

1 Use of carbon - chlorine dual isotope analysis to  
2 assess the degradation pathways of  
3 1,1,1-trichloroethane in groundwater

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15

16 **Abstract**

17 Compound-specific isotope analysis (CSIA) is a powerful tool to track contaminant fate in  
18 groundwater. However, the application of CSIA to chlorinated ethanes has received little  
19 attention so far. These compounds are toxic and prevalent groundwater contaminants of  
20 environmental concern. The high susceptibility of chlorinated ethanes like  
21 1,1,1-trichloroethane (1,1,1-TCA) to be transformed via different competing pathways (biotic  
22 and abiotic) complicates the assessment of their fate in the subsurface. In this study, the use of  
23 a dual C-Cl isotope approach to identify the active degradation pathways of 1,1,1-TCA is  
24 evaluated for the first time in an aerobic aquifer impacted by 1,1,1-TCA and trichloroethylene  
25 (TCE) with concentrations of up to 20 mg/L and 3.4 mg/L, respectively. The reaction-specific  
26 dual carbon-chlorine (C-Cl) isotope trends determined in a recent laboratory study illustrated  
27 the potential of a dual isotope approach to identify contaminant degradation pathways of  
28 1,1,1-TCA. Compared to the dual isotope slopes ( $\Delta\delta^{13}\text{C}/\Delta\delta^{37}\text{Cl}$ ) previously determined in the  
29 laboratory for dehydrohalogenation / hydrolysis (DH/HY,  $0.33 \pm 0.04$ ) and oxidation by  
30 persulfate ( $\infty$ ), the slope determined from field samples ( $0.6 \pm 0.2$ ,  $r^2 = 0.75$ ) is closer to the  
31 one observed for DH/HY, pointing to DH/HY as the predominant degradation pathway of  
32 1,1,1-TCA in the aquifer. The observed deviation could be explained by a minor contribution  
33 of additional degradation processes. This result, along with the little degradation of TCE  
34 determined from isotope measurements, confirmed that 1,1,1-TCA is the main source of the  
35 1,1-dichloroethylene (1,1-DCE) detected in the aquifer with concentrations of up to 10 mg/L.  
36 This study demonstrates that a dual C-Cl isotope approach can strongly improve the  
37 qualitative and quantitative assessment of 1,1,1-TCA degradation processes in the field.

38

39 **Keywords:** volatile organic contaminants, chlorinated solvents, compound-specific isotope  
40 analysis, contaminant fate, groundwater remediation, abiotic degradation.

## 41 1. INTRODUCTION

42 Groundwater contamination by chlorinated aliphatic hydrocarbons (CAHs) is a major  
43 environmental problem and it has an adverse impact on water resources (Moran et al. 2007).  
44 1,1,1-trichloroethane (1,1,1-TCA) and trichloroethene (TCE) are toxic and persistent  
45 contaminants commonly found in polluted aquifers because of their widespread use as  
46 solvents (ATDSR 2003, 2006a). TCE is frequently a co-contaminant in aquifers with  
47 1,1,1-TCA due to their similar industrial applications and both compounds are considered as  
48 priority pollutants by the United States Environmental Protection Agency (USEPA 2013). In  
49 groundwater, 1,1,1-TCA may be transformed by multiple biotic and abiotic reactions (Fig. 1)  
50 (Scheutz et al. 2011), making it challenging to elucidate active degradation pathways. This  
51 knowledge is necessary to evaluate contaminant degradation and potential formation of toxic  
52 intermediates. Identifying pathways is further complicated in sites contaminated by mixed  
53 CAHs because some products of 1,1,1-TCA such as 1,1-dichloroethylene (1,1-DCE) can be  
54 formed from different precursors (Fig. 1). Hence, identification of pathways based solely on  
55 substrate-product concentration relationships may lead to erroneous interpretations.  
56 Therefore, development of innovative strategies for 1,1,1-TCA degradation pathways  
57 elucidation and evaluation in the field is warranted.

58 In groundwater, 1,1,1-TCA is abiotically degraded to 1,1-DCE and acetic acid (HAc)  
59 via dehydrohalogenation and hydrolysis (DH/HY), respectively (Scheutz et al. 2011) (Fig. 1).  
60 Thermal enhancement of DH/HY has been recently proposed for in situ remediation of 1,1,1-  
61 TCA contamination (Suthersan et al. 2012). Generally, reductive dechlorination of TCE  
62 (biotic and abiotic) also results in the formation of 1,1-DCE as minor product via  
63 hydrogenolysis (Arnold and Roberts 2000, Field and Sierra-Alvarez 2004). However, Zhang  
64 et al. 2006 found 1,1-DCE as the predominant intermediate in microcosm degradation  
65 experiments of TCE prepared with a microbial culture derived from a landfill site. In addition,

66 1,1-DCE may also result from dehydrohalogenation of 1,1,2-trichloroethane (1,1,2-TCA)  
67 (Pagan et al. 1998) and from dihaloelimination of 1,1,1,2-tetrachloroethane (1,1,1,2-TeCA)  
68 (Culubret et al. 2001, O'Loughlin and Burris 2004) (Fig. 1). 1,1-DCE is also a contaminant of  
69 environmental concern because it may be transformed to vinyl chloride (VC) in anaerobic  
70 conditions (Fig. 1), a confirmed carcinogenic compound (ATDSR 2006b). In anaerobic  
71 conditions, 1,1,1-TCA may undergo metal catalyzed reduction either by naturally occurring  
72 reductants such as iron sulfide (Butler and Hayes 2000) and iron hydroxides (O'Loughlin and  
73 Burris 2004) or by zero valent iron (Fe(0)) in engineered systems (Fennelly and Roberts  
74 1998). Reduction of 1,1,1-TCA by Fe(0) leads to the formation of 1,1-dichloroethane (1,1-  
75 DCA), ethene and ethane in parallel pathways (Fennelly and Roberts 1998) (Fig. 1).  
76 Biodegradation of 1,1,1-TCA under both aerobic and anaerobic conditions has been reported  
77 in a number of studies (Field and Sierra-Alvarez 2004, Scheutz et al. 2011). Dehalorespiration  
78 of 1,1,1-TCA by the anaerobic bacterium *Dehalobacter* sp. strain TCA1 was demonstrated by  
79 Sun et al. (2002). 1,1,1-TCA was transformed into chloroethane (CA) with transient  
80 formation of 1,1-DCA as intermediate. In aerobic conditions, cometabolic oxidation of 1,1,1-  
81 TCA has been observed in several studies with pure and enrichment cultures (Field and  
82 Sierra-Alvarez 2004, Yagi et al. 1999).

