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# Combining multi-phase flow and pathway-specific reactive transport modeling to investigate the impact of water table fluctuations on dichloromethane biodegradation

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1           **Combining multi-phase flow and pathway-specific reactive transport**  
2           **modeling to investigate the impact of water table fluctuations on**  
3           **dichloromethane biodegradation**

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21

22 **Abstract**

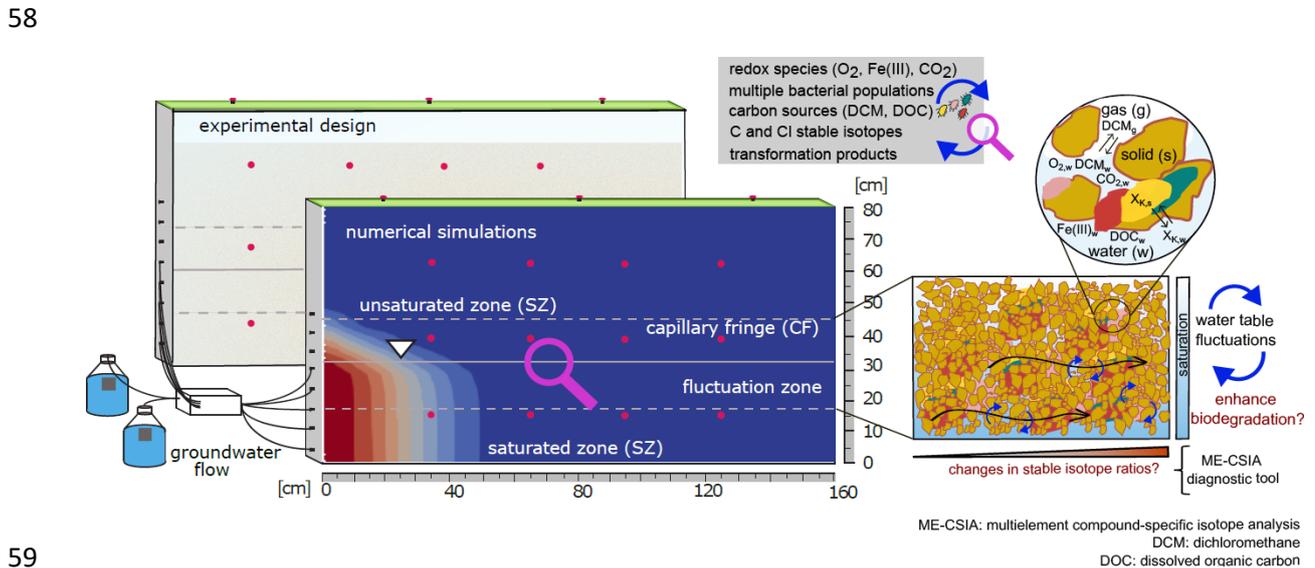
23 Water table fluctuations play a significant role in the redistribution of chemical species,  
24 microorganisms and organic pollutants in aquifers by creating favorable zones for reactive  
25 processes. This in turn is expected to affect the extent and pathways of biodegradation of  
26 halogenated contaminants such as dichloromethane (DCM). Here, a multi-phase flow reactive  
27 transport model (RTM) was developed to identify the main drivers of DCM biodegradation in  
28 aquifers under steady-state and transient conditions. The RTM includes a description of multi-  
29 phase flow, redox conditions, four characteristic bacterial populations and stable isotopologues  
30 (i.e.,  $^{13}\text{C}/^{12}\text{C}$  and  $^{37}\text{Cl}/^{35}\text{Cl}$ ) of DCM according to pathway-specific reactions. Dissolved organic  
31 carbon (DOC) was also included as a carbon source for non-DCM degrading populations  
32 yielding a more realistic heterotrophic groundwater microbial community. Numerical  
33 simulations of eight model scenarios were compared with experimental results from laboratory  
34 aquifers. Key biogeochemical processes involved in DCM biodegradation were captured,  
35 particularly across the capillary fringe. Only model scenarios of DCM degradation by multiple  
36 bacterial metabolisms were able to reproduce the dynamics observed in the laboratory aquifers.  
37 The observed and computed enrichment of  $^{13}\text{C}$  and  $^{37}\text{Cl}$  isotopes over time confirmed enhanced  
38 DCM biodegradation during water table fluctuations and highlighted interactions between  
39 different bacterial metabolisms. In particular, our RTM suggested that heterotrophic  
40 groundwater bacteria played a key role in regulating  $\text{O}_2$ -depletion and redox conditions across  
41 fluctuation zones during parallel DOC mineralization. The produced  $\text{CO}_2$  favored metabolic  
42 activity of anaerobic DCM degraders requiring  $\text{CO}_2$  for their specific fermentative pathways.  
43 This study underscores the added value of integrating multi-phase flow, stable isotopes and  
44 distinct bacterial populations to understand natural attenuation of DCM in contaminated  
45 groundwater.

46 **Keywords:** multi-phase flow, reactive transport modeling, CSIA, bacterial populations, DCM,  
47 water table fluctuations

48 **Highlights**

- 49 • A multi-phase flow reactive transport model was developed to examine DCM  
50 biodegradation
- 51 • The model captured biogeochemical processes during DCM biodegradation under  
52 steady-state and transient conditions
- 53 • Heterotrophic groundwater bacteria favored anaerobic DCM degraders requiring  $\text{CO}_2$
- 54 • DCM degradation by multiple bacterial populations reproduced the DCM dynamics  
55 observed in laboratory aquifers

56  
57 **Graphical abstract**



## 60 1. Introduction

61 Reactive transport in aquifers involves a complex interplay between multi-phase flow,  
62 transport of elements and pollutants, chemical reactions and microbial activities (Carrera et al.,  
63 2022; Meile & Scheibe, 2019). Water table fluctuations can affect the transfer of volatile  
64 organic pollutants (McCarthy & Johnson, 1993; Werner & Höhener, 2002), distribution of  
65 redox species (Haberer et al., 2012; Rezanezhad et al., 2014; Seybold et al., 2002) and  
66 composition of microbial communities (Jost et al., 2015a; Pronk et al., 2020; Rühle et al., 2015).  
67 These biogeochemical variations can support reactive processes in aquifers that may alter the  
68 degradation extent and pathways of common organic pollutants (Prieto-Espinoza et al., 2021).  
69 However, variations of biogeochemical conditions and pollutant transformation during water  
70 table fluctuations remain difficult to predict quantitatively under field conditions, restricting the  
71 accuracy of long-term monitoring of contaminated sites (Zhang & Furman, 2021).

72 Natural attenuation in contaminated aquifers largely relies on the ability of  
73 microorganisms to oxidize organic pollutants while reducing available electron acceptors (e.g.,  
74  $O_2$ ,  $NO_3^-$ ,  $Fe^{3+}$ ) (Meckenstock et al., 2015). Monitoring hydrochemical conditions including  
75 contaminant concentrations and contaminant-degrading bacterial populations may help to  
76 identify active contaminant transformation processes and prevailing degradation pathways in  
77 aquifers. The extent of degradation is nonetheless difficult to determine owing to the  
78 simultaneous effect of degrading and nondegrading (i.e., dilution, volatilization, sorption)  
79 processes on overall contaminant mass dissipation. An additional line of evidence to identify  
80 the occurrence of *in-situ* degradation may be obtained with compound-specific isotope analysis  
81 (CSIA). CSIA has proven useful to quantify degradation processes of halogenated contaminants  
82 under both laboratory (Audí-Miró et al., 2013; Hermon et al., 2018; Renpenning et al., 2014)  
83 and field conditions (Blázquez-Pallí et al., 2019; Palau et al., 2016; Wiegert et al., 2012). CSIA  
84 relies on changes in stable isotope ratios (e.g.,  $^{13}C/^{12}C$ ) produced during a given degradation

85 reaction in which bond cleavage of molecules containing light isotopes occurs preferentially  
86 over those bearing one or more heavy isotopes (Elsner, 2010), resulting in an enrichment of  
87 heavy isotopes in the remaining contaminant fraction. When changes of stable isotope ratios of  
88 two elements involved in the same bond cleavage reaction (e.g.,  $^{13}\text{C}/^{12}\text{C}$  and  $^{37}\text{Cl}/^{35}\text{Cl}$ ) are  
89 compared in a dual plot, the resulting slope ( $\Delta$ ) provides a quantitative parameter of the  
90 corresponding contaminant transformation pathway or mechanism (Höhener & Imfeld, 2020;  
91 Ojeda et al., 2020).

92 The relevance of multi-element CSIA (ME-CSIA) was recently demonstrated when  
93 investigating the effect of water table fluctuations on dichloromethane (DCM) degradation in  
94 laboratory aquifers fed with anoxic contaminated groundwater (Prieto-Espinoza et al., 2021).  
95 DCM ( $\text{CH}_2\text{Cl}_2$ ) is a toxic industrial halogenated solvent frequently detected in multi-  
96 contaminated aquifers, and included in the list of priority pollutants of the U.S. (ASTDR, 2019)  
97 and the European Commission (European Commission, 2013). ME-CSIA of DCM (i.e.,  $^{13}\text{C}/^{12}\text{C}$   
98 and  $^{37}\text{Cl}/^{35}\text{Cl}$ ) evidenced larger extent of DCM degradation in laboratory aquifers during water  
99 table fluctuations compared to steady-state conditions, and suggested distinct anaerobic DCM  
100 degradation pathways. Bacterial populations belonging to the anaerobic *Peptococaceae* family  
101 such as *Desulfosporosinus*, *Dehalobacterium* and *Dehalobacter* spp. which include DCM-  
102 degrading strains displayed higher relative population size during transient conditions and  
103 localized across the fluctuation zone and CF. This suggested oxygen tolerance of these taxa  
104 during water table fluctuations and emphasized the role of different bacterial populations during  
105 DCM biodegradation (Blázquez-Pallí et al., 2019; Hermon et al., 2018; Wright et al., 2017).

106 To understand the complex interplay between biogeochemical variations and  
107 contaminant degradation in aquifers subjected to water table fluctuations, multi-phase flow  
108 reactive transport models (RTMs) may integrate CSIA data and distinct bacterial populations  
109 directly or indirectly involved in contaminant degradation. Several RTMs relying on CSIA data

110 have been successfully applied to evidence contaminant degradation, particularly for  
111 chlorinated ethenes (CEs), further demonstrating the promising insights of CSIA in RTMs  
112 (Antelmi et al., 2021; Badin et al., 2018; D’Affonseca et al., 2011; Höhener, 2016; Hunkeler et  
113 al., 2009; Kuder et al., 2016; Prommer et al., 2009). Nevertheless, such RTMs are often applied  
114 in oversimplified configurations (e.g., batch case, steady-state, single bacteria, etc.) compared  
115 to field complexity. While advanced numerical approaches have enabled capturing dynamics  
116 across the CF (Hron et al., 2015), RTMs accounting for ME-CSIA are still needed to unravel  
117 the effect of water table fluctuations on common halogenated solvents at contaminated sites.

118         Here, we developed and implemented a mathematical formalism describing multi-  
119 component and multi-phase flow reactive transport of DCM in aquifers, including the  
120 description of six DCM isotopologues (i.e., accounting for  $^{13}\text{C}/^{12}\text{C}$  and  $^{37}\text{Cl}/^{35}\text{Cl}$ ) and distinct  
121 DCM-degrading and nondegrading bacterial populations. The objective of this study was to  
122 apply the developed integrative RTM on the experimental setup described in Prieto-Espinoza  
123 et al. (2021). Eight model scenarios, including abiotic and biotic conditions, were examined to  
124 distinguish the contribution of volatilization and degradation processes on DCM mass  
125 dissipation across the aquifer during steady-state (i.e., static water table) and transient (i.e.,  
126 fluctuating water table) conditions. For all biotic scenarios, DCM degradation was examined  
127 using different combinations of bacterial degrading populations, including heterotrophic  
128 groundwater bacterial populations, and numerical results were assessed using experimental  
129 data.

## 130 **2. Model formalisms**

### 131 **2.1 Experiment**

132 Experiments were conducted in two laboratory aquifers with inner dimensions of 1.60  
133 m × 0.80 m × 0.05 m (length × height × width) and filled with sterile quartz sand (grain size:  
134 0.4 - 0.6 mm). A detailed description of the experimental setup is provided in Prieto-Espinoza  
135 et al. (2021) and as Supporting Information (SI, Section A). Briefly, aquifers were covered by  
136 a top glass mounted with 3 sampling ports opened to the atmosphere (Figure 1). Both aquifers  
137 were continuously fed with O<sub>2</sub>-depleted contaminated groundwater (O<sub>2</sub> < 1 mg/L) and kept  
138 under constant N<sub>2</sub>-flux. Prior to the experiments, an incubation period was established by  
139 operating both aquifers with a continuous flow of DCM-spiked groundwater collected from a  
140 former industrial site (Hermon et al., 2018). This allowed to reach initial steady-state conditions.  
141 The water table was positioned at a depth of 0.33 m from the bottom of both aquifers. During  
142 the experimental phase, one aquifer remained under steady-state conditions while the second  
143 aquifer underwent two water table fluctuation events (Figure 1). Hydrochemical species, DCM,  
144 stable isotopes and bacteria were followed before and after each water table fluctuation event,  
145 and at sampling ports located at 0.15 m and 0.40 m from the bottom. For a detailed description  
146 of chemical, isotopes and bacterial analyses we refer to Prieto-Espinoza et al. (2021).

