Radiocarbon Assessment of Aerobic Petroleum Bioremediation in the Vadose Zone and Groundwater at an AS/SVE Site

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Radiocarbon (^{14}C) measurement from vadose zone air and groundwater is an alternative technique to quantitatively estimate aerobic microbial CO_2 production from petroleum mineralization. The objectives of this study were (1) to demonstrate the effectiveness of radiocarbon in providing direct quantified evidence of *in situ* aerobic petroleum hydrocarbon biodegradation from vadose zone CO_2 and groundwater DIC at a gasoline-contaminated site being remediated by an air sparging/soil vapor extraction (AS/SVE) system and (2) to quantify the microbial contribution to vadose zone CO_2 and DIC production. Vadose zone CO_2 and groundwater DIC were collected from soil vapor probes, SVE wells, AS/SVE stack exhaust, and groundwater monitoring wells. Analogous gas and groundwater samples were collected from a geologically similar uncontaminated site. Vadose zone CO_2 and DIC extracted from groundwater were concentrated using NaOH traps and sent to analytical laboratories for conventional ^{14}C analysis using β counters. ^{14}C values ranged from 15.9 to 47.7 percent modern carbon (PMC) and demonstrated isotopic depletion from aerobic microbial mineralization of the petroleum. Aerobic biodegradation was calculated to account for 59−87% of the CO_2 produced. ^{14}C analysis clearly illustrated biodegradation from one time point and even in samples of low CO_2 content after CO_2 was concentrated.

Introduction

Detecting and quantifying biodegradation of petroleum products (gasoline, diesel, jet fuel, heating oils, etc.) in the vadose zone and in groundwater has been a focus of many recent investigations (*1*−*4*). Monitoring hydrocarbon mineralization and demonstrating that microbial remediation is occurring in the subsurface is a vital part of a remediation program. It is a difficult task, however, to show that the disappearance of hydrocarbons is attributable to microbial degradation instead of physical processes such as sorption, volatilization, transformation, or dilution. Providing evidence of microbial degradation under field conditions is commonly achieved by monitoring soil gas CO_2, O_2, and contaminant concentrations over time (*5*, *6*). An increase in CO_2 in conjunction with a decrease in O_2 and contaminant concentrations over time has been used to estimate rates of petroleum biodegradation for bioremediation, bioventing, and soil vapor extraction (*7*, *8*). A disadvantage of soil gas monitoring is that it may require many data points over time to show that microbial mineralization is occurring in the subsurface. It may be difficult or impossible to differentiate microbially derived CO_2 from geochemical or plant-derived soil gas CO_2 if concentrations are near natural background or atmospheric levels.

Recently, stable carbon isotopes have been employed to verify petroleum biodegradation by comparing the $\delta^{13}C$ values in a remediation area to uncontaminated $\delta^{13}C$ values (*9*−*13*). Petroleum-hydrocarbon $\delta^{13}C$ signatures are between −34 and −20‰ and gasoline has a $\delta^{13}C$ value of approximately −27 ± 2.3‰ (*14*). During aerobic mineralization of petroleum hydrocarbons to CO_2, ^{13}C has been suggested to fractionate approximately 3‰ heavier than the source material (*12*).

The sources of $\delta^{13}C$ in the subsurface include C_3 and C_4 plants and the atmosphere. Most plants use the Calvin (C_3) cycle of photosynthesis which produces root respired CO_2 values and organic matter with $\delta^{13}C$ values within the range −25 ± 5‰ (*15*−*17*). Plants that use the Hatch−Slack (C_4) photosynthetic pathway generally inhabit deserts and tropical or subtropical grasslands and yield $\delta^{13}C$ values ranging from −5.6 to −18.6‰ (*18*). Atmospheric CO_2 has stable carbon isotopic values of −7.4 to −12‰ diurnally shifting and depending on the amount of air pollution (*19*).

Employment of stable carbon isotopes may become problematic when they are applied to a site located in a temperate, nonarid region where C_3 vegetation dominates and where petroleum hydrocarbons are aerobically biodegraded. Stable carbon isotope results may be difficult to interpret because of the overlap between $\delta^{13}C$ values characteristic of aerobic petroleum biodegradation and those of root respiration or natural degradation of soil organic matter in temperate, nonarid regions.

In areas where C_3 vegetation dominates, radiocarbon (^{14}C) measurement of vadose zone CO_2 and groundwater dissolved inorganic carbon (DIC) is an alternative technique which better identifies the origin of microbial CO_2 and gives a quantitative estimate of microbial CO_2 production from aerobic petroleum mineralization. The primary source of ^{14}C is the transitional region between the stratosphere and the troposphere where ^{14}C is formed through a nuclear reaction between secondary cosmic ray neutrons and nitrogen nuclei. The ^{14}C atoms oxidize to form $^{14}CO_2$ molecules and become mixed within atmospheric CO_2 and subsequently enter the bio- and hydrosphere. Atmospheric CO_2 has been highly contaminated with artificial ^{14}C since the mid-1950s from above-ground nuclear weapons tests. Maximum increases in atmospheric ^{14}C content occurred in 1961 and 1962 (*20*), and ^{14}C concentrations in plant tissue reached a peak (up to ca. 200 PMC) in 1964 and declined thereafter (*21*). Atmospheric ^{14}C is depleting approximately 6.1% per year (*20*) which relates to the exchange of contaminated atmospheric $^{14}CO_2$ into large reservoirs with long storage such as oceanic DIC. ^{14}C contents in the two CO_2 sources (petroleum and plants/soil) do not overlap and are vastly dissimilar, so these sources can be differentiated readily. CO_2 solely of petroleum-degradation origin will contain no detectable ^{14}C because of the ancient origin of petroleum. This contrasts strongly with the modern plants and the recent humus found in most soils which are equilibrated metabolically with ^{14}C in CO_2 in the atmosphere (≥100 PMC).

