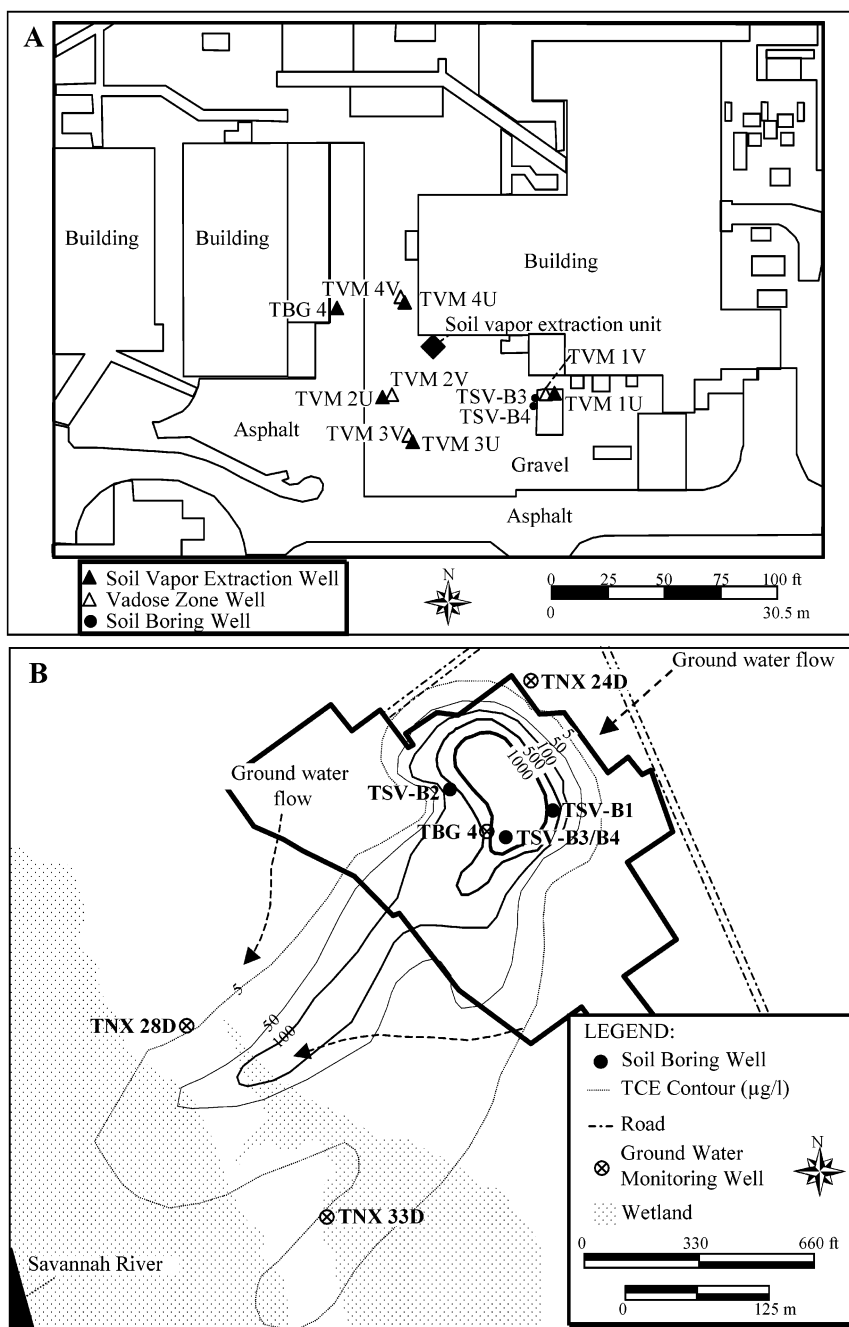


FIGURE 1. Site map of the TNX area at the Savannah River Site showing (A) the soil vapor extraction unit and well locations and (B) sediment boring locations and trichloroethylene (TCE) contours (29).

$\delta^{13}\text{C}$  of CHCs) to assess in situ CHC mineralization in vadose zone sediments and groundwater.

## Methods

**Site Characterization.** The field site was located in the TNX area at the Department of Energy's Savannah River Site (SRS) near Aiken, SC. Wastewater disposed in the TNX area contaminated shallow groundwater and sediments with a complex mixture of CHCs (Figure 1). TCE and CT are the major contaminants, and PCE, DCE, MC, CF, and trichlorofluoromethane (TCFM) (CFC-11) are detectable in many of the wells at lower concentrations (Table 1). The 200-m wide and 700-m long TCE plume flows southeast and discharges into the Savannah River located one-quarter mile from the TNX facilities (Figure 1B). No petroleum hydrocarbons have been detected in the study area. The TNX facility

is covered with asphalt, concrete, or gravel, and surrounding areas are a mixture of grasses and trees. The down-gradient flood plain is virtually an uncontaminated forested area containing various wetland shrubs and trees.

