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A field test of $\delta^{13}C$ as a tracer of aerobic hydrocarbon degradation

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Abstract

The controlled release of a mixture simulating jet fuel was conducted to determine the effects of transport and degradation on the $\delta^{13}C$ of reactants and products in a field setting. Benzene, toluene, naphthalene, xylenes, and decane were mixed with native soil and placed 4 m below ground into a surficial aquifer. The $\delta^{13}C$ values of the hydrocarbons prior to and after emplacement were measured and no significant isotopic fractionation was observed following migration and partial degradation. The $\delta^{13}C$ values of the compounds within the hydrocarbon mixture prior to addition and following 40 and 164 days in the field ranged from −26.6 to −30.0, −26.3 to −30.4 and −26.5 to −29.4‰, respectively. Also measured were the concentrations and $\delta^{13}C$ values of respiration endproducts CO_2 and CH_4 in groundwater samples prior to and 164, 278, 468, 642 and 831 days after the contamination of the surficial aquifer. Endproduct concentrations were clearly elevated relative to pre-emplacement values indicating microbial respiration of the added hydrocarbon mixture. Isotope mass balance calculations yielded similar $\delta^{13}C$ values for the sum of the respiration products and the added hydrocarbon mixture. Our results indicate that the products of hydrocarbon respiration reflect the $\delta^{13}C$ of the substrates, and in less controlled contaminated field settings DIC isotopic values may be useful for estimating hydrocarbon degradation when DIC up-gradient of the spill has a $\delta^{13}C$ value different from that of the contaminants.

1. Introduction

The use of stable carbon isotopes ($\delta^{13}C$‰) as a tracer of hydrocarbon degradation relies on two important factors. First, there should not be significant isotopic fractionation of the hydrocarbon during its decomposition. While the degradation of complex, high molecular weight carbon compounds does not result in significant isotopic fractionation (Peterson and Fry, 1987; Lajtha and Michener, 1994; Hoefs, 1997; Lapham et al., 1999; Proctor et al., 2001), the fate of smaller hydrocarbons is less certain. Second, it is critical that there be a difference in the carbon isotopic composition of the native organic matter relative to the exotic hydrocarbon.

Previous reports of isotopic fractionation during laboratory and field studies of hydrocarbon biodegradation are somewhat inconsistent. Under aerobic conditions, fractionations reported in the literature vary from nil (Sherwood Lollar et al., 1999) to 2 to 3‰ (Meckenstock et al., 1999) to as much as 6‰ (Stehmeier

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et al., 1999). Under anaerobic conditions, toluene fractionation may be in the order of 2–3‰ (Stehmeier et al., 1999; Meckenstock et al., 1999) or less (Ahad et al., 2000). Benzene degradation under aerobic conditions varies between 1 and 3‰ (Hunkeler et al., 2001; Stehmeier et al., 1999) and is less important under anaerobic conditions (Stehmeier et al., 1999). Mansuy et al. (1997) observed no isotopic difference in the $\delta^{13}C$ values between unweathered and weathered oils. Hammer et al. (1998) reported the lack of isotopic fractionation in the $\delta^{13}C$ values of petroleum hydrocarbons (PAH) in a creosote-contaminated site within a 3-month period.

The first objective of this study was to determine the effects of transport and degradation on jet fuel hydrocarbon carbon isotopic composition in a field setting. Little isotopic fractionation occurs in the partitioning of jet fuel hydrocarbons from a non-aqueous phase to an aqueous one or in the volatilization of hydrocarbons (Dempster et al., 1997; Harrington et al., 1999; Slater et al., 1999). To achieve this objective of evaluating the conservative nature of jet fuel hydrocarbon $\delta^{13}C$ values under in situ conditions of transport and degradation, the $\delta^{13}C$ values of a hydrocarbon mixture prior to and after its emplacement in the field at Columbus Air Force Base in Mississippi, USA were measured.

The second objective of this study was to compare $\delta^{13}C$ of respiration products with $\delta^{13}C$ of the hydrocarbon mixture via a mass balance approach. The increase of dissolved inorganic carbon (DIC) and CH_4 concentrations over pre-emplacement background values and their $\delta^{13}C$ values were monitored over time.

It was hypothesized that similar to compounds with higher molecular weights, there would be no significant isotopic fractionation during transport and bacterial uptake and respiration of the hydrocarbons. It was hypothesized that the $\delta^{13}C$ values of the weighted means of the endproducts CO_2 and CH_4 in excess over pre-existing background concentrations would mirror those of the hydrocarbon mixture.

2. Methods

The controlled release of a mixture simulating jet fuel was conducted at a US Air Force Base at Columbus, Mississippi (CAFB). The CAFB site is underlain by a surficial aquifer with an average thickness of 11 m and comprised of alluvial terrace deposits. The aquifer contains primarily gravelly sand and sandy gravel with small amounts of silt and clay. The sandy gravel ranges from poorly sorted to well sorted. Groundwaters flow in a northerly direction. The aquifer system at Columbus is heterogeneous, possessing a large range in hydraulic conductivities from 10^{-3} cm s^{-1} to 1–2 orders larger in magnitude (Adams and Gelhar, 1992; Boggs et al., 1992; Rehfeldt et al., 1992). The site has been the subject of a number of other studies (MacIntyre et al., 1991; Adams and Gelhar, 1992; Boggs et al., 1992; Rehfeldt et al., 1992; MacIntyre et al., 1993; Stauffer et al., 1994).

The hydrocarbon "source" occupied a "trench" with a volume of 15×1×2 m (L×W×H) at 59–61 msl (meters above sea level) in the upgradient section of a field where over 300 multi-level sampling (i.e., MLS) devices had been installed (Boggs et al., 1988). The land surface is approximately 65 msl while the water table is approximately 62.5 msl. The added hydrocarbon mixture (~1601 kg) was composed of benzene (0.01% by weight), toluene (6.25%), ethylbenzene (6.25%), *p*-xylene (6.24%), and naphthalene (6.25%) that were dissolved in decane (75%). The hydrocarbons were mixed with the native soil and replaced. The "source-trench" was constructed in Oct. 1995 and release of the hydrocarbons into the groundwater flow commenced in Nov. 1995. The $\delta^{13}C$ values of the hydrocarbon compounds in the original mixture that was added to soil to form the "source" were measured and compared to the $\delta^{13}C$ values of hydrocarbons recovered from the subsurface downgradient of the "source" after 40 and 164 days.

2.1. Hydrocarbon sampling and analysis

Water samples for hydrocarbon concentration and hydrocarbon-$\delta^{13}C$ analyses were collected from multi-level well samplers downgradient of the source. A 10-channel peristaltic pumps, was used to fill 40-ml VOA vials to the brim (leaving no headspace), and 2 drops of concentrated HCl added for preservation. Water samples were refrigerated prior to analysis.

