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Tracking in situ Biodegradation of 1,2-Dichloroethenes in a Model Wetland

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The spatial and temporal biogeochemical development of a model wetland loaded with *cis*- and *trans*-1,2-dichloroethene contaminated groundwater was characterized over 430 days by hydrogeochemical and compound-specific isotope analyses (CSIA). The hydrogeochemistry dramatically changed over time from oxic to strongly reducing conditions as emphasized by increasing concentrations of ferrous iron, sulfide, and methane since day 225. $\delta^{13}C$ values for *trans*- and *cis*-DCE substantially changed over the flow path and correlated over time with DCE removal. The carbon enrichment factor values (ϵ) retrieved from the wetland became progressively larger over the investigation period, ranging from -1.7 ± 0.3‰ to -32.6 ± 2.2‰. This indicated that less fractionating DCE oxidation was progressively replaced by reductive dechlorination, associated with a more pronounced isotopic effect and further confirmed by the detection of vinyl chloride and ethene since day 250. This study demonstrates the linkage between hydrogeochemical variability and intrinsic degradation processes and highlights the potential of CSIA to trace the temporal and spatial changes of the dominant degradation mechanism of DCE in natural or engineered systems.

Introduction

Wetlands are widespread ecotones covering interfaces between terrestrial and aquatic ecosystems. These biogeochemically heterogeneous systems can be hydrologically connected to an underlying contaminated aquifer. Therefore, they may play a crucial role in the control of water quality of surface water systems (*1*). However, knowledge about biogeochemical processes associated with transformation of contaminants in wetlands remains limited. Simulating natural wetlands, constructed wetlands (CW) may capitalize intrinsic physical, chemical, and biological processes for the purpose of water remediation. Recently, several attempts have been made to extend the application of CW for treating chlorinated solvents (*2–5*).

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For instance, the *cis*- and *trans*-1,2-dichloroethene isomers (DCE) often accumulate in anaerobic aquifers and are considered as priority pollutants due to their widespread presence and toxicity (*6*). During microbial degradation, DCE can serve either as electron donor or as electron acceptor mainly depending on the geochemical conditions and the intrinsic microbial ecology. In aerobic systems, oxidation of DCE has been observed to occur metabolically (*7, 8*) or cometabolically (*9*). Alternatively, anaerobic oxidation of DCE has been demonstrated in laboratory experiments under Mn(IV)-, Fe(III)-, or SO_4-reducing conditions (*10, 11*). Under strongly reducing conditions, biotic reductive dechlorination may result in DCE transformation to nontoxic ethene via carcinogenic vinyl chloride as intermediate compound (*12*). Thus, DCE transformation is feasible by several pathways mirroring the prevailing biogeochemical characteristics of an environmental system. DCE can then be used as model compounds to follow-up the spatio-temporal variability of environmental conditions with respect to the dominant biodegradation mechanism in complex environmental systems.

Physico-chemical and biological processes associated with natural attenuation of organic contaminants are often monitored in contaminated groundwater systems but more rarely in wetlands. The hydrogeochemical conditions in the subsurface can generally be delineated and statistically analyzed over time along with variations in contaminant and metabolites concentrations (*13, 14*). Furthermore, compound specific isotope analysis (CSIA) allows a qualitative and/or quantitative integrative assessment of biological transformation of organic contaminants over a flow path in environmental system (*15*). The mechanism of a chemical reaction results in a characteristic isotope effect, and isotope fractionation analysis can then be used to identify biodegradation pathways. The characterization of biodegradation processes in environmental systems generally relies on laboratory enrichment factors (ϵ) obtained from cultures with known degradation pathways (*16*). For instance, the reported enrichment factors determined for cometabolic aerobic *cis*- and *trans*-DCE isomers degradation (*17, 18*) were found to significantly differ from those determined for anaerobic degradation via reductive dechlorination (*19, 20*). Attributing these variations to mechanistically different degradation pathways, CSIA can help in distinguishing DCE oxidation from reductive dechlorination in complex environmental systems experiencing spatial and temporal variability of redox conditions.

