Environmental Forensics

Radiocarbon and Stable Carbon Isotope Analysis to Confirm Petroleum Natural Attenuation in the Vadose Zone

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CO_2 and CH_4 radiocarbon and stable carbon isotope ratios were used to assess natural attenuation at a fuel-contaminated soil site at the Norfolk Navy Base, Norfolk, VA (USA). Soil gas samples were collected spatially over a monitoring network in October 2002 and in March 2003. CO_2 and CH_4 from regions with high petroleum concentrations were ^{14}C-depleted relative to uncontaminated areas. ^{14}C-depleted methane suggested methanogenic hydrocarbon degradation. The difference in CO_2 age between background and plume-influenced areas indicated that approximately 90% of the CO_2 at the latter was petroleum derived, making contaminant the primary source of carbon for the microbial assemblage.

Keywords: natural attenuation, vadose zone, groundwater, carbon isotopes, carbon dioxide, methane, radiocarbon, stable carbon isotope analysis, soil gas, petroleum, methanogenesis

Introduction

Evaluating in situ biodegradation of organic contaminants requires an efficient, precise, and cost-effective monitoring strategy. Predicting environmental remediation timescales requires understanding contaminant turnover with respect to additional source introduction, contaminant transport and (bio)degradation. Multiple sources and their mixing may complicate remedial actions at many subsurface fuel-contaminated sites (Atmadja and Bagtzoglou, 2001; Macdonald and Kavanaugh, 1994; Marryott et al., 2002; Renner, 1998). Anthropogenic organic compound turnover depends on organic contaminant availability and inherent lability, nutrient availability, natural organic carbon concentrations, and seasonal physicochemical variability. Thus, defining the parameters necessary to substantiate natural attenuation is difficult and not without uncertainty (cf. Renner, 2000).

Stable carbon and radiocarbon isotope analysis ($\delta^{13}C$ and $\Delta^{14}C$, respectively) have been extensively used to assess basic biogeochemical roles in natural carbon cycling (Cherrier et al., 1999; Coffin et al., 1990; Druffel et al., 1992; Hullar et al., 1996; Peterson and Fry, 1987). More recently, stable carbon isotope analysis has been applied to identify contaminant carbon

(Aggarwal and Hinchee, 1991) as well as biodegradation byproducts and residual contamination (Dempster et al., 1997; Lollar et al., 1999; Slater et al., 2001). Stable isotope techniques have also been applied to field settings by analyzing fractionation factors (Elsner et al., 2005; Lollar et al., 2001), addition of stable isotope labeled tracers (Fischer et al., 2006), and evaluating efficacy of active bioremediation strategies through monitoring production of contaminant-derived CO_2 (Mueller et al., 1995). In addition, as a biomarker approach, bacterial nucleic acid stable carbon isotopes were analyzed from beaches contaminated with oil from the Valdez oil spill to confirm bacterial hydrocarbon biodegradation in Prince William Sound, Alaska (Coffin et al., 1997). Aside from measuring fractionation factor(s) of residual contaminant pools, monitoring hydrocarbon biodegradation respiration products (e.g., CH_4 and CO_2) to confirm biodegradation may require the least analytical effort. This strategy has been applied to groundwater and vadose zone gases (Conrad et al., 1999).

However, definitive biodegradation confirmation may be hindered when contaminant and natural organic carbon stable isotope ratios overlap; for example, under aerobic conditions, when the CO_2 produced from biodegradation has "the same" isotopic ratios as both the contaminant and the background organic matter. In a system driven anaerobic by contaminant input, methane production yields ^{13}C-depleted CH_4 and ^{13}C-enriched CO_2 (Lollar et al., 2001). Without ancillary analyses, the similarity of ^{13}C content in atmospheric CO_2 ($\delta^{13}C$ approximately -7%) and CO_2 produced from organic matter degradation under methanogenic

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conditions may interfere with the ability of stable carbon isotope analysis to aid in confirming natural attenuation, particularly if one is trying to link the $\delta^{13}C$ value of CO_2 to the contaminant $\delta^{13}C$ value or cannot accurately estimate the atmospheric contribution to CO_2 in the vadose zone. Recent reviews provide considerable information on the application of stable isotope methods for observing and confirming contaminant biodegradation (Elsner et al., 2005; Meckenstock et al., 2004).

Clearly, when identification of hydrocarbon source(s) and fate(s) using stable isotope analyses is problematic due to an inability to distinguish between contaminant and natural organic carbon of overlapping isotope ranges, another strategy to confirm biodegradation must be undertaken. Natural abundance radiocarbon analysis is a complementary approach for tracing carbon assimilation and respiration through the microbial assemblage. ^{14}C-content can be used to distinguish between carbon sources of different age (Bauer et al., 1992, 1995, 1990; Cherrier et al., 1999). With a half-life of approximately 5700 years, petroleum sources are radiocarbon-free and thus provide a definitive end-member when analyzed against photosynthetic-based carbon sources, which contain modern CO_2 from the atmosphere. The percentage modern carbon (pMC) of living photosynthetic biomass is approxiamtely 110% because of nuclear bomb tests, which peaked before 1965 (Aelion et al., 1997). In contrast, petroleum carbon and CO_2 derived from its biodegradation have no ^{14}C and are thus 0 pMC. Given the two extreme end-members, one can readily differentiate the relative contributions of petroleum and plant biomass degradation in the total respired CO_2 pool.

