Evidence for extensive anaerobic dechlorination and transformation of the pesticide chlordecone ($C_{10}Cl_{10}O$) in soil from Guadeloupe

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Abbreviations:
CLD Chlordecone
MHCLD Monohydrochlordecone
DHCLD Dihydrochlordecone
THCLD Trihydrochlordecone
HCLD Hydrochlordecone
PCIN Polychloroindene
CPCIN Carboxylated polychloroindene
TCE Trichloroethene
VC Vinyl Chloride
MC Microcosm
GC Gas Chromatograph
LC Liquid Chromatograph
MS Mass Spectrometry
eeq Electron Equivalents
SI Supporting Info

ABSTRACT

The historic use of chlordecone ($C_{10}Cl_{10}O$) as a pesticide to control banana weevil infestations has resulted in pollution of large land areas in the French West Indies. Although currently banned, chlordecone persists because it adsorbs strongly to soil and its complex bis-homocubane structure is stable, particularly under aerobic conditions. Abiotic chemical transformation catalyzed by reduced vitamin B12 has been shown to break down chlordecone by opening the cage structure to produce C9 polychloroindenes. More recently these C9 polychloroindenes were also observed as products of anaerobic microbiological transformation. To investigate the anaerobic biotransformation of chlordecone by microbes native to the French West Indies, microcosms were constructed anaerobically from chlordecone impacted Guadeloupe soil and sludge. Original microcosms and transfers were incubated over a period of 8 years, during which they were repeatedly amended with chlordecone and electron donor (ethanol and acetone). Using LC-MS, chlordecone and degradation products were detected in all the biologically active microcosms. Observed products in active but not sterile incubations included monohydro-, dihydro- and trihydrochlordecone derivatives ($C_{10}Cl_{10-n}O_2H_n$; n= 1,2,3), as well as "open cage" C9 polychloroindene compounds ($C_9Cl_{5-n}H_{3+n}$ n=0,1,2) and C10 carboxylated polychloroindene derivatives ($C_{10}Cl_{4-n}O_2H_{4+n}$, n=0-3). Products with as many as 9 chlorine atoms removed were detected. Chlordecone concentrations decreased in active microcosms as concentrations of products increased, but quantification was hampered by a lack of standards. Mono- and dihydrochlordecones and pentachloroindenes were also detected in field samples, indicating that anaerobic dechlorination processes have occurred intrinsically in situ. Therefore it may be possible to stimulate anaerobic dechlorination through donor addition to contaminated soils. Microbial community analysis in microcosms showed enrichment of anaerobic fermenting and acetogenic microbes possibly involved in anaerobic chlordecone biotransformation.

INTRODUCTION

Chlordecone (CLD) was used to control insect pests (mainly the banana Weevil) in banana plantations in the Caribbean, particularly in Guadeloupe and Martinique from 1971 to 1993, despite being banned in the United States since 1974 (ATSDR 1995) (Cabidoche *et al.,* 2009). Chlordecone ($C_{10}Cl_{10}O$) is a bis-homocubane, comprising a cage structure with 10 chlorine atoms and a ketone functionality. It was commercialized under the brand names Kepone® and

69 Curlone® and was spread at the foot of each banana plant as a solution delivering 1.5 grams of
70 chlordecone per plant. With 800 banana plants per hectare and 2.5 applications per year, the
71 resulting dosage was a significant 3 kg chlordecone per hectare per year. As a consequence of
72 such intensive application, 8–9% of the cultivated areas of Guadeloupe have CLD concentrations
73 higher than 1 mg/kg in topsoil, and some banana fields have concentrations higher than 9 mg/kg
74 (Cabidoche *et al.,* 2009). CLD adsorbs strongly to soils rich in organic matter, and its
75 partitioning coefficient (log K_{oc}) has been reported in the range of 3.3-3.41 (Bonvallot and Dor
76 2004) (ATSDR 1995). CLD is only slightly soluble in water (2.7 mg/L at pH 7 and 25ºC (Kilzer
77 *et al.,* 1979) and tends to bioaccumulate in fatty tissues of living organisms (Howard 1991,
78 Dromard *et al.,* 2019). CLD is also neurotoxic, hepatotoxic and spermatotoxic to most living
79 organisms (Multigner *et al.,* 2016). In 2009, CLD was added to the list of persistent organic
80 pollutants in the Stockholm Convention, banning its production and use worldwide. Studies
81 suggest exposure to CLD may be linked to higher occurrence of prostate cancer and to impaired
82 cognitive and motor development in young children (Multigner *et al.,* 2010). Watershed
83 modelling predicts that CLD will remain in soils for decades or even centuries because of its
84 stability and affinity for soil organic matter, providing a long term source of contamination to the
85 aquatic environment (Cabidoche *et al.,* 2009). Surface water, ground water, sediments and
86 receiving coastal wasters have all been impacted. CLD has also been found to accumulate in
87 certain animals and plants used for food, including tubers and other vegetables grown in soil
88 (Devault *et al.,* 2016). Effective methods to decontaminate soil and protect downgradient
89 environments, food and water supply are clearly needed.
90
91 Few researchers have investigated CLD transformation. Early detailed studies by (Schrauzer and
92 Katz 1978) revealed that reduced vitamin B12 could dechlorinate chlordecone into products
93 including monohydrochlordecone (MHCLD), dihydrochlordecone (DHCLD) and
94 pentachloroindene. More recently, Ranguin *et al.,* (2017) were also able to show abiotic
95 dechlorination using reduced vitamin B12. The abiotic reduction of CLD with B12, zerovalent
96 iron or sodium sulphide led to formation of hydrochlordecones (HCLDs) and polychloroindenes
97 (PCINs) (Chevallier 2017). Very little data exists on the microbial degradation of CLD. Orndorff
98 and Colwell (1980) showed that *Pseudomonas aeruginosa strain K03* was able to partially
99 transform CLD to 15% MHCLD and 5% DHCLD. However, the HCLDs detected could have

been impurities and not biodegradation products (George and Claxton 1988). Under methanogenic conditions, Jablonski (1996) reported extensive dechlorination and formation of apolar and polar metabolites in a culture of *Methonosarcina thermophila* at 50ºC. According to this work, 86% of labelled [14C] CLD was dechlorinated. Very slow, natural transformation of CLD to 5-b-hydrochlordecone was documented in polluted soils indicating the possible natural biotransformation of chlordecone (Devault *et al.,* 2016). More recently, the formation of MHCLD, DHCLD, trihydrochlordecone (THCLD) and indene metabolites $C_9Cl_5H_3$ and $C_9Cl_4H_4$ was detected in a mixed bacterial consortium as well as by an isolated *Citrobacter* strain (Chaussonnerie *et al.,* 2016, Chevallier 2017), and a third group of metabolites, carboxylated polycholoroindenes (CPCIN) was also reported (Chevallier 2017). The genome of the Citrobacter strain in these studies contained no reductive dehalogenase genes and thus transformation was likely cometabolic (Chaussonnerie *et al.,* 2016). A very recent paper by the same team further identified and characterized many chlordecone transformation products (Chevallier *et al.,* 2019), using NMR, chemical reduction and organic synthesis to elucidate isomer structures. This work enabled quantification of these transformation products in soil samples from Martinique, documenting evidence for some natural transformation in the environment.

The objective of this work was to evaluate anaerobic microbial transformation of CLD in batch bottle microcosms prepared from impacted soils from Guadeloupe. A major challenge was to develop sampling and analytical techniques to detect, identify and track CLD and metabolites repeatedly over time in small (~1 ml) samples from microcosms. An LC-Orbitrap MS method was eventually developed for small volume samples, where we found that the choice of extractive co-solvent was critical to the detection of both polar and non-polar metabolites. The isotopic fingerprint of compounds with multiple chlorines enabled tentative identification of metabolites despite lack of authentic standards. In microcosms maintained under strictly anaerobic conditions, we were able to document slow but extensive dechlorination of CLD to products with up to 9 chlorine atoms removed. In field samples, MHCLD, DHCLD and pentachloroindene metabolites were convincingly detected, indicating that these dechlorinating reactions can also proceed *in situ*, particularly where anaerobic field conditions can be found.

131 **MATERIALS AND METHODS**

132 **Collection of Field Samples.** Field samples were collected twice, first in the fall of 2010 for
133 microcosm setup, and second in the spring of 2018 to analyze CLD and potential degradation
134 products by LC/MS using a refined protocol. In 2010, samples were collected from seven
135 locations in the south of Basse-Terre island, Guadeloupe (Table S1A): three andosol samples
136 from agricultural areas used for banana production (soil); three fluvisol samples from river banks
137 near a banana production area (soil and water); and one sludge sample from an anaerobic
138 digester at a sugar cane distillery plant. In 2018, nine different locations were sampled (Table
139 S1B): six from CLD-impacted agricultural areas (as soil and water slurries); and three activated
140 carbon sludge samples from a water treatment plant that handles chlordecone-contaminated
141 water. All agricultural soil samples were collected from 0-20 cm depth. In 2010, soil, water and
142 sludge samples were collected separately in polypropylene sampling bottles, while in 2018, a
143 mix of soil and water was placed into 1-liter glass sampling jars that were filled to the top and
144 sealed. All samples were shipped from Guadeloupe to University of Toronto for analysis where
145 they were stored at 4°C until use.

146

147 **Chemicals.** CLD (neat) and CLD standard (analytic standard 1mg/mL in MeOH) were
148 purchased from Accustandard (New Haven, USA). Hexanes and acetone (Fisher), water and
149 methanol (Caledon Laboratory Chemicals), and ethanol (Commercial Alcohols, Brampton,
150 Canada) were all of HPLC grade. TCE was purchased from Sigma and had a purity of 99.5%.
151 Metabolite B1 was a gift from researchers at Genoscope (Évry, France).

