TITLE: Long-Term Impacts on Groundwater and Reductive Dechlorination following Bioremediation in a Highly Characterized Trichloroethene DNAPL Source Area

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High resolution soil and groundwater monitoring was performed to assess the long-term impacts of bioremediation using bioaugmentation with a dechlorinating microbial consortium (and sodium lactate as the electron donor) in a well-characterized trichloroethene (TCE) dense non-aqueous phase liquid (DNAPL) source area. Monitoring was performed up to 3.7 years following active bioremediation using a high-density monitoring network that included several discrete interval multi-level sampling wells.

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Abstract

High resolution soil and groundwater monitoring was performed to assess the long-term impacts of bioremediation using bioaugmentation with a dechlorinating microbial consortium (and sodium lactate as the electron donor) in a well-characterized trichloroethene (TCE) dense non-aqueous phase liquid (DNAPL) source area. Monitoring was performed up to 3.7 years following active bioremediation using a high-density monitoring network that included several discrete interval multi-level sampling wells. Results showed that, despite the absence of lactate, lactate fermentation transformation products, or hydrogen, biogeochemical conditions remained favorable for the reductive dechlorination of chlorinated ethenes. In locations where soil data showed that TCE DNAPL sources persisted, local contaminant rebound was observed in groundwater, whereas no rebound or continuous decreases in chlorinated ethenes were observed in locations where DNAPL sources were treated. While ethene levels measured 3.7 years after active treatment suggested relatively low (2 to 30%) dechlorination of the parent TCE and daughter products, carbon stable isotope analysis showed that the extent of complete dechlorination was much greater than indicated by ethene generation, and that the estimated first-order rate constant describing the complete dechlorination of TCE at 3.7 years following active bioremediation was approximately 3.6 yr^{-1}. Overall, results of this study suggest that biological processes may persist to treat TCE for years after cessation of active bioremediation, thereby serving as an important component of remedial treatment design and long-term attenuation.

Introduction

In situ bioaugmentation to facilitate the reductive dechlorination of chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethene (TCE) has been widely demonstrated (Major et al., 2002; Hood et al., 2008; Schaefer et al., 2010; Pérez-de-Mora et al., 2014). Despite many documented successes, the presence of low permeability zones and/or DNAPL sources can serve as barriers to achieving groundwater remedial goals. When contaminant mass present in low permeability zones or as residual DNAPL remains after active treatment (i.e., after depletion of electron donor amendment), observed increases in contaminant concentrations, commonly referred to as "rebound", can occur (Manoli et al., 2012).

McGuire et al. (2016) have shown that reductive dechlorination can be sustained long after active treatment, but that the rate of reductive dechlorination in some cases is not sufficient to prevent observed contaminant rebound. Tillotson and Borden (2017) also showed that reductive dechlorination can persist after active treatment, and that the slow rate of decline in dechlorination rates is not correlated to total organic carbon levels in the aquifer. Tillotson and Borden further showed that dechlorination transformation products such as *cis*-1,2,-dichloroethene (DCE) and vinyl chloride (VC) often remain at elevated concentrations after treatment. A recent assessment of bioaugmentation in a fractured rock DNAPL source area showed that reductive dechlorination persisted for 10-months following lactate injection, and that continued biotransformation of PCE to ethene masked its rebound from the remaining DNAPL sources (Schaefer et al., 2017).

While the studies noted above suggest that extended dechlorination can occur following active bioremediation, a detailed field assessment of the long-term (> 3 years)

post active treatment impacts within a heterogeneous DNAPL source area has yet to be performed. Specifically, the long-term post treatment biogeochemical conditions in both high and low permeability zones, the extent to which DNAPL mass impacts rebound, and the post treatment rates of complete dechlorination have not been systematically studied in a well-characterized DNAPL source area. Such information and assessment are needed so that any long-term enhanced attenuation in treated DNAPL source areas can be appropriately incorporated within conceptual site models. The objective of this study was to assess the long-term dechlorination and groundwater quality (chlorinated solvent and biogeochemical conditions) at a well-characterized DNAPL source area approximately 2 to 3.7 years following active bioremediation, with the intent of using this information for improved remedial design by taking advantage of beneficial long-term treatment processes.

Experimental

Site Description and Key Historical Characterization / Remediation Activities

The post bioremediation field study was performed within Plume 4-1 (operable unit 2B) at Alameda Point, CA. This residual TCE DNAPL source area associated with this site was highly studied and characterized prior to implementing bioaugmentation. The source area characterization, described in Wang et al. (2014), included a combination of soil core sampling, membrane interface probes, hydraulic profiling tools, multi-level sampling (MLS) wells, passive flux meters, and partitioning tracer testing to define and characterize the TCE DNAPL source area and corresponding groundwater flow field. The layout of the source area is shown in Figure 1, and a conceptual cross-section of the

source area is provided in Figure 2. The conceptual model developed for this test area (Wang et al., 2014) consisted of an overlying silty sand zone that was (based on tracer flow testing) approximately 3 to 4 times less permeable than the underlying sandy zone. The ambient groundwater velocity through the higher permeability sandy zone was calculated to be approximately 0.013 m/day. Both partitioning tracer testing and soil core data confirmed that the residual DNAPL was primarily present in the overlying lower permeability (silty sand) zone.

The MLS wells, both within the DNAPL source (SMLS wells) and in the adjacent downgradient plume (PMLS wells), were installed in 2011 using the Solinst CMT Multilevel System. The original intent of these wells was to facilitate the DNAPL zone characterization, and to facilitate the assessment of bioaugmentation during the active remediation phase (i.e., when amendments were being delivered and when groundwater was being recirculated). The system utilized the CMT 7 channel system that allowed the installation of 7 discrete sampling intervals within a single wellbore.

Previously performed *in situ* bioremediation to treat residual DNAPL sources was implemented from July 18, 2012 to November 19, 2012 (CB&I, 2013). Bioaugmentation was implemented during bioremediation by injecting sodium lactate, nutrients (diammonium phosphate and yeast extract), and a commercially available microbial consortium containing *Dehalococcoides* mccartyi (SDC-9; Vainberg et al., 2009; Schaefer et al., 2009); a total of 3 x 10^{12} *Dehalococcoides* mccartyi cells were injected during bioaugmentation. A total of approximately 1,400 L of 60% sodium lactate solution was injected during overall treatment implementation. Amendments were delivered and recirculated throughout the DNAPL source area using the 3 injection wells (SIW-01

though SIW-03) and 4 extraction wells shown in Figure 1. Monitoring during the bioremediation study performed in 2012 was conducted immediately before, during, and up to 4 months following active treatment (Shaw Environmental, Inc., 2013).

Long-Term Post Bioremediation Monitoring

The long-term post bioremediation monitoring performed as part of this post treatment study was initiated in March 2015 (approximately 2.3 years following completion of active bioremediation). Three rounds of groundwater sampling at selected monitoring locations were performed at t= 2.3, 3.1, and 3.7 years following active treatment. Sampling was performed at SMLS wells, PMLS wells, extraction wells, and monitoring wells. Monitoring locations and depth intervals are provided in Table 1. Only source area SMLS monitoring locations that were monitored during active bioremediation and appreciably (>1 mg/L chlorinated ethenes) impacted with chlorinated solvents prior to bioaugmentation, and only the extraction (PEW) and PMLS wells that were in the core of the downgradient plume, were selected for evaluation in this study.

