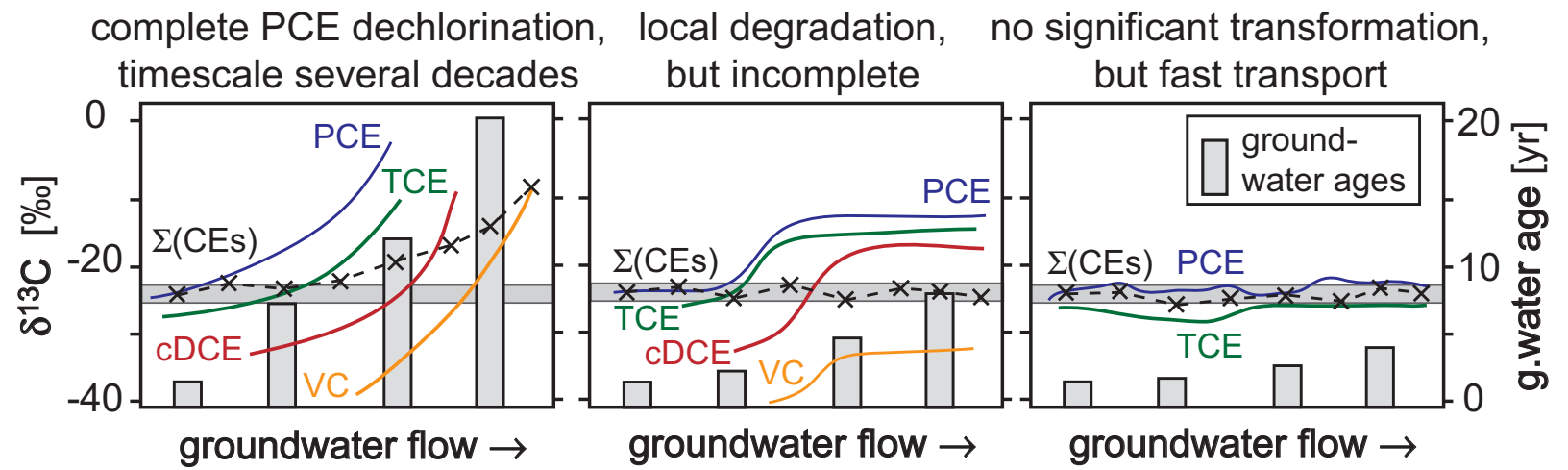


Research highlights

- Chlorinated ethenes (CEs) are among the most notorious groundwater contaminants
- Methods enabling the quantification of time scales of in-situ degradation are needed
- To this end we combined compound-specific isotope analysis and groundwater dating
- We tested this approach at five hydrogeochemically different CE spill sites
- Our approach allowed to characterize extent and time scale of CE transformation

Natural attenuation potential assessed for typical PCE/TCE spill sites scenarios



**Assessing the Transformation of Chlorinated Ethenes in Aquifers With
Limited Potential for Natural Attenuation: Added Values of Compound-
Specific Carbon Isotope Analysis and Groundwater Dating**

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Abstract

The evaluation of the biotransformation of chlorinated ethenes (CEs) in contaminated aquifers is challenging when variable redox conditions and groundwater flow regime are limiting factors. By using compound-specific stable carbon isotope analysis (C-CSIA) and ^3H - ^3He based groundwater dating, we assessed three CE-contaminated field sites that differed in groundwater flow velocities, redox conditions, and level of contamination. CE isotopic signatures and carbon isotopic mass balances were applied to quantify CE transformation, whereas groundwater dating allowed determining probable degradation-process timescales and assessing hydrodynamic regimes. The combination of these techniques enabled to indicate, at all field sites, zones within the aquifers where CE dechlorination preferably occurred, sometimes even to the metabolites of no toxic concern. However, the natural transformation processes were insufficient to mitigate the entire CE contamination in the studied sites. Such situations of limited transformation are worldwide far more common than sites where optimal natural (mainly redox) conditions are enabling complete CEs degradation. Despite such constraints for natural transformation, this study showed that even under non-favorable biogeochemical CEs degradation, the combination of CSIA and groundwater dating provide valuable information to the understanding of the fate of the CEs, thus, being an important contribution in the definition of efficient remediation measures to the given biogeochemical conditions.

Keywords: spill sites, groundwater contamination, transformation, remediation, isotopic mass balance, field studies.

1. Introduction

Compound-specific stable carbon isotope analysis (C-CSIA) is a proven tool for the *in situ* identification and quantification of natural transformation of chlorinated ethenes (CEs) in contaminated groundwaters (e.g., Hunkeler et al., 1999; Sherwood Lollar et al., 2001; Morrill et al., 2005; Aeppli et al., 2010a, and references cited therein). The C-CSIA approach is based on the $^{13}\text{C}/^{12}\text{C}$ isotopic fractionation that occurs during transformation of contaminants (e.g., Meckenstock et al., 2003; Schmidt et al., 2004; Hofstetter and Berg, 2011), such as the sequential microbial reductive dechlorination of tetrachloroethene (PCE) to trichloroethene (TCE), *cis*-1,2-dichloroethene (cDCE), vinyl chloride (VC) and finally ethene. Thus, quantification of the (bio)transformation steps, which is often not feasible from concentration data alone, is essential if a remediation scheme based on natural attenuation of contamination is to be implemented at contaminated field sites (Wiedemeier et al., 1999). In addition, determining groundwater residence times by means of ^3H - ^3He groundwater dating (Tolstikhin and Kamenskiy, 1969) proved to be highly valuable to assess time scales of natural attenuation processes in contaminated aquifers, as, e.g., demonstrated by Amaral et al. (2009) and Aeppli et al. (2010a). In the latter study, the use of CE isotopic mass balances furthermore allowed quantifying complete CE dechlorination, leading to non-toxic products. Thus, combining CSIA and groundwater dating applied to field sites leads to a comprehensive understanding of the CE natural transformation.

So far, most of CSIA-based assessments of CE transformation were carried out at field sites where predominantly homogeneous reducing conditions prevailed, leading to relatively uniform CE biotransformation throughout the investigated aquifers. However, far more common are PCE contaminated aquifers under oxic or nitrate-reducing conditions (Wiedemeier et al., 1999; Squillace et al., 2004), which may cause spatially limited CE

transformation. At such sites, the heterogeneous distribution of reactive and non-reactive zones might lead to over- or underestimation of CSIA based quantification of natural attenuation (Fischer et al., 2007).

Although CE contaminated sites with limited biodegradation are usually not suitable for natural attenuation as a sole remediation strategy, engineered approaches focused on enhancing on-going CE biodegradation are often economically attractive and more environmentally friendly than other remediation measures (Lemming et al., 2010). Hence, there is a need for methods and tools to identify reactive zones even at field sites with limited natural CE biodegradation.

Since there is a lack of CSIA based studies investigating such sites in current literature, the aim of our study was to test the methodology of combining CSIA, groundwater dating, and CE isotopic mass balance for assessing contaminated field sites with spatially limited CE biodegradation. For this purpose, three representative situations of CE groundwater contamination in the urban and industrialized Swiss midlands were chosen, all located on glaciofluvial deposits of sand and gravel sediments. These field sites mainly differed in their groundwater flow velocities (30 to 800 m y⁻¹), redox conditions (oxic to sulfate reducing), and on the levels of maximum CE concentrations (1 to 73 µM). We investigated the added values of CSIA and groundwater dating, and showed that CSIA and groundwater dating are excellent tools for stakeholders to elaborate and implement proper remediation measures to mitigate CE contamination in aquifers.

2. Description of the field sites

2.1. Site I: shallow semi-confined sub-aerobic aquifer

Site I (Fig. 1A) is located in an industrial area where solvents and fuel have been stored and

shipped since 1969. The site consists of a semi-confined sub-aerobic aquifer composed of gravels and sands that are partly overlain by a turf-silt layer (0.5 m thick). Monitoring wells tap the entire aquifer thickness, which is between 4-5 m below surface upstream of the CEs source, and 8-10 m in the downstream wells. Unknown quantities of CEs and of fuel were discharged into the subsurface due to several accidental spills at different locations on this site.

2.2. Site II: deep unconfined aerobic aquifer

On Site II (Fig. 2A) several small-scale PCE using facilities were in operation before the entire site was rebuilt during the 1970-80ies. The quantity of solvents consumed cannot be reconstructed, as little historical data is available. The site consists of an unconfined gravel aquifer of 18 to 30 m thickness. River water recharging the aquifer at the Southeastern boundary forces the groundwater to flow inland towards Northwest. Several monitoring wells tap the aquifer between 7 and 9 m below surface.

2.3. Site III: shallow unconfined aerobic aquifer

A degreasing-processing facility operated between 1964-1983 at Site III (Fig. 3A), and an estimated CEs contamination of >5,000 tons PCE occurred through leaking concrete tanks and pipes. The aquifer is unconfined and consists of a 6 to 8 m thick permeable sequence of pebbles, gravels and sands overlaying molassic bedrock (i.e., 'old' sand and gravel rock). Monitoring wells tap the aquifer between 4-6 m depth (2 m screens) near the CE source, and throughout the entire aquifer thickness upstream and downstream of the source. To remediate the site, the contaminated soil was removed at the source zone (zone SRC; Fig. 3A) by excavation of approx. 700 m³ aquifer material, followed by a groundwater pump-and-treat remediation scheme, which was in operation during the presented site investigations.

3. Materials and Methods

3.1. Groundwater Sampling

Prior to groundwater sampling the well volumes were flushed at least three times using a submersible pump. Samples for groundwater dating as well as for analyses of redox sensitive compounds were collected by submersible pumps at flow rates of about 10 L min⁻¹. The pump was always submersed to about two-thirds of the total well depth. Samples for ³H-³He groundwater dating were taken into copper tubes according to Beyerle et al. (2000), and samples for nitrate, sulfate, and Fe(II) were collected in polyethylene bottles. Water samples for concentration and isotopic analysis of organic compounds were taken by a peristaltic pump connected to PTFE hoses at a flow rate of approximately 2 L min⁻¹, collected in 120 mL amber glass bottles, and sealed without headspace using PTFE-lined screw caps.