83 For a given compound, different degradation pathways are sometimes related to  
84 distinct subsurface redox environments and, therefore, redox conditions may help elucidating  
85 reaction pathways. However, for 1,1,1-TCA different reaction pathways may be active under  
86 the same redox conditions. For instance, 1,1,1-TCA biodegradation, via either reductive  
87 dechlorination or cooxidation, and DH/HY can occur simultaneously in anaerobic or aerobic  
88 conditions, respectively, complicating their evaluation. In addition, redox zones  
89 characterization may be difficult due to the presence of micro-redox environments and/or  
90 strong redox gradients with depth within the contaminant plume (Christensen et al. 2000). In

91 this case, groundwater samples collected from conventional long screen wells may be a  
92 mixture of water from different parts of the plume with distinct redox conditions.

93 Compound-specific isotope analysis (CSIA) is an innovative tool to investigate  
94 degradation pathways of organic contaminants because the extent of isotope fractionation  
95 ( $\epsilon_{\text{bulk}}$ ) during compound transformation is highly reaction-specific (Hirschorn et al. 2004,  
96 Hunkeler et al. 2005, Vanstone et al. 2008). The isotope fractionation of the substrate can be  
97 quantified in laboratory studies using the Rayleigh equation, which can be approximated by  
98 the following expression, Eq. (1):

$$99 \delta^{\text{h}}\text{E}_S \approx \delta^{\text{h}}\text{E}_{S0} + \epsilon_{\text{bulk}} (\text{‰}) \cdot \ln f \quad (1)$$

100 where  $\delta^{\text{h}}\text{E}_S$  is the isotopic composition of element E at a remaining fraction (f) and  $\delta^{\text{h}}\text{E}_{S0}$  is  
101 the initial isotopic composition.

102 In aquifers, transformation-induced isotope fractionation is generally larger than the  
103 one related to phase transfer processes such as sorption or volatilization (Braeckevelt et al.  
104 2012). While isotope fractionation of one element alone (e.g.  $\epsilon_{\text{bulk}}^{\text{C}}$ ) could provide pathway  
105 distinction in laboratory experiments (Elsner et al. 2007), this is not possible under field  
106 conditions. Here, contaminant concentration changes related to processes other than its  
107 transformation (such as sorption and dispersion) cannot be excluded, preventing accurate  
108 calculation of  $\epsilon_{\text{bulk}}$  values. However, contaminant degradation pathways differentiation in the  
109 field may be addressed using a dual isotope approach (Whiticar 1999, Zwank et al. 2005).  
110 Recent development of analytical methods for online CI-CSIA, either by continuous flow gas  
111 chromatography isotope ratio mass spectrometry (GC-IRMS) (Shouakar-Stash et al. 2006) or  
112 GC-quadrupole mass spectrometry (GC-qMS) (Aeppli et al. 2010, Bernstein et al. 2011, Jin et  
113 al. 2011, Palau et al. 2014a, Sakaguchi-Soder et al. 2007) has facilitated the measurement of  
114 chlorine isotope ratios in chlorinated ethenes and ethanes. These novel methods open new

115 possibilities for a dual C-Cl isotope approach, which has not yet been applied to investigate  
116 the fate of chlorinated ethanes in the field.

117         During the course of a reaction, combined changes in isotope ratios (e.g.  $\Delta\delta^{13}\text{C}$  vs.  
118  $\Delta\delta^{37}\text{Cl}$ ) for a given reactant generally yield a linear trend in a dual element isotope plot (Abe  
119 et al. 2009, Cretnik et al. 2013, Palau et al. 2014a). The dual element isotope slope ( $\Lambda =$   
120  $\Delta\delta^{13}\text{C}/\Delta\delta^{37}\text{Cl} \approx \varepsilon_{\text{bulk}}^{\text{C}}/\varepsilon_{\text{bulk}}^{\text{Cl}}$ ) reflects isotope effects of both elements and, thus, different  
121 slopes may be expected for distinct transformation mechanisms involving different bonds  
122 with distinct elements (Elsner 2010). Following this approach, dual isotope slopes observed in  
123 the field can be compared to the slopes determined in laboratory experiments to identify  
124 degradation pathways. A significant advantage of the dual isotope approach is that the  $\Lambda$   
125 value often remains constant, regardless of the occurrence of transport and retardation  
126 processes (Thullner et al. 2013). The reason is that such processes are generally non- or  
127 slightly-isotope-fractionating so that both elements are affected similarly. In this case, by  
128 taking the ratio of the isotope shift for the two elements (e.g.,  $\Delta\delta^{13}\text{C}/\Delta\delta^{37}\text{Cl}$ ) their effect is  
129 cancelled out (Elsner et al. 2005). In addition, if a given contaminant is simultaneously  
130 degraded by two different pathways, the dual isotope approach could allow determining the  
131 portion of reaction occurring through each pathway (Centler et al. 2013, van Breukelen 2007).  
132 For 1,1,1-TCA, distinctly different dual C-Cl isotope trends were determined during oxidation  
133 with persulfate, reduction by Fe(0) and DH/HY in a recent laboratory study (Palau et al.  
134 2014b), illustrating the potential of this approach for 1,1,1-TCA degradation pathways  
135 differentiation. The dual C-Cl isotope approach has been applied to a limited number of  
136 chlorinated ethenes contaminated sites (Badin et al. 2014, Hunkeler et al. 2011, Lojkasek-  
137 Lima et al. 2012a, Lojkasek-Lima et al. 2012b, Wiegert et al. 2012) but, to our knowledge,  
138 not to sites with chlorinated ethanes.

