Field Evidence for Co-Metabolism of Trichloroethene Stimulated by Addition of Electron Donor to Groundwater

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Abstract

For more than 10 years, electron donor has been injected into the Snake River aquifer beneath the Test Area North site of the Idaho National Laboratory for the purpose of stimulating microbial reductive dechlorination of trichloroethene (TCE) in groundwater. This has resulted in significant TCE removal from the source area of the contaminant plume and elevated dissolved CH_4 in the groundwater extending 250 m from the injection well. The $\delta^{13}C$ of the CH_4 increases from -56‰ in the source area to -13‰ with distance from the injection well, whereas the $\delta^{13}C$ of dissolved inorganic carbon decreases from 8‰ to -13‰, indicating a shift from methanogenesis to methane oxidation. This change in microbial activity along the plume axis is confirmed by PhyloChip microarray analyses of 16S rRNA genes obtained from groundwater microbial communities, which indicate decreasing abundances of reductive dechlorinating microorganisms (e.g., *Dehalococcoides ethenogenes*) and increasing CH_4-oxidizing microorganisms capable of aerobic co-metabolism of TCE (e.g., *Methylosinus trichosporium*). Incubation experiments with ^{13}C-labeled TCE introduced into microcosms containing basalt and groundwater from the aquifer confirm that TCE co-metabolism is possible. The results of these studies indicate that electron donor amendment designed to stimulate reductive dechlorination of TCE may also stimulate co-metabolism of TCE.

Introduction

Trichloroethene (TCE) is a suspected human carcinogen and one of the most widespread and persistent groundwater contaminants in many industrialized nations (*1*). Remediation of TCE with pump-and-treat or vapor-stripping techniques is difficult and expensive because of its low solubility (1100 mg/L in water) and high density (1.46 g/cm^3), especially in deep groundwater aquifers. As a result, there has been significant interest in developing in situ options for degrading TCE. Reductive dechlorination of TCE to ethene and chloride has emerged as one of the most promising methods for accomplishing this goal. In essence, under anaerobic conditions when suitable electron donors (e.g., lactate) are available, microorganisms can utilize TCE as an electron acceptor, sequentially removing chlorine atoms from TCE to form dichlorethene (DCE), then vinyl chloride (VC), and finally ethene. This process occurs naturally at sites where the appropriate conditions exist (*2-6*).

In TCE-contaminated aquifers, reductive dechlorination can be encouraged by adding electron donor and/or other nutrients to the groundwater to produce an anoxic environment (*7-9*). At some sites, addition of electron donor does not induce complete reductive dechlorination by the natural microbial populations (leading to accumulation of DCE). Supplementation with microbial consortia enriched from sites where complete reductive dechlorination does occur (bioaugmentation), has been shown to stimulate complete reduction to ethene (*10-13*).

Another promising method of bioremediation of chlorinated solvents involves co-metabolism of the contaminants by aerobic microorganisms in groundwater and soils (*15, 16*). Several aerobic microorganisms have been demonstrated to be capable of doing this, including methane oxidizers (*17-19*), phenol-degraders (*20*), and toluene-degraders (*21*). Unlike reductive dechlorination, the chlorinated compounds are completely mineralized to CO_2 and chloride with

no intermediates making co-metabolism an attractive alternative where it can be sustained. However, the microorganisms gain no energy from these processes, limiting the ability of cells to co-metabolize chlorinated compounds (*22, 23*). This, together with the difficulties and high costs of maintaining substrate and an oxic environment, have led to limited field-scale application of co-metabolism for solvent degradation.

In this study, we present data from a field site that has been undergoing long-term electron donor addition to stimulate reductive dechlorination of TCE in groundwater. The primary effect of the electron donor addition has been significant decreases in TCE concentrations, especially within 50 m of the injection well. Changes in the chemistry and microbiology of the groundwater (*8, 10, 14*) include an increase in the dissolved CH_4 content of the groundwater as far as 250 m down-gradient from the injection well. This suggests that oxidation of the CH_4 by methanotrophic bacteria could lead to enhanced co-metabolism of the chlorinated solvents in the groundwater, providing a secondary mechanism for TCE removal.

Materials and Methods

Field Site and Sample Collection. Test Area North (TAN) is an Idaho National Laboratory (INL) facility located on the Snake River Plain of eastern Idaho (Figure 1). TAN consists of several experimental and support facilities used for research and development on reactor performance and nuclear safety (*24*). From 1953 to 1972, liquid waste consisting primarily of industrial and sanitary wastewater was pumped into the upper aquifer through a 93 m deep injection well, TSF-05 (Figure 1). This resulted in a TCE plume (defined by TCE concentrations >5 ppb or 38 nM) in the upper aquifer extending 2 km down-gradient from TSF-05. In 1999, a pilot study was conducted to explore the potential for enhancing in situ reductive dechlorination

of TCE through injection of Na-lactate (*8*). Following favorable results from the pilot test, electron donor addition has been continued through the present.

The geology at TAN consists of highly fractured, permeable basalt flow units ranging in thickness from 1 to 15 m. Between some of the basalt flows are low-permeability sedimentary interbeds composed of fluvial, lacustrine and aeolian deposits (*25*). The depth to groundwater at the TAN site is approximately 70 m. The upper aquifer is approximately 65 m thick and is separated from the main Snake River Plain Aquifer by a continuous impermeable sedimentary interbed.

In May 2007, groundwater and biomass samples were collected from 8 wells in the TAN plume for this project (Figure 1). The samples were collected with dedicated down-hole pumps following purging of the groundwater using a low-flow technique to minimize wastewater. Most of these wells have screened or uncased intervals through most of the upper aquifer (Table S.1 in the Supporting Information contains the screened intervals). This is a potential source of variability for the sampling data; however, this is minimized by the low-flow sampling technique.

From each well, duplicate samples were collected in 40 mL vials for measurements of the concentrations and isotopic compositions of chlorinated solvents. For CH_4 and dissolved inorganic carbon (DIC) analyses, approximately 400 mL was collected in a custom glass flow-through vessel fitted with Teflon valves at the inflow and outflow ends that could be sealed after the vessel was flushed and filled with groundwater. Biomass samples were filtered from 0.25 to 20 L of groundwater for molecular analysis of the microbial community structure (more detail on the biomass sampling procedures is contained in the Supporting Information).

To provide material for laboratory incubation experiments to test for the ability of the

endogenous microbial community to co-metabolize TCE, six flow-through in situ bioreactors (ISBRs) containing crushed basalt from core collected from the TAN site were suspended in the upper aquifer in TAN-35 for 8 months (November, 2006 through July, 2007) to allow microorganisms from the TAN groundwater to colonize the basalt. Each ISBR was 1 m in length with an inner diameter of 2.5 cm. Water from the aquifer was pumped through the ISBRs at flow rates of 0.1 m/day in three reactors and 1.0 m/day in the other three, representing the minimum and maximum flow rates in the aquifer. Following removal of the ISBRs from the aquifer, the groundwater was drained from the reactors and collected in airtight containers. The basalt was then removed from the reactors for the incubation experiments.

Analytical Techniques. The dissolved CH_4 concentrations were measured by replacing a known volume of sample with helium gas in bottles containing a sample collected with no headspace. The CH_4 concentration in the helium bubble was then analyzed and converted back to a dissolved CH_4 concentration using the Henry's Law constant for CH_4 in water.

The $\delta^{13}C$ values of the DIC and CH_4 from the groundwater and incubation experiments were analyzed at the Lawrence Berkeley National Laboratory (LBNL). The $\delta^{13}C$ values of DIC in samples collected during the 1999 pilot test were measured by injecting an aliquot of the sample into an evacuated tube containing phosphoric acid. The $\delta^{13}C$ of CO_2 evolved was analyzed with the Prism Series II isotope ratio mass spectrometer at the Center for Isotope Geochemistry (CIG) at LBNL. The isotopic compositions of the DIC in the 2007 groundwater samples and the samples from the incubation experiments were analyzed following the procedure outlined by Torn et al. (*26*). For CH_4 isotopic measurements, the samples were prepared in the manner outlined above for the CH_4 concentration measurements. Using a Micromass Trace Gas system, CH_4 in the headspace bubble was converted to CO_2 that was then fed into a Micromass JA Series

Isoprime isotope ratio mass spectrometer for $\delta^{13}C$ analysis. For all samples, the isotopic compositions are reported as per mil (‰) variations from Vienna Pee Dee Belemnite (V-PDB) using the conventional δ notation:

$$\delta^{13}C = ((R_{sample}/R_{standard}) - 1) \times 1000 \qquad \text{(eq. 1)}$$

where $R = {}^{13}C/{}^{12}C$. The reproducibility of reference standards analyzed with the unknown samples is ±0.3‰ (1σ) for both techniques used for DIC isotopic analyses and ±0.5‰ (1σ) for CH_4 analyses.

Microbial Community Analyses. DNA was extracted from the biomass filtered from the monitoring wells at LBNL. This DNA was amplified using PCR analysis of the microbial community structure using the PhyloChip. The PhyloChip is a high-density oligonucleotide microarray capable of detecting the presence and relative abundances of almost 9000 bacterial and archeal taxa (*27, 28*). More detail on the DNA extraction and amplification and the PhyloChip analyses are given in the Supporting Information for this paper.

Results and Discussion

Groundwater Geochemistry. Electron donor added to the groundwater at TAN is plotted in Figure 2A (in kg/week, calculated as a 16-week moving average). There was considerable variability in both the amount and injection strategies (e.g., variable electron donor concentrations were tested, tests of continuous versus pulsed addition of electron donor). In addition, electron donor was injected into other wells besides TSF-05 (TAN-31 beginning in January of 2006 and TAN-1859 beginning in July of 2006; see Figure 1). In August 2004, the electron donor was switched from Na-lactate to whey powder.

Eight-week moving average concentrations of dissolved CH_4 concentrations for TAN-37A and TAN-29 are plotted against time in Figure 2B. CH_4 data for TAN-25 and TAN-28 (the other 2 wells sampled that contain significant dissolved CH_4) are plotted in the Supporting Information, S.1. Also included in the Supporting Information are plots of the concentrations of chlorinated solvents versus time (Supporting Information, Figures S.2-S.6).