3.2. Analytical Methods

CEs concentrations were determined by direct aqueous injection gas chromatography-mass spectrometry (GC-MS) according to Aeppli et al. (2008), with typical errors of <10%. Nitrate and sulfate concentrations were analyzed by ion chromatography, and those of Fe(II) by inductively coupled plasma atomic emission spectroscopy (ICP-OES) of on-site acidified and filtered (0.45 μm) water samples. Dissolved oxygen, electrical conductivity, pH and temperature were measured *in situ* by multi-parameter electrodes (WTW, Weilheim, Germany).

The ¹³C/¹²C ratios of CEs were determined using a purge-and-trap pre-concentrator coupled to a gas chromatograph – combustion - isotope ratio mass spectrometer, as described in Zwank et al. (2003), and are reported as deviation from the international standard VPDB:

$$\delta^{13}\text{C} = (R_{\text{sample}} / R_{\text{VPDB}} - 1) \cdot 1000\text{‰} \quad (1)$$

where R_{sample} and R_{VPDB} are the $^{13}\text{C}/^{12}\text{C}$ ratios of the sample and of VPDB, respectively. The analytical errors in $\delta^{13}\text{C}$ values were $<0.5\text{‰}$.

Quantification of ^3H and tritiogenic ^3He ($^3\text{He}_{trit}$) for groundwater dating (Tolstikhin and Kamenskiy, 1969) was performed following standard procedures (Beyerle et al., 2000). The ^3H - ^3He method is based on the decay of ^3H to the noble gas ^3He . Large amounts of ^3H were released to the atmosphere during the 1950-1960's due to the thermonuclear bomb tests. As part of the water molecule, ^3H enters the saturated part of the aquifer and the resulting decay gas ^3He starts to accumulate in the aquifer along the groundwater flow while ^3H decreases in concentration. Thus, by quantifying both elements, the time of groundwater recharge or groundwater age τ , can be estimated according to Eq. (2) (Schlosser et al., 1988):

$$\tau = \frac{T_{1/2}}{\ln(2)} \cdot \ln \left(1 + \frac{[^3\text{He}_{trit}]}{[^3\text{H}]} \right) \quad (2)$$

where, $^3\text{He}_{trit}$ and ^3H are the respective aqueous concentrations of the ^3He and ^3H , and $T_{1/2}$ is the 4500 days half-life of ^3H (Lucas and Unterweger, 2000). The average analytical uncertainties were ± 1 tritium unit (TU: where 1 TU = 1 atom of ^3H in 10^{18} atoms of H, (Ferronsky and Polyakov, 1982), corresponding in most cases to age uncertainties of ± 0.5 y.

3.3. Calculation of CEs transformation and C-isotopic mass balance

The isotope-based PCE degradation (B_{PCE}) was calculated assuming a Rayleigh fractionation process (Bloom et al., 2000; Sherwood Lollar et al., 2001; Vieth et al., 2003):

$$B_{PCE} = 1 - \left(\frac{\delta^{13}\text{C}_{PCE} + 1000\text{‰}}{\delta^{13}\text{C}_{PCE}^{source} + 1000\text{‰}} \right)^{1000\text{‰}/\epsilon_{PCE}} \quad (3)$$

where, $\delta^{13}\text{C}_{PCE}$ and $\delta^{13}\text{C}_{PCE}^{source}$ are the isotopic PCE signatures at a specific well and of the contamination source, respectively, and ϵ_{PCE} is the isotopic enrichment factor for the observed dechlorination reaction. In this study, ranges of B_{PCE} were calculated using ϵ_{PCE} -values of -

2‰ and -7‰, which span a typical range for microbiological cultures enriched from CE contaminated aquifer sediment (Hunkeler et al., 1999; Slater et al., 2001; Liang et al., 2007). If original $\delta^{13}\text{C}_{\text{PCE}}^{\text{source}}$ values were non accessible, they were reconstructed according to Eberts et al. (2008) using isotopic mass balances (Eq. 4, see below). This reconstruction was not possible for wells where dechlorination beyond VC occurred. Instead, PCE- $\delta^{13}\text{C}$ values of the wells located nearest to the putative CE contamination source were used as conservative estimates.

The isotopic mass balance of all CEs were calculated as previously published (Aeppli et al., 2010a):

$$\delta^{13}\text{C}_{\Sigma(\text{CEs})} = \frac{\delta^{13}\text{C}_{\text{PCE}} \cdot [\text{PCE}] + \delta^{13}\text{C}_{\text{TCE}} \cdot [\text{TCE}] + \delta^{13}\text{C}_{\text{cDCE}} \cdot [\text{cDCE}] + \delta^{13}\text{C}_{\text{VC}} \cdot [\text{VC}]}{[\Sigma(\text{CEs})]} \quad (4)$$

where $[i]$ is the aqueous concentration of compound i and $[\Sigma(\text{CEs})]$ is the total CE concentration. The maximum uncertainty of $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ was $\pm 1.2\text{‰}$, as calculated by error propagation. Quantitatively, $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ -values were used to estimate CE dechlorination beyond VC (e.g., to the non-toxic ethene). This degree of biotransformation, $B_{\Sigma(\text{CEs})}$, was calculated according to Aeppli et al. (2010a), in analogy to Eq. (3):

$$B_{\Sigma(\text{CEs})} = 1 - \left(\frac{\delta^{13}\text{C}_{\Sigma(\text{CEs})} + 1000\text{‰}}{\delta^{13}\text{C}_{\Sigma(\text{CEs})}^{\text{source}} + 1000\text{‰}} \right)^{1000\text{‰}/\epsilon_{\Sigma(\text{CEs})}} \quad (5)$$

where, $\delta^{13}\text{C}_{\Sigma(\text{CEs})}^{\text{source}}$ is the isotopic mass balance at the source, and $\epsilon_{\Sigma(\text{CEs})}$ is the isotopic enrichment factor for the whole sequence of PCE-to-ethene reductive dechlorination. Since PCE was the original contamination at all investigated field sites, the $\delta^{13}\text{C}_{\text{PCE}}^{\text{source}}$ value (see above) was used for $\delta^{13}\text{C}_{\Sigma(\text{CEs})}^{\text{source}}$, and a range of $\epsilon_{\Sigma(\text{CEs})}$ -values between -22‰ and -51‰ was

inserted into Eq. (5) to estimate maximal and minimal $B_{\Sigma(\text{CEs})}$, respectively. These ε -values span a typical range for complete dehalogenation at field sites, as shown in Aeppli et al. (2010a). According to the literature (Schwarzenbach et al., 1983; Domenico and Schwarz, 1998) the organic carbon fraction (f_{oc}) of typical glaciofluvial deposits in Switzerland lies at or below 0.01%, which means the retardation of the individual CEs is negligible. For this case, and a typical porosity of 0.3 (sand and gravels), the most retarded CE (PCE) exhibits a maximum retardation factor of only 1.06. Since the three sites in discussion are located on such type of sedimentary deposits, no relevant CE retardation is expected, and the travel time of the contaminants within two sampling points was considered to be that of the water travel time as determined from groundwater ages.

4. Results and Discussion

4.1. Site I: partly favorable reductive conditions for CE transformation

CE transformation near the reported contamination source area was qualitatively detected from concentration data alone. The total CE concentration dropped significantly between KB5 and KB2, where the relative amount of CEs shifted towards less chlorinated products (Fig. 1B). This clearly demonstrated reductive dechlorination of PCE and TCE. Reducing conditions that favored this transformation were presumably induced by a co-spill of fuel that lead to a groundwater contamination by benzene, toluene, ethylbenzene, xylene (BTEX), totaling $>100 \mu\text{mol L}^{-1}$ at KB2 (data not shown). Aerobic BTEX degradation was likely the reason of the anoxic (KB5) to iron- and sulfate-reducing (KB2) conditions near the source zone (Fig. 1D). Oxidic conditions prevailed 300 m downstream the plume at well KB8, where

>70% of the total CE mass was VC (Fig. 1B). Although this suggested reductive dechlorination of cDCE along the plume, the persistence or transformation of the very toxic VC cannot be assessed by concentration data alone.

Isotope data revealed efficient CE mass removal near the source zone. As indicated by $\delta^{13}\text{C}$ shifts between KB5 and KB2 towards isotopically heavier cDCE, VC, and $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$, the local anoxic conditions obviously favored reductive CE dechlorination (Fig. 1C). In these two wells, even CE mass removal occurred, i.e., dechlorination beyond VC, as the $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ -values were enriched by 8‰ to 18‰ relative to an assumed source isotopic signature of -26.2±0.5‰. The $\delta^{13}\text{C}_{\text{PCE}}$ value in KB5 was used as a conservative estimate for the PCE source, since CE dechlorination beyond VC (as indicated by $\delta^{13}\text{C}_{\Sigma(\text{CEs})} > \delta^{13}\text{C}_{\text{PCE}}$) excluded a reconstruction of $\delta^{13}\text{C}_{\text{PCE}}^{\text{source}}$ at KB5 using the isotopic mass balance. Using Eq. (5), the observed enrichment in $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ translated to an estimated $B_{\Sigma(\text{CEs})}$ of 27±14% (KB5) to 44±17% (KB2) of the original PCE mass that was transformed to ethene. Note that the uncertainty associated with this calculation is mainly associated with the variability of ϵ -values of different dehalogenating bacteria (Cichocka et al., 2008). Although we were not able to verify the calculated $B_{\Sigma(\text{CEs})}$ -values by ethene analysis (because ethene is rapidly converted to other compounds, thus difficult to sample and analyze for), the isotopic data strongly suggested efficient CE attenuation leading to non-toxic products in the anoxic zone around KB2. Since this transformation was very fast, as reflected by almost identical groundwater ages around the source (Fig. 1B), it was not possible to calculate PCE transformation rates for this site.