### 147 **2.2 Main model assumptions**

148 The model formalism includes a state-of-the-art description of three DCM-degrading  
149 bacterial populations with partly known DCM degradation pathways (Chen et al., 2018, 2020;  
150 Holland et al., 2019; Kleindienst et al., 2017, 2019; Mägli et al., 1996, 1998) and associated  
151 stable carbon and chlorine isotope fractionation (Chen et al., 2018; Lee et al., 2015) (Table 1).  
152 The model only considers anaerobic DCM-degrading bacteria (*Peptococaceae* family) as O<sub>2</sub>-  
153 depleted conditions prevailed in the laboratory aquifers (Prieto-Espinoza et al., 2021). Abiotic  
154 DCM degradation is not considered in the model due to substantial evidence of biodegradation

155 as the main DCM dissipation process in contaminated aquifers (Blázquez-Pallí et al., 2019;  
156 Hermon et al., 2018; Wright et al., 2017). A non-DCM degrading bacterial population which  
157 mineralizes dissolved organic carbon (DOC) and produces CO<sub>2</sub> was additionally included, as  
158 CO<sub>2</sub> production by heterotrophic groundwater microorganisms may impact DCM degradation  
159 (Chen et al., 2020). Hereafter, this generic population will be named ‘heterotrophic’ population  
160 as it may use many different organic compounds (DOC) as carbon sources.

161 Simulations were carried out for both steady-state and transient conditions. Mass  
162 exchange of six DCM isotopologues was implemented assuming the same rate of gas-phase  
163 partitioning and volatilization. A first-order kinetic exchange was also assumed between the gas  
164 and water phases for O<sub>2</sub> and CO<sub>2</sub> (Hron et al., 2015). Isotope fractionation of DCM caused by  
165 diffusion was not accounted for in the model as it is typically negligible compared to  
166 degradation processes (Bouchard et al., 2008; Jeannotat and Hunkeler, 2013). Similarly, DCM  
167 sorption and its associated isotope fractionation was not accounted for in the model due to its  
168 low log K<sub>ow</sub> (<1.25) (Merlin et al., 1992). Consumption of all dissolved chemical species was  
169 assumed to occur in the water phase only and following a dual-Monod approach.  
170 Transformation products of each pathway-specific reaction were also followed, including Cl<sup>-</sup>,  
171 acetate and formate (Chen et al., 2020). Finally, interactions of dissolved chemical species  
172 including electron acceptors (EA) and transformation products (TP) with the solid phase were  
173 neglected.

### 174 **2.3 Multi-phase flow and transport**

175 The model considers two phases ( $\alpha$ ): a gaseous phase  $g$  and an aqueous phase  $w$  (for  
176 water). The two phases are considered incompressible. Each of these phases have their own  
177 volumetric mass density  $\rho_\alpha$  [kg/m<sup>3</sup>] and saturation  $s_\alpha$ . The saturations must satisfy the closure  
178 relation  $\sum_\alpha s_\alpha = 1$ . The mass conservation equation for the flow of each  $\alpha$  phase is:

179  $\partial_t(\phi s_\alpha) + \nabla \cdot (\mathbf{v}_\alpha) = \mathbf{Q}_\alpha$  (1)

180 where  $\mathbf{v}_\alpha$  is the mean pore velocity [m/s] expressed by the Darcy Muskat's law following

181  $\mathbf{q}_\alpha = \phi s_\alpha \mathbf{v}_\alpha$ ,  $\phi$  is the porosity [-], and  $\mathbf{Q}_\alpha$  is a source term [m<sup>3</sup>/s].

182 For each component  $i$  present in  $\alpha$  phase, the advection-dispersion equations describing  
 183 reactive transport and mass transfer exchange are written as:

184  $\partial_t(\epsilon_\alpha c_{\alpha,i}) + \nabla \cdot (\epsilon_\alpha c_{\alpha,i} \mathbf{v}_\alpha + \epsilon_\alpha \mathbf{j}_{\alpha,i}) = -\epsilon_\alpha r_{\alpha,i} + \sum_{\beta \neq \alpha} e_{\alpha,i}^{\alpha\beta}$  (2)

185 where  $c_{\alpha,i}$  is the mass concentration of component  $i$  in the  $\alpha$  phase [kg/m<sup>3</sup>],  $\mathbf{j}_{\alpha,i}$  is the intrinsic  
 186 mass dispersion vector [kg/m<sup>2</sup>/s],  $r_{\alpha,i}$  is the reactive rate [kg/m<sup>3</sup>/s] and  $e_{\alpha,i}^{\alpha\beta}$  is the rate of mass  
 187 transfer of component  $i$  into the  $\alpha$  phase at the  $\alpha\beta$  interface (dissolution) [kg/m<sup>3</sup>/s]. The volume  
 188 fraction occupied by the  $\alpha$  phase is defined as  $\epsilon_\alpha = \phi s_\alpha$ .

189 The Fick's law was used to describe diffusion and dispersion fluxes  $\mathbf{j}_{\alpha,i}$  together with  
 190 the second model of Millington and Quirk for the dependence of the effective diffusion  
 191 coefficient on phase saturation (Millington & Quirk, 1961). For mechanical interactions of the  
 192 fluid with the porous media causing mixing of solutes across the two phases, a  
 193 diffusion/dispersion tensor was used to estimate the corresponding fluxes:

194  $\mathbf{j}_{\alpha,i} = -\left( s_\alpha^{10/3} \phi^{4/3} D_{\alpha,i}^{mol} \mathbf{I} + \alpha_t |\mathbf{q}_\alpha| \mathbf{I} + (\alpha_l - \alpha_t) \frac{\mathbf{q}_\alpha \mathbf{q}_\alpha}{|\mathbf{q}_\alpha|} \right) \nabla c_{\alpha,i}$  (3)

195 where  $\alpha_l$  and  $\alpha_t$  are the longitudinal and transverse dispersivities [m], respectively, and  $D_{\alpha,i}^{mol}$  is  
 196 the molecular diffusion of component  $i$  in  $\alpha$  phase [m<sup>2</sup>/s].

197 Due to capillary effects, average pressure fields of each phase are not equal. A  
 198 macroscale capillary pressure  $p_c$ , depending on the water saturation  $s_w$ , was thus defined as  $p_c$   
 199  $= p_n - p_w$ , where  $p_n$  is the pressure of the non-wetting phase and  $p_w$  the pressure of the wetting  
 200 phase [Pa]. The  $p_c$  values were obtained following the Brooks-Corey model (Brooks & Corey,

201 1965). A description of mass-momentum conservation equations for the CF is provided as SI  
 202 (Section B).

## 203 2.4 Mass exchange across the capillary fringe

204 The porous media is considered partially saturated with water and gas phases with a  
 205 mixture of DCM, O<sub>2</sub> and CO<sub>2</sub>. In the case of local non-equilibrium, volatilization of component  
 206 *i* from the aqueous (*w*) to the gaseous (*g*) phase (and conversely the leaching of component *i*  
 207 present in the gas phase by re-solubilization), corresponding to the adimensional Henry's  
 208 constant, is given by (Hron et al., 2015):

$$209 \quad e_{g,i}^{wg} = \epsilon_g \Gamma_{volat,i} \left( p_i^{sat} \frac{M_{mol,i}}{c_i^{eq} RT} \rho_w \omega_{w,i} - \rho_g \omega_{g,i} \right) = -e_{w,i}^{wg} \quad (4)$$

210 where  $\Gamma_{volat,i}$  is the mass transfer coefficient by volatilization [1/s],  $c_i^{eq}$  is the water solubility  
 211 [kg/m<sup>3</sup>],  $M_{mol,i}$  is the molar mass [kg/mol] and  $p_i^{sat}$  is the saturated vapor pressure of component  
 212 *i* (pure form) [Pa]. Phase composition (i.e., mass concentration) is relative to the mass fraction  
 213 of component *i* in  $\alpha$  phase (i.e.,  $\omega_{\alpha,i}$ ) and the mass phase density (i.e.,  $\rho_w$  and  $\rho_g$ ).

## 214 2.5 Pathway-specific reaction rates and dual isotope fractionation

215 Evolution of carbon and chlorine stable isotopes of DCM during biodegradation  
 216 reactions was simulated accounting for dual-element isotopologues. The relative abundance of  
 217 the six DCM isotopologues, considering the occurrence of both stable C and Cl isotopes, can  
 218 be calculated with a binomial distribution (Jin et al., 2018):

$$219 \quad A_j = \binom{1}{a} X^a (1 - X)^{1-a} \binom{2}{b} Y^b (1 - Y)^{2-b} \quad (5)$$

220 where  $A_j$  is the relative abundance of the  $j^{\text{th}}$  DCM isotopologues containing  $a$  <sup>13</sup>C out of a total  
 221 of one carbon atom and  $b$  <sup>37</sup>Cl out of a total of two chlorine atoms.  $X$  and  $Y$  are the relative  
 222 abundances of heavy C and Cl isotopes, respectively. Double layer brackets represent classical  
 223 binomial coefficients. The initial C-Cl DCM isotopologues abundance was computed according

224 to initial isotopic signatures of DCM of  $\delta^{13}\text{C}_0 = -46.38 \pm 0.37\text{‰}$  and  $\delta^{37}\text{Cl}_0 = 3.68 \pm 0.10\text{‰}$ ,  
 225 and the international standards of C (VPDB,  $R_{VPDB}^0 = 0.0112372$ ) and Cl (SMOC,  $R_{SMOC}^0 =$   
 226  $0.319766$ ) isotope ratios (Coplen, 2011; Kaufmann et al., 1984):

$$227 \quad R_{E_0}^h = \left( \frac{\delta^h E_0}{1000} + 1 \right) R_{E,std}^0 \quad (6)$$

$$228 \quad A_{E_0}^h = \frac{R_{E_0}^h}{1 + R_{E_0}^h} \quad (7)$$

229 where  $R_{E_0}^h$  is the initial DCM isotope ratio,  $\delta^h E_0$  is the initial DCM isotopic signature, and  
 230  $R_{E,std}^0$  is the international standard of element  $E$ .  $A_{E_0}^h$  is the relative abundance of stable heavy  
 231 isotopes of element  $E$ , so that the abundance of light isotopes of  $E$  is given by  $A_{E_0}^l = 1 - A_{E_0}^h$ .  
 232 Note that the terms  $X$  and  $Y$  in equation 5 correspond to the abundances of heavy C and Cl  
 233 isotopes, respectively, calculated with equation 7.

234 During a given reaction of an organic contaminant the stable isotopes present in the bond  
 235 cleavage are primarily affected (position-specific isotopes). Position-specific isotope  
 236 fractionation factors for the  $j^{\text{th}}$  DCM isotopologues are thus calculated to account for the number  
 237 of atoms at the reactive position (Elsner, 2010):

$$238 \quad \alpha_E^\kappa = \frac{1}{AKIE_E^\kappa} \approx 1 + \frac{n_E}{z_E} \varepsilon_E^\kappa \quad (8)$$

239 where  $\alpha_E^\kappa$  is the fractionation factor of element  $E$  (i.e., C and Cl) at the reactive position.  $\varepsilon_E^\kappa$  is  
 240 here referred to as the *bulk* enrichment factor [in ‰],  $n_E$  is the number of atoms of element  $E$ ,  
 241 and  $z_E$  is the number of atoms at the reactive position. In the case of DCM, chemically composed  
 242 by 1 C atom and 2 Cl atoms, we consider that  $n_C = a = 1$  and  $n_{Cl} = b = 2$ . Reactive positions will  
 243 thus be affected with  $z_C = 1$  and  $z_{Cl} = 2$ .

244 Let's  $\kappa$  be the  $\kappa$ -bacterial population involved in the pathway-specific degradation  
 245 reaction of DCM (section 2.6). The C and Cl isotope fractionation factors for each  $\kappa$ -bacterial

246 population are assigned to pathway-specific degradation reactions (Jin et al., 2018). These  
 247 fractionation factors affect overall reaction rates of each  $j^{\text{th}}$  position-specific isotopologue of  
 248 DCM (see Eq. 17).

$$249 \quad \alpha_j^\kappa = \alpha_c^{\kappa,a_j} \alpha_{Cl}^{\kappa,b_j} \quad (9)$$

250 The  $\Lambda^{C/Cl}$  values associated with changes of C *versus* Cl isotope signatures provide  
 251 evidence of the corresponding DCM degradation pathway or mechanism by the  $\kappa$ -bacterial  
 252 population, and can be calculated as (Höhener & Imfeld, 2021; Ojeda et al., 2020):

$$253 \quad \Lambda^{C/Cl} = \frac{\ln[(\delta^{13}C_t/1000+1)/(\delta^{13}C_0/1000+1)]}{\ln[(\delta^{37}Cl_t/1000+1)/(\delta^{37}Cl_0/1000+1)]} \approx \frac{\varepsilon_{bulk}^C}{\varepsilon_{bulk}^{Cl}} \quad (10)$$

254 Finally, the degradation extent of DCM ( $B$ , in %) was estimated according to Van  
 255 Breukelen (2007) using a modified version of the Rayleigh equation in which the rate ratio of  
 256 two competing degradation pathways can be accounted for.

$$257 \quad B (\%) = \left( 1 - \left( \frac{\delta^h E_t + 1000}{\delta^h E_0 + 1000} \right)^{\frac{1000}{(F\varepsilon_E^{\kappa,1} + (1-F)\varepsilon_E^{\kappa,2})}} \right) \times 100 \quad (11)$$

258 Here,  $F$  is the contribution factor of a degradation pathway concomitantly transforming DCM  
 259 as a function of the apparent  $\Lambda^{C/Cl}$  and  $\varepsilon_E^\kappa$  values of element  $E$  associated with the two pathways  
 260 (Van Breukelen, 2007):

$$261 \quad F = \frac{\Lambda^{C/Cl} \varepsilon_{Cl}^{\kappa,2} - \varepsilon_C^{\kappa,2}}{(\varepsilon_C^{\kappa,1} - \varepsilon_C^{\kappa,2}) - \Lambda^{C/Cl} (\varepsilon_{Cl}^{\kappa,1} - \varepsilon_{Cl}^{\kappa,2})} \quad (12)$$

## 262 **2.6 DCM biodegradation by distinct $\kappa$ -bacterial populations**

263 Three distinct anaerobic DCM degradation pathways were considered to represent the  
 264 activity of co-occurring DCM-degraders in polluted aquifers (Table 1) (Blázquez-Pallí et al.,  
 265 2019; Hermon et al., 2018; Lee et al., 2015; Wright et al., 2017). These correspond to anaerobic  
 266 DCM degradation by simulated populations of (i) *Dehalobacter*-containing culture under iron-

267 reducing conditions (Lee et al., 2015) Dhber ( $X_1$ ), (ii) *Dehalobacterium formicoaceticum* (Chen  
268 et al., 2020; Kleindienst et al., 2019) Dhbium ( $X_2$ ), and (iii) *Candidatus Dichloromethanomonas*  
269 *elyunquensis* (Chen et al., 2018, 2020; Kleindienst et al., 2019) Diely ( $X_3$ ). Mineralization of  
270 DOC was also considered and mediated by heterotrophic non-DCM degrading bacteria denoted  
271  $B_{DOC}$  ( $X_4$ ) under both aerobic and iron-reducing conditions. Such microorganisms produce  $CO_2$   
272 as final product, which can be further utilized during DCM metabolism by Dhbium and Diely  
273 (Chen et al., 2020).