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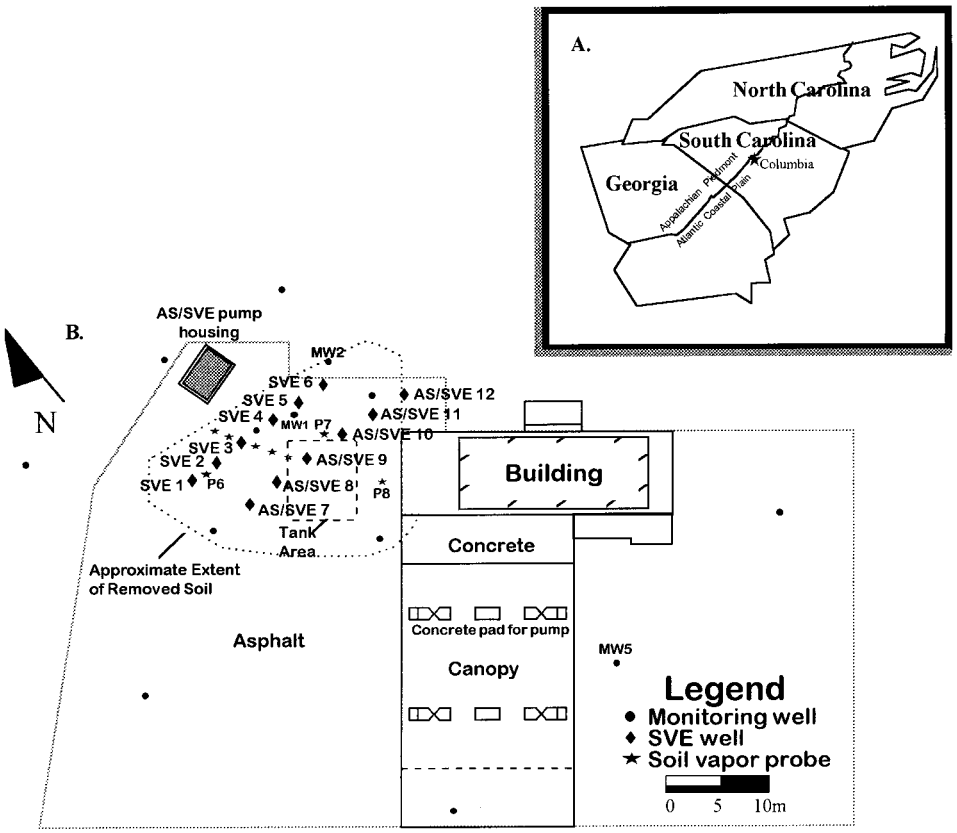

FIGURE 1. (A) Map showing the geographic area of the study site in Columbia, SC, and (B) the site map showing locations of the tank area, air sparging and soil vapor extraction wells, soil vapor probes, and groundwater monitoring wells.

The incorporation of ^{14}C into plant organic matter, vadose zone CO_2, and groundwater DIC (H_2CO_3, HCO_3^{-1}, etc.) permits the use of radiocarbon as a tracer of aerobic petroleum biodegradation in the vadose and saturated zone. Suchomel *et al.* (*11*) reported depleted ^{14}C values from soil gas CO_2 from the biodegradation of trichloroethylene (TCE) (7.7−8.6 PMC) and suggested that organic solvents derived from petroleum may introduce CO_2 devoid of ^{14}C into the soil atmosphere. We present the first quantitative study of radiocarbon analysis of the vadose zone CO_2 and groundwater DIC associated with an air sparging/soil vapor extraction (AS/SVE) petroleum remediation system, which provides evidence of aerobic petroleum biodegradation and overcomes the problems encountered in soil gas monitoring or the use of stable carbon isotopes in this and similar regions.

The objectives of this study were (1) to demonstrate the effectiveness of radiocarbon in providing direct quantified evidence of *in situ* aerobic petroleum hydrocarbon biodegradation in soil CO_2 and groundwater DIC at a gasoline-contaminated site and thus (2) to quantify the microbial contribution of soil CO_2 and DIC production.

Methods

Site Description. This study was performed between 1993 and 1996 at a former gas station in Columbia, SC, where an undetermined amount of petroleum (gasoline) leaked into the subsurface. The site is located in a transition zone between the Atlantic Upper Coastal Plain and the Appalachian Piedmont in low permeability, clayey soil. It contains three general soil strata: (1) an upper stratum (0−4.6 m) dominated by clay, (2) a middle stratum (4.6−6.7 m) containing sandy clay, and (3) a lower stratum (6.7−10.7 m) that is predominantly sandy clay loam with intermittent quartz pebbles. Air permeability increases from 10^{-9} to 10^{-8} cm^2 in the upper zone and to $1-3 \times 10^{-7}$ cm^2 in the lower strata (*22*). The saturated conductivity varies between 1.8×10^{-6} and 3.5×10^{-4} cm s^{-1} from the upper to lower strata, respectively. The water table is located at approximately 6.7 m and groundwater flow is estimated between 1.0×10^{-6} and 4.8×10^{-6} cm s^{-1}. The groundwater is acidic with pH values ranging from 3.8 to 6.3. Preliminary soil analyses from the contaminated source area revealed total petroleum hydrocarbon (TPH) and benzene, toluene, ethylbenzene, and xylene (BTEX) concentrations ranging from undetectable to 7400 mg/kg and undetectable to 793 mg/kg, respectively (*22*). The monitored area and much of the surrounding area is covered with asphalt, with some surrounding area that is landscaped. Sedimentary carbonates, which would influence ^{14}C values, are considered to be low to essentially absent from these acidic geologic materials.