152

153 **Microcosm Setup and Enrichment.** In December 2010, a microcosm study consisting of 13
154 different conditions each in triplicate for a total of 39 microcosms was initiated (Table S2A).
155 Microcosms contained soil and water samples from Guadeloupe and were augmented with
156 artificial groundwater (recipe in Supplemental Method Details 1) as there was not enough field
157 water. The microcosms were prepared in 160 ml serum glass bottles (Fisher Scientific) sealed
158 with blue butyl stoppers (Bellco Glass Inc.) with 22.5 ml soil and 80 ml water. Rezasurin (1
159 mg/l) was added to one microcosm from each triplicate set as an indicator of anaerobic
160 conditions. At setup, 15 microcosms (5 different soil samples in triplicate) were poisoned by
161 adding mercuric chloride (0.05%) and sodium azide (0.02%). The 15 poisoned control

162 microcosms and 18 of the 24 active microcosms were amended with CLD at a target
163 concentration of 10 mg/l (which is above the solubility of ~2 mg/L (Howard 1991, ATSDR
164 1995), therefore a separate phase of CLD was expected). Three other active microcosms were
165 amended with both CLD and trichloroethene (TCE) at target concentrations of 10 mg/l each, and
166 three microcosms were left unamended. TCE was added as a positive control for reductive
167 dechlorination. All 39 microcosms received an initial dose of an electron donor mix of acetone
168 and ethanol, each at ~80 mg/L. The ratio of donor (acetone/ethanol) to CLD (acceptor) in terms
169 of electron equivalents (eeq) was around 100:1, assuming 20 eeq/mol CLD for complete
170 dechlorination. This ratio is very high, and higher than typically used for other chlorinated
171 electron acceptors like TCE (5:1) because more acetone and ethanol were needed to dissolve
172 CLD (solid powder) into the feeding stock solution (Table S3). The microcosms were incubated
173 in the dark, unshaken in an anaerobic glovebox (Coy Lab Products, Grass Lake, MI, USA) for
174 about 8 years. During the first 2.5 years, all the active microcosms were re-amended with CLD
175 twice and electron donor five times. Those microcosms that also received TCE were re-amended
176 with TCE twice during this period. Six active microcosms were transferred into a defined pre-
177 reduced anaerobic mineral medium (Edwards and Grbic-Galic 1994) in slightly larger bottles:
178 one after 1 year, one after 1.5 years, and four after 2 1/2 years (Figure S1 and Table S2B). These
179 transfers were made in 250 ml Boston Round glass bottles (VWR) to a total liquid volume of 200
180 ml and sealed with screw-cap Mininert^{TM} valves (Chromatographic specialties). Subsequently,
181 these transferred bottles (GT5, GT20, GT33, GT4, GT15, GT3) were re-amended with donor and
182 acceptor regularly. All the other microcosms were not maintained after 2.5 years, but some were
183 sampled a few times for comparison with the transfers. The amounts and frequency of
184 amendments to original microcosms and to transfers over the full study are shown in Table S4.
185 Two bottles with medium only (Medium1, Medium2) were prepared in 2016 for use as controls
186 for LC/MS analysis. These were set up in 250 ml Boston Round glass bottles with 200 ml
187 mineral medium (no culture) and amended with CLD feeding stock to CLD concentrations of 10
188 and 20 mg/l (Table S2C).
189
190 **Microcosm Sampling and Analysis.** For the first 1.5 years, liquid samples (1 ml) from
191 microcosms were sampled regularly and analyzed by GC/FID for methane, ethene, ethane and
192 chlorinated ethenes. Beyond this time, only the transferred microcosms were analyzed routinely.

193 Microcosms were sampled using glass syringes and a 1 ml sample was added to a 10 ml
194 headspace autosampler vials (Agilent) containing 5 ml of acidified deionized water (pH 2). The
195 vials were then sealed with Teflon-coated septa and aluminum crimp caps (Chromatographic
196 Specialties) for automated headspace injection onto the CG (see below). To measure chloride,
197 nitrite, nitrate, sulfate and phosphate concentrations, samples (1 ml) were filtered through 0.2 μm
198 nylon filters (Fisher Scientific) and analyzed by IC (Dionex) as described below. These analyses
199 were only done a few times over the course of the study. One ml liquid samples were also taken
200 for "in-syringe" pH analysis using an Oakton pH spear (Oakton Instruments, Vernon Hills,
201 USA). If needed, pH was adjusted with Na-bicarbonate or HCl to pH 7.
202
203 When the microcosms were first established, we did not have a good method to measure CLD or
204 its daughter products. Nevertheless, during the first 1.5 years, 1 ml liquid samples (in duplicate)
205 were taken every 1-2 months from each microcosm and archived frozen at -20°C for later
206 analysis. Eventually, an LC/MS method was developed and CLD and daughter product analyses
207 by LC/MS were performed approximately once per year, and more frequently in the last two
208 years. The transfers (GT5, GT20, GT33, GT4, GT15, GT3) were analyzed most often, and some
209 poisoned controls, medium controls and some of the original active microcosms were analyzed
210 occasionally for comparison. Sampling and sample preparation procedures for the LC/MS
211 analysis are described below.
212
213 **Sampling and Sample Preparation for CLD Analysis by LC/MS.** Sampling procedures,
214 sample preparation methods and chromatographic and MS methods for analysis of CLD and
215 dechlorinated products were improved progressively (explained in Supplemental Method Details
216 2). Eventually, two satisfactory sample preparation methods were developed for the microcosms,
217 one for sampling liquid only (method 3) and one for sampling a soil/water slurry (method 4).
218 Samples were always taken using glass syringes (Hamilton Company, Reno, USA) and stored in
219 glass vials with caps with PTFE lined septa (Agilent) to minimize sorption. All samples and
220 standards were filtered through 0.2 μm Millex PTFE syringe filters (Millipore, Burlington,
221 USA). For the liquid samples (method 3), bottles were shaken and left to settle overnight. The
222 next day 0.75 ml liquid was carefully sampled (avoiding collecting any solids) and was placed
223 into a 2 ml glass vial. The sample was then centrifuged for 5 minutes at 3000 rpm, and 0.5 ml of

224 the supernatant was transferred into a new vial containing 0.5 ml methanol. After mixing, the
225 sample was filtered into a final 2 ml glass sampling vial. For the slurry samples (method 4),
226 bottles were shaken and a 1 ml sample was taken with syringe immediately before the solids
227 settled to get a representative sample of the soil/water slurry. The slurry sample was added to a
228 vial containing 1 ml methanol, shaken gently for 10 min and left sitting for 30 min. The sample
229 was then centrifuged for 5 minutes at 3000 rpm, and the supernatant was filtered into the final 2
230 ml sampling vial. The addition of methanol to the sample had three purposes; to help extract
231 compounds associated with the solids, to help clean up the sample matrix by inducing
232 precipitation of salts from the sample matrix, and to reduce sorption of compounds to filter
233 membranes. For some of the samples the dry weight of solids was measured after drying the
234 sample in a drying oven at 105°C for several hours to estimate sorbed mass.
235
236 The field samples taken in 2018 were expected to have much lower concentrations of CLD and
237 daughter products than the microcosms, therefore a different extraction and concentration
238 procedure was used. Two different sample volumes (5 ml and 20 ml) were extracted by liquid-
239 liquid extraction using a mix of 15% acetone and 85% hexane (methods 5 & 6). The extracts
240 were evaporated to dryness under a stream of nitrogen and the samples were re-dissolved in 1ml
241 and 0.5ml methanol and filtered. Sample preparation procedures, LC/MS instrumentation and
242 methods were in constant development over the course of the study. Only the final optimized
243 method, used from April 9th 2018, is described in detail here. The history and development of the
244 protocol is explained in Supplemental Method Details 2, and the optimum LC/MS method is
245 described below.
246
247 **Liquid Chromatography Mass Spectrometry (LC/MS) Analysis.** Chromatography was
248 carried out on a ZORBAX Rapid Resolution High Definition Phenyl-Hexyl column (50mm x
249 3.0mm, 1.8um) (Agilent, Santa Clara, USA) equipped with a guard column, using a Thermo
250 Scientific Ultimate 3000 UHPLC (Thermo Fisher Scientific, Waltham, MA). The column
251 temperature was 40°C and the flow rate was set to 300 $\mu L.min^{-1}$. The eluents used were water
252 (A) and methanol (B), and both eluents contained 5 mM of ammonium acetate. The gradient
253 started at 50% B , followed by a linear gradient to 100% B over 8 min, then a hold at 100% B for
254 4 min, then a return to 50% B over 1min, and finally a re-equilibration under the initial