274 **Table 1.**  $\kappa$ -bacterial populations involved in anaerobic DCM degradation, and corresponding pathway-specific reactions based on stable carbon  
 275 and chlorine isotope fractionation values. The results of laboratory aquifers under steady-state and transient conditions are also provided for  
 276 comparison.

277

Name	$\kappa$ -bacterial populations	$\epsilon_C$ (‰) <sup>a</sup>	$\epsilon_{Cl}$ (‰) <sup>a</sup>	$\Lambda^{Cl}$ <sup>a</sup>	Degradation pathway	Redox reactions	Source
<b><i>Simulated anaerobic DCM degrading populations</i></b>							
X <sub>1</sub> (Dhber)	<i>Dehalobacter</i> containing culture	-15.5±1.5	-5.3±0.1 <sup>b</sup>	2.92 <sup>c</sup>	Fermentation	$CH_2Cl_2 + 2H_2O + Fe^{3+} \rightarrow CO_2 + Fe^{2+} + 6H^+ + 6Cl^-$	Lee et al., 2012, 2015
X <sub>2</sub> (Dhbium)	<i>Dehalobacterium formicoaceticum</i>	-42.4±0.7	-5.3±0.1	7.89±0.12	Fermentation harboring WLP <sup>d</sup>	$3CH_2Cl_2 + 4H_2O + CO_2 \rightarrow 2HCOO^- + CH_3COO^- + 9H^+ + 6Cl^-$	Chen et al., 2018, 2020
X <sub>3</sub> (Diely)	Consortium RM harboring <i>Ca. Dichloromethanomonas elyunquensis</i>	-18.3±0.2	-5.2±0.1	3.40±0.03	Mineralization (acetogenesis required) <sup>e</sup>	$CH_2Cl_2 + 2H_2O \rightarrow CO_2 + 2H_2 + 2Cl^- + 2H^+$ $4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+$	Chen et al., 2018, 2020
<b><i>Simulated heterotrophic groundwater bacterial population (DOC assimilation)</i></b>							
X <sub>4</sub> (BDOC)	Heterotrophic bacteria	-	-	-	Aerobic respiration	$CH_2O + O_2 \rightarrow CO_2 + H_2O$	Rolle et al., 2008
		-	-	-	Iron reduction <sup>f</sup>	$CH_2O + Fe^{3+} + H_2O \rightarrow Fe^{2+} + CO_2 + 4H^+$	Rolle et al., 2008
<b><i>Laboratory aquifers</i></b>							
Transient conditions	<i>Dehalobacter, Dehalobacterium,</i>	-11.8±2.0	-3.1±0.6	3.58±0.42	Prevailing anaerobic	n.a. <sup>h</sup>	Prieto-Espinoza et al., 2021
Steady-state conditions	<i>Desulfosporosinus</i> <sup>g</sup>	-23.6±3.2	-8.7±1.6	1.92±0.30	Prevailing anaerobic	n.a.	Prieto-Espinoza et al., 2021

<sup>a</sup> Uncertainties of  $\epsilon$  and  $\Lambda$  values correspond to the 95% confidence interval (CI).

<sup>b</sup> Assumed  $\epsilon_{Cl}$  (‰) value for a *Dehalobacter* containing culture.

<sup>c</sup> Estimation of  $\Lambda^{Cl}$  was based on assumed Cl isotope fractionation.

<sup>d</sup> Wood Ljungdhal Pathway (Ragsdale et al., 2008).

<sup>e</sup> DCM mineralization by *Ca. D. elyunquensis* requires the presence of H<sub>2</sub>-consuming partner populations.

<sup>f</sup> This model assumes the presence of aqueous Fe<sup>3+</sup> species.

<sup>g</sup> Most abundant taxa observed in the laboratory aquifers.

<sup>h</sup> n.a.: values were not analyzed/derived.

278 Stoichiometry of redox reactions were used to approximate biomass growth and  
 279 evolution of redox species (Barry et al., 2002; Rittmann, 2006) based on pathway-specific  
 280 degradation reactions of DCM (Chen et al., 2018, 2020; Lee et al., 2015). All bacterial  
 281 populations relied on the availability of dissolved O<sub>2</sub>, Fe(III) and/or CO<sub>2</sub> species. Growth  
 282 equations (Table 2) were estimated assuming that 50% of the carbon sources (i.e., DCM and  
 283 CO<sub>2</sub>) were required for the synthesis of new biomass (C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N) (Eckert et al., 2012). A detailed  
 284 description of growth stoichiometry reactions is given in the SI (Section C).

285

286 **Table 2.** Estimated growth reactions of  $\kappa$ -bacterial populations for DCM and DOC degradation.

Name	Growth reactions
X <sub>1</sub> (Dhber)	$\text{CH}_2\text{Cl}_2 + 2\text{Fe}^{3+} + 1.2\text{H}_2\text{O} + 0.1\text{NH}_4^+ \rightarrow 0.5\text{CO}_2 + 0.1\text{C}_5\text{H}_7\text{O}_2\text{N} + 2\text{Fe}^{2+} + 4.1\text{H}^+ + 2\text{Cl}^-$
X <sub>2</sub> (Dhbium)	$\text{CH}_2\text{Cl}_2 + 0.9\text{H}_2\text{O} + 0.15\text{CO}_2 + 0.1\text{NH}_4^+ \rightarrow 0.1\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.35\text{HCOO}^- + 0.15\text{CH}_3\text{COO}^- + 2.7\text{H}^+ + 2\text{Cl}^-$
X <sub>3</sub> (Diely)	$\text{CH}_2\text{Cl}_2 + 0.88\text{H}_2\text{O} + 0.1\text{NH}_4^+ \rightarrow 0.1\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.17\text{CO}_2 + 0.17\text{CH}_3\text{COO}^- + 0.33\text{H}_2 + 2.29\text{H}^+ + 2\text{Cl}^-$
X <sub>4</sub> (B <sub>DOC,ox</sub> )	$\text{CH}_2\text{O} + 0.5\text{O}_2 + 0.1\text{NH}_4^+ \rightarrow 0.5\text{CO}_2 + 0.1\text{C}_5\text{H}_7\text{O}_2\text{N} + 0.1\text{H}^+ + 0.8\text{H}_2\text{O}$
X <sub>4</sub> (B <sub>DOC,anoX</sub> )	$\text{CH}_2\text{O} + 2\text{Fe}^{3+} + 0.2\text{H}_2\text{O} + 0.1\text{NH}_4^+ \rightarrow 0.5\text{CO}_2 + 0.1\text{C}_5\text{H}_7\text{O}_2\text{N} + 2\text{Fe}^{2+} + 2\text{H}^+$

287

## 288 2.7. Modeling of bacterial dynamics

289 Active bacterial growth was set in both the aqueous and on the solid (s) phases, and  
 290 depended on the presence of carbon substrates (i.e., DCM and DOC) and electron acceptors  
 291 (i.e., O<sub>2</sub>, Fe(III), CO<sub>2</sub>). Total bacterial concentration ( $c_{X_\kappa}$ ) in the aquifer was thus defined as:

$$292 \quad c_{X_\kappa} = c_{w,X_\kappa} + c_{s,X_\kappa} \frac{\epsilon_s}{\epsilon_w} \quad (13)$$

293 where  $c_{w,X_\kappa}$  and  $c_{s,X_\kappa}$  are the bacterial concentrations in the aqueous phase (i.e., planktonic)  
 294 [kg/m<sup>3</sup>] and on the solid phase (i.e., attached) [kg/m<sup>3</sup>], respectively. Planktonic biomass in the  
 295 aqueous phase was considered using the classical advection-dispersion formulation (Eq. 2). The  
 296 biomass bound to the solid matrix was assumed immobile and its volume sufficiently low to  
 297 avoid changes in the permeability and clogging. The solid phase mass balance equation for  $\kappa$ -  
 298 bacterial solid concentrations  $c_{s,X_\kappa}$  is given by Eq. 14. Evolution of biomass on the solid phase

299 is affected by bacterial attachment-detachment processes (Section 2.7.1) and growth kinetics  
 300 (Section 2.7.2; Eq. 25).

$$301 \quad \frac{\partial \epsilon_s c_{s,X_\kappa}}{\partial t} = -r_{s,X_\kappa} \quad (14)$$

### 302 **2.7.1 Bacterial attachment-detachment in porous media**

303 In order to evaluate  $\kappa$ -bacterial transport and attachment in the saturated and unsaturated  
 304 porous media, bacterial adhesion to sand grains was included following a reversible formulation  
 305 involving an adhesion term  $a_{w,X_\kappa}$  from the aqueous phase (Hron et al., 2015):

$$306 \quad a_{w,X_\kappa} = -\epsilon_w k_\kappa^{att} \psi_\kappa c_{w,X_\kappa} + \epsilon_s k_\kappa^{det} c_{s,X_\kappa} \quad (15)$$

307 where  $k_\kappa^{att}$  and  $k_\kappa^{det}$  are the first order deposition and detachment coefficients for the  $\kappa$ -bacterial  
 308 population [1/s], respectively.  $\psi_\kappa$  is a dimensionless [-] deposition function that relates adhesion  
 309 to solid surface with water saturation (Hron et al., 2015):

$$310 \quad \psi_\kappa = 1 - \frac{c_{s,X_\kappa}}{s_w c_{s,X_\kappa}^{max}} \quad (16)$$

311 where  $c_{s,X_\kappa}^{max}$  is the  $\kappa$ -specific maximum attainable bacterial concentration on the solid phase  
 312 [kg/m<sup>3</sup>].

### 313 **2.7.2 Bacterial growth kinetics**

314 Growth kinetics of  $\kappa$ -bacterial populations are limited by the availability of dissolved  
 315 oxygen ( $c_{w,O_2}$ ), ferric iron ( $c_{w,Fe}$ ), carbon dioxide ( $c_{w,CO_2}$ ), DOC ( $c_{w,DOC}$ ) and bioconvertible  
 316  $j^{\text{th}}$  isotopologues of DCM ( $c_{w,j}$ ). Both planktonic and attached bacteria are able to consume  
 317 substrates. New biomass is considered immediately mobile in the aqueous phase and can attach  
 318 onto solid grains. Specific bacterial growth rates were based on the Contois model (Contois,  
 319 1959). The Contois modification describes variations of half-saturation constants in Monod  
 320 models during one growth cycle (i.e., the dependence of  $\mu_{max}$  on the biomass density)

321 (Kovárová-Kovar & Egli, 1998). Pathway-specific reaction rates based on C and Cl isotopes  
 322 were also introduced for  $\kappa$ -bacterial populations involved in DCM degradation ( $\kappa = 1, 2, 3$ ) (see  
 323 Section 2.5).

$$324 \quad r_j^\kappa = \mu_{max}^k \frac{\alpha_C^{\kappa,a_j} \alpha_{Cl}^{\kappa,b_j} c_{w,j}}{c_{X_\kappa} B_{DCM}^\kappa + c_{w,DCM}} \frac{c_{w,EA_\kappa}}{c_{X_\kappa} B_{EA}^\kappa + c_{w,EA_\kappa}} \quad (17)$$

325 where  $\mu_{max}^k$  is the specific growth rate [1/s] of the  $\kappa$ -specific degradation pathway involving  
 326 iron-reduction, fermentation or anaerobic mineralization of DCM (Table 3).  $c_{w,DCM}$  is the total  
 327 DCM concentration, such as  $c_{w,DCM} = \sum_j^N c_{w,j}$ , where  $j=1, \mathcal{N}$  with  $\mathcal{N}=6$  is the total number of  
 328 DCM isotopologues. The constant  $B$  [-] multiplied by  $c_{X_\kappa}$ , introduced as the Contois  
 329 modification, corresponds to the half-saturation constant in Monod kinetics. Electron acceptors  
 330 (EA) for each degradation pathway are introduced with a dual Monod term. Oxygen inhibition  
 331 terms were not accounted for in the model due to high abundance (up to 80%) of putative  
 332 anaerobic bacterial populations in the fluctuation zone and CF of the laboratory aquifers (Prieto-  
 333 Espinoza et al., 2021). This high bacterial abundance coincided with DCM metabolic activity,  
 334 indicative of tolerance to temporal  $O_2$  variations associated with water table fluctuations.