139 In this study, dual C-Cl isotope analysis of 1,1,1-TCA in groundwater samples was  
140 performed for the first time with the purpose of elucidating the fate of 1,1,1-TCA in a  
141 contaminated aquifer. In order to evaluate the potential of the multi-isotope analysis and the  
142 dual C-Cl isotope slopes to identify degradation pathways of 1,1,1-TCA in the field, the  
143 isotope ratios of 1,1,1-TCA ( $\delta^{13}\text{C}$  and  $\delta^{37}\text{Cl}$ ) and 1,1-DCE ( $\delta^{13}\text{C}$ ) from field samples, in  
144 conjunction with concentration data, were compared to the isotope patterns determined from a  
145 previous laboratory experiment of 1,1,1-TCA transformation by DH/HY (Palau et al. 2014b).  
146 In addition, the isotopic composition of TCE ( $\delta^{13}\text{C}$  and  $\delta^{37}\text{Cl}$ ) detected in the aquifer was also  
147 determined to assess its transformation.

## 148 2. FIELD SITE

149 The dual C-Cl isotope approach was evaluated at a site where the subsurface is impacted by a  
150 mixture of CAHs. A detailed hydrogeological site characterization and complementary  
151 information about subsurface contamination are available in the supplementary material (SI).  
152 The origin of the contamination was related to an industrial plant where 1,1,1-TCA and TCE  
153 were used as solvents for cleaning and degreasing metal parts since the 60's. In the late 80's,  
154 an environmental survey at the site revealed important subsurface contamination in the north-  
155 eastern part of the plant, where the waste disposal and the delivery zones were located (Fig.  
156 2).

157 The lithology at the site consists of, from top to bottom, Quaternary loess deposits  
158 (from 5 to 18 m thick), a layer of flint conglomerate resulting from chalk alteration and  
159 dissolution (from 4 to 8 m thick), Senonian chinks forming the fractured bedrock aquifer  
160 (thickness of ~ 30 m) and Campanian smectite clay corresponding to the low permeability  
161 basis of the aquifer. The chalk unit can be considered as a dual porosity aquifer composed of  
162 high matrix porosity (up to 45%) and much lower fracture porosity (on the order of 1-5%)  
163 (Brouyère et al. 2004, Orban et al. 2010). Despite the relatively low fracture porosity, its

164 contribution to the hydraulic conductivity is predominant (see SI). In the studied area, the  
165 chalk aquifer is unconfined and the groundwater table is found between 16.9 and 28.6 m  
166 below ground surface, showing an annual fluctuation of up to 2 m and inter-annual variations  
167 of approximately 5 m. Groundwater flows towards north-west and the average hydraulic  
168 gradient is  $\sim 1\%$  (Fig. 2). According to the hydraulic conductivity range determined at the site  
169 by pumping tests (see SI) and assuming an effective porosity of 0.01 (Orban et al. 2010), the  
170 groundwater seepage velocity can be estimated to be  $0.3 - 8.6 \text{ m}\cdot\text{d}^{-1}$  (SI).

171

## 172 **3. MATERIAL AND METHODS**

### 173 **3.1 Groundwater sampling**

174 The sampling methods are described in detail in the SI. Briefly, the field site is equipped with  
175 a groundwater monitoring network consisting of 30 wells situated along the CAHs plume.  
176 Water samples from selected wells (18 wells, Fig. 2A) were collected for chemical and  
177 isotope analysis in February 2011 (first campaign) and March 2013 (second campaign). Prior  
178 to samples collection, monitoring wells were purged (3-5 well volumes) until temperature,  
179 pH, redox potential, electrical conductivity and dissolved oxygen (DO) stabilized. Samples  
180 for CAHs concentration and isotope analysis were collected in 40 mL glass vials closed  
181 without headspace using screw caps with Teflon coated septa, preserved at  $\text{pH} \sim 2$  with  $\text{HNO}_3$   
182 (10%) and stored at  $4^\circ\text{C}$  in the dark until analysis. Concentration analysis of CAHs was  
183 performed within 48h after samples collection.

### 184 **3.2 Chemical and isotope analysis**

185 Detailed descriptions of analytical methods are available in the SI. The DH/HY experiments  
186 preparation and analysis for concentration and isotope ratios are thoroughly described in  
187 Palau et al. (2014b) (see a summary in the SI). Concentration analysis of CAHs in



188 groundwater samples was performed by GC-MS in an accredited commercial laboratory. The  
189 analysis of redox sensitive species in groundwater samples was performed by ion  
190 chromatography (nitrate and sulfate) and atomic absorption spectrometry (iron and  
191 manganese) at the University of Liège.

192 Carbon isotope ratios (i.e.  $^{13}\text{C}/^{12}\text{C}$ ) of 1,1,1-TCA, TCE and 1,1-DCE were determined  
193 by GC-IRMS, whereas chlorine isotope ratios (i.e.  $^{37}\text{Cl}/^{35}\text{Cl}$ ) of 1,1,1-TCA and TCE were  
194 measured by GC-MS (Bernstein et al. 2011, Palau et al. 2014b) at the University of Neuchâtel  
195 (see SI). Isotope ratios of individual compounds were reported using the delta notation, Eq.  
196 (2),

$$197 \quad \delta \text{ } ^h\text{E}_{\text{sample}} = \frac{R(^h\text{E}/\text{}^l\text{E})_{\text{sample}}}{R(^h\text{E}/\text{}^l\text{E})_{\text{standard}}} - 1 \quad (2)$$

198 where R is the isotope ratio of heavy ( $^h\text{E}$ ) to light ( $^l\text{E}$ ) isotopes of an element E (e.g.,  $^{13}\text{C}/^{12}\text{C}$   
199 and  $^{37}\text{Cl}/^{35}\text{Cl}$ ). The  $\delta$  values are usually expressed in per mil. For chlorine, the raw  $\delta^{37}\text{Cl}$   
200 values were obtained by referencing against two external laboratory standards of 1,1,1-TCA  
201 and TCE according to Eq. (2). These standards were dissolved in water and measured  
202 similarly to the samples interspersed in the same sequence (Aeppli et al. 2010). Samples and  
203 standards were diluted to a similar concentration and each of them was measured ten times.  
204 Further details about samples and standards analysis scheme as well as raw  $\delta^{37}\text{Cl}$  values (two-  
205 point) calibration to the standard mean ocean chloride (SMOC) scale are available in the SI.  
206 Precision ( $1\sigma$ ) of the analysis was 0.3‰ for  $\delta^{13}\text{C}$  and 0.4‰ for  $\delta^{37}\text{Cl}$ .