255 conditions of 50% B for 5 min (total runtime 18 min). Liquid samples (10 µL) were injected
256 using an Ultimate 3000 UHPLC autosampler, with autosampler temperature of 8ºC. Compounds
257 were detected and quantified using a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher
258 Scientific) equipped with a Heated Electrospray Ionization (HESI II) probe, operating in
259 negative ionization mode. Mass spectra were acquired over an m/z range from 80 to 750 with the
260 mass resolution set to 140k, and common setting parameters were as follows; AGC Target: 1E6,
261 max injection time 200 ms, spray voltage 4 kV, capillary temperature 320ºC, sheath gas 15, aux
262 gas 5, spare gas 2, and s-lens RF level 55. Data from the LC-MS were processed through
263 Xcalibur Qual Browser (Thermo Fisher Scientific). Calibration standard solutions of CLD (0.02,
264 0.2, 1.7, 2.6, 3.6 mg/l) were prepared from successive dilutions of a purchased analytical
265 standard (1 mg/mL CLD in MeOH). Standards of pentachlorindene metabolite B1 (0.02, 0.1, 0.2,
266 0.5 and 1 mg/l) were prepared similarly but from a 0.4 mg/ml acetonitrile stock solution of B1.
267 All the standards were diluted in a 50/50% mix of methanol and mineral medium to simulate the
268 matrix of the microcosm samples. Standard dilutions were made fresh the day before or on the
269 same day as they were run.
270
271 **Other Analyses: Gas Chromatography (GC) and Ion Chromatography (IC).** Methane,
272 ethene, ethane, and chlorinated ethene measurements were carried out as headspace analysis
273 using an Agilent 7890A gas chromatograph (GC) equipped with a flame ionization detector
274 (FID), a G1888 headspace autosampler and a J&W GS-Q column (30 m x 0.53 mm) (Agilent,
275 Santa Clara, CA, USA). Helium was the carrier gas (11 ml/min) and the oven program was as
276 follows: Hold at 35°C for 1.5 min, increase to 100°C at the rate of 15°C/min, then increase to
277 185°C at the rate of 5°C/min, hold 10 min, then increase to 200 °C at the rate of 20°C/min, hold
278 10 min (total runtime 43.6 min). The GC had a packed inlet and a 3 ml sample loop. Headspace
279 operating temperatures for oven, loop and transfer line were 70, 80 and 90ºC respectively, while
280 vial equilibration time, pressurization time, loop fill time, loop equilibration time and injection
281 time were 40, 0, 0.2, 0 and 3 min respectively. During equilibration in oven, the samples were
282 shaken at low speed. Data from the GC was integrated using ChemStation (Agilent). Calibration
283 standards were prepared in the concentration range 0.2 to 2 mg/l (liq) for methane and ethene,
284 and 1 to 20 mg/l for chlorinated ethenes.
285

286 Chloride, nitrite, nitrate, sulphate, phosphate and acetate measurements were performed using a
287 Dionex ICS 2100 ion chromatograph with a Dionex IonPac AS18 analytical column (4x250 mm)
288 and an ASRS 500 suppressor (Thermo Fisher Scientific). The samples were run isocratically at
289 23 mM KOH, 57 mA current and with a flow rate of 1 ml/min. Data from the IC was integrated
290 using Chromelion (Thermo Fisher Scientific). Standards were prepared in the range 0.005 to 0.5
291 mM.
292
293 **DNA Extraction, Amplicon Sequencing and Quantitative Polymerase Chain Reaction**
294 **(qPCR).** Samples for DNA extraction were taken from the 6 transfers (GT5, GT20, GT33, GT4,
295 GT15, GT3) about 7 years into the study for microbial community analysis. Slurry samples (1
296 ml) were taken, centrifuged at 10 000 rpm for 20 min, and cell pellets were frozen at -80°C for
297 future DNA extraction. DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Hilden,
298 Germany) according to manufacturer's protocol with some modifications (explained in
299 Supplemental method details 3). DNA extracts were stored at -80°C. Microbial community
300 composition was assessed by small subunit (SSU) rRNA gene fragment sequencing and
301 Quantitative Polymerase Chain Reaction (qPCR) analysis (see Supplemental method details 3).
302
303 **RESULTS AND DISCUSSION**
304 **Overview of Microcosm Data.** CLD is a challenging molecule to quantify, particularly in small
305 volume samples from a microcosm. It took us several years after microcosm set up to develop a
306 suitable method, and until that time, we periodically amended microcosms with CLD without
307 knowing if it was being degraded or not. We rationalized that dechlorination would be slow and
308 limited by the low solubility of CLD. Therefore, provided that we had good controls, we would
309 eventually be able to identify activity by comparing active microcosms to poisoned and medium
310 controls.
311
312 To provide an overview of how each set of microcosms was treated over the course of about
313 3000 days (~8 years), cumulative CLD and electron donor (acetone and ethanol) amendments
314 were plotted over time (Figure S2; with data in Table S4). We monitored methane production,
315 sulfate consumption and TCE dechlorination (where added), as indications of anaerobic
316 microbial activity. All active microcosms except for those made from distillery sludge samples

317 (G63 series) produced methane during the first 1.5 years (Figure S3). Beyond 1.5 years, we
318 continued to monitor and amend only six of the microcosms. Two (GT4 and GT3) were 20%
319 transfers from original bottles into anaerobic medium, that received CLD and donor (GT4) or
320 CLD+TCE and donor (GT3). Transfer GT4 contained none of the original microcosm soil. Four
321 other transfers were made by pouring the entire contents of the original microcosm into slightly
322 larger bottles sealed with Teflon Mininert caps, topped up with medium and amended with CLD
323 and donor (GT5, GT20 and GT33) or CLD+TCE and donor (GT15) (Figure S1). Transfers GT5,
324 GT20 and GT15 continued to produce methane after being transferred, GT33 stopped producing
325 methane after the first re-amendment of CLD, while GT3 and GT4 did not produce any methane
326 after the transfer (Figure S2). In microcosms that received TCE in addition to CLD, TCE was
327 partially dechlorinated to trans- and cis-DCE, with possibly small amounts of ethane produced
328 (Figure S4), but the 20% transfer (GT3) no longer dechlorinated TCE. No ethene was ever found
329 in any of the microcosms (Table S5). In the transfers that did not produce methane (GT33, GT3
330 and GT4), we observed production of acetate (Table S5) although not enough to explain
331 complete transformation of donor. As a result, pH dropped to below 6 in these microcosms (and
332 was occasionally re-adjusted), which probably subsequently eliminated methanogens (Table S5).
333 Sulfate (~1.2 mM initially) was present in all soil microcosms but was depleted within the first
334 year and was not added to transfers (Table S5). We suspect that iron(III) carried over from soil
335 may have also served as an acceptor in some bottles. High chloride background (>12 mM) was
336 present in the microcosms, precluding using chloride increase as a proxy for dechlorination. In
337 summary, we established that all of the active (non-poisoned) microcosms were microbially
338 active initially as well as after 8 years of incubation, despite repeated additions of chlordecone
339 and donor (acetone or ethanol), but that there were significant differences in their extent of
340 methanogenesis depending on particular treatment history.
341
342 **Development of an LC/MS Method for Chlordecone and metabolites.** To enable a time-
343 course analysis and mass balance in microcosms, we needed to develop a CLD analysis method
344 suitable for repeated sampling in microcosms using small sample volumes. CLD analyses are
345 challenging for many reasons, including strong sorption of CLD to soil (and to rubber stoppers),
346 low water solubility of CLD and certain metabolites, lack of authentic standards for degradation
347 products, poor/variable or inconsistent ionization, as well as matrix and solvent effects. We

348 hoped that the high sensitivity and mass accuracy of Orbitrap MS technology would facilitate
349 identification of compounds despite lack of standards. The various early trials and final method
350 are described in Supplemental Method Details 2. Moriwaki and Hasegawa (2004) were first to
351 report use of LC-MS for CLD; they used a water/methanol gradient and negative ionization
352 mode, although only standards in methanol were tested. Durimel *et al.,* (2013) also detected CLD
353 by LC-MS using a water/acetonitrile gradient, also in negative mode. Cimetiere *et al.,* (2014)
354 included formic acid (pH 2) to the eluent and observed adduct (M+HCOOH+OH) formation.
355 These authors recommended addition of a co-solvent (acetonitrile, methanol) to avoid adsorption
356 of CLD to filters and to desorb CLD from suspended matter (acetonitrile). They concluded that
357 the presence of salts in sample matrixes weaken the CLD MS signal, which made us realize the
358 importance of preparing standards in the same matrix as the samples.
359
360 Based on these prior studies, standards were prepared in 50% medium and 50% methanol.
361 Methanol was added to samples before filtration to decrease sorption and to precipitate salts.
362 Methanol addition proved later to be a good solvent to mix with water to recover compounds
363 from soil particles. As first described by Harless *et al.,* (1978) and observed by others (Bichon *et*
364 *al.,* 2015, Chevallier 2017), CLD actually exists as a gem-diol (hydrate) in water or a hemiacetal
365 in methanol and can also form various adducts in the presence of compounds often used in LC
366 eluents, such as acetate and formate. In this study chromatographic separation, ionization and
367 signal intensity were maximized when ammonium acetate was included in the water and
368 methanol eluents. We observed many CLD adducts (hydrate, formate, acetate) and hemiacetal in
369 our analyses (Figure S5), that required careful data interpretation. Hydrochlordecones (MHCLD,
370 DHCLD and THCLD) also showed similar adduct formation. In this study we chose to calibrate
371 and report the hydrate forms of CLD, MHCLD, DHCLD and THCLD. All of the LC/MS data
372 collected during this study are compiled in Table S6. We initially only sampled the liquid phase
373 (centrifuged and filtered) to avoid variability related to sorption to solids in the microcosms.
374 However, sorption to soil in the microcosm was very strong, precluding a satisfactory mass
375 balance when considering only liquid phase samples. We eventually had to adapt the method to
376 include and quantify CLD on solids. Owing to the lack of standards for compounds other than
377 CLD (and later B1), the runs with different methods could not be compared easily. Despite all
378 these challenges, dechlorination products were clearly observed, as described in the next section.