In 1993, researchers from the University of South Carolina and the SC Department of Health and Environmental Control installed an AS/SVE system, monitoring wells, and soil vapor probes in the vadose zone as part of a pilot study of AS/SVE effectiveness in low-permeability soil (Figure 1). The AS/SVE system was composed of 12 SVE wells screened over a 3 m interval at 4.6−7.6 m below the surface. The SVE wells were constructed of 100 mm diameter PVC and were connected to a 5.22 kw vacuum pump. Six AS wells were installed 1.5−2.1 m below the water table and connected to a 2.24 kw air compressor. The soil vapor probes were constructed from 38 mm PVC as a support for a cluster of four 6 mm polyethylene sampling tubes (Parflex) installed at 4.9, 5.5, 6.1, and 6.7 m. Bentonite, an expanding clay, was placed between sampling depths during installation to isolate them. Soil vapor probes P6 and P7 were located in the contaminant plume and within the AS/SVE system's radius of influence. Soil vapor probe P8 was located approximately 5.4 m away from an AS/SVE well but was within the radius of influence of the AS/SVE system. The AS/SVE system was operated between 5/18/95 and 8/2/95 (prior to soil gas

TABLE 1. Values of ^{14}C, $\delta^{13}C$, CO_2, O_2, and pH from the Uncontaminated Well

well	medium	date sampled	depth (m)	lab #	^{14}C (PMC)	^{13}C (‰)	CO_2 (% v/v)	DIC (mg/L)	O_2 (% v/v)	pH
GW-H	groundwater	Feb/96	8.2−11.3	Beta-93011[b]	115.1 ± 0.7	−22.9		60−65	7.6 mg/L	5.82
P9[a]	vadose-zone air	Mar/96	4.0−6.1	Beta-94059[b]	117.9 ± 1.0	−23.9	2.9		18.3	
P9	vadose-zone air	Mar/96	4.0				4.6		16.3	
P9	vadose-zone air	Mar/96	5.2				2.2		18.5	
P9	vadose-zone air	Mar/96	6.1				2.0		20.2	

[a] Depth integrated sample [b] Beta = Beta Analytic.

monitoring) to ensure complete oxygenation of the subsurface and to stimulate aerobic biodegradation of the hydrocarbon contamination and then was shut off for the duration of the study.

Soil Gas Monitoring. The soil gas monitoring system consisted of two soil vapor probes and three SVE wells modified for gas sampling. Samples were collected from 8/2/95 to 1/2/96 and analyzed for O_2, CO_2, and total combustible hydrocarbons (TCH). Samples were drawn with a small vacuum pump into 1 L or 5 L Tedlar bags and analyzed within 24 h of collection. Soil gas samples were analyzed using a CO_2 meter (GasTech RA411A; range: 0−4.975%) and a hydrocarbon/O_2 meter (GasTech GT201; range 0−10 000 ppm, 0−100% LEL). The hydrocarbon meter uses a platinum catalyst-type sensor calibrated against a 2.5% methane standard. O_2 and CO_2 values were verified using a Varian 3400 gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) and Carboxen 1000 1/8 in. ss column. In previous studies, we showed a strong correlation between soil air measurements using the portable hand meters and GC values (*23*), thus numerical values presented here are from hand-meter measurements.

Sampling and Analysis. Standard methods for ^{14}C dating of groundwater (*24*−*26*) including the CO_2 extraction and trapping techniques were used. Preliminary soil air samples were collected from two groundwater monitoring wells (MW1 and MW2) in 1993 before AS/SVE installation. The wells were sealed at the top of the casing pipe and pumped slowly by a small vacuum pump to extract soil air. Air samples came from about 7 m below ground surface at the bottom of the unsaturated zone. In September 1995, a preliminary sample was collected from SVE 2, one month after the AS/SVE system was shut off. In 1996, vadose soil air and groundwater DIC were collected from soil vapor probes, SVE wells, AS/SVE stack exhaust, and from groundwater monitoring wells. Before sampling soil air, wells were purged to remove at least 3 well volumes of soil gas. Soil air samples were routed through a flow meter then to a CO_2 trap which consisted of an air-tight bottle containing 500 mL of repurified 5 N sodium hydroxide. The repurified sodium hydroxide solution was prepared the day before sample collection by adding a small amount of $BaCl_2$ solution to precipitate any contaminant CO_3^{-2} as $BaCO_3$ and decanting before use (*16*). Decanting was performed quickly to minimize exposure to atmospheric CO_2. The soil vapor was pumped through a fritted bubbler submerged in the CO_2-absorbing solution. The trap was run in series to capture all CO_2 from the soil air. The approximate volumes of sample gas required were first determined by measuring the CO_2 concentration of the extracted air using a CO_2 meter. Approximately 4 g of carbon were desired for conventional radiometric analysis.

Groundwater samples were collected with a submersible electric pump (Redi-Flo2, Grundfos) from contaminated wells into a 55 gallon drum. The groundwater was acidified to approximately pH 2.0 to inhibit microbes for the several days of storage and to help release the dissolved CO_2 (*15, 24*). A closed system was used to strip and concentrate the CO_2 which consisted of a vacuum pump pulling the CO_2-laden air from the drum headspace and into a NaOH trap. The CO_2-free gas was then routed to the bottom of the drum through a fritted bubbler to strip CO_2 from the groundwater. The groundwater samples were extracted for approximately 10 h to ensure that almost all CO_2 was removed (*15*). DIC concentrations were measured by field titration (Hack CA-DT kit) to ensure a sufficient volume of groundwater was collected to capture 4 g of carbon for ^{14}C analysis.