Radiocarbon analysis of soil gas carbon dioxide was applied to study contaminant degradation and migration in the vadose zone of an aquifer contaminated with organic solvents (Suchomel et al., 1990). The results demonstrated distinct CO_2 radiocarbon signatures from control and contaminated sites. In a subsequent study, radiocarbon was used to investigate petroleum degradation in groundwater and soil gas (Conrad et al., 1997). In this study, definitive demonstration of petroleum biodegradation by stable carbon isotope analysis was hindered by CO_2 generated from methanogenesis. Radiocarbon analysis was able to confirm petroleum biodegradation. Radiocarbon was also used to survey groundwater contaminant degradation at a gasoline-contaminated site undergoing remediation with an air sparging/ soil vapor extraction system (Aelion et al., 1997). The CO_2 radiocarbon signature in the soil gas and groundwater-dissolved inorganic carbon suggested aerobic petroleum biodegradation produced 59–87% of the total CO_2. The approach was then applied to a chlorinated solvent plume wherein biodegradation was confirmed through the observation of the fossil end-member in soil gas CO_2 (Kirtland et al., 2003, 2005). These studies provide strong support for applying and coupling stable carbon and radiocarbon analyses to confirm and quantify natural attenuation.

This study focuses on the isotopic (^{13}C and ^{14}C) composition and concentration of CO_2 and CH_4 from the vadose zone of a site contaminated by fuel leakage at the Navy Ship Yard in Norfolk, VA, to evaluate petroleum natural attenuation in the soil and groundwater. The vadose zone is capped by an asphalt parking lot where 11 shallow and deep soil gas monitoring points (MPs) were installed. Recent evaluation of the petroleum plume was conducted by the Norfolk Navy Base to determine concentrations and transport rates. Data from late summer/early fall and late winter/ early spring sampling events are compared here. Our results support the application of CO_2 radiocarbon analysis to assess natural attenuation of contaminated soil and groundwater.

Methods and Materials

Site Description

The Norfolk Naval Station at Sewell's Point in Hampton Roads, VA, is the world's largest naval station and home port to 78 ships and 133 aircraft. There are 14 piers along a 4-mile waterfront, 15 aircraft hangars, and other military support facilities on the 3,400-acre area. Extensive refueling operations have and continue to release hydrocarbons into the subsurface environment at several locations. Groundwater flow adjacent to fuel plumes may leach soluble components and transport contaminants down gradient; in this case, towards the Elizabeth River, a tributary of the Chesapeake Bay. Fueling operations around the northwestern end of the base have released Bunker C and other fuels into the subsurface. A network of soil gas MPs was installed for monitoring petroleum concentrations in the soil and groundwater (Figure 1). Soil gas MPs 2, 3, and 11 are outside of the contaminated soil region and, therefore, were selected as control sites (Figure 1).

Vadose Zone Gas Sampling

The soil gas MPs are adjacent to those previously used by the base to survey the contaminated groundwater plume. Each MP had sampling ports at 72.6 (28.6 in) and 135 cm (53.2 in) below the soil surface (Figure 2). Vadose gas was collected using a 1-L gas-tight syringe after a 1-L purge and were transferred into Cali-5 1-L gas-tight bags (Calibrated Instruments, Inc., Hawthorne, NY, USA). Sampling was conducted in October 2002 and March 2003.

CO_2 Concentration

Carbon dioxide concentrations in the vadose gas samples were determined with a UIC Model 5011 coulometer (UIC, Inc., Joliet, IL, USA). The gas samples were injected into a UHP He stream (80 mL min^{-1}) at a rate 1 mL/ min^{-1} to avoid saturating the cathode solution of the coulometric cell. The percentage of carbon dioxide in the injected volume was calculated by multiplying the coulometer counts by a calibration factor determined with a 100% CO_2 standard (MG Industries, Malvern, PA, USA). Analytical precision of the standards and unknowns was <1.5% and <3.0%, respectively, based on triplicate analysis.
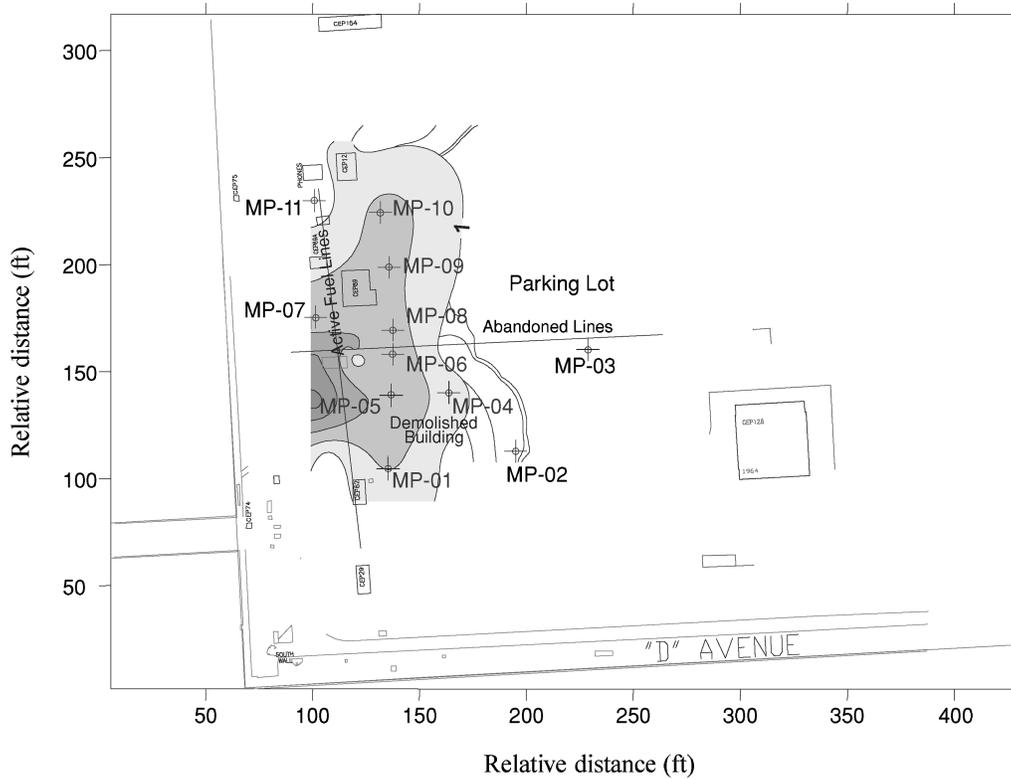
Figure 1. Soil gas monitoring point (MP) distribution. Shading represents petroleum contaminated soil regions. Points 2, 3, and 11 were used as control stations. Generalized groundwater flow is from right to left (east to west).