379

380 **Detection of CLD Metabolites using best method.** A series of metabolites were observed in all
381 the active original microcosms and transfers over the course of the study (Figure 1 with data in
382 Table S6). Despite not having standards, the accuracy of the Orbitrap MS enabled identification
383 of metabolites by matching first or highest m/z values of the mass spectrum to the presumed
384 structure, and further evaluating the full characteristic isotope patterns for these multi-chlorinated
385 transformation products (details below). Table 1 lists all observed metabolites with their
386 measured and theoretical masses, and Figure 2 illustrates the characteristic mass spectra for these
387 metabolites. Details of observed and theoretical spectra are provided in Figure S6. The identified
388 metabolites were assigned to three different groups of metabolites, group A; hydrochlordecones
389 (HCLD), group B; polychloroindenes (PCIN) and group C; carboxylated polychloroindenes
390 (CPCIN). Group assignments A, B and C were chosen to be consistent with the work of
391 Chaussonnerie, Chevalier *et al.,* (Chaussonnerie *et al.,* 2016, Chevallier 2017, Chevallier *et al.,*
392 2018) who observed many of the same metabolites in their studies. A total of 19 different
393 dechlorination products were detected in the microcosms in this study. Some metabolites showed
394 multiple isomers, identified by having exactly the same mass but varying retention time.
395 Retention times were consistent with the relative polarity of each compound. Compounds with
396 retention times 9.3, 8.88, and 8.05 min and mass to charge ratios m/z 468.7264, m/z 434.7661
397 and m/z 400.8042 respectively, could be attributed to the hydrate forms of MHCLD (A1)
398 $[C_{10}Cl_9O_2H_2]^-$, DHCLD (A2) $[C_{10}Cl_8O_2H_3]^-$ and THCLD (A3) $[C_{10}Cl_7O_2H_4]^-$ obtained by the
399 loss of 1, 2 and 3 chlorine atoms from CLD (Table 1). Three metabolites, less polar than CLD
400 and with longer retention times at 12.19, 11.44 and 10.34 min, respectively, exhibiting mass to
401 charge ratios of m/z 284.8616, m/z 250.9006 and m/z 216.9382 were identified as
402 pentachloroindene (B1) $[C_9Cl_5H_2]^-$, tetrachloroindene (B2) $[C_9Cl_4H_3]^-$ and trichloroindene (B3)
403 $[C_9Cl_3H_4]^-$ (Table 1). Thirteen (13) metabolites more polar that CLD were assigned to group C,
404 and observed at retention times ranging from 2.02 to 6.69 min (Table 1). Compounds C1 and C2,
405 detected respectively at retention times of 5.47 and 5.02 min, and with a corresponding m/z of
406 294.8899 could be attributed to two isomers of a carboxylated tetrachloroindene compound
407 $[C_{10}Cl_4O_2H_3]^-$ obtained by the loss of 6 chlorine atoms. At retention times between 3.03 and 6.69
408 min, 5 isomers (C3-C7) with a corresponding m/z of 260.9287 were detected and attributed to a
409 carboxylated trichloroindene compound $[C_{10}Cl_3O_2H_4]^-$ obtained by the loss of 7 chlorine atoms.

410 Four isomers (C8-C11) obtained by the loss of 8 chlorine atoms were detected at retention times
411 between 2.02 and 5.25 min and had m/z of 226.9677, assigned to be a carboxylated
412 dichloroindene $[C_{10}Cl_2O_2H_5]^-$. Two last isomers (C12 and C13) with retention times at 2.5 and
413 2.95 min and m/z of 193.0064 were classified as carboxylated monochloroindene $[C_{10}ClO_2H_6]^-$,
414 obtained by the loss of 9 chlorine atoms from CLD. An example chromatogram from one of the
415 samples, bottle GT20 sampled June 29th 2018, is shown in Figure 3. Chlordecone and 17 of the
416 19 observed dechlorination products detected in active microcosms are shown, including the
417 non-polar B compounds (polychloroindenes, PCINs) showing longer retention times than CLD,
418 and the more polar C compounds (carboxylated polychloroindenes, CPCINs) with much shorter
419 retention times (Figure 3).
420
421 In summary, CLD metabolites observed in the microcosms could be classified into 3 families of
422 compounds: group A compounds including mono-, di- and trihydrochlordecone derivatives with
423 proposed neutral formula $[C_{10}Cl_{10-n}OH_n$ n= 1,2,3]; group B non-polar "open cage" structures
424 including three polychloroindene compounds with proposed neutral formula $[C_9Cl_{5-n}H_{3+n}$
425 n=0,1,2]; and group C polar "open cage" structures consisting of carboxylated polychloroindene
426 derivatives with neutral formula $[C_{10}Cl_{4-n}O_2H_{4+n}$, n=0-3].
427
428 Due to a number of challenges with and changes in sample preparation and LC-MS analysis over
429 time, it was difficult to get an accurate picture of concentration changes of CLD over time.
430 Therefore, when we finally settled on a good method, we re-analyzed some frozen archived
431 samples to get a comparable set of data. Results from a selection of samples from GT20 with
432 comparable sample preparation (liquid phase only, no soil) and analytical methods are shown in
433 Figure 4 (raw data in Table S7). Results show that none of the monitored metabolites were
434 detected in the sample taken two weeks into the experiment, but by 8 months into the study, we
435 observed MHCLD, DHCLD, and two CPCINs with loss of 8 and 9 chlorines. Later sampling
436 time points showed increasing concentrations of MHCLD, DHCLD, and four CPCINs with loss
437 of 6 to 9 chlorines. Pentachloroindene (B1) only showed up in the last sampling point, however it
438 was found to sorb strongly to soil, and we would therefore not expect to see it in these liquid
439 phase samples analyzed here. Also, because we kept adding more CLD to the bottles over time,
440 we could not use aqueous CLD concentration changes as a measure of degradation rate. Despite

441 not seeing a clear decrease in CLD concentrations in the liquid phase, highly dechlorinated
442 metabolites with up to 9 chlorine removed were observed in the active bottles but not in the
443 controls indicating that biological processes were involved in the dechlorination of CLD into
444 HCLD, PCIN and CPCIN metabolites.
445
446 **Effect of Sorption.** Strong sorption of CLD and non-polar PCID metabolites to soil particles
447 made it difficult to evaluate the fate of CLD in the bottles by sampling the aqueous phase only.
448 We therefore changed our approach to also extract soil in our samples. By analyzing the same
449 samples by two different sample preparation methods, one in which only the liquid concentration
450 was measured (filtered sample) and another in which a mixture of soil and water was analyzed,
451 and quantifying the amount of soil (dry mass) in the sample, we were able to calculate and
452 estimate a distribution coefficient, K_d (l/kg) equal to the ratio of sorbed concentration (mg/kg) to
453 dissolved concentration (mg/l) for most of the analytes (Table 2; raw data in Table S8). This
454 analysis confirmed that CLD and mono- and dihydro- CLD, with K_d values of 130 ± 57, 52 ± 12
455 and 28 ± 6 ml/g (or l/kg), respectively, absorb quite strongly to the Guadeloupe soil used, while
456 the K_d value for B1 was much higher at 5700 ± 220 l/kg, and thus absorb even more strongly.
457 The carboxylated chloroindenes had much lower K_d values ranging from 2 to 11 l/kg, and were
458 found in the aqueous phase. The estimated K_d values correspond well to the retention times by
459 reverse phase LC (Table 2). Our estimated distribution coefficient for chlordecone is in the same
460 range (60-330 l/kg) as one previous report (Fernández-Bayo *et al.,* 2013). A distribution
461 coefficient based on organic content, K_{oc}, of 2,500 l/kg has also been reported (Howard 1991),
462 corresponding to a K_d of 250 l/kg assuming a fraction of organic carbon of 10%. We were not
463 able to find any reports of sorption coefficients for any CLD metabolites.
464
465 **Quantification of the Extent of Transformation in Microcosms.** To quantify the extent of
466 transformation of CLD added to the microcosms over the course of the study, we used data from
467 well-mixed slurry samples from 13 active, 7 poisoned controls, 2 unamended microcosms and
468 two medium controls (Table 3). The well-mixed slurry samples were analyzed because they
469 capture mass from both the liquid and solid phases, so that we could better compare final
470 measured mass recovered to the total amount of CLD that had been added to the bottles. To
471 attempt a mass balance, MHCLD, DHCLD, and THCLD concentrations were estimated based on

472 the response factor for CLD as no standards were available for these metabolites. Researchers
473 from Genoscope (France) kindly provided us with a small sample of pentachloroindene (B1) that
474 they had managed to chemically purify. As a result, we were able to quantify the amount of B1
475 produced in active microcosms. B2 concentrations were estimated using the response factor for
476 B1. For the carboxylated polychlorinated indenes (C group), we have no proxy for calibration;
477 however, to get a very rough idea of possible concentrations we also used the response factor for
478 B1 for these compounds.
479
480 Using these estimated response factors applied to the areas determined by LC/MS analysis of
481 well-mixed slurry samples, we could calculate total moles recovered per bottle by multiplying
482 concentrations by the total slurry volume. We then compared the CLD and metabolite moles
483 recovered in the bottles after 8 years to the initial amount of CLD added (Table 3; calculations in
484 Table S9). The three different groups of microcosms, poisoned controls, active original
485 microcosms, and active microcosm transfers, did indeed show differences in CLD recovery. In
486 the group of seven poisoned controls, two microcosms (G1 and G3) produced a lot of methane
487 and thus were biologically active, despite having been poisoned. These bottles also exhibited
488 extensive metabolite production, unlike remaining controls (Figure 1 and Table 3). Therefore, for
489 the mole balance analysis, we included those two microcosms into the group of active original
490 microcosms. We were able to recover 63±6% of added CLD in the poisoned controls after 8
491 years, 44±11% in the original microcosms that only receive electron donor in the first 2 years,
492 and only 32±3% of added CLD in the transfers amended regularly with donor and CLD (Table
493 3). The loss of ~37% in the poisoned control group likely results from sorption to glass and
494 stoppers, poor extraction from soils during sample preparation, losses from volatilization, and
495 some minimal losses (<1%) from sample removal. Losses in microbially-active bottles are
496 greater and can be explained by the contribution of biological transformation processes.
497
498 We estimated the total moles recovered as metabolites in the various treatments. Metabolites
499 were not detected in the un-amended slurry microcosms, nor in the medium controls. The
500 inactive poisoned controls had only trace amounts of MHCLD (0.001-0.004 μmoles) and no
501 other metabolites. The active bottles had significantly higher concentrations of metabolites,
502 especially in the transfers that received more CLD and donor. The sum of moles of metabolites

