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
IC Ion 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

70 Curlone® and was spread at the foot of each banana plant as a solution delivering 1.5 grams of
71 chlordecone per plant. With 800 banana plants per hectare and 2.5 applications per year, the
72 resulting dosage was a significant 3 kg chlordecone per hectare per year. As a consequence of
73 such intensive application, 8–9% of the cultivated areas of Guadeloupe have CLD concentrations
74 higher than 1 mg/kg in topsoil, and some banana fields have concentrations higher than 9 mg/kg
75 (Cabidoche *et al.,* 2009). CLD adsorbs strongly to soils rich in organic matter, and its
76 partitioning coefficient (log K_{oc}) has been reported in the range of 3.3-3.41 (Bonvallot and Dor
77 2004) (ATSDR 1995). CLD is only slightly soluble in water (2.7 mg/L at pH 7 and 25ºC (Kilzer
78 *et al.,* 1979) and tends to bioaccumulate in fatty tissues of living organisms (Howard 1991,
79 Dromard *et al.,* 2019). CLD is also neurotoxic, hepatotoxic and spermatotoxic to most living
80 organisms (Multigner *et al.,* 2016). In 2009, CLD was added to the list of persistent organic
81 pollutants in the Stockholm Convention, banning its production and use worldwide. Studies
82 suggest exposure to CLD may be linked to higher occurrence of prostate cancer and to impaired
83 cognitive and motor development in young children (Multigner *et al.,* 2010). Watershed
84 modelling predicts that CLD will remain in soils for decades or even centuries because of its
85 stability and affinity for soil organic matter, providing a long term source of contamination to the
86 aquatic environment (Cabidoche *et al.,* 2009). Surface water, ground water, sediments and
87 receiving coastal wasters have all been impacted. CLD has also been found to accumulate in
88 certain animals and plants used for food, including tubers and other vegetables grown in soil
89 (Devault *et al.,* 2016). Effective methods to decontaminate soil and protect downgradient
90 environments, food and water supply are clearly needed.
91
92 Few researchers have investigated CLD transformation. Early detailed studies by (Schrauzer and
93 Katz 1978) revealed that reduced vitamin B12 could dechlorinate chlordecone into products
94 including monohydrochlordecone (MHCLD), dihydrochlordecone (DHCLD) and
95 pentachloroindene. More recently, Ranguin *et al.,* (2017) were also able to show abiotic
96 dechlorination using reduced vitamin B12. The abiotic reduction of CLD with B12, zero-valent
97 iron or sodium sulphide led to formation of hydrochlordecones (HCLDs) and polychloroindenes
98 (PCINs) (Chevallier 2017). Very little data exists on the microbial degradation of CLD. Orndorff
99 and Colwell (1980) showed that *Pseudomonas aeruginosa strain K03* was able to partially
100 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.

132 **MATERIALS AND METHODS**

133 **Collection of Field Samples.** Field samples were collected twice, first in the fall of 2010 for
134 microcosm setup, and second in the spring of 2018 to analyze CLD and potential degradation
135 products by LC/MS using a refined protocol. In 2010, samples were collected from seven
136 locations in the south of Basse-Terre island, Guadeloupe (Table S1A): three andosol samples
137 from agricultural areas used for banana production (soil); three fluvisol samples from river banks
138 near a banana production area (soil and water); and one sludge sample from an anaerobic
139 digester at a sugar cane distillery plant. In 2018, nine different locations were sampled (Table
140 S1B): six from CLD-impacted agricultural areas (as soil and water slurries); and three activated
141 carbon sludge samples from a water treatment plant that handles chlordecone-contaminated
142 water (sampling details in Supplemental Method Details (SMD) 1A)
143

144 **Chemicals.** CLD (neat) and CLD standard (analytic standard 1mg/mL in MeOH) were
145 purchased from Accustandard (New Haven, USA). Hexanes and acetone (Fisher), water and
146 methanol (Caledon Laboratory Chemicals), and ethanol (Commercial Alcohols, Brampton,
147 Canada) were all of HPLC grade. TCE was purchased from Sigma and had a purity of 99.5%. A
148 pentachloroindene standard (2,4,5,6,7-pentachloro-1H-indene) referred to as B1, was kindly
149 provided by researchers at Genoscope (Évry, France).
150