This study focuses on the spatial and temporal development of biogeochemical processes in a model wetland treating *cis*- and *trans*-1,2-dichloroethene contaminated groundwater with emphasis on the characterization of degradation pathways. While the system as a whole is dynamic, specific geochemical processes may dominate on a spatial and/or temporal scale and control the contaminant transformation mechanism. Therefore, the variability of hydrogeochemical indicators and carbon stable isotope composition of *cis*- and *trans*-DCE in pore water was assessed at high spatial resolution across the wetland over 430 days. Multivariate analysis was used to retrieve additional insight into the spatial and temporal trends of biogeochemical development and the relationships between the hydrogeochemical variables. To our knowledge, this is the first report of detailed characterization of biogeochemical processes associated with degradation of DCE compounds in a wetland system.

Materials and Methods

System Design. A general scheme of the model constructed wetland and detailed experimental design information are provided in Supporting Information (SI) Figures S1 and S2. The model horizontal subsurface flow wetland consisted of a stainless steel tank (201 × 60 × 5 cm), filled with quartz sand and planted with the common rush (*Juncus effusus*, L.). The supplied groundwater was collected bimonthly from a local, well-characterized contaminated aquifer (*21, 22*) in 50 L tanks and maintained under anaerobic conditions at constant N_2 pressure. The hydrogeochemistry of the groundwater was constant over time and *cis*- and *trans*-1,2-DCE were the dominant contaminants. Detailed information regarding the groundwater quality is provided in the SI (section 1.2 and table S1). Additional amounts of contaminant were spiked into the tanks during the groundwater sampling to reach average concentrations of 6.5 and 1.5 mg L^{-1} for *cis*- and *trans*-DCE, respectively. During five months preceding the investigation as well as during the investigation period, the system was operated in a flow-through mode with a rate of 1.8 L d^{-1} contaminated groundwater, corresponding to a retention time of approximately 15 days.

Sampling Procedure. Pore water samples were collected 18 times at regular intervals (two or three weeks) over 430 days for hydrogeochemical investigation, concentration and isotopic composition analysis of chlorinated ethenes. Pore water samples were retrieved at the inflow chamber, at 12 sampling ports across the sand compartment and at the pond (SI, Figure S1 and section 1.3). Four vertical profiles across the sand compartment located at 6, 49, 94, 139 cm from the inflow, each consisting of three sampling ports at 20, 32, and 44 cm depth below the surface, were systematically investigated. In situ oxygen measurements were performed in parallel at discrete resolution ($\Delta \sim 6$ cm) along the four vertical profiles across the soil compartment.

Pore Water Geochemistry and Contaminant Concentration Analysis. The sampling and analytical methods are described in SI section 1.4.

Compound Specific Isotope Analysis and Definitions. Stable carbon isotope compositions of the chlorinated ethenes were measured using a gas chromatography-combustion-isotope ratio mass spectrometry system (GC-C-IRMS) as described earlier (*22, 23*). The detailed procedure of stable isotope analysis and definitions are provided in the SI (section 1.5). Briefly, estimates of the wetland isotope fractionation factor α were derived from the slope of the standard linear regression plot applying the log-based Rayleigh equation according to Mariotti et al. (*24*). Fractionation factors were then converted into carbon enrichment factors (ϵ). Two data treatment were performed. First, a single and integrative carbon enrichment factor, further referred as "mean isotope enrichment factor", was derived for each DCE isomer at each sampling date, taking the inflow chamber values as the initial values. For this purpose, discrete-depth concentrations and isotopic composition values retrieved at each of the four vertical profiles across the sand compartment of the wetland were separately averaged before a mean enrichment factor was calculated. Second, assuming laminar flow in the system, isotope composition and concentration data were also evaluated separately for each investigated depths. Three enrichment factors were then retrieved for each specific flow path in the sand compartment per sampling date and per DCE isomer, taking the inflow chamber values as initial values.

Statistical Analysis. The significance of the correlation of the standard linear regression for further derivation of the carbon enrichment factor ϵ was evaluated based on the coefficient of determination (R^2) of the linear regression curve and on *F*-statistics (ANOVA).

The hydrogeochemical and isotope data from day 0 to 127 and from day 127 to 430 were separately subjected to principal component analysis (PCA). PCA were performed on the basis of the correlation matrix and the numerical data matrices were converted using the program R (R: Copyright 2005, The R Foundation for Statistical Computing, Version 2.1.1).