The effectiveness of SVE at the TNX site is being assessed as a remediation option for unsaturated sediment contamination. The SVE system utilized groundwater wells TVM-1U and 4U as vacuum extraction wells. Sediments collected by SRS personnel as part of this investigation were composed of interbedded sand, silty sand, and relatively thin clay layers (28). Sandy clay lenses were interspersed throughout the 21-m borings. Unsaturated sediments were comprised of densely packed stratified sands (well-sorted medium to fine grain) which exhibited relatively low intrinsic permeability ranging from 5.4 to 18.6 darcies ( $5.3 \times 10^{-8}$  to  $1.8 \times 10^{-7}$   $\text{cm}^2$ ) (29) (Table 2). The majority of TCE contamination in sediment

**TABLE 1. Groundwater Monitoring Results Collected from an Up-Gradient Control Well (TNX-24D), Source (TBG-4), and Relatively Uncontaminated Down-Gradient Wells (TNX-28D and 33D) at the TNX Area of the Savannah River Site**

parameter	units	TBG-4	TNX-24D	TNX-28D	TNX-33D
date of collection		6/7/2001	6/28/2001	6/28/2001	6/28/2001
dissolved O <sub>2</sub>	mg/L	2.4	9.2	7.1	7.7
dissolved inorganic carbon (DIC)	mg CO <sub>2</sub> /L	43	65	52	81
alkalinity	mg/L-CaCO <sub>3</sub>	0	12	8	5
ammonia	mg/L (NH <sub>4</sub> -N)	0.02	0.00	0.00	0.01
nitrate	mg/L (NO <sub>3</sub> -N)	21.70	1.60	0.56	3.81
nitrite	mg/L (NO <sub>2</sub> -N)	0.005	0	0.001	0.001
total iron	mg/L	0.01	0.11	0	0.05
ferrous iron	mg/L	0	0	0	0
ferric iron	mg/L	0.01	0.11	0	0.05
sulfate	mg/L	0	39.2	34.1	29.9
sulfide	mg/L	0.008	0	0	0
chloride	mg/L	3.07	11.48	1.95	9.24
conductivity	μS/cm	305	99	44	112
pH		4.5	5.4	5.3	5.3
pE <sup>a</sup>	mV	210	158	151	157
temperature	°C	25.0	25.1	19.8	19.2
PCE	μg/L	18.70 ± 1.4	0.00	0.02 ± 0.03	0.00
TCE	μg/L	149.80 ± 17.5	0.02 ± 0.01	0.10 ± 0.01	10.10 ± 0.2
<i>cis</i> -1,2-DCE	μg/L	22.30 ± 3.5	0.00	0.00	0.08 ± 0.02
1,1-DCE	μg/L	0.17 ± 0.01	0.00	0.00	0.00
<i>trans</i> -1,2-DCE	μg/L	0.39 ± 0.05	0.00	0.00	0.00
VC	μg/L	0.11 ± 0.01	0.00	0.00	0.00
CT	μg/L	33.8 ± 1.8	0.00	0.00	0.013 ± 0.004
CF	μg/L	0.00	0.00	0.00	0.00
MC	μg/L	0.00	0.00	0.00	0.00
ethene	μg/L	0.00	0.00	0.00	0.00
ethane	μg/L	0.01	0.00	0.00	0.00
methane	μg/L	0.045 ± 0.0035	0.10	0.00	0.00
<sup>14</sup> C-DIC	pMC	76	121	106	113
δ <sup>13</sup> C-DIC	‰	-20.1	-21.1	-21.3	-22.8

<sup>a</sup> Measured > 24 h after collection.

**TABLE 2. Subsurface Hydrogeological Parameters at the Savannah River Site (28, 29)**

parameter	units	value
<b>Shallow Groundwater</b>		
horizontal hydraulic conductivity ( $K_h$ )	cm/s	$2.3 \times 10^{-2}$
vertical hydraulic conductivity ( $K_v$ )	cm/s	$1.1 \times 10^{-2}$
specific yield ( $S_y$ )		0.29
storativity (S)		0.067
effective porosity		0.15
pore velocity	cm/s	$1.1 \times 10^{-3}$
horizontal gradient	ft/ft	0.007
sand:silt:clay	%	89:00:11
soil texture		sand
bulk density	g/mL	1.56
particle density	g/mL	2.65
pore space	%	41
<b>Unsaturated Sediment</b>		
intrinsic permeability (average)	Darcies	8.42
vertical permeability ( $K_z$ )	Darcies	1.68
transmissivity	ft <sup>2</sup> /min	0.023–0.076
SVE radius of influence	m	12–33
water table	m	13
sand:silt:clay	%	90:00:10
soil texture		sand
bulk density	g/mL	1.35
particle density	g/mL	2.63
pore space	%	49

was 14–18 m below ground surface (bgs) (data not shown). Sedimentary carbonates that can influence <sup>14</sup>C values were absent in these shallow sediments (30).