151 **Microcosm Setup and Enrichment.** In December 2010, a microcosm study consisting of 13
152 different conditions each in triplicate for a total of 39 microcosms was initiated (Table S2A).
153 Microcosms contained soil and water samples from Guadeloupe and were augmented with
154 artificial groundwater (recipe in SMD 1B) as there was not enough field water. The microcosms
155 were prepared in 160 ml serum glass bottles (Fisher Scientific) sealed with blue butyl stoppers
156 (Bellco Glass Inc.) with 22.5 ml soil and 80 ml water. Rezasurin (1 mg/l) was added to one
157 microcosm from each triplicate set as an indicator of anaerobic conditions. At setup, 15
158 microcosms (5 different soil samples in triplicate) were poisoned by adding mercuric chloride
159 (0.05%) and sodium azide (0.02%). The 15 poisoned control microcosms and 18 of the 24 active
160 microcosms were amended with CLD at a target concentration of 10 mg/l (which is above the
161 solubility of ~2 mg/L (Howard 1991, ATSDR 1995), therefore a separate phase of CLD was
162 expected). Three other active microcosms were amended with both CLD and trichloroethene

163 (TCE) at target concentrations of 10 mg/l each, and three microcosms were left unamended. TCE
164 was added as a positive control for reductive dechlorination. All 39 microcosms received an
165 initial dose of an electron donor mix of acetone and ethanol, each at ~80 mg/L. The ratio of
166 donor (acetone/ethanol) to CLD (acceptor) in terms of electron equivalents (eeq) was around
167 100:1, assuming 20 eeq/mol CLD for complete dechlorination. This ratio is very high, and higher
168 than typically used for other chlorinated electron acceptors like TCE (5:1) because more acetone
169 and ethanol were needed to dissolve CLD (solid powder) into the feeding stock solution (Table
170 S3). The microcosms were incubated in the dark, unshaken in an anaerobic glovebox (Coy Lab
171 Products, Grass Lake, MI, USA) for about 8 years. During the first 2.5 years, all the active
172 microcosms were re-amended with CLD twice and electron donor five times. Those microcosms
173 that also received TCE were re-amended with TCE twice during this period. Six active
174 microcosms were transferred into a defined pre-reduced anaerobic mineral medium (Edwards
175 and Grbic-Galic 1994) in slightly larger bottles: one after 1 year, one after 1.5 years, and four
176 after 2 1/2 years (Figure S1 and Table S2B). These transfers were made in 250 ml Boston Round
177 glass bottles (VWR) to a total liquid volume of 200 ml and sealed with screw-cap Mininert™
178 valves (Chromatographic specialties). Subsequently, these transferred bottles (GT5, GT20,
179 GT33, GT4, GT15, GT3) were re-amended with donor and acceptor regularly. All the other
180 microcosms were not maintained after 2.5 years, but some were sampled a few times for
181 comparison with the transfers. The amounts and frequency of amendments to original
182 microcosms and to transfers over the full study are shown in Table S4. Two bottles with medium
183 only (Medium1, Medium2) were prepared in 2016 for use as controls for LC/MS analysis. These
184 were set up in 250 ml Boston Round glass bottles with 200 ml mineral medium (no culture) and
185 amended with CLD feeding stock to CLD concentrations of 10 and 20 mg/l (Table S2C).
186
187 **Microcosm Sampling and Analysis.** For the first 1.5 years, liquid samples (1 ml) from
188 microcosms were sampled regularly and analyzed for methane, ethene, ethane, and chlorinated
189 ethenes by Gas Chromatography with Flame Ionization Detector (GC-FID), but beyond this
190 time, only the transferred microcosms were analyzed routinely.
191
192 Anion analysis (IC) and pH measurements were made a few times over the course of the study.
193 Samples for DNA extraction were taken from the 6 transfers (GT5, GT20, GT33, GT4, GT15,