To limit perturbations to the ambient flow field, particularly for monitoring locations within the lower permeability materials, groundwater sampling was performed at the SMLS and PMLS using a peristaltic pump, purging 1 L of water volume (100 to 300 mL/min) before sample collection; the tubing volume was less than 0.25 L, so the 1 L purge volume was sufficient for purging any water in the tubing. In the other monitoring locations (Table 1), groundwater sampling was conducted using a standard 1.5 inch (nominal) diameter down-hole bladder pump. Field chemistry parameters (dissolved oxygen, pH, oxidation-reduction potential) were measured during the purge process and sampling was

initiated once parameters stabilized. Collected groundwater samples were analyzed for chlorinated ethenes (PCE, TCE, *cis* and *trans* 1,2-dichloroethene, and VC), reduced gases (methane, ethane, ethene, acetylene, and propane), volatile fatty acids (lactic, acetic, propionic, formic, butyric, pyruvic, valeric), total organic carbon, pH, dissolved iron, dissolved hydrogen, and anions (chloride, sulfate, bromide, nitrate, nitrite, ortho phosphate). In addition, *Dehalococcoides* mccartyi (DHC) cell abundance and compound-specific isotopic analysis (CSIA) of carbon in TCE, *cis*-1,2-dichloroethene (DCE), and VC were performed at select locations over the three rounds of groundwater sampling. These combined data were used to assess biogeochemical conditions, contaminant rebound, and reductive dechlorination relative to conditions immediately before and after bioaugmentation.

In addition to the groundwater sampling described above, a limited soil investigation was performed in April 2015 (approximately 29 months after cessation of active bioremediation). The purpose of the soil sampling, which entailed collection of four soil cores, was to determine if the DNAPL sources identified during site characterization (Wang et al., 2014) persisted. The four soil core locations within the source area are identified in Figure 1. The soil cores were collected using direct push technology, and targeted the interval from 4.6 to 7.6 m below ground surface (bgs). Collected cores were visually inspected and screened using a photoionization detector (PID) along the length of the core interval. The PID screening, along with information obtained from the previous DNAPL investigations (Wang et al., 2014) were used to identify probable locations of elevated chlorinated ethene levels. Soil samples (~ 10 g) for chlorinated ethene analysis

were placed in pre-weighed jars with methanol as an extractant; this methodology was selected to be consistent with the soil sampling method used prior to bioaugmentation.

Analytical Methods

Groundwater was analyzed for reduced gases (ethene, ethane, methane, acetylene, and propane) using the headspace method RSK-175 on a Varian 3900 gas chromatograph with a flame ionization detector (Kampbell et al., 1989). Analysis of the chlorinated compounds including VC, DCE, and TCE were analyzed by gas chromatography (GC) with mass spectrometry (MS) detection (Agilent GC-5890/MS-5971). Anions were analyzed by ion chromatography on a Dionex ICS-2000 IC chromatograph, using a 2mm Dionex AS18 column and a Dionex DS6 conductivity detector, via USEPA Method 300.0 (USEPA, 1993). Volatile fatty acids (lactic acid, acetic acid, propionic acid, formic acid, butyric acid, pyruvic acid, and valeric acid) were analyzed by a Dionex DX-600 chromatograph, using a 4 mm AS11-HC column and a Dionex DS3 conductivity detector. Dissolved hydrogen was analyzed by R SK-175 headspace method on a Varian 3800GC equipped with a pulsed discharge ionization detector. Total organic carbon (TOC) was analyzed on a Tekmar/Dormann Appollo 9000 TOC analyzer. DHC analysis was performed by Microbial Insights, Inc. (Knoxville, TN) using the quantitative polymerase chain reaction (qPCR) based Quant-Array-Chlor analysis (https://www.microbe.com/quantarray-chlor/), while CSIA sample analysis was performed by Pace Analytical (Pittsburgh, PA) using GC/combustion chamber/isotope ratio mass spectrometry (GC/C/IRMS). Groundwater samples (filtered using a 0.45 micron filter in the field, then acidified with nitric acid) were

sent to Chemtech (Mountainside, NJ) for dissolved iron analyses using inductively coupled plasma atomic emission spectroscopy.

Results and Discussion

Soil Cores

Soil core results from each of the 4 source area locations are shown in Figure 3. While both TCE and DCE were observed at each core location, only at location DPC-20 were concentrations indicative of residual TCE DNAPL, indicating that TCE source remains in this portion of the source area. The depth interval of the high concentration TCE source in Figure 3 corresponds to the lower permeability material (Figure 2). The TCE concentrations and depths present in DPC-20 are similar (within 20%) to those measured in an adjacent soil boring collected at SPW-3-2 (Figure 1) several months prior to the dissolution testing and bioaugmentation performed prior to and in 2012 (Wang et al., 2014). These data suggest that only a small fraction of the DNAPL mass likely was removed in this portion of the source area, although estimating partial DNAPL mass removal via soil cores is difficult due to the DNAPL heterogeneous distribution.

In contrast, TCE and DCE concentrations at DPC-21 through DPC-23 suggest that DNAPL is not present (or, present at trace levels) at these locations. Soil data at DPC-17 (located directly adjacent to DPC-23, as shown in Figure 1), collected several months prior to the dissolution testing and bioaugmentation performed in 2012, showed TCE concentrations up to 590 mg/kg at a depth of 6.3 m bgs. This historical elevated TCE soil concentration, located within the lower permeability material, is indicative of residual TCE DNAPL. Thus, in the vicinity of DPC-23, substantial removal of DNAPL sources

appears to have occurred following the dissolution and bioaugmentation testing. Assessment of groundwater data in the following sections was used to further evaluate the potential presence of remaining DNAPL sources.

Groundwater Biogeochemical Conditions

Groundwater monitoring results showed that VFAs were below the analytical detection limit of 1 mg/L at all locations, indicating that (as expected) the lactate and subsequent fermentation products present during active treatment were no longer present in the source area. Dissolved hydrogen levels also were below the analytical detection limit of 0.0084 µg/L.

Despite the absence of any measurable VFAs or dissolved hydrogen in the source area, other biogeochemical indicators suggest that conditions favorable to the biological reductive dechlorination of chlorinated ethenes persisted up to 3.7 years following active treatment. The pH generally ranged from 6.3 to 7.6, which is in the range conducive for the reductive dechlorination of chlorinated ethenes. Naturally-occurring TOC (which can potentially serve as electron donor) (Lyon et al., 1995) in the source area generally ranged from 10 to 30 mg/L, which were consistent with levels measured upgradient and downgradient of the treatment area. Dissolved iron concentrations generally ranged from 2 to 8 mg/L. More convincingly, comparison of methane, sulfate, and DHC cell abundance measured 3.7 years after active treatment to those measured just prior to bioaugmentation (Table 2) suggest that biogeochemical impacts from active remediation conducive to reductive dechlorination persist in the source area. Dissolved methane levels in the lower permeability materials remained approximately 1 to 2 orders of magnitude

greater compared to pre-bioaugmentation levels; in the higher permeability zone, methane levels ranged from 4- to 22-times greater than pre-bioaugmentation levels. Furthermore, methane levels showed little dissipation (less than a factor of 2) since immediately after active treatment, even in the higher permeability materials where approximately 3 pore volumes of groundwater have passed through since the end of active treatment. These methane results suggest that methanogenic activity, especially in the low permeability materials, has been sustained 3.7 years following treatment using lactate as the electron donor. Previous studies (Sleep et al., 2005; Adamson and Newell, 2009) suggest persistent reducing conditions in the absence of amended electron donor can be due to decay of endogenous bacteria that amassed during active treatment. We speculate that the abundant delivery of electron donor and DHC (which facilitated biomass growth) during the relatively brief active bioremediation phase of the study, and then the subsequent decay of this biomass, were factors that contributed to the long-term persistence of reducing conditions and (as discussed later in the text) biotic dechlorination at the site.