TAN-37A and TAN-29 are located 44 and 154 m down-gradient from TSF-05, respectively (Figure 1). Neither well developed significant concentrations of CH_4 during the 1999 pilot test. Shortly following resumption of electron donor addition in early 2000, dissolved CH_4 concentrations began to rise in TAN-37A. Approximately one year later, elevated CH_4 concentrations were observed in TAN-29. Although highly variable, dissolved CH_4 concentrations remained high in TAN-37A through at least late 2007, averaging 920 μmol/L. CH_4 in TAN-29 fluctuated between background and 500 μmol/L with spikes to greater than 1000 μmol/L (between April 2001 and September 2007 it averaged 160 μmol/L). The source of the variability in CH_4 in these wells is weakly correlated with the changes in electron donor addition, but there may be other causes such as seasonal recharge of oxygenated water to the aquifer.

The $\delta^{13}C$ of DIC in four of the wells sampled during 2007 are plotted versus time in Figure 2C. This includes data for two of the wells from an early survey of isotopic compositions in the TAN groundwater from 1997, monthly sampling during the pilot test for three of the wells, and the 2007 samples that we collected for this study. Also shown is the general range of background DIC $\delta^{13}C$ values for the upper aquifer at TAN (-8 to -11.5‰). These data are clear indicators of the evolution of the TAN groundwater during electron donor addition. In TAN-25, located 15 m from TSF-05, the $\delta^{13}C$ of the DIC increases significantly following the beginning of the pilot test and was even higher in 2007. Inorganic carbon produced from microbial

fermentation of acetate by acetoclastic methanogens is highly enriched in ^{13}C relative to the substrate (*29*). The significant increase in the $\delta^{13}C$ of DIC observed in TAN-25 could only be produced by development of a highly anoxic zone of sustained methanogenic activity near the electron donor injection well. Other microbial processes that are common in anoxic groundwater systems such as iron reduction and sulfate reduction produce inorganic carbon with $\delta^{13}C$ values lower than the substrate and could not be a significant source of DIC in TAN-25. High dissolved CH_4 and low levels of TCE in TAN-25 (data in Supporting Information file) provide evidence that ideal conditions for microbial reductive dechlorination are being maintained in this region of the plume. In TAN-37A, the $\delta^{13}C$ of the DIC remained within the background range during the pilot test but increased to -4.6‰ by 2007. This is consistent with the CH_4 concentration data that did not increase until after resumption of electron donor addition following the pilot test. This indicates that methanogenesis is the dominant process in this region of the plume and that the zone of active reductive dechlorination now extends to TAN-37A.

In TAN-29, the $\delta^{13}C$ of DIC remained in the background range during the pilot test, but dropped below that range to -13.3‰ in 2007. This shift from high to low carbon isotope ratios of DIC between TAN-37A and TAN-29 indicates a change from methanogenic conditions around TAN-37A to conditions favoring microbial CH_4 oxidation in the area of TAN-29. Microbial oxidation of CH_4 produces DIC with $\delta^{13}C$ values lower than the CH_4 substrate (*30*). Coupled with the low $\delta^{13}C$ values of the dissolved CH_4, CH_4 oxidation can cause significant negative shifts in the $\delta^{13}C$ of DIC (*31*). To produce the shift from -4.6‰ to -13.3‰ observed between TAN-37A and TAN-29 would require that between 10% and 15% of the DIC in TAN-29 be derived from CH_4 oxidation (assuming a $\delta^{13}C$ value of -70‰ for inorganic carbon from CH_4 oxidation). This estimation is consistent with the significant drop in dissolved CH_4

concentrations (from 1.2 mmol in TAN-37A to 0.1 mmol in TAN-29) relative to the total DIC concentration in TAN-29 (6 mmol). This interpretation is simplified and ignores other factors that undoubtedly affect dissolved CH_4 and DIC concentrations in the groundwater such as dilution during transport or contributions from other sources of DIC (e.g., oxidation of organic acids by iron or sulfate reducing organisms), but it does demonstrate that significant CH_4 oxidation occurs between TAN-37A and TAN-29. A similar value for the $\delta^{13}C$ value of DIC was observed in TAN-28 (-13.1‰) during 2007. Although no data for dissolved O_2 or oxidation-reduction potential is available for these samples, limited data from other sampling periods does suggest that occasional increases in both of these parameters do occur in TAN-28 and TAN-29. In addition, nitrate and sulfate concentrations returned to background levels in these wells and ferrous iron drops below detection (data in Table S.1 in Supporting Information).

Also plotted in Figure 2C are data for the $\delta^{13}C$ of DIC from TAN-36, one of the down-gradient monitoring wells (Figure 1) sampled during 2007. This well was also sampled during 1997, and the $\delta^{13}C$ value of the DIC was in the background range then and in 2007. The other wells located more than 300 m from TSF-05 (TAN-42, TAN-44 and TAN-33) also had $\delta^{13}C$ values for DIC in the background range, suggesting that electron donor addition has not had a significant effect at distances further than 300 m from the injection point.

The carbon isotope data for DIC and CH_4 for all of the groundwater samples collected during 2007 are plotted versus distance from TSF-05 in Figure 3. In addition to the eight samples collected during May 2007, data are also included for TAN-35 for samples collected when the ISBRs were removed from the well in July 2007. The data for DIC clearly show the trends discussed above, with very high $\delta^{13}C$ values near the injection wells shifting to values less than background between 80 and 160 m down-gradient before returning to background carbon isotope

compositions at 300 m from TSF-05. The $\delta^{13}C$ values of dissolved CH_4 are around -55‰ in TAN-25 and TAN-37A, the two wells closest to the injection wells and are directly impacted by electron donor additions. -55‰ is a typical $\delta^{13}C$ value for CH_4 produced from fermentation of acetate by methanogenic microorganisms (*29*) and probably represents a good approximation for the initial carbon isotope composition for CH_4 produced in this system. In TAN-28 and TAN-29, the samples with low $\delta^{13}C$ DIC, the $\delta^{13}C$ values of the CH_4 are much higher (-28‰ and -36‰, respectively). This shift is consistent with the significant levels of CH_4 oxidation in this part of the plume indicated by the low $\delta^{13}C$ DIC values. Preferential oxidation of $^{12}CH_4$ by methanotrophic bacteria results in higher $\delta^{13}C$ values in the residual CH_4. The $\delta^{13}C$ for dissolved CH_4 from TAN-35 (at 219 m from TSF-05) is shifted to an even higher value (-13‰) indicating a greater amount of oxidation at this point in the plume. This is reasonable given that this well typically has very low CH_4 with occasional spikes to higher concentrations and appears to be the approximate limit of where CH_4 generated from electron donor addition extends into the plume. CH_4 is detected in the monitoring wells beyond this point, but generally at concentrations of less than 20 μmol/L, which are within the background range for the Snake River aquifer (*32*).

Microbial Ecology. Figure 4 shows changes in the relative abundances of *Dehalococcoides ethenogenes*, an acetoclastic methanogen (clone S30-29, accession number AJ236538 (*33*)) within the Methanosarcinaceae (hereafter referred to as "*Methanosarcinaceae* spp.") and a Type II methane oxidizer (*Methylosinus trichosporium*) for the May 2007 groundwater samples determined using the PhyloChip. The data are given in terms of relative fluorescence units and although hybridization efficiencies vary between probe-sets (taxa), a change of 1000 intensity units is about an order of magnitude change in the gene copy number for a given taxon (*34*).

The samples from TAN-25 and TAN-37A (the 2 wells closest to TSF-05) had the highest

relative abundances of both *D. ethenogenes* and *Methanosarcinaceae* spp., reflecting the methanogenic conditions and high levels of reductive dechlorination of TCE observed in those wells. The next two downstream wells, TAN-28 and TAN-29, are characterized by increasing abundances of *M. trichosporium*, a CH_4-oxidizing organism, and lower, but still significant abundances of both *D. ethenogenes* and *Methanosarcinaceae* spp., suggesting that this region of the plume represents a transition for anoxic to oxic conditions. It should also be noted that these data represent DNA obtained from cells filtered from groundwater samples and therefore represent the pelagic microbial population. The PhyloChip detects 16S rRNA gene fragments amplified from the samples and only indicates that the organisms were present in the groundwater but not necessarily active at the time of sampling. Therefore, it is possible that microbes transported from up-gradient sites or from mixing of groundwater from different depths within the wells will be detected, leading to mixing of microbes from zones of different activity. In the distal wells, *Methanosarcinaceae* spp. was present at lower relative abundances in all but TAN-33, which had concentrations similar to those observed in TAN-29. The significance of the relatively higher relative abundances of *Methanosarcinaceae* spp. in this well is not clear at this time. *D. ethenogenes* was also present in the distal wells at relatively low levels.

The abundances of *M. trichosporium* provide further evidence for the transition from methanogenesis to CH_4 oxidation with increasing distance from the TSF-05 as indicated by the geochemical data. *M. trichosporium* is present at very low concentrations in TAN-25 and TAN-37A. It is higher in TAN-28 and peaks in TAN-29 at abundances approximately 3 orders of magnitude higher than in TAN-25 and TAN-37A. This is likely because of the combination of abundant CH_4 in the groundwater and elevated concentrations of dissolved oxygen, conditions ideal for aerobic methanotrophs. In the wells further from TSF-05, there are still significant

abundances of *M. trichosporium*, but the numbers are lower than in TAN-29, likely due to the limited amount of CH_4 in the groundwater.

Co-Metabolism of TCE. The geochemical conditions between TAN-29 and TAN-42 combined with the higher relative abundance of *M. trichosporium*, a Type II methanotroph known to be capable of co-metabolism of TCE (*18*), suggest aerobic co-metabolism of TCE should be occurring in the groundwater in this part of the TAN plume. To test this, ^{13}C-labeled TCE was added to microcosm experiments containing basalt chips and groundwater from the ISBRs incubated in well TAN-35 with a headspace of air. The concentration of dissolved CH_4 in the microcosms was 0.8 μmol/L, which is lower than the concentration in the groundwater likely because of the brief exposure to air when the water was drained from the ISBRs. It is also significantly lower than the concentration of TCE in the microcosms (ranging between 4 and 20 μmol/L), meaning that methanotrophs in the microcosms will be exposed to more TCE than CH_4 which will inhibit their activity (*22,23*).