335 For DOC assimilation, it was assumed that bacterial growth occurs in the presence of  
 336 both  $O_2$  and ferric iron (Hron et al., 2015).

$$337 \quad r_{w,DOC}^{ox} = \mu_{max}^{ox} \frac{c_{w,DOC}}{c_{X_4} B_{DOC}^4 + c_{w,DOC}} \frac{c_{w,O_2}}{c_{X_4} B_{O_2}^4 + c_{w,O_2}} \quad (18)$$

$$338 \quad r_{w,DOC}^{anox} = \mu_{max}^{anox} \frac{c_{w,DOC}}{c_{X_4} B_{DOC}^4 + c_{w,DOC}} \frac{c_{w,Fe}}{c_{X_4} B_{Fe}^4 + c_{w,Fe}} \quad (19)$$

339 The global reactive terms for the  $j^{\text{th}}$  isotopologues of DCM and DOC and the  
 340 consumption of EA during degradation of both carbon sources by the corresponding  $\kappa$ -bacterial  
 341 population have the following form:

$$342 \quad r_{w,j} = \sum_{\kappa=1}^3 \frac{r_j^\kappa}{Y_{X_\kappa/j}} c_{X_\kappa} \quad (20)$$

$$343 \quad r_{w,DOC} = \left( \frac{r_{w,DOC}^{ox}}{Y_{X_4/DOC}^{ox}} + \frac{r_{w,DOC}^{anox}}{Y_{X_4/DOC}^{anox}} \right) c_{X_4} \quad (21)$$

$$344 \quad r_{w,EA} = \sum_{\kappa=1}^3 E_{EA/j}^\kappa \frac{r_j^\kappa}{Y_{X_\kappa/j}} c_{X_\kappa} + \left( E_{EA/DOC}^{4,ox} \frac{r_{w,DOC}^{ox}}{Y_{X_4/DOC}^{ox}} + E_{EA/DOC}^{4,anox} \frac{r_{w,DOC}^{anox}}{Y_{X_4/DOC}^{anox}} \right) c_{X_4} \quad (22)$$

345 For each transformation product (i.e.,  $Cl^-$ , acetate, formate and  $CO_2$ ), the corresponding  
 346 reactive term has the form:

$$347 \quad r_{w,TP} = - \sum_j^N \sum_\kappa Y_{TP/j}^\kappa \frac{r_j^\kappa}{Y_{X_\kappa/j}} c_{X_\kappa} - \left( Y_{TP/DOC}^{4,ox} \frac{r_{w,DOC}^{ox}}{Y_{X_4/DOC}^{ox}} + Y_{TP/DOC}^{4,anox} \frac{r_{w,DOC}^{anox}}{Y_{X_4/DOC}^{anox}} \right) c_{X_4} \quad (23)$$

348 where yield factor coefficients  $Y_{X_\kappa/j}$  [biomass  $X_\kappa$  produced in g per mass of organic substrate  $j$   
 349 biodegraded in g],  $E_{EA/j}^\kappa$  [g of electron acceptor ( $EA$ ) consumed per mass of organic substrate  $j$   
 350 biodegraded in g] and  $Y_{TP/j}$  [g of transformation product (TP) produced per mass of organic  
 351 substrate  $j$  biodegraded in g] are associated with pathway-specific reaction rates (Table 3). The  
 352 second terms of Eq. 22 and Eq. 23 are only valid for species affected by  $B_{DOC}$  ( $X_4$ ; Table 3).

353 The multi-phase flow reactive transport equations of DCM biodegradation by three  
 354 distinct DCM-degrading populations and in the presence of heterotrophic groundwater bacterial  
 355 populations are presented in the SI (Section D). The reactive terms of the  $\kappa$ -bacterial  
 356 populations, denoting biomass growth and decay ( $k_\kappa^{dec}$ ; constant rate) in the aqueous and on  
 357 the solid phases, are given by:

$$358 \quad r_{w,X_\kappa} = -\epsilon_w \sum_j^N r_j^\kappa \left( 1 - \frac{c_{X_\kappa}}{c_{X_\kappa}^{max}} \right) - a_{w,X_\kappa} \quad (24)$$

$$359 \quad r_{s,X_\kappa} = \epsilon_s k_\kappa^{dec} c_{s,X_\kappa} + a_{w,X_\kappa} \quad (25)$$

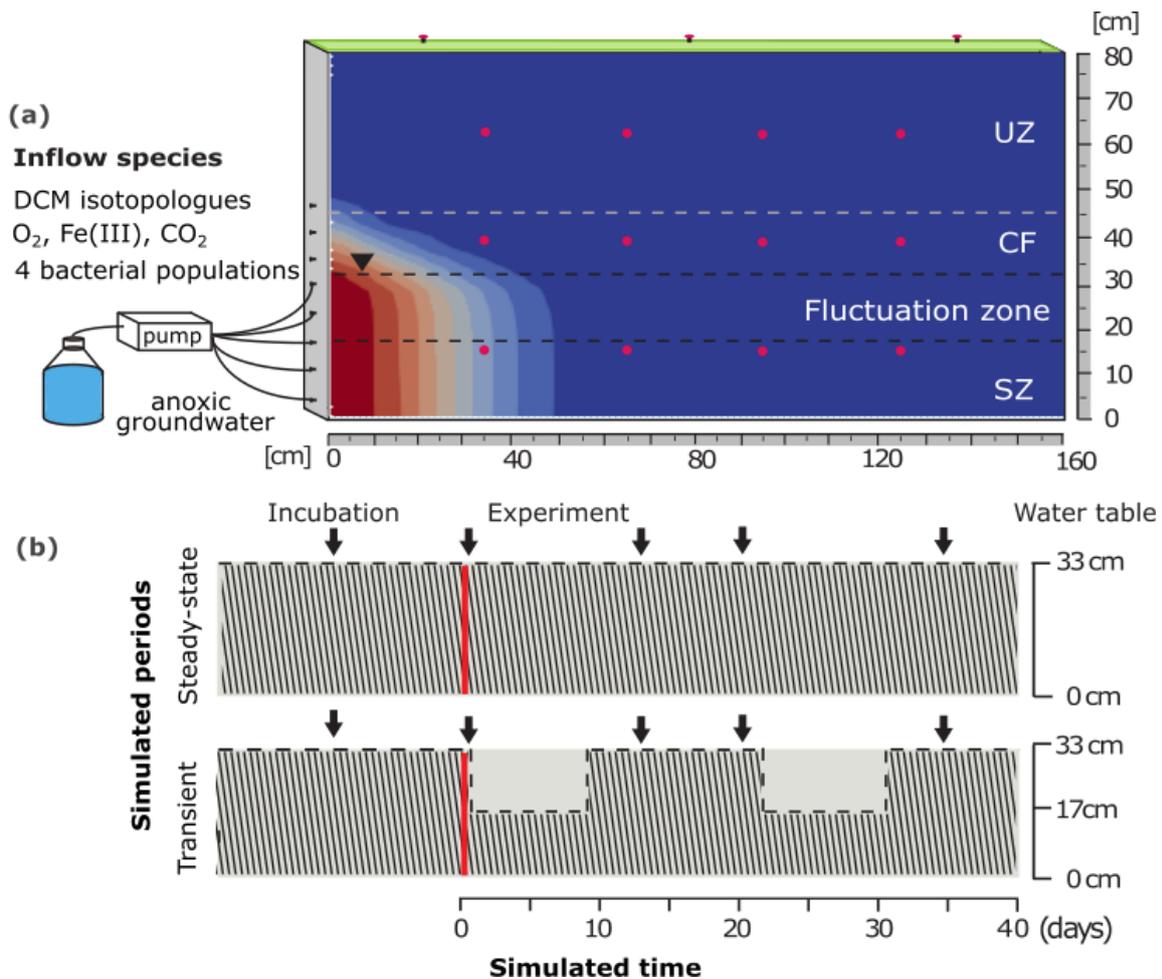
### 360 **3. Modeling approach**

#### 361 **3.1 Numerical resolution**

362 A detailed description of the numerical resolution is described in the SI (Section E). The  
363 flow is solved by an IMPES scheme (IMplicit in gas Pressure and Euler Explicit in water  
364 Saturation) with a finite volume discretization centered on the nodes (CVFE). The linear solver  
365 for the gas phase is a preconditioned conjugate gradient (PCG). A Courant criterion is imposed  
366 for saturation (0.5) allowing higher time steps ( $\sim 2.7$  sec) for explicit resolution of water  
367 saturation and to take capillary diffusion and gravity effects into account. Following the Method  
368 Of Line (MOL) framework, the transport (non-symmetric matrix) is solved with a large-scale  
369 preconditioned differential-algebraic solver using a Krylov method (DASPK) (Brown et al.,  
370 1994) and by performing an adaptive time-step strategy based on interpolation of previous time  
371 solutions (Backward Differentiation Formula ‘BDF’ scheme). A linear solver for the non-  
372 symmetric matrix is used following a preconditioned biconjugate gradient method  
373 (PBiCGStab). A variant called cubicIsotopFoam integrating a distributed parallelization is  
374 implemented (PStream library of OpenFoam 9 solver; Weller et al., 1998) to handle massively  
375 parallel cases. The numerical code is integrated in a user interface coded in python (3.10.2) and  
376 based on the Qt 15.2 library (Qt group, 2023) initially coded with DISLIN (Michels, 1998).

#### 377 **3.2 Model domain and boundary conditions**

378 Model simulations were carried out in 2D on a tank with the same dimensions as the  
379 laboratory aquifers (Figure 1 and Section 2.1). Hydrodynamic parameters (porosity, capillary  
380 parameters,  $K_{sat}$ ) were determined using  $k_r-p_c$  curves (data not shown). The modeled tank had  
381 1808 elements of size  $5 \times 5$  cm. Coordinates along x-axis of the headspace ports were 0.20 m,  
382 0.80 m and 1.4 m, with 0.05 m width. Atmospheric pressure and equilibrium gas concentrations  
383 of O<sub>2</sub> and CO<sub>2</sub> were imposed at the top of the domain.



384

385 Figure 1. Simulated model domain based on the laboratory aquifers setup. (a) Schematic  
 386 overview of the aquifers fed with anoxic groundwater spiked with DCM (flow path from left to  
 387 right), indicating the saturated zone (SZ), unsaturated zone (UZ), fluctuation zone, position of  
 388 sampling ports (red dots), and top boundary layer (green) with three sampling ports opened to  
 389 the atmosphere. (b) Simulated steady-state and transient periods. Water table level is indicated  
 390 as dotted lines. The end of the incubation period (total 60 days) is marked with a red line.  
 391 Simulations of the two flow regimes (experimental phase) are performed for the period between  
 392 0 and 40 days. Black arrows show sampling events of water and gas samples in the experiments  
 393 at 0, 13, 20 and 35 days.

394

395 Initial conditions were set by simulating a free drainage starting from a situation where  
 396 the porous medium was saturated with anoxic water free of DCM (isotopologues) and  $O_2$ .  
 397 Dissolved biomass, ferric iron and DOC were initially present only during biotic simulations to  
 398 initiate DCM biodegradation across the CF. Initial concentrations of all species corresponded  
 399 to those imposed at the inflow of the laboratory aquifers. The biomass attached onto the solid

400 phase was initially set to zero. The saturation level at the top of the domain was maintained at  
401 a residual saturation  $s_{wr}$  of 8.7%. The fluctuation zone was set in the geometry with boundaries  
402 positioned at 0.17 m from the bottom, corresponding to the lower water level kept in the  
403 experiments (Figure 1). All domain boundaries, excluding inlet and outlet sides, were  
404 impermeable for the aqueous phase (i.e., zero Neumann boundary condition). For the gas phase,  
405 all but the top sides were impermeable.

406 Parameters for all simulations and inflow concentrations followed the experimental  
407 setup (Table 3 and SI, Section F). A total of 100 days were simulated including an incubation  
408 phase of 60 days followed by an experimental phase of 40 days (Figure 1). The simulated time  
409 is described from day 0 (i.e., end of the 60-day incubation period) until 40 days (i.e., end of the  
410 experiment) (Figure 1b). Continuous injection of DCM (40 mg/L), DOC (100 mg/L), ferric iron  
411 (3 mg/L), O<sub>2</sub> (0.25 mg/L) and biomass  $X_x$  (0.01 mg/L) were set at the inflow of the aquifer.  
412 After the incubation period, simulations of steady-state consisted on a continuous DCM  
413 injection for 40 days (Figure 1b). For transient conditions, the first lowering of the water table  
414 took place at day 0 (i.e., end of incubation period) for 1 day, followed by injection of DCM and  
415 dissolved species for 7 days at a now low water table ( $z = 0.17$  m). The water table was then  
416 raised to its original position ( $z = 0.33$  m) for 1 day and injection of dissolved species was kept  
417 until day 21 (Figure 1b). A similar second water table fluctuation occurred from days 21 to 30  
418 and continuous injections followed until the end of the experiment.

419 Table 3. Kinetic parameters of pathway-specific reactions of DCM degradation according to  $j^{\text{th}}$   
 420 isotopologues of DCM and electron acceptors (EA) and during concomitant DOC assimilation.  
 421 When a  $\kappa$ -bacterial population is absent its corresponding aqueous concentration was set to  
 422 zero.