503 ranged from 13% to 98% of the CLD remaining after 8 years in active microcosms. When the
504 sum of all measured metabolites was included in the mole balance, overall recoveries after 8
505 years were more similar regardless of treatment, 63±6%, 60±14%, and 50±13% for controls,
506 originals and transfers, respectively. Given the length of the study and approximations in
507 calibration factors, these results were very reassuring and provided confidence in the
508 measurements.
509
510 **Microbial Community Analysis qPCR and Sequencing Analysis.** While DNA samples were
511 collected and analyzed at various times throughout the 8 years, preliminary analyses have not
512 revealed any clear trends to date. A snap shot of the microbial community and abundance after
513 77 months is shown in Figure 5 (raw data are provided in Tables S10 and S11). The microbial
514 community of the 6 transfers reveals an abundance of fermentative and syntrophic anaerobes,
515 consistent with the electron donor mix (acetone and ethanol) provided. Microcosms GT33 and
516 GT4 produced little to no methane, and contain few or no methanogens. GT4 is the most
517 extensively transferred microcosm and no longer contains soil. It also has the lowest bacterial
518 cell numbers inferred from qPCR of 16S rRNA copies per ml. No particular trends are
519 discernable at this time. Perhaps, as concluded by Chaussonnerie *et al.,* (2016), the microbial
520 transformation is cometabolic, and really only dependent on sufficient availability of reduced
521 vitamin B12. Further studies are clearly warranted.
522
523 **Analysis of Field Samples from 2018.** We wondered if metabolites identified in the microcosms
524 could also be detected in field samples, therefore we returned to Guadeloupe and analyzed 9
525 more samples. (Table 4; raw data in Table S12). Anticipating quite low concentrations in the
526 field samples, we decided to sample larger 5 and 20 ml volumes and perform a liquid-liquid
527 extraction with a concentration step (method 5 and 6, Supplemental Method Details 2) in
528 addition to our already established 1 ml slurry sample preparation method (method 4,
529 Supplemental Method Details 2). Analysis of 6 soil samples from banana plantations in
530 Guadeloupe revealed CLD concentrations in the range 120 to 1000 ng/g soil, or 0.12 to 1.0
531 mg/kg. These values are pretty typical of Guadeloupe soils: a recent survey (Crabit *et al.,* 2016)
532 reported CLD concentrations in mg/kg in soil of 0.03 (minimum), 2.00 (median), 3.39 (mean),
533 and 24.2 (maximum). MHCLD was detected in all soil samples and ranged from 1 to 8% of

534 CLD based on area from LC/MS analysis. Most soil samples also showed DHCLD, but area
535 counts were 10 to 100 times lower than those of MHCLD. Pentachloroindene (B1)
536 concentrations ranged from 0.5 to 24 ng/g of solid. B2 metabolites were also detected in the soil
537 samples, with slightly lower area counts than those of B1. Two of the 3 activated carbon sludge
538 samples showed quite high CLD concentrations, between 6,000 and 8,500 ng/g soil. MHCLD
539 concentrations were also significant, between 4 and 10% of CLD based on area counts.
540 Interestingly, no PCIN was detected in the activated carbon sludge samples – presumably too
541 aerobic. We did not detect CPCIN metabolites in any of the field samples. Due to their
542 hydrophilicity, the CPCIN metabolites would likely not be captured using the liquid-liquid
543 extraction/concentration method (methods 5 and 6) used, and we did not have an alternative
544 method for concentration of these compounds. Perhaps due to their hydrophilic nature and low
545 sorption, these compounds were already washed out from the soils by rain. These field data
546 confirm that anaerobic ring opening and dechlorinating processes do occur *in situ* in Guadeloupe
547 soils. A more extensive analysis is thus warranted to determine locations and conditions for
548 highest intrinsic activity on the islands and if rates could be accelerated by inducing anaerobic
549 conditions, such as through organic amendment. The mechanism of CLD biotransformation also
550 needs further investigation.
551
552 **ASSOCIATED CONTENT**
553 **Supporting Information.** Method details including recipe for artificial groundwater, LC/MS
554 analysis procedure and sample preparation methods, DNA extraction, amplicon sequencing, and
555 qPCR analysis procedures. Figures showing microcosm transfers and their origins, history of
556 microcosms (CLD added, donor added, methane produced), methane production in active CLD
557 amended MCs during the first 1.5 years of monitoring, TCE and its dechlorination products in a
558 CLD and TCE amended MC (G15) during the first 1.5 years of monitoring, LC/MS scan analysis
559 of CLD (CLD hydrate, hemi-acetal and other observed CLD adducts), and chromatograms and
560 spectra for each of the 19 observed metabolites. Tables showing Guadeloupe field sample
561 details, treatment table for Guadeloupe microcosm study, details of feeding stock preparation,
562 details of CLD and donor amendments, details of GC-FID and IC analysis and pH
563 measurements, results of all LC/MS analysis, normalized LC/MS area counts in GT20, sorption

564 calculations and plots, mass balance calculations, sequencing analysis and qPCR analysis, and
565 concentration calculations for CLD and its degradation products in Guadeloupe field samples.
566

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575

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653
654

655 **TABLES FOLLOW**

656 **Then FIGURES with LEGENDS for review**

Table 1: Chlordecone and dechlorinated metabolites identified in microcosm samples. Mass to charge (m/z) values and retention times are from GT20 slurry sample taken June 29th 2018, except as noted.

Compound ID*	m/z theoretical (monoisotopic)	m/z observed (monoisotopic)	delta ppm	Proposed formula (neutral)	Proposed ion formula (negative mode)	Used for calibration and reporting m/z (theoretical)	Molecular formula	Compound structure*	# of chlorines removed	Retention time (min)	Isomer
A1	468.7257	468.7264	-1.49	$C_{10}Cl_9O_2H_3$	$[C_{10}Cl_9O_2H_2]^-$	472.7198	$C_{10}{}^{35}Cl_7{}^{37}Cl_2O_2H_2$	Monohydrochlorodecone hydrate	1	9.3	Isomer 1
A2[α]	434.7647	434.7661	-3.22	$C_{10}Cl_8O_2H_4$	$[C_{10}Cl_8O_2H_3]^-$	438.7588	$C_{10}{}^{35}Cl_6{}^{37}Cl_2O_2H_4$	Dihydrochlorodecone hydrate	2	8.88	Isomer 1
A3	400.8036	400.8042 [β]	-1.50	$C_{10}Cl_7O_2H_5$	$[C_{10}Cl_7O_2H_4]^-$	402.8007	$C_{10}{}^{35}Cl_6{}^{37}ClO_2H_2$	Trihydrochlorodecone hydrate	3	8.05	Isomer 1
B1	284.8605	284.8616	-3.86	$C_9Cl_5H_3$	$[C_9Cl_5H_2]^-$	286.8575	$C9^{35}Cl_4{}^{37}ClH_3$	pentachloroindene	5	12.19	Isomer 1
B2	250.8994	250.9006	-4.78	$C_9Cl_4H_4$	$[C_9Cl_4H_3]^-$	252.8965	$C9^{35}Cl_3{}^{37}ClH_4$	tetrachloroindene	6	11.44	Isomer 1
B3	216.9384	216.9382 [γ]	0.92	$C_9Cl_3H_5$	$[C_9Cl_3H_4]^-$	216.9384	$C_9Cl_3H_5$	trichloroindene	7	10.34	Isomer 1
C1	294.8893	294.8899	-2.03	$C_{10}Cl_4O_2H_4$	$[C_{10}Cl_4O_2H_3]^-$	294.8893	$C_{10}Cl_4O_2H_4$	carboxylated tetrachloroindene	6	5.47	Isomer 1
C2	294.8893	294.8899	-2.03	$C_{10}Cl_4O_2H_4$	$[C_{10}Cl_4O_2H_3]^-$	294.8893	$C_{10}Cl_4O_2H_4$	carboxylated tetrachloroindene	6	5.02	Isomer 2
C3	260.9282	260.9287	-1.92	$C_{10}Cl_3O_2H_5$	$[C_{10}Cl_3O_2H_4]^-$	260.9282	$C_{10}Cl_3O_2H_5$	carboxylated trichloroindene	7	6.69	Isomer 1
C4	260.9282	260.9287	-1.92	$C_{10}Cl_3O_2H_5$	$[C_{10}Cl_3O_2H_4]^-$	260.9282	$C_{10}Cl_3O_2H_5$	carboxylated trichloroindene	7	6.13	Isomer 2
C5	260.9282	260.9288	-2.30	$C_{10}Cl_3O_2H_5$	$[C_{10}Cl_3O_2H_4]^-$	260.9282	$C_{10}Cl_3O_2H_5$	carboxylated trichloroindene	7	3.88	Isomer 3
C6	260.9282	260.9287	-1.92	$C_{10}Cl_3O_2H_5$	$[C_{10}Cl_3O_2H_4]^-$	260.9282	$C_{10}Cl_3O_2H_5$	carboxylated trichloroindene	7	3.41	Isomer 4
C7	260.9282	260.9287	-1.92	$C_{10}Cl_3O_2H_5$	$[C_{10}Cl_3O_2H_4]^-$	260.9282	$C_{10}Cl_3O_2H_5$	carboxylated trichloroindene	7	3.03	Isomer 5
C8	226.9672	226.9677	-2.20	$C_{10}Cl_2O_2H_6$	$[C_{10}Cl_2O_2H_5]^-$	226.9672	$C_{10}Cl_2O_2H_6$	carboxylated dichloroindene	8	5.25	Isomer 1
C9	226.9672	226.9677	-2.20	$C_{10}Cl_2O_2H_6$	$[C_{10}Cl_2O_2H_5]^-$	226.9672	$C_{10}Cl_2O_2H_6$	carboxylated dichloroindene	8	4.57	Isomer 2
C10	226.9672	226.9676	-1.76	$C_{10}Cl_2O_2H_6$	$[C_{10}Cl_2O_2H_5]^-$	226.9672	$C_{10}Cl_2O_2H_6$	carboxylated dichloroindene	8	2.21	Isomer 3
C11	226.9672	226.9674	-0.88	$C_{10}Cl_2O_2H_6$	$[C_{10}Cl_2O_2H_5]^-$	226.9672	$C_{10}Cl_2O_2H_6$	carboxylated dichloroindene	8	2.02	Isomer 4
C12	193.0062	193.0064	-1.04	$C_{10}ClO_2H_7$	$[C_{10}ClO_2H_6]^-$	193.0062	$C_{10}ClO_2H_7$	carboxylated chloroindene	9	2.95	Isomer 1
C13	193.0062	193.0064	-1.04	$C_{10}ClO_2H_7$	$[C_{10}ClO_2H_6]^-$	193.0062	$C_{10}ClO_2H_7$	carboxylated chloroindene	9	2.5	Isomer 2
CLD	502.6867	502.6879	-2.39	$C_{10}Cl_{10}O_2H_2$	$[C_{10}Cl_{10}O_2H]^-$	506.6808	$C_{10}{}^{35}Cl_8{}^{37}Cl_2O_2H_2$	chlordecone hydrate	0	9.86	Isomer 1