Samples were sent to analytical laboratories for standard radiocarbon dating analysis where the carbonate samples were converted to CO_2 by acidification before ^{14}C measurement using β counters. The ^{14}C laboratories took a small aliquot of each sample to use for standard ^{13}C analysis. Preliminary samples went to the University of Waterloo (Ontario) or to Geochron Laboratories (Cambridge, MA) and subsequent samples were sent to Beta Analytic (Miami, FL). ^{14}C dating laboratories measure and report the low activities of ambient ^{14}C relative to a low-activity standard as

$$\mathrm{PMC} = (^{14}\mathrm{C}/\mathrm{C}_{\mathrm{sample}})/(^{14}\mathrm{C}/\mathrm{C}_{\mathrm{standard}}) \quad (1)$$

The ^{14}C standard is defined as 0.95 times the activity of National Institute of Standards and Testing oxalic acid. The artificial standard represents wood (referenced as −25‰) grown in AD 1950 remote from industrial influence. The precision for the ^{14}C analyses was approximately ±1 PMC (Tables 1 and 2).

^{13}C isotopic ratios are reported relative to that of the PeeDee belemnite (PDB) standard in per mil as

$$\delta^{13}\mathrm{C} = ((R_{\mathrm{sample}} - R_{\mathrm{standard}})/R_{\mathrm{standard}}) \times 1000 \quad (2)$$

where R is the ratio of ^{13}C to ^{12}C. The analytical precision of ^{13}C analysis for our samples was approximately ±0.1‰.

In quantifying the percent of vadose zone CO_2 or DIC produced from the aerobic mineralization of gasoline, the following equation was used:

$$(\mathrm{CO}_{2\mathrm{soil}})(\mathrm{PMC}_{\mathrm{unc}}) + (\mathrm{CO}_{2\mathrm{pet}})(\mathrm{PMC}_{\mathrm{pet}}) = \mathrm{PMC}_{\mathrm{sample}} \quad (3)$$

where CO_{2soil} is the proportion of sample CO_2 coming from natural soil contribution, PMC_{unc} is the measured PMC value from the uncontaminated site, CO_{2pet} is the proportional contribution from petroleum mineralization, PMC_{pet} is the PMC value of petroleum (0 PMC), and PMC_{sample} is the measured PMC value of the sample. Assuming 0 PMC for gasoline, the equation becomes

$$\mathrm{CO}_{2\mathrm{soil}} = \mathrm{PMC}_{\mathrm{sample}}/\mathrm{PMC}_{\mathrm{unc}} \quad (4)$$

The proportion of the CO_2 sample produced from aerobic gasoline mineralization (CO_{2pet}) is related to the natural soil contribution (CO_{2soil}) at this site by

$$\mathrm{CO}_{2\mathrm{pet}} = 1 - \mathrm{CO}_{2\mathrm{soil}} \quad (5)$$

Therefore, incorporating eqs 4 and 5 yields the percent of CO_2 produced from aerobic mineralization of the gasoline

TABLE 2. Values of ^{14}C, $\delta^{13}C$, CO_2, O_2, and Total Combustible Hydrocarbons (TCH) from Soil Vapor Extraction Wells, Soil Vapor Probes, and Groundwater Monitoring Wells from the Petroleum-Contaminated Site

well	medium	date sampled	depth (m)	lab #	^{14}C (PMC)	^{13}C (‰)	$\%CO_2$ (v/v)	$\%O_2$ (v/v)	TCH (ppm)	pH
				Monitoring Wells						
MW 1	vadose-zone air	Aug/93	6.7	WAT-2747[a]	17.7 ± 0.2	−35.9	1.3			
MW 2	vadose-zone air	Aug/93	6.7	WAT-2746	33.5 ± 0.4	−34.4	1.0			
				SVE Wells						
SVE 2	vadose-zone air	Sep/95	4.6−7.6	GX-21304[b]	33.9 ± 1.2	−24.8	4.6	6.6	11 549	
SVE 1	vadose-zone air	May/96	4.6−7.6	Beta-94049[c]	15.9 ± 0.4	−27.1	9.4	1.1	10 975	
SVE 7	vadose-zone air	May/96	4.6−7.6	Beta-94051	18.8 ± 0.4	−25.5	12.1	0.7	21 885	
SVE 8	vadose-zone air	May/96	4.6−7.6	Beta-94050	17.1 ± 0.3	−25.7	11.6	0.3	28 775	
				Soil Vapor Probes						
P6-22	vadose-zone air	Apr/96	6.7	Beta-94055	19.2 ± 0.2	−26.3	13.3	1.2	37 388	
P6-16	vadose-zone air	May/96	4.9	Beta-94056	24.5 ± 0.3	−26.4	3.3	14.2	1787	
P7-22	vadose-zone air	May/96	6.7	Beta-94059	35.0 ± 0.5	−22.8	5.6	7.5	89 067	
P8	vadose-zone air	May/96	4.9−7.6	Beta-94058	24.6 ± 0.2	−27.9	7.8	9.7	27 053	
				System Exhaust						
EXHT	vadose-zone air	May/96	4.6−7.6	Beta-94052	21.8 ± 0.3	−24.8	16.6	1.0	10 975	
				Groundwater Wells						
MW2	groundwater	Mar/96	7.3−10.4	Beta-94053	47.7 ± 0.5	−22.0	120−130 mg/L	0.5 mg/L	100 mg/L	5.3
MW5	groundwater	Mar/96	7.3−10.4	Beta-94054	30.7 ± 0.3	−23.3	160−165 mg/L	1.4 mg/L	66 mg/L	4.8