Kinetic parameters	$\kappa$ -bacterial populations DCM degradation			Facultative heterotroph for DOC assimilation	
	Dhber (X <sub>1</sub> )/Fe <sup>3+</sup>	Dhbium (X <sub>2</sub> )/CO <sub>2</sub>	Diely (X <sub>3</sub> )/CO <sub>2</sub>	B <sub>DOC</sub> (X <sub>4,ox</sub> )/O <sub>2</sub>	B <sub>DOC</sub> (X <sub>4,anox</sub> )/Fe <sup>3+</sup>
$\mu_{max}^{\kappa}$ [1/s]	$2.0 \times 10^{-6}$	$1.0 \times 10^{-6}$	$1.0 \times 10^{-6}$	$1.0 \times 10^{-5}$	$1.0 \times 10^{-7}$
$k_{\kappa}^{dec}$ [1/s]	$2.3 \times 10^{-9}$	$2.3 \times 10^{-9}$	$2.3 \times 10^{-9}$	$2.3 \times 10^{-9}$	$2.3 \times 10^{-9}$
$k_{\kappa}^{att}$ [1/s]	$3.0 \times 10^{-4}$	$3.0 \times 10^{-4}$	$3.0 \times 10^{-4}$	$3.0 \times 10^{-4}$	$3.0 \times 10^{-4}$
$k_{\kappa}^{det}$ [1/s]	$6.2 \times 10^{-6}$	$6.2 \times 10^{-6}$	$6.2 \times 10^{-6}$	$6.2 \times 10^{-6}$	$6.2 \times 10^{-6}$
$E_{EA,i}$ [g of EA consumed/g of C source degraded]	1.315	0.078	0.088	0.533	3.723
$Y_{X,\kappa/i}$ [g of biomass/g of C source degraded]	0.133	0.133	0.133	0.027	0.027
$Y_{CO_2/i}$ [g of CO <sub>2</sub> produced/g of C source degraded]	0.965	-	-	0.733	0.733
$Y_{Cl,j}$ [g of Cl <sup>-</sup> produced/g of $j^{\text{th}}$ DCM degraded]	0.835	0.835	0.835	-	-
$Y_{HCOO^-}$ [g of HCOO <sup>-</sup> produced/g of $j^{\text{th}}$ DCM degraded]	-	0.243	0.118	-	-
$Y_{CH_3COO^-}$ [g of CH <sub>3</sub> COO <sup>-</sup> produced/g of $j^{\text{th}}$ DCM degraded]	-	0.080	-	-	-
$B_i^{\kappa}$ [-]	3	3	3	-	-
$B_{DOC}^{\kappa}$ [-]	-	-	-	1.8	1.8
$B_{EA}^{\kappa}$	0.019	0.019	0.019	0.019	0.019

423

### 424 3.3 Modeled scenarios

425 Multi-phase flow and transport – without reactions – was first tested under steady-state  
 426 (model A) and transient (model B) conditions to examine the ability of the model to reproduce  
 427 spatial and temporal dynamics of DCM and O<sub>2</sub>. These simulations allowed to quantify the  
 428 extent of DCM volatilization from the saturated zone (SZ) to the unsaturated zone (UZ) under  
 429 both flow regimes. For simulations where reactions were present, anaerobic DCM degradation  
 430 was tested by one or several of the  $\kappa$ -bacterial degrading populations in the presence of the non-  
 431 DCM degrader B<sub>DOC</sub> (Table 1). These simulations allowed to examine (i) redox dynamics  
 432 driven by individual  $\kappa$ -bacterial degrading populations under steady-state conditions (models  
 433 C, D and E), (ii) concomitant DCM degradation under steady-state conditions (models F and  
 434 G), and (iii) concomitant DCM degradation under transient conditions (models H and I). The  
 435 main kinetic parameters and modeled scenarios are provided in Tables 3 and 4, respectively.  
 436 Numerical simulations are successively presented in the results section from scenarios A to I.

437 Table 4. Description of the eight modeled scenarios. Models A and B were compared with O<sub>2</sub>  
 438 data from the laboratory aquifers, while models G and I were compared with experimental data  
 439 of DCM concentrations and stable C and Cl isotopes of DCM.

<b>Transport scenarios</b>			
<b>Scenario</b>	<b>Conditions</b>	<b>k-bacterial population</b>	<b>Purpose</b>
Model A	Steady	abiotic transport	Multi-phase flow of O <sub>2</sub> and CO <sub>2</sub> across the capillary fringe and DCM volatilization
Model B	Transient	abiotic transport	
<b>Reactive transport scenarios</b>			
<b>Scenario</b>	<b>Conditions</b>	<b>k-bacterial population</b>	<b>Purpose</b>
Model C	Steady	Dhber and B <sub>DOC</sub>	Linking redox conditions, heterotrophic groundwater bacteria and DCM biodegradation by $\kappa$ -bacterial populations under steady-state
Model D	Steady	Dhbium and B <sub>DOC</sub>	
Model E	Steady	Diely and B <sub>DOC</sub>	Insights from multiple $\kappa$ -bacterial populations and heterotrophic groundwater bacteria during DCM biodegradation under static vs. water table fluctuations
Model F	Steady	Dhbium, Diely and B <sub>DOC</sub>	
Model G	Steady	Dhber, Dhbium, Diely and B <sub>DOC</sub>	
Model H	Transient	Dhbium, Diely and B <sub>DOC</sub>	
Model I	Transient	Dhber, Dhbium, Diely and B <sub>DOC</sub>	

440

### 441 3.4 Evaluation strategy and model validation

442 The numerical methods implemented in this work allow to obtain mechanistic details on  
 443 multi-phase flow and reactive transport processes affecting DCM dissipation in the laboratory  
 444 aquifers and particularly across the CF and the fluctuation zone. In total, the 2D-model included  
 445 29 non-linear coupled equations with 74 parameters, accounting for the evolution of  $\alpha$  phase  
 446 saturation, capillary pressure, 8 reactive species in both aqueous and gas phases (i.e., the six  
 447 DCM isotopologues, O<sub>2</sub> and CO<sub>2</sub>), 9 species in the aqueous phase (i.e., Fe(III), DOC, Cl<sup>-</sup>,  
 448 acetate, formate, and 4 planktonic bacterial populations), and 4 bacterial populations on the  
 449 solid phase (attached biomass).

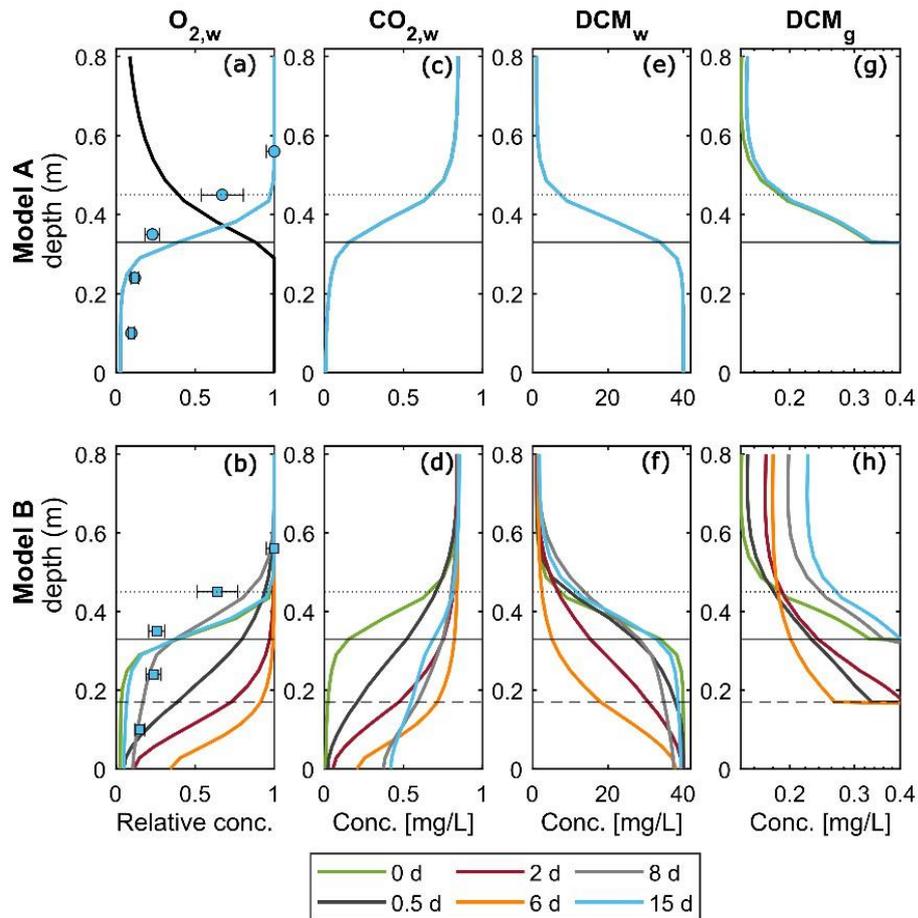
450 The calibration of this model based on experimental results presented in Prieto-Espinoza  
 451 et al., (2021) was beyond the scope of this work. This would require long-term investment  
 452 owing to the large number of parameters, sensitivity of model outputs, and constraints of CPU  
 453 time (Execution time: 7186 s). Nevertheless, comparisons between numerical model outputs  
 454 and laboratory experiments without model calibration relating to multi-phase flow across the  
 455 CF are suitable for a first optimization (Hron et al., 2015), which is the scope of the present  
 456 study. The model was thus validated based on its ability to capture the trends of key variables

457 observed in previous laboratory experiments. This may help to understand the processes  
458 affecting DCM biodegradation during water table fluctuations.

## 459 **4. Results**

### 460 **4.1 Multi-phase flow across the capillary fringe**

461 Mass exchange across the CF was examined for O<sub>2</sub>, CO<sub>2</sub> and DCM for the non-reactive  
462 transport cases under both steady-state (model A) and transient (model B) conditions. The  
463 water-retention curve of the aquifer model was well described according to the Brooks and  
464 Corey parametrization (Brooks and Corey, 1965), resulting in a sharp increase of the water  
465 content across the CF (black line; Figure 2a). The numerical model captured aqueous O<sub>2</sub> profiles  
466 formed during O<sub>2</sub> diffusion from the top of the aquifer toward the steady anoxic SZ (Figure 2a).  
467 Limited O<sub>2</sub> transfer was observed at high water saturation levels. Contrastingly, water table  
468 fluctuations resulted in a re-oxygenation of the underlying groundwater after drainage,  
469 establishing saturated O<sub>2</sub> concentrations across the fluctuation zone ( $z = 0.17\text{-}0.33$  m; Figure  
470 2b) from 0 to 6 days. Conversely, the inflow of anoxic groundwater then allowed to re-establish  
471 both the water table to its original position and the anoxic conditions in the SZ (day 8 onwards;  
472 Figure 2b). On the other hand, vertical CO<sub>2</sub> profiles were similar to that of O<sub>2</sub> under steady-  
473 state conditions (Figure 2c). During transient conditions, downward diffusion of CO<sub>2</sub> was  
474 observed after drainage. However, dissolved CO<sub>2</sub> concentrations in the SZ remained up to 0.5  
475 mg/L (Figure 2d) when the water table reached its initial position, likely due to its higher  
476 solubility than that of O<sub>2</sub>.



477

478 Figure 2. Depth profiles of (a, b)  $O_2$ , (c, d)  $CO_2$ , (e, f) DCM aqueous concentrations, and (g, h)  
 479 DCM gas-phase concentrations in simulated laboratory aquifers under steady-state (top; model  
 480 A) and transient (bottom; model B) conditions. Depth profiles were retrieved at  $x = 0.65$  m and  
 481 correspond to one water table fluctuation event (0-15 d). (a) Black line represents the water  
 482 saturation profile at 15 d, and (a, b) symbols represent data measurements of  $O_2$  aqueous  
 483 concentrations under steady-state (circles) and transient (squares) conditions at 15 d. Horizontal  
 484 lines represent the initial position of the water table ( $z = 0.33$  m). Dashed horizontal lines  
 485 represent the position of the low water table during fluctuation events ( $z = 0.17$  m). Dotted lines  
 486 represent the upper layer of the CF ( $z = 0.45$  m).

487

488 Under steady-state conditions, steep DCM aqueous concentration gradients were also  
 489 established across the CF after continuous horizontal flow of DCM for 60 days. The upper layer  
 490 of the CF ( $z = 0.45$  m) featured a drop in DCM concentrations by approximately 90% compared  
 491 to the SZ (Figure 2e). DCM gas concentrations remained low ( $< 50 \mu\text{g/L}$ ) and constant across  
 492 the upper UZ (Figure 2g). On the other hand, simulated transient conditions showed that as  
 493 DCM aqueous concentrations dropped across the fluctuation zone after drainage ( $z = 0.17$ - $0.33$

494 cm), an increase in DCM gas concentrations occurred above the CF ( $< 300 \mu\text{g/L}$ ; Figure 2h).  
495 However, they rapidly decreased when the water table was raised (Figure 2f), indicating that  
496 diffusive volatilization was temporarily present during transient conditions and restricted by the  
497 slow horizontal groundwater flow.

498 Monitoring of DCM aqueous concentrations and stable isotopes during abiotic and  
499 biotic scenarios (models C-I) allowed to distinguish the contribution of DCM volatilization and  
500 biodegradation processes. Overall, volatilization accounted for 10% of DCM mass dissipation  
501 during steady-state conditions (models C-G) and approximately 16% during water table  
502 fluctuations (models H-I; Table 5). These results are consistent with estimations of  
503 volatilization in the laboratory aquifers coinciding with undetected gaseous DCM across the  
504 UZ (Prieto-Espinoza et al., 2021).