CH_4 Concentration

Methane concentrations were determined using a Shimadzu GC-14A gas chromatograph (GC) equipped with a flame ionization detector (FID). The gas was introduced through a manual gas-sampling valve and GC separation was achieved with a Poropak-Q column (8′ × 1/8″; Alltech Associates, Inc., Deerfield, IL, USA) at isothermal conditions (50°C). Methane concentrations were determined against certified gas standards (Scott Gas, Plumsteadville, PA, USA). Injection volumes ranged

Figure 2. Soil gas monitoring point (MP) and sample port design.

from 2 to 10 mL of gas. Analytical precision from 3-mL standard injections was 0.2%.

Stable Carbon Isotope Analysis

Stable carbon isotope analysis was conducted on methane and CO_2 from the vadose zone. Sample volumes ranging between 0.5–10 μL were injected into a Trace GC Ultra (Thermo Electron, Waltham, MA, U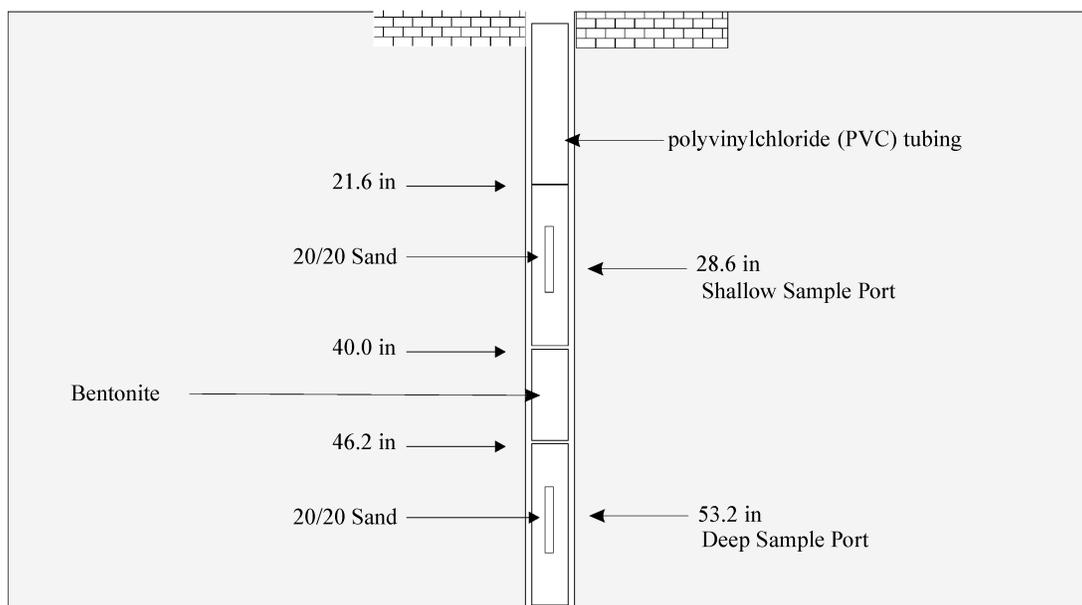SA) containing a PoraPLOT Q 25 m × 0.32 μm capillary column (Varian, Inc., Palo Alto, CA, USA). Runs were isothermal at 50°C. Separated analytes (CH_4 and CO_2) were run through a Thermo-Finnigan GC Combustion III (Thermo-Fisher Scientific, Waltham, MA) interface and transferred to a Finnigan Delta Plus XP (Thermo-Fisher Scientific, Waltham, MA) isotope ratio mass spectrometer. Calibration was performed by co-injection of a laboratory standard CO_2, which in turn was calibrated against NBS-22 (National Institute of Standards and Technology [NIST, Gaithersburg, MD]). Stable carbon isotope ratios were expressed as:

$$\delta^{13}C = \left[\frac{Rs}{Rstd} - 1 \right] \times 1000 \, (\permil) \qquad (1)$$

where $\delta^{13}C$ is the carbon isotope ratio, R_s is the $^{13}C/^{12}C$ for the unknown, and R_{std} is the $^{13}C/^{12}C$ for standard Pee Dee Belemnite (PDB). The detection limit was approximately 1 mg C, and the precision was ±0.3‰, based on triplicate measurements.

Radiocarbon Analysis

Gas samples for radiocarbon analysis of CO_2 and CH_4 were injected into an oxygen-enriched He carrier stream and cryogenically separated with a series of stainless steel loops immersed in dry ice/ethanol and liquid nitrogen. Methane was oxidized on-line to CO_2 over alumina pellets at 650°C and purified by cryogenic distillation. The CO_2 from each trap was cryogenically transferred to reactors and converted to graphite by iron catalyzed reduction with hydrogen (Pohlman et al., 2000; Vogel et al., 1987). The graphite was pressed into aluminum targets and analyzed with a 3-MV Pelletron tandem accelerator mass spectrometer with an multi-cathode source of negative ions by cesium sputtering MC-SNICS ion source (Grabowski et al., 2000). Radiocarbon values are reported as pMC and as the $D^{14}C$ notation relative to the NIST oxalic acid II standard (Stuiver and Polach, 1977).