### 207 **3.3. Calculation of substrate remaining fraction**

208 In order to evaluate if the observed isotope pattern of primary compounds and potential  
209 metabolites is related to reactive processes, measured concentrations are transformed to  
210 relative concentrations taking into account reaction equations and related to isotope ratios in  
211 analogy to the Rayleigh equation (Eq. 1). As several reactive processes might occur

212 simultaneously, the slope of such a plot will not necessarily correspond to a specific  
213 laboratory enrichment factor ( $\epsilon_{\text{bulk}}$ ). The substrate remaining fraction (f) at a certain well is  
214 estimated according to Eq. (3) and (4) for 1,1,1-TCA and TCE, respectively:

$$215 \quad f_{1,1,1\text{-TCA}} = \frac{[1,1,1\text{-TCA}]}{[1,1,1\text{-TCA} + \text{HAc} + 1,1\text{-DCE}]} = \frac{[1,1,1\text{-TCA}]}{[1,1,1\text{-TCA} + 3.6 \times 1,1\text{-DCE}]} \quad (3)$$

$$216 \quad f_{\text{TCE}} = \frac{[\text{TCE}]}{[\text{TCE} + 1,1\text{-DCE}]} \quad (4)$$

217 where [1,1,1-TCA] and [TCE] are the aqueous concentration of 1,1,1-TCA and TCE,  
218 respectively, and [1,1,1-TCA + HAc + 1,1-DCE] and [TCE + 1,1-DCE] are the total  
219 concentration of 1,1,1-TCA, TCE and their respective products for the DH/HY and  
220 hydrogenolysis pathways, respectively (Fig. 1). Mole fractions are used instead of absolute  
221 concentrations as the first take into account the effect of dilution. Regarding the  
222 hydrogenolysis products of TCE, *cis*-1,2-DCE is not considered in Eq. (4) as its concentration  
223 in groundwater (up to 14  $\mu\text{g/L}$ ) is much smaller than that of 1,1-DCE (up to 10  $\text{mg/L}$ ). For  
224 1,1,1-TCA, HAc produced by hydrolysis was not analyzed in groundwater samples. In the  
225 aquifer, HAc is readily biodegraded because it is used as electron donor and carbon source by  
226 the microorganisms. Therefore, for  $f_{1,1,1\text{-TCA}}$  the expression  $[1,1,1\text{-TCA}] / [1,1,1\text{-TCA} + 3.6 \times$   
227  $1,1\text{-DCE}]$  is used, which accounts for the produced HAc. The yield of HAc (hydrolysis  
228 product) relative to 1,1-DCE (dehydrohalogenation product) was estimated by first order  
229 curve fitting of concentration-time data series obtained in a previous laboratory study (Palau  
230 et al. 2014b) (see SI). Previous studies showed that hydrolysis of 1,1-DCE in water is  
231 negligible at environmental conditions (Gerken and Franklin 1989, Jeffers et al. 1989). The  
232 uncertainty of the calculated  $f_{1,1,1\text{-TCA}}$  and  $f_{\text{TCE}}$  in the field, i.e. 39% and 17%, respectively,  
233 was estimated by error propagation in Eq. (3) and (4), and an uncertainty of 10% was  
234 assumed for commercial concentration analysis of volatile organic compounds (Hunkeler et  
235 al. 2008).

236

## 237 4. RESULTS AND DISCUSSION

### 238 4.1 Field geochemical conditions and CAHs concentration

239 High DO and nitrate concentrations were measured in groundwater, ranging between 2.6 and  
240 8.4 mg/L for DO and from 51.6 to 94.4 mg/L for nitrate, which indicate the presence of  
241 aerobic conditions in the aquifer. Concentrations of dissolved Mn and dissolved Fe are low ( $\leq$   
242 0.01 and  $\leq$  0.07 mg/L, respectively), which is in agreement with the presence of oxygen.  
243 Aerobic conditions are unfavorable for microbial reductive dechlorination of CAHs.

244 The main CAHs present in groundwater, i.e. 1,1,1-TCA, TCE and 1,1-DCE, are  
245 detected at concentrations  $> 1$  mg/L, reaching a value of up to 20 mg/L for 1,1,1-TCA in the  
246 source area (well E in February 2011) and forming a CAHs plume spreading northwest (see  
247 the 1,1,1-TCA plume in Fig. 2A). Several compounds are detected at lower concentrations,  
248 including 1,1,2-TCA (up to 500  $\mu\text{g/L}$ ), 1,1-DCA (up to 140  $\mu\text{g/L}$ ), 1,2-dichloroethane (1,2-  
249 DCA, up to 270  $\mu\text{g/L}$ ) and *cis*-1,2-dichloroethene (*cis*-1,2-DCE, up to 14  $\mu\text{g/L}$ ), and their  
250 mole fractions relative to the total concentration of chlorinated ethanes and ethenes are  $< 7\%$ .  
251 The presence of 1,1,1-TCA and TCE reductive dechlorination products such as 1,1-DCA and  
252 *cis*-1,2-DCE, respectively (Fig. 1), could be related to the occurrence of micro-anaerobic  
253 environments in the aquifer. The contribution of 1,1,2-TCA dehydrohalogenation to 1,1-DCE  
254 concentration (Fig. 1) is probably very small according to the low molar concentration of  
255 1,1,2-TCA relative to 1,1,1-TCA ( $< 8\%$ ).