**Sample Collection and Analysis.** Vadose zone soil gas samples were collected between 12/18/00 and 4/11/01 and coincided with SVE shut-down. Samples were collected from source wells TVM-1V, 1U, 2V, 2U, 3U, 4V, 4U, and TNX-28D

and 33D twice weekly initially, followed by at least twice monthly for 114 days (Figure 1A). TVM-2U data were excluded due to a leaking well cap discovered on day 30. The groundwater monitoring wells designated “U” have 3-m screens with approximately 1.5 m in the vadose zone at 12-m bgs and 1.5 m in the saturated zone. Vadose zone monitoring wells labeled “V” have 0.3-m screens located approximately 10-m bgs. Flood plain groundwater wells TNX-28D and 33D were installed at a total depth of 4.6 m with a screened section between 1.5- and 4.6-m bgs. An increase in groundwater elevation prevented vadose zone sampling of the flood plain wells after day 58, and TVM-3U was only periodically sampled due to processing time constraints.

Vadose zone air samples were collected by attaching a PVC cap fitted with a 1/4-in. Swagelok fitting to the top of the well. A small vacuum pump was attached to the PVC cap, and at least three well volumes of air were purged before collection of the sample into 1- or 5-L Tedlar bags. Soil air samples were analyzed for O<sub>2</sub>, CO<sub>2</sub>, methane, ethene, ethane, PCE, TCE, DCEs, VC, CT, CF, MC, and TCFM or processed for δ<sup>13</sup>C and <sup>14</sup>C of CO<sub>2</sub> in our laboratory within 12 h after collection. Also, gas samples were screened at each time point for petroleum hydrocarbons that may interfere with carbon isotopic ratios, but no petroleum hydrocarbons were detected in the TNX area. Detection limits for benzene, toluene, ethylene benzene, and xylene were 1 ppb (v/v).

O<sub>2</sub> and CO<sub>2</sub> concentrations were measured using a Varian CP-3800 gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) and a 60/80 Carboxen 1000 column (15 ft × 1/8 in. ss, Supelco). Detection limits were approximately 0.1 and 0.03% (v/v), respectively. Methane, ethane, and ethene concentrations were measured using a Varian 3700 GC equipped with a flame ionization detector (FID) and a 60/80 Carbopack B column packed with 1% SP 1000 (8 ft × 1/8 in. ss, Supelco). The analysis of chlorinated

and petroleum hydrocarbons was performed on a Varian 3800 GC/Saturn 2000 mass spectrometer (MS) with a Varian CP-8200 autosampler equipped for solid-phase micro-extraction (SPME) with a SPME fiber coated with 75- $\mu$ m Carboxen/poly(dimethylsiloxane) (Supelco, Bellefonte, PA). Details of all analyses are reported in Supporting Information.

Groundwater samples were collected by SRS personnel from TBG 4 located in the source area on 6/07/01 (Figure 1A). Control well TNX-24D and down-gradient wells TNX-28D and 33D were sampled on 6/27/01 (Figure 1B). Field measurements by SRS personnel included dissolved O<sub>2</sub>, pH, conductivity, and temperature. The following day in our laboratory, groundwater DIC was extracted and purified for isotopic analysis, and geochemical parameters DIC, nitrate, nitrite, sulfate, sulfide, total iron, iron(II), iron(III), chloride, and ammonia were measured using colorimetric analysis (Hack Company; Loveland, CO).

Sediment samples were processed for carbon isotopic analysis using quartz tubes and copper oxide wire (31, 32). The resulting CO<sub>2</sub> from sediment combustion, vadose zone CO<sub>2</sub>, and CO<sub>2</sub> stripped from groundwater samples was released into a purification line in a He stream and purified cryogenically and chemically using liquid nitrogen (-196 °C), pentane slush (-129 °C), ethyl alcohol slush traps (-117 °C), and magnesium perchlorate. The purified CO<sub>2</sub> was measured using a Baratron (MKS Instruments) capacitance manometer and sealed under vacuum in 6-mm Pyrex ampules and sent to an analytical isotope laboratory for  $\delta^{13}\text{C}$  or  $^{14}\text{C}$  analysis. The mean  $\delta^{13}\text{C}$  value for a known CO<sub>2</sub> isotopic standard ( $-44.06 \pm 0.04\text{‰}$ ) processed identically as the vadose zone samples was  $-43.99 \pm 0.07$  (relative percent recovery =  $99.85 \pm 0.16$ ;  $n = 6$ ) and verified that limited fractionation occurred during sample processing. Stable carbon isotope ratios were reported in the standard  $\delta$  notation in per mil (‰) as

$$\delta(\text{‰}) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \quad (1)$$

where  $R$  is the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  and the  $\delta^{13}\text{C}$  standard is the belemnite fossil of the Cretaceous Peedee formation of South Carolina (PDB). The  $^{14}\text{C}$  activity was expressed as pMC and is the ratio of the activity of the sample to the corrected activity of the oxalic acid standard (33).  $^{14}\text{C}$  precision was  $\pm 0.9$  pMC.

Vadose zone air samples collected from wells TVM-1U, 2V, 2U, and 4V on day 70 and TVM-1V and 4V collected on day 80 were sent to the University of Waterloo, Canada, for  $\delta^{13}\text{C}$  analysis of PCE, TCE, DCE, and CT. A combination of SPME-GC-isotope ratio MS allowed  $\delta^{13}\text{C}$  analysis of low concentrations of CHCs in vadose zone air (Supporting Information).