194 GT3) about 7 years into the study and the microbial community composition was assessed by
195 small subunit (SSU) rRNA gene amplicon sequencing and Quantitative Polymerase Chain
196 Reaction (qPCR) analysis. Method details of GC, IC, pH and microbial community analysis are
197 described in SMD 2.
198
199 When the microcosms were first established, we did not have a good method to measure CLD or
200 its daughter products. Nevertheless, during the first 1.5 years, 1 ml liquid samples (in duplicate)
201 were taken every 1-2 months from each microcosm and archived frozen at -20°C. Once an
202 appropriate LC/MS method was developed, analyses by LC/MS were performed approximately
203 once per year, and more frequently in the last two years. The transfers (GT5, GT20, GT33, GT4,
204 GT15, GT3) were analyzed most often, and some poisoned controls, medium controls and some
205 of the original active microcosms were analyzed occasionally for comparison. Sampling and
206 sample preparation procedures for the LC/MS analysis are described below with further details
207 in SMD 2.
208
209 **Sampling and Sample Preparation for CLD Analysis by LC/MS.** Sampling procedures,
210 sample preparation methods and chromatographic and MS methods for analysis of CLD and
211 dechlorinated products were improved progressively (explained in SMD 2). Eventually, two
212 satisfactory sample preparation methods were developed for the microcosms, one for sampling
213 liquid only (method 3) and one for sampling a soil/water slurry (method 4) (details in SMD 2).
214 Samples were always taken using glass syringes (Hamilton Company, Reno, USA) and stored in
215 glass vials with caps with PTFE lined septa (Agilent) to minimize sorption. All samples and
216 standards were filtered through 0.2 μm Millex PTFE syringe filters (Millipore, Burlington,
217 USA). For the liquid samples (method 3), bottles were shaken and left to settle overnight. The
218 next day 0.75 ml liquid was carefully sampled (avoiding collecting any solids) and was placed
219 into a 2 ml glass vial. The sample was then centrifuged for 5 minutes at 3000 rpm, and 0.5 ml of
220 the supernatant was transferred into a new vial containing 0.5 ml methanol. After mixing, the
221 sample was filtered into a final 2 ml glass sampling vial. For the slurry samples (method 4),
222 bottles were shaken and a 1 ml sample was taken with syringe immediately before the solids
223 settled to get a representative sample of the soil/water slurry. The slurry sample was added to a
224 vial containing 1 ml methanol, shaken gently for 10 min and left sitting for 30 min. The sample

225 was then centrifuged for 5 minutes at 3000 rpm, and the supernatant was filtered into the final 2
226 ml sampling vial. The addition of methanol to the sample had three purposes; to help extract
227 compounds associated with the solids, to help clean up the sample matrix by inducing
228 precipitation of salts from the sample matrix, and to reduce sorption of compounds to filter
229 membranes. For some of the samples the dry weight of solids was measured after drying the
230 sample in a drying oven at 105°C for several hours to estimate sorbed mass.
231
232 The field samples taken in 2018 were expected to have much lower concentrations of CLD and
233 daughter products than the microcosms, therefore a different extraction and concentration
234 procedure was used. Two different sample volumes (5 ml and 20 ml) were extracted by liquid-
235 liquid extraction using a mix of 15% acetone and 85% hexane (methods 5 & 6). The extracts
236 were evaporated to dryness under a stream of nitrogen and the samples were re-dissolved in 1ml
237 and 0.5ml methanol and filtered. Sample preparation procedures, LC/MS instrumentation and
238 methods were in constant development over the course of the study. Only the final optimized
239 method, used from April 9th 2018, is described in detail here. The history and development of the
240 protocol is explained in SMD 2, and the optimum LC/MS method is described below.
241
242 **Liquid Chromatography Mass Spectrometry (LC/MS) Analysis.** Chromatography was
243 carried out on a ZORBAX Rapid Resolution High Definition Phenyl-Hexyl column (50mm x
244 3.0mm, 1.8um) (Agilent, Santa Clara, USA) equipped with a guard column, using a Thermo
245 Scientific Ultimate 3000 UHPLC (Thermo Fisher Scientific, Waltham, MA). The column
246 temperature was 40°C and the flow rate was set to 300 $\mu L \cdot min^{-1}$. The eluents used were water
247 (A) and methanol (B), and both eluents contained 5 mM of ammonium acetate. The gradient
248 started at 50% B , followed by a linear gradient to 100% B over 8 min, then a hold at 100% B for
249 4 min, then a return to 50% B over 1min, and finally a re-equilibration under the initial
250 conditions of 50% B for 5 min (total runtime 18 min). Liquid samples (10 µL) were injected
251 using an Ultimate 3000 UHPLC autosampler, with autosampler temperature of 8°C. Compounds
252 were detected and quantified using a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher
253 Scientific) equipped with a Heated Electrospray Ionization (HESI II) probe, operating in
254 negative ionization mode. Mass spectra were acquired over an m/z range from 80 to 750 with the
255 mass resolution set to 140k, and common setting parameters were as follows; AGC Target: 1E6,