Consistent with the methane data, Table 2 also shows that sulfate-reducing conditions persisted in the source area, although measurable sulfate (43 to 208 mg/L) was present at all monitoring locations shown in Table 2. Thus, with dissolved iron also present, the formation of iron sulfides (especially near the injection wells which became fouled during active treatment) was possible; persistence of these iron sulfide minerals could likely have contributed to the long-term reducing conditions.

Finally, aqueous DHC cell abundance in the lower permeability materials remained several orders of magnitude greater than those observed immediately prior to

bioaugmentation; only one monitoring location was monitored for DHC cell abundance in the higher permeability materials prior to bioaugmentation, so while over a 400-fold increase was observed, the limited comparison precludes a definitive assessment for the high flow zone. The persistence of the elevated DHC cell abundance suggests the potential for enhanced dechlorination, but the dechlorinating activity of the DHC and contributions from other dechlorinators cannot be readily obtained from this comparison. However, when the methane, sulfate, and DHC data are collectively assessed, the data suggest that conditions conducive to the continued dechlorination of TCE sources persisted, especially in the lower permeability zones.

Groundwater Chlorinated Ethenes and Ethene

Potential chlorinated ethene abiotic dechlorination products acetylene and propane (He et al., 2015; Schaefer et al., 2015) were not detected in any of the groundwater samples. These results suggest that abiotic dechlorination likely was not playing a dominant role at this site, although biotic transformation of any acetylene or propane that was formed may have masked their generation.

Groundwater chlorinated ethene and ethene results for SMLS locations screened within the lower permeability materials are shown in Figure 4. Consistent with the soil results (Figure 3), chlorinated ethene concentrations at SMLS 1-3 were elevated and consistent with the presence of nearby DNAPL sources. SMLS locations in the adjacent intervals above, and approximately downgradient (SMLS 4 intervals) also show substantial rebound following bioaugmentation treatment; such rebound is not unexpected considering that nearby DNAPL sources persisted. In contrast, monitoring

intervals at SMLS 7 showed an absence of contaminant rebound and/or continued decreases in chlorinated ethene levels following bioaugmentation. This result also is consistent with the soil core data which show an absence of DNAPL sources in the vicinity and upgradient of SMLS 7, thus the observed rebound appears to be related to the persistence of DNAPL sources.

The discrete interval sampling results collected at the final sampling event during active remediation (day 61) and from the first post treatment rebound sample (day 110) are consistent with the longer- term (>900 days in Figure 4) groundwater monitoring and soil sampling performed as part of this study. Total molar chlorinated ethenes + ethene concentrations increased at MLS 1-3 by approximately a factor of 2.5 between the end of active remediation and 49 days after active remediation (day 110), indicating that source DNAPL mass likely is still present, which results in the accumulation of these compounds in the aqueous phase as the DNAPL slowly dissolves. In contrast, total molar chlorinated ethenes + ethene decreased by approximately a factor of two at MLS 7-3 between the end of active remediation and 49 days after active remediation, suggesting that appreciable removal of DNAPL source has likely occurred. These results highlight the usefulness of discrete interval monitoring locations placed within suspected lower permeability DNAPL source areas for performance monitoring during and shortly after active bioremediation.

Groundwater chlorinated ethene and ethene results for SMLS and extraction well locations screened within the higher permeability materials are shown in Figure 5. With respect to contaminant rebound, the trends for monitoring locations screened in the higher permeability materials are similar to those observed for those screened in the

lower permeability materials, with locations near and downgradient of SMLS 1 showing rebound and/or contaminant concentrations greater than or equal to those observed prior to bioaugmentation. SMLS 4 and PEW03, which are not adjacent to or downgradient of SMLS-1, do not exhibit any measurable rebound and concentrations remain substantially lower than prior to bioaugmentation. The greatly elevated vinyl chloride concentration in PEW03 at 1176 days is considered anomalous.

SMLS locations 4-2 through 4-4 show an increasing trend in total chlorinated ethene molar concentrations during the last three monitoring events performed in this study. The reason for this increasing trend is unclear, as increasing trends were not observed in upgradient monitoring locations.

While the molar fraction of ethene compared to the other chlorinated ethenes present was small, both during and after bioaugmentation (Table S1), the molar fraction of ethene remained 1 to 2 orders of magnitude greater than baseline (prior to bioaugmentation) at most monitoring locations. Thus, the complete reductive dechlorination of the chlorinated ethenes continued for 3.7 years following active treatment. If there was no subsequent transformation of ethene, and if all chlorinated ethene removal proceeded through ethene, these results would suggest that (based on the typical molar fraction of ethene) only 2 to 30% of the dissolved chlorinated ethene mass was fully dechlorinated within the source area by the end of the rebound monitoring period; further evaluation of complete dechlorination will be assessed via CSIA analyses.

To verify that these ethene levels were due to ongoing generation, rather than slow release from the source area due to generation during active treatment only, groundwater in the three injection wells (Figure 1) was monitored. The injection wells were screened

similarly to the extraction wells, but located immediately upgradient of the source area. The injection wells showed a substantial (> 75%) loss of permeability, presumably due to biofouling, as noted during the final stage of active treatment and during draw-down testing. While chlorinated ethene levels in the injection wells were all less than 1 µg/L in the 2 to 3.7 years following active treatment, strongly reducing conditions (as evidenced by sulfate reduction and elevated methane levels) persisted. However, ethene was below the analytical detection limit of 5 µg/L in all 3 injection wells, which was substantially less than the ~ 250 µg/L observed at the end of the active remediation phase. Thus, in the absence of chlorinated ethene sources and limited groundwater flow, ethene did not persist in the source area, thereby confirming that the ethene observed in the source area monitoring locations (Figure 1) likely was due to ongoing reductive dechlorination.

Monitoring locations PMLS 3-2 and 4-2, which are approximately downgradient from PEW-03 and screened in the higher permeability material, show that total chlorinated ethenes + ethene continued to decrease in the downgradient plume (Figure S1) following active treatment. These results are consistent with the trends observed in the SMLS 7 intervals (Figures 4 and 5). Based on the calculated groundwater velocity in the shallow lower permeability zone (0.0037 m/day), water treated during active bioremediation was expected to migrate to PMLS 3-1 and 4-1 by approximately 900 days (2.5 years) assuming no retardation. This migration provides a possible explanation for the decreasing trend in chlorinated ethenes observed over the last three monitoring events, as treated water may be just beginning to arrive at these locations. Despite the observed impacts of source area treatment on the downgradient PMLS wells, there is no measurable increase in ethene concentrations from the SMLS 7 intervals to the PMLS 3

and 4 intervals (data not shown). This result suggests that continued complete

dechlorination did not occur downgradient of the source area, that dilution/dispersion of

any ethene generated between the source area and PMLS wells masked the ethene

generation, and/or that the ethene was subsequently transformed and not accumulated as

a final dechlorination product. This issue is further discussed in the CSIA Analyses

section.

