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

Richard B. Coffin \(^a\); John W. Pohlman \(^b\); Kenneth S. Grabowski \(^c\); David. L. Knies \(^c\); Rebecca E. Plummer \(^d\); Robert W. Magee \(^e\); Thomas J. Boyd \(^a\)

\(^a\) Marine Biogeochemistry, United States Naval Research Laboratory, Washington, DC, USA
\(^b\) United States Geographic Survey, Wood Holes Science Center, Wood Holes, MA, USA
\(^c\) Accelerator Mass Spectrometry, United States Naval Research Laboratory, Washington, DC, USA
\(^d\) SAIC, Marine Biogeochemistry, Washington, DC, USA
\(^e\) Naval Facilities Engineering Command - Atlantic, Norfolk, VA, USA

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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$_2$ ($^{13}C$ approximately $-7\%$) and CO$_2$ produced from organic matter degradation under methanogenic
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 δ13C value of CO2 to the contaminant δ13C value or cannot accurately estimate the atmospheric contribution to CO2 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. 14C-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 radioactively free and thus provide a definitive end-member when analyzed against photosynthetic-based carbon sources, which contain modern CO2 from the atmosphere. The percentage modern carbon (pmC) of living photosynthetic biomass is approximately 110% because of nuclear bomb tests, which peaked before 1965 (Aelion et al., 1997).

In contrast, petroleum carbon and CO2 derived from its biodegradation have no 14C 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 CO2 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 CO2 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 CO2 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/solvent vapor extraction system (Aelion et al., 1997). The CO2 radiocarbon signature in the soil gas and groundwater-dissolved inorganic carbon suggested aerobic petroleum biodegradation produced 59–87% of the total CO2. 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 CO2 (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 (13C and 14C) composition and concentration of CO2 and CH4 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 CO2 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.

CO2 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% CO2 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).

**CH₄ 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...
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 CO₂ from the vadose zone. Sample volumes ranging between 0.5–10 µL were injected into a Trace GC Ultra (Thermo Electron, Waltham, MA, USA) 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 (CH₄ and CO₂) 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 CO₂, which in turn was calibrated against NBS-22 (National Institute of Standards and Technology [NIST, Gaithersburg, MD]). Stable carbon isotope ratios were expressed as:

\[
\delta^{13}C = \left( \frac{R_{s}}{R_{std}} - 1 \right) \times 1000 \text{ (‰)}
\]

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 CO₂ and CH₄ 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 CO₂ over alumina pellets at 650°C and purified by cryogenic distillation. The CO₂ 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**

CO₂ and CH₄ 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 CO₂. In October 2002, the percentage CO₂ 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 downstream 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, CO₂ concentrations were substantially lower (0.1–17.6%). CH₄ 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 CO₂ concentrations lower in March 2003. During both samplings the percentage CO₂ relative to percentage CH₄ 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 CO₂ and CH₄ concentrations determined during this study. CO₂ and CH₄ concentrations in control MPs were lower than the contaminated wells during both samplings. CH₄ in the control points was below detection (Table 1).

\[\delta^{13}C_{\text{OF CO}_2 \text{ and CH}_4} = \frac{\delta^{13}C_{\text{OF CO}_2}}{\delta^{13}C_{\text{OF CO}_2}}\]

\[\delta^{13}C_{\text{OF CO}_2} = \text{from } -30.9 \text{ to } 22.7\% \text{ in October 2002 and from } -27.3 \text{ to } 9.99\% \text{ in March 2003.} \]

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, CO₂ was generally enriched in \(^{13}C\) over \(^{14}C\). CO₂ was also \(^{13}C\)-enriched in October relative to March (Table 1).

**Percent Modern Carbon of CO₂ and CH₄**

In October 2002, CO₂ 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 CO₂ concentrations coincided with a higher pMC (Table 1). CO₂ radiocarbon values at the control sites from both samplings were enriched in \(^{14}C\) relative to plume values; however, control soil gas CO₂ 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 CO₂ at contaminated MPs, although it was never greater than 1.7 pMC. The CH₄ concentrations at the control sites were not high enough to measure radiocarbon.

**Discussion**

**Gas Concentrations**

The percentage CO₂ and CH₄ 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. CO₂ concentration in the vadose zone is a function of microbial respiration, plant root
respiration, horizontal gas transport, and atmospheric CO2 exchange. Because the study area was paved over, concentration equilibration with the atmosphere and CO2 root respiration were likely minimal relative to other processes. The range in percentage CO2 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 CO2 measured at greater than another study where values ranged from 1–16% for the October and March samples (0.1–23.2%) was likely minimal relative to other processes. The range in percentage CH4 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 CO2 and CH4 values plotted in the same region of the graph (Figure 4).