Samples for hydrocarbon concentration were analyzed by the Air Force Research Laboratory, Tyndall AFB, FL using a purge and trap system connected to a gas chromatograph. Compound specific $\delta^{13}C$ analysis of hydrocarbon compounds (Hammer et al., 1998) was conducted using a purge and trap system connected to a gas chromatograph that was then coupled to an isotope ratio mass spectrometer via a combustion interface. These analyses were conducted at the EPA Laboratory in Gulf Breeze, FL. Hydrocarbons within the original mixture that was added to the Columbus aquifer, samples extracted from 6 cores collected downfield of the source 40 days after emplacement, and samples collected from 27 multi-level sampling wells located downfield of the source 164 days after emplacement were analyzed. Following the hydrocarbon-$\delta^{13}C$ method of analysis in Kelley et al. (1997), only $\delta^{13}C$ values from samples that registered 1–9 V on the GC/IRMS (Gas Chromatography, Isotope Ratio Mass Spectrometry) system were used. Kelley et al. (1997) reported that standard deviations of replicate samples were less than 0.5‰. Ethylbenzene $\delta^{13}C$ values are not reported because the compound was poorly resolved from an interfering peak in our chromatographic method.

2.2. DIC (dissolved inorganic carbon) and CH_4 sampling and analysis

Water samples were collected at multi-level sampling wells before and after source emplacement. Waters were purged at a rate of 10–20 ml min^{-1}, discarding the first 100 ml before samples were collected in 10-ml syringes (*B-D*) equipped with 3-way stopcocks. Air bubbles were removed from within the syringe and the samples, filtered through 0.45 μm Whatman GF/F filter, were injected directly into 20-ml evacuated Pierce hypovials that were fitted with red rubber stoppers (Wheaton #06-447K). Vials were stored inverted on ordinary ice during transport from the field, and were stored frozen and inverted in the laboratory.

During analysis, thawed samples were re-pressurized to ambient pressure using nitrogen gas and a splitter. Samples were then acidified with CO_2-free 30% H_3PO_4, shaken for 2 min and then placed in a ~30 °C constant temperature water bath. Standard solutions were made up from $NaHCO_3$ with a known $\delta^{13}C$ value using CO_2-free deionized distilled water, and the same size vials and solution volumes as the field samples. DIC concentration and $\delta^{13}C$ values were measured by direct injection, GC/IRMS. Typically 0.1–0.5 ml of headspace gas was injected. Each sample was injected twice. The $\delta^{13}C$ values of CH_4 were also recorded when CH_4 was observed. Lombardi (1999) observed that linear calibration curves ($r^2 = 0.999$) can be generated by either using the 44 cup peak voltage or area, therefore making the GC/IRMS method a quick way of determining concentrations (Fig. 1). Final sample DIC concentrations were calculated from the integrated sample voltages using measured concentrations corrected for injected volume, total headspace and aqueous volumes, and CO_2 solubility relative to a standard curve generated from known bicarbonate standards. Bicarbonate standards of 2–6 mM with a $\delta^{13}C$ value of -21.3 ± 0.3‰ were used. To determine analytical and sampling variability, 28 samples were collected in duplicate at random field locations in August 1995. Each of these 56 individual samples was analyzed twice. Analytical and sample reproducibility will be compared.

Most water samples had dissolved methane concentrations that were too low for measurement with the GC/IRMS system (<500 ppmv). Therefore, following DIC analysis, headspace gas from the same sample vials were injected onto a gas chromatograph (Shimadzu 14A) with a flame ionization detector at column and detector temperatures of 40 and 110 °C, respectively. Column packing was made up of HayeSep Q. Sample concentrations were calculated and corrected for aqueous and headspace volumes, and methane solubility. The detection limit is 0.5 nM. Laboratory standards containing 1.812 ppmv, 99 ppmv and 1% CH_4 and calibrated against NIST standards were used.

2.3. Soil incubations

Laboratory experiments were conducted to measure the $\delta^{13}C$ values of evolved CO_2 under more controlled conditions. The incubations were conducted in 1L

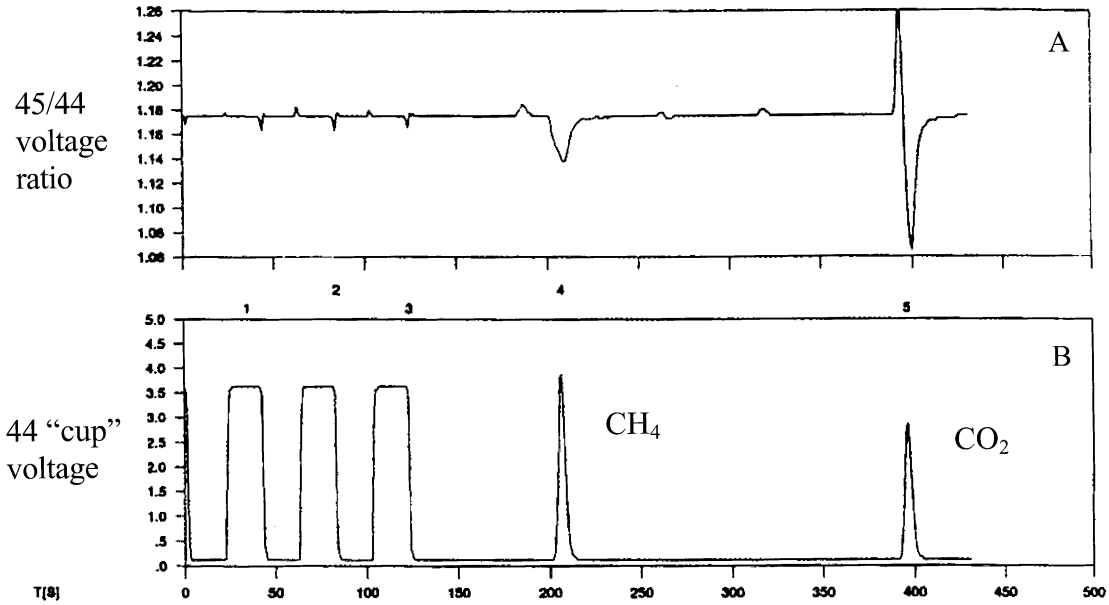

Fig. 1. Sample chromatogram from the GC/IRMS. Graph A shows the mass ratio 45/44 while graph B shows the voltage in the 44 cup. The x axis is time in seconds. Peaks 1–3 represent reference standards. Peaks 4 and 5 represent CH_4 and CO_2, respectively. The 44 cup measures voltage which is proportional to the amount of $^{12}C^{16}O_2$, while the 45 cup quantifies $^{13}C^{16}O_2$.

Erlenmeyer flasks and contained 40–60 g of moist soil from the site. Flasks were stoppered and purged with either air or nitrogen to simulate aerobic or anaerobic conditions respectively. To half of the flasks a 0.5 ml subsample of the hydrocarbon mixture originally applied to the source was added. Flasks were incubated in the dark at room temperature for 100 days. Similar to the analysis for DIC concentration, headspace CO_2 concentration and $\delta^{13}C$ were determined by direct injection GC/IRMS. CO_2 standards ranging from 0.25 to 2% by volume were used and produced a linear calibration curve for integrated area under the 44 mass voltage peak (Fig. 1). Incubation concentrations that are reported represent the accumulated CO_2 in vol.% in the incubation vessel headspace at end of the experiment. Increases in CO_2 were linear over time in these incubations (Bugna, 1999).