Furthermore, we investigated whether VC downstream the source area was reduced,

oxidized, or a persistent final product. Since VC was only slightly enriched at KB8 relative to the assumed $\delta^{13}\text{C}$ of the PCE-source ($\delta^{13}\text{C}$ values of -22.4‰ and -26.2‰, respectively), significant VC reductive dechlorination seems not to occur. Owing the large carbon isotopic enrichment factor of VC reductive dechlorination (ϵ -values from -22‰ to -31‰, see e.g. Abe et al. (2009) and references cited therein), this similar $\delta^{13}\text{C}$ -values can be interpreted as VC being the final, persistent CE reduction product. Likewise, the isotopic mass balance at KB8 did not point to major dechlorination beyond VC, as $B_{\Sigma(\text{CEs})}$, calculated according to Eq. (5), was only $12 \pm 9\%$. Hence, isotopic data, and the fact that reducing conditions at KB2 were not observed at KB8 any more (Fig. 1D), led to conclude that reductive dechlorination was not an attenuation process of VC in the contamination plume. However, VC might be oxidized under such oxic conditions (Bradley, 2000). In contrast to VC reduction, the (slight) ^{13}C enrichment in VC does not a priori exclude VC oxidation, since only small carbon ϵ -values of -3 to -8‰ are associated with this process (Abe et al., 2009). However, a $B_{\Sigma(\text{CEs})}$ value of only $16 \pm 11\%$ for this scenario, as well as the high VC concentration of $9 \mu\text{mol L}^{-1}$ KB8, which is 30 times the WHO guideline value for drinking water (WHO, 2004), reflects the overall inefficiency of the occurring VC attenuating processes.

Finally, CSIA suggested that PCE found at KB8 originated from plumes that traveled from the source area without being degraded, and that multiple plumes mixed at KB8. Since the PCE dechlorination product VC and PCE itself had very similar $\delta^{13}\text{C}$ values at KB8 (-22.4‰ and -25.7‰, respectively), these two compounds cannot belong to the same plume. Rather, VC has to originate from zones with reductive PCE-to-VC dechlorination (although very likely not directly from the zone at KB5, where VC is isotopically heavier than at KB8). In contrast, PCE appears to originate from a plume that traveled exclusively through oxic parts

of the aquifer, as seen by the similar PCE- $\delta^{13}\text{C}$ value at the source area and at KB8 (-26.2‰ and -25.7‰, respectively), signifying no degradation. Since several spill locations were identified at Site I, the mixing of several CE plumes at KB8 appears to be plausible.

In summary, combining CSIA and groundwater dating allowed to identify local reducing conditions at the CE source zone that subsequently fostered significant CEs natural attenuation. However, the oxic conditions downstream limited an efficient PCE transformation. Furthermore, isotopic data showed that VC was not efficiently attenuated in the downstream part of the plume, and that multiple CE plumes from different spill events are very likely. Moreover, groundwater dating (Fig. 1C) revealed that the slow groundwater flow of maximal 30 m y⁻¹ would hamper a fast cleanup of the 300 m long contamination plume, even if the CE source would cease polluting the aquifer.

4.2. Site II: near-source reductive CE dechlorination and downstream cDCE oxidation

The CE concentration pattern implies on-going CE degradation at Site II: from the highest CE contamination tapped by wells P11 and P4, a plume with decreasing CE concentrations developed along the groundwater flow. The variation in the PCE levels along that plume suggested CE transformation (Fig. 2B).

Isotopic data showed that no PCE and TCE transformation occurred in the studied part of the aquifer. Although the CE concentrations decreased significantly, isotope ratios of all individual CEs remained within 1.2‰ (1 σ) along the investigated flow path (Fig. 2C). Such constant $\delta^{13}\text{C}$ -values suggest that dilution rather than CE degradation was responsible for the decrease of the CE concentrations along the investigated flow path. However, compared to the other wells, the relative cDCE concentrations were considerably lower at P3 and PS. We hypothesize that cDCE oxidation occurred in these two wells, where oxic groundwater was

tapped ($O_2 > 3 \text{ mg L}^{-1}$; Fig. 2D). Under such conditions, PCE and TCE are persistent (Bradley, 2000), thus leading to constant PCE and TCE isotopic signatures. The cDCE concentration at P3 and PS were too low for CSIA, and did not allow for detecting possible isotopic shifts in cDCE due to hypothesized oxidative degradation.

Constant isotopic signatures of individual CEs suggested the presence of a single contamination source, as well as a near-source zone of on-going reductive dechlorination. As the investigated wells do not reach the aquitard, a dense non-aqueous phase liquid (DNAPL) of CEs at the bottom of the aquifer can be hypothesized. Furthermore, the isotopic enrichment of the CE isotopic mass balances in all wells ($\delta^{13}C_{\Sigma(CEs)} = -19.4\text{‰}$ to -18.3‰) compared to the assumed source values of $-23.5 \pm 0.5\text{‰}$ indicated the presence of a zone of continuous reductive CE mass removal. Using Eq. (5) we calculated that in average $17 \pm 11\%$ of the CEs mass was degraded, i.e., dechlorinated beyond VC, when CEs were passing this anoxic zone. For this calculation, the PCE- $\delta^{13}C$ of the well with highest PCE concentration, P11, was used as source $\delta^{13}C$ -value. This local reductive dechlorination happened before the groundwater turned oxic due to river infiltration (indicated by higher O_2 levels and lower electrical conductivities measured at wells closer to the river; Fig. 2D), and was tapped by the monitoring wells.

Finally, the origin of PCE at a pumping station (PS) located 1 km downstream was investigated. The PCE- $\delta^{13}C$ at well PS had the same $\delta^{13}C$ -values as in the main contaminated area ($-23.1 \pm 0.5\text{‰}$ and -24.4 to -22.8‰ , respectively; Fig. 2C). Admitting a groundwater flow line between Site II and well PS, this finding suggests that PCE in PS might originate from the investigated area. However, this requires PCE to remain isotopically unaltered, i.e. to be unaffected by PCE degradation during the travel time for water to reach well PS, of approx. 3

years as determined by groundwater dating (Fig. 2C). The oxic conditions of the aquifer (Fig. 2D), observed in most wells, as well as the fast groundwater flow of approx. 300 m y⁻¹, would make such non-reactive PCE transport possible. Note, however, that additional PCE input from other identified contamination plumes in the catchments at PS could not be excluded, as the PCE-δ¹³C value from PS (-23.1±0.5‰) was in the range of commonly used PCE, with has typical values of -37.2‰ to -23.2‰ (van Warmerdam et al., 1995).

In summary, CSIA allowed to exclude that efficient reductive dechlorination was ongoing in the investigated part of the aquifer. However, based on CE isotopic mass balance, a zone with favorable conditions for PCE and TCE degradation could be hypothesized at lower parts of the aquifer. Additionally, isotopic data suggested that the contamination at Site II might be responsible for PCE found in a pumping station located 3 years of groundwater traveling time downstream the main contamination.

4.3. Site III: unfavorable conditions for CE transformation

Despite the rapid CE concentrations decrease along the 250 m long contamination plume, suggesting natural attenuation, the low abundance of TCE (<2% of total CE mass in average) and the occasional detection of cDCE (mainly at KB00/7) demonstrated insignificant and/or very local CE degradation (Fig. 3B).

In line with this observation from concentration data, constant PCE-δ¹³C-values along the entire investigated flow path (-25.3±0.8‰ in average) confirmed that the decrease of PCE concentration was mainly due to dilution (Fig. 3C). Such non-reactive PCE transport was supported by the observed sub-oxic to oxic groundwater conditions, under which PCE is persistent (Fig. 3D).

CSIA allowed assessing the sources of TCE and cDCE. The similar isotopic composition

of TCE ($-25.0 \pm 0.7\%$) and PCE ($-25.3 \pm 0.8\%$) suggested that TCE might be solely a technical impurity of PCE, since industrially produced TCE was shown to have similar $\delta^{13}\text{C}$ values as PCE (van Warmerdam et al., 1995). In contrast, a more depleted TCE- $\delta^{13}\text{C}$ relative to PCE would be expected for TCE as PCE hydrogenolysis product, at least in wells SRC and KB00/1 where no cDCE was formed. Unlike TCE, cDCE detected at KB00/7 seems to be a dechlorination product, as indicated by the enrichment in PCE- $\delta^{13}\text{C}$ of 1.7% compared to the source zone. Using Eq. (4), this isotopic shift translated to a degree of PCE reductive dechlorination B_{PCE} of $42 \pm 33\%$ at KB00/7 (using $\delta^{13}\text{C}_{\Sigma(\text{CEs})}$ at SRC as source PCE- $\delta^{13}\text{C}$). In fact, reducing conditions at this well ($\text{O}_2 < 1 \text{ mg L}^{-1}$, low sulfate concentrations) allowed the PCE biotransformation observed (Fig. 3D). These conditions were probably induced by input of organic carbon through well KB00/7 located inside the industrial fat-extraction operation building. The isotopic enrichment of cDCE relative to PCE was probably caused by the cDCE-to-VC transformation, resulting in ^{13}C -depleted VC. However, since VC was not included in the analytical program at Site III, this hypothesis was not verified. Downstream of KB00/7, non-degraded PCE from the source area dissolved into the groundwater and dominated the CE mass.

Isotopes of He (^3He and ^4He), used for groundwater dating, additionally provided evidences of the bedrock (Molasse) being fractured, which seems to affect the distribution of the PCE in the subsurface. Near-CEs-source wells, reaching down to the Molasse showed $^3\text{He}/^4\text{He}$ ratios of $(1.05\text{--}1.30) \times 10^{-6}$, which were lower than the atmospheric ratio of 1.39×10^{-6} . This suggested the emanation of terrigenic (non-atmospheric origin) ^4He from the bedrock, suggesting the presence of some sort of structural discontinuities (such as fractures) in the bedrock that allowed ^4He to emanate and dissolve in the groundwater. Thus, it seems

reasonable to assume that these fractures also allowed PCE (in the form of DNAPL) to penetrate into the bedrock and to form a local reservoir of the detected PCE contamination, which cannot be removed by the chosen pump-and-treat remediation scheme. However, if all CE sources were removed by other appropriate remediation measures, the fast groundwater flow of approx. 400 to 800 m y⁻¹ (determined by groundwater dating; Fig. 3C) would move the CE plume out of the investigated area within months or years. This would further allow two important groundwater pumping stations located approx. 1.5 km downstream of the source to be operated again.