The $\delta^{13}C$ values of the DIC in the day 1 samples were variable, but leveled off for the day 2 through day 4 samples, suggesting that the maximum capacity of the bacteria in the microcosms to degrade TCE was achieved after 2 days. The average and range of DIC $\delta^{13}C$ values for the day 2 through day 4 samples are plotted versus the amount of ^{13}C TCE added in Figure 5 (see Table S.2 in the Supporting Information for data for the individual samples). Because of the differing amounts of ^{13}C TCE added to the samples and the relatively high concentration of TCE in the groundwater (500 μg/L with a $\delta^{13}C$ value of -30‰), the $^{13}C/^{12}C$ of the TCE in the microcosms ranged from 0.058 to 3.859, resulting in a significant range of expected $\delta^{13}C$ values for the DIC produced from co-metabolism of the TCE. The result was an increase of almost 2‰ in the average $\delta^{13}C$ value of DIC in the samples with 2000 μg/L of ^{13}C TCE added relative to the

samples with only 25 μg/L ^{13}C TCE added. There are large ranges in the data, but this is to be expected given the highly variable nature of microorganisms expected on the basalt chips added to the microcosms. However, it is important to note that there is no overlap between the range of data for the 25 and 50 μg/L of added TCE samples (-8.0 to -8.6‰) and the range of data for the 1000 and 2000 μg/L of added TCE samples (-4.7 to -7.3‰).

Also plotted in Figure 5 are calculated trends in the $\delta^{13}C$ values of DIC at varying concentrations of added ^{13}C TCE added to the microcosms assuming a constant mass of TCE was degraded per liter of groundwater. A description of how these calculations were made is included in the Supporting Information. Considering the analytical uncertainty of the measurements (±0.3‰), most of the data (~80%) fall between the calculated lines for 10 μg/L and 20 μg/L of TCE co-metabolically degraded with the average $\delta^{13}C$ values for the different amounts of ^{13}C TCE added matching very well with the calculated trend for 15 μg/L of TCE co-metabolically degraded. Similar calculations assuming degradation of a constant proportion of the available TCE do not match the data nearly as well as the constant mass calculations do.

In essence, the microcosms were capable of co-metabolizing TCE, but only for a limited amount of time because of the relatively low amounts of CH_4 available. This, coupled with the high concentrations of TCE in the aqueous phase in the microcosms, suggests that the microorganisms were primarily degrading the TCE and not CH_4, which is likely the cause of the apparent lack of activity after the first few days. In laboratory experiments, Oldenhuis et al. (22) and Alvarez-Cohen et al. (23) found that methane-oxidizing microorganisms have a finite capacity for co-metabolism of TCE.

The results of these experiments, combined with the geochemical and molecular data demonstrating that methanotrophic bacteria are present and active in the TAN groundwater,

strongly suggest that CH_4 generated from metabolism of electron donor injected to stimulate reductive dechlorination of TCE is also causing co-metabolism of TCE in the down-gradient, more aerobic portions of the plume. This could be a significant mechanism for degrading TCE within aerobic portions of plumes located down-gradient from source areas undergoing stimulated reductive dechlorination of chloroethenes.

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Supporting Information Available

Plots of the concentrations of dissolved CH_4 and chlorinated solvents, available chemical and isotopic data for the May 2007 samples, additional information on sampling preparation and analyses of microbial community data with the PhyloChip, data for the incubation experiments, and the calculation of predicted $\delta^{13}C$ values for the incubation experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Figure Captions

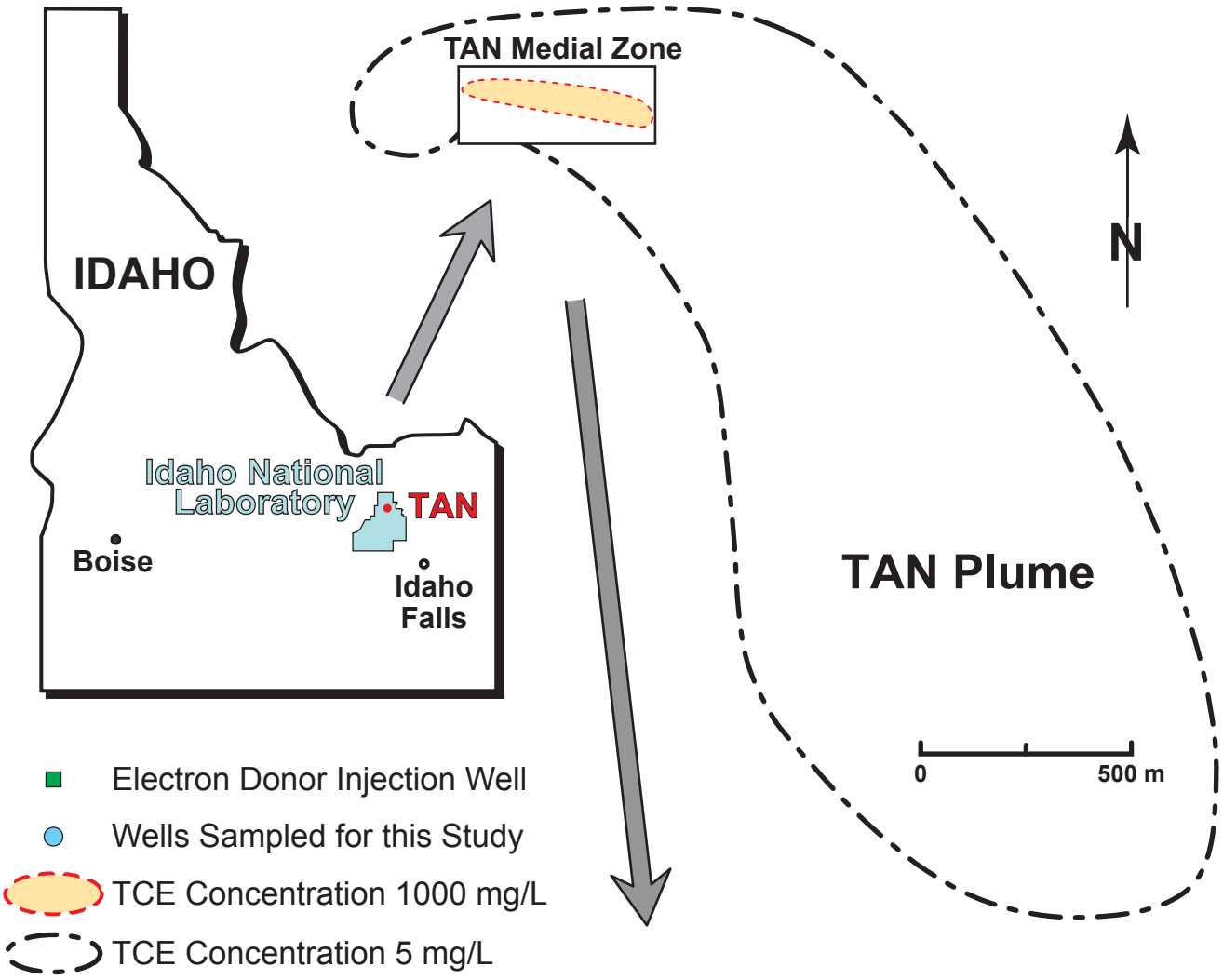
Figure 1. Map showing the location of the INL and TAN with an expanded view of the core area ("medial zone") of the TAN plume showing the locations of the monitoring wells sampled for this study (light blue circles) and the wells used for electron donor injection (green squares).

Figure 2. Plot versus time of (A) time-averaged electron donor addition to the groundwater (B), time-averaged dissolved methane concentrations in wells TAN-37A and TAN-29, and (C) carbon isotope compositions of dissolved inorganic carbon in groundwater samples from selected wells in the TAN plume. Time averaging was done on a 16-week moving average.

Figure 3. $\delta^{13}C$ of DIC and CH_4 in TAN groundwater samples collected during May, 2007 plotted versus distance from TSF-05 (the primary injection well).

Figure 4. Relative abundances of *Dehalococcoides ethenogenes* (a representative microorganism capable of complete reductive dechlorination of TCE), *Methanosarcinaceae* spp. (an acetoclastic methanogen) and *Methylosinus trichosporium* (a representative Type II methane oxidizer known to co-metabolically degrade TCE) determined using Phylochip analyses of 16S rRNA genes extracted from TAN groundwater wells sampled May 2007. A change in 1000 relative fluorescence units is approximately equivalent to an order of magnitude change in the relative abundance of a given organism (due to differences in hybridization efficiency, the relative intensity varies between organisms).

Figure 5. Change in $\delta^{13}C$ values of DIC in short-term (two to four day) incubation experiments performed using basalt chips that had colonized for eight months in TAN-35 and groundwater from TAN-35 plotted versus the amount of ^{13}C-labeled TCE added to the experiments (in addition to 500 ppb of unlabeled TCE in the groundwater). Vertical bars represent the range of values measured for 3-6 replicates that contained the same amount of added ^{13}C TCE. Also plotted are changes in DIC carbon isotope ratios calculated for varying amounts of total TCE degradation (dashed and lowed solid curves).


TAN Medial Zone
IDAHO
Idaho National Laboratory
TAN
Boise
Idaho Falls
N
TAN Plume
0
500 m
Electron Donor Injection Well
Wells Sampled for this Study
TCE Concentration 1000 mg/L
TCE Concentration 5 mg/L

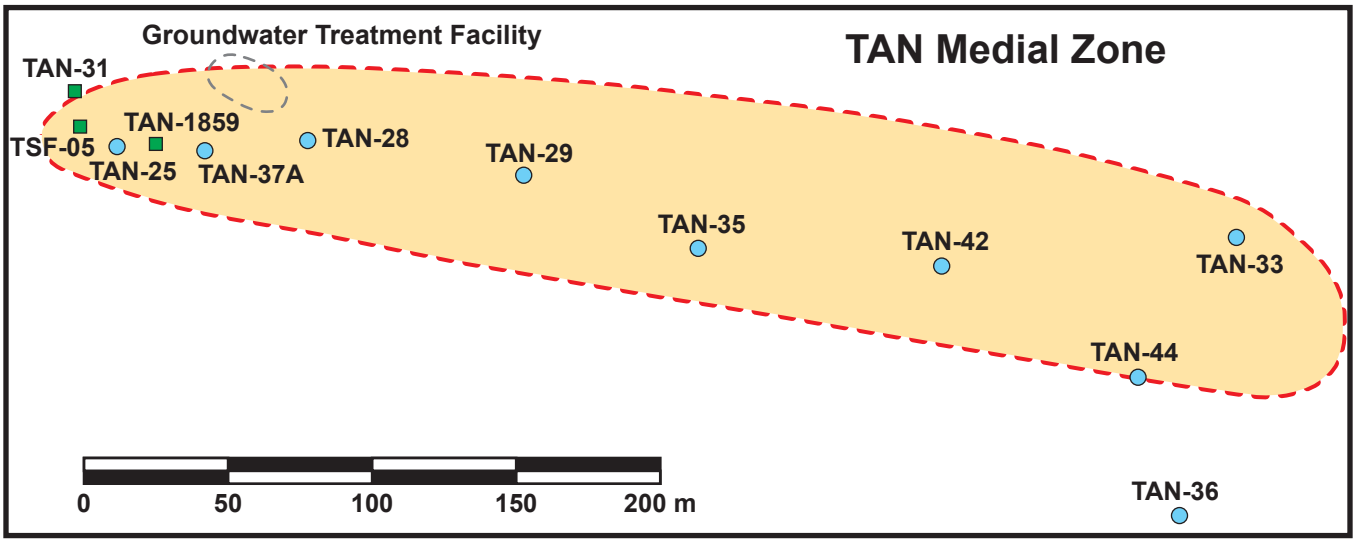

Groundwater Treatment Facility
TAN Medial Zone
TAN-31
TAN-1859
TSF-05
TAN-25
TAN-37A
TAN-28
TAN-29
TAN-35
TAN-42
TAN-33
TAN-44
TAN-36
0
50
100
150
200 m