Results and Discussion

Biogeochemical Development of the Wetland. Two main phases could be clearly distinguished over the investigation period based on the hydrogeochemical analysis (SI Table S1). During the first phase (from day 0 to day 127), oxic conditions prevailed in the system, as inferred from the mean dissolved oxygen concentration >1.5 mg L^{-1} and low concentrations of reduced redox-sensitive constituents. The oxygen concentration globally decreased over depth and increased over the flow path, indicating the occurrence of spatial gradients within the system (SI Figure S2). Oxygen diffusion and/or advection through the water column and sand combined with oxygen transport in the subsurface via plant tissues likely contributed to the establishment of the aerobic-anaerobic interfaces. During the second phase (from day 127 to 430), lower mean dissolved oxygen concentrations (<0.5 mg L^{-1}) in the system indicated overall anoxic conditions. Moreover, the increase of reduced species concentration values (NH_4^+, Fe^{2+}, sulfide, and CH_4) suggested parallel activity of several microbially mediated redox reactions in the wetland. This period also coincided with intense precipitation of iron sulfide minerals. Since day 199, the precipitates formed a front of black patches in the sand compartment. The front progressed from the inflow region along the flow path at about 1.5 cm d^{-1} (SI Figure S2). This clearly points to the prevalence of anoxic conditions in the sand compartment.

To highlight the spatio-temporal variability of hydrogeochemical patterns across the wetland and to support the interpretation of biogeochemical processes, the hydrogeochemical data sets corresponding to each phase were separately subjected to principal component analysis (Figure 1). In the two analyses, the percent of variance amounted within the data for the first (PC1) and second (PC2) principal components was >50%. Isotopic composition values of DCE showed negative correlation with DCE concentration values (Pearson's product-moment correlation < −0.34). This strongly suggests a mechanistic relationship between stable isotope fractionation and contaminant depletion. Moreover, the enrichment of ^{13}C in the residual *cis*- and *trans*-DCE fractions provides a qualitative evidence of microbiological degradation as a mechanism contributing to DCE removal in the model wetland over time.