[a] WAT = University of Waterloo. [b] GX = Geochron Laboratories. [c] Beta = Beta Analytic.

contamination in the vadose zone air and groundwater samples as

$$CO_{2pet} = (1 - (PMC_{sample}/PMC_{unc})) \times 100 \qquad (6)$$

Uncontaminated Site. Ideally, an identical background site should be selected adjacent to the area of study, with no petroleum hydrocarbon contamination. Because of the large contaminant plume and location of the study site in a commercial and residential area, a nearby similarly developed but uncontaminated location was not available for background measurements. Uncontaminated groundwater and soil CO_2 samples were collected from Harbison State Forest, a natural area located 3.6 km from the field site. The uncontaminated site was of predominately C_3 type vegetation, typical of forested areas of this region. Vadose zone sample ports were attached to the monitoring well and soil CO_2 was collected from 4.0, 5.2, and 6.1 m depths. Low groundwater well yields and low dissolved CO_2 levels from this uncontaminated site required collection and analysis methods alternative to those used at wells from the contaminated site. After well purging, groundwater was carefully bailed and sealed in 2.5 L containers to minimize exposure to atmospheric CO_2. Repurified 5 N NaOH and $BaCl_2$ were added to the sample to precipitate the carbonate as $BaCO_3$ (*16*). The small precipitate sample was kept isolated from the atmosphere and sent to Beta Analytic for ^{14}C analysis using an accelerator mass spectrometer (AMS).

Results and Discussion

Vadose Zone Monitoring. Vadose zone CO_2 and O_2 measurements suggested that microbial activity was abundant, with the greatest CO_2 and O_2 fluctuations occurring at the unsaturated-saturated zone interface. Soil vapor probe P6 at 6.7 m showed the strongest response with a sharp decrease in vadose zone O_2 negatively correlated with an increase in CO_2 levels ($r^2 = 0.96$) (data not shown). O_2 was consumed 2.2 times more than the production of CO_2, which may indicate that carbon was incorporated into biomass or incomplete microbial mineralization of the contaminants occurred. The aerobic microbial degradation signal at P6−6.7 m contrasted with the response observed at P6−4.9 m which had limited CO_2 and O_2 fluctuations, reaching levels of 1.4 and 16.6% (v/v), respectively. These levels are within the range of values in uncontaminated soils; thus, petroleum biodegradation at this depth was inconclusive. It is possible that microbes were not actively degrading the contaminant at 4.9 m or mineralization of a larger quantity of contamination is required for soil gas monitoring to be effective in detecting gasoline biodegradation. No detectable decrease in TCH occurred at 6.7 or 4.9 m. Microbial activity was not evident in soil vapor probe P7 at 6.7 m during the 153 day monitoring period. Vadose zone CO_2 and O_2 levels were near atmospheric values with measurements of 0.5 and 19.8% (v/v), respectively.

Unlike the heterogenous biological response observed at discrete depths in the soil vapor probes, the soil vapor extraction wells, which are depth-integrated samples screened over 4.6−7.6 m depth, showed consistent microbial biodegradation over the monitoring area (data not shown). At the end of the monitoring period, vadose zone CO_2 and O_2 levels within the three SVE wells averaged 18.9 ± 11.9% (v/v) and 1.3 ± 1.6% (v/v), respectively. Vadose zone CO_2 production was negatively correlated with O_2 depletion in the three monitored SVE wells ($r^2 = 0.82$), indicating that aerobic biodegradation was occurring in the vadose zone. Slight decreases in TCH concentration were observed in the SVE wells, but only after a 30 day time period after AS/SVE shutdown in which subsurface hydrocarbon equilibration may have masked any small decreases in TCH concentration. Despite the differences in TCH concentrations in the three SVE wells, similar trends of biodegradation were observed from monitoring soil CO_2 and O_2 over time.

Uncontaminated Vadose Zone Air and Groundwater ^{13}C and ^{14}C Results. The $\delta^{13}C$ value of the vadose zone CO_2 (−23.9‰) (Table 1) was slightly heavier than the value typically assumed for the soil zone of C_3 plants (−25.0‰) (*24*−*26*). This may represent normal variation among sites or it may involve some small component in the local groundcover vegetation of C_4 metabolism plants which produce $\delta^{13}C$ values substantially higher than C_3 plants. The groundwater DIC $\delta^{13}C$ value was slightly higher still (−22.9‰) and possibly represents some minor alteration of original values derived from vadose zone CO_2. The $\delta^{13}C$ value differences are in the direction expected for isotopic equilibration between vadose zone CO_2 and groundwater or minor reaction of groundwater with sedimentary carbonates. Other $\delta^{13}C$ values from uncontaminated acidic groundwater DIC in SC are −23.4, −25.6,

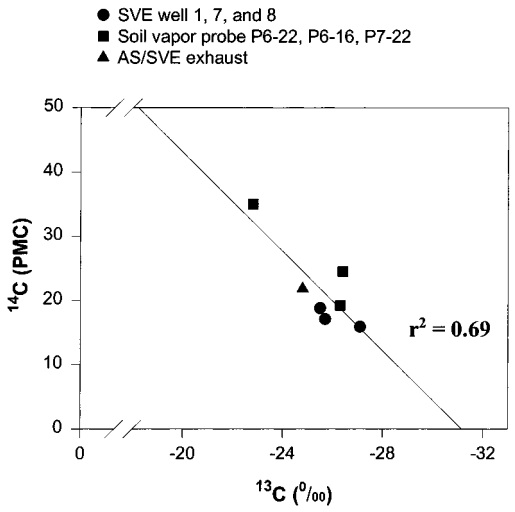

FIGURE 2. Regression of ^{14}C and δ^{13}C values from the soil vapor probes and soil vapor extraction wells within the influence of the air sparging/soil vapor extraction system.