Figure 1

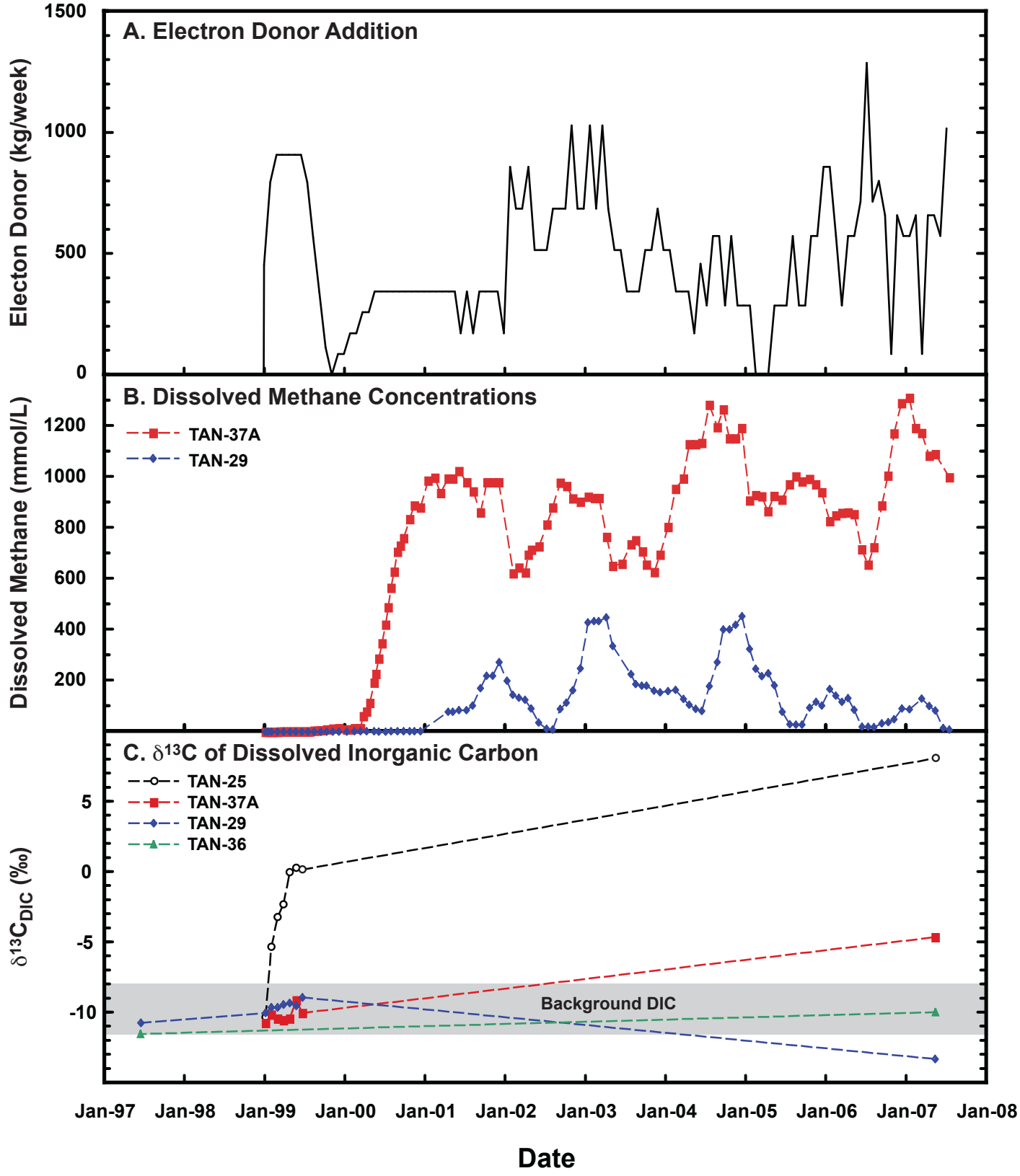

A. Electron Donor Addition
Electon Donor (kg/week)
1500
1000
500
0
B. Dissolved Methane Concentrations
TAN-37A
TAN-29
Dissolved Methane (mmol/L)
1200
1000
800
600
400
200
C. δ13C of Dissolved Inorganic Carbon
TAN-25
TAN-37A
TAN-29
TAN-36
δ13CDIC (‰)
5
0
-5
-10
Background DIC
Jan-97
Jan-98
Jan-99
Jan-00
Jan-01
Jan-02
Jan-03
Jan-04
Jan-05
Jan-06
Jan-07
Jan-08
Date

Figure 2

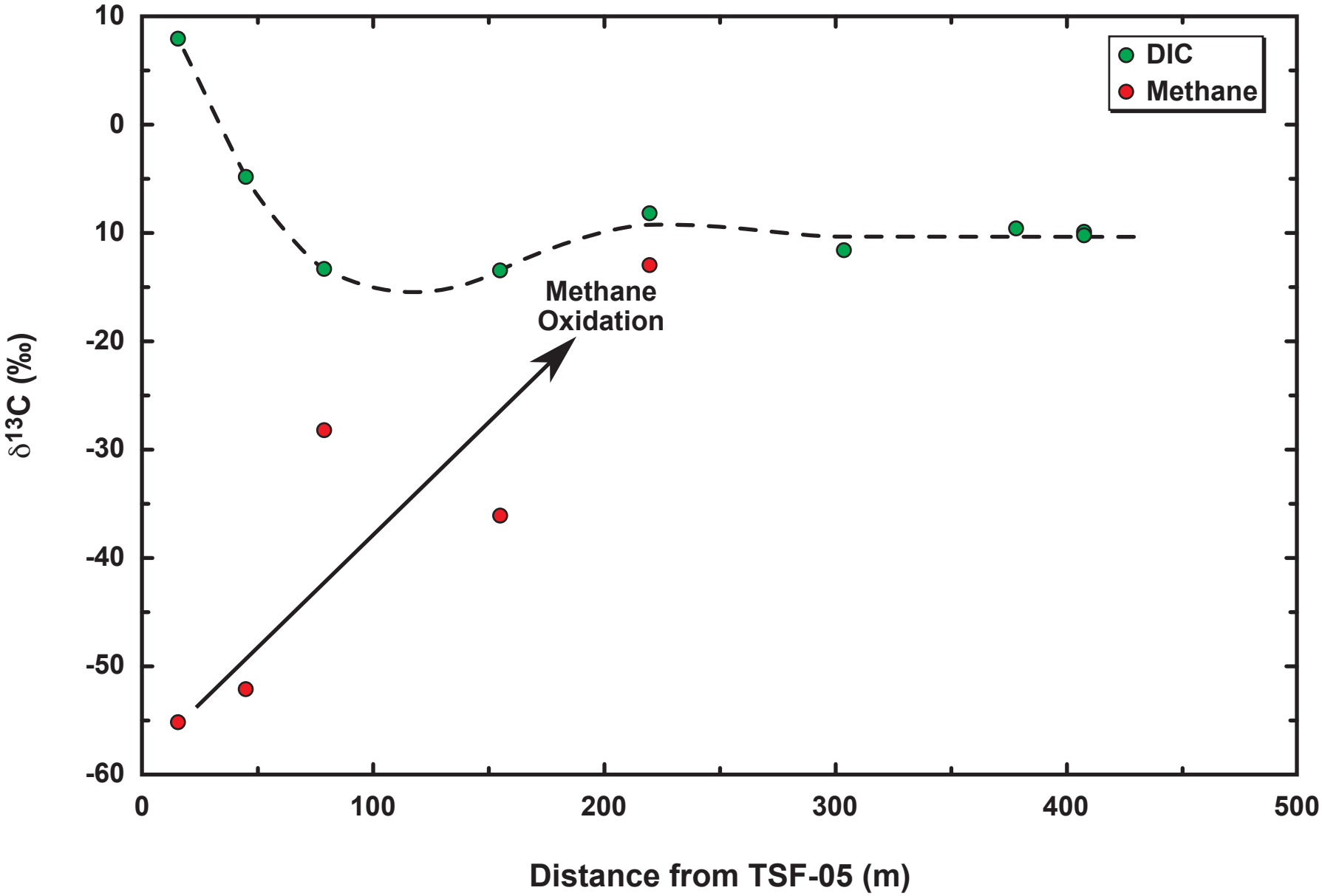

DIC
Methane
Methane
Oxidation
δ13C (‰)
Distance from TSF-05 (m)
10
0
-10
-20
-30
-40
-50
-60
0
100
200
300
400
500