2.4. Stable carbon isotopes, $\delta^{13}C$

The standard notation relative to PDB (Lajtha and Michener, 1994; Hoefs, 1997).

$$\delta^{13}C(‰) = \left(\frac{R_{sample}}{R_{PDB}} - 1\right) \times 1000 \quad (1)$$

where $R = {}^{13}C/{}^{12}$ and $R_{PDB} = 0.0112372$ (Hoefs, 1997) was used. Samples that contain more ^{13}C relative to the standard have $\delta^{13}C$ values that are positive and are termed enriched. Samples containing proportionally less ^{13}C than the standard have $\delta^{13}C$ values that are negative and are termed depleted (Lajtha and Michener, 1994).

2.5. Isotope ratio measurements

Isotopic analysis and DIC concentrations were conducted using a Finnigan MAT delta S GC/IRMS (Gas Chromatograph–Isotope Ratio Mass Spectrometer). A headspace volume of 0.1–0.5 ml of the sample or standard was injected into a Hewlett-Packard 5890 gas chromatograph equipped with a PoraPLOT Q column operated at 40 °C. Ultrapure helium was used as carrier gas. The GC effluent passed across a 940 °C non-porous alumina tube containing braided wires of copper, nickel and platinum to combust hydrocarbons to CO_2 (Habfast, 1991; Merritt and Hayes, 1995). Nafion membrane was used to prevent water from reaching the ion source. Ions of CO_2 were collected in the 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$), and 46 ($^{12}C^{16}O^{18}O$) faraday cups where they produced a voltage which was recorded, amplified, converted to frequency and counted (Schimel, 1993). Sample isotopic ratio was calculated relative to the isotopic ratio of a CO_2 standard with a known $\delta^{13}C$ value that was introduced to the ion source via an independent capillary with each run. Check standards, consisting of 1% CO_2 and bicarbonate standards made up from 2 to 6 mM with a $\delta^{13}C$ value of -21.3 ± 0.3‰ were run daily. Fig. 1 shows a chromatogram of a CO_2- and CH_4-containing sample run by GC/IRMS.

3. Results and discussion

3.1. Field hydrocarbon $\delta^{13}C$ values

To address the issue of ^{13}C fractionation of the hydrocarbon compounds during in-situ degradation and transport, the $\delta^{13}C$ values of the original hydrocarbon mixture that was added to the field were compared to the $\delta^{13}C$ values of the hydrocarbon from field samples recovered in January and May 1996. For hydrocarbons that were measured in the original mixture and later observed in field samples, reproducibility of the analysis varied from 0.2 to 0.5‰ for xylene, from 0.5 to 0.9‰ for toluene, and from 0.4 to 1.1‰ for benzene. Hydrocarbon $\delta^{13}C$ values did not vary with hydrocarbon concentration in groundwaters collected in May (after 164 days) from a number of CAFB sampling wells (Fig. 2A–D). Respiration of hydrocarbon compounds did occur over the 164-day period as DIC concentrations more than doubled in groundwater (see below).

The $\delta^{13}C$ values of the hydrocarbon compounds of the mixture comprising the source and those collected from the soils (after 40 days) and groundwaters (after 164 days) at CAFB ranged from -26.6 ± 0.2 to -30.0 ± 0.9‰, -26.3 ± 0.2 to -30.4 ± 0.5‰ and -26.5 ± 0.5 to -29.4 ± 0.9‰, respectively (Table 1). Statistical analyses showed no significant difference in the means of the $\delta^{13}C$ values of individual hydrocarbon compounds prior to dispersal and collected under different conditions at $P < 0.05$; indicating no isotopic fractionation of hydrocarbon $\delta^{13}C$ during transport and microbial degradation within a 164 day (~5.5 month) period. These results are consistent with the first of the two conditions under which $\delta^{13}C$ analyses can be used to trace hydrocarbon degradation. The lack of fractionation in hydrocarbon mixtures during transport and degradation indicates that the sum of the respiration products produced from these substrates should have similar $\delta^{13}C$ values, from -26 to -30‰. This idea will be tested in the section below.

The observed values of BTEX hydrocarbons (-26 to -30‰, Table 1) were similar to the range of $\delta^{13}C$-hydrocarbon values observed by Dempster et al. (1997) (~-26 to -30‰) and Harrington et al. (1999) (-24 to -29‰) who analyzed hydrocarbon compounds straight from the suppliers. Dempster et al. (1997) also reported a lack of isotopic fractionation in the partitioning of BTEX between the aqueous phase and free product. Our field results are consistent with the results of laboratory incubations conducted by Sherwood Lollar et al. (1999) who showed a lack of significant isotopic