**Statistical Analysis.** All statistical analyses were performed using SAS (34), with 0.05 significance level. A mixed model was utilized to perform an analysis of covariance (ANACOVA) to determine if there were significant differences in the rate of CO<sub>2</sub> production and O<sub>2</sub> depletion between source wells. The mixed model is a general linear model modified to account for repeated sampling from each well. A general linear model also was utilized to determine the regression coefficients for O<sub>2</sub> depletion and CO<sub>2</sub> production. All correlations were calculated using Pearson's correlation.

## Results and Discussion

**Sediment Characterization.** Geochemical and isotopic analysis of sediment organic matter (SOM) suggested that SOM mineralization to vadose zone CO<sub>2</sub> would be insignificant at the monitored depth (Table 3). Total organic carbon was low (8–136 mg/kg) and averaged  $22 \pm 3$  mg/kg at 9-m bgs.  $\delta^{13}\text{C}$

**TABLE 3. Geochemical Analysis of Vadose Zone and Saturated (>13 m-bgs) Sediment Collected in the Source Area**

well	depth (m-bgs)	total organic carbon (mg/kg)	$\delta^{13}\text{C}$ (‰)	$^{14}\text{C}$ (pMC)
TSV-B1	9	20	-24.5	
TSV-B2	3	136	-24.7	
TSV-B2	6	66	-25.3	
TSV-B2	9	21 $\pm$ 3	-23.5	174
TSV-B2	12	43	-25.3	
TSV-B2	15	14	-25.7	
TSV-B2	18	8 $\pm$ 1	-25.1	88
TSV-B3	3	14	-21.1	
TSV-B3	6	2	-21.3	
TSV-B3	9	23 $\pm$ 6	-24.8	168
TSV-B3	12	27	-26.1	
TSV-B3	15	99	-25.3	
TSV-B4	9	8 $\pm$ 9	-26.8	

of SOM averaged  $-24.6 \pm 1.7\text{‰}$  which is typical of C<sub>3</sub> vegetation.  $^{14}\text{C}$  values of SOM in the vadose zone were significantly higher than SOM in the saturated zone at 18 m-bgs (Table 3). SOM- $^{14}\text{C}$  values at 9-m bgs (168–174 pMC) resembled atmospheric levels from the 1960s when atmospheric CO<sub>2</sub> was highly contaminated with artificial  $^{14}\text{C}$  from above-ground nuclear weapons tests. Maximum increases in atmospheric  $^{14}\text{C}$  content occurred in 1961 and 1962 (35) and  $^{14}\text{C}$  concentrations in plant tissue reached a peak (up to ca. 200 pMC) in 1964 and declined thereafter (36). It also is possible that these elevated  $^{14}\text{C}$  values reflect releases of radiocarbon from SRS during its period of nuclear materials production. The  $^{14}\text{C}$  value of SOM in the saturated zone (88 pMC) corresponded to an age of  $1060 \pm 45$  yrs old and suggested that the limited amount of sediment organic carbon present was recalcitrant and not a significant contributor to DIC.

**Groundwater Analysis.** Geochemical, contaminant, and carbon isotopic analysis suggested that reductive dechlorination of chlorinated solvent and complete mineralization to CO<sub>2</sub> was occurring in groundwater located in the source area (Table 1). Available electron acceptors were O<sub>2</sub> and nitrate. Groundwater DO in the source area (TGB-4) was significantly depleted (2.4 mg/L) compared to control well TNX-24D which was near saturation (9.2 mg/L). Nitrate, a reported contaminant at this site (28), was elevated in TBG-4 (21.7 mg/L), and nitrite was measurable compared to control well TNX-24. Chloride was not a good indicator of reductive dechlorination with greater chloride concentrations in the control well (11.5 mg/L) than the source or down-gradient wells (1.9–9.2 mg/L).

DCEs accounted for 10.2% of CHC concentration, and trace levels of ethane in TBG-4 suggested complete but limited reductive dechlorination. CT concentrations in all wells were relatively low (0.01–33.8  $\mu\text{g/L}$ ) compared to vadose zone concentrations (up to 3.3 ppm in TVM-1V), and no CT metabolites were detected.

A depleted  $^{14}\text{C}$ -DIC value of 76 pMC in TBG-4 compared to 121 pMC for the control well DIC indicated that CHC mineralization was occurring in groundwater within the source area.  $\delta^{13}\text{C}$  was also enriched by 1.0 ‰ in TBG-4 compared to TNX-24D, but more data from groundwater wells in the source area are necessary to assess the variation of  $\delta^{13}\text{C}$ -DIC values.