#### 505 **4.2 DCM biodegradation by individual DCM-degraders in the presence of a heterotrophic** 506 **bacterial population under steady-state conditions**

507 DCM biodegradation by individual  $\kappa$ -bacterial populations was examined under steady-  
508 state flow conditions along with the evolution of redox conditions. During the simulated  
509 incubation period (i.e., 60 days of continuous DCM injection to initiate the experimental and  
510 model domain), DCM mass dissipation in the SZ accounted for up to 12% of DCM mass  
511 removal for Dhber (model C), 17% for Dhbium (model D) and 17% for Diely (model E). Here,  
512 planktonic biomass of Dhber increased by 13%, while both Dhbium and Diely increased by  
513 25% ( $x = 1.60 \text{ m}$ ). These numerical results thus support the notion that an incubation period  
514 prior to the experiments allows for the development of an acclimated degrading bacterial  
515 community in the laboratory aquifers (Prieto-Espinoza et al., 2021, 2023). During the  
516 experimental phase, numerical results showed a gradual increase of DCM mass dissipation to  
517 up to 27% at day 35 for both Dhbium ( $X_2$ ) and Diely ( $X_3$ ). However, DCM mass dissipation by  
518 Dhber ( $X_1$ ) remained low  $<12\%$  (Table 5). This indicated that slow degradation of DCM

519 occurred via the X<sub>1</sub>-pathway which was restricted by low Fe(III) availability (inflow: 3 mg/L)  
 520 and competition with the heterotrophic bacterial population B<sub>DOC</sub> for this EA during DOC  
 521 assimilation.

522

523 Table 5. Experimental results and simulated DCM degradation extent (%) and corresponding C  
 524 and Cl isotopes fractionation for the different model scenarios at day 35 (experimental phase),  
 525 including the contribution of the incubation phase. Degradation pathways and modeled  
 526 scenarios are provided in Tables 1 and 4, respectively.

	DCM mass dissipation (%) <sup>a</sup>	Extent of DCM degradation (%) <sup>b</sup>	$\Delta\delta^{13}\text{C}$ (‰) <sup>c</sup>	$\Delta\delta^{37}\text{Cl}$ (‰) <sup>c</sup>	$\varepsilon_{\text{C}}$ (‰) <sup>d</sup>	$\varepsilon_{\text{Cl}}$ (‰) <sup>d</sup>	$\Lambda^{\text{C/Cl}}$ <sup>e</sup>
<b>Modeled scenarios</b>							
Model C	12	2	0.3	0.1	-15.1	-5.1	2.98
Model D	25	18	7.3	1.0	-41.5	-5.2	8.00
Model E	27	18	3.4	1.0	-17.8	-5.1	3.51
Model F	41	35	11.5	2.1	-24.9	-4.4	5.59
Model G	43	36	11.9	2.2	-25.1	-4.4	5.62
Model H	92	76	37.4	6.7	-10.9	-1.9	5.76
Model I	95	79	39.4	6.2	-11.3	-2.0	5.70
<b>Laboratory aquifers (experiments)</b>							
Steady-state	42	(15-51) <sup>f</sup>	11.6	6.3	-23.6	-8.7	1.92
Transient	95	(48-91) <sup>f</sup>	49.2	12	-11.8	-3.1	3.58

<sup>a</sup> Estimations of DCM mass dissipation based on mass balance between inflow and outflow at  $z = 0.15$  m.

<sup>b</sup> Extent of DCM degradation based on stable C isotopes observed at the inflow and outflow of the domain ( $z = 0.15$  m) and according to  $\kappa$ -bacterial populations. For concomitant DCM degradation, two main competing pathways were considered for Dhhbium and Diely (Eq. 11).

<sup>c</sup> Based on inflow and outflow observations ( $z = 0.15$  m).

<sup>d</sup> According to Eq. 8.

<sup>e</sup> According to Eq. 10.

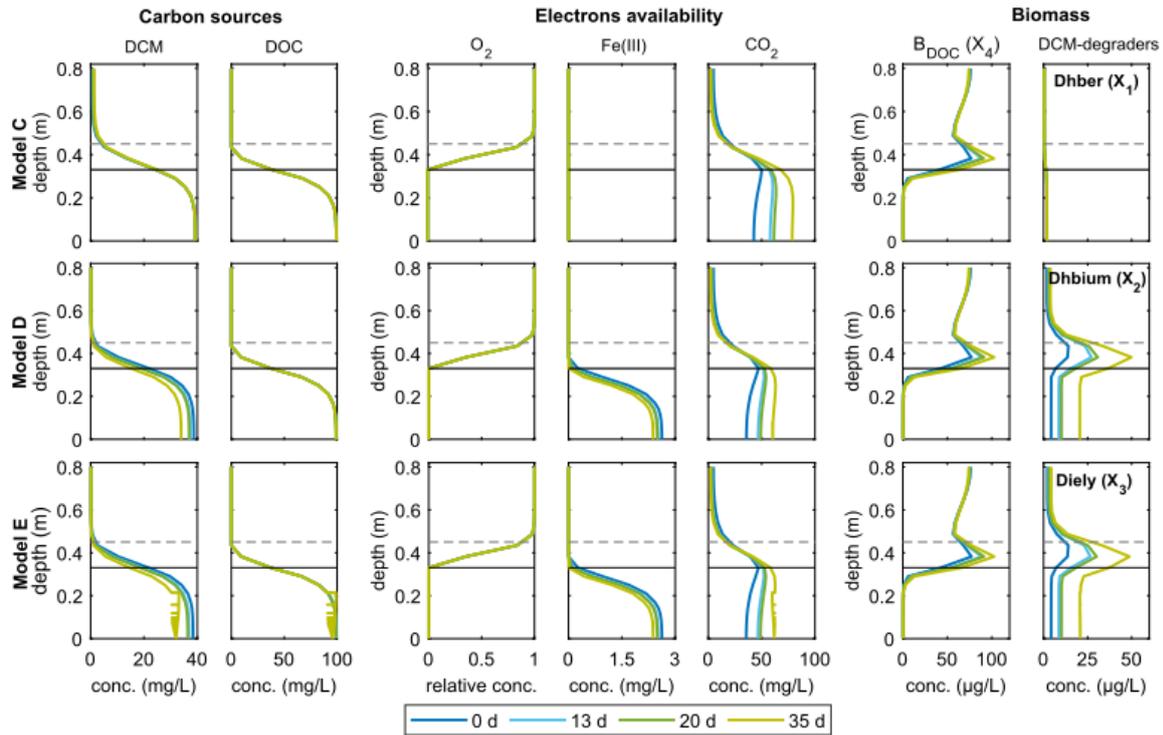
<sup>f</sup> A range of the extent of DCM degradation in laboratory aquifers was provided using reported  $\varepsilon_{\text{C}}$  and  $\varepsilon_{\text{Cl}}$  for both aerobic and anaerobic degradation pathways (Prieto-Espinoza et al., 2021).

527

528 The simulated changes in stable C and Cl isotope ratios of DCM according to distinct  
 529  $\kappa$ -bacterial populations (models C-E) were in accordance with initial  $\varepsilon_{\text{C}}$  and  $\varepsilon_{\text{Cl}}$  values  
 530 associated with anaerobic DCM degradation. This confirmed a good model description of  
 531 pathway-specific reaction rates (Tables 1 and 5). DCM degradation by Dhhbium and Diely  
 532 evidenced slight enrichment in <sup>13</sup>C and <sup>37</sup>Cl isotopes over time and according to distance from  
 533 inflow compared to experimental results (SI, Section G). Nonetheless, models C-E  
 534 underestimated DCM biodegradation by approximately 50% compared to experimental results  
 535 (Table 5). This indicates that DCM biodegradation by a single simulated DCM-degrading

536 bacterial population is likely insufficient to capture the trends of DCM dissipation in the  
537 laboratory aquifers. The wide diversity of experimentally observed taxa potentially involved in  
538 DCM biodegradation further supports this result and justifies the need for model scenarios  
539 accounting for multiple bacterial metabolisms during DCM biodegradation.

540 The heterotrophic bacterial population  $B_{DOC}$  ( $X_4$ ) showed a central role in regulating  
541 redox conditions and supporting DCM metabolism.  $B_{DOC}$  is capable of utilizing both  $O_2$  and  
542 ferric iron as terminal electron acceptor during assimilation of DOC. Under steady-state, higher  
543 relative abundance of  $B_{DOC}$  was observed near the water table and across the CF for models C,  
544 D and E (Figure 3). Dissolved DOC was also established across the CF and followed similar  
545 concentration gradients as DCM (Figure 3). Hence, the constant supply of dissolved  $O_2$  across  
546 the CF favored DOC mineralization resulting in an increase of  $B_{DOC}$  over time (Figure 3). The  
547 higher abundance of  $B_{DOC}$  across the CF can also be explained by its higher degradation rate  
548 during aerobic ( $\mu_{max}^{BDOC,ox} = 1.0 \times 10^{-5}$  [1/s]) than during anaerobic degradation ( $\mu_{max}^{BDOC,anox} =$   
549  $1.0 \times 10^{-7}$  [1/s]) (Table 3), consistent with thermodynamic effects (Meckenstock et al., 2015).  
550 Similarly, the high abundance of *Dhbium* and *Diely* across the CF coincided with high  $CO_2$   
551 production by  $B_{DOC}$  (models D and E; Figure 3). Nevertheless, despite lower bacterial  
552 abundance of  $B_{DOC}$  across the SZ, dissolved Fe(III) (< 3 mg/L) and  $O_2$  depletion (< 0.25 mg/L)  
553 were sufficient to maintain dissolved  $CO_2$  concentrations across the SZ and thus to trigger DCM  
554 biodegradation by *Dhbium* and *Diely* across this zone (models D and E; Figure 3).



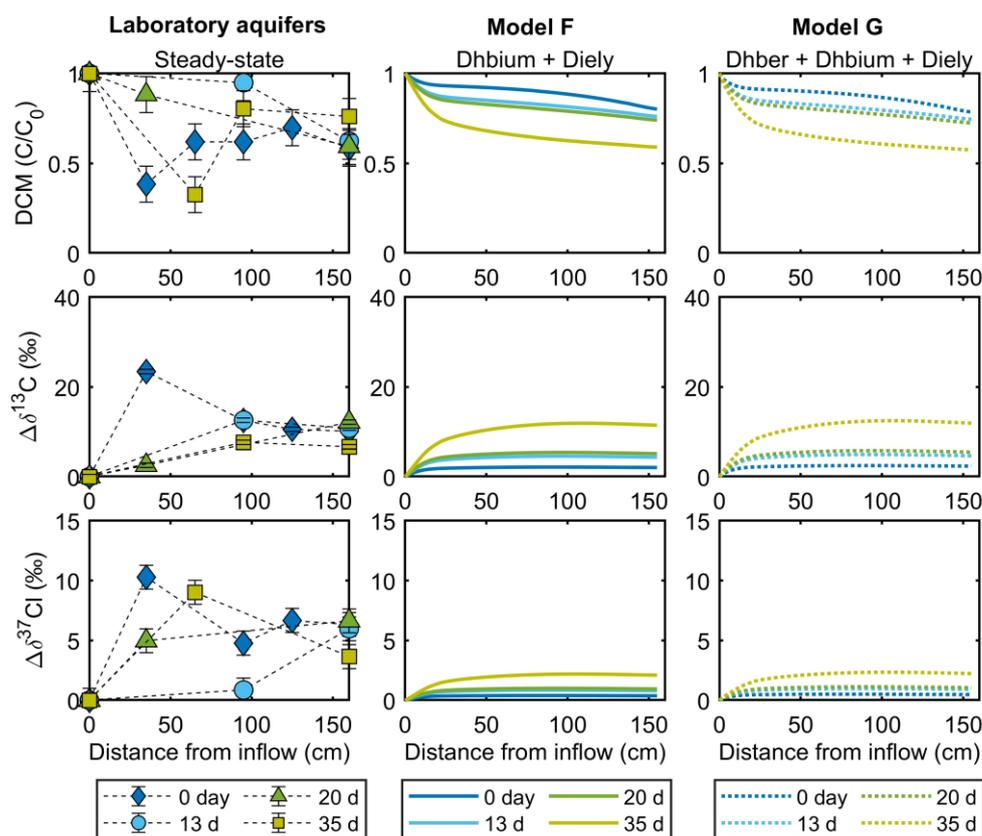
555  
 556 Figure 3. Carbon sources (DCM, DOC), redox species (O<sub>2</sub>, Fe(III), CO<sub>2</sub>) and biomass of four  
 557 bacterial populations over time during simulations of DCM biodegradation by individual DCM  
 558 degraders (Dhber, Dhbium and Diely in models C, D and E, respectively), and in the presence  
 559 of heterotrophic groundwater bacteria (DOC assimilator B<sub>DOC</sub>) under steady-state conditions.  
 560 Horizontal lines represent the position of the water table ( $z = 0.33$  m) and horizontal dashed  
 561 lines represent the upper layer of the CF. Vertical profiles were retrieved at  $x = 0.65$  m and at  
 562 0, 13, 20 and 35 days.

563

### 564 4.3 Concomitant DCM degradation by multiple bacterial populations under steady-state 565 conditions

566 DCM biodegradation via multiple degradation pathways was tested accounting for  
 567 active metabolism of Dhbium and Diely with and without the presence of Dhber, corresponding  
 568 to models F and G, respectively (Table 4). The role of Dhber on DCM biodegradation was of  
 569 interest as iron-reducing conditions prevailed in laboratory aquifers. Numerical simulations  
 570 were also carried out in the presence of B<sub>DOC</sub>. Overall, model simulations of concomitant DCM  
 571 biodegradation under steady-state conditions (models F and G) evidenced larger DCM mass  
 572 dissipation over time (simulated 43%; Figure 4) than simulations of metabolic activity by  
 573 individual populations (Table 5). This agrees well with the experimentally observed DCM

574 dissipation (up to 42%; Table 5), and further supports the occurrence of multiple DCM  
 575 degradation pathways in laboratory aquifers. Comparisons between simulations in the absence  
 576 or presence of Dhber (models F and G, respectively) confirmed insignificant DCM degradation  
 577 under iron-reducing conditions (model G) compared to DCM fermentation or mineralization  
 578 (Figure 4 and Table 5). Iron-reducing conditions in the laboratory aquifers may thus be assigned  
 579 to the activity of heterotrophic bacteria not directly involved in DCM biodegradation.