The PCA plot of variables corresponding to the first phase (day 0−127) permitted the geochemical variables associated with changes in the isotopic signatures to be highlighted (Figure 1A). $\delta^{13}C$ values for *trans*- and *cis*-DCE are positively correlated with chlorine, sulfate, redox potential, and oxygen. This suggests a possible linkage between these geochemical indicators and degradation activity. At wetland zones displaying relatively higher redox potential, DCE oxidation reaction may preferentially be linked to oxygen or, at lower redox conditions, to sulfate, with concomitant release of chlorine. However, high background concentration of chlorine in the supplied groundwater (127 ± 9 mg L^{-1}) hindered a concentration-based sensitive analysis of the fraction released during DCE degradation. Furthermore, significant isotope fractionation under prevailing oxic geochemical conditions (Table S1) with no detection of reductive dechlorination metabolites (i.e., VC, ethene) supports the occurrence of oxidative degradation processes. In the PCA plot of sampling locations (Figure 1B), no clusters could be distin-
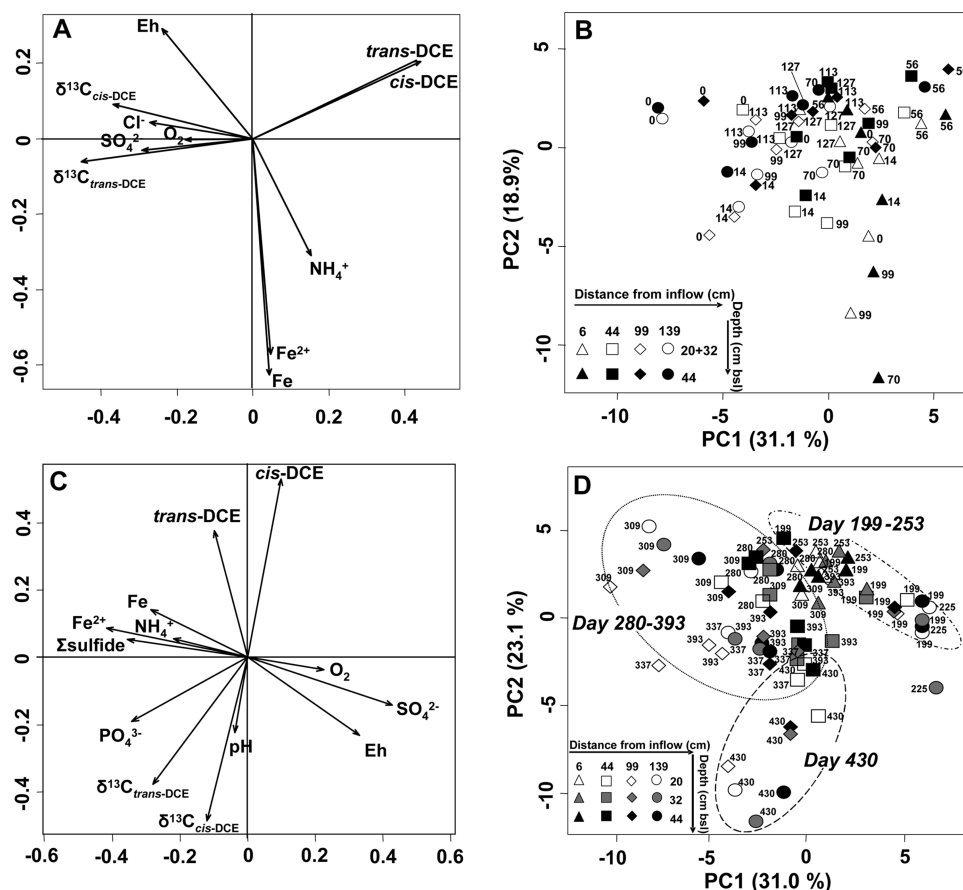
FIGURE 1. PCA ordination plot of hydrogeochemical characteristics and isotope composition values of porewater samples collected from the model constructed between day 0 and 127 (A, B) and between day 127 and 430 (C, D) of the investigation period. Values on the axes indicate % of total variation explanation by the corresponding axis (PC 1, principal component axis 1; PC 2, principal component axis 2). Description vectors correspond to: $\delta^{13}C_{DCE}$ = carbon isotope signature of *cis*- or *trans*-DCE; Fe^{2+} = ferrous iron; Fe = tot. Total iron; SO_4^{2-}, sulfate; Σsulfide = total sulfide; Cl^-, chloride; O_2 = oxygen; NO_3^- = nitrate; NH_4^+ = ammonium; PO_4^{3-} = phosphate; Eh = redox potential. Objects corresponds to the samples retrieved at different sampling days from sampling ports at 6 ($\triangle$), 44 ($\square$), 99 ($\diamond$), and 139 ($\bigcirc$) cm from the inflow of the wetland and at 20 (white) and/or 32 (gray) and 44 (black) cm below the surface level (cm bsl).

guished and samples mainly grouped close to the origin of the biplot, independently on their spatial or temporal origin. This emphasizes no clear spatio-temporal trends in the evaluated biogeochemical processes and relatively homogeneous hydrogeochemical conditions between day 0 and 127.

In the PCA plot of sampling locations corresponding to the second phase (day 127–430), sampling locations from the early anoxic (day 199–253), intermediate (day 280–393), and late sampling (day 430) periods could be distinguished (Figure 1D). This suggests an overall temporal variation of the hydrogeochemical patterns. Higher PC1 and PC2 scores indicated lower extent of fractionation and less reducing conditions. Scores of PC1 correlated positively to sulfate, redox potential and oxygen (loadings >0.2) and negatively to ferrous iron, sulfide and ammonium (loadings < −0.2) in the plot of variables (Figure 1C). This emphasizes the importance of redox sensitive indicators to distinguish clusters of sampling locations and substantiates the expected negative correlation over space and time between reduced and oxidized redox species. In the second phase, sampling locations from day 199 to 253 globally clustered positively along PC1 and PC2 and were associated with higher contaminant concentrations and indicators of less-reducing conditions (higher O_2 concentrations and redox potential values). Indeed, this transient period corresponded to the beginning of iron- and sulfate-reduction processes and moderate reducing conditions. From day 225–430, reductive