In summary, CSIA allowed to assess the occurrence of CE transformation at Site III. First, the constant PCE isotopic compositions along the contaminant plume showed that CEs transformation was not expected to act efficiently, although PCE metabolites were detected in some wells and CE concentrations rapidly decreased along the contamination plume. Second, the detected metabolites were shown to originate either from technical impurities (TCE) or from PCE transformation at a local reducing zone in the aquifer (cDCE). Finally, He isotopes implied that most probably several CE DNAPLs existed within the molassic bedrock. Such lingering PCE sources are thus expected to continuously contaminate the groundwater, if not removed.

5. General Applicability and Outlook

We demonstrated that the combined application of CSIA and groundwater dating allows characterizing CE transformation at field sites exhibiting various hydrogeochemical conditions. The general benefits of the presented approach are summarized in Table 1. Applying CSIA and groundwater dating in the assessment of contaminated sites leads to an improved understanding of the nature and time scales of the processes occurring in the

subsurface, and hence, will greatly assist in the design and implementation of appropriate remediation measures. Whereas monitoring the natural attenuation processes (MNA) might be the remediation strategy of choice for predominantly anoxic plumes with efficient CE transformation, sites where CEs are only degraded in localized regions of the plume might call for other approaches, such as, e.g., enhanced bioremediation schemes. Finally, excavation or hydraulic isolation of the CE source zone might be a remediation strategy to opt for at spill sites lacking natural attenuation potential. In any of the above cases, CSIA can also be a powerful tool for the apportionment of multiple sources of CE contamination at a given field site (environmental forensics; (Eberts et al., 2008; Blessing et al., 2009)).

Recent progress in multi-element CSIA is expected to further improve CSIA-based assessment of CE biodegradation. First, the uncertainties associated with the isotope-based calculation of CE mass removal (Eqs. 3-5), mainly caused by the rather large range of ϵ -values that need to be assumed for calculating degrees of biodegradation, ought to be lowered. It was suggested that this can be achieved by *in situ* determination of the site-specific ϵ -values using CSIA of two elements, i.e., chlorine and carbon (Hofstetter et al., 2007; Abe et al., 2009). Second, including the chlorine isotopic system ($^{37}\text{Cl}/^{35}\text{Cl}$) in the analysis would also make CSIA more impactful as an environmental forensics tool, as this would allow for better apportionment of multiple contamination sources. In this context it is promising that a simple analytical method for stable chlorine isotopes of CEs and other organochlorine compounds was recently developed (Aeppli et al., 2010b).

This study showed in particular that the combination of CSIA and groundwater dating provide valuable information to the understanding of the fate of the CEs, even for field sites with limited natural attenuation potential. Therefore, this approach is of considerable added value in the definition and design of efficient remediation measures to remediate the umpteen

thousands of sites that pose a threat to groundwater resources around the globe.

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References

- Abe, Y., Aravena, R., Zopfi, J., Shouakar-Stash, O., Cox, E., Roberts, J.D., Hunkeler, D., 2009. Carbon and chlorine isotope fractionation during aerobic oxidation and reductive dechlorination of vinyl chloride and cis-1,2-dichloroethene. *Environ. Sci. Technol.* 43, 101-107.
- Aeppli, C., Berg, M., Hofstetter, T.B., Kipfer, R., Schwarzenbach, R.P., 2008. Simultaneous quantification of polar and non-polar volatile organic compounds in water samples by direct aqueous injection-gas chromatography/mass spectrometry. *J. Chromatogr. A* 1181, 116-124.
- Aeppli, C., Hofstetter, T.B., Amaral, H.I.F., Kipfer, R., Schwarzenbach, R.P., Berg, M., 2010a. Quantifying in situ transformation rates of chlorinated ethenes by combining compound-specific stable isotope analysis, groundwater dating, and carbon isotope mass balances. *Environ. Sci. Technol.* 44, 3705-3711.
- Aeppli, C., Holmstrand, H., Andersson, P., Gustafsson, Ö., 2010b. Direct compound-specific stable chlorine isotope analysis of organic compounds with quadrupole gc/ms using standard isotope bracketing. *Anal. Chem.* 82, 420-426.
- Amaral, H.I.F., Fernandes, J., Berg, M., Schwarzenbach, R.P., Kipfer, R., 2009. Assessing

412 TNT and DNT groundwater contamination by compound-specific isotope analysis and
 413 ^3H - ^3He groundwater dating: a case study in Portugal. *Chemosphere* 77, 805-812.
 414 Beyerle, U., Aeschbach-Hertig, W., Imboden, D.M., Baur, H., Graf, T., Kipfer, R., 2000. A
 415 mass spectrometric system for the analysis of noble gases and tritium from water
 416 samples. *Environ. Sci. Technol.* 34, 2042-2050.
 417 Blessing, M., Schmidt, T.C., Dinkel, R., Haderlein, S.B., 2009. Delineation of multiple
 418 chlorinated ethene sources in an industrialized area - A forensic field study using
 419 compound-specific isotope analysis. *Environ. Sci. Technol.* 43, 2701-2707.
 420 Bloom, Y., Aravena, R., Hunkeler, D., Edwards, E.A., Frape, S.K., 2000. Carbon isotope
 421 fractionation during microbial dechlorination of trichloroethene, cis-1,2-
 422 dichloroethene, and vinyl chloride: Implications for assessment of natural attenuation.
 423 *Environ. Sci. Technol.* 34, 2768-2772.
 424 Bradley, P.M., 2000. Microbial degradation of chloroethenes in groundwater systems.
 425 *Hydrogeo. J.* 8, 104-111.
 426 Cichocka, D., Imfeld, G., Richnow, H.H., Nijenhuis, I., 2008. Variability in microbial carbon
 427 isotope fractionation of tetra- and trichloroethene upon reductive dechlorination.
 428 *Chemosphere* 71, 639-648.
 429 Domenico, P.A., Schwarz, F.W., 1998. *Physical and Chemical Hydrogeology*. John Wiley &
 430 Sons, Inc.
 431 Eberts, S.M., Braun, C., Jones, S., 2008. Compound-specific isotope analysis: questioning the
 432 origins of a trichloroethene plume. *Environ. Forensics* 9, 85-95.
 433 Ferronsky, V.I., Polyakov, V.A., 1982. *Environmental Isotopes in the Hydrosphere*. John
 434 Wiley & Sons, Chichester, New York.
 435 Fischer, A., Theuerkorn, K., Stelzer, N., Gehre, M., Thullner, M., Richnow, H.H., 2007.
 436 Applicability of stable isotope fractionation analysis for the characterization of
 437 benzene biodegradation in a BTEX-contaminated aquifer. *Environ. Sci. Technol.* 41,
 438 3689-3696.
 439 Hofstetter, T.B., Berg, M., 2011. Assessing transformation processes of organic contaminants
 440 by compound-specific stable isotope analysis. *TrAC, Trends Anal. Chem.* 30, 618-
 441 627.
 442 Hofstetter, T.B., Reddy, C.M., Heraty, L.J., Berg, M., Sturchio, N.C., 2007. Carbon and
 443 chlorine isotope effects during abiotic reductive dechlorination of polychlorinated

ethanes. *Environ. Sci. Technol.* 41, 4662-4668.

Hunkeler, D., Aravena, R., Butler, B.J., 1999. Monitoring microbial dechlorination of tetrachloroethene (PCE) in groundwater using compound-specific stable carbon isotope ratios: Microcosm and field studies. *Environ. Sci. Technol.* 33, 2733-2738.

Lemming, G., Hauschild, M.Z., Chambon, J., Binning, P.J., Bulle, C., Margni, M., Bjerg, P.L., 2010. Environmental impacts of remediation of a trichloroethene-contaminated site: life cycle assessment of remediation alternatives. *Environ. Sci. Technol.* 44, 9163-9169.

Liang, X., Dong, Y., Kuder, T., Krumholz, L.R., Philp, R.P., Butler, E.C., 2007. Distinguishing abiotic and biotic transformation of tetrachloroethylene and trichloroethylene by stable carbon isotope fractionation. *Environ. Sci. Technol.* 41, 7094-7100.

Lucas, L.L., Unterwieser, M.P., 2000. Comprehensive review and critical evaluation of the half-life of tritium. *J. Res. Nat. Inst. Stand. Technol.* 105, 541-549.

Meckenstock, R.U., Morasch, B., Griebler, C., Richnow, H.H., 2003. Stable isotope fractionation analysis as a tool to monitor biodegradation in contaminated aquifers. *J. Contam. Hydrol.* 75, 215-255.

Morrill, P.L., Lacrampe-Couloume, G., Slater, G.F., Sleep, B.E., Edwards, E.A., McMaster, M.L., Major, D.W., Lollar, B.S., 2005. Quantifying chlorinated ethene degradation during reductive dechlorination at Kelly AFB using stable carbon isotopes. *J. Contam. Hydrol.* 76, 279-293.

Schlosser, P., Stute, M., Dörr, C., Sonntag, C., Münnich, K.O., 1988. Tritium/He-3 dating of shallow groundwater. *Earth Planet. Sci. Lett.* 89, 353-362.

Schmidt, T.C., Zwank, L., Elsner, M., Berg, M., Meckenstock, R.U., Haderlein, S.B., 2004. Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges. *Anal. Bioanal. Chem.* 378, 283-300.

Schwarzenbach, R.P., Giger, W., Hoehn, E., Schneider, J.K., 1983. Behavior of organic compounds during infiltration of river water to groundwater. Field studies. *Environ. Sci. Technol.* 17, 472-479.

Sherwood Lollar, B., Slater, G.F., Sleep, B., Witt, M., Klecka, G.M., Harkness, M., Spivack, J., 2001. Stable carbon isotope evidence for intrinsic bioremediation of

tetrachloroethene and trichloroethene at area 6, Dover Air Force Base. *Environ. Sci. Technol.* 35, 261-269.

Slater, G.F., Sherwood Lollar, B., Sleep, B.E., Edwards, E.A., 2001. Variability in carbon isotopic fractionation during biodegradation of chlorinated ethenes: Implications for field applications. *Environ. Sci. Technol.* 35, 901-907.

Squillace, P.J., Moran, M.J., Price, C.V., 2004. VOCs in shallow groundwater in new residential/commercial areas of the United States. *Environ. Sci. Technol.* 38, 5327-5338.