256 max injection time 200 ms, spray voltage 4 kV, capillary temperature 320°C, sheath gas 15, aux
257 gas 5, spare gas 2, and s-lens RF level 55. Data from the LC-MS were processed through
258 Xcalibur Qual Browser (Thermo Fisher Scientific). Calibration standard solutions of CLD (0.02,
259 0.2, 1.7, 2.6, 3.6 mg/l) were prepared from successive dilutions of a purchased analytical
260 standard (1 mg/mL CLD in MeOH). Similarly, a sample of pentachloroindene B1 (2,4,5,6,7-
261 pentachloro-1H-indene) (from Genoscope, France), stored as a 0.4 mg/ml acetonitrile stock
262 solution, was diluted to make five concentration level (0.02, 0.1, 0.2, 0.5 and 1 mg/l) and used as
263 standards for estimates of CLD metabolite concentrations. The dilutions of CLD and B1 were
264 prepared in a 50/50% mix of methanol and mineral medium. Standard dilutions were made fresh
265 the day before or on the same day as they were run.
266
267
268 **RESULTS AND DISCUSSION**
269 **Overview of Microcosm Data.** CLD is a challenging molecule to quantify, particularly in small
270 volume samples from a microcosm. It took us several years after microcosm set up to develop a
271 suitable method, and until that time, we periodically amended microcosms with CLD without
272 knowing if it was being degraded or not. We rationalized that dechlorination would be slow and
273 limited by the low solubility of CLD. Therefore, provided that we had good controls, we would
274 eventually be able to identify activity by comparing active microcosms to poisoned and medium
275 controls.
276
277 To provide an overview of how each set of microcosms was treated over the course of about
278 3000 days (~8 years), cumulative CLD and electron donor (acetone and ethanol) amendments
279 were plotted over time (Figure S2; with data in Table S4). We monitored methane production,
280 sulfate consumption and TCE dechlorination (where added), as indications of anaerobic
281 microbial activity. All active microcosms except for those made from distillery sludge samples
282 (G63 series) produced methane during the first 1.5 years (Figure S3). Beyond 1.5 years, we
283 continued to monitor and amend only six of the microcosms. Two (GT4 and GT3) were 20%
284 transfers from original bottles into anaerobic medium, that received CLD and donor (GT4) or
285 CLD+TCE and donor (GT3). Transfer GT4 contained none of the original microcosm soil. Four
286 other transfers were made by pouring the entire contents of the original microcosm into slightly

287 larger bottles sealed with Teflon Mininert caps, topped up with medium and amended with CLD
288 and donor (GT5, GT20 and GT33) or CLD+TCE and donor (GT15) (Figure S1). Transfers GT5,
289 GT20 and GT15 continued to produce methane after being transferred, GT33 stopped producing
290 methane after the first re-amendment of CLD, while GT3 and GT4 did not produce any methane
291 after the transfer (Figure S2). In microcosms that received TCE in addition to CLD, TCE was
292 partially dechlorinated to trans- and cis-DCE, with possibly small amounts of ethane produced
293 (Figure S4), but the 20% transfer (GT3) no longer dechlorinated TCE. No ethene was ever found
294 in any of the microcosms (Table S5). In the transfers that did not produce methane (GT33, GT3
295 and GT4), we observed production of acetate (Table S5) although not enough to explain
296 complete transformation of donor. As a result, pH dropped to below 6 in these microcosms (and
297 was occasionally re-adjusted), which probably subsequently eliminated methanogens (Table S5).
298 Sulfate (~1.2 mM initially) was present in all soil microcosms but was depleted within the first
299 year and was not added to transfers (Table S5). We suspect that iron(III) carried over from soil
300 may have also served as an acceptor in some bottles. High chloride background (>12 mM) was
301 present in the microcosms, precluding using chloride increase as a proxy for dechlorination. In
302 summary, we established that all of the active (non-poisoned) microcosms were microbially
303 active initially as well as after 8 years of incubation, despite repeated additions of chlordecone
304 and donor (acetone or ethanol), but that there were significant differences in their extent of
305 methanogenesis depending on particular treatment history.
306
307 **Development of an LC/MS Method for Chlordecone and metabolites.** To enable a time-
308 course analysis and mass balance in microcosms, we needed to develop a CLD analysis method
309 suitable for repeated sampling in microcosms using small sample volumes. CLD analyses are
310 challenging for many reasons, including strong sorption of CLD to soil (and to rubber stoppers),
311 low water solubility of CLD and certain metabolites, lack of authentic standards for degradation
312 products, poor/variable or inconsistent ionization, as well as matrix and solvent effects. We
313 hoped that the high sensitivity and mass accuracy of Orbitrap MS technology would facilitate
314 identification of compounds despite lack of standards. The various early trials and final method
315 are described in SMD 2. Moriwaki and Hasegawa (2004) were first to report use of LC-MS for
316 CLD; they used a water/methanol gradient and negative ionization mode, although only
317 standards in methanol were tested. Durimel *et al.,* (2013) also detected CLD by LC-MS using a