Figure 3

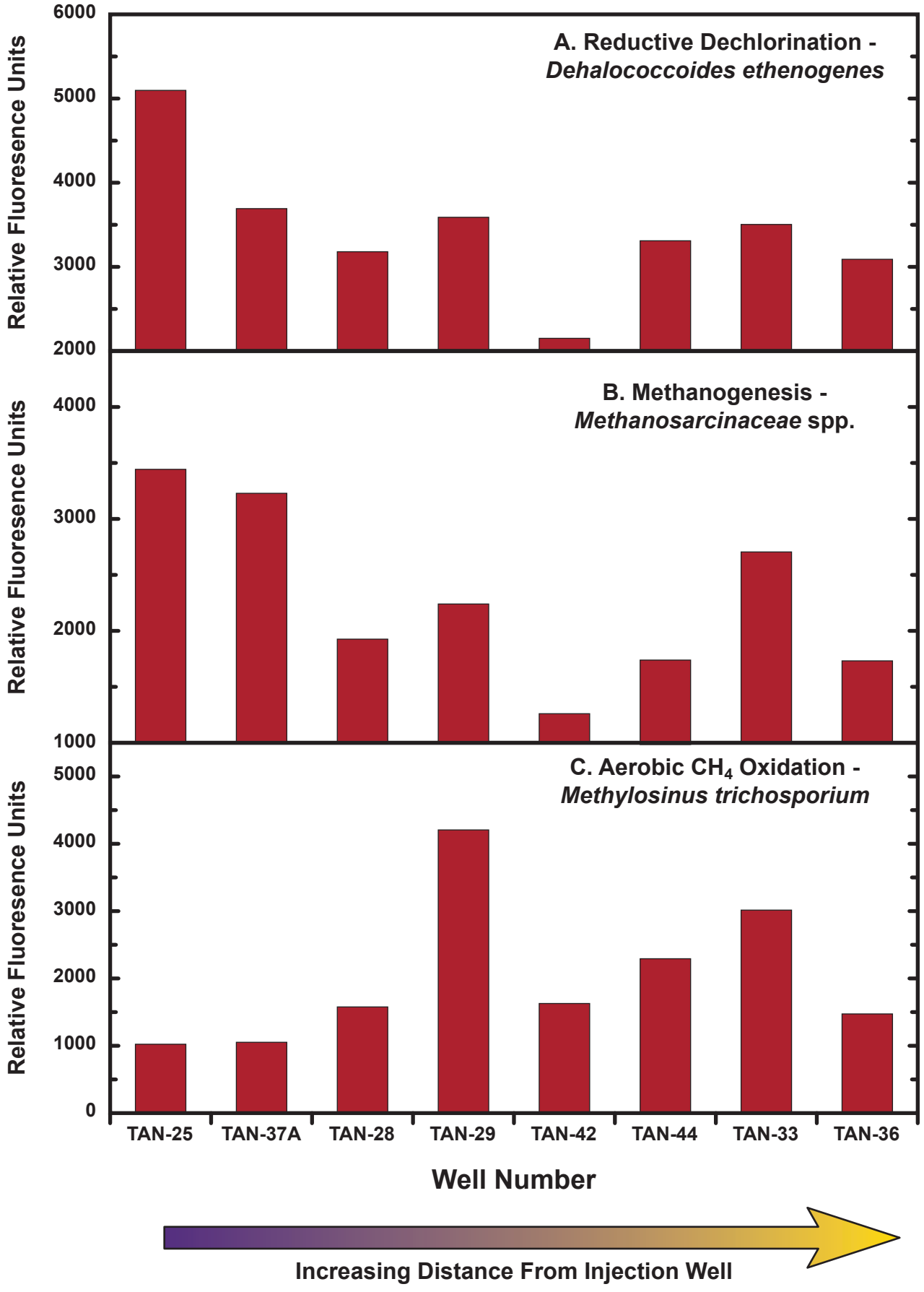

A. Reductive Dechlorination -
Dehalococcoides ethenogenes
B. Methanogenesis -
Methanosarcinaceae spp.
C. Aerobic CH_4 Oxidation -
Methylosinus trichosporium
Relative Fluoresence Units
6000
5000
4000
3000
2000
1000
0
TAN-25
TAN-37A
TAN-28
TAN-29
TAN-42
TAN-44
TAN-33
TAN-36
Well Number
Increasing Distance From Injection Well

Figure 4

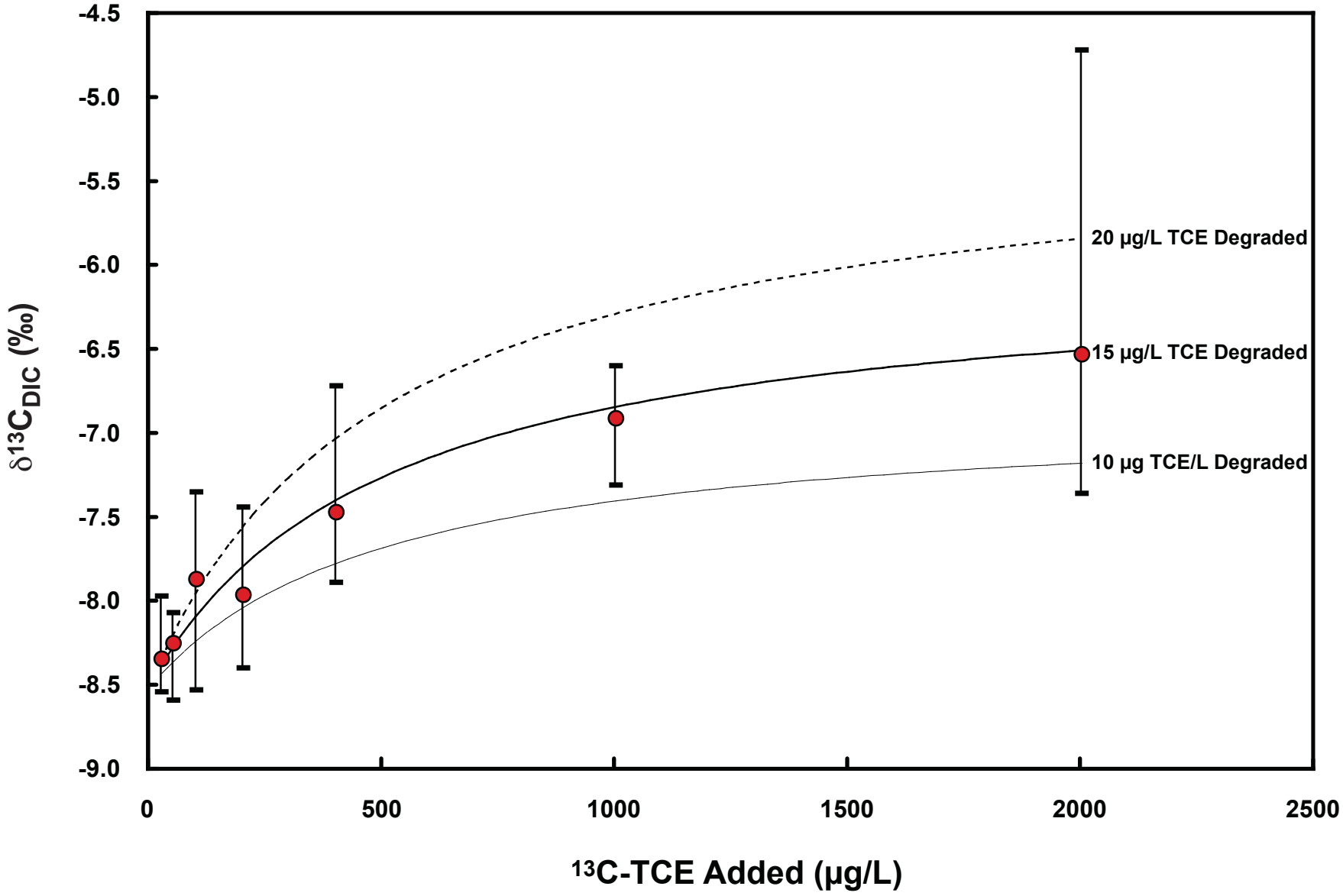

-4.5
-5.0
-5.5
-6.0
-6.5
-7.0
-7.5
-8.0
-8.5
-9.0
δ13CDIC (‰)
0
500
1000
1500
2000
2500
13C-TCE Added (µg/L)
20 µg/L TCE Degraded
15 µg/L TCE Degraded
10 µg TCE/L Degraded

Figure 5

Supporting information for the manuscript:

Field Evidence for Co-Metabolism of TCE Stimulated by Addition of Electron Donor to Groundwater

Environmental Science & Technology Manuscript# es-2009-03535j

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Number of pages: 6

Number of tables: 2

Number of figures: 6

Concentration Data for Methane and Chlorinated Solvents

Data collected for the concentrations of dissolved CH_4, trichloroethene (TCE), cis-dichloroethene (cDCE), vinyl chloride (VC) and ethene are presented in Figures S.1 through S.6. Due to the large number of data collected by the site operators over the period of interest (December 1998 through December 2007), the data are given in graphic form rather than in a table. Data for replicate samples taken on a given date have been averaged. In addition, each point on the Figures S.1 through S.5 represents an average of all analyses from within ±4 weeks of the given date (the nominal sampling interval for the site has been every 2-4 weeks) to help smooth out short-term variability and present the general concentration trends. Figure S.1 includes dissolved CH_4 concentrations for the four wells closest to TSF-05 (the contaminant injection well), TAN-25, TAN-37A, TAN-28 and TAN-29, for comparison. CH_4 concentrations for the other wells involved with this study are significantly lower (<40 μmol/L) and have not been plotted. Figures S.2 through S.5 contain the combined chloroethene data for each of the four closest well. The TCE concentrations for the wells further from TSF-05 (TAN-33, TAN-36, TAN-40 and TAN-44) are plotted on Figure S.6. The concentrations of the other chlorinated compounds were lower than 0.6 μmol/L and have not been plotted.

Chemical and Isotopic Data for May 2007 Samples

Table S.1 contains available chemical isotopic data for the groundwater samples collected during May 2007 for this project and groundwater collected during retrieval of the ISBRs in TAN-45 during July of 2007. Also included in this table are the relative distances of the sampled wells from the injection well (TSF-05) and depths of the screened or open intervals in each of the wells.

PhyloChip Analyses

The PhyloChip is a high-density oligonucleotide microarray containing 297,851 probes targeting 16S rRNA genes capable of detecting up to 8,741 bacterial and archaeal taxa following hybridization of fragmented 16S rRNA gene amplicons. Biomass was collected by passing up to 20 L of groundwater through a 142 mm diameter, 0.2 μm Supor filters (Pall, NY) during groundwater sampling. For several of the TAN wells, the filters became clogged before 20 L could be passed through them (in the case of TAN-25, the filter clogged after only 250 mL of water was passed through it). Following sampling, the filters were frozen and transported on dry ice to LBNL for DNA extraction and PhyloChip analysis.

DNA Extraction and Amplification. For DNA extraction, filters were ground while frozen over dry-ice in sterile whirlpak bags. The same area of filter was extracted for each sample after homogenization to a powder on dry ice (approximately half of the filter, 0.32g). This step normalized for unequal biomass on different areas of the filters. The samples were then weighed into Lysing matrix E tubes (MP Biomedicals, OH). Nucleic acid extraction and DNA purification from the filters were carried out using a modified CTAB protocol coupled with an AllPrep purification (Qiagen, CA) as described previously (*27*). DNA was quantified by absorbance at 260nm using a NanoDrop ND-1000.