Results

CO₂ and CH₄ Concentrations

CO_2 and CH_4 concentrations from the deep and surface soil gas MPs were generally not significantly different ($P < 0.05$), although some exceptions were observed (Table 1). The similarity in the vertical gas concentrations was likely a result of the asphalt cover over the contaminated zone, restricting exchange with atmospheric CO_2. In October 2002, the percentage CO_2

in vadose zone samples ranged from 4.6 to 23.2% (Table 1). The lowest values were found at control MPs 2 and 3 located at the upstream edge of the groundwater sampling region and control MP 11 at the down stream contaminant plume boundary (Figure 1). Methane was a substantially higher percentage of the vadose zone gas, with a range from non-detect to 60% (Table 1). In March 2003, CO_2 concentrations were substantially lower (0.1–17.6%). CH_4 concentrations were lower at MPs 1 and 10 and higher at MP 6, but otherwise similar to October (0.0–60%). In March, samples from MPs 2, 3, 10, and 11 had the lowest concentrations of both gases. Seasonal variation was observed in the vadose zone for MPs outside the contaminant plume, with CO_2 concentrations lower in March 2003. During both samplings the percentage CO_2 relative to percentage CH_4 was more evenly distributed than seasonal concentrations.

MPs 2, 3, and 11 were selected as control sites for this study based on previous groundwater contaminant distribution surveys conducted by the Navy base (Figure 1) and CO_2 and CH_4 concentrations determined during this study. CO_2 and CH_4 concentrations in control MPs were lower than the contaminated wells during both samplings. CH_4 in the control points was below detection (Table 1).

δ¹³C of CO₂ and CH₄

$\delta^{13}CO_2$ ranged from −30.9 to 22.7‰ in October 2002 and from −27.3 to 9.99‰ in March 2003. Methane $\delta^{13}C$ ranged from –42.09 to −37.43‰ in October and −44.19 to −25.52‰ in March (Table 1). For soil gas stations over the plume, where there was detectable methane, CO_2 was generally enriched in ^{13}C. CO_2 was also ^{13}C-enriched in October relative to March (Table 1).

Percent Modern Carbon of CO₂ and CH₄

In October 2002, CO_2 pMC ranged from 0 to 24.9 (Table 1). A similar range was observed for March 2003 with values from 1.5 to 27.8 pMC. There was variation in values observed for each individual MP between the two samplings. For both sampling events, lower CO_2 concentrations coincided with a higher pMC (Table 1). CO_2 radiocarbon values at the control sites from both samplings were enriched in ^{14}C relative to plume values; however, control soil gas CO_2 was only 30 pMC or approximately 10,000 years old. Methane pMC for March was in a similar range of the values as vadose zone CO_2 at contaminated MPs, although it was never greater than 1.7 pMC. The CH_4 concentrations at the control sites were not high enough to measure radiocarbon.

Discussion

Gas Concentrations

The percentage CO_2 and CH_4 in October 2002 and March 2003 provides petroleum biodegradation evidence in the contaminated regions of the study site because concentrations were elevated relative to background values. CO_2 concentration in the vadose zone is a function of microbial respiration, plant root

Table 1. *Vadose zone data collected at the Norfolk Navy Base, Norfolk VA during October 2002 and March 2003. The standard deviations for percent carbon dioxide and methane and stable carbon isotope ratios of carbon dioxide and methane are calculated from 3 replicate runs. The standard deviation for pMC for CO_2 and CH_4 averaged 0.22 and 0.42, respectively. Not determined (ND) represents either no analysis was made (i.e. October 2002 methane) or gas concentrations below ~5% of soil gas which provided too little sample to obtain stable carbon or radiocarbon isotopes.*

	% CO_2	% CH_4	$\delta^{13}C\ CO_2$	$\delta^{13}C\ CH_4$	pMC CO_2	pMC CH_4
October 2002 Wells						
1S	19.6 ± 0.05	44.6 ± 0.88	8.36 ± 0.56	-37.4 ± 0.29	1.25	ND
1D	22.3 ± 0.12	52.9 ± 0.49	16.7 ± 0.24	-39.1 ± 0.71	-0.13	ND
2S	13.0 ± 0.17	0.05 ± 1.27	-30.9 ± 0.23	ND	ND	ND
2D	12.5 ± 0.13	0.00 ± 0.00	-30.3 ± 0.10	ND	ND	ND
3S	4.60 ± 0.25	0.00 ± 0.00	-22.3 ± 0.20	ND	13.8	ND
5S	22.6 ± 0.31	56.5 ± 5.39	22.7 ± 0.20	-40.7 ± 0.37	-0.20	ND
5D	23.2 ± 0.53	59.9 ± 0.87	22.7 ± 0.46	-40.3 ± 0.14	0.30	ND
6S	18.1 ± 0.39	18.4 ± 5.13	13.6 ± 1.90	-40.4 ± 0.16	1.56	ND
7S	19.5 ± 0.06	43.5 ± 5.04	6.84 ± 0.33	-41.8 ± 0.06	1.92	ND
8S	22.1 ± 0.25	56.2 ± 3.86	18.4 ± 0.35	-41.7 ± 0.58	0.90	ND
8D	22.5 ± 0.54	58.0 ± 4.05	19.7 ± 0.60	-42.5 ± 0.42	0.60	ND
10S	17.2 ± 0.31	50.6 ± 1.46	7.37 ± 0.19	-42.1 ± 0.44	2.67	ND
10D	18.1 ± 0.19	60.5 ± 1.89	20.6 ± 0.60	-42.1 ± 1.45	2.08	ND
11S	6.91 ± 0.34	0.08 ± 0.00	-28.7 ± 0.80	ND	22.7	ND
11D	7.04 ± 0.29	0.00 ± 0.00	-29.6 ± 0.39	ND	24.9	ND
March 2003 Wells						
1S	8.98 ± 0.02	21.30 ± 0.04	-20.7 ± 0.08	-25.5 ± 0.12	5.53	0.22
1D	14.50 ± 0.05	39.50 ± 0.08	-25.6 ± 0.30	-31.4 ± 0.13	1.90	1.52
2D	7.95 ± 0.06	0.00 ± 0.00	ND	ND	8.61	ND
3S	2.12 ± 0.03	0.01 ± 0.00	-13.2 ± 0.34	ND	27.8	ND
4D	11.70 ± 0.07	3.11 ± 0.00	ND	ND	4.09	1.62
5S	17.6 ± 0.05	59.2 ± 0.12	6.00 ± 0.43	-34.9 ± 0.11	ND	1.58
5D	17.40 ± 0.22	58.2 ± 0.12	-25.4 ± 0.32	-34.9 ± 0.14	1.48	0.66
6S	16.30 ± 0.04	51.1 ± 0.10	-3.86 ± 0.23	-36.9 ± 0.18	ND	0.68
7S	11.70 ± 0.12	36.9 ± 0.07	-4.48 ± 0.29	-33.7 ± 0.33	3.48	0.41
7D	14.3 ± 0.10	44.1 ± 0.09	ND	ND	2.56	0.85
8S	16.00 ± 0.01	54.1 ± 0.11	2.19 ± 0.08	-38.8 ± 0.22	1.73	0.98
8D	16.70 ± 0.14	53.6 ± 0.11	2.57 ± 0.22	-39.1 ± 0.26	1.69	1.48
9S	11.60 ± 0.02	22.4 ± 0.04	-6.67 ± 0.45	-39.5 ± 0.04	3.63	1.24
9D	11.80 ± 7.72	21.8 ± 0.04	-5.61 ± 0.83	39.9 ± 0.04	2.77	0.98
10S	7.72 ± 0.07	6.65 ± 0.01	9.99 ± 0.32	-44.2 ± 0.11	2.68	1.43
10D	0.11 ± 0.00	0.00 ± 0.00	ND	ND	ND	ND
11S	1.26 ± 0.02	0.06 ± 0.00	ND	ND	ND	ND
11D	2.05 ± 0.01	0.00 ± 0.00	-27.3 ± 0.23	ND	14.52	ND