Although CH4 to CO2 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 CH4 and CO2 under field conditions.

### Isotope Analyses

δ13C for CO2 and CH4 fit well with the trends observed in percentage CO2 and percentage CH4. At MP5s with high percent CO2 and 13C-enriched CO2 signatures, CH4 was consistently depleted in 13C (Figures 5 and 6). In October 2002, CO2 was more 13C-enriched in contaminated areas than in March (Figures 5 and 6). March 2003 had more 13C-depleted values for both CO2 and CH4. These 13C-depleted signatures correspond to lower percentage CO2 and percentage CH4 and suggested

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>% CO2</th>
<th>% CH4</th>
<th>δ13C CO2</th>
<th>δ13C CH4</th>
<th>pMC CO2</th>
<th>pMC CH4</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 2002 Wells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1S</td>
<td>19.6 ± 0.05</td>
<td>44.6 ± 0.88</td>
<td>8.36 ± 0.56</td>
<td>−36.4 ± 0.29</td>
<td>1.25</td>
<td>ND</td>
</tr>
<tr>
<td>1D</td>
<td>22.3 ± 0.12</td>
<td>52.9 ± 0.49</td>
<td>16.7 ± 0.24</td>
<td>−39.1 ± 0.71</td>
<td>−0.13</td>
<td>ND</td>
</tr>
<tr>
<td>2S</td>
<td>13.0 ± 0.17</td>
<td>0.05 ± 1.27</td>
<td>−30.9 ± 0.23</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2D</td>
<td>12.5 ± 0.13</td>
<td>0.00 ± 0.00</td>
<td>−30.3 ± 0.10</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3S</td>
<td>4.60 ± 0.25</td>
<td>0.00 ± 0.00</td>
<td>−22.3 ± 0.20</td>
<td>ND</td>
<td>ND</td>
<td>13.8</td>
</tr>
<tr>
<td>5S</td>
<td>22.6 ± 0.31</td>
<td>56.5 ± 5.39</td>
<td>22.7 ± 0.20</td>
<td>−40.7 ± 0.37</td>
<td>−0.20</td>
<td>ND</td>
</tr>
<tr>
<td>5D</td>
<td>23.2 ± 0.53</td>
<td>59.9 ± 0.87</td>
<td>22.7 ± 0.46</td>
<td>−40.3 ± 0.14</td>
<td>0.30</td>
<td>ND</td>
</tr>
<tr>
<td>6S</td>
<td>18.1 ± 0.39</td>
<td>18.4 ± 5.13</td>
<td>13.6 ± 1.90</td>
<td>−40.4 ± 0.16</td>
<td>1.56</td>
<td>ND</td>
</tr>
<tr>
<td>7S</td>
<td>19.5 ± 0.06</td>
<td>43.5 ± 5.04</td>
<td>6.84 ± 0.33</td>
<td>−41.8 ± 0.06</td>
<td>1.92</td>
<td>ND</td>
</tr>
<tr>
<td>8S</td>
<td>22.1 ± 0.25</td>
<td>56.2 ± 3.86</td>
<td>18.4 ± 0.35</td>
<td>−41.7 ± 0.58</td>
<td>0.90</td>
<td>ND</td>
</tr>
<tr>
<td>8D</td>
<td>22.5 ± 0.54</td>
<td>58.2 ± 4.05</td>
<td>19.1 ± 0.60</td>
<td>−42.5 ± 0.42</td>
<td>0.60</td>
<td>ND</td>
</tr>
<tr>
<td>10S</td>
<td>17.2 ± 0.31</td>
<td>50.6 ± 1.46</td>
<td>7.37 ± 0.19</td>
<td>−42.1 ± 0.44</td>
<td>2.67</td>
<td>ND</td>
</tr>
<tr>
<td>10D</td>
<td>18.1 ± 0.19</td>
<td>60.5 ± 1.89</td>
<td>20.6 ± 0.60</td>
<td>−42.1 ± 1.45</td>
<td>2.08</td>
<td>ND</td>
</tr>
<tr>
<td>11S</td>
<td>6.91 ± 0.34</td>
<td>0.08 ± 0.00</td>
<td>−28.7 ± 0.80</td>
<td>ND</td>
<td>22.7</td>
<td>ND</td>
</tr>
<tr>
<td>11D</td>
<td>7.04 ± 0.29</td>
<td>0.00 ± 0.00</td>
<td>−29.6 ± 0.39</td>
<td>ND</td>
<td>24.9</td>
<td>ND</td>
</tr>
</tbody>
</table>