For each microarray, the same amount of 16S rRNA gene amplicons was hybridized regardless of the yield of the PCR reactions. The 16S amplicons were spiked with a mixture containing a known quantity of non-16S molecules (spike-in controls) that were treated identically from this point onwards. Subsequent technical variation due to minor differences in target fragmentation, fragment labeling with biotin, array hybridization, washing, staining and scanning were accounted for by normalizing fluorescence across arrays using the internal non-

16S spikes. Therefore the PhyloChip data can be considered an accurate measurement of the differences in relative abundances between samples. 16S rRNA gene amplification was performed using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') for bacteria and 1492R combined with 4Fa (5'-TCCGGTTGATCCTGCCRG-3') for archaea. Each PCR reaction contained 1x Ex Taq buffer (Takara Bio Inc., Japan), 1.25 U Ex Taq polymerase, 200 μM each dNTP, 2.5 μg BSA, 300 nm each primer and ~17 ng DNA template in a final volume of 50 μl. PCR conditions were 95°C (3 min), followed by 25 cycles 95°C (30 s), 48-58°C (25 s), 72°C (2 min), followed by a final extension 72°C (10 min). Each DNA extract was amplified in 8 replicate 50 μl reactions spanning a range of annealing temperatures between 48-58°C for both archaea and bacteria. Amplicons from the eight reactions were pooled separately for bacteria and archaea, precipitated with isopropanol, washed with ethanol and resuspended to 50 μl.

High-density Oligonucleotide Array Analyses. PhyloChip data, like most microbial community analysis data, are reported in relative units. Our procedures attempt to reduce variance associated with the process. From each pool of 16S rRNA gene amplicons, 500 ng of bacterial 16S rRNA gene amplicons and 50 ng of archaeal 16S rRNA gene amplicons were prepared for hybridization to the PhyloChip as previously described (*28*). Target fragmentation, biotin labeling, PhyloChip hybridization, scanning and staining, background subtraction, noise calculation, and detection quantification criteria were as reported in Brodie et al. (*27*) with some minor exceptions. For a probe pair to be considered positive, the difference in intensity between the perfect match (PM) and mismatch (MM) probes must be at least 130 times the squared noise value (N). A taxon was considered present when 90% or more of the probe pairs for its corresponding probe set were positive (positive fraction, ≥0.90).

Incubation Experiments

To test the capacity of the TAN groundwater system to aerobically degrade TCE, a set of incubation experiments was conducted with varying concentrations of ^{13}C-labeled TCE added (25, 50, 100, 200, 400, 1000 and 2000 μg/L) were conducted with groundwater and basalt collected from the in situ bioreactors after they were retrieved from TAN-35. ^{13}C TCE was used to be able to distinguish small contributions of inorganic carbon resulting from aerobic degradation of TCE during the incubations from the high background of DIC in the groundwater (4 mM). For the experiments, ~2 cm^3 of basalt, 3 ml of groundwater and 3 ml of air were added to 8 ml crimp-top vials sealed with a blue butyl septa. Four vials at each concentration of ^{13}C TCE were prepared (the 2000 μg/L treatment was done in duplicate). Each day for 4 days following the beginning of the experiment, 1 vial for each treatment was killed by addition of Na-azide. Following addition of Na-azide, the samples were refrigerated until they could be shipped to the LBNL for isotopic analyses. The carbon isotopic data for DIC and dissolved CH_4 (only for the microcosms with 2000 μg/L added ^{13}C TCE) for these experiments is given in Table S.2.

The expected $\delta^{13}C$ values for DIC following co-metabolism of a given amount of ^{13}C TCE were calculated by converting the $\delta^{13}C$ values of the starting DIC and TCE to molar concentrations of ^{13}C using the following formula to convert the measured $\delta^{13}C$ value to the ratio of ^{13}C to ^{12}C in the sample:

$$(^{13}C/^{12}C)_{sample} = (d^{13}C_{sample}/1000 + 1) \times (^{13}C/^{12}C)_{VPDB} \quad (1)$$

where $(^{13}C/^{12}C)_{VPDB}$ is 0.011. This ratio is then converted to the molar concentration of ^{13}C ($[^{13}C]$) using formula (2):

$$[^{13}C]_{sample} = (^{13}C/^{12}C)_{sample}/\{1 + (^{13}C/^{12}C)_{sample}\} \quad (2)$$

For the average background $\delta^{13}C$ value of -8.5‰ for DIC in the microcosms, $[^{13}C]_{BG}$ is equal to 0.01079 and $[^{13}C]_{TCE}$ 0.058 to 3.859 (versus 0.011 for the background DIC). To calculate the predicted $[^{13}C]_{DIC}$ in the microcosms for a given amount of co-metabolism of TCE, the following mass balance calculation was used:

$$[^{13}C]_{DIC} = (CM \times [^{13}C]_{TCE} + BD \times [^{13}C]_{BG}) / (CM + BD) \quad (3)$$

where CM is the amount of DIC produced from TCE co-metabolism in moles/L and BD is the background concentration of DIC in the microcosms (4 mmol/L). The calculated values were then converted back to $\delta^{13}C$ values using formulas (1) and (2) and plotted on Figure 5 in the manuscript.

Table S.1. Chemical and isotopic data for May 2007 groundwater samples. For comparison, available data for TAN-35 groundwater collected in July 2007 during removal of the in situ bioreactors is also given.

Well #	Distance from TSF-05 (m)	Bottom of sampling interval (m bgs)	Top of sampling interval (m bgs)	Nitrate (μmol/L)	Ferrous Iron (μmol/L)	Sulfate (μmol/L)	Dissolved Inorganic Carbon (mmol)	$\delta^{13}C_{DIC}$ (‰)
TAN-25	15	66	91	2.2	100	378	52.3	8.1
TAN-37A	44	62	127	0.6	82	0.6	22.4	-4.6
TAN-28	78	67	79	1.6	ND	246.8	7.6	-13.1
TAN-29	154	68	80	47.3	ND	263.6	5.9	-13.3
TAN-35	219	59	128	-	-	-	4.0	-8.2
TAN-42	303	60	134	23.1	-	223.0	3.2	-11.4
TAN-44	378	59	135	22.7	-	232.3	2.7	-9.4
TAN-33	407	70	134	19.8	-	227.5	4.0	-9.6
TAN-36	407	60	135	22.8	-	231.3	3.1	-10.0

Well #	TCE (nmol/L)	$\delta^{13}C_{TCE}$ (‰)	cDCE (nmol /L)	$\delta^{13}C_{cDCE}$ (‰)	VC (nmol /L)	$\delta^{13}C_{VC}$ (‰)	Ethene (nmol /L)	$\delta^{13}C_{eth}$ (‰)	CH_4 (μmol/L)	$\delta^{13}C_{CH4}$ (‰)
TAN-25	38	-27.0	ND	ND	ND	-19.4	ND	-19.8	538	-56.0
TAN-37A	15	-27.9	<100	-19.4	65	ND	ND	-13.8	1066	-51.9
TAN-28	7669	-28.5	914	-28.3	42	-22.0	ND	ND	38	-28.0
TAN-29	2934	-27.3	407	-27.1	ND	ND	ND	ND	24	-35.9
TAN-35	4069	-	-	-	-	-	-	-	-	-21.9
TAN-42	2189	-29.1	75	ND	ND	ND	-	ND	-	ND
TAN-44	999	-28.5	<100	-27.7	ND	ND	-	ND	-	ND
TAN-33	1071	-29.4	<100	-28.6	ND	ND	-	ND	-	ND
TAN-36	917	-28.4	<100	-28.5	ND	ND	-	ND	-	ND

ND – Not detected.

Table S.2. Carbon isotope compositions for dissolved inorganic carbon and CH_4 from microcosm experiments. Day corresponds to the day that each individual microcosm was sacrificed by addition of Na-azide.

^{13}C TCE added (μg/L)	$\delta^{13}C_{DIC}$ (Day1)	$\delta^{13}C_{DIC}$ (Day2)	$\delta^{13}C_{DIC}$ (Day3)	$\delta^{13}C_{DIC}$ (Day4)	Average (Days 2-4)
25 DIC	-7.1	-8.5	-8.5	-8.0	-8.4
50 DIC	-7.4	-8.1	-8.1	-8.6	-8.2
100 DIC	-7.4	-7.7	-8.5	-7.3	-7.9
200 DIC	-7.2	-8.0	-7.4	-8.4	-8.0
400 DIC	-7.5	-7.9	-7.8	-6.7	-7.5
1000 DIC	-6.1	-7.3	-6.6	-6.8	-6.9
2000.1 DIC	-8.4	-7.0	-6.7	-4.7	-6.1
2000.2 DIC	-6.3	-6.4	-7.4	-7.0	-6.9
2000.1 CH_4	-17.7	-17.4	-17.6		
2000.2 CH_4	-17.8	-17.8	-17.2		