* Compound groups A, B, C are consistent with work of Chevalier et al. Assumed compound structures, based on observed m/z values and retention times, and comparison with work previously reported (Chaussonnerie *et al.,* 2016, Chevallier *et al.,* 2018 and Chevallier 2017)

[α] Referred to as A3 in Chaussonnerie *et al.,* 2016

[β] Observed m/z value from sample GT3 March 7th 2019 (metabolite A3 was not found in GT20 June 29th 2018)

[γ] Observed m/z value from sample G19 March 7th 2019 (metabolite B3 was not found in GT20 June 29th 2018)

Table 2: Estimated Sorption coefficients for CLD and some dechlorinated metabolites in Guadeloupe soils

Compound	Measured Kd* (ml/g)	Kd from literature (ml/g)	MW (g/mol)	LC/MS retention time
B1	5700 ± 220	not available	288.39	12.19 min
B2	NA	not available	253.94	11.44 min
CLD	**130 ± 57**	**~ 60-330****	**490.64**	**9.86 min**
MHCLD	52 ± 12	not available	456.19	9.3 min
DHCLD	28 ± 6	not available	421.75	8.88 min
C1-C2	5.6 ± 1.0	not available	297.95	5.02 - 5.46 min
C3-C7	11 ± 2.0	not available	263.5	3.03 - 6.69 min
C8-C11	9.8 ± 4.4	not available	229.06	2.02 - 5.25 min
C12-C13	2.1 ± 1.0	not available	194.61	2.5 - 2.95 min

*See Table S8 for calculations and plots. Kd values are slope ± standard error of the slope (n=4)
** Fernandez-Bayo, J.D., *et al.,* Science of the Total Environment, 2013. **463**: p. 395-403.
NA: Not applicable (Kd could not be calculated because compound was not detected in liquid phase)

Table 3: Extent of transformation of CLD in microcosms and transfers (µmol per bottle) after 8 years of incubation. Samples were taken March 7th 2019 (1 ml slurry samples; sample preparation method 4). Raw data and calculations are shown in Table S9.

	Sample	Total CLD added to date (µmol)	CLD remaining after 8 years (µmol)	CLD recovery %	MHCLD, DHCLD and THCLD* (µmol)	PCINs (B1 and B2)** (µmol)	CPCINs (C1-C13)** (µmol)	Sum of all products (µmol)	Sum all after 8 yrs (incl. CLD) (µmol)	Products/CLD remaining %	Total recovery after 8 years %
CONTROLS	G17_unamended	0.00	0.00	NA	ND	ND	ND	0.0	0.00	0%	NA
	G18_unamended	0.00	0.00	NA	ND	ND	ND	0.0	0.00	0%	NA
	Medium1	4.08	4.02	99%	ND	ND	ND	0.0	4.01	0%	98%
	Medium2	8.15	1.69	21%	ND	ND	ND	0.0	1.69	0%	21%
	G28_poisoned	1.63	1.13	69%	0.002	ND	ND	0.002	1.13	0%	69%
	G30_poisoned	1.63	0.967	59%	0.001	ND	ND	0.001	0.97	0%	59%
	G41_poisoned	1.63	1.06	65%	0.004	ND	ND	0.004	1.06	0%	65%
	G50_poisoned	1.63	0.899	55%	0.002	ND	ND	0.002	0.90	0%	55%
	G59_poisoned	1.63	1.07	66%	0.001	ND	ND	0.001	1.07	0%	65%
AVERAGE poisoned controls		***1.63***	***1.03***	***63%***	***0.002***	***ND***	***ND***	***0.002***	***1.02***	***0.2%***	***63%***
Standard Dev (n=5)		***0.00***	***0.09***	***6%***	***0.001***	***-***	***-***	***0.001***	***0.09***	***0.1%***	***6%***
ORIGINAL MICROCOSMS	G1_poisoned #	1.63	0.831	51%	0.092	0.030	0.10	0.22	1.05	27%	64%
	G3_poisoned #	1.63	0.835	51%	0.079	0.061	0.08	0.22	1.05	26%	64%
	G4	3.26	1.36	42%	0.139	0.047	0.11	0.30	1.65	22%	51%
	G19	3.26	1.75	54%	0.451	0.125	0.24	0.81	2.55	47%	78%
	G31	3.26	1.73	53%	0.130	0.045	0.05	0.22	1.94	13%	60%
	G45	3.26	0.826	25%	0.237	0.031	0.05	0.32	1.15	39%	35%
	G54	3.26	1.18	36%	0.507	0.038	0.10	0.64	1.82	55%	56%
	G63	3.26	1.81	56%	0.034	0.011	0.77	0.82	2.63	45%	81%
	G14 +TCE	3.26	1.05	32%	0.615	0.095	0.20	0.91	1.95	87%	60%
AVERAGE originals		***2.90***	***1.39***	***44%***	***0.30***	***0.06***	***0.22***	***0.57***	***1.96***	***40%***	***60%***
Standard Dev (n=9)		***0.72***	***0.39***	***11%***	***0.22***	***0.04***	***0.26***	***0.29***	***0.51***	***22%***	***14%***
TRANSFERS AMENDED REGULARLY	GT15	17.9	5.60	31%	2.43	0.689	1.07	4.19	9.77	75%	54%
	GT3	15.8	5.70	36%	4.11	0.115	1.37	5.60	11.3	98%	71%
	GT5	23.6	7.37	31%	1.28	0.507	1.12	2.91	10.3	40%	43%
	GT20	25.7	8.42	33%	2.70	1.02	1.12	4.84	13.2	58%	52%
	GT33	19.6	6.11	31%	1.54	0.231	1.14	2.90	8.99	48%	46%
	GT4	22.4	5.99	27%	0.475	0.159	0.63	1.26	7.24	21%	32%
AVERAGE transfers		***20.8***	***6.53***	***32%***	***2.09***	***0.45***	***1.07***	***3.62***	***10.1***	***57%***	***50%***
Standard Dev (n=6)		***3.7***	***1.12***	***3%***	***1.28***	***0.35***	***0.24***	***1.57***	***2.04***	***27%***	***13%***

Microcosm G1 and G3 were amended with mercuric chloride and sodium azide initially, yet produced similar amounts of methane (and products) as un-poisoned original microcosms

*Concentrations were estimated using response factor for CLD

**Concentrations were estimated using response factor for B1

NA: Not applicable; ND: Not detected

Table 4: Chlordecone and dechlorinated metabolites detected in field samples (agricultural soils and treatment plant activated carbon-AC) taken in 2018 from Guadeloupe. No carboxylated polychloroindenes were detected in these samples. Numbers are maximum area from the LC/MS analysis of two different slurry sample sizes (5 and 20 ml). Raw data in Table S12. CLD, MHCLD and DHCLD are calibrated and reported as CLD hydrate, MHCLD hydrate and DHCLD hydrate.

	Chlordecone		**Hydrochlordecones**			**Polychloroindenes**			
	CLD		**MHCLD (A1)**	**DHCLD (A2)**	**A1+A2**	**$C_9Cl_5H_3$ (B1)**		**$C_9Cl_4H_4$ (B2)**	
	area/g solids	ng/g solids	area/g solids	area/g solids	% CLD area/g solids	area/g solids	ng/g solids*	area/g solids	% B1 area/ g solids
AgSoil1	1.4E+07	120	8.7E+05	2.1E+04	6%	6.3E+03	0.48	4.1E+03	65%
AgSoil2	2.7E+07	130	1.9E+06	1.6E+04	7%	3.0E+04	2.3	5.9E+03	20%
AgSoil3	1.9E+08	1000	5.3E+06	8.0E+04	3%	3.1E+04	2.3	9.9E+02	3.2%
AgSoil4	6.2E+07	270	5.1E+06	8.8E+04	8%	3.1E+05	24	4.2E+04	14%
AgSoil5	5.7E+07	130	4.2E+06	2.2E+04	7%	1.1E+05	8.8	2.6E+04	23%
AgSoil6	3.5E+07	430	5.0E+05	0.0E+00	1%	1.5E+04	1.1	1.0E+04	70%
AC-1	1.5E+07	24	1.5E+06	7.0E+05	10%	ND	ND	ND	ND
AC-2	3.6E+09	6000	2.8E+08	1.8E+07	8%	ND	ND	ND	ND
AC-3	5.1E+09	8400	1.9E+08	5.8E+06	4%	ND	ND	ND	ND