318 water/acetonitrile gradient, also in negative mode. Cimetiere *et al.,* (2014) included formic acid
319 (pH 2) to the eluent and observed adduct (M+HCOOH+OH) formation. These authors
320 recommended addition of a co-solvent (acetonitrile, methanol) to avoid adsorption of CLD to
321 filters and to desorb CLD from suspended matter (acetonitrile). They concluded that the presence
322 of salts in sample matrixes weaken the CLD MS signal, which made us realize the importance of
323 preparing standards in the same matrix as the samples.
324
325 Based on these prior studies, standards were prepared in 50% medium and 50% methanol.
326 Methanol was added to samples before filtration to decrease sorption and to precipitate salts.
327 Methanol addition proved later to be a good solvent to mix with water to recover compounds
328 from soil particles. As first described by Harless *et al.,* (1978) and observed by others (Bichon *et*
329 *al.,* 2015, Chevallier 2017), CLD actually exists as a gem-diol (hydrate) in water or a hemiacetal
330 in methanol and can also form various adducts in the presence of compounds often used in LC
331 eluents, such as acetate and formate. In this study chromatographic separation, ionization and
332 signal intensity were maximized when ammonium acetate was included in the water and
333 methanol eluents. We observed many CLD adducts (hydrate, formate, acetate) and hemiacetal in
334 our analyses (Figure S5), that required careful data interpretation. Hydrochlordecones (MHCLD,
335 DHCLD and THCLD) also showed similar adduct formation. In this study we chose to calibrate
336 and report the hydrate forms of CLD, MHCLD, DHCLD and THCLD. All of the LC/MS data
337 collected during this study are compiled in Table S7. We initially only sampled the liquid phase
338 (centrifuged and filtered) to avoid variability related to sorption to solids in the microcosms.
339 However, sorption to soil in the microcosm was very strong, precluding a satisfactory mass
340 balance when considering only liquid phase samples. We eventually had to adapt the method to
341 include and quantify CLD on solids. Owing to the lack of standards for compounds other than
342 CLD (and later B1), the runs with different methods could not be compared easily. Despite all
343 these challenges, dechlorination products were clearly observed, as described in the next section.
344
345 **Detection of CLD Metabolites using best method.** A series of metabolites were observed in all
346 the active original microcosms and transfers over the course of the study (Figure 1 with data in
347 Table S7). Despite not having standards, the accuracy of the Orbitrap MS enabled identification
348 of metabolites by matching first or highest m/z values of the mass spectrum to the presumed

349 structure, and further evaluating the full characteristic isotope patterns for these multi-chlorinated
350 transformation products (details below). Table S6 lists all observed metabolites with their
351 measured and theoretical masses (and IDs), and Figure S6 illustrates the characteristic mass
352 spectra for these metabolites (observed and theoretical). The identified metabolites were assigned
353 to three different groups: group A - hydrochlordecones (HCLD); group B - polychloroindenes
354 (PCIN); and group C - carboxylated polychloroindenes (CPCIN) to be consistent with previous
355 studies detailing the masses and NMR structures of CLD metabolites (Chaussonnerie *et al.,*
356 2016, Chevallier 2017, Chevallier *et al.,* 2018, Chevallier et al., 2019). In this study, a total of 19
357 different dechlorination products were detected by LC-MS in the microcosms. Multiple isomers
358 of certain metabolites were found having exactly the same mass but varying retention time;
359 complete structural elucidation of observed metabolites was not attempted in this study.
360 Retention times were consistent with the relative polarity of each compound. An example
361 chromatogram from one of the samples, bottle GT20 sampled June 29th 2018, is shown in Figure
362 2. Chlordecone and 17 of the 19 observed dechlorination products detected in this active
363 microcosm are shown, including the non-polar B compounds (polychloroindenes, PCINs)
364 showing longer retention times than CLD, and the more polar C compounds (carboxylated
365 polychloroindenes, CPCINs) with much shorter retention times (Figure 2). Compounds with
366 retention times 9.3, 8.88, and 8.05 min and mass to charge ratios m/z 468.7264, m/z 434.7661
367 and m/z 400.8042 respectively, could be attributed to the hydrate forms of MHCLD (A9a)
368 $[C_{10}Cl_9O_2H_2]^-$, DHCLD (A8a) $[C_{10}Cl_8O_2H_3]^-$ and THCLD (A7a) $[C_{10}Cl_7O_2H_4]^-$ obtained by the
369 loss of 1, 2 and 3 chlorine atoms from CLD (Figure 2 and Table S6). Three metabolites, less
370 polar than CLD and with longer retention times at 12.19, 11.44 and 10.34 min, respectively,
371 exhibiting mass to charge ratios of m/z 284.8616, m/z 250.9006 and m/z 216.9382 were
372 identified as pentachloroindene (B5a) $[C_9Cl_5H_2]^-$, tetrachloroindene (B4a) $[C_9Cl_4H_3]^-$ and
373 trichloroindene (B3a) $[C_9Cl_3H_4]^-$ (Figure 2 and Table S6). Thirteen (13) metabolites more polar
374 that CLD were assigned to group C, and observed at retention times ranging from 2.02 to 6.69
375 min (Figure 2 and Table S6). Compounds C4a and C4b, detected respectively at retention times
376 of 5.47 and 5.02 min, and with a corresponding m/z of 294.8899 could be attributed to two
377 isomers of a carboxylated tetrachloroindene compound $[C_{10}Cl_4O_2H_3]^-$ obtained by the loss of 6
378 chlorine atoms. At retention times between 3.03 and 6.69 min, 5 isomers (C3a-C3e) with a
379 corresponding m/z of 260.9287 were detected and attributed to a carboxylated trichloroindene