dechlorination occurred in the system as inferred from increasing vinyl chloride and ethene concentrations (<5 to 518 ± 13, and <5 to 102 ± 5 μg L^{-1}, respectively). Microbial reductive dechlorination guilds require a sustainable source of electron donors such as fermentable organic compounds (*25*). Average outflow/inflow COD, TOC and DOC concentration ratios higher than 2 in the second phase (SI Table S3) indicated the production of organic compounds in the wetland. This was likely due to plant leached organic carbon and supports the occurrence of conducive conditions for reductive dechlorination. Sampling locations from day 280 to 393 clustered negatively along PC1 and were associated with larger amounts of reduced species (Fe^{2+}, NH_4^+ and sulfide). During this period, enrichment in ^{13}C coincided with reductive dechlorination of DCE and the simultaneous occurrence of various reduction processes. Finally, samples from day 430 clustered separately and were associated with higher $\delta^{13}C_{DCE}$ values. This emphasizes a substantial change in the magnitude of the isotope fractionation coinciding with evidence for reductive dechlorination activity in the system. Overall, a predominant temporal variability of biogeochemical processes over spatial variability was observed in the second phase. However, various biogeochemical reactive zones occurred across the system putatively influencing transformation of DCE over the flow path. It mainly explains the differences between hydrogeochemical patterns within each cluster of sampling locations. Nevertheless, the converging geochemical evolution of discrete wetland zones over
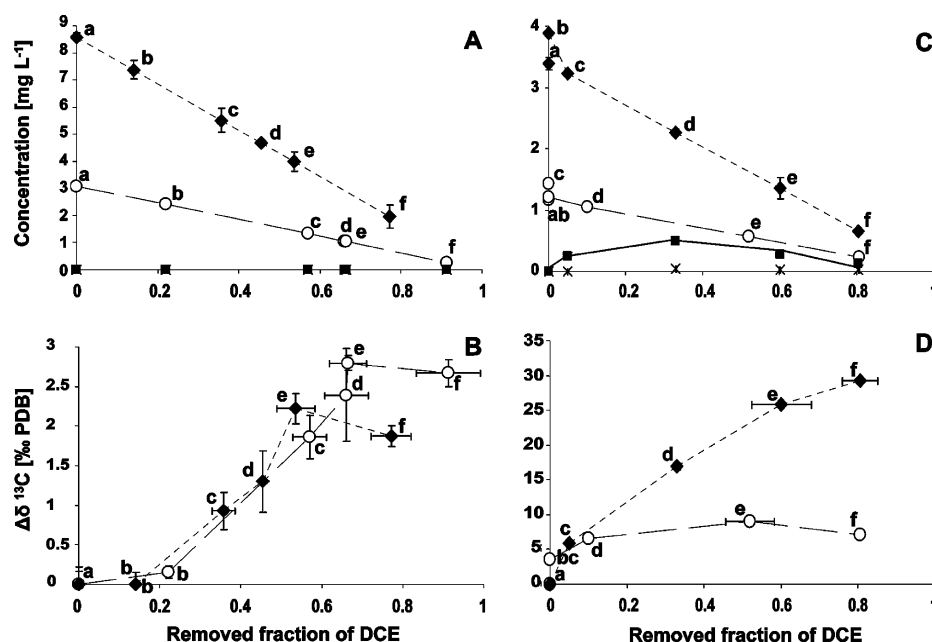
FIGURE 2. Change in concentrations (A, C) of *cis*-DCE (♦), *trans*-DCE (○) and their products vinyl chloride (■) and ethene (×) and shift in carbon isotopic composition (B, D) of *cis*-DCE (♦), *trans*-DCE (○) in the model wetland as a function of the removed DCE fraction over the flow path at 44 cm depth. A and B correspond to day 14 (first phase) and C and D to day 430 (second phase) of the investigation period. Letters *a* to *e* correspond to the investigated distances across the wetland: a: 0 cm (inflow); b: 6 cm; c: 49 cm; d: 94 cm; e: 139 cm; and f: 175 cm (pond).

time toward more reducing conditions favored a prevailing degradation mechanism at the system scale.

PCA were performed on the basis of complex data sets consisting of a large number of samples and variables collected at multiple regular intervals over a reasonably long period. This strategy permitted a robust and integrative evaluation of dynamic biogeochemical processes occurring in the wetland system. In particular, the combination of both hydrogeochemistry and isotope data in a single analysis allowed proposing mechanistic interpretation of contaminant degradation.