−22.4, −23.2, and −25.7‰ (P. A. Stone, 1997, unpublished data).

Unsaturated zone CO_2 and shallow groundwater DIC from the uncontaminated forest site showed the expected effect of nuclear weapons ^{14}C contamination (>100 PMC: 117.9 ± 1.0 and 115.1 ± 0.7 PMC, respectively) (Table 1). This small difference between vadose zone air and groundwater may result from reaction of a small portion of the dissolved CO_2 in the saturated zone with sparse carbonates in the aquifer which would move the values in the observed direction of lower PMC in groundwater. Sedimentary carbonates ($CaCO_3$) are low to essentially devoid of ^{14}C (to 0 PMC) and virtually all are isotopically heavier than soil CO_2 (0 to −12‰). Though overall considered to be characterized by siliceous rocks, acidic soils, and soft waters, the Piedmont does contain some carbonate in places.

Contaminated Vadose Zone Air ^{13}C and ^{14}C Results. δ^{13}C values for vadose zone CO_2 from MW1 and MW2 were the lowest of any sample measured with values of −34.4 and −35.9‰ (Table 2). It is unclear whether these δ^{13}C values represent biodegradation of an isotopically lighter portion of gasoline or if isotopic fractionation occurred during sample collection. The preliminary samples were collected using only one CO_2 trap while all other samples used two traps in series.

δ^{13}C values from vadose zone CO_2 samples taken from soil vapor probes, SVE wells, and AS/SVE exhaust gases after AS/SVE covered a fairly wide range (−22.8 to −27.9‰) (Table 2). They correlated with ^{14}C values (r^2 = 0.69), indicating a relation of lower δ^{13}C values with aerobic petroleum biodegradation (Figure 2). Overall, the δ^{13}C values indicated that petroleum biodegradation was occurring in the vadose zone with most δ^{13}C values lower than the uncontaminated δ^{13}C value of −23.9‰. Only one vadose zone sample (P7−22 at −22.8‰) was less negative than the uncontaminated δ^{13}C value, but vadose zone samples from the exhaust stack and SVE 2 were only 0.9‰ lower than the uncontaminated site (Figure 3A). SVE 7 and SVE 8 were sites of the highest biological signal based on soil air monitoring and were only 1.6−1.8‰ less than the uncontaminated site with ^{13}C values of −25.5 and −25.7‰, respectively. In these four samples, radiocarbon was a better indicator of petroleum biodegradation because of the wide difference in the activities of natural organic matter and petroleum (Figure 3A).

The ^{14}C values from the preliminary vadose CO_2 samples taken from groundwater monitoring wells MW1 and MW2 in August, 1993, showed that gasoline biodegradation was occurring in the unsaturated zone. The measured ^{14}C activities for the two wells were 33.5 ± 0.4 and 17.7 ± 0.2 PMC (Table 2) and were much lower than what would be expected from natural soil CO_2 at uncontaminated sites (≥100 PMC). We estimated (using eq 6) that approximately 72 and 85% of the sampled CO_2 came from petroleum biodegradation for the two samples, respectively (Table 3).

The ^{14}C values of SVE wells 1, 7, and 8 were similar ranging from 15.9 ± 0.4 to 18.8 ± 0.4 PMC (Table 2) and these SVE wells contained elevated soil CO_2 levels ranging 9.4−12.1% (v/v). The earliest SVE sample, SVE 2, was collected one month after AS/SVE shut-down, and displayed a higher ^{14}C value of 33.9 ± 1.2 PMC and a lower CO_2 concentration of 4.6% (v/v) compared to the latter (1996) SVE samples. The higher PMC value in SVE 2 was impacted by CO_2 from surrounding less contaminated areas that was pulled into the monitoring area by the AS/SVE system in the previous months. The similar ^{14}C values in SVE wells 1, 7, and 8 suggested that the PMC value continued to decrease as microbial mineralization of the gasoline continued through time. But even this higher PMC value in SVE 2 after one month of bioremediation clearly demonstrated the majority of soil CO_2 was from aerobic petroleum biodegradation (71%). The proportion of vadose zone CO_2 from gasoline mineralization in the SVE wells was estimated to be between 84 and 86% (Table 3). The ^{14}C results from the SVE wells demonstrated the ability of radiocarbon to detect petroleum biodegradation in the vadose zone from samples with varying concentrations of CO_2 and contamination levels and that the vadose zone consistently contained a high percent of soil CO_2 originating from gasoline biodegradation.

^{14}C dilution representing pronounced additions of ancient carbon from petroleum biodegradation was exhibited by vadose zone air samples from the soil vapor probes (Table 2). ^{14}C values from the soil vapor probes ranged from 19.2 ± 0.2 to 35.0 ± 0.5 PMC (Table 2). ^{14}C results from soil vapor probe P8, less affected by the AS/SVE system, showed the same level of isotopic dilution compared to those probes located in areas undergoing aerobic biostimulation. From the two vadose samples collected at 6.7 m, P7−22 had a higher PMC value, suggesting less petroleum mineralization in this region which is in agreement with soil gas monitoring data. The low microbial activity in P7−22 was not detectable by monitoring vadose zone CO_2 and O_2 levels using hand meters and GC analysis during the 153 day monitoring period, but ^{14}C values distinctly showed that gasoline mineralization was occurring with approximately 70% of vadose zone CO_2 present from petroleum mineralization. Petroleum mineralization was not conclusive from monitoring soil gas in soil vapor probe P6−16 (4.9 m) but radiocarbon results with a PMC value of 24.5 ± 0.3 demonstrated that even the lower CO_2 levels were produced predominately from petroleum biodegradation.