CSIA Analyses

To assess the extent to which complete dechlorination of TCE is occurring at the

site, the net isotopic enrichment for TCE, DCE, and VC is calculated as follows:

$$\delta^{13}C = (\chi_{TCE}\ \delta^{13}C_{TCE} + \chi_{DCE}\ \delta^{13}C_{DCE} + \chi_{VC}\ \delta^{13}C_{VC}) \qquad\qquad \text{Eq. 1}$$

where $\delta^{13}C$ is the molar weighted isotopic carbon enrichment, χ_i is the mole fraction of

compound i, and δ^{13} is the ^{13}C isotopic level in either the TCE, DCE, or VC (‰). Use of

an isotopic mass balance similar to Eq. 1 for multiple transforming species has been

previously employed for chlorinated ethenes and ethene (Mundle et al., 2012; Schaefer et

al., 2018). Figure 6a shows $\delta^{13}C$ as a function of total chlorinated ethene molar

concentration emanating from the DNAPL sources in the lower permeability materials;

Figure 6b shows the corresponding values for the underlying higher permeability

material. In the higher permeability materials, chlorinated ethene enrichment and

attenuation are clearly observed. The enrichment factor of -1.87±0.18‰ observed in the

higher permeability materials is less negative than enrichment factors (-26 to -2.2‰)

observed for reductive TCE, DCE, or VC biodegradation (Bloom et al., 2000), which

suggests that mechanisms other than biotic reductive dechlorination may be responsible for the attenuation of the chlorinated ethenes. The relative absence of enrichment and attenuation observed in the lower permeability materials likely is due to the fact that treated water has not yet fully migrated through the downgradient (PMLS) monitoring locations, consistent with the data in Figure S1, so isotopic levels do not yet reflect changes due to bioremediation.

The isotopic and concentration data in Figure 6b suggest that substantial complete dechlorination of TCE, DCE, and VC occurred, which is in apparent contradiction to the ethene data presented in Table S1 that showed ethene (the presumed complete dechlorination transformation product) represented only a small fraction of the molar chlorinated ethenes + ethene. While CSIA analysis was not performed on ethene, the low levels of ethene present likely would not have resolved the isotopic balance, as the ethene δ^{13}C required to complete the isotopic mass balance would be (based on the inclusion of ethene in Eq. 1) approximately -400‰, which is not plausible (Bloom et al., 2000). This apparent discrepancy suggests that ethene was further transformed, and/or that vinyl chloride transformation proceeded without formation of ethene. Only trace (approximately 10-times less than ethene) ethane was generated, so continued reduction of ethene to ethane cannot explain this discrepancy.

Previous studies have shown that trace levels of oxygen can result in the aerobic transformation of vinyl chloride and ethene (Abe et al., 2009; Gossett, 2010), with enrichment factors that are more in-line with (but still ~4-times greater than) those observed in Figure 6b. Others have shown that anaerobic oxidation of ethene can occur via sulfate as an electron acceptor (Fullerton et al., 2013). Either, or both, of these

oxidative processes readily explains the observed chlorinated ethene fractionation in absence of appreciable stoichiometric quantities of ethene or ethane.

The CSIA data are used to estimate a first-order complete dechlorination rate constant (k), based on the net overall dechlorination of the chlorinated ethenes defined in Eq. 1, using the following expression (ITRC, 2013):

$$k = \frac{v(\delta_0^{13}C - \delta^{13}C_0)}{\epsilon d} \qquad \text{Eq. 2}$$

where $\delta^{13}C_0$ refers to the initial (upgradient DNAPL source) isotopic enrichment (Eq. 1), v is the ambient superficial velocity through the higher permeability material (0.013 m/day), ε is the enrichment factor (slope in Figure 6b) and d is the distance (5.4 m from the DNAPL source to the PMLS wells). The average isotopic enrich of the PMLS wells is used for $\delta^{13}C$. Eq. 2 assumes retardation of the chlorinated ethenes due to adsorption to the higher permeability soil is negligible, which is a reasonable assumption based on the absence of measurable retardation of hydrophobic tracers previously observed (Wang et al., 2014). For evaluation over this distance, Eq. 2 yields a first order overall dechlorination rate constant of 3.6 yr^{-1} (half life = 0.19 yr). Assuming that this rate constant has been maintained since the end of active treatment, and assuming a constant chlorinated ethene concentration (maintained by the presence of DNAPL) near SMLS 1-3 of 1,600 µM, the chlorinated ethene DNAPL near SMLS 1-3 would have decreased by approximately 500 mg/kg since the end of active bioremediation via the biodegradation-enhanced dissolution into the surrounding groundwater. This decrease represents approximately 25% of the DNAPL soil core concentration currently near SMLS 1-3 and soil boring location DPC-20, and suggests that biotic dechlorination remains a significant attenuation mechanism 3.7 years after active bioremediation.

Conclusions

This study showed that enhanced biotic reductive dechlorination is ongoing 3.7 years after completion of active bioremediation. While significant improvements in groundwater quality were only observed where DNAPL sources had been substantially removed, biogeochemical indicators suggested that conditions remained more conducive to reductive dechlorination than prior to bioremediation. Finally, while ethene levels were low, isotopic data showed that the complete dechlorination of the chlorinated ethenes was ongoing at a rate that not only mitigated downgradient migration, but that also may be significantly attenuating DNAPL source mass. Biological oxidation of the vinyl chloride and/or the ethene likely is masking ethene accumulation; detailed assessment of a broader range of biomarkers than those employed for this study may have provided further insight on this process. The long-term remedial enhancement observed in this study should be taken into consideration when designing and implementing site remedies. Such consideration may result in reduced timeframes and costs associated with active bioremediation.

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References

Abe, Y., R. Aravena, J. Zopfi, O. Shouakar-Stash, E. Cox, J.D. Roberts, and D. Hunkeler. 2009. Carbon and chlorine isotope fractionation during aerobic oxidation and reductive dechlorination of vinyl chloride and *cis*-1,2-dichloroethene. *Environmental Science & Technol*ogy 43:101-107.

Adamson, D.T. and C.J. Newell. 2009. Support of source zone bioremediation through endogenous biomass decay and electron donor recycling. *Bioremediation Journal* 13:29-40.

Bloom, Y., R. Aravena, D. Hunkeler, E. Edwards, and S.K. Frape. 2000. Carbon isotope fractionation during microbial dechlorination of trichloroethene, *cis*-1,2-dichloroethene, and vinyl chloride: implications for assessment of natural attenuation. *Environmental Science & Technol*ogy 34:2768-2772.

CB&I, 2013. Treatability study report on enhanced bioremediation source area treatment at Plume 4-1. submitted to NAVFAC.

Fullerton, H., M. Crawford, A. Bakenne, D.L. Freedman, and S.H. Zinder. 2013. Anaerobic oxidation of ethene coupled to sulfate reduction in microcosms and enrichment cultures. *Environmental Science & Technol*ogy 47:12374-12381.

Gossett, J.M. 2010. Sustained aerobic oxidation of vinyl chloride at low oxygen concentrations. *Environmental Science & Technology* 44:1405-1411.

He, Y.T., J.T. Wilson, and R.T. Wilkin. 2015. Review of Abiotic Degradation of Chlorinated Solvents by Reactive Iron Minerals in Aquifers. *Ground Water Monitoring & Remediation* 35:57-75.

Hood, E.D., D.W. Major, J.W. Quinn, W.S. Yoon, A. Gavaskar, and E.A. Edwards. 2008. Demonstration of enhanced bioremediation in a TCE source area at Launch Complex 34, Cape Canaveral Air Force Station. *Ground Water Monitoring & Remediation* 28:98-107.

ITRC (Interstate Technology & Regulatory Council). 2013. Environmental Molecular Diagnostics. Washington, D.C.

Kampbell, D.H., J. T. Wilson, and S. A. Vandergrift. 1989. Dissolved Oxygen and Methane in Water by a GC Headspace Equilibration Technique. Environmental Analytical Chemistry, 36(4):249-257.

Lyon, W.G., C.C. West, M.L. Osborn, and G.W. Sewell. 1995. Microbial utilization of vadose zone organic carbon for reductive dichlorination of tetrachloroethene. *J. Environ. Sci. Health*, Part A. 30(7): 1627-1639.