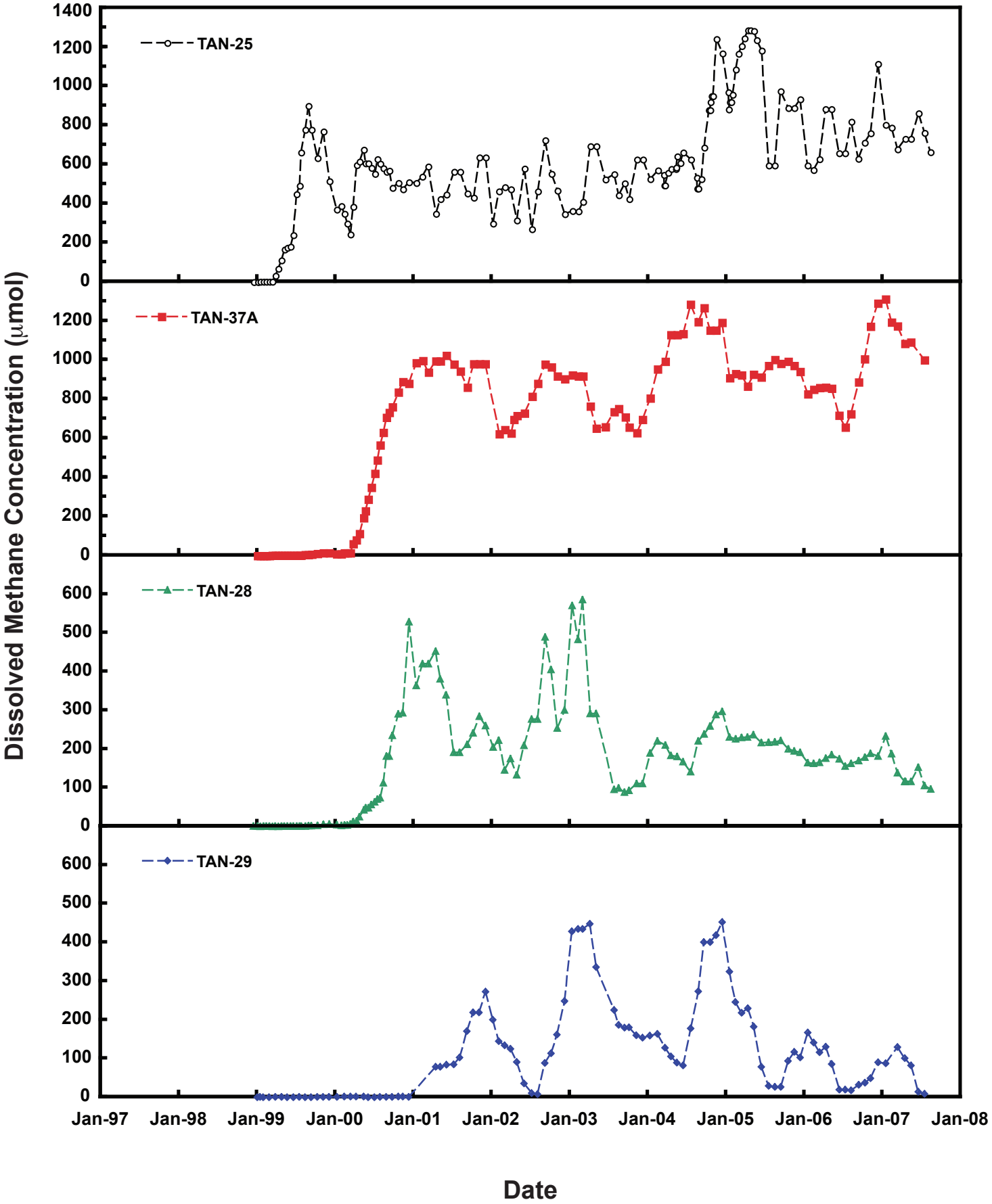

Figure S.1. - Dissolved CH_4 concentrations for TAN wells with significant CH_4. All data plotted are 8-week moving averages.

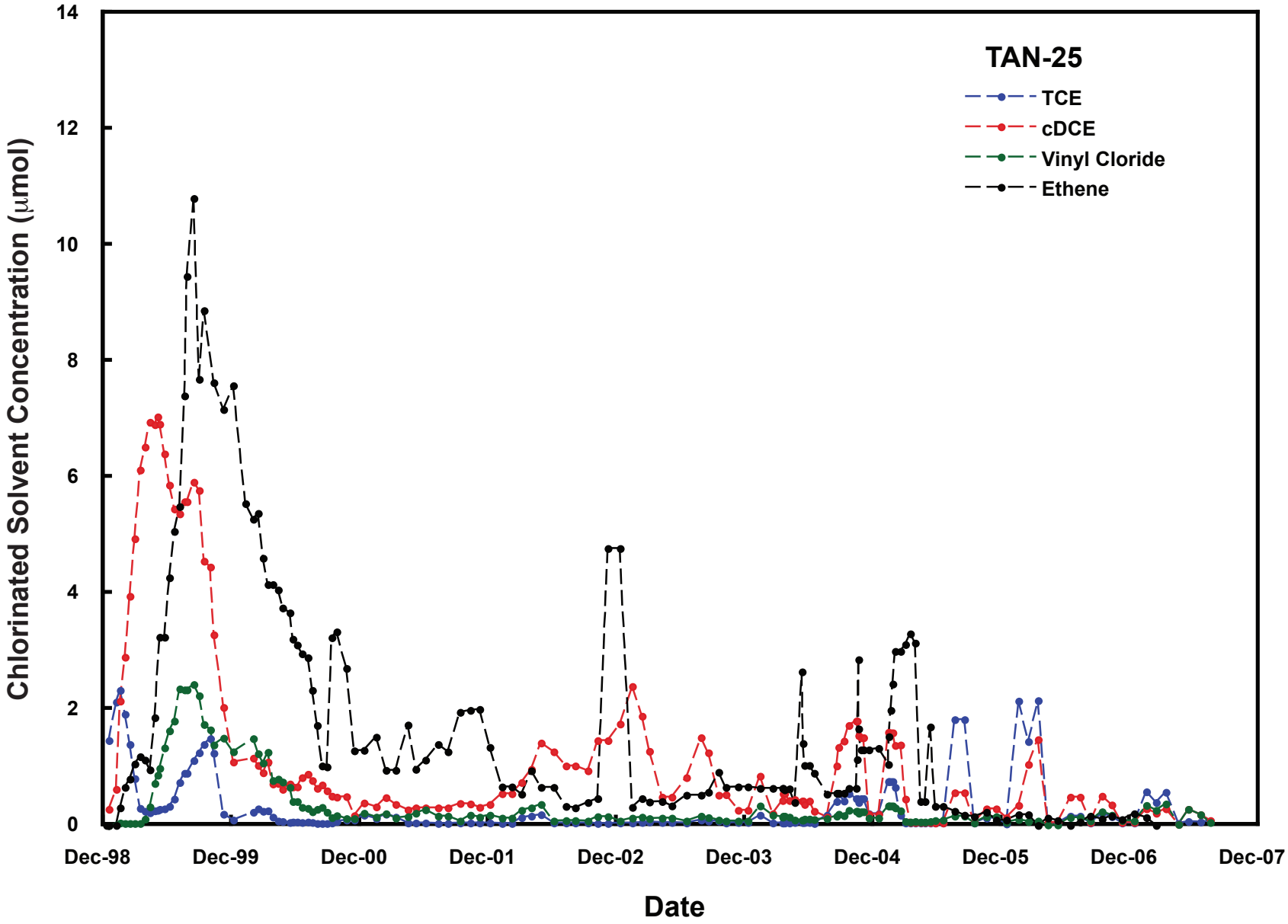

Figure S.2. 8-week moving average concentrations of chlorinated solvents in well TAN-25.

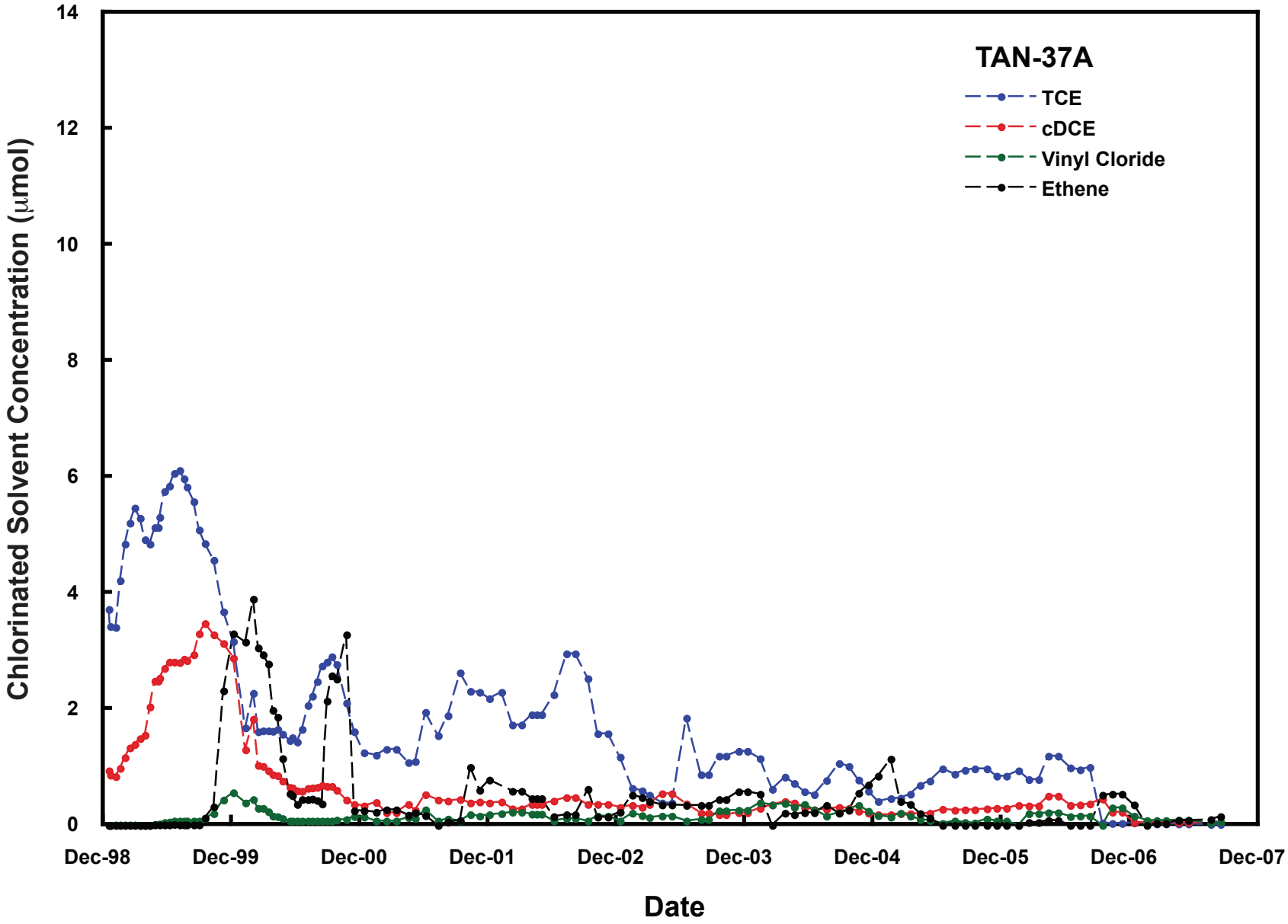

Figure S.3. 8-week moving average concentrations of chlorinated solvents in well TAN-37A.