256 Concentrations of 1,1,1-TCA, TCE and 1,1-DCE show a similar distribution in the  
257 aquifer and a large concentration range of two orders of magnitude is observed for all of them  
258 in the wells situated close to the plume centerline (Fig. S1, SI). High concentrations of 1,1-  
259 DCE are already present in the wells located in the source area, up to 10 mg/L in well E in

260 February 2011 (Fig. 2A and Fig. S1). Changes in aqueous CAH concentrations in the plume  
261 can be related to transformation processes but also to non-degradative processes such as  
262 hydrodynamic dispersion and sorption. To account for dispersion, relative variations in  
263 1,1,1-TCA, TCE and 1,1-DCE concentrations along the plume can be expressed as mole  
264 fractions. Increasing mole fractions of 1,1-DCE downgradient from the source would be  
265 indicative of 1,1,1-TCA and/or TCE degradation during transport. However, the mole  
266 fractions of 1,1-DCE in several wells located close to the plume centerline show a small  
267 variation relative to [1,1,1-TCA+1,1-DCE], from 0.41 to 0.60 (Fig. 2B and Fig. S2), and the  
268 fractions of 1,1-DCE relative to [TCE+1,1-DCE] are higher for the wells situated close to the  
269 source, i.e. wells E and C (Fig. 2C and Fig. S2). Therefore, additional data is necessary to  
270 confirm the contribution of degradation processes to the observed changes in mole fractions.

#### 271 **4.2 Isotope patterns of 1,1,1-TCA, TCE and 1,1-DCE in the aquifer**

272 The chlorine isotope composition of 1,1,1-TCA in groundwater range from +2.4 to +7.6‰. In  
273 previous studies, chlorine isotope ratios of pure phase 1,1,1-TCA from different  
274 manufacturers showed values ranging from -3.54 to +2.03‰ (Shouakar-Stash et al. 2003).  
275 Compared to the manufacturers' range, the higher range of  $\delta^{37}\text{Cl}$  values in groundwater  
276 suggests that 1,1,1-TCA could be affected by degradation processes. Similarly, the carbon  
277 isotopic composition of 1,1,1-TCA in groundwater, which ranges from -21.1 to -25.1‰ (with  
278 the exception of the value of -26.3‰ measured in the well E in February 2011), is also higher  
279 than the manufacturers' range, which varies between -25.5 and -31.6‰ (Hunkeler and  
280 Aravena 2010), supporting 1,1,1-TCA transformation in the aquifer. To evaluate in more  
281 detail whether the variations of isotope ratios of 1,1,1-TCA in groundwater are due to  
282 degradation,  $\delta^{37}\text{Cl}$  and  $\delta^{13}\text{C}$  values are related to the concentration data according to the  
283 Rayleigh equation (Eq. 1) in Figure 3C-D. Chlorine and carbon isotope ratios of 1,1,1-TCA  
284 exhibit an enrichment in heavy isotopes (i.e.  $^{37}\text{Cl}$  and  $^{13}\text{C}$ ) with decreasing mole fractions,

285 with the exception of data from wells A, E and G (red markers in Fig. 3C-D), confirming that  
286 isotope variations of 1,1,1-TCA are related to its degradation. The  $\delta^{13}\text{C}$  values of 1,1-DCE in  
287 groundwater, from -18.5 to -25.3‰, are generally depleted in  $^{13}\text{C}$  compared to those of 1,1,1-  
288 TCA (Fig. 3D), which is consistent with the abiotic formation of 1,1-DCE from 1,1,1-TCA  
289 via dehydrohalogenation. In addition, this isotope pattern also suggests that 1,1-DCE is not  
290 further degraded in most of the wells.

291 In well A, carbon and chlorine isotopes ratios of 1,1,1-TCA are significantly enriched  
292 in both  $^{13}\text{C}$  and  $^{37}\text{Cl}$ . These higher values could be explained either by a distinct source of  
293 1,1,1-TCA with a heavier isotope composition or by the effect of biodegradation. Relatively  
294 low DO values varying from 1.0 to 1.7 mg/L were measured in this well between 2005 and  
295 2008, which could indicate that micro-anaerobic environments favorable to microbial  
296 reductive dechlorination of 1,1,1-TCA took place at that time and that 1,1,1-TCA affected by  
297 biodegradation is still present in the vicinity of well A. In contrast, for wells E and G,  $\delta^{13}\text{C}_{1,1,1\text{-TCA}}$   
298 values are slightly depleted in  $^{13}\text{C}$  (up to -26.3‰ in E-February 2011), while  $\delta^{37}\text{Cl}_{1,1,1\text{-TCA}}$   
299 values are lightly enriched in  $^{37}\text{Cl}$  (up to +5.8‰ in G-March 2013). Such behavior could be  
300 related to the effect of vaporization and diffusion processes on the residual 1,1,1-TCA  
301 contamination in the unsaturated zone (Jeannotat and Hunkeler 2012). Wells E and G are  
302 located in the vicinity of the source area (Fig. 2A) and previous reports at the site showed that,  
303 when the water level rises, it sometimes reaches highly contaminated parts of the unsaturated  
304 zone in the source area (see SI), leading to a direct input of residual contaminants into the  
305 aquifer. For the remaining 15 out of 18 wells investigated (i.e. B-D, F and H-R), observed  
306 variations with regard to both Cl and C isotope values are well described by a linear trend ( $r^2$   
307  $\geq 0.75$ , Fig. 3C-D). The intercepts of the correlation lines, i.e.  $-0.7 \pm 1.9\%$  for Cl and  $-27 \pm$   
308  $1\%$  for C (the uncertainties were estimated by error propagation in the regression equations  
309 for Cl and C isotope data indicated in Fig. 3C-D), can be considered as an estimate of the

310 initial isotopic composition of 1,1,1-TCA ( $\delta^{37}\text{Cl}_0$  and  $\delta^{13}\text{C}_0$ , respectively, Eq. 1), which agree  
311 very well with the ranges reported for pure 1,1,1-TCA from different manufacturers, i.e.  
312 between -3.54 and +2.03‰ for Cl (Shouakar-Stash et al. 2003) and between -25.5 and -  
313 31.6‰ for C (Hunkeler and Aravena 2010).