**Vadose Zone Geochemical Analysis.** O<sub>2</sub> and CO<sub>2</sub>. Vadose zone O<sub>2</sub> and CO<sub>2</sub> were measured after SVE shut-down in an effort to detect microbial chlorinated solvent mineralization. The vadose zone within the source area was oxygenated to near atmospheric levels either naturally or induced by previous SVE operation (Figure 1, Supporting Information). CO<sub>2</sub> concentrations at day 0 were elevated between 2.0 and

TABLE 4. Regression and Correlation Results of Vadose Zone O<sub>2</sub>, CO<sub>2</sub>, <sup>14</sup>C, and δ<sup>13</sup>C Production and Consumption Rates Measured over Time in the TNX Area Source Wells and Relatively Uncontaminated Peripheral Flood Plain Wells at the Savannah River Site

well ID	parameter	CO <sub>2</sub> or O <sub>2</sub> (% d <sup>-1</sup> )	CO <sub>2</sub> or O <sub>2</sub> reg. (r)	O <sub>2</sub> -CO <sub>2</sub> corr. (r)	<sup>14</sup> C (pMC d <sup>-1</sup> )	<sup>14</sup> C reg. (r)	δ <sup>13</sup> C (‰ d <sup>-1</sup> )	δ <sup>13</sup> C reg. (r)	δ <sup>13</sup> C-CO <sub>2</sub> corr. (r)
Source Wells									
TVM-1V	O <sub>2</sub>	-0.007	-0.29						
	CO <sub>2</sub>	0.003	0.09	-0.94 <sup>a</sup>	-0.13	-0.90	0.010	0.71	0.69 <sup>a</sup>
TVM-1U	O <sub>2</sub>	-0.006	-0.55						
	CO <sub>2</sub>	0.003	0.40	-0.73 <sup>a</sup>	-0.07	-0.89	0.001	0.09	0.84 <sup>a</sup>
TVM-2V	O <sub>2</sub>	-0.007	-0.51						
	CO <sub>2</sub>	0.004	0.76	-0.71 <sup>a</sup>	-0.23	-0.99	-0.015	-0.77	-0.60
TVM-3U	O <sub>2</sub>	-0.002	-0.11						
	CO <sub>2</sub>	0.005	0.56	-0.73	-0.28	1.00	-0.011	-0.57	0.92
TVM-4V	O <sub>2</sub>	0.003	0.24						
	CO <sub>2</sub>	-0.006	-0.64	-0.60 <sup>a</sup>	-0.09	-0.45	0.003	0.33	-0.02
TVM-4U	O <sub>2</sub>	-0.003	-0.46						
	CO <sub>2</sub>	-0.001	0.32	-0.94 <sup>a</sup>	-0.15	-0.60	-0.0003	-0.24	0.53
Flood Plain Wells									
TNX-28D	O <sub>2</sub>	0.006	0.40						
	CO <sub>2</sub>	0.002	0.63	-0.45	-0.10	-0.98	-0.001	-0.44	-0.32
TNX-33D	O <sub>2</sub>	-0.069	-0.92						
	CO <sub>2</sub>	0.013	0.68	-0.90 <sup>a</sup>	-0.05	-0.73	-0.024	-0.59	-0.49

<sup>a</sup> *p*-value < 0.05.

4.1% in the source wells and suggested that the surrounding vadose zone air that was pulled into the monitored area via SVE was elevated in CO<sub>2</sub> concentration (Figure 1A–C, Supporting Information). CO<sub>2</sub> concentrations and δ<sup>13</sup>C values (discussed below) were typical of plant root respiration (37, 38) and probably the main source of CO<sub>2</sub> within these sandy sediments with limited organic matter.

CO<sub>2</sub> production and a concurrent depletion of O<sub>2</sub> were statistically measured in all but three wells (TVM-4V, TVM-4U, and TNX-28D) (Table 4). A statistical difference in the production of CO<sub>2</sub> was not detected between individual wells (*p*-value = 0.45) or wells grouped by depth (*p*-value = 0.83). CO<sub>2</sub> production rates were low (0 to 0.013% (v/v) d<sup>-1</sup>) and suggested aerobic biodegradation of contaminants was either limited or that contaminant concentrations were too low for this GC monitoring technique to effectively measure their oxidation to CO<sub>2</sub>.

**Contaminant Monitoring.** TCE metabolites (DCE, VC, ethene, and ethane) and CT metabolites (CF and MC) were detected in low concentration in the majority of source wells and suggested that the indigenous microbial community was capable of anaerobically metabolizing the CHC mixture via reductive dechlorination under bulk aerobic conditions (Figure 2 and Figures 2 and 3, Supporting Information). This field site was not ideal for reductive dechlorination with low organic matter and oxygenated sandy sediments. Reductive dechlorination probably only occurred within anaerobic microenvironments of sediment pores. Ellis et al. (39) also described a field study where anaerobic processes occurred in dominantly aerobic groundwater, and they concluded that the anaerobic processes potentially occurred at the micropore level. Minimal methanogenesis was detected at this site with the highest average methane concentration in TVM-2V of 4.5 ppm (v/v) (data not shown).