Major, D.W., M.L. McMaster, E.E. Cox, E.A. Edwards, S.M. Dworatzek, E.R. Hendrickson, M.G. Starr, J.A. Payne, and L.W. Buonamici. 2002. Field demonstration of a successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene. *Environmental Science & Technol*ogy 36:5106-5116.

Manoli, G., J.C. Chambon, J.C., P.L. Bjerg, C. Scheutz, P.J. Binning, and M.M. Broholm. 2012. A remediation performance model for enhanced metabolic reductive dechlorination of chloroethenes in fractured clay till. *Journal of Contaminant Hydrology* 131:64-78.

McGuire, T.M., D.T. Adamson, M.S. Burcham, P.B. Bedient, and C.J. Newel. 2016. Evaluation of long-term performance and sustained treatment of anaerobic bioremediation sites. *Ground Water Monitoring & Remediation* 36:32-44.

Mundle, S.O.C., T. Johnson, G. Lacrampe-Coulourne, A. Pérez-de-Mora, M. Duhamel, E.A. Edwards, M.L. McMaster, E. Cox, K. Révész, and B.S. Lollar. 2012. Monitoring biodegradation of ethene and bioremediation of chlorinated ethenes at a contaminated site using compound-specific isotope analysis (CSIA). *Environmental Science & Technol*ogy 46:1731-1738.

Pérez-de-Mora, A., A. Zila, M.L. McMaster, and E.A. Edwards. 2014. Bioremediation of chlorinated ethenes in fractured bedrock and associated changes in dechlorinating and nondechlorinating microbial populations. *Environmental Science & Technol*ogy 48:5770-5779.

Schaefer, C.E., C.W. Condee, S. Vainberg, and R.J. Steffan. 2009. Bioaugmentation for Chlorinated Ethenes using *Dehalococcoides* sp.: Comparison between batch and column experiments, *Chemosphere* 75:141-148.

Schaefer, C.E., D.R. Lippincott, and R.J. Steffan. 2010. Field-scale evaluation of bioaugmentation dosage for treating chlorinated ethenes. *Ground Water Monitoring & Remediation* 30:113-124.

Schaefer, C.E., R.M. Towne, D.R. Lippincott, P. Lacombe, M.E. Bishop, and H. Dong. 2015. Abiotic Dechlorination in Rock Matrices Impacted by Long-Term Exposure to TCE. *Chemosphere* 119:744-749.

Schaefer, C.E., G.M. Lavorgna, E.B. White, and M.D. Annable. 2017. Bioaugmentation in a well-characterized fractured rock DNAPL source area. *Ground Water Monitoring and Remediation* 37:35-42.

Schaefer, C.E., D.R. Lippincott, H. Klammler, and K. Hatfield. 2018. Evidence of rock matrix back-diffusion and abiotic dechlorination using a field testing approach. *Journal of Contaminant Hydrology* 209:33-41.

Shaw Environmental, Inc. 2013. Treatability study report on enhanced bioremediation source area treatment at Plume 4-1. submitted to the US Navy, Document Control Number: SHAW-0807-0007-0487.

Sleep, B.E., A.J. Brown, and B.S. Lollar. 2005. Long-term tetrachlorethene degradation sustained by endogenous cell decay. *Journal of Environmental Engineering Science* 4:11-17.

Tillotson, J.M. and R.C. Borden. 2017. Rate and extent of chlorinated ethene removal at 37 ERD sites. *Journal of Environmental Engineering* 143(8).

United States Environmental Protection Agency (USEPA). 1993. Determination of inorganic anions by ion chromatography. EPA/600/R-93/100

Vainberg, S., C.W. Condee, and R.J. Steffan. 2009. Large-scale production of bacterial consortia for remediation of chlorinated solvent-contaminated groundwater. *J. Industrial Micobiol. Biotechnol.* 36:1189-1197.

Wang, F., M.D. Annable, C.E. Schaefer, T.D. Ault, J. Cho, and J.W. Jawitz. 2014. Enhanced Aqueous Dissolution of a DNAPL Source to Characterize the Source Strength Function. *Journal of Contaminant Hydrology*. 169:75-89.

TABLES

Table 1. Monitoring locations used in this study. bgs = below ground surface.

Well Identification	Screen Interval (m bgs)	Type of Well
Low Permeability		
SMLS 1-1	5.0 – 5.2	
SMLS 1-2	5.3 – 5.5	
SMLS 1-3	5.6 – 5.8	
SMLS 4-2	5.3 – 5.5	
SMLS 4-3	5.6 – 5.8	Discrete Interval – Source
SMLS 7-1	5.0 – 5.2	
SMLS 7-2	5.3 – 5.5	
SMLS 7-3	5.6 – 5.8	
PMLS 3-1	5.2 – 5.3*	Discrete Interval – Plume
PMLS 4-1	5.2 – 5.3*	
SPW-1	5.2 – 6.7	
SPW-3-1	5.2 – 5.9	Monitoring well
High Permeability		
SMLS 1-4	5.9 – 6.1	
SMLS 4-4	5.9 – 6.1	Discrete Interval - Source
SMLS 4-5	6.2 – 6.4	
SMLS 7-4	5.9 – 6.1	
PEW02	4.6 – 7.6	Extraction Well**
PEW03	4.6 – 7.6	
PMLS 3-2	6.1 – 6.2***	Discrete Interval - Plume
PMLS 4-2	6.1 – 6.2***	
SPW-3-2	5.9 – 6.9	Monitoring Well

* Sand back interval is from 4.9 to 5.5 m bgs

** Extraction wells are screened across both the low and high permeability zones

*** Sand back interval is from 5.8 to 6.4 m bgs

Table 2. Ratios of the final (post bioremediation) measured groundwater concentrations to those measured prior to bioremediation. Ratios are shown at the monitoring locations listed below for methane, sulfate, and aqueous phase *Dehalococcoides* mccartyi (DHC).

Well Identification	Methane Ratio	Sulfate Ratio	DHC Ratio
Low Permeability			
SMLS 1-1	46	0.48	NA
SMLS 1-2	40	0.50	4700
SMLS 1-3	48	0.46	1100
SMLS 4-2	23	0.68	1700
SMLS 4-3	124	0.48	2000
SMLS 7-1	15	0.34	73000
SMLS 7-2	48	0.23	830
SMLS 7-3	19	0.23	140
High Permeability			
SMLS 1-4	22	0.56	438
PEW-02	4.4	0.64	NA
PEW-03	8.6	0.80	NA