respiration, horizontal gas transport, and atmospheric CO_2 exchange. Because the study area was paved over, concentration equilibration with the atmosphere and CO_2 root respiration were likely minimal relative to other processes. The range in percentage CO_2 for the October and March samples (0.1–23.2%) was greater than another study where values ranged from 1–16% (Aelion et al., 1997). The higher percentage CO_2 measured at the Norfolk site may be a result of vadose zone gas trapping under the pavement or rapid petroleum biodegradation rates. There was a difference in the percentage CO_2 between October and March samplings (Table 1) with higher values observed in October. This difference was assumed to be seasonal. Microbial production and respiration rates were higher under warmer conditions before and at the time of the October sampling. CH_4 concentration in the soil gas was routinely in excess of 40% in contaminated region MPs during both samplings (Table 1). CH_4 was not detected in samples from the control MPs. There was a significant linear correlation ($P < 0.01$) between the percentage CO_2 and percentage CH_4 in the vadose zone with goodness of fit statistic

(r^2) values of 0.93 for March (Figure 3). Although a significant correlation was not evident for October, the highest percentage CO_2 and CH_4 values plotted in the same region of the graph (Figure 4).

Although CH_4 to CO_2 ratios were never as high as the literature methanogenic value of 4:1 (Anderson and Lovley, 2000), the ratios ranged from 2 to 3, which is indicative of hydrocarbon conversion to CH_4 and CO_2 under field conditions.

Isotope Analyses

$\delta^{13}C$ for CO_2 and CH_4 fit well with the trends observed in percentage CO_2 and percentage CH_4. At MPs with high percent CO_2 and ^{13}C-enriched CO_2 signatures, CH_4 was consistently depleted in ^{13}C (Figures 5 and 6). In October 2002, CO_2 was more ^{13}C-enriched in contaminated areas than in March (Figures 5 and 6). March 2003 had more ^{13}C-depleted values for both CO_2 and CH_4. These ^{13}C-depleted signatures correspond to lower percentage CO_2 and percentage CH_4 and suggested
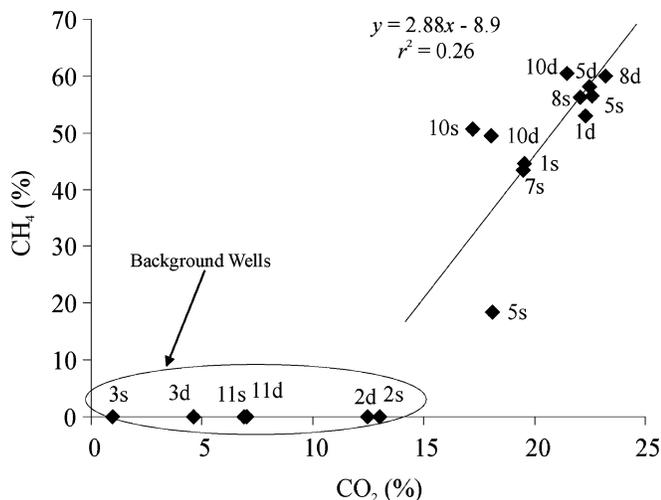
Figure 3. Vadose zone gas comparison with percentage CH_4 plotted relative to percentage CO_2 for the March 2003 sampling.