The highest concentrations of contaminants were measured in source wells TVM-1U, 4V, and 4U with maximum TCE and CT concentrations of 12.7 ppm in TVM-1U (Figure 2B, Supporting Information) and 3.3 ppm in TVM-4V (Figure 3C, Supporting Information), respectively. A strong positive correlation between TCE, DCE, and CT concentrations over time in TVM-1V and 1U (*r*<sup>2</sup> = 0.61–0.93, *p*-values < 0.0015) suggested these compounds were volatilizing into the soil air after SVE shut-down at day 0 (Figure 2, Supporting Information). This physical diffusion process, termed con-

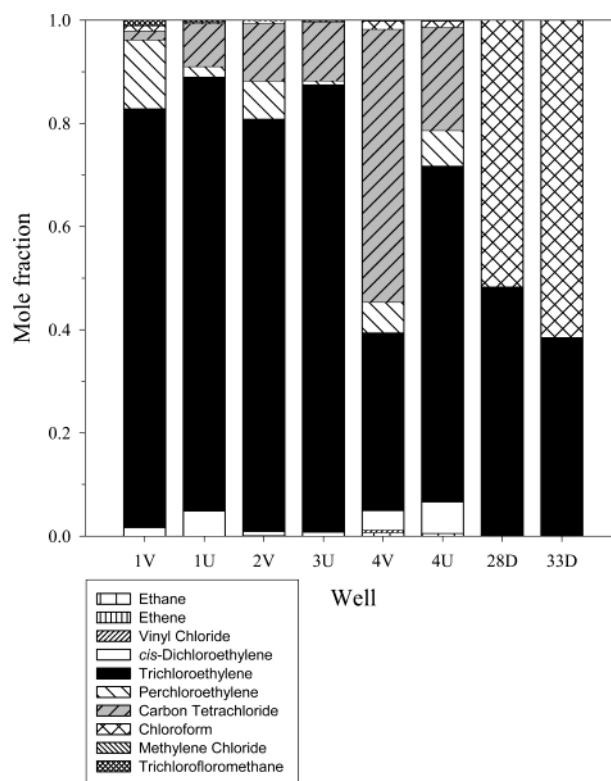


FIGURE 2. Molar fractions of CHC and metabolite concentrations within each vadose zone well on day 114. The mole fractions were calculated by dividing the concentration of each compound by the total concentration of chlorinated hydrocarbons and metabolites.

taminant “rebound”, is common during SVE shut-down at petroleum contaminated sites (40). Similar positive statistical correlations between TCE, DCE, and CT were detected in all source wells (data not shown). Thus, measurement of the reduction of CHC concentrations over time due to biodegradation was inhibited due to the physical diffusion of contaminants.

The extent of TCE and CT reductive dechlorination was examined by comparing the ratio of each contaminant to the total CHC concentration of its known biodegradative

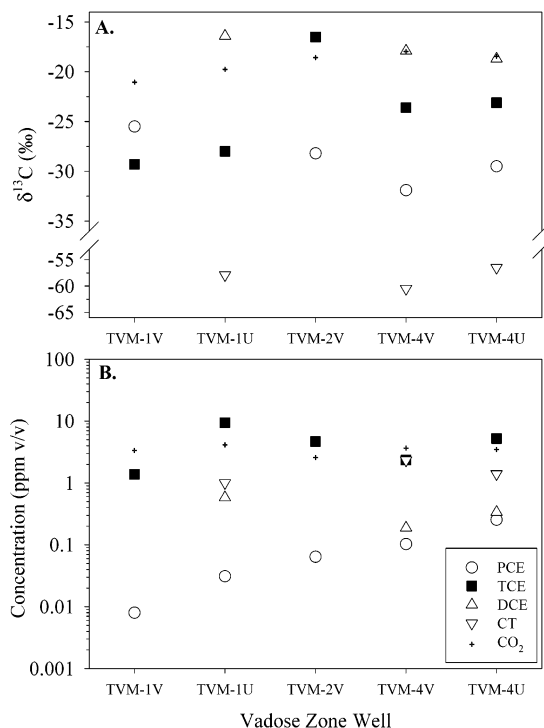


FIGURE 3. (A)  $\delta^{13}\text{C}$  values of perchloroethylene (PCE), trichloroethylene (TCE), *cis*-1,2-dichloroethylene (DCE), carbon tetrachloride (CT), and carbon dioxide ( $\text{CO}_2$ ) and (B) PCE, TCE, DCE, CT, and  $\text{CO}_2$  concentrations in vadose zone wells measured on day 70 or 80 (TVM-1V and TVM-4V) at the Savannah River Site.

pathway. Reductive dechlorination of TCE and CT was minimal with the metabolites accounting for less than 10% of total CHC concentration (Figure 2). CF was the main metabolite of CT reductive dechlorination and accounted for 4–5% of the total CT, CF, plus MC concentration, whereas MC accounted for only 0.04–0.07%. Similar TCE dechlorination ratios were measured with DCE accounting for 5–8% of the total TCE, DCE, VC, ethene, plus ethane concentration, whereas VC accounted for 0.01–0.8%. VC was detected on day 37 and complete transformation to ethene and ethane was not detected until day 98 when contaminant concentrations were highest after contaminant revolatilization (data not shown). Undetectable or low VC concentrations suggested either limited anaerobic transformation after DCE was produced, or rapid oxidation of VC to  $\text{CO}_2$ , both of which have been reported in laboratory studies (1).