NA = data not available

FIGURES
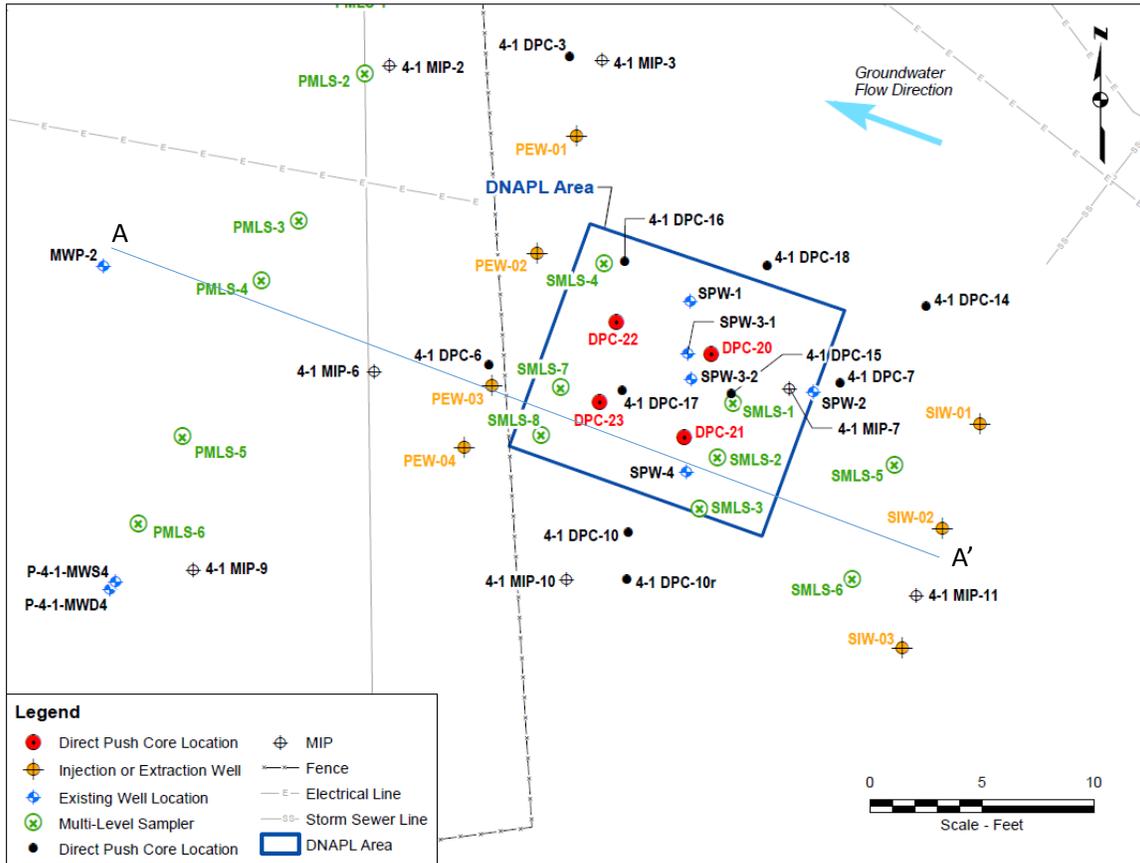
Figure 1. Demonstration location at Alameda Point.
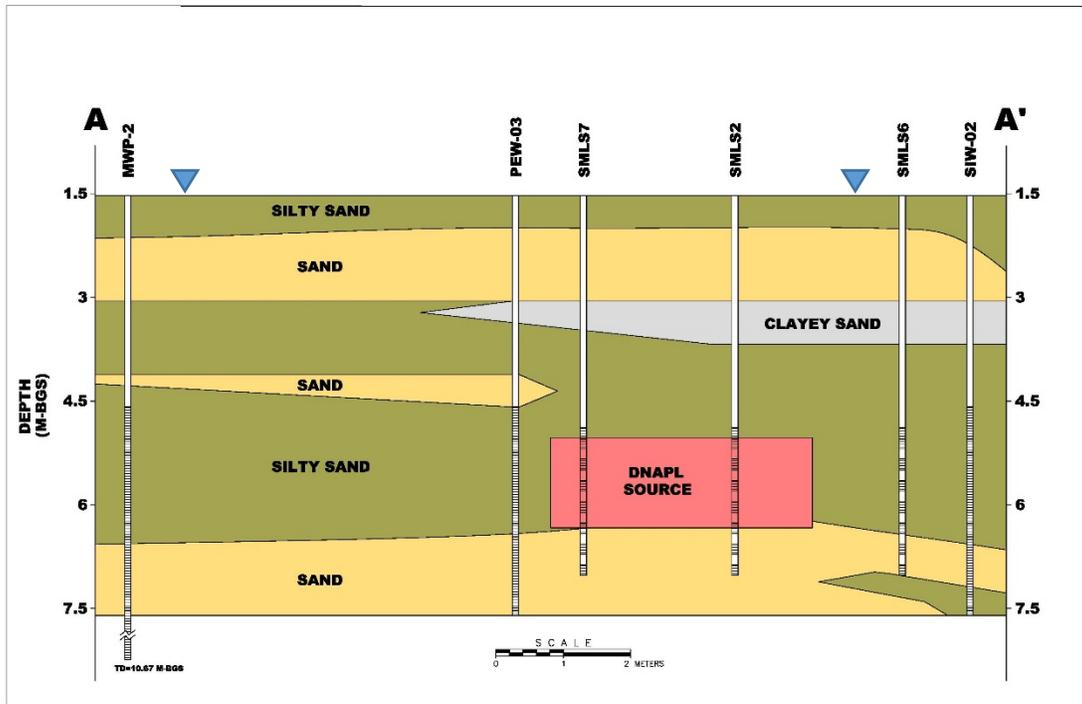
Figure 2. Conceptualized cross-section of the demonstration area, showing the location of monitoring wells and DNAPL sources prior to bioaugmentation treatment. The A-A' transect is shown in Figure 1. The water table is approximately 1.5 m below ground surface (bgs).
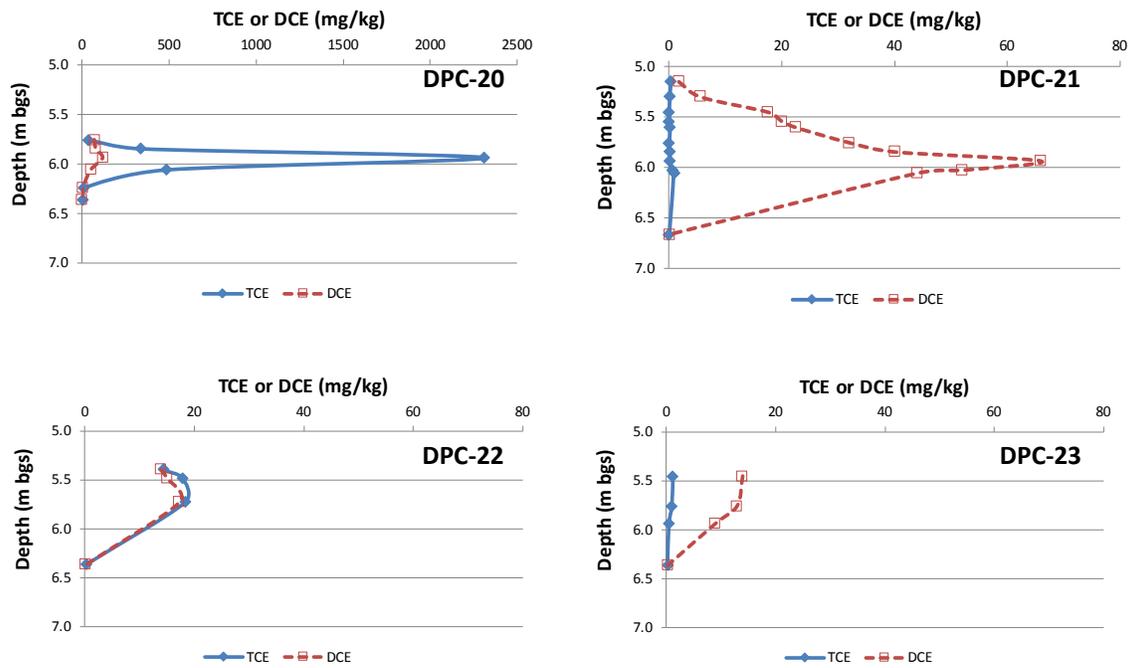
Figure 3. TCE and DCE soil concentrations measured in the four soil cores (Figure 1). The x-axis scale for location DPC-20 has been increased due to the relatively large TCE concentrations at this location, which are indicative of DNAPL.
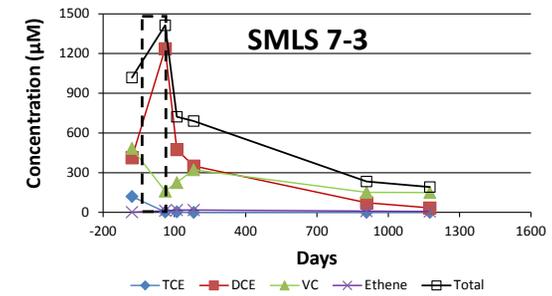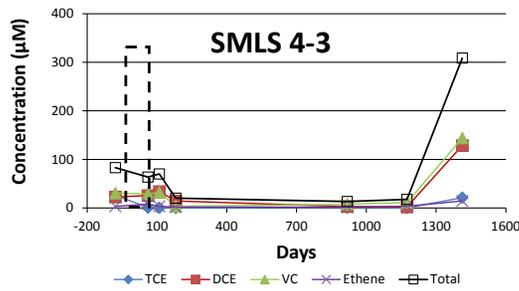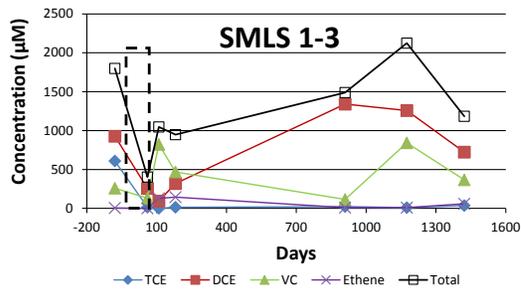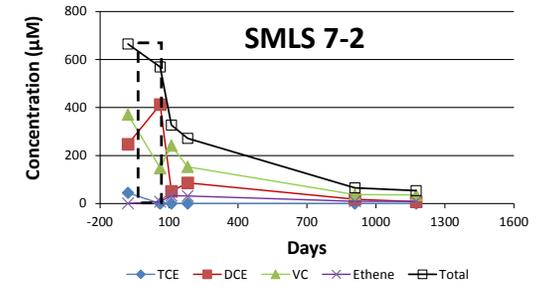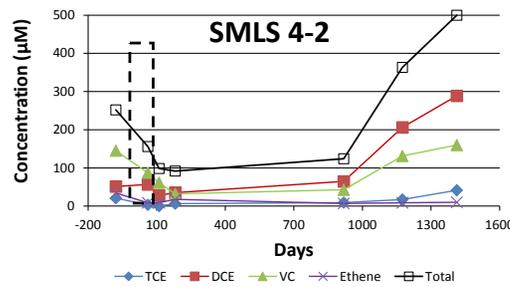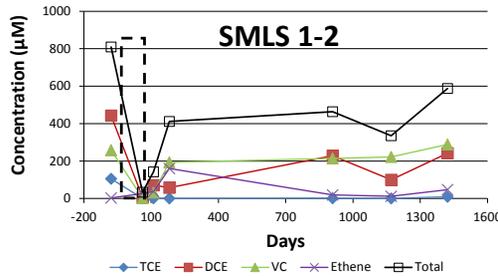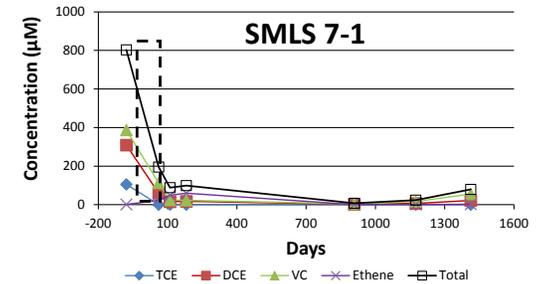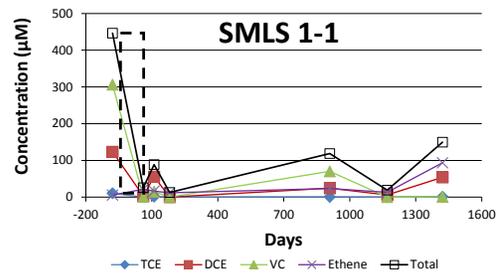