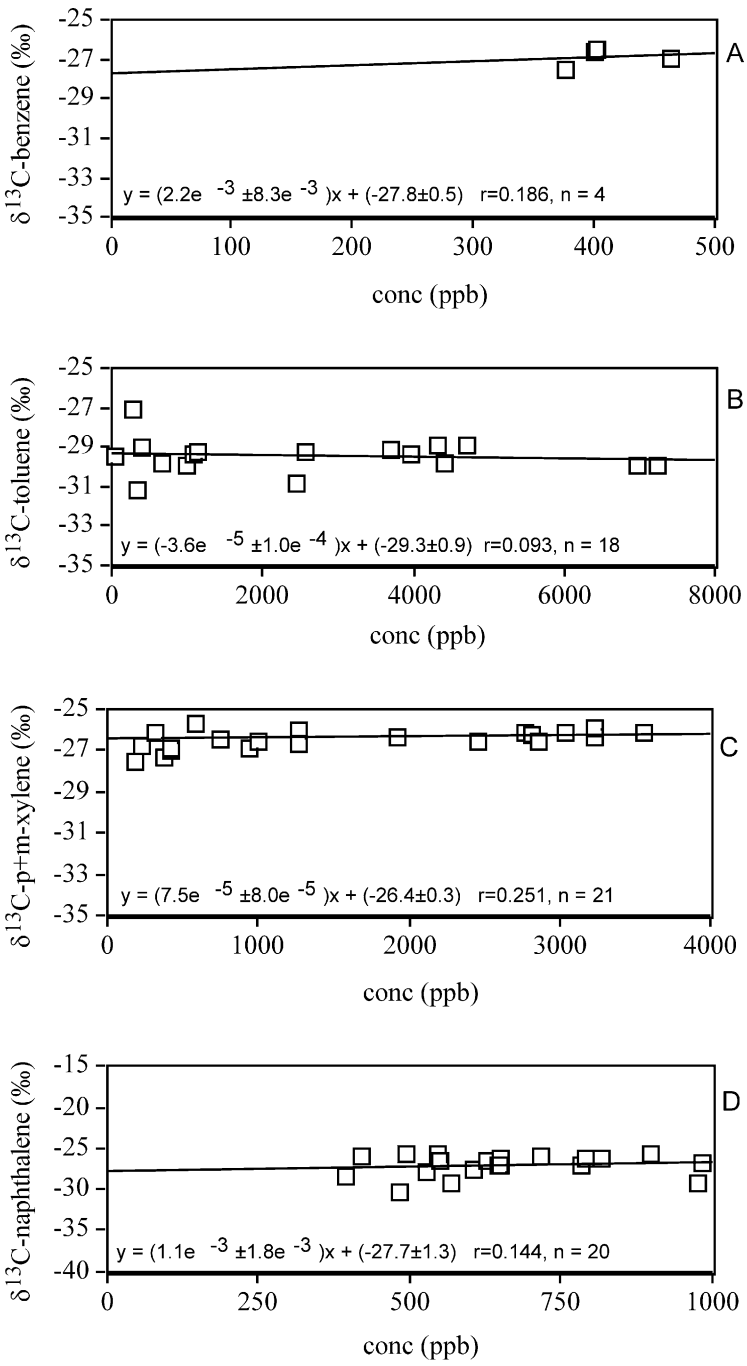

Fig. 2. There was no correlation between BTX (A—benzene, B—toluene, C—$p+m$-xylene) and naphthalene (D) concentrations and their $\delta^{13}C$ values in ground water samples collected in May 1996 at Columbus AFB, indicating little isotopic fractionation in the degradation and dispersion of these compounds below ground.

Table 1
The $\delta^{13}C$ values of hydrocarbons placed in the "source-trench" at Columbus AFB (CAFB), Mississippi. Soil and water samples were collected in January and May 1996, 40 and 164 days after placement. Errors represent 1 σ for repeated runs on the hydrocarbon mixture or for the number of field samples indicated. N.A. denotes no available data

Petroleum hydrocarbon	$\delta^{13}C$ (‰)		
	Hydrocarbon mixture	CAFB soils	CAFB groundwaters
Benzene	−27.5±1.1 (3)	N.A.	−26.8±0.4 (4)
Toluene	−30.0±0.9 (7)	−30.4±0.5 (6)	−29.4±0.9 (18)
p+*m*-Xylene	−26.6±0.2 (5)	−26.3±0.2 (6)	−26.5±0.5 (21)
Naphthalene	N.A.	N.A.	−27.0±1.3 (20)
Decane	−26.8±1.9 (18)	N.A.	N.A.

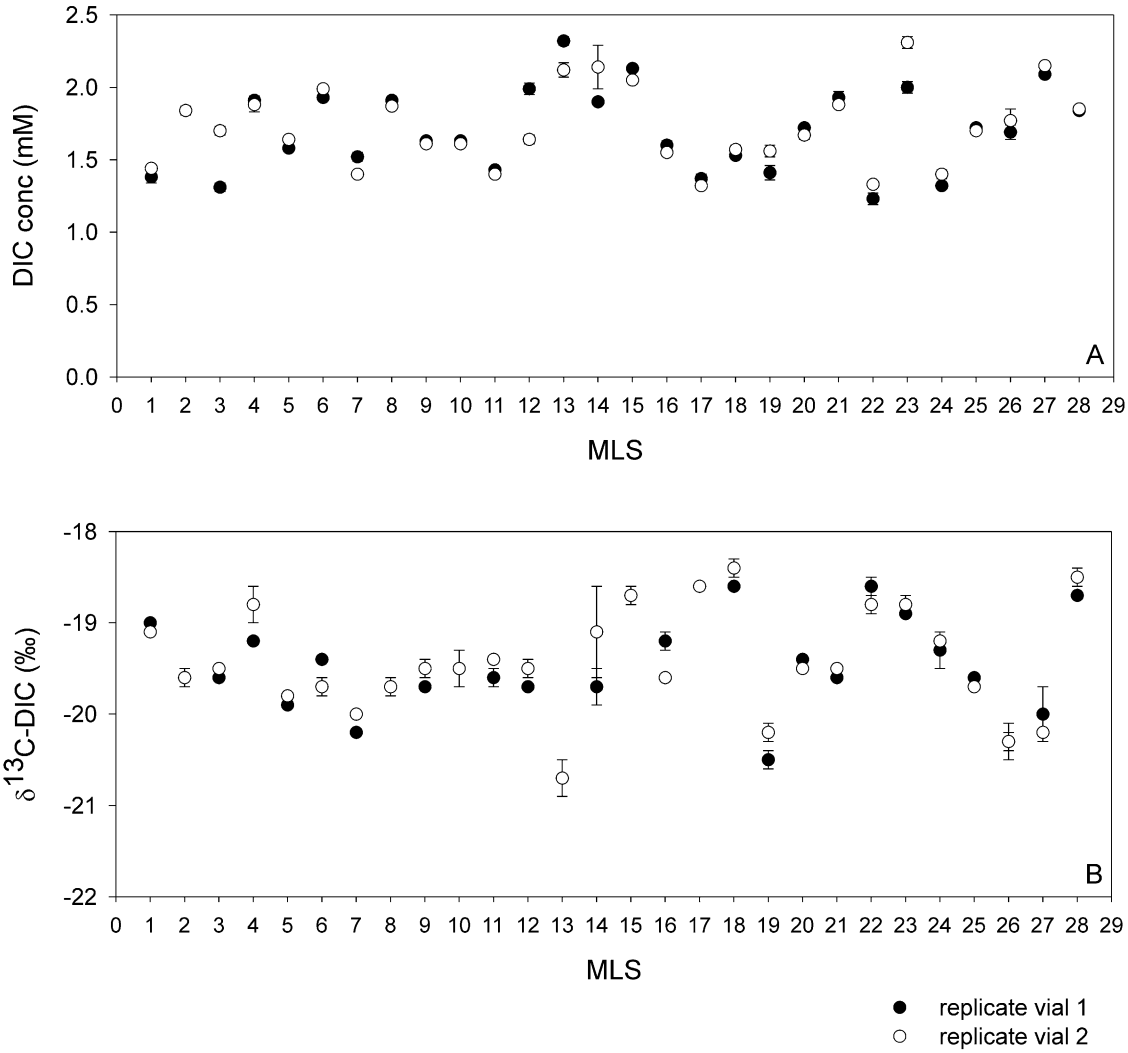

Fig. 3. Reproducibility in DIC concentrations (A) and $\delta^{13}C$ values (B) measured from 28 samples each obtained from a different MLS (multi level sampler). For each water sample duplicate vials were collected and each vial was analyzed twice. Error bars represent 1/2 range and every symbol has error bars although some may not be distinguishable from the symbol. In eight cases the open symbol lies exactly over the filled symbol, obscuring it.

fractionation in the $\delta^{13}C$ of toluene with decreased concentrations during aerobic biodegradation.