**Vadose Zone Isotope Analysis.**  $\delta^{13}\text{C}$  of Contaminants. Assessment of in situ reductive dechlorination was accomplished by measuring the  $\delta^{13}\text{C}$  of individual chlorinated compounds in the vadose zone. Monitoring contaminant concentration alone was successful in detecting reductive dechlorination of TCE and CT but was limited in assessing the extent of biodegradation.  $\delta^{13}\text{C}$  measurement of contaminants showed that DCE was always heavier than TCE by approximately 4–12‰ (Figure 3A). This suggested that a substantial amount of DCE had been degraded either via oxidation in the bulk aerobic vadose zone or via reductive dechlorination in the anaerobic microniches followed by oxidation of VC, ethene, and ethane in the bulk aerobic vadose zone. Microcosm studies have shown the initial DCE produced is depleted in  $^{13}\text{C}$  relative to its parent compound (13–15). Over time, the carbon of DCE and metabolites becomes enriched in  $^{13}\text{C}$  during extensive reductive dechlorination. Thus, extensive reductive dechlorination of DCE was indicated by  $\delta^{13}\text{C}$  analysis, and DCE concentration may not be a good quantitative indicator for the progress of biodegradation.

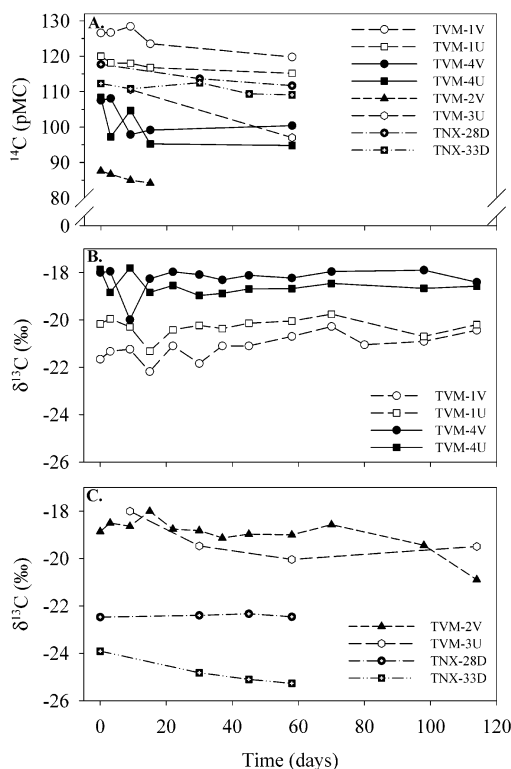


FIGURE 4. Vadose zone  $\text{CO}_2$  values of (A)  $^{14}\text{C}$  (in percent modern carbon (pMC)), and (B) and (C)  $\delta^{13}\text{C}$  measured over 58 or 114 days in source (TVM) and flood plain (TNX) wells at the Savannah River Site.

$\delta^{13}\text{C}$  of PCE ranged from  $-31.9$  to  $-25.5$ ‰ and was inversely correlated with concentration ( $r = -0.53$ ) with higher  $\delta^{13}\text{C}$  values measured in wells with lower PCE concentrations (Figure 3A,B). PCE is a “parent” compound in that no other compound is being degraded to produce PCE, similar to CT at this site. Thus, as microbes discriminate against the  $^{13}\text{C}$ -PCE molecule during reductive dechlorination, the residual PCE becomes enriched in  $^{13}\text{C}$  (13–15). The measured  $\delta^{13}\text{C}$  enrichment of PCE suggested that substantial reductive dechlorination of PCE was occurring at this site.

CT was highly depleted in  $^{13}\text{C}$  relative to PCE, TCE, and DCE and measured between  $-51.4$  and  $-60.5$ ‰ (Figure 3A).  $\delta^{13}\text{C}$  increased as CT concentration decreased ( $r = -0.80$ ) suggesting the concentration decrease may be attributed to biodegradation. No previous measurement of  $\delta^{13}\text{C}$ -CT has been reported in the literature to the authors’ knowledge. It was unclear whether microbial processes were solely responsible for the overall depletion of  $^{13}\text{C}$  or if depletion was also due to the manufacturing process. We measured the  $\delta^{13}\text{C}$  value of commercial CT (Supelco, Bellefonte, PA) and found relatively depleted values ( $-39.1 \pm 0.3$ ‰) compared to reported TCE ( $-31$  to  $-27$ ‰) and PCE ratios ( $-37$  to  $-23$ ‰ (41)) which suggested that a proportion of  $^{13}\text{C}$ -CT depletion may be attributed to the manufacturing process.