Figure 4. Groundwater chlorinated ethene and ethene results for SMLS locations screened within the lower permeability materials. The dashed boxes represent the time intervals where active bioremediation occurred; the high density of data collected during active treatment was omitted for clarity.
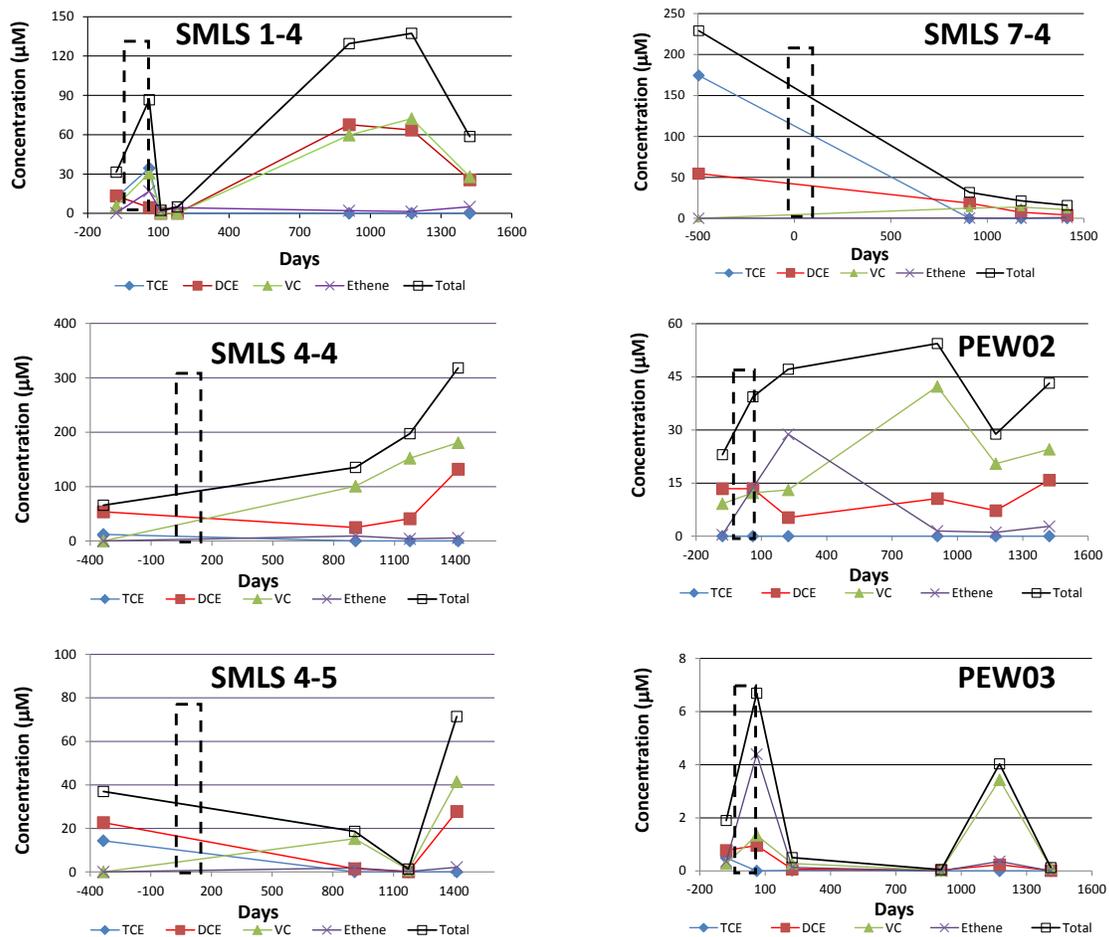
Figure 5. Groundwater chlorinated ethene and ethene results for SMLS and extraction well locations screened within the higher permeability materials. The dashed boxes represent the time intervals where active bioremediation occurred; the high density of data collected during active treatment was omitted for clarity.
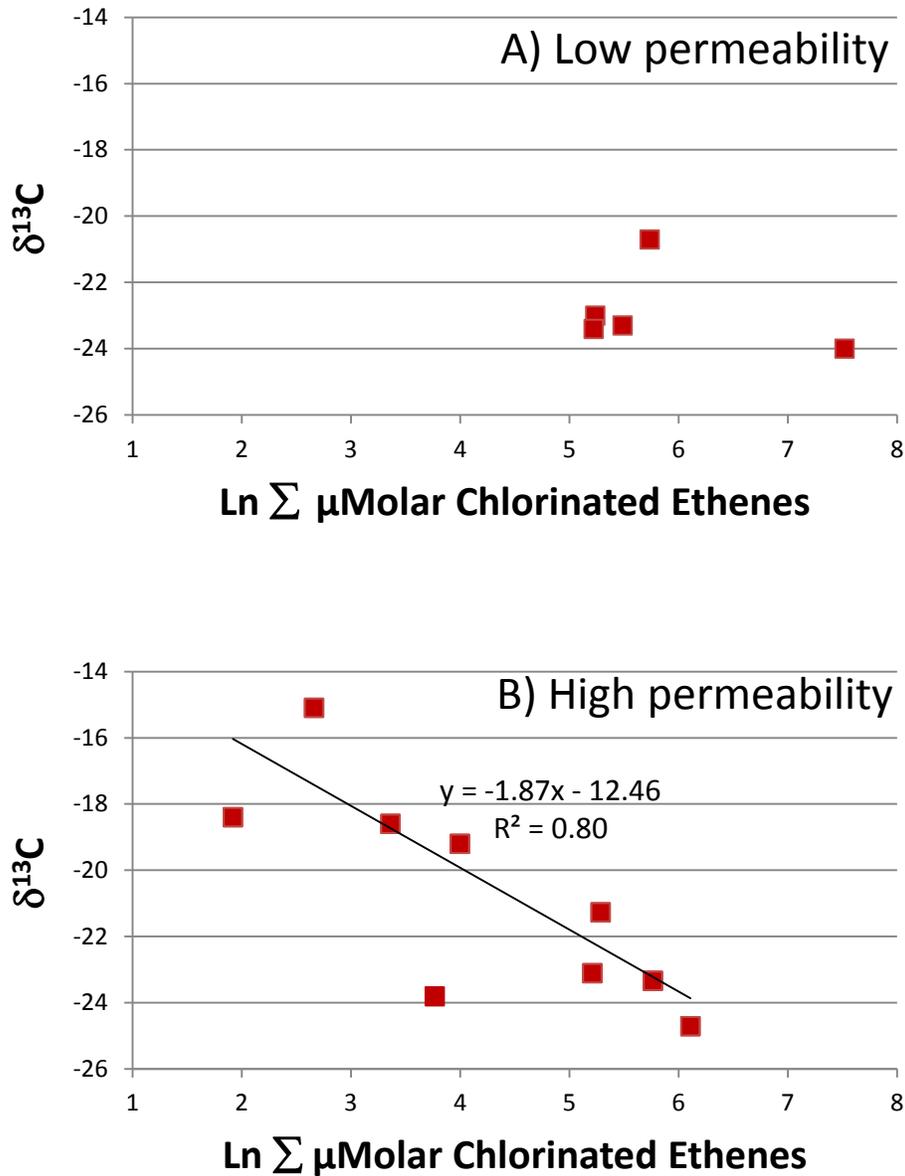
Figure 6. Carbon isotopic enrichment measured as a function of the total chlorinated ethene concentration in both the low (top) and high (bottom) permeability materials emanating from the existing DNAPL sources through the PMLS wells. The 95% confidence interval on the regressed slope is 0.18. The visibly outlying data point in the bottom figure (3.8 on the x-axis) was not used in the linear regression.