a lower microbial petroleum cycling rate (most likely due to lower temperature). Seasonal shifts in microbial metabolism rates in temperate aquatic environments are well documented (cf. Lomas et al., 2002). In MPs 2, 3, and 11, CH_4 concentrations were below the $\delta^{13}C$ limits of detection At these control sites the CO_2 was ^{13}C-depleted relative to the other sample MPs (Table 1). This depletion is consistent with aerobic hydrocarbon or soil organic matter degradation as the primary source(s) of respired soil CO_2, particularly so given the lack of significant root respiration or atmospheric CO_2 input. In other studies, vadose zone CO_2 was substantially depleted in ^{13}C relative to the data described here. For contaminated groundwater at a gas station in Columbia, South Carolina, the range for $\delta^{13}CO_2$ was -22.0 to $-35.9‰$ (Aelion et al., 1997). Similar results are observed for a PCB-contaminated Savannah River site, where the range for vadose zone CO_2 over the contaminated region was -26.8 to $-21.1‰$ (Kirtland et al., 2003). At these sites, the stable carbon
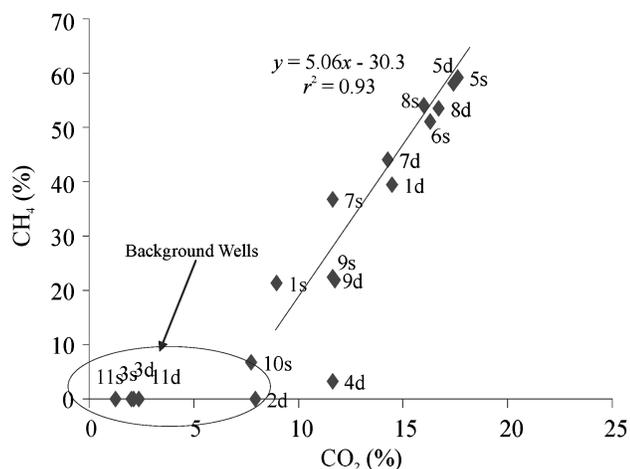

Figure 4. Vadose zone gas comparison with percentage CH_4 plotted relative to percentage CO_2 for the October 2002 sampling.

isotope signature was determined to be a function of plant root respiration, atmospheric input, and microbial respiration.

^{14}C isotope analysis can be used to differentiate between contemporary carbon respiration relative to the petroleum-derived carbon respiration. The technique is very sensitive because there is considerable analytical resolution between end-members (approximately 1200‰); for instance, carbon in atmospheric CO_2 is modern with pMC values up to 120 ($\Delta^{14}C = +200‰$) or even greater due to the influence of bomb-released ^{14}C, whereas carbon in fossil fuels is "radiocarbon dead" with a pMC of 0 ($\Delta^{14}C = -1000‰$). ^{14}C-depleted CO_2 and CH_4 occurred where CO_2 and CH_4 concentrations were elevated (Figure 7). For October 2002 and March 2003, CO_2 from the control MPs ranged from 8.6 to 27.8 pMC, which suggests isolation from atmospheric exchange and microbial degradation of aged plant material if one assumes there was no residual or lateral contributions from petroleum degradation.

Samples taken in March 2003 from MPs above the petroleum plume were more modern and variable with values ranging from 1.48 to 4.09 pMC compared with October 2002 when values were lower with pMC ranging from 0 to 2.06. This change in CO_2 pMC between the sampling events coincides with the variation in the vadose zone percentage CO_2. While the range in variation between the two sample dates was small, the differences were taken as evidence of slower microbial petroleum degradation in March.

Comparing CO_2 pMC and $\delta^{13}C$ reveals that ^{13}C-enriched values were coincident with depleted ^{14}C (Figure 8). In the control MPs (3 and 11), we observed lower $\delta^{13}C$ corresponding to greater pMC. Under aerobic biodegradation, one might expect incorporated carbon and $\delta^{13}CO_2$ to be similar to that of the hydrocarbon contaminant (cf. Pelz et al., 1998). In March 2003, MPs 1D and 5D had $\delta^{13}CO_2$ values in a range typical of fuel hydrocarbons (*cf.* Boyd et al., 2006; Mansuy et al., 1997), perhaps indicating aerobic hydrocarbon biodegradation (Figure 8). Fossil CO_2 with ^{13}C-enrichment indicates anaerobic hydrocarbon degradation under methanogenic conditions (see circled area in Figure 8) as preferential utilization of lighter CO_2 to produce CH_4 leaves a heavier residual CO_2 pool (*cf.* Anderson and Lovley, 2000; Kaplan et al., 1997).

CH_4 was consistently depleted in ^{14}C in the March 2003 sampling (Table 1). These data suggest that the CO_2 respired during petroleum degradation served as a terminal electron acceptor for methanogenesis. There were no clear trends (i.e., a significant regression model) when comparing radiocarbon content and stable carbon isotope values for CH_4 (Figure 9). The lightest $\delta^{13}CH_4$ value was greater than $-45‰$, whereas most values were between -40 and $-30‰$ (Table 1). These values were heavier than expected for biogenic methane production (*e.g.*, Kaplan et al., 1997) unless the CO_2 source signature was ^{13}C-enriched (Grossman et al., 2002). Because the site was covered by an asphalt parking lot and background CO_2 was low in radiocarbon (approximately 25 pMC), atmospheric exchange and root respiration are not likely to impact CO_2 age in the vadose zone. A greater likelihood is that the CO_2 source available
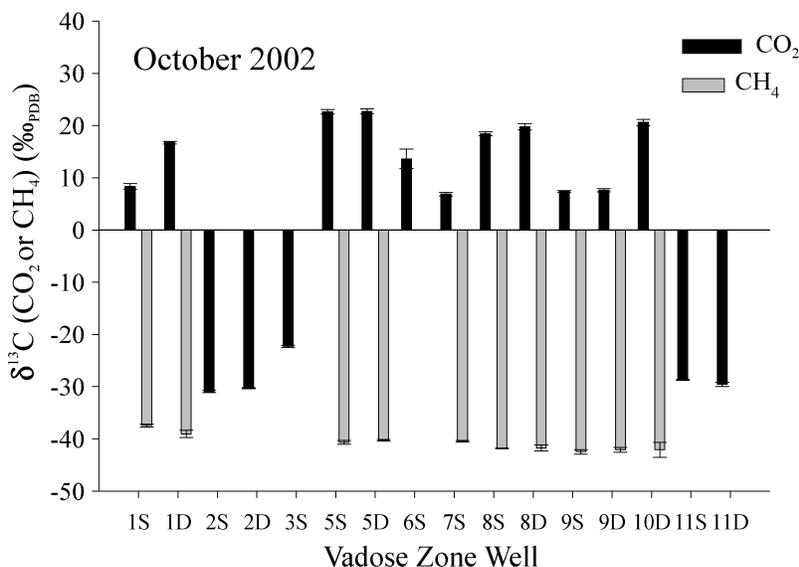
Figure 5. $\delta^{13}C$ of vadose zone CO_2 and CH_4 in October 2002.