$^{14}\text{C}$  and  $\delta^{13}\text{C}$  of Vadose Zone  $\text{CO}_2$ .  $^{14}\text{C}$  values of vadose zone  $\text{CO}_2$  decreased over time in all contaminated source and flood plain wells (Figure 4A). This measured depletion in  $^{14}\text{C}$  suggested chlorinated solvents were completely aerobically or anaerobically biodegraded to  $\text{CO}_2$  over time because no other significant depleted carbon source, such as carbonate minerals or petroleum hydrocarbons, was detected at this site. Also, fractionation due to carbon isotopic exchange or equilibrium processes would be minimal in these acidic sediments (42) and thus not account for measured  $^{14}\text{C}$  reductions over time (up to 12 pMC in TVM-3U). To our

knowledge, these results are the first to provide qualitative evidence of complete in situ biodegradation of CHCs to CO<sub>2</sub>.

<sup>14</sup>C values varied between well locations and ranged from 84 to 128 pMC in TVM-2V and TVM-1V, respectively (Figure 4A). Overall, <sup>14</sup>C values were near or above modern activity and suggested that the majority of vadose zone CO<sub>2</sub> was from natural background inputs, particularly plant root respiration as also suggested by SOM geochemical and isotopic analysis. <sup>14</sup>C-CO<sub>2</sub> values in TVM-1V and 1U were initially elevated above measured atmospheric levels at this site (108 pMC). The source of elevated <sup>14</sup>C activity was unclear with numerous natural and anthropogenic possibilities. Ideally, several nearby control wells installed in uncontaminated areas could provide further insight into CO<sub>2</sub> dynamics, but a relatively large contaminant plume and strict drilling and sampling restrictions at this DOE facility limited available control sites.

<sup>14</sup>C-CO<sub>2</sub> measurement was effective in detecting chlorinated solvent mineralization even though CO<sub>2</sub> was naturally elevated which may have diluted CO<sub>2</sub> derived from contaminant mineralization. TVM-2V and 3U had markedly lower CO<sub>2</sub> concentrations (Figure 1B, Supporting Information) and had between 1.5 and 4 times higher rates of <sup>14</sup>C depletion over time compared to other source wells (Table 4). Small inputs of contaminant-derived CO<sub>2</sub> (0 pMC) produced during biodegradation would have a greater effect in TVM-2V and 3U compared to the other source wells which have greater CO<sub>2</sub> concentrations.

<sup>δ13</sup>C-CO<sub>2</sub> trends over time were less pronounced compared to <sup>14</sup>C values (Figure 4B,C). <sup>δ13</sup>C-CO<sub>2</sub> values from natural contributions were elevated at this site and may have masked contaminant-derived CO<sub>2</sub>. <sup>δ13</sup>C of TCE (−16 to −29‰) and DCE (−16 to −19‰) was similar to the range of <sup>δ13</sup>C-CO<sub>2</sub> from root-respired plants (−5.6 to −33‰ (26, 27)). The clearest indication of contaminant mineralization was measured in TVM-1V and 1U with a statistically significant, positive correlation between <sup>δ13</sup>C values and CO<sub>2</sub> production over time (r = 0.69 and 0.84, respectively) (Table 4). This <sup>δ13</sup>C-CO<sub>2</sub> correlation corresponded to the measured depletion of <sup>14</sup>C over the same time scale indicating that a <sup>14</sup>C depleted carbon source with a relatively enriched <sup>13</sup>C value was being mineralized. Laboratory studies have demonstrated DCE and VC may become enriched in <sup>δ13</sup>C through extensive microbial reductive dechlorination processes producing <sup>δ13</sup>C values of these metabolites near or greater than 0‰ (12–15). <sup>δ13</sup>C measurement of DCE in TVM-1U (−16‰) confirmed <sup>13</sup>C enrichment relative to CO<sub>2</sub> (Figure 3A) and thus DCE oxidation would match this scenario assuming limited DCE fractionation during mineralization.

<sup>14</sup>C-CO<sub>2</sub> measurement over time identified in situ biodegradation of chlorinated solvents to CO<sub>2</sub> which had previously only been detected in laboratory studies. <sup>14</sup>C analysis was more effective than geochemical measurements (CO<sub>2</sub> and O<sub>2</sub>) in detecting biodegradation and was successful even though CO<sub>2</sub> was naturally elevated which may dilute CO<sub>2</sub> derived from contaminant mineralization. <sup>δ13</sup>C measurement of contaminants provided further insight into the extent of biodegradation over that of traditional contaminant monitoring alone. Monitoring <sup>14</sup>C and the <sup>δ13</sup>C of contaminants worked effectively in vadose zone wells with varying contaminant and CO<sub>2</sub> concentrations demonstrating that it was a valuable field monitoring technique for microbial degradation of chlorinated solvents. The continued development of these field monitoring techniques may provide direct measures of microbial degradation of chlorinated pollutants in groundwater and subsurface sediments and improve remediation strategies at contaminated facilities.

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## Supporting Information Available

Method details for analysis of O<sub>2</sub>, CO<sub>2</sub>, methane, ethene, ethane, CHC, and <sup>δ13</sup>C of CHCs; O<sub>2</sub> and CO<sub>2</sub> concentration over time (Figure 1 SI), CHC concentration measured over time in vadose zone wells TVM-1V and 1U and TVM-4V and 4U (Figures 2 and 3 SI, respectively). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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