δ^{13}C Values. To interpret in more details the observed hydrogeochemical changes with respect to DCE biodegradation, isotope fractionation accompanying contaminant depletion over the flow path was analyzed. The relation between isotope fractionation and DCE removal over the flow path is exemplified for two representative sampling dates (day 14 and 430) in Figure 2. During the investigation period, the removed fraction of DCE was similar for both isomers and generally increased across the flow path with respect to the initial concentrations. The removed fraction of DCE ranged between 0 and 60% in the sand compartment (SI Figure S3), whereas it reached 74 ± 16% (*n* = 18) at the pond. A parallel significant isotopic shift for *trans*- and *cis*-DCE (δ^{13}C > 0.5 ‰) could be observed between the inflow and the end of the sand compartment for 9 and 11 sampling campaigns, respectively. The largest shifts in the DCE isotopic composition were observed at day 393 and 430, and reached 8.1 ± 3.0‰ and 28.8 ± 1.5‰ for *trans*- and *cis*-DCE, respectively. These values were 7–50 fold larger compared to previous sampling dates for a similar fraction of DCE removal. This change in the fractionation patterns coincided with the detection of VC and ethene since day 225, but concentrations were generally too low for determining the isotopic composition. However, at day 430, a broad range of isotopic signatures for vinyl chloride existed across the wetland (VC = −43.8 ± 0.1 to −23.1 ± 0.2‰). This suggested various stages of DCE conversion *via* reductive dechlorination across the wetland. Indeed, the newly formed VC would be initially lighter than nondegraded DCE (*cis*-DCE = −22.5 ± 0.2; *trans*-DCE = −17.9 ± 0.2‰) and may then become

heavier in the course of DCE degradation as well as through further dechlorination to ethene (*20*).

The ability of CSIA to distinguish between mass losses due to biodegradation versus mass loss due to nondegradative processes permitted assessing DCE biodegradation in the wetland. Indeed, nondestructive processes such as dissolution, volatilization, and sorption of chlorinated ethenes were reported to cause no significant isotope fractionation (*26–29*). Moreover, biological degradation of DCE is very likely to largely predominate in the wetland and hypothetical abiotic transformation to proceed at very low rate (*30*). However, mass balancing of contaminants in wetland system is often complicated because the sorbed, volatilized, and biodegraded fractions of DCE can hardly be differentiated in situ. The relative contribution of sorption to the overall contaminant removal is assumed to be negligible, as DCE display a relatively low K_{ow} and very likely reached sorption equilibrium after five months of groundwater inoculation preceding the investigation. Maximal DCE removal was observed at the pond and amounted to about 3/4 of the initial contaminant load. The removed fraction integrates both volatilization and degradation losses. However, very low DCE volatilization rates are likely occurring in the sand compartment due to very slow flow regimes (*4*) and slow diffusion rates through the unsaturated zone. Furthermore, a wetland study based on the more volatile TCE suggests low transfer of DCE in the atmosphere (*2*). This is also supported by the absence of substantial DCE removal at day 56, 70, 113, and 127 (SI Figure S3) and the distribution of removed DCE fraction over the depth profiles (SI Table S4).