314 In order to compare the field and laboratory isotope patterns, the isotope data of 1,1,1-  
315 TCA and 1,1-DCE measured during 1,1,1-TCA transformation by DH/HY in the laboratory  
316 (Palau et al. 2014b) were reevaluated in this study according to Eq. (1) and (3) (Fig. 3A-B). In  
317 general, the field  $\delta^{13}\text{C}$  values of 1,1,1-TCA and 1,1-DCE (Fig. 3D) exhibit a pattern similar to  
318 the one observed in the laboratory batch experiment (Fig. 3B), providing further evidence for  
319 1,1,1-TCA dehydrohalogenation in the aquifer. Compared to the laboratory experiment, the  
320 correlation lines for field isotope data show a smaller slope for Cl, i.e.  $-3.3 \pm 0.8\text{‰}$  (field, Fig.  
321 3C) and  $-4.8 \pm 0.2\text{‰}$  (laboratory, Fig. 3A), and a larger slope for C, i.e.  $-2.5 \pm 0.5\text{‰}$  (field,  
322 Fig. 3D) and  $-1.6 \pm 0.2\text{‰}$  (laboratory, Fig. 3B). However, when taking their uncertainty into  
323 consideration, the slopes for field and laboratory data are relatively similar for both elements.  
324 The larger slope obtained from field carbon isotope data compared to the laboratory DH/HY  
325 experiment can be associated with the simultaneous occurrence of biodegradation processes  
326 of 1,1,1-TCA in addition to DH/HY in the field, which is further investigated using a dual  
327 isotope approach (section 4.3).

328 For TCE, several groundwater samples with different  $\delta^{13}\text{C}$  values (data points labelled  
329 in Fig. 3F) were selected for chlorine isotope analysis, showing similar  $\delta^{37}\text{Cl}$  values (from  
330  $+1.3 \pm 0.4\text{‰}$  to  $+2.1 \pm 0.4\text{‰}$ , Fig. 3E). The  $\delta^{37}\text{Cl}$  values of TCE in groundwater fall within  
331 the reported range of pure TCE from different manufacturers which varies between -3.19 and  
332 +3.90‰ (Hunkeler and Aravena 2010), suggesting little transformation of TCE. The carbon  
333 isotopic composition of TCE varied from -21.6 to -30.0‰, with an average of  $-27 \pm 2\text{‰}$  ( $\pm$   
334  $1\sigma$ ,  $n = 24$ ), except for the wells A (-18.1‰) and K (-34.9‰) on March 2013. As observed for

335 chlorine, most of the  $\delta^{13}\text{C}_{\text{TCE}}$  values fall within the range of TCE from different  
336 manufacturers, i.e. between -24.5 and -33.5‰ (Hunkeler and Aravena 2010), supporting little  
337 degradation of TCE in groundwater. Contrary to the isotope patterns of 1,1,1-TCA,  $\delta^{37}\text{Cl}_{\text{TCE}}$   
338 and  $\delta^{13}\text{C}_{\text{TCE}}$  values do not show any enrichment in  $^{37}\text{Cl}$  and  $^{13}\text{C}$  with decreasing mole  
339 fractions of TCE (Fig. 3E-F), confirming that TCE is not significantly degraded in the aquifer.  
340 This result is in agreement with the aerobic conditions determined in the aquifer. In addition,  
341 the  $\delta^{13}\text{C}$  values of 1,1-DCE are generally enriched in  $^{13}\text{C}$  compared to TCE (Fig. 3F).  
342 According to the normal carbon isotope fractionation of TCE during reductive dechlorination  
343 (Hunkeler and Morasch 2010), the  $\delta^{13}\text{C}$  values of produced 1,1-DCE would be lower than  
344 those of TCE. Therefore, for most of the samples, the observed changes in  $\delta^{13}\text{C}_{\text{TCE}}$  can  
345 probably be associated with some variability in the carbon isotopic composition of source  
346 TCE.

#### 347 **4.3 Dual C-Cl isotope approach to investigate degradation pathways in the field**

348 Carbon and chlorine  $\delta$  isotope values of 1,1,1-TCA in groundwater samples were combined in  
349 a dual isotope plot (Fig. 4). Isotope values from wells A, E and G are not included because, as  
350 indicated above (section 4.2), isotope data from these wells could be affected by processes  
351 different than compound transformation. The plotted data show a linear trend ( $r^2 = 0.75$ ) with  
352 a dual isotope slope ( $\Lambda = \Delta\delta^{13}\text{C}/\Delta\delta^{37}\text{Cl} \approx \epsilon_{\text{bulk}}^{\text{C}}/\epsilon_{\text{bulk}}^{\text{Cl}}$ ) of  $0.6 \pm 0.2$ , confirming that  
353 transformation of 1,1,1-TCA is an important process in the aquifer. This field  $\Lambda$  value is very  
354 different from that determined in a recent laboratory study for oxidation (Fig. 4), clearly  
355 indicating that oxidation cannot be the main process involved (Palau et al. 2014b). In contrast,  
356 the field slope is closer to the one determined for 1,1,1-TCA transformation via DH/HY in the  
357 laboratory ( $0.33 \pm 0.04$ , Fig. 4) (Palau et al. 2014b). The significant difference between the  
358 dual isotope slopes determined for the field and the DH/HY experiment (ANCOVA,  $P =$