3.2. Field DIC and CH_4 $\delta^{13}C$ values

Groundwater samples were collected before and after contamination and analyzed for DIC and CH_4 concentrations and $\delta^{13}C$ values. Sample sets were collected from the well field twice prior to hydrocarbon emplacement and 164, 278, 462, 642 and 831 days following emplacement. To save space, only a representative subset of the data will be shown here. The complete data set may be found in Bugna (1999).

In August 1995, a time prior to the emplacement of the hydrocarbon when we expected fairly uniform conditions, duplicate field sample vials were collected and analyzed twice to determine inter- and within- bottle precision for the sampling and analysis of DIC concentration and $\delta^{13}C$ (Fig. 3). Duplicate analysis of single samples indicated mean errors of 0.03 ± 0.02 mM and 0.1 ± 0.1‰ ($n = 56$) for concentration and $\delta^{13}C$, respectively. Comparison between duplicate sample vials indicated mean errors of 0.05 ± 0.05 mM and 0.1 ± 0.1‰ ($n = 28$). These values represent the mean and standard deviation of the error for analytical precision and sample collection precision respectively, where individual

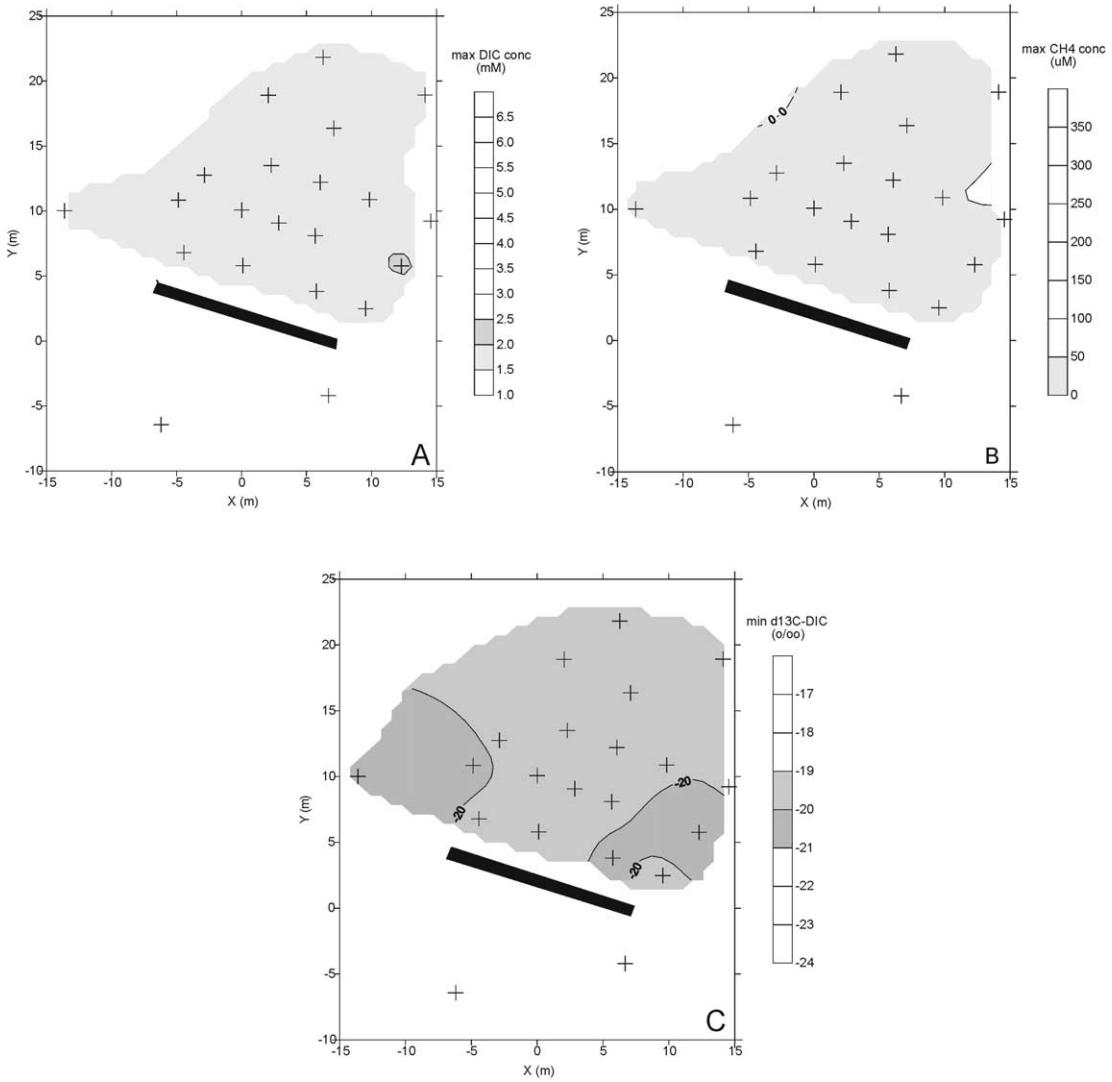

Fig. 4. Contour maps showing DIC (A) and CH_4 (B) concentrations and DIC $\delta^{13}C$ (C) in groundwater in September 1995, 100 days prior to hydrocarbon contamination. The black rectangular box located in the upgradient section of field represents the trench that was filled with hydrocarbons in November 1995.