Carbon Enrichment Factors. Carbon isotopic enrichment factors were derived over the investigation period to integrate the isotope composition changes as a function of contaminant mass depletion over the flow path in the wetland. The mean isotope enrichment factors for *cis*- and *trans*-DCE ranged from −1.7 ± 0.3‰ to −32.6 ± 2.2‰ and became progressively larger over time (Figure 3 and SI Table S5). While factors for *cis*- and *trans*-DCE were generally in the same range during the first investigation period, fractionation patterns of *trans*-DCE more rarely fitted a Rayleigh model. Differences in aerobic degradation patterns between similar
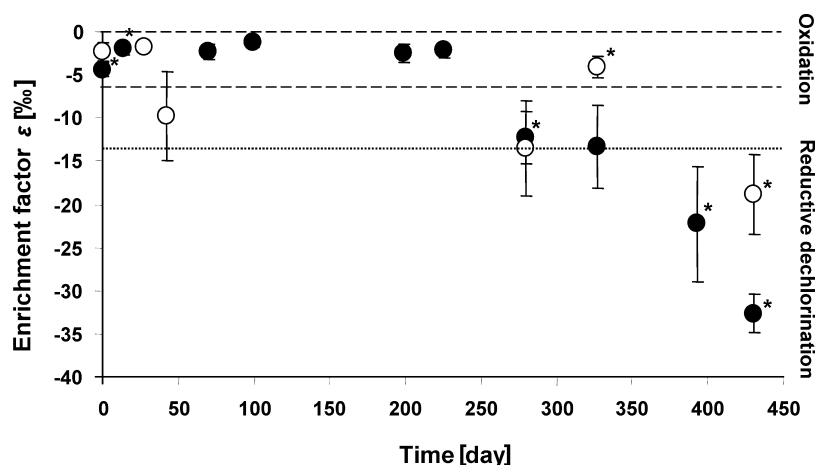
FIGURE 3. Temporal changes in carbon enrichment factors of *cis*-1,2-DCE (•) and *trans*-1,2-DCE (○) obtained by fitting a Rayleigh model to concentration and isotope composition data. The enrichment factors correspond to mean data retrieved from transects across the wetland at 20, 32, and 44 cm depth below the surface. The plotted enrichment factors were retrieved when the coefficient of determination (R^2) of the linear regression curve of the respective Rayleigh model was >0.5 and (*) indicates values significantly fitting the model at $P < 0.05$. Dashed lines delimit the ranges of enrichment factors reported in the literature for DCE degradation *via* oxidation pathway (—) and reductive dechlorination ($\cdot\cdot\cdot$). Error bars show the standard error of the regression.

isomeric forms of chlorinated compounds have also been noted previously (*31, 32*). From day 0 to 225, stable isotope enrichment factors for *cis*-DCE reproducibly ranged between -1.7 ± 0.3‰ and -4.4 ± 0.8‰. This corresponds to the range of enrichment factor values obtained under aerobic cometabolic degradation for DCE in laboratory experiments (*17, 18*). Together with the data of the hydrogeochemical analysis, these results indicate prevailing occurrence of degradation via oxidative pathways during this period. Since day 225, gradually larger mean enrichment factors for *cis*-DCE were retrieved. Since day 337, the mean isotope enrichment factors were in the characteristic range of values obtained under reductive dechlorination of *cis*- and *trans*-DCE by enrichment or pure cultures (range from -14.1 to -30.1‰, refs *19, 20, 33*). The occurrence of reductive dechlorination was further evidenced by the systematic detection of *Dehalococcoides* sp. related DNA in the sand compartment between day 225 and 430 (data not shown). Considering depth-discrete flow paths, larger enrichment factors for *cis*-DCE were found at lower depths at days 225, 337, and 393, whereas an opposite pattern was found at day 430 (SI Figure S4 and Table S6). This can be explained by the occurrence of more pronounced oxic-anoxic interfaces before day 430. Indeed, dissolved oxygen concentration values were slightly higher than 0.5 mg L^{-1} until day 393 at about one-third of the 32 measured locations of the wetland (data not shown). In the CW, stronger fractionating reductive dechlorination may occur in anaerobic zones adjacent to aerobic zones where less fractionating oxidative processes occur. Indeed, oxygen diffusion may longer contribute to DCE degradation *via* oxidative pathways in the upper layers of the CW. This results in lower enrichment factors than in deeper sediment layers.

Overall, the progressive change toward more pronounced isotope fractionation over the investigation period or with increasing depth mirrored the development of geochemical conditions. It also emphasized the succession of degradation mechanism of DCE on both a spatial and temporal scale. The range of kinetic isotope fractionation in the wetland may offer further characterization on the mechanism of the degradation reaction. One the one hand, the reductive dehalogenation reaction includes a C−Cl bond cleavage, which generally results in significant isotope effects (*23, 33*). On the other hand, the oxidative degradation of DCE and vinychloride is suggested to be initiated by an epoxidation reaction of the C=C bond (*19, 34, 35*), which results in lower

fractionation factors (*36*). Thus, the observed fractionation pattern in complex systems would mainly depend on the respective contribution of degradation reactions occurring in oxic and anoxic compartments intercepting the flow path. However, a quantitative estimation of the relative contribution of each DCE degradation pathway based on an intermediate enrichment factor integrating both reactions remains limited. Indeed, preliminary knowledge about the enrichment factors corresponding to each pathway before their co-occurrence would be required. Furthermore, the existence of micro- and macroheterogeneity sustaining strongly varying local reaction conditions in the wetland affects the relationship between measured isotope signatures and the extent of contaminant degradation. This hinders a direct quantitative estimation of biodegradation by means of the Rayleigh equation holding for homogeneous reaction media.