on-site for methanogenesis is highly "recycled." Much of the CO_2 in the soil gas was ^{13}C-enriched (Table 1). As this CO_2 is converted to CH_4, the resultant $\delta^{13}CH_4$ values should be heavier than expected (e.g., Kaplan et al., 1997). This is further substantiated by the contrast of Norfolk vadose zone samples with those of other recently reported isotopic field studies. In the vadose zone of a petroleum-contaminated site in Columbia, SC, $\delta^{13}CO_2$ ranged from -35.9 to $-22‰$ (Aelion et al., 1997). At a TCE-contaminated site in Georgia $\delta^{13}CO_2$ in the vadose zone ranged from -26.8 to $-21.1‰$ (Kirtland et al., 2003). The ^{13}C-enriched CO_2 and CH_4 from this study likely indicated considerable carbon recycling in this "atmospherically closed" environment and simplified the interpretation of natural attenuation. Probably also due to the "closed" nature of the presently studied system, the

range of pMC values (0.3 to 24.9) was ^{14}C-depleted relative to other studies (Aelion et al., 1997; Conrad et al., 1997; Kirtland et al., 2003, 2005). Others have reported pMC values for contaminated and uncontaminated vadose zones ranging from 14 to 128 pMC reflecting CO_2 input from the atmosphere and plant root respiration.

Biodegradation Quantification

A two-component radiocarbon mass balance approach was employed in an attempt to constrain the relative contaminant and natural organic matter contributions:

$$\Delta^{14}CO_2 = (\Delta^{14}C_{pet} \times f_{pet}) + [\Delta^{14}C_{NOM} \times (1 - f_{pet})] \quad (2)$$
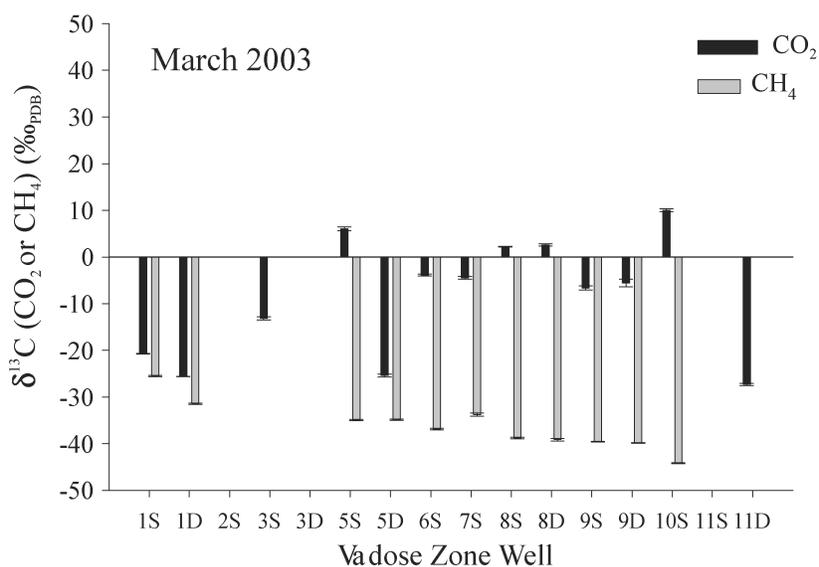

Figure 6. $\delta^{13}C$ of vadose zone CO_2 and CH_4 in March 2003.
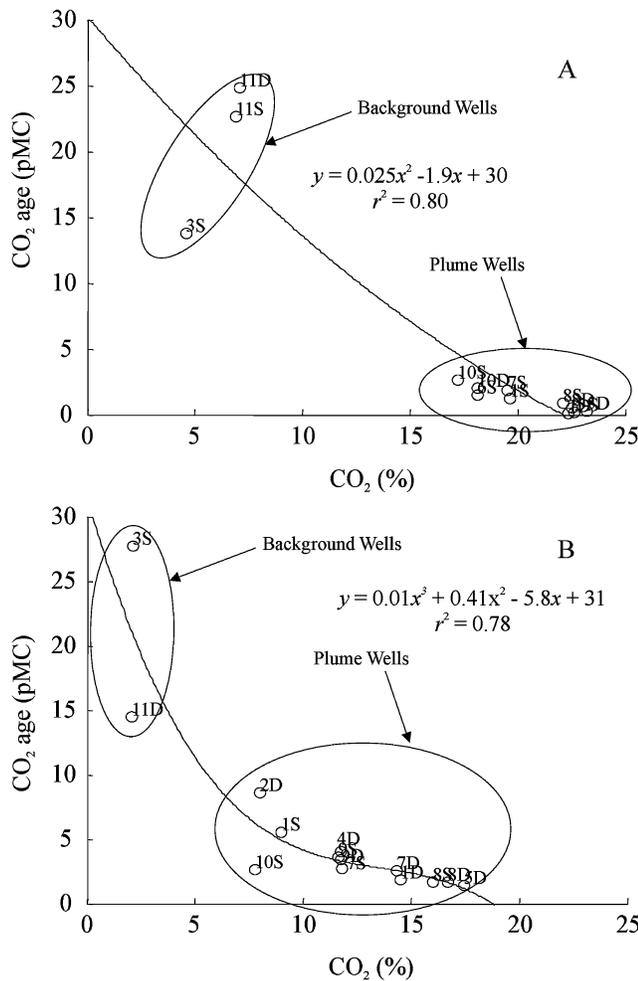
Figure 7. Percentage modern carbon (pMC) soil gas CO_2 relative percentage to CO_2 for A) October 2002 and B) March 2003.
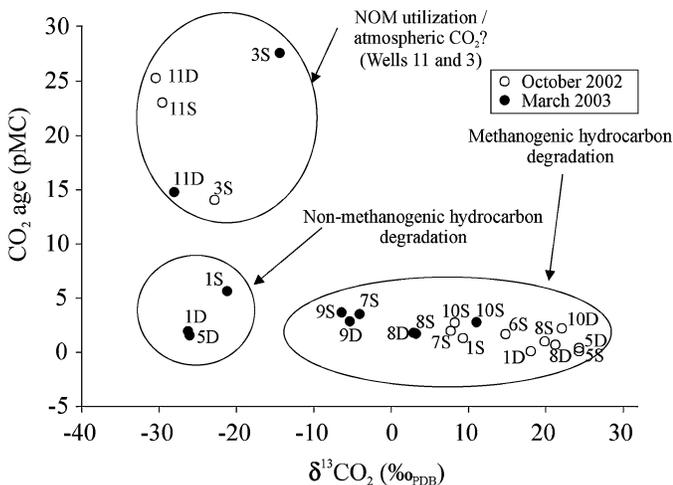