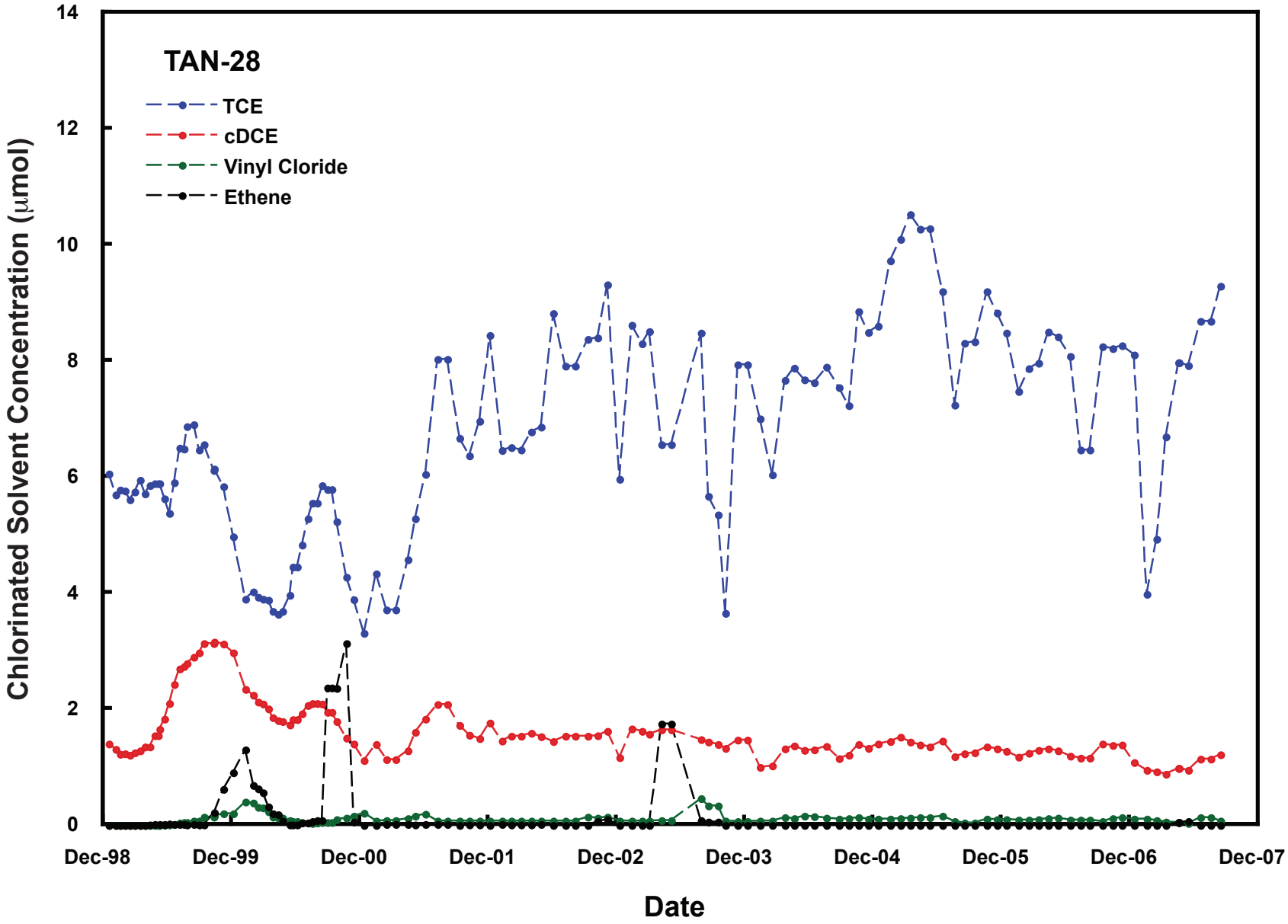

Figure S.4. 8-week moving average concentrations of chlorinated solvents in well TAN-28.

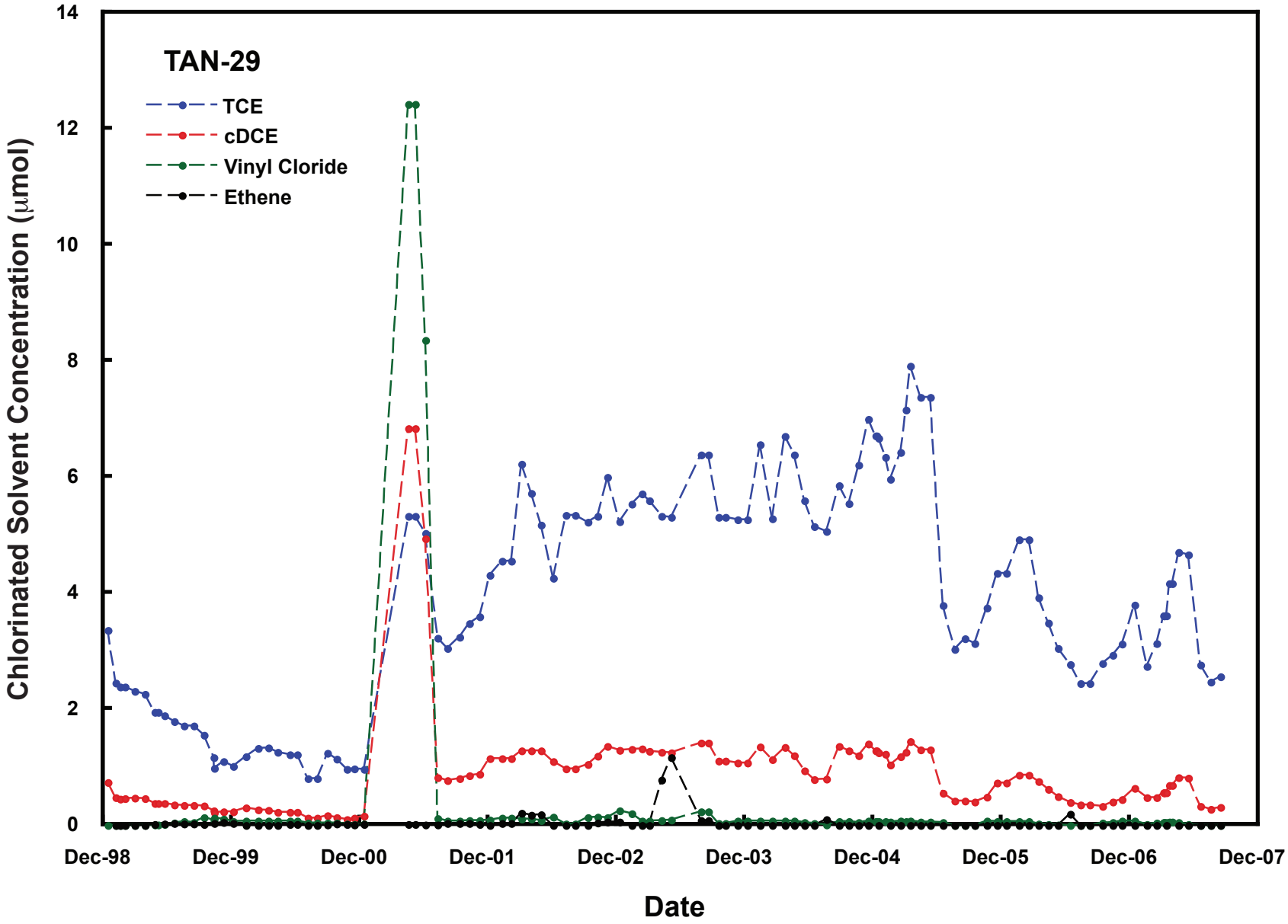

Figure S.5. 8-week moving average concentrations of chlorinated solvents in well TAN-29.

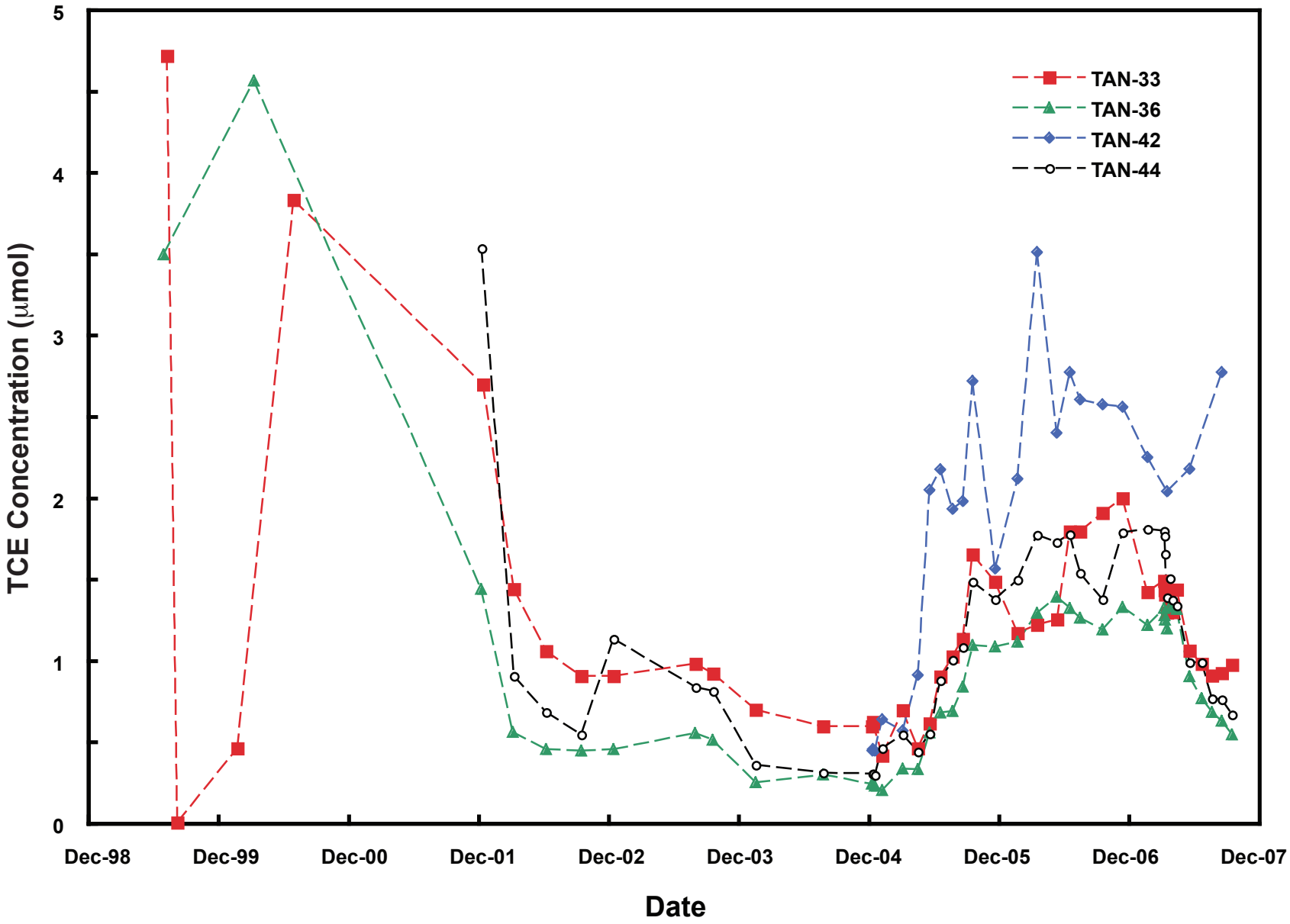

Figure S.6. TCE concentrations for distal wells sampled in TAN plume. Concentrations of other chlorinated solvents were low in these wells (<0.6 μmol/L).