Tolstikhin, I.N., Kamenskiy, I.L., 1969. Determination of ground-water ages by the T-He-3 method. *Geochem. Int.* 6, 810-811.

van Warmerdam, E.M., Frape, S.K., Aravena, R., Drimmie, R.J., Flatt, H., Cherry, J.A., 1995. Stable chlorine and carbon isotope measurements of selected chlorinated organic solvents. *Appl. Geochem.* 10, 547-552.

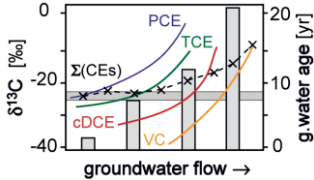
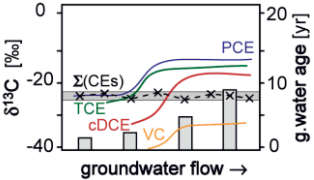
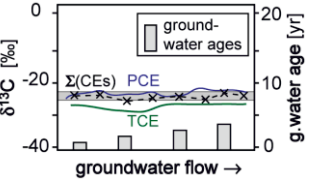
Vieth, A., Muller, J., Strauch, G., Kastner, M., Gehre, M., Meckenstock, R.U., Richnow, H.H., 2003. In-situ biodegradation of tetrachloroethene and trichloroethene in contaminated aquifers monitored by stable isotope fractionation. *Isot. Environ. Health Stud.* 39, 113-124.

WHO, 2004. Guidelines for Drinking-Water Quality. World Health Organization, Geneva.

Wiedemeier, T.H., Rifai, H.S., Newell, C.J., Wilson, J.T., 1999. Natural Attenuation of Fuels and Chlorinated Solvents in the Subsurface. John Wiley & Sons.

Zwank, L., Berg, M., Schmidt, T.C., Haderlein, S.B., 2003. Compound-specific carbon isotope analysis of volatile organic compounds in the low-microgram per liter range. *Anal. Chem.* 75, 5575-5583.

Table 1. Added value of CSIA and groundwater dating for the assessment of CE transformation in contaminated aquifers, illustrated for three typical cases

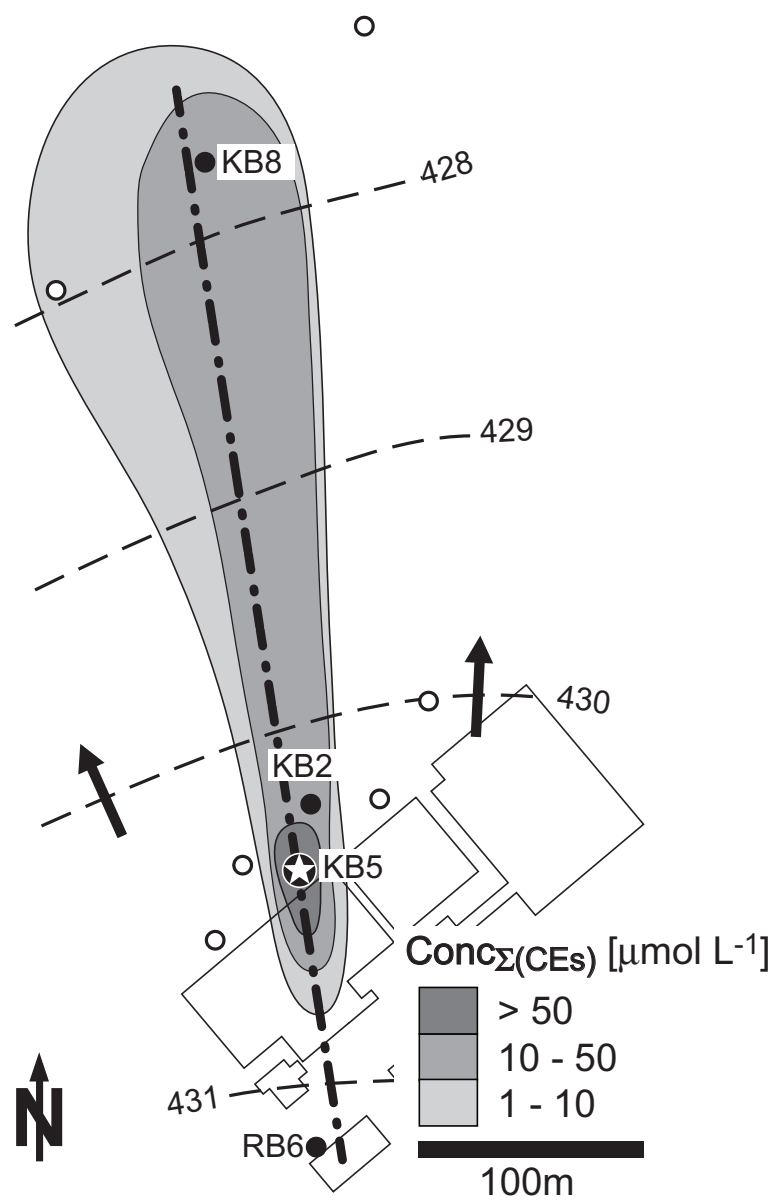
	CE Transformation	Limited CE transformation	No CE transformation
	Anoxic groundwater throughout the plume, slow groundwater flow	Heterogeneous redox conditions, medium flow velocities	Oxic and fast flowing groundwater
Typical evolution of CE- $\delta^{13}\text{C}$, isotopic mass balance and groundwater ages			
Added value of CSIA	Quantification of degree of PCE-to-TCE and complete PCE-to-ethene degradation	Identification of reactive zones for CE transformation	Apportionment of multiple contamination sources (environmental forensics)
Added value of dating	<i>In-situ</i> determination of transformation rates	Time scale of local CE transformation	Time scale of plume remediation after CE source stop
Natural attenuation potential	PCE-to-ethene transformation, but lasts decades to centuries; VC accumulation critical	CE degradation incomplete; moderate groundwater flow; stimulated natural attenuation worthwhile to evaluate	No CE degradation, but fast CE transport along plume; site requires source zone remediation
Example	Aeppli et al. (2010a)	This study ^{a)}	This study ^{b)} Blessing et al. (2009)

a) Field Site I, and zone upstream the main contamination of Field Site III.

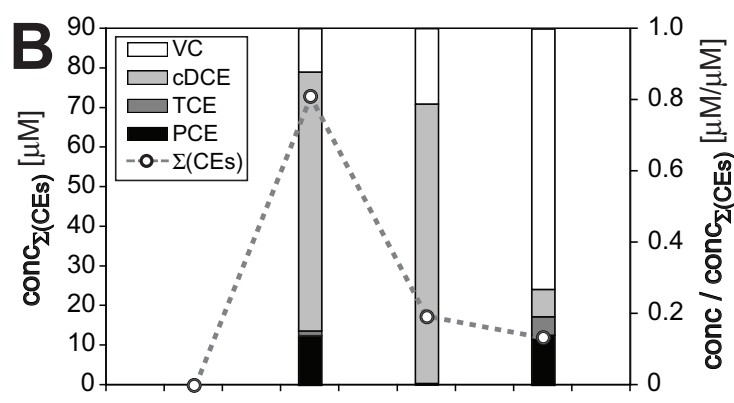
b) Field Site II, and zone downstream the main contamination of Field Site III.