*estimated values based on analysis of B1 standards run in 2019

ND: Not detected

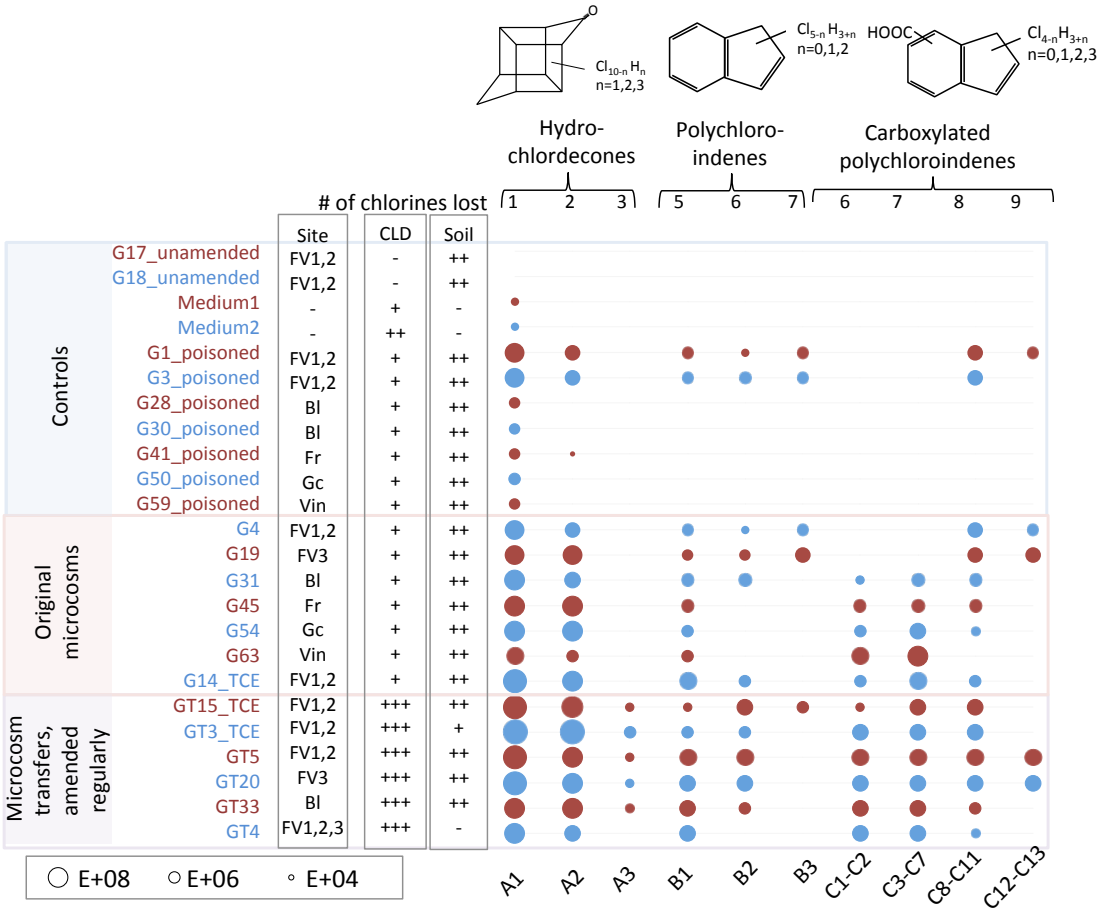


Figure 1: Dechlorinated metabolites observed in anaerobic microcosms constructed from Guadeloupe soil and water. The size of each circle is proportional to the area count from LC/MS analysis of slurry samples performed March 7th 2019 (see Table S6 for raw data).

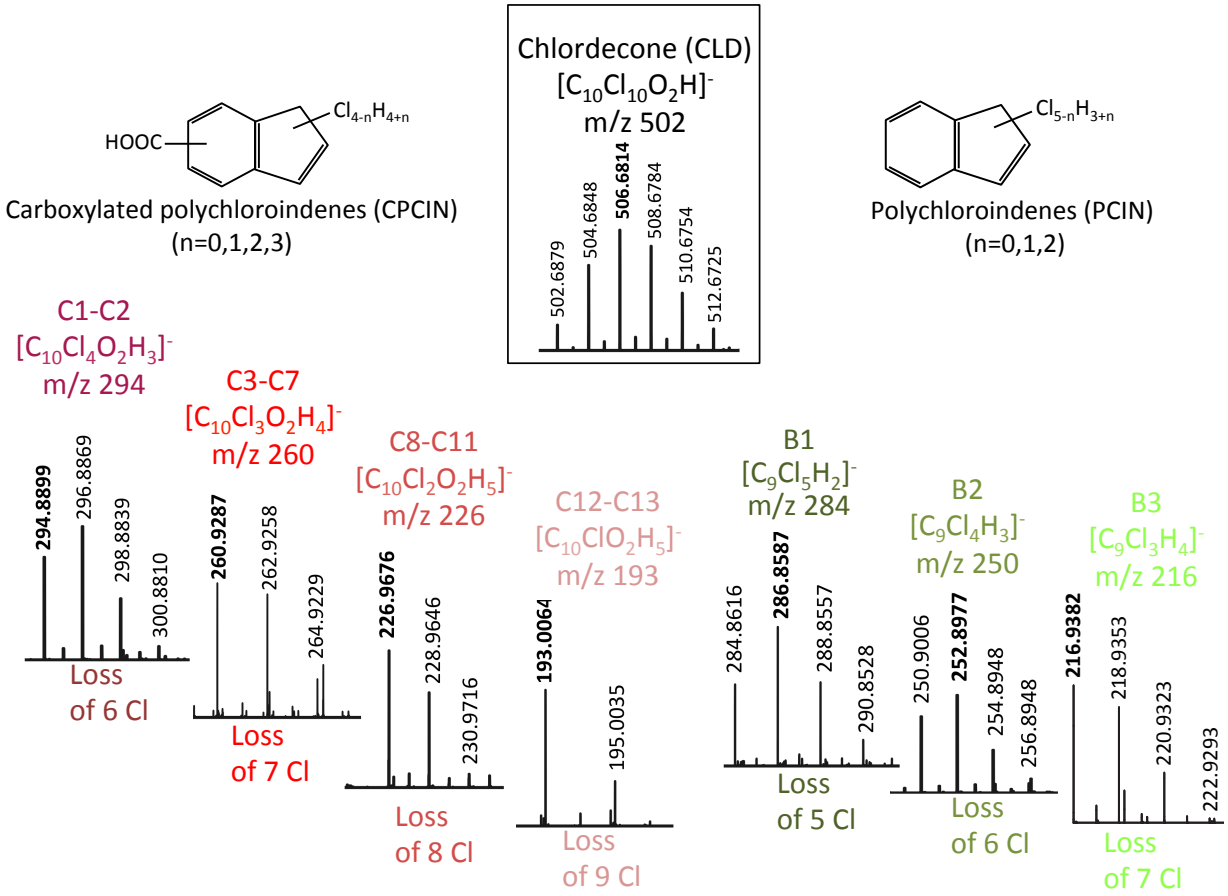


Figure 2: Mass spectra of observed "open cage" dechlorinated metabolites. Mass spectra are from sample GT20 June 29th 2018 (CLD, B1, B2, C1-C13) and sample G19 March 7th 2019 (B3) (slurry samples). Full spectra are shown in Figure S6. Isotope m/z values in bold were the masses used for calibration and reporting. Chlordecone is calibrated and reported in the form of chlordecone hydrate.