359 0.0003) suggests that additional degradation processes of 1,1,1-TCA likely occur in the  
 360 aquifer, as pointed out by the carbon isotope patterns in Figure 3. A higher  $\Lambda$  value ( $1.5 \pm 0.1$ )  
 361 associated with the reduction of 1,1,1-TCA by zero-valent iron was previously reported  
 362 (Palau et al. 2014b), however, significant biotic and/or abiotic reductive dechlorination of  
 363 1,1,1-TCA are discarded due to the aerobic conditions in the aquifer. On the other hand, in  
 364 aerobic conditions, microbial cooxidative degradation of 1,1,1-TCA to 2,2,2-trichloroethanol  
 365 via C-H bond cleavage in the first reaction step has been reported in several studies (Field and  
 366 Sierra-Alvarez 2004, Yagi et al. 1999). The occurrence of microbial oxidation of 1,1,1-TCA  
 367 would be consistent with the different slopes determined from  $\delta^{13}\text{C}_{1,1,1\text{-TCA}}$  data for the field  
 368 and the DH/HY experiment in Figure 3. As observed during abiotic oxidation of 1,1,1-TCA in  
 369 a recent study (Palau et al. 2014b), a much higher isotope effect associated with C-H bond  
 370 cleavage is expected for C compared to Cl. This might explain, taking as a reference the  
 371 slopes determined from the laboratory experiment, the higher slope obtained for C,  $-2.5 \pm$   
 372  $0.5\text{‰}$  (field) and  $-1.6 \pm 0.2\text{‰}$  (laboratory) (Fig. 3B,D), compared to the smaller slope  
 373 observed for Cl,  $-3.3 \pm 0.8\text{‰}$  (field) and  $-4.8 \pm 0.2\text{‰}$  (laboratory) (Fig. 3A,C). Therefore, a  
 374 combination of DH/HY and microbial oxidation may be taking place.

375 In this case, oxidation and DH/HY pathway-specific contributions to total 1,1,1-TCA  
 376 degradation may be estimated using the expression derived by van Breukelen (2007), Eq. (5),

$$377 \quad F = \frac{\Lambda \cdot \varepsilon_{\text{O}}^{\text{Cl}} - \varepsilon_{\text{O}}^{\text{C}}}{(\varepsilon_{\text{D/H}}^{\text{C}} - \varepsilon_{\text{O}}^{\text{C}}) - \Lambda (\varepsilon_{\text{D/H}}^{\text{Cl}} - \varepsilon_{\text{O}}^{\text{Cl}}} \quad (5)$$

378 where  $F$  is the distribution of DH/HY and oxidation pathways,  $\varepsilon_{\text{D/H}}^{\text{C}}$  and  $\varepsilon_{\text{D/H}}^{\text{Cl}}$  are the C and Cl  
 379 isotope fractionation values during DH/HY of 1,1,1-TCA and  $\varepsilon_{\text{O}}^{\text{C}}$  and  $\varepsilon_{\text{O}}^{\text{Cl}}$  correspond to the C  
 380 and Cl isotope fractionation values for 1,1,1-TCA oxidation. For this equation, in addition to  
 381 the  $\varepsilon_{\text{bulk}}$  values of 1,1,1-TCA for both reactions involved, only the dual isotope slope  
 382 determined from field data ( $\Lambda = 0.6 \pm 0.2$ ) is necessary. The  $\varepsilon_{\text{bulk}}^{\text{C}}$  and  $\varepsilon_{\text{bulk}}^{\text{Cl}}$  values of 1,1,1-



383 TCA during DH/HY and oxidation reactions were reported in a recent study (Palau et al.  
384 2014b), showing values of  $-1.6 \pm 0.2\text{‰}$  and  $-4.7 \pm 0.1\text{‰}$  (DH/HY),  $-4.0 \pm 0.2\text{‰}$  and no  
385 chlorine isotope fractionation (Oxidation). In this previous study, the isotope fractionation  
386 values of 1,1,1-TCA during oxidative C-H bond cleavage were determined abiotically by  
387 reaction with persulfate. Chlorine isotope fractionation values for microbial oxidation of  
388 1,1,1-TCA are still not available in the literature, however, isotope fractionation values  
389 determined from abiotically mediated oxidation may be used as a rough approximation. In  
390 fact, isotope fractionation values from abiotic reactions are often considered closest to the  
391 intrinsic isotope effects (Lollar et al. 2010). According to the reported reaction-specific  $\epsilon_{\text{bulk}}$   
392 values, the contribution of DH/HY was of  $80 \pm 10\%$  (the uncertainty was estimated by error  
393 propagation in Eq. (5)). This result indicates a relatively small contribution of the oxidation  
394 pathway, provided that the  $\epsilon_{\text{bulk}}$  values for microbial oxidation of 1,1,1-TCA by indigenous  
395 microorganisms at the site are confirmed in future biodegradation studies. Eq. (5) assumes  
396 simultaneous activity of both pathways, which is a likely assumption in our case judging by  
397 the good linear correlation between  $\delta^{37}\text{Cl}$  and  $\delta^{13}\text{C}$  values (Fig. 4).

398 The expected rate of 1,1,1-TCA degradation by DH/HY at the measured groundwater  
399 temperature can be estimated using the Arrhenius equation, Eq. (6),

$$400 \quad k = A \cdot \exp(-E_a/RT) \quad (6)$$

401 where  $k$  is the first order rate constant ( $\text{s}^{-1}$ ),  $A$  is the frequency factor ( $\text{s}^{-1}$ ),  $R$  is the gas  
402 constant ( $8.314 \times 10^{-3} \text{ kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ ),  $E_a$  is the activation energy ( $\text{kJ} \cdot \text{mol}^{-1}$ ) and  $T$  is the absolute  
403 temperature (K). According to the  $E_a$  ( $122.8 \text{ kJ} \cdot \text{mol}^{-1}$ ) and  $A$  ( $8.7 \times 10^{13} \text{ s}^{-1}$ ) values determined  
404 by Gauthier and Murphy (2003) from several previous studies and the average groundwater  
405 temperature at the site ( $284 \pm 1 \text{ K}$ ,  $\pm 1\sigma$ ,  $n = 34$ ), the transformation rate is estimated to be  
406  $1.95 \times 10^{-4} \text{ d}^{-1}$  (i.e. half-life of around 10 years). This slow reaction rate contrasts with the  
407 relatively fast groundwater seepage velocity in the saturated zone (up to  $8.6 \text{ m} \cdot \text{d}^{-1}$ ), suggesting

408 that significant contaminant retardation would be necessary to explain the high concentrations  
409 of 1,1-DCE in the source area. In this site, owing to the high chalk matrix porosity (up to  
410 45%), 1,1,1-TCA is probably subject to retardation by diffusion into the matrix pore water  
411 (Parker et al. 1997). In addition to degradation of 1,1,1-TCA in the saturated zone,  
412 dehydrohalogenation of 1,1,1-TCA to 1,1-DCE might also occur in the unsaturated part of the  
413 aquifer (up to 28 m thick). Here, downward migration for dissolved compounds in  
414 groundwater was estimated at  $\sim 1 \text{ m}\cdot\text{y}^{-1}$  by different studies (Brouyère et al. 2004, Orban et al.  
415 2010). Degradation of 1,1,1-TCA in the unsaturated zone is supported by the detection of 1,1-  
416 DCE in relatively high concentrations in soil samples from the unsaturated zone analyzed in  
417 previous reports (see SI).