| March 2003 Wells |           |           |          |          |         |         |
| 1S       | 8.99 ± 0.03 | 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 | 0.01 ± 0.00 | −13.2 ± 0.34 | ND        | ND      | 8.61    |
| 3S       | 2.12 ± 0.03 | 3.11 ± 0.00 | ND        | ND        | 4.09    | 1.62    |
| 4D       | 11.70 ± 0.07 | 3.11 ± 0.00 | ND        | ND        | 27.8    | ND      |
| 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 | 56.2 ± 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 ± 0.67 | 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      |
Figure 3. Vadose zone gas comparison with percentage CH4 plotted relative to percentage CO2 for the March 2003 sampling.

Figure 4. Vadose zone gas comparison with percentage CH4 plotted relative to percentage CO2 for the October 2002 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, CH4 concentrations were below the δ13C limits of detection. At these control sites the CO2 was 13C-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 CO2, particularly so given the lack of significant root respiration or atmospheric CO2 input. In other studies, vadose zone CO2 was substantially depleted in 13C relative to the data described here. For contaminated groundwater at a gas station in Columbia, South Carolina, the range for δ13CO2 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 CO2 over the contaminated region was −26.8 to −21.1‰ (Kirtland et al., 2003). At these sites, the stable carbon isotope signature was determined to be a function of plant root respiration, atmospheric input, and microbial respiration.

14C 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 CO2 is modern with pMC values up to 120 (Δ14C = +200‰) or even greater due to the influence of bomb-released 14C, whereas carbon in fossil fuels is “radiocarbon dead” with a pMC of 0 (Δ14C = −1000‰). 14C-depleted CO2 and CH4 occurred where CO2 concentrations were elevated (Figure 7). For October 2002 and March 2003, CO2 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.

Comparing CO2 pMC and δ13C reveals that 13C-enriched values were coincident with depleted 14C (Figure 8). In the control MPs (3 and 11), we observed lower δ13C corresponding to greater pMC. Under aerobic biodegradation, one might expect incorporated carbon and δ13CO2 to be similar to that of the hydrocarbon contaminant (cf. Pelz et al., 1998). In March 2003, MPs 1D and 5D had δ13CO2 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 CO2 with 13C-enrichment indicates anaerobic hydrocarbon degradation under methanogenic conditions (see circled area in Figure 8) as preferential utilization of lighter CO2 to produce CH4 leaves a heavier residual CO2 pool (cf. Anderson and Lovley, 2000; Kaplan et al., 1997).

CH4 was consistently depleted in 14C in the March 2003 sampling (Table 1). These data suggest that the CO2 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 CH4 (Figure 9). The lightest δ13CH4 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 CO2 source signature was 13C-enriched (Grossman et al., 2002). Because the site was covered by an asphalt parking lot and background CO2 was low in radiocarbon (approximately 25 pMC), atmospheric exchange and root respiration are not likely to impact CO2 age in the vadose zone. A greater likelihood is that the CO2 source available...
on-site for methanogenesis is highly “recycled.” Much of the CO₂ in the soil gas was ¹³C-enriched (Table 1). As this CO₂ is converted to CH₄, the resultant δ¹³CH₄ 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, δ¹³CO₂ ranged from −35.9 to −22‰ (Aelion et al., 1997). At a TCE-contaminated site in Georgia, δ¹³CO₂ in the vadose zone ranged from −26.8 to −21.1‰ (Kirtland et al., 2003). The ¹³C-enriched CO₂ and CH₄ 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 ¹⁴C-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 CO₂ 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:

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

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**Figure 5.** δ¹³C of vadose zone CO₂ and CH₄ in October 2002.

**Figure 6.** δ¹³C of vadose zone CO₂ and CH₄ in March 2003.
where $\Delta^{14}CO_2$, $\Delta^{14}C_{pet}$, $\Delta^{14}C_{NOM}$ are the soil gas $CO_2$, petroleum, and natural organic matter $^{14}C$ 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 $CO_2$ $^{14}C$ from the control sites. The assumption that the $CO_2$ from the control sites accurately represents natural organic matter (NOM) $^{14}C$ 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 $^{14}C$ 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 CO₂ and CH₄ 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 CO₂ 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.

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References


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