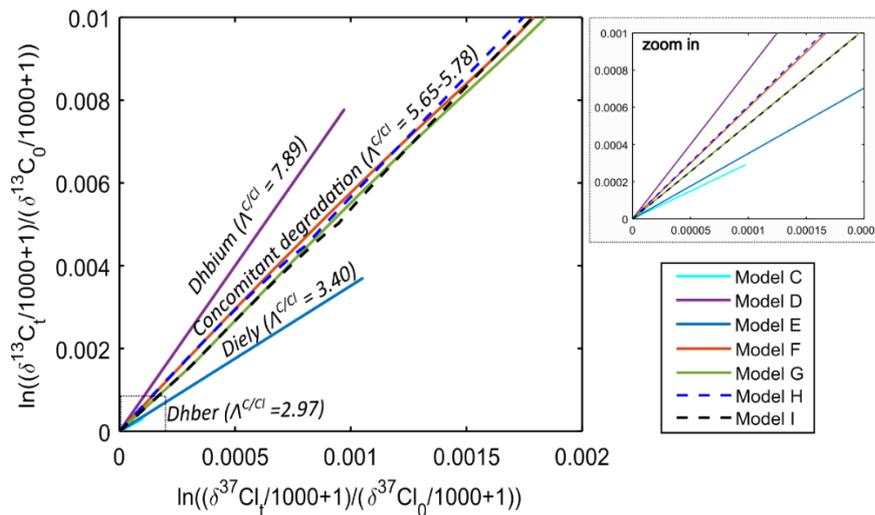


580

581 Figure 4. Concomitant DCM degradation by Dhber ( $X_1$ ), Dhbium ( $X_2$ ) and Diely ( $X_3$ ) under  
 582 steady-state conditions (static water table) over time and distance from the aquifer inflow. From  
 583 left to right: measured data from the laboratory aquifers at  $z = 0.15$  m (symbols), simulated  
 584 concomitant DCM degradation by Dhbium ( $X_2$ ) and Diely ( $X_3$ ) only (Model F), and simulated  
 585 concomitant DCM degradation by Dhber ( $X_1$ ), Dhbium ( $X_2$ ) and Diely ( $X_3$ ) (Model G).  
 586 Heterotrophic groundwater bacteria  $B_{DOC}$  ( $X_4$ ) was active in all models. From top to bottom:  
 587 DCM concentrations, evolution of C isotope fractionation ( $\Delta\delta^{13}C$  in ‰), and evolution of Cl  
 588 isotope fractionation ( $\Delta\delta^{37}Cl$  in ‰). All models had an incubation period of 60 days. The water  
 589 table was positioned at  $z = 0.33$  m. Error bars are associated with DCM concentrations and  
 590 stable isotope values and represent standard errors with  $n = 3$ .

592 Simulated DCM removal during concomitant degradation by Dhber, Dhbium and Diely  
593 was accompanied by changes in C and Cl isotope ratios over time and with distance from inflow  
594 (Figure 4), with  $\Delta\delta^{13}\text{C}$  of up to 12‰ and  $\Delta\delta^{37}\text{Cl}$  of < 3‰ in the presence or absence of Dhber.  
595 In particular, changes in C isotope ratios followed the trends observed in laboratory aquifers  
596 ( $\Delta\delta^{13}\text{C}$  of 12‰). This further supports the hypothesis that concomitant degradation of DCM  
597 likely occurred by multiple degradation pathways in the laboratory aquifers due to activity of a  
598 wide range of DCM degraders (Prieto-Espinoza et al., 2021). Dual plots of concomitant DCM  
599 degradation showed  $\Lambda^{\text{C/Cl}}$  values distinct from those of individual  $\kappa$ -bacterial populations  
600 (Figure 5), thus reflecting the contribution of multiple DCM degradation pathways on overall  
601 DCM isotope signatures (Van Breukelen, 2007). The  $\Lambda^{\text{C/Cl}}$  values computed from numerical  
602 simulations further indicated that bacterial populations Dhbium and Diely were the major  
603 contributors to DCM biodegradation. This was also supported by the increase of the  
604 transformation products acetate and formate over time (SI, Section H), which are the main  
605 transformation products during anaerobic DCM degradation by Dhbium and Diely (Chen et al.,  
606 2020). Overall, the similar  $\Lambda^{\text{C/Cl}}$  values between models F and G confirmed the marginal role  
607 of Dhber during DCM biodegradation in model G (Table 5).

608



609

610 Figure 5. Simulated evolution of dual-element isotopes ( $\Delta^{13}\delta\text{C}$  versus  $\Delta^{37}\delta\text{Cl}$ ) during DCM  
 611 biodegradation according to models C-I. Each line corresponds to simulations of distinct DCM  
 612 degradation pathways by Dhber (model C), Dhbium (model D) and Diely (model E), as well as  
 613 for concomitant DCM biodegradation by Dhbium and Diely under steady-state (model G) and  
 614 transient (model I) conditions. Models F and H correspond to concomitant DCM biodegradation  
 615 by Dhber, Dhbium and Diely under steady-state and transient conditions, respectively. For all  
 616 modeled scenarios, heterotrophic groundwater bacteria  $B_{\text{DOC}}$  were present. Insert shows the low  
 617 C and Cl isotope fractionation produced by Dhber due to limited metabolic activity during DCM  
 618 biodegradation in the aquifer (model C).

619

#### 620 **4.4 Concomitant DCM degradation by multiple bacterial populations under transient** 621 **conditions**

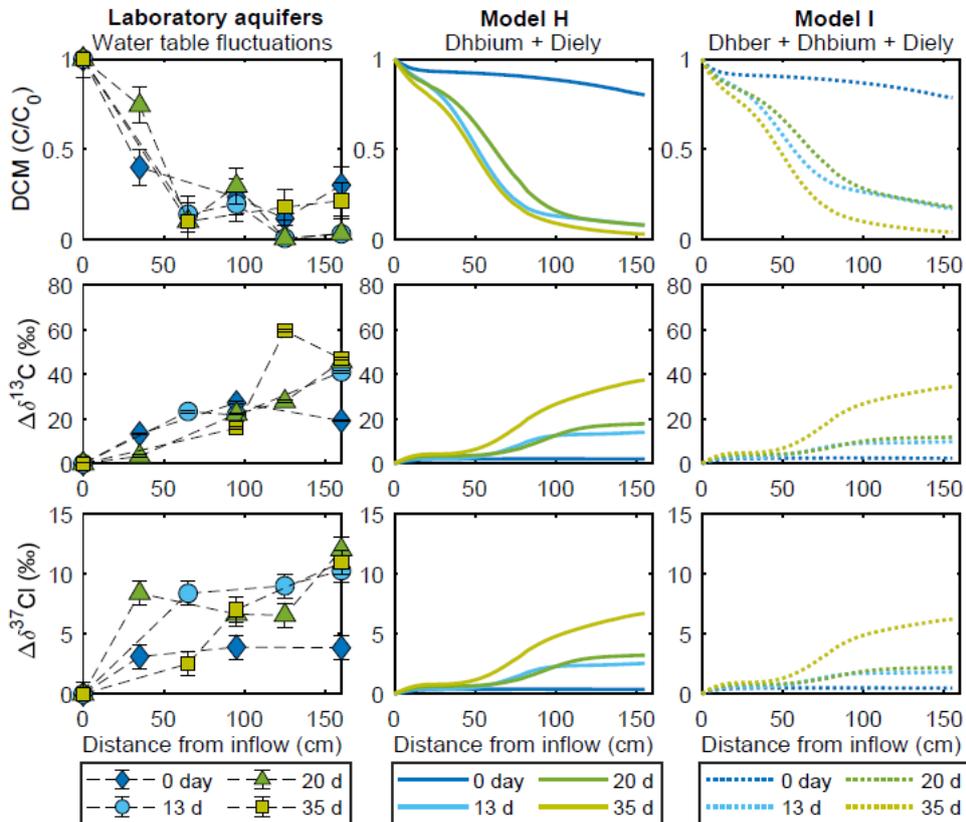
622 Models H and I (i.e., in the presence and absence of Dhber, respectively) strongly  
 623 suggested that biogeochemical dynamics created by water table fluctuations favor DCM  
 624 removal in contaminated aquifers. Evolution of DCM concentrations and stable isotopes were  
 625 followed in the SZ during water table fluctuation events ( $z = 0.15$  m). Accordingly, the  
 626 simulated mass removal of DCM was higher under transient flow conditions (95%) (upper  
 627 panel; Figure 6) than under steady-state (43%) (upper panel; Figure 4). Numerical results of  
 628 models H and I were consistent with experiments showing up to 95% DCM mass removal in  
 629 laboratory aquifers (Table 5). Simulated DCM mass removal was also accompanied with an  
 630 enrichment of  $^{13}\text{C}$  and  $^{37}\text{Cl}$  isotopes over time and distance from inflow (Figure 6). In models  
 631 H and I, C isotope fractionation ( $\Delta\delta^{13}\text{C}$ ) was up to 37‰ and 39‰, respectively, at the outlets

632 (x = 1.6 m) after 35 days, in agreement with experimental results (middle panel; Figure 6). Cl  
633 isotope fractionation resulted in  $\Delta\delta^{37}\text{Cl}$  values of up to 7‰ (lower panel; Figure 6).

634 Simulated  $\Lambda^{\text{C/Cl}}$  values during transient conditions showed similar values as those from  
635 steady-state conditions ( $\Lambda^{\text{C/Cl}}$  range: 5.62-5.78) for all scenarios (models F-I; Figure 5). This  
636 indicated limitations of the numerical model to reproduce shifts in DCM biodegradation  
637 pathways triggered by water table fluctuations, as observed experimentally (steady state:  $\Lambda^{\text{C/Cl}}$   
638 = 1.92 vs. transient:  $\Lambda^{\text{C/Cl}} = 3.52$ ; Table 5). These limitations are likely associated with the  
639 proposed reactive framework in which  $\kappa$ -bacterial populations are mainly dependent on  $\text{CO}_2$   
640 availability (i.e., for Dhbium and Diely metabolism) produced by heterotrophic groundwater  
641 bacterial populations. Moreover, based on ME-CSIA data, the contribution of Dhbium and  
642 Diely on DCM degradation was 28% and 72%, respectively, for both flow regimes, and resulted  
643 in an overall extent of DCM degradation of approximately 35% and 80% under steady-state and  
644 transient conditions, respectively. These numerical results support the fact that closely related  
645 microorganism to Diely such as *Desulfosporosinus* sp. (Kleindienst et al., 2019) were likely  
646 implicated in DCM biodegradation under both flow regimes.

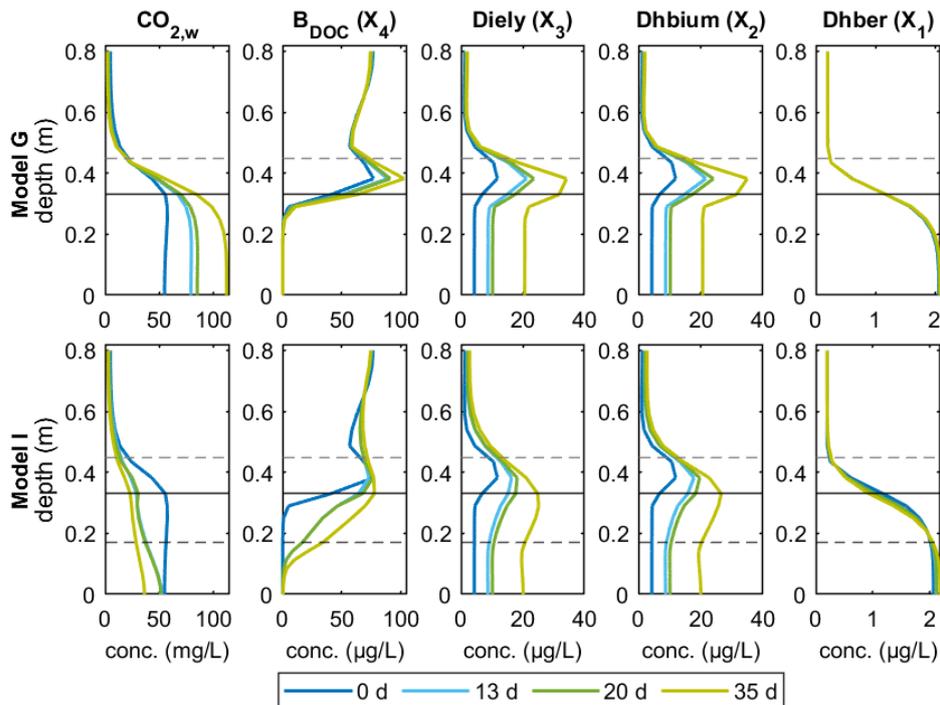
647 Numerical results also showed an increase in bacterial abundance across the fluctuation  
648 zone (z = 0.17-0.33 m; Figure 7). Under transient conditions, the observed increase of  $B_{\text{DOC}}$   
649 across this zone was associated with  $\text{O}_2$  diffusing deeper into the underlying groundwater during  
650 drainage events as shown in (abiotic) model B (Figure 2). This was beneficial for DOC  
651 assimilation by  $B_{\text{DOC}}$  upon oxygen respiration while maintaining  $\text{O}_2$ -depleted conditions during  
652 water table fluctuations (Figure 7). Overall, this supports that the dynamics of heterotrophic  
653 bacterial populations in laboratory aquifers likely controlled  $\text{O}_2$  depletion during drainage and  
654 imbibition events (blue symbols; Figure 2). This was also coherent with the observed decrease  
655 in DOC with increasing distance from inflow in laboratory aquifers (Prieto-Espinoza et al.,  
656 2021). Moreover, approximately 80% of DCM was removed at the outlet after 13 days and

657 removal increased to up to 95% until the end of the experiment (model I; Figure 6),  
 658 corresponding to the first and second water fluctuation event, respectively.