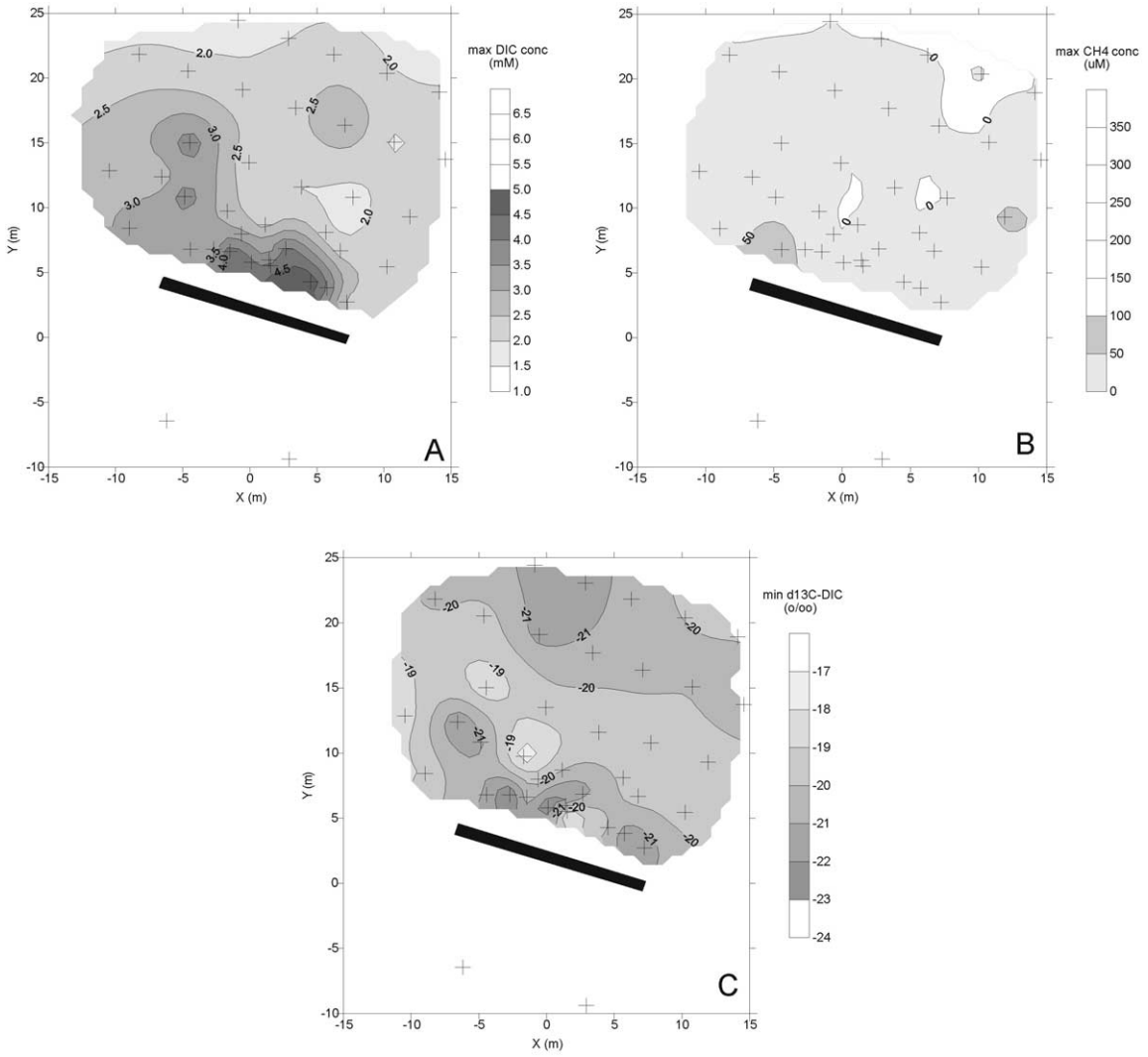

Fig. 5. Contour maps showing DIC (A) and CH_4 (B) concentrations and DIC $\delta^{13}C$ (C) in groundwater in September 1996, 278 days after the November 1995 hydrocarbon contamination. Maximum concentrations and minimum isotopic signatures were used to represent maximum extent of degradation. The black rectangular box located in the upgradient section of field represents the trench containing the hydrocarbons.

Table 2
Calculation of $\delta^{13}C$ values of DIC carbon in excess of ambient concentration using Eq. (2)

Time (days)	Max $[DIC]_{final}$ (mM)	Min $\delta^{13}C$-DIC_{final} (‰)	$[DIC]_{excess}$ (mM)[a]	$\delta^{13}C$-DIC_{excess} (‰)
164	3.8	−22.2	2.1	−24.3
278	4.3	−23.5	2.6	−26.1
462	4.8	−23.3	3.1	−25.3
642	5.8	−22.6	4.1	−23.9
831	6.1	−23.4	4.4	−24.8

Maximum DIC concentrations and minimum $\delta^{13}C$-DIC values were applied in the calculations to yield the maximum extent of degradation. Ambient DIC and $\delta^{13}C$ values of 1.71 mM and −19.65‰, respectively, were used.

[a] $[DIC]_{excess} = [DIC]_{final} - [DIC]_{initial}$.

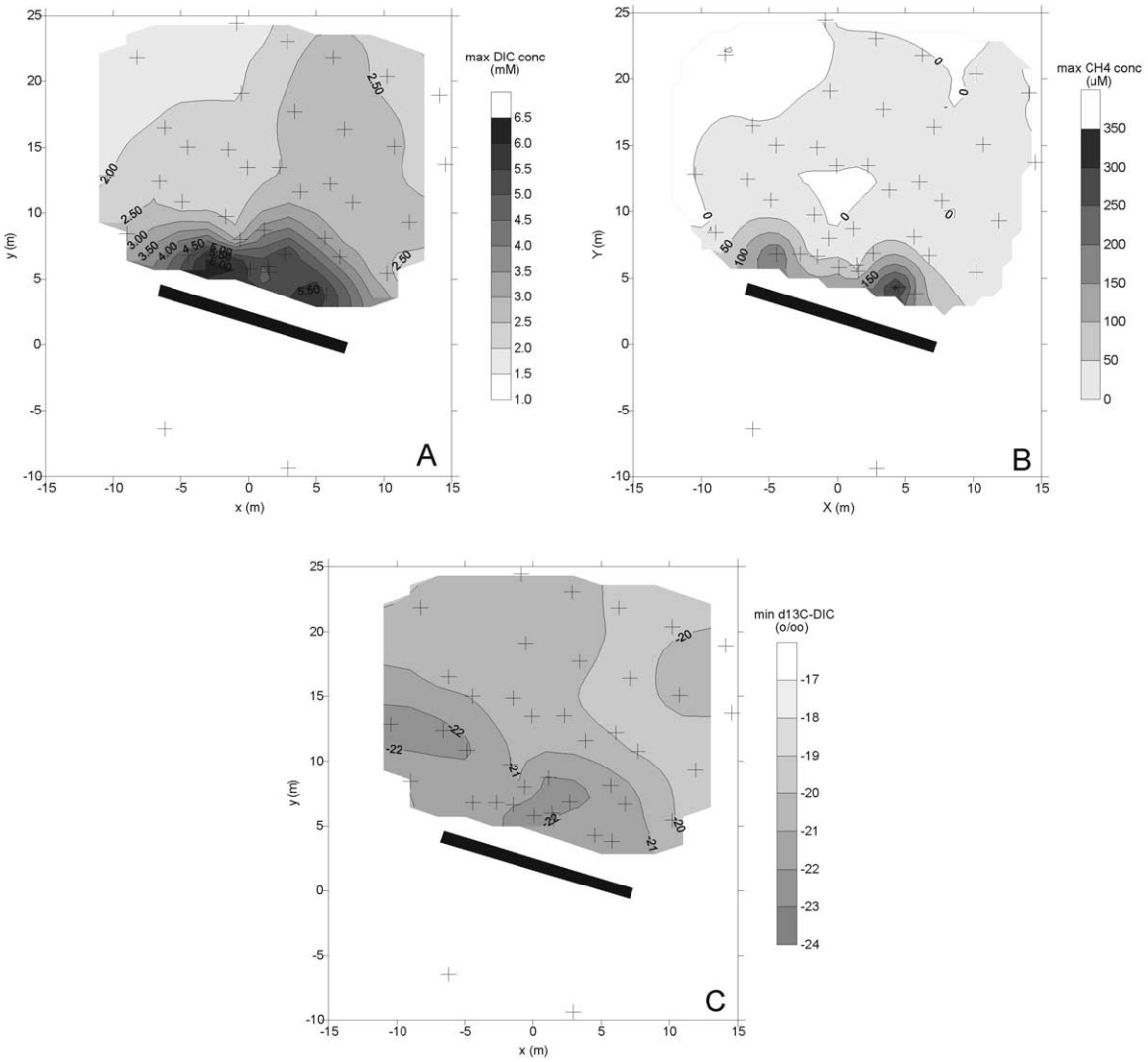

Fig. 6. Contour maps showing DIC (A) and CH_4 (B) concentrations and DIC $\delta^{13}C$ (C) in groundwater in March 1998, 831 days after contamination. Maximum concentrations and minimum isotopic signatures were used to represent maximum extent of degradation. The black rectangular box located in the upgradient section represents the trench containing the hydrocarbons.