SUPPORTING INFORMATION

TITLE: Long-Term Impacts on Groundwater and Reductive Dechlorination following Bioremediation in a Highly Characterized Trichloroethene DNAPL Source Area

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Table S1. Molar fraction of ethene to chlorinated ethenes measured prior to bioaugmentation, and at the last measured rebound event.

Well Identification	Prior to Bioaugmentation	Final Rebound Sampling
Low Permeability		
SMLS 1-1	0.013	1.7
SMLS 1-2	0.0035	0.088
SMLS 1-3	0.0026	0.052
SMLS 4-2	0.16	0.021
SMLS 4-3	0.042	0.28[*]
SMLS 7-1	0.0020	0.031
SMLS 7-2	0.0018	0.22
SMLS 7-3	0.0024	0.038
High Permeability		
SMLS 1-4	0.0099	0.093
PEW-02	0.017	0.069
PEW-03	0.23	0.097

[*] the last rebound event appeared anomalously high, so the second to last rebound event was used.
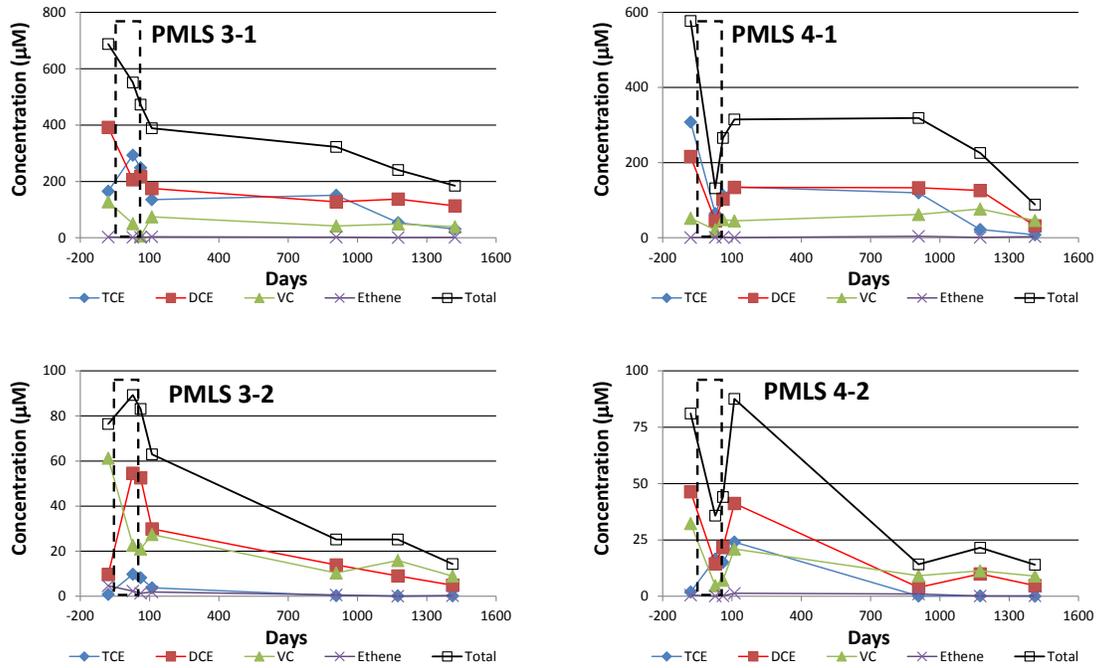
Figure S1. Groundwater chlorinated ethene and ethene results for PMLS well locations screened within the higher permeability materials. The dashed boxes represent the time intervals where active bioremediation occurred.