Figure 1 revised black/white

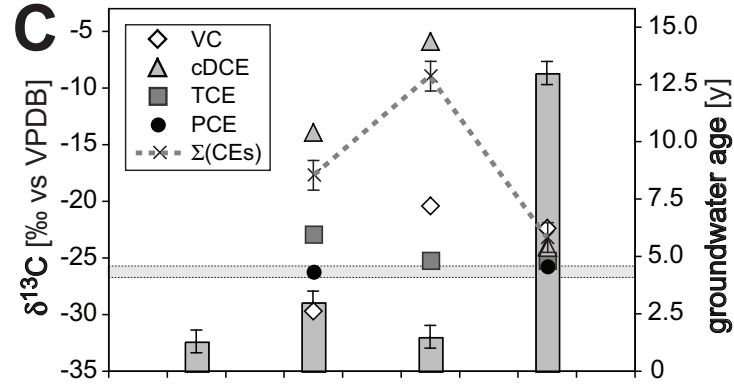
A



B



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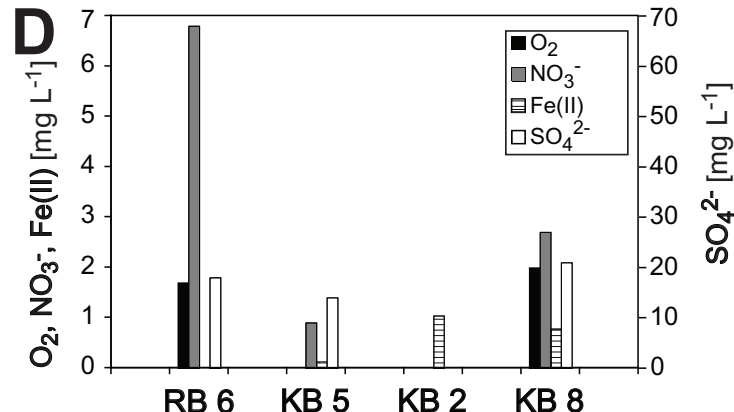
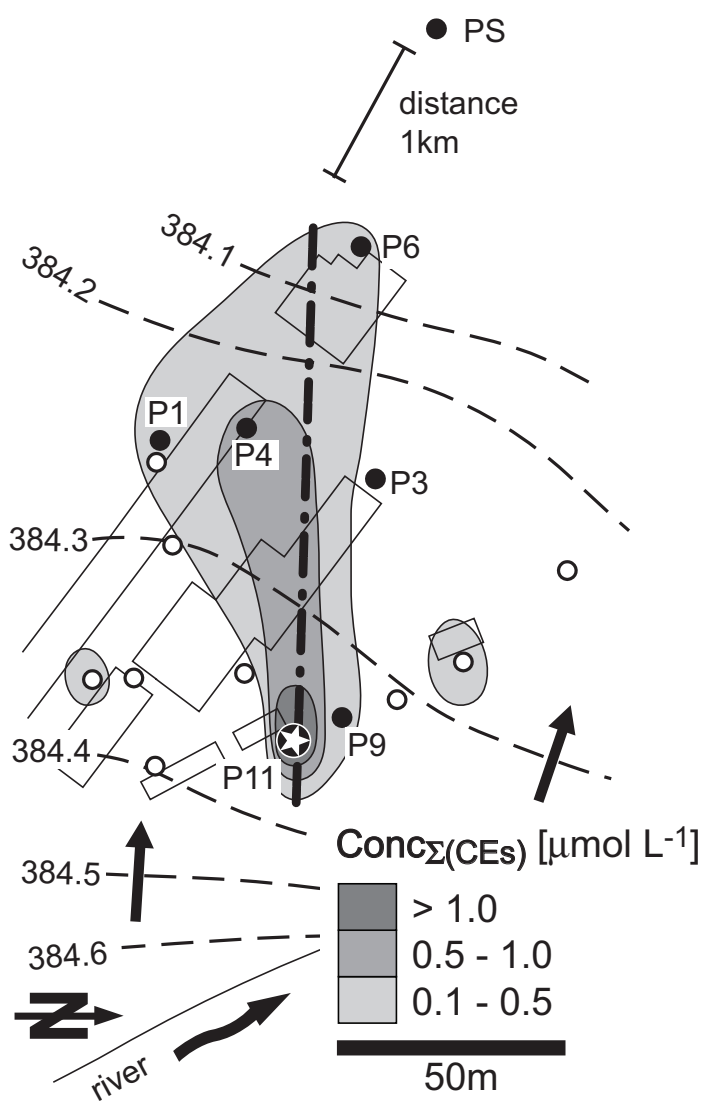
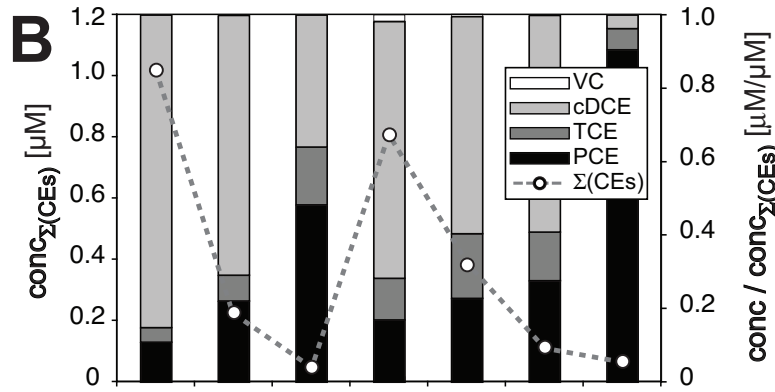


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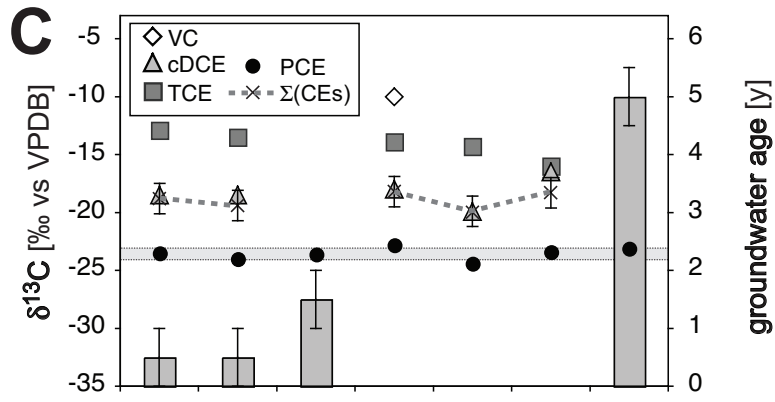
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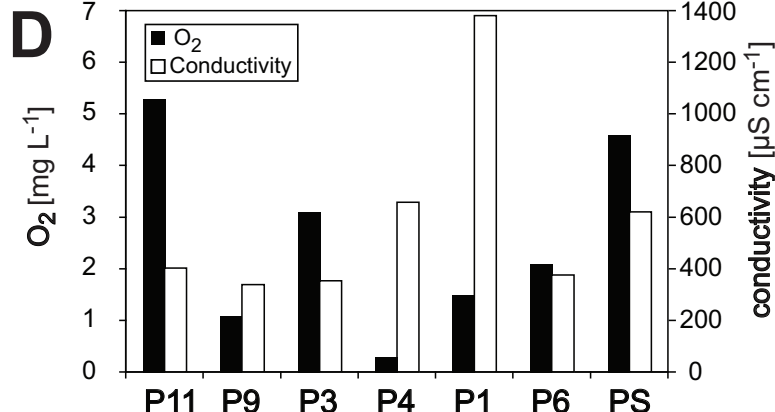
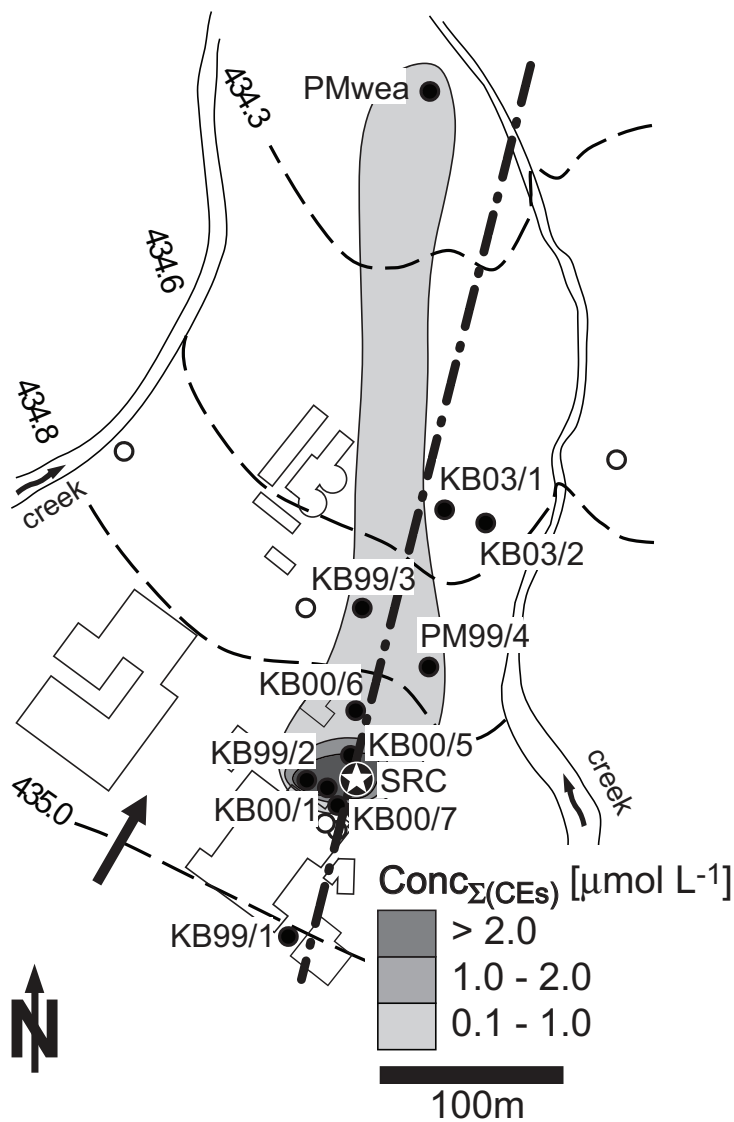
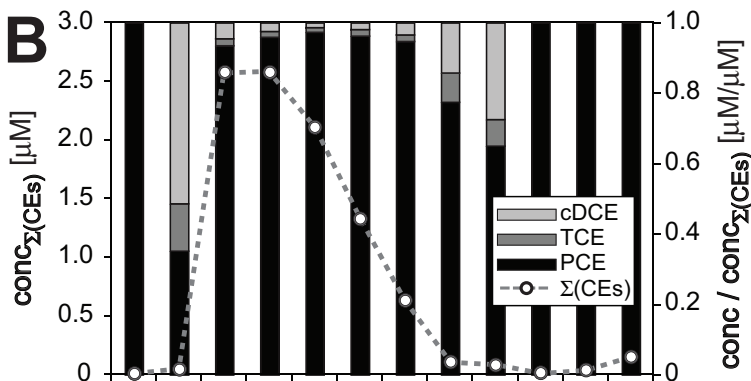