Figure 8. Percentage modern carbon (pMC) variation relative to the $\delta^{13}C$ in the vadose zone CO_2.
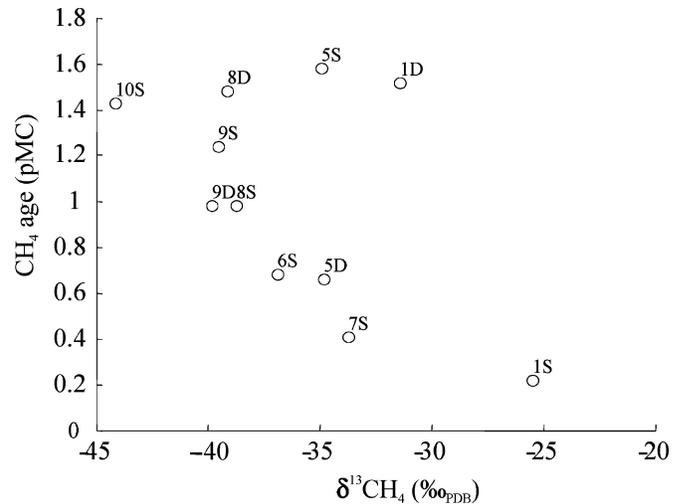

Figure 9. Comparison of radio and stable carbon isotope analysis of methane sampled from soil gas monitoring points (MPs). pMC, percentage modern carbon.

where $\Delta^{14}CO_2$, $\Delta^{14}C_{pet}$, $\Delta^{14}C_{NOM}$ are the soil gas CO_2, petroleum, and natural organic matter $\Delta^{14}C$ signatures, respectively, and f_{pet} is the fraction of CO_2 derived from petroleum. The $\Delta^{14}CO_2$ was measured directly (Table 1), the $\Delta^{14}C_{pet}$ was assumed to be -1000‰, and the $\Delta^{14}C_{NOM}$ was assumed to be represented by the average CO_2 $\Delta^{14}C$ from the control sites. The assumption that the CO_2 from the control sites accurately represents natural organic matter (NOM) $\Delta^{14}C$ no contaminant-derived CO_2 was present at those sites. If the average background radiocarbon CO_2 age (20.7 pMC) and the average plume CO_2 radiocarbon age (2.3 pMC) are converted to $\Delta^{14}C$ notation ($\Delta^{14}C = [pMC -100] * 10$) and placed in the mass balance model, f_{pet} can be calculated as approximately 0.90, indicating that approximately 90% of the CO_2 found in vadose zone gas above the plume is derived from petroleum sources. Using the lowest background pMC value (8.6), the most conservative estimate of f_{pet} is 73%, still indicating that the majority of onsite soil gas CO_2 is derived from the petroleum end-member. This estimate is in line with the site in Columbia, SC, where a range from 58 to 86% was reported (Aelion et al., 1997). Although the overall CO_2 production rate is not currently known, its measurement would give a degradation rate for the hydrocarbon pool in the soil.

Conclusions

This study indicates that fuel hydrocarbons are a significant carbon source to in situ bacteria and that fuel hydrocarbon respiration resulted in the production of ^{14}C-depleted CO_2 (and ultimately CH_4) with stable carbon isotope ratios indicative of microbial methanogenesis. However, the most weathered samples indicated there is considerable recycling of carbon because $d^{13}C$ values for methane were uncharacteristically heavy (-45 to -26‰). The radiocarbon ages of CO_2 and CH_4 were definitive in showing that petroleum carbon makes up approximately 90%

of the carbon utilized by the in situ microbial assemblage. The radiocarbon measurement alone inconclusively demonstrates biodegradation is occurring on-site. Stable isotope ratios alone would likely fail to convey the importance of petroleum carbon to the CO_2 and CH_4 pools as considerable recycling led to relatively heavier than expected values (20–40‰). Coupling of radiocarbon and stable carbon isotope measurements allows a better understanding of onsite biogeochemical conditions (cf. Figure 8). These data provide strong support for the use of carbon isotope analyses to monitor natural attenuation and warrants more extensive seasonal and spatial sampling to better understand the dynamics of petroleum degradation under anoxic conditions. While sample analysis costs for radiocarbon are still relatively high (approximately $600 per sample), the installation of relatively inexpensive (approximately $150 each) soil gas MPs coupled with the results of stable isotope analyses may provide not only a definitive answer as to whether biodegradation is occurring but may provide considerable in situ biogeochemical information. Commercial laboratories are able to cryogenically separate CO_2 from "raw" soil gas samples (i.e., in Cali-5 bags). Methane analysis would likely add approximately $125 to the $600 per sample radiocarbon analysis. Again, though this seems high, several plume and background MPs could be installed, sampled, and analyzed for less than $5K, a fairly low cost to so definitely confirm onsite hydrocarbon biodegradation.

Acknowledgement

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