The average PMC value from the SVE wells and soil vapor probes (23.6 PMC) was similar to the value from the AS/SVE system exhaust gas which is an integrated sample (21.8 ± 0.3 PMC). Approximately 82% of the extracted CO_2 from the AS/SVE system at the time of sampling came from petroleum biodegradation. This suggests that radiocarbon measurement of CO_2 exhaust from an AS/SVE system may provide a relatively quick and simple diagnostic monitoring method to demonstrate or quantify microbial biodegradation of petroleum-based contaminants. By monitoring stack gas CO_2 it would be possible to determine the rate and amount of contaminant removal by microbial mineralization or how an AS/SVE system affected microbial activity in the vadose or saturated zone. For example, at the time of sample collection, TCH concentration was 10 975 ppm and the exhaust stack CO_2 level was 16.6% (v/v). Radiocarbon results showed that approximately 81.5% of the exhaust CO_2 was from petroleum mineralization; therefore, 13.5% (v/v) [or 81.5% of 16.6% (v/v)] of exhaust CO_2 was from petroleum mineralization at the time of sample

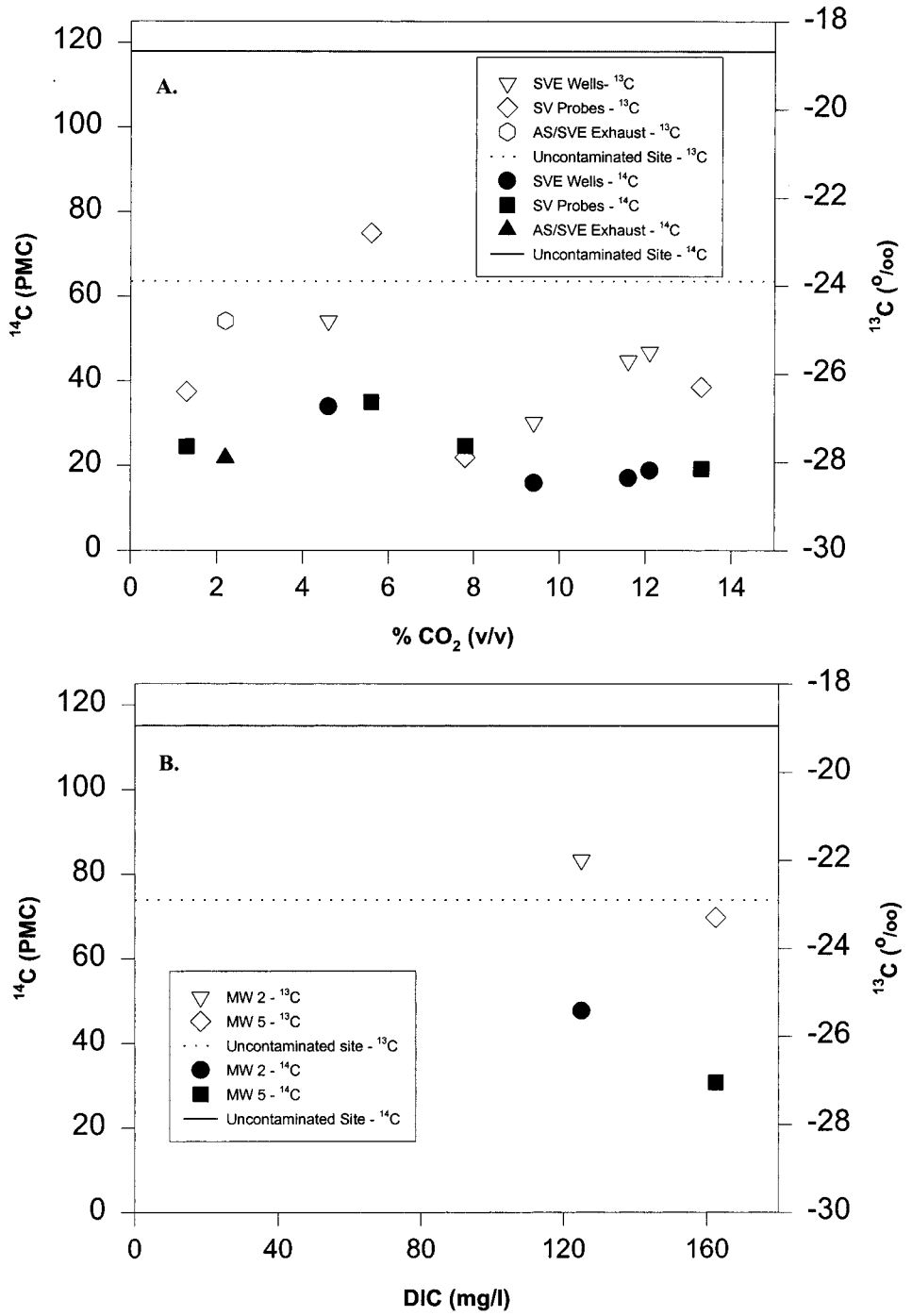

FIGURE 3. (A) Plot of the ^{14}C and $\delta^{13}C$ values versus CO_2 from all of the vadose zone air samples compared to the uncontaminated ^{14}C and $\delta^{13}C$ values and (B) plot of the ^{14}C and $\delta^{13}C$ values versus DIC from the groundwater samples compared to the uncontaminated ^{14}C and $\delta^{13}C$ values.

collection. Thus, for every cubic meter of exhaust air, there were 86 g of hydrocarbon microbially degraded and 42 g physically removed by the AS/SVE system (using hexane as a standard hydrocarbon molecular weight for weathered gasoline at standard temperature and pressure). At the exhaust sampling time point, biodegradation was responsible for twice the hydrocarbon removal compared to physical extraction alone which is what would be expected at the initial start-up of the AS/SVE system after a lengthy shut-down period.