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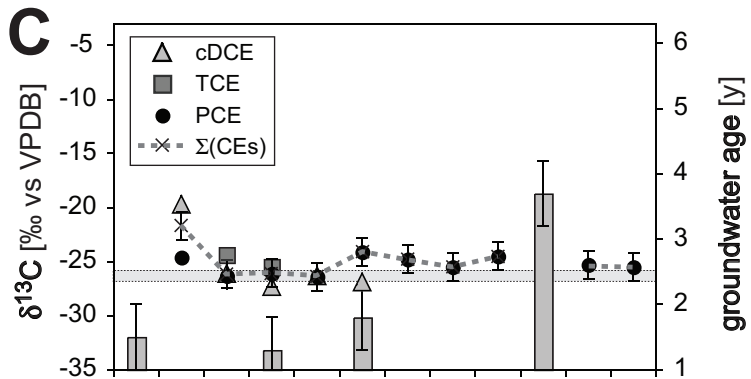
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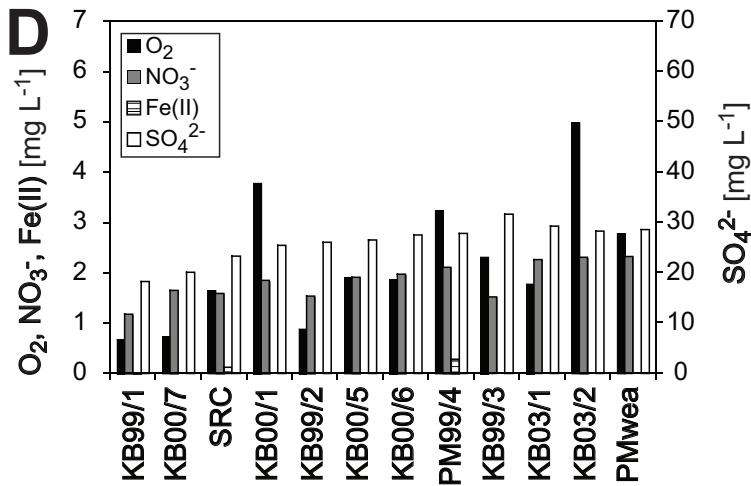
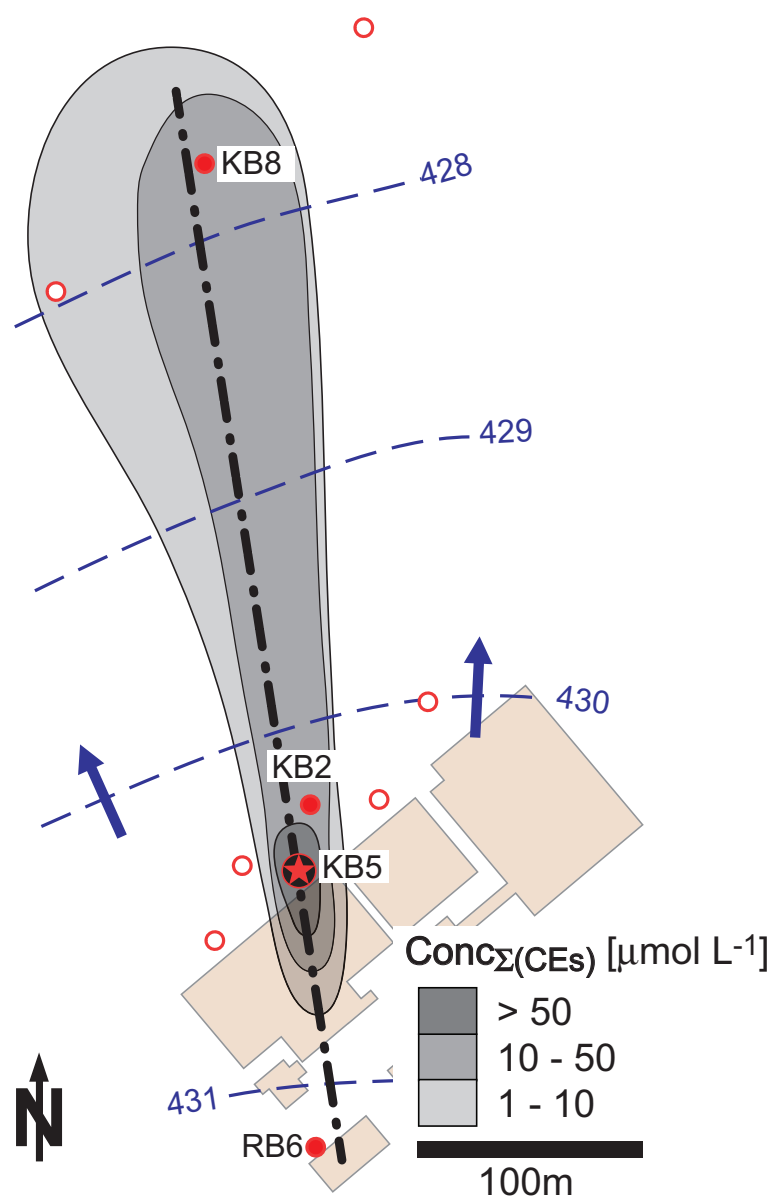
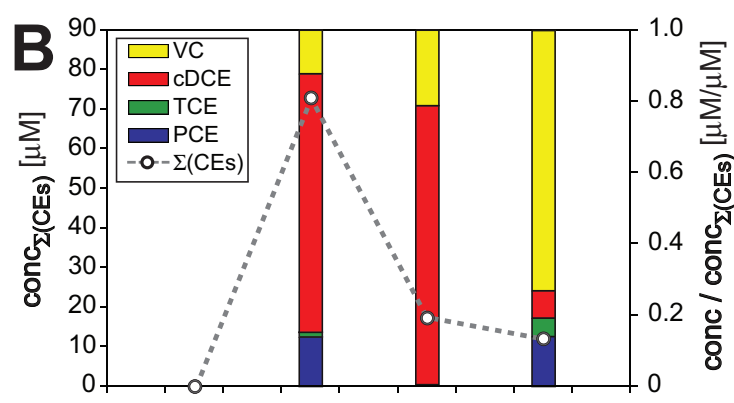


Figure 1 revised color

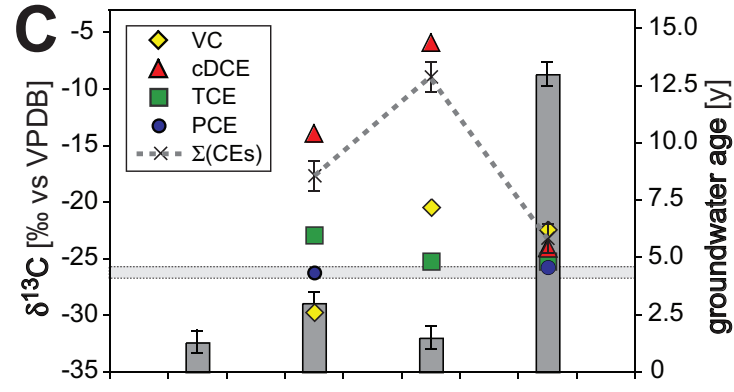
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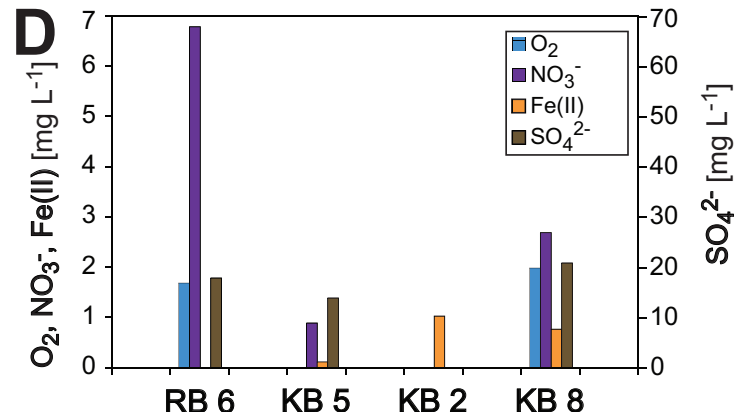
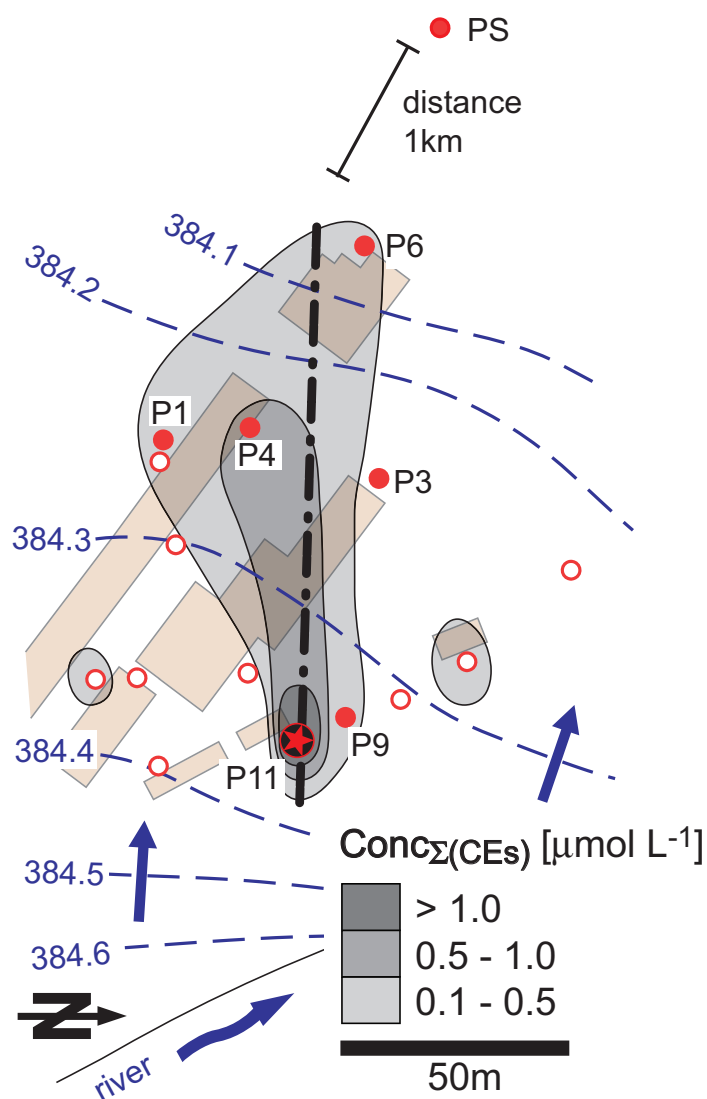
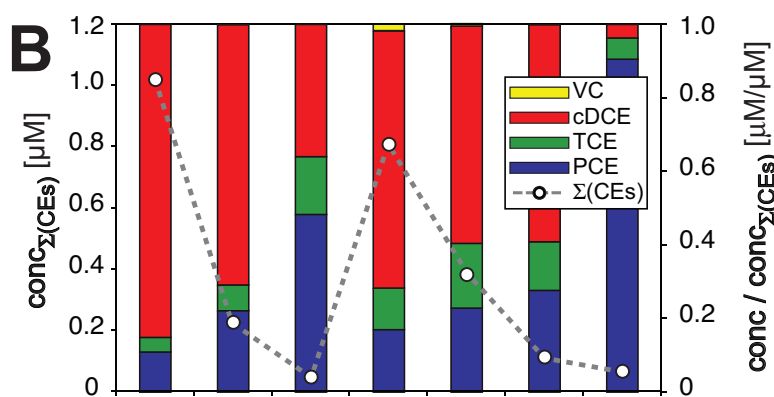