CSIA allow distinguishing degradation pathways if the respective ranges of enrichment factors associated with oxidative degradation and with reductive dechlorination of DCE differ from each other under field conditions. However, wetlands may sustain dynamic microbial communities with a widely distributed potential among a variety of microorganisms for both metabolically or cometabolically DCE mineralization and reductive dechlorination (*37, 38*). Therefore, when one single pathway predominates in the system, diverse DCE degrading microbial populations may generate different fractionation patterns. This would result in an intermediate enrichment factor integrating the co-occurring fractionation patterns. For instance, large differences in carbon isotope fractionation were already observed during aerobic cometabolic degradation of TCE (*18, 39*) as well as during reductive dechlorination of PCE and TCE by different dehalorespiring bacteria (*23*). These examples emphasize the possible extent of the ranges of variation of DCE enrichment factors for oxidative degradation and reductive dechlorination. An overlapping between these ranges would complicate the interpretation and would require detailed insight into the hydrogeochemistry, intrinsic degrading community, and/or reaction mechanism. Moreover, stable isotope fractionation patterns potentially associated with the hypothetical oxidative transformation of chloroethenes under anaerobic conditions is not currently understood and may differ to that associated with oxidative dechlorination under aerobic conditions. Further studies are clearly necessary to understand and delineate the possible range of variation of enrichment factors associated with each major DCE deg-

radation pathway. Nevertheless, our results support the relevance of CSIA coupled with hydrogeochemical analysis to assist in identifying over space and time the prevailing DCE degradation pathway in hydraulically well-characterized engineered or natural field settings subjected to variation of geochemical conditions. In this case, the change of isotope enrichment factors characterizes the transition from aerobic to anaerobic conditions and reflects a compartmentalized environment leading to heterogeneous reaction conditions.

Characterizing Degradation of Organic Contaminants in Wetlands. Wetland systems may sustain on a spatial and/or temporal scale degradation of organic chemicals subjected to both anaerobic and aerobic pathways. However, the potential for organic chemicals degradation over the system lifespan is determined by the respective contribution and significance of occurring elimination pathways. Therefore, differentiating pathways is crucial as transformation rates and toxicities of the intermediate metabolites may vary according to the prevailing pathway. In this study, CSIA permitted capturing information on biogeochemical variability occurring in the saturated zone of a model wetland. This provided an opportunity to elucidate possible underlying causes. In particular, CSIA permitted identifying and tracing the prevailing mechanism of DCE degradation over space and time in a complex environmental system. It supports that degradation of DCE occurs in wetlands via both oxidative and reductive pathways, with relative contribution mainly depending on the variability of hydrogeochemical conditions. Application strategies including quantification aspects could be conceived in environmental systems if future research permits delineating mechanism-specific ranges of enrichment factors for DCE degradation. Furthermore, the combination of hydrogeochemical analysis and CSIA aided with multivariate statistics represents a promising approach for interpreting wetlands biogeochemical development with respect to organic chemicals degradation. Indeed, the evaluation of spatial and temporal trends is critical for evaluating and predicting the biodegradation potential over the system lifespan since the hydrogeochemistry may control and/or reflect specific patterns of contaminant degradation. Finally, coupling isotope fractionation and hydrogeochemical analysis is of interest to understand factors affecting the transformation of contaminant in natural or constructed wetlands. Knowledge about processes may then support optimization of wetland systems with respects to natural attenuation of organic contaminants.

Acknowledgments

Supporting Information Available

Detailed schemes of the model wetland. Pore water geochemical analysis procedure and hydrogeochemical analysis. Detailed carbon isotope enrichment factors over the investigation period. This material is available free of charge via the Internet at http://pubs.acs.org.

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