418

## 419 5. CONCLUSIONS

420 The demonstration and evaluation of CAHs degradation processes is necessary to predict their  
421 fate and long-term impact on contaminated sites. The chlorine and carbon isotopic  
422 composition of 1,1,1-TCA exhibited clear correlations with its varying mole fractions,  
423 revealing the contribution of degradation processes of 1,1,1-TCA in the aquifer. Dual C-Cl  
424 isotope data showed that, while the slope obtained from field samples is very different from  
425 that seen in the laboratory for oxidation, the field  $\Lambda$  value is closer to the one determined for  
426 DH/HY, pointing to DH/HY as the dominant degradation pathway. In addition, the observed  
427 deviation from the dual isotope trend expected for DH/HY suggests the occurrence of  
428 additional degradation processes of 1,1,1-TCA in groundwater. A minor contribution of  
429 microbial cooxidation of 1,1,1-TCA via C-H bond cleavage could be a feasible explanation  
430 according to the isotope results and the aerobic conditions of the aquifer. Contrary to 1,1,1-  
431 TCA, the chlorine and carbon isotopic composition of TCE suggest little degradation, which  
432 is in agreement with the aerobic conditions and the product concentration analysis.

433           Considering the time scale of cost-efficient contaminant remediation strategies like  
434 monitored natural attenuation, low rate abiotic reactions such as DH/HY have the potential to  
435 contribute significantly to 1,1,1-TCA attenuation in contaminated sites. However, low rate  
436 transformation processes are typically difficult to monitor and to evaluate based on  
437 concentration measurements only. This study shows that the dual C-Cl isotope analysis is a  
438 valuable tool to assess degradation pathways of 1,1,1-TCA in the field. Such information is  
439 crucial to improve contaminant attenuation estimates and to delineate adequate remediation  
440 strategies.

441

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633

634 **Figure captions:**

635 **Fig. 1** Degradation pathways of chlorinated ethanes and ethenes. Aerobic biodegradation  
636 pathways are not shown. In grey color are indicated the compounds and pathways that are not  
637 mentioned explicitly in the text. Compounds in *bold* represent the main contaminants  
638 investigated in this study. *Dotted lines* show reactions that can be both abiotically or biotically  
639 mediated whereas *solid lines* indicate biodegradation pathways (Culubret et al. 2001, Field  
640 and Sierra-Alvarez 2004) and *dashed lines* abiotic transformations catalyzed by iron bearing  
641 minerals (Butler and Hayes 2000, Han et al. 2012, Lee and Batchelor 2002) and zero valent  
642 iron (Arnold and Roberts 2000, Hara et al. 2005, Song and Carraway 2005). For chlorinated  
643 ethanes, abiotic hydrolysis and dehydrohalogenation in water are included (Jeffers et al. 1989,  
644 Scheutz et al. 2011). Chlorinated and non-chlorinated acetylenes and higher molecular  
645 compounds (i.e. C<sub>4</sub>-C<sub>6</sub>) produced during metal catalyzed reactions are not shown. (a)  
646 hydrogenolysis, (b) dihaloelimination, (c) dehydrohalogenation, (d) hydrolysis, (e)  
647 hydrogenation and (f) reactions that proceed via  $\alpha$ -elimination. (d\*) Transformation of CA to  
648 HAc evolves via hydrolysis of CA to ethanol and subsequent fermentation to HAc.

649  
650 **Fig. 2** (A) Site map and groundwater monitoring wells network. Dashed lines correspond to  
651 the groundwater surface (in m above sea level) and contour lines depict the 1,1,1-TCA  
652 concentrations in the aquifer. (B) Total concentration (i.e. 1,1,1-TCA+1,1-DCE) (right y-axis,  
653 line) and concentration of 1,1,1-TCA and 1,1-DCE normalized by the total concentration (left  
654 y-axis, bars). (C) Total concentration (i.e. TCE+1,1-DCE) (right y-axis, line) and  
655 concentration of TCE and 1,1-DCE normalized by the total concentration (left y-axis, bars).  
656 The data indicated were obtained in March 2013 but similar concentration patterns were  
657 observed in February 2011 (Fig. S2, SI).

658  
659 **Fig. 3** A-B) Isotopic composition of 1,1,1-TCA ( $\delta^{37}\text{Cl}$  and  $\delta^{13}\text{C}$ ) and 1,1-DCE ( $\delta^{13}\text{C}$ ) during  
660 DH/HY of 1,1,1-TCA in batch experiments. Data from duplicate experiments are combined  
661 (i.e. rhombus and circle symbol marks). C-D) Isotopic composition of 1,1,1-TCA ( $\delta^{37}\text{Cl}$  and  
662  $\delta^{13}\text{C}$ ) and 1,1-DCE ( $\delta^{13}\text{C}$ ) in groundwater samples. Dashed lines correspond to the 95%  
663 confidence intervals of regression parameters. E-F) Isotopic composition of TCE ( $\delta^{37}\text{Cl}$  and



664  $\delta^{13}\text{C}$ ) and 1,1-DCE ( $\delta^{13}\text{C}$ ) in groundwater samples. For field samples, data from both  
665 sampling campaigns are combined, i.e. rhombus (February 2011) and circles (March 2013).

666 **Fig. 4.** Dual C-Cl isotope trends during transformation of 1,1,1-TCA in the investigated test  
667 site and in two experimental systems. Data from both campaigns are combined (blue circles).  
668  $\Lambda$  values ( $\pm 95\%$  C.I.) are given by the slope of the linear regressions and the black dashed  
669 lines correspond to the 95% C.I. Shaded areas (95% C.I) indicate exclusive occurrence of  
670 either one of the two pathways.