Figure 2 revised color

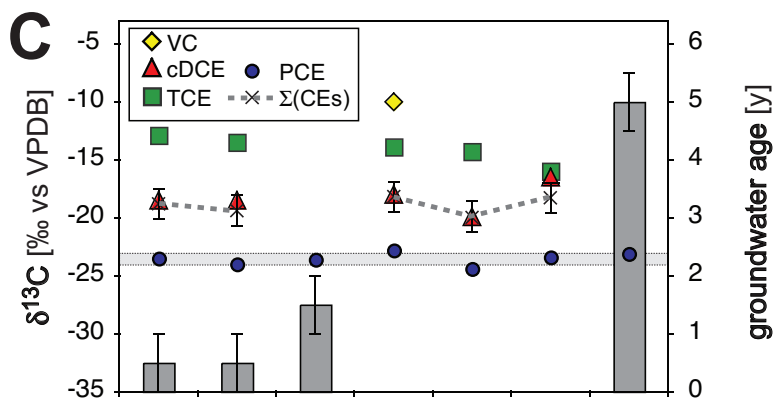
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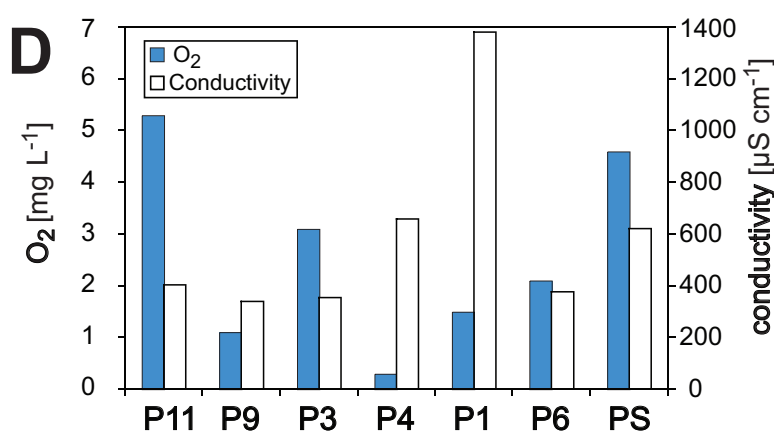
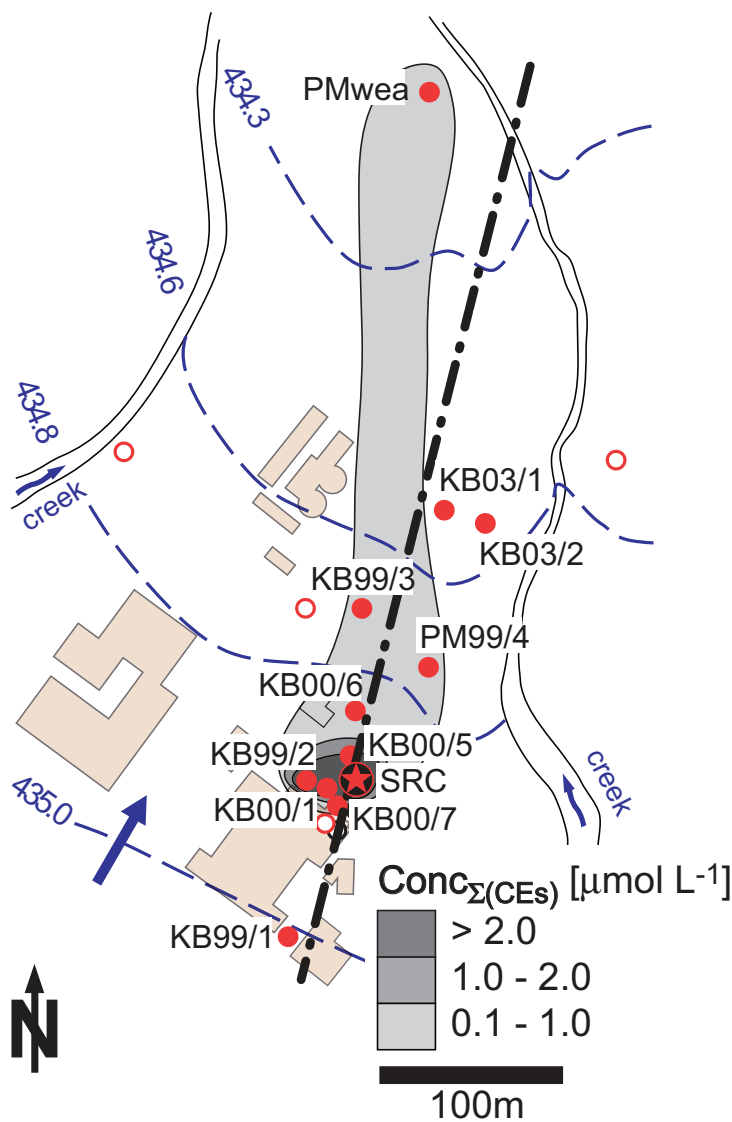
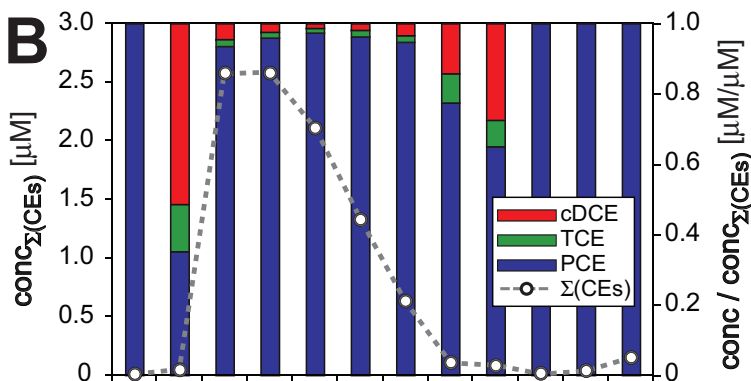


Figure 3 revised color

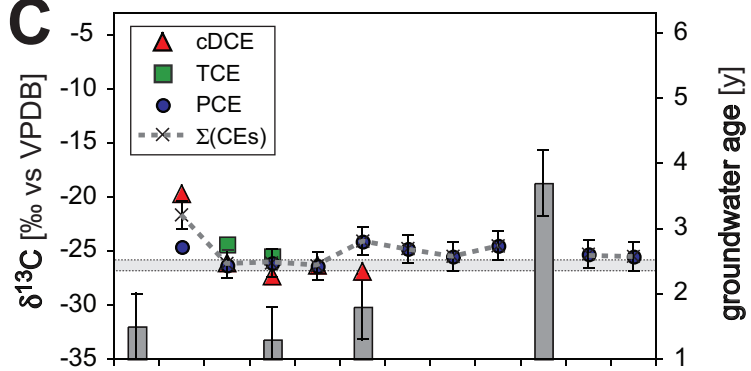
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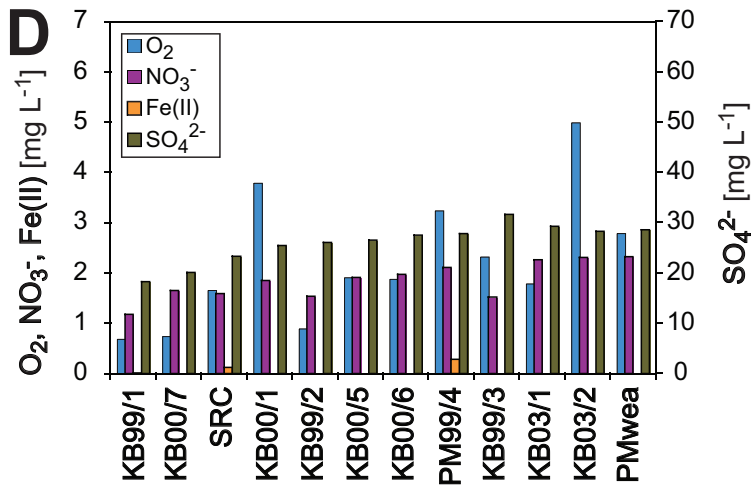


Figure Captions

Fig 1. Field Site I. (A) Map of the field site indicating the monitoring wells (circles), buildings (polygons), and groundwater head (dashed line, in m.a.s.l.) and general groundwater flow (arrows). Full circles are the monitoring wells along the investigated transect (dashed-dotted line). The shaded contours represent the total CE concentration, $\text{Conc}_{\Sigma(\text{CEs})}$ (sum of PCE, TCE, cDCE and VC). The star marks the assumed contamination source zone. (B) Total CE concentration (circles, left y-axis) and concentration of the individual CEs normalized to $\text{Conc}_{\Sigma(\text{CEs})}$ (bars, right y-axis) along the transect depicted in panel A. (C) Carbon isotopic signatures of CEs (symbols, left y-axis; errors are smaller than symbols) and isotopic mass balance (x symbols and dashed line, left y-axis) as well as groundwater residence times (bars, right y-axis). The horizontal grey bar represents the assumed source $\delta^{13}\text{C}$ value of $-26.2 \pm 0.5\text{‰}$ (D) Concentration of redox-sensitive compounds.

Fig 2. Field Site II. (A) Map of the field site and contamination plume. See caption of Fig. 1A for details. The distance from P6 to PS (1 km) is not in scale. (B) Total CE concentration ($\text{conc}_{\Sigma(\text{CEs})}$, circles, left y-axis) and concentration of the individual CEs normalized to the $\text{conc}_{\Sigma(\text{CEs})}$ (bars, right y-axis) along the transect depicted in panel A. (C) Carbon isotopic signatures of CEs (symbols, left y-axis; errors are smaller than symbols) and isotopic mass balance (x symbols and dashed line, left y-axis) and groundwater residence times (bars, right y-axis). The horizontal grey bar represents the assumed source $\delta^{13}\text{C}$ value of $-23.5 \pm 0.5\text{‰}$ (D) Concentration of dissolved oxygen (black bars, left y-axis) and measured electric conductivity (white bars, right y-axis).

Fig 3. Field Site III, situation after remediation by soil excavation followed by pump-and-treat. (A) Map of the field site. See caption of Fig. 1A for details. (B) Total CE concentration ($\text{conc}_{\Sigma(\text{CEs})}$, circles, left y-axis) and concentration of the individual CEs normalized to $\text{Conc}_{\Sigma(\text{CEs})}$ (bars, right y-axis) along the transect. (C) Carbon isotopic signatures of CEs (symbols, left y-axis; errors are smaller than symbols) and isotopic mass balance (x symbols and dashed line, left y-axis) as well as groundwater residence times (bars, right y-axis). The horizontal grey bar represents the assumed source $\delta^{13}\text{C}$ value of $-26.3 \pm 0.5\text{‰}$ (D) Concentration of redox-sensitive compounds.