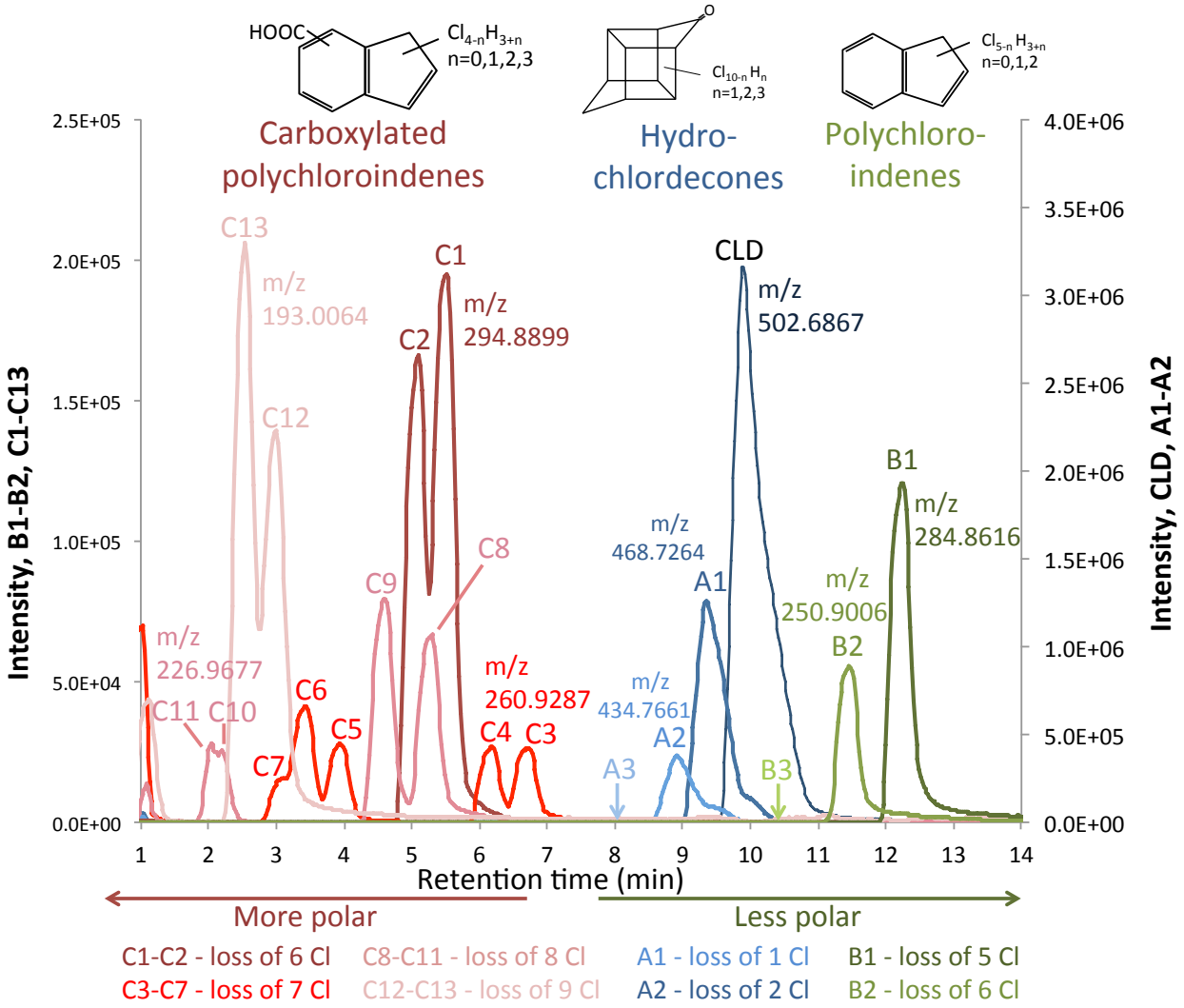


Figure 3: Chromatograms of chlordecone and its dechlorinated metabolites in sample GT20 June 29th 2018 (slurry sample). Results are from LC/MS, equipped with ESI, in negative mode. Observed m/z values (monoisotopic) for the different compounds were; 502.6879 (CLD), 468.7264 (A1), 434.7661 (A2), 284.8616 (B1), 250.9006 (B2), 294.8899 (C1-C2), 260.9287 (C3-C7), 226.9677 (C8-C11), 193.0064 (C12-C13) (see details in Table 1). Metabolites m/z 400.8042 (A3) and 216.9382 (B3) were not observed in the illustrated sample but in some other samples in the study. Arrows indicate observed retention times for these two metabolites. CLD, MHCLD, DHCLD and THCLD are calibrated and reported in the forms of CLD hydrate, MHCLD hydrate, DHCLD hydrate and THCLD hydrate.

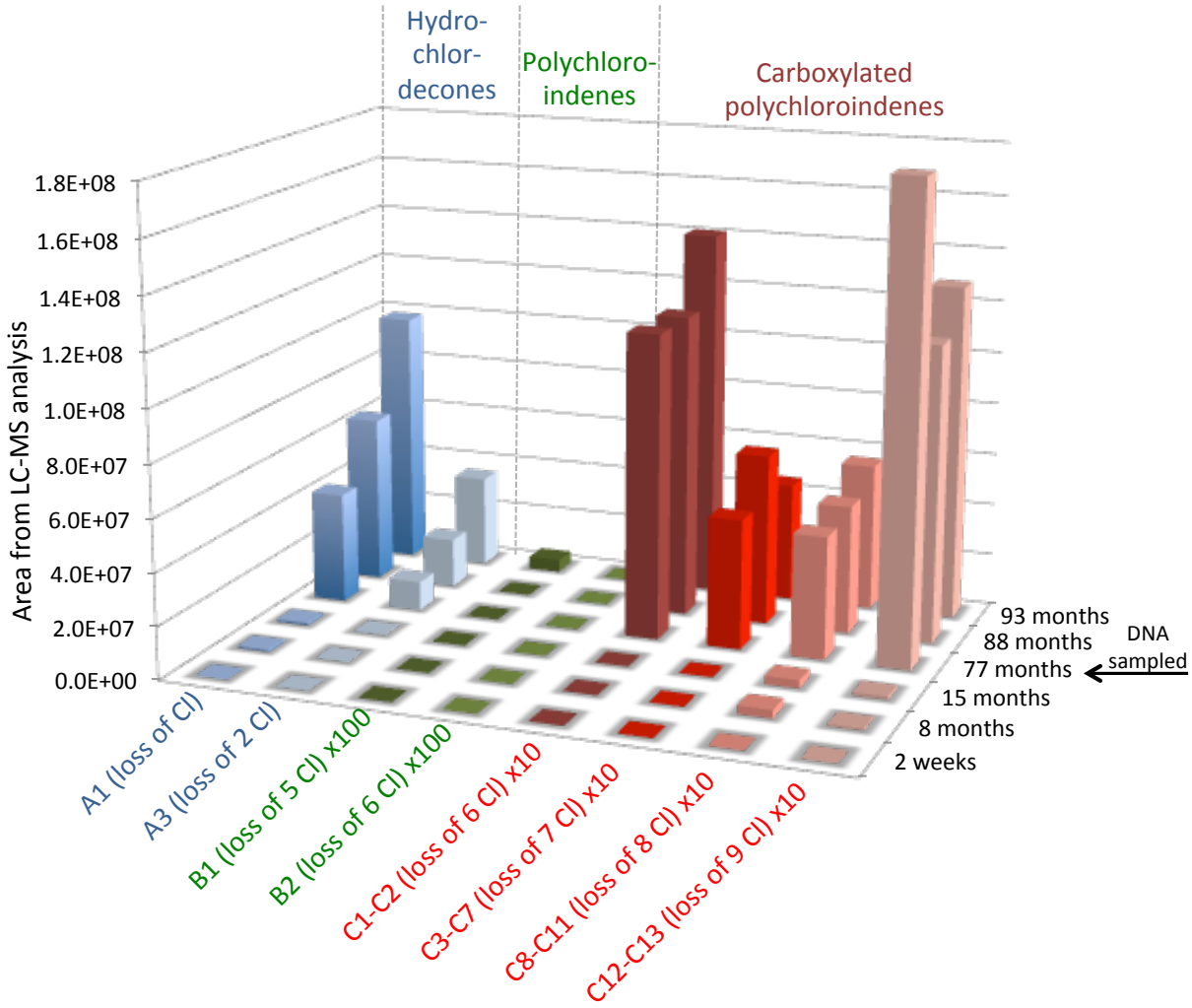
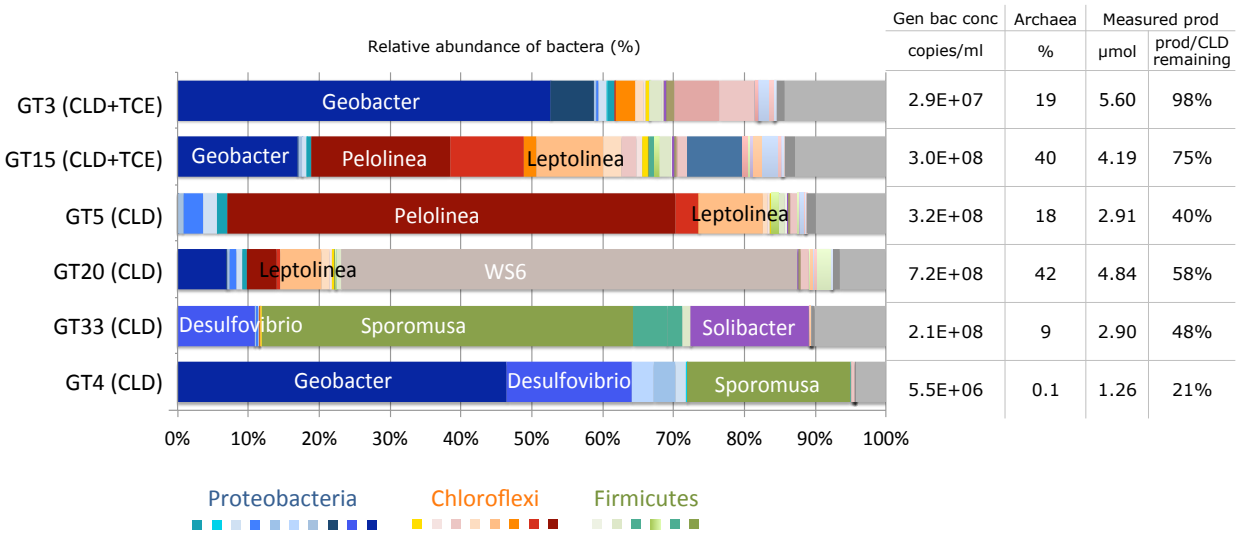


Figure 4: Dechlorinated metabolites from chlordecone over time in one of the anaerobic microcosms, transfer GT20. Only samples that were prepared the same way are included in this graph (sample preparation method 3, liquid phase only, no soil, see supplemental method details). Areas of B compounds were multiplied with 100, and areas of C compounds were multiplied with 10 for better display of all metabolites in the same graph. All areas were normalized (raw data in Table S7).



	Gen bac conc	Archaea	Measured prod	
	copies/ml	%	µmol	prod/CLD remaining
GT3 (CLD+TCE)	2.9E+07	19	5.60	98%
GT15 (CLD+TCE)	3.0E+08	40	4.19	75%
GT5 (CLD)	3.2E+08	18	2.91	40%
GT20 (CLD)	7.2E+08	42	4.84	58%
GT33 (CLD)	2.1E+08	9	2.90	48%
GT4 (CLD)	5.5E+06	0.1	1.26	21%

Figure 5: Microbial community in microcosm transfers. DNA was sampled 77 months after microcosm setup. The bar chart includes bacteria only. Table to the right shows concentration of general bacteria measured by qPCR (copies/ml), % archaea, and measured products (estimated) (total, µmol and product/CLD remaining, %) for each bottle. Raw data of sequencing results and qPCR measurements can be found in Table S10.