Table 3
Calculation of $\delta^{13}C$ values of total respired carbon using Eqs. (2) and (3)

Time (days)	$[DIC]_{excess}$ (mM)	$\delta^{13}C\text{-}DIC_{excess}$ (‰)	Max $[CH_4]$ (mM)	Min $\delta^{13}C\text{-}CH_4$ (‰)	$\delta^{13}C_{respired}$ (‰)
164	2.1	−24.3	0.0673	−91.2	−26.3
278	2.6	−26.1	0.1064	−92.3	−28.7
462	3.1	−25.3	0.1740	−81.1	−28.2
642	4.1	−23.9	0.1607	−74.2	−25.8
831	4.4	−24.8	0.3338	−78.1	−28.5
mean					-27.5 ± 1.4 ($n = 5$)

DIC_{excess} concentrations and $\delta^{13}C\text{-}DIC_{excess}$ values were taken from Table 2.

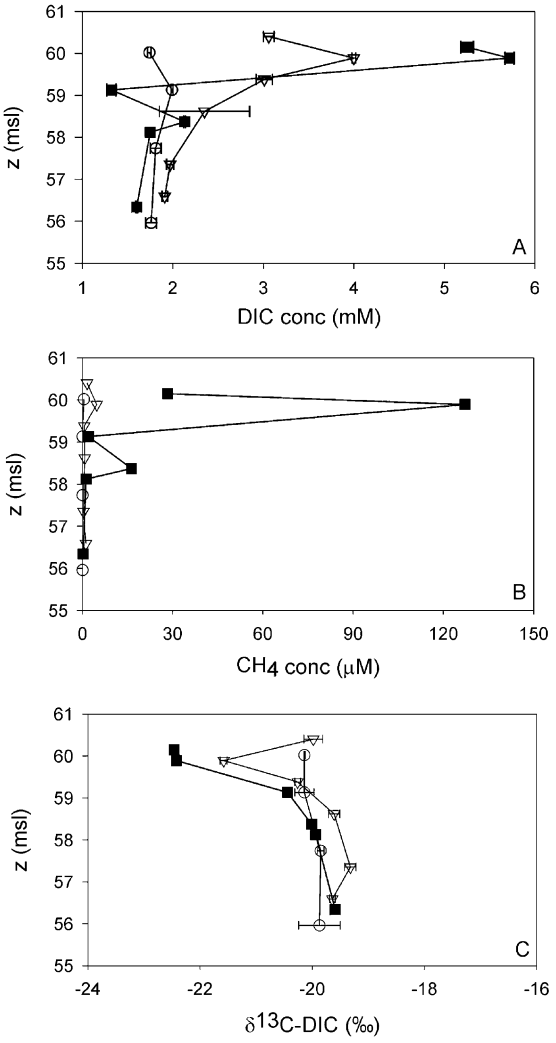

Fig. 7. Temporal vertical profiles of DIC and CH_4 concentrations and $\delta^{13}C$ at a multi-level sampler located 4.2 m down-gradient of the hydrocarbon source (M013). The vertical axis is in terms of msl (meters above sea level) with land surface located at 65 msl. Groundwater contamination was initiated in November 1995. Open circles represent samples collected in September 1995, 100 days prior to contamination; open triangles, September 1996, 278 days after contamination; and filled squares, March 1998, 831 days after contamination.

error for duplicate analysis on the same vial or for companion vials was calculated as 1/2 the range of the two measurements. For comparisons of companion vials, the average of the two analytical determinations was compared. Given that the analytical replication of single samples was so similar to replication of duplicate samples, we decided to collect only single samples and analyze each one twice as a standard protocol. Over the course of the entire study, 2104 DIC samples were analyzed twice and precision averaged 0.05±0.06 mM and again the observed precision for $\delta^{13}C$ was 0.1±0.1‰. Accuracy was determined comparing our working standards, which were run with each set of samples, with NIST NBS-19 calcite. The expected value of 1.95±0.02‰ (Coplen, 1996) was identical to the measured value of 1.88±0.32‰ ($n=3$ individual determinations) within the stated uncertainty.

In September 1995, one hundred days prior to emplacement of the hydrocarbon source, DIC and methane concentrations were relatively low and uniform (Fig. 4). DIC had a mean concentration of 1.7±0.2 mM while methane had a mean concentration of 0.2±0.4 μM. The mean $\delta^{13}C$ DIC value under ambient, pre-placement conditions was −19.7±0.7‰, while jet fuel hydrocarbons have $\delta^{13}C$ values that range from −26 to −30‰ (Table 1) and were not fractionated during their decomposition. Thus the second condition in employing isotopes to trace hydrocarbon decomposition is met, i.e., there was a difference in the $\delta^{13}C$ of DIC produced from native organic matter and that produced from exotic hydrocarbon degradation.

Following emplacement of the hydrocarbon source, in November 1995, DIC and methane concentrations increased and DIC isotopic values became more negative (Figs. 5 and 6). From pre-emplacement to 831 days following emplacement in March 1998, DIC increased from 1.7 mM to as high as 6.1 mM while CH_4 concentrations increased from 0.2 μM to as high as 334 μM (Tables 2 and 3). Although some methane was produced, degradation at the Columbus site was predominantly aerobic. Measurements of dissolved oxygen in groundwaters never indicated oxygen depletion so apparently methane production occurred in reduced microzones (Libelo, unpublished data, 1997). DIC $\delta^{13}C$ values became more depleted (as low as −23.4‰) over the same time period. In addition, vertical profiles of individual wells located 4.2 m down-gradient of the hydrocarbon source showed increases in concentration and depletion in DIC $\delta^{13}C$ (Fig. 7) at a depth coincident with the depth of the hydrocarbon source (59–61 msl).