659

660 Figure 6. Concomitant DCM degradation by Dhber ( $X_1$ ), Dhbiium ( $X_2$ ) and Diely ( $X_3$ ) under  
 661 transient conditions (fluctuating water table) over time and distance from the aquifer inflow.  
 662 From left to right: measured data from the laboratory aquifers at  $z = 0.15$  m (symbols), simulated  
 663 concomitant DCM degradation by Dhbiium ( $X_2$ ) and Diely ( $X_3$ ) (Model H), and simulated  
 664 concomitant DCM degradation by Dhber ( $X_1$ ), Dhbiium ( $X_2$ ) and Diely ( $X_3$ ) (Model I).  
 665 Heterotrophic groundwater bacteria  $B_{DOC}$  ( $X_4$ ) was active in all models. From top to bottom:  
 666 DCM concentrations, evolution of C isotope fractionation ( $\Delta\delta^{13}C$  in ‰), and evolution of Cl  
 667 isotope fractionation ( $\Delta\delta^{37}Cl$  in ‰). All models had an incubation period of 60 days. The water  
 668 table was positioned at  $z = 0.33$  m and dropped to 0.17 m. For comparisons with experimental  
 669 results, model simulations follow the first water table fluctuation (corresponding to 0 to 13 d)  
 670 and the second water table fluctuation (corresponding to 20 to 35 d). Error bars are associated  
 671 with DCM concentrations and stable isotope values and represent standard errors with  $n = 3$ .



672

673 Figure 7. Change of CO<sub>2</sub> and active biomass concentrations over time during concomitant DCM  
 674 degradation by Dhber (X<sub>1</sub>), Dhbium (X<sub>2</sub>) and Diely (X<sub>3</sub>) in the presence of the non-DCM  
 675 degrader B<sub>DOC</sub> (X<sub>4</sub>). Top: steady-state conditions (model G). Bottom: transient conditions  
 676 (model I). Horizontal lines represent the position of the water table (0.33 m from the bottom).  
 677 Upper dashed horizontal lines represent the upper limit of the CF. Lower dashed horizontal  
 678 lines represent the position of the water table after the fluctuation events (0.17 m from the  
 679 bottom). All depth profiles were retrieved at x = 0.65 m.

680

## 681 **5. Discussion**

682 This study demonstrates that previously obtained experimental results on bacterial DCM  
683 degradation, and in particular the intriguing impact of water table fluctuations, can indeed be  
684 modelled and confirmed numerically. Our work with contrasting abiotic and biotic scenarios  
685 show the key role of the capillary fringe on DCM biodegradation upon groundwater table  
686 fluctuations and highlight heterotrophic groundwater bacteria as key players. Further, only  
687 modelling scenarios taking redox conditions into account and in which multiple modes of DCM  
688 metabolism are present are compatible with experimental results, and highlight the added value  
689 of integrating CSIA data in RTMs .

### 690 **5.1 The role of the capillary fringe in DCM biodegradation**

691 In abiotic scenarios (models A and B), numerical simulations indicated that DCM  
692 volatilization across the CF was not a significant mechanism of DCM removal in laboratory  
693 aquifers under both flow regimes (Figure 2). Comparisons with biotic scenarios (models C-I)  
694 confirmed that DCM volatilization accounted for less than 10% and 16% under steady-state and  
695 transient conditions, respectively (Table 5), based on DCM aqueous concentrations and  
696 associated stable isotope fractionation. The steep water saturation levels formed across the CF  
697 restricted mass exchange of DCM due to low diffusion in water compared to the gas-phase  
698 (Jeannotat & Hunkeler, 2013). Under transient conditions, however, DCM volatilization across  
699 the fluctuation zone ( $z = 0.17\text{-}0.33$  m) was suggested during drainage events (model B; Figure  
700 2f) as a result of the residual contaminated water exposed to the gas phase (McCarthy & Johnson  
701 1993; Werner & Höhener, 2002).

702 Under transient conditions, model B exhibited rapid oxygenation of the SZ after  
703 drainage ( $z = 0.17$  m; Figure 2b). This contradicts the O<sub>2</sub>-depleted conditions observed in  
704 laboratory aquifers at a low water table (Prieto-Espinoza et al., 2021). Haberer et al., (2012)  
705 showed that reoxygenation of an underlying anoxic groundwater is directly related to the speed

706 and magnitude of the water table drop driving advective O<sub>2</sub> fluxes in the vertical direction.  
707 However, comparisons between O<sub>2</sub> transport during water table fluctuations (model B) and  
708 experimental results of the laboratory aquifers suggest the likely presence of aerobic bacteria  
709 consuming dissolved O<sub>2</sub> in aquifers. We thus propose that heterotrophic groundwater bacteria  
710 across the CF contribute to limit O<sub>2</sub> during water table fluctuations, as suggested previously  
711 (Jost et al., 2015b) and evidenced in models H and I for transient conditions (SI, Section H).

## 712 **5.2 Interplay of redox conditions and multiple groundwater bacterial populations on** 713 **DCM biodegradation**

714 Our numerical simulations highlighted the complex interplay of multiple bacterial  
715 populations on DCM biodegradation. Steady-state models C, D and E underestimated DCM  
716 degradation by either Dhber, Dhbiom or Diely, respectively, compared to experimental results  
717 (Table 5). However, simulations of concomitant DCM degradation by Dhbiom together with  
718 Diely and/or Dhber (models F and G) yielded better fits to experimental results (Figures 4 and  
719 6). This strongly suggests that different bacterial populations with different pathways are  
720 simultaneously active in degrading DCM under both flow regimes. Thus, RTMs that account  
721 for multiple bacterial metabolisms associated with different populations may be better suited  
722 for accurate predictions of biodegradation in DCM-contaminated aquifers (Chen et al., 2017;  
723 Trueba-Santiso et al., 2020).

724 Moreover, inclusion of heterotrophic bacteria (B<sub>DOC</sub>) in all models was important due to  
725 the role of such organisms in shaping redox conditions across the aquifer and regulating O<sub>2</sub>-  
726 depleted conditions across the CF and near the water table (Figure 3 and SI, Section H). Indeed,  
727 previous studies conclusively showed the importance of such metabolism across the CF (Hron  
728 et al., 2015; Jost et al., 2015a, 2015b; Rezanezhad et al., 2014). Our results thus supports that  
729 multi-phase flow and mass exchange processes play a key role in shaping dynamic  
730 biogeochemical conditions and contaminant biodegradation across fluctuation zones.

731 Our modelling also demonstrated that heterotrophic groundwater bacteria affected DCM  
732 biodegradation, particularly for pathways requiring CO<sub>2</sub> (Figures 3 and 7). In particular, an  
733 increase in anaerobic DCM-degraders Dhhium and Diely across the CF was confirmed due to  
734 available CO<sub>2</sub> produced by B<sub>DOC</sub> upon DOC degradation (up to 50 mg/L; Figures 3 and 7)  
735 compared to abiotic conditions (<0.5 mg/L; Figure 2). Nevertheless, simulated Dhhium and  
736 Diely were present across this zone despite the presence of dissolved O<sub>2</sub> (> 1 mg/L; Figures 3),  
737 contrasting their strictly anaerobic metabolism (Holliger et al., 1998; Mägli et al., 1996). O<sub>2</sub>  
738 tolerance for some anaerobic DCM degraders such as *Desulfosporosinus* strains and  
739 *Desulfovibrio* spp. have been suggested (Agostino et al., 2020; Mardanov et al., 2016; Ramel  
740 et al., 2015), despite limited experimental evidence (Lueders, 2017). This may enable their  
741 establishment across moderately oxygenated niches such as the CF. In the future, implementing  
742 O<sub>2</sub> inhibition terms and bacterial dormancy in RTMs may help more accurately predict the  
743 establishment of anaerobic degrader niches across oxic/anoxic redox gradients near the water  
744 table and CF (Eckert et al., 2015), and improve integrative RTMs for in-depth understanding of  
745 DCM metabolism across oxic/anoxic redox gradients.

### 746 **5.3 Water table fluctuations enhance DCM degradation in laboratory aquifers**

747 Numerical results of DCM biodegradation by multiple  $\kappa$ -bacterial populations (models  
748 H and I) showed that dispersive mixing driving biogeochemical dynamics is necessary to  
749 enhance DCM biodegradation during water table fluctuations, and also that mutualistic  
750 relationships among groundwater bacteria should no longer be overlooked. Indeed, models H  
751 and I showed that DCM mass dissipation increased during water table fluctuations (Figure 6)  
752 compared to steady-state conditions (models F and G; Figure 4). Here, downward O<sub>2</sub> diffusion  
753 was key to sustain DCM degradation in the fluctuation zone due to interactions with  
754 heterotrophic bacteria (B<sub>DOC</sub>). Nevertheless, numerical results also showed that a single water  
755 fluctuation event (from 0 to 13 days; Figure 6) is sufficient to produce a strong effect on DCM

756 biodegradation (80% at 13 d;  $x = 1.60$  m). This resulted in a simulated increase of DHBium and  
757 Diely by approximately 15% across this zone (Figures 7), enough to enhance DCM degradation.

758 Our model also showed that biomass,  $O_2$  and  $CO_2$  are key parameters to be measured to  
759 capture biogeochemical variations caused by water table fluctuations in relation to DCM  
760 degradation. In particular, future flow-through experiments with a high-resolution monitoring  
761 of  $CO_2$  could help to shed light on the relevance of heterotrophic groundwater bacteria during  
762 DCM biodegradation. Moreover, direct competition experiments between known anaerobic  
763 DCM degraders requiring  $CO_2$  such as DHBium and Diely have not yet been reported. However,  
764 our model offers a flexible framework for corresponding processes during DCM degradation  
765 by such *Peptococcaceae* bacteria. It has recently been proposed that known anaerobic DCM  
766 degraders from the *Peptococcaceae* family share a unique anaerobic gene cassette, containing  
767 proteins *mecE* and *mecF* (for methylene chloride catabolism) (Murdoch et al., 2022). In the  
768 future, these genes may provide another line of evidence to monitor anaerobic DCM  
769 biodegradation in contaminated groundwater, and help further extend implementations of  
770 genome-scale metabolic RTMs (Scheibe et al., 2009).

771 It is worth stressing that high-resolution hydrochemical monitoring, ME-CSIA of DCM  
772 and biomolecular tools (16S rRNA gene) from the laboratory aquifers enabled the development  
773 of the reactive transport framework presented in this work. This framework underscored the  
774 interplay of redox conditions and several groundwater bacterial populations to enhance DCM  
775 biodegradation during water table fluctuations. In particular, the integration of stable isotopes  
776 of DCM (i.e., six isotopologues) in our multi-phase RTM proved useful to (i) quantify the  
777 biodegradation extent of DCM across different biotic scenarios and degradation pathways, and  
778 (ii) distinguish the effect of volatilization on the overall DCM mass dissipation.

779 An inverse modelling approach may further improve the applicability of our model to  
780 obtain a more suitable parametrization, particularly for bacterial-mediated processes. This could

781 be further extended to a sensitivity analysis on parameters related to bacterial kinetics,  
782 fundamental for appropriate descriptions of bacterial development during biodegradation  
783 reactions. Indeed, limitations of the dataset to test dissolved CO<sub>2</sub> and DCM transformation  
784 products (e.g., acetate and formate) were encountered, restricting in-depth interpretations of  
785 DCM biodegradation in the SZ and CF of the laboratory aquifers. Furthermore, anaerobic DCM  
786 degradation pathways and mechanisms are still poorly understood, which calls for future studies  
787 to elucidate the possibility of concomitant anaerobic DCM degradation at contaminated sites.

## 788 **6. Conclusions**

789         The proposed mathematical formalism describing multi-phase flow, multi-component  
790 and pathway-specific reaction rates of DCM proved suitable for shedding light on the multiple  
791 processes affecting reactive transport of DCM during water table fluctuations. In particular, the  
792 integration of ME-CSIA and pathway-specific reactions of four bacterial populations provided  
793 a reasonable understanding of the interplay between biogeochemical conditions and multiple  
794 bacterial metabolisms associated with DCM biodegradation under both steady-state and  
795 transient conditions. Most notably, only numerical simulations in which DCM biodegradation  
796 originated from multiple bacterial populations reproduced the DCM dynamics observed in  
797 laboratory aquifers. In addition, the contribution of heterotrophic groundwater bacteria (non-  
798 DCM degraders) on the overall DCM biodegradation was essential for providing CO<sub>2</sub> sources  
799 for fermentative pathways while maintaining O<sub>2</sub>-depleted conditions across fluctuation zones.  
800 This highlights the importance of interactions within bacterial communities with regard to  
801 natural attenuation of DCM in contaminated groundwater, in contrast to the metabolic activity  
802 of a single bacterial population. The implemented model formalism with regard to DCM  
803 biodegradation highlights the added value of integrative RTMs in the evaluation of natural  
804 attenuation of DCM and other VOCs in contaminated groundwater.

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809

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812 Data curation, Visualization, Formal analysis, Writing - original draft, Writing - review &  
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819

820 **Declaration of Competing Interests**

821 The authors declare that they have no known competing financial interests or personal  
822 relationships that could have appeared to influence the work reported in this paper.

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