Contaminated Groundwater ^{13}C and ^{14}C Results. The $\delta^{13}C$ values from the contaminated groundwater samples were −22.0 and −23.3‰. Therefore, the $\delta^{13}C$ values did not definitively indicate aerobic petroleum biodegradation compared to the uncontaminated groundwater $\delta^{13}C$ value of −22.9‰ (Figure 3B).

The measured ^{14}C values for the two contaminated groundwater samples were 47.7 ± 0.5 and 30.7 ± 0.3 PMC, which are higher isotopically diluted compared to the control well (115.1 ± 0.7) (Table 2). This suggests that approximately 59 and 73% of the DIC came from petroleum biodegradation, respectively. The dissolved CO_2 levels in the contaminated groundwater wells (120−165 mg/L) were at least twice the level of the uncontaminated site. The depleted ^{14}C values clearly showed that gasoline biodegradation was occurring in the saturated zone and demonstrated the usefulness of radiocarbon in monitoring biodegradation of petroleum based contaminants in groundwater.

TABLE 3. Calculated Percent CO_2 from Microbial Mineralization of Gasoline Based on ^{14}C Values Using Eq 6

medium	well	%CO_2
vadose-zone air	SVE 1	86.5
vadose-zone air	SVE 2	71.2
vadose-zone air	SVE 7	84.1
vadose-zone air	SVE 8	85.5
vadose-zone air	EXHT	81.5
vadose-zone air	P6-22	83.7
vadose-zone air	P6-16	79.2
vadose-zone air	P7-22	70.3
vadose-zone air	P8	79.1
vadose-zone air	MW 2	71.6
vadose-zone air	MW 1	85.0
groundwater	MW2	58.6
groundwater	MW5	73.3

Although extremely useful under many conditions, radiocarbon analysis has several limitations. While ^{13}C requires small amounts of carbon (micro- to milligram ranges), standard radiometric analysis requires ca. 1−4 g of carbon and this limits spatial resolution if you are taking single batch samples. AMS is an alternative method of radiocarbon analysis which requires much less carbon for analysis (0.001−0.3 g) but is twice the cost of standard radiometric analysis (approximately $600 versus $245 per sample, respectively). Carbonates in geologic formations, which are a principal source of carbon isotope dilution, require further computational corrections, especially for groundwater ^{14}C. Reactions of H_2CO_3 with mineral $CaCO_3$ adds another ^{14}C depleted dilutant component to DIC (*27*) and by exchange likely also to CO_2 in vadose zone atmosphere. The two end-member mixing approach used here is then insufficient. However, in analyzing either CO_2 or DIC, this complication can be handled using background values from a uncontaminated area and accounting for the further reactions. If there is incomplete reaction of contaminant-produced CO_2 with $CaCO_3$, a correction could involve accounting for the separate DIC species (H_2CO_3, HCO_3^{-1}) and their source implications, similar to that used for groundwater dating (*27, 28)*. Other limitations include oxidizing ancient organic matter (peaty or lignitic matter), older groundwater, and the presence of gasohol (methanol, ethanol) in petroleum products, none of which was pertinent to this study. Most of these limitations could be overcome by corrections and modifications to the equations, but with some loss of accuracy.

The ^{14}C results in this study demonstrated that measuring radiocarbon was a more diagnostic and sensitive technique compared to measuring soil gas composition or stable carbon isotopes for providing evidence of aerobic petroleum biodegradation in temperate, nonarid regions. Vadose zone CO_2 levels from the groundwater monitoring wells measured approximately 1% (v/v). This is within the range reported as typical for uncontaminated soils so there was no overt evidence of CO_2 enrichment from contaminant degradation by CO_2 concentration alone. Low concentrations of soil CO_2 have been reported in other petroleum biodegradation studies (*6*). Our results showed that ^{14}C measurement of vadose zone CO_2 was an effective technique to demonstrate aerobic petroleum biodegradation even when CO_2 levels were low. $\delta^{13}C$ was a good indicator of biodegradation of petroleum hydrocarbon for most, but not all samples. On the basis of the limited number of groundwater and vadose zone samples in this study, radiocarbon was a better indicator of aerobic petroleum mineralization in the subsurface at this site than $\delta^{13}C$ because of a greater differential between the contaminated and uncontaminated values.

Radiocarbon analysis undoubtedly has many applications in monitoring and quantifying bioremediation of contaminants in soil and groundwater. Radiocarbon analysis of DIC and soil CO_2 may easily detect microbial mineralization and quantify the microbial contribution of CO_2 production from any petroleum-synthesized contaminant such as jet fuel, heating oil, diesel, trichloroethylene, or any other petroleum-derived solvent. Radiocarbon should work equally well on CO_2 from anaerobic degradation using sulfate or nitrate reduction. It can be used to detect the CO_2 produced in methanogenic degradation of petroleum hydrocarbons, but would then be a qualitative test because the CH_4 component would be missed. Radiocarbon analysis may be a valuable technique in aerobically bioengineered operations (AS/SVE, bioventing, etc.) as bioremediation is increasingly relied upon to treat contaminated soil and groundwater.

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