A simple mixing model was used to verify that the source of excess DIC in the system was due to the degradation of the hydrocarbons

$$[\mathrm{DIC}]_{\mathrm{excess}} * \delta^{13}\mathrm{C\text{-}DIC}_{\mathrm{excess}} = [\mathrm{DIC}]_{\mathrm{final}} * \delta^{13}\mathrm{C\text{-}DIC}_{\mathrm{final}} - [\mathrm{DIC}]_{\mathrm{initial}} * \delta^{13}\mathrm{C\text{-}DIC}_{\mathrm{initial}} \quad (2)$$

where $[\mathrm{DIC}]_{\mathrm{initial}}$ and $\delta^{13}\mathrm{C\text{-}DIC}_{\mathrm{initial}}$ are mean ambient values obtained from the data of all sampled wells before the source was emplaced; $[\mathrm{DIC}]_{\mathrm{final}}$ and $\delta^{13}\mathrm{C\text{-}DIC}_{\mathrm{final}}$ are values obtained afterwards; and $[\mathrm{DIC}]_{\mathrm{excess}}$ and $\delta^{13}\mathrm{C\text{-}DIC}_{\mathrm{excess}}$ are the concentration and $\delta^{13}C$ of the additional DIC source ($[\mathrm{DIC}]_{\mathrm{excess}} = [\mathrm{DIC}]_{\mathrm{final}} - [\mathrm{DIC}]_{\mathrm{initial}}$). Using Eq. (2), values for $\delta^{13}\mathrm{C\text{-}DIC}_{\mathrm{excess}}$ can be determined. The premise of the above equation is that when

organic materials are biodegraded (whether they be of native or anthropogenic origin), the end products are in the form of CO_2 that has the same isotopic signature as the starting materials. This is essentially true in aerobic and certain anaerobic conditions such as nitrate, sulfate and iron reducing conditions wherein the major end product is only CO_2. However, in methanogenic conditions, when biodegradation products include not only CO_2 but CH_4 as well, an isotopic fractionation occurs because of the preferential formation of $^{12}CH_4$ thereby causing the isotopic signatures of the CO_2 to be enriched in ^{13}C. Whether the methane production uses either the acetate fermentation or the CO_2 reduction pathways, the result is the ^{13}C enrichment of the isotopic signature of the DIC (Barker and Fritz, 1981; McMahon and Chapelle, 1991a; Aravena et al., 1995). In such a case, an additional equation is used to correct for this enrichment.

$$[DIC]_{excess} * \delta^{13}C\text{-}DIC_{excess} + [CH_4] * \delta^{13}C\text{-}CH_4 = [C]_{respired} * \delta^{13}C_{respired\ carbon} \quad (3)$$

where $[DIC]_{excess}$ and $\delta^{13}C\text{-}DIC_{excess}$ are the DIC concentration and the isotopic signature of the additional DIC source [Eq. (2)], and $[C]_{respired}$ and $\delta^{13}C_{respired\ carbon}$ are the total respired carbon concentration and isotope ratio that were corrected for the presence of methane, respectively. For the calculation of $\delta^{13}C_{respired}$, pre-placement ambient DIC concentration and $\delta^{13}C$-DIC values of 1.71 mM and −19.65‰ were used in Eq. (2), and $[DIC]_{excess}$ and $\delta^{13}C_{excess}$ were calculated. The most depleted $\delta^{13}C\text{-}CH_4$ value from each sampling snapshot was used. In assuming the maximum extent of biodegradation, the maximum DIC and CH_4 concentrations and minimum $\delta^{13}C$-DIC values were used to represent post contamination in Eqs. (2) and (3) (Tables 2 and 3).

McMahon and Chapelle (1991a,b) suggested that a CH_4 concentration that is less than 100 μM does not significantly affect the isotopic signature of the excess DIC. In 4 out of 5 sampling snapshots between 164 and 831 days, the maximum CH_4 concentrations ranged between 106 and 334 μM with the lowest concentration of 67 μM found at 164 days. It is therefore reasonable to include the CH_4 contribution in the calculation of the respired carbon $\delta^{13}C$ values. The $\delta^{13}C_{respired\ carbon}$ values that were calculated for each sampling snapshot ranged from −25.8 to −28.7‰, with a mean of −27.5 ± 1.4‰ ($n = 5$, Table 3). These values are comparable to the isotopic signatures of hydrocarbon compounds added to make up the source (Table 1).

3.3. Field decane contribution

Groundwater samples collected immediately down gradient of the source showed decane levels at detection limits (10 ppb). While decane solubility is 4 orders of magnitude less than the other hydrocarbon contaminants, it can be degraded under many environmental conditions (Caldwell et al., 1998; Rabus et al., 1999; So and Young, 1999; Zengler et al., 1999; Kropp et al., 2000). Rabus et al. (1999) observed degradation of C_5–C_{12} chains with denitrifying bacteria enriched from freshwater sediments when the bacteria formed aggregates, adhered to the crude oil layer and emulsified the oil. Decane could have been degraded within the "source-trench" of our study area and the endproducts carried downstream.

3.4. Laboratory incubations

Aerobic and anaerobic incubations of soil from the site showed significantly greater CO_2 production in the hydrocarbon containing flasks, indicating degradation of the hydrocarbons (Table 4). As observed in the field setting, the CO_2 produced in hydrocarbon containing incubations was higher in concentration and was ^{13}C depleted relative to CO_2 produced from respiration of the native soil. The $\delta^{13}C$ of excess CO_2 produced in the incubations (Table 4) was between −28 to −30‰ similar to the range of $\delta^{13}C$ values of the added hydrocarbons (−26 to −30‰, Table 1). These results are consistent with a lack of fractionation during degradation and suggest that the $\delta^{13}C$ of respiration products should mirror the $\delta^{13}C$ of the original substrate in the field experiment.

Table 4
Concentrations and $\delta^{13}C$ values of CO_2 evolved in laboratory incubations of soil collected following 100 days of jet fuel contamination

Incubation	Con CO_2%	Con $\delta^{13}C$‰	H–CO_2%	H–$\delta^{13}C$‰	Excess CO_2%	Excess $\delta^{13}C$‰
Aerobic-1	0.42	−25.8	8.4	−30.0	8.0	−30.2
Aerobic-2	0.46	−26.0	13.4	−29.0	12.9	−29.1
Aerobic-3	0.40	−25.7	1.8	−29.4	1.4	−30.5
Anaerobic 2	0.27	−22.3	1.1	−26.8	0.80	−28.3
Anaerobic-3	0.40	−25.0	1.2	−27.2	0.75	−28.4

"Con" represents control or soil only incubations and "H" represents hydrocarbon amended incubations. "Excess" represents the evolved CO_2 and $\delta^{13}C$ attributed to hydrocarbon degradation corrected for CO_2 evolution in controls. $\%CO_2$ is the concentration of CO_2 in the incubation flask as the % of the headspace by volume. Excess $\delta^{13}C$ values were calculated using Eq. (2).

4. Conclusions

In our field and laboratory study, there was no evidence for significant carbon isotopic fractionation in the migration and oxic and suboxic degradation of the hydrocarbon compounds found in jet fuel. The weighted average $\delta^{13}C$ of the products of bacterial respiration ($-27.5 \pm 1.4‰$) was within the range of measured hydrocarbon $\delta^{13}C$ values (-26 to $-30‰$) and of CO_2 produced when they were incubated in the laboratory (-28 to $-30‰$). When differences in isotopic composition exist between the respiration products produced from the decomposition of native organic matter and exotic hydrocarbons, then the $\delta^{13}C$ values of respiration end-products may be a useful tracer of hydrocarbon biodegradation.

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