380 compound $[C_{10}Cl_3O_2H_4]^-$ obtained by the loss of 7 chlorine atoms. Four isomers (C2a-C2d)
381 obtained by the loss of 8 chlorine atoms were detected at retention times between 2.02 and 5.25
382 min and had m/z of 226.9677, assigned to be a carboxylated dichloroindene $[C_{10}Cl_2O_2H_5]^-$. Two
383 last isomers (C1a and C1b) with retention times at 2.5 and 2.95 min and m/z of 193.0064 were
384 classified as carboxylated monochloroindene $[C_{10}ClO_2H_6]^-$, obtained by the loss of 9 chlorine
385 atoms from CLD.
386
387 In summary, CLD metabolites observed in the microcosms could be classified into 3 families of
388 compounds: group A compounds including mono-, di- and trihydrochlordecone derivatives with
389 proposed neutral formula $[C_{10}Cl_{10-n}OH_n$, n= 1,2,3]; group B non-polar "open cage" structures
390 including three polychloroindene compounds with proposed neutral formula $[C_9Cl_{5-n}H_{3+n}$,
391 n=0,1,2]; and group C polar "open cage" structures consisting of carboxylated polychloroindene
392 derivatives with neutral formula $[C_{10}Cl_{4-n}O_2H_{4+n}$, n=0-3].
393
394 Due to a number of challenges with and changes in sample preparation and LC-MS analysis over
395 time, it was difficult to get an accurate picture of concentration changes of CLD over time.
396 Therefore, when we finally settled on a good method, we re-analyzed some frozen archived
397 samples to get a comparable set of data. Results from a selection of samples from GT20 with
398 comparable sample preparation (liquid phase only, no soil) and analytical methods are shown in
399 Figure 3 (raw data in Table S8). Results show that none of the monitored metabolites were
400 detected in the sample taken two weeks into the experiment, but by 8 months into the study, we
401 observed MHCLD, DHCLD, and two CPCINs with loss of 8 and 9 chlorines. Later sampling
402 time points showed increasing concentrations of MHCLD, DHCLD, and four CPCINs with loss
403 of 6 to 9 chlorines. Pentachloroindene (B5a) only showed up in the last sampling point, however
404 it was found to sorb strongly to soil, and we would therefore not expect to see it in these liquid
405 phase samples analyzed here. Also, because we kept adding more CLD to the bottles over time,
406 we could not use aqueous CLD concentration changes as a measure of degradation rate. Despite
407 not seeing a clear decrease in CLD concentrations in the liquid phase, highly dechlorinated
408 metabolites with up to 9 chlorine removed were observed in the active bottles but not in the
409 controls indicating that biological processes were involved in the dechlorination of CLD into
410 HCLD, PCIN and CPCIN metabolites.

411

412 **Effect of Sorption.** Strong sorption of CLD and non-polar PCID metabolites to soil particles
413 made it difficult to evaluate the fate of CLD in the bottles by sampling the aqueous phase only.
414 We therefore changed our approach to also extract soil in our samples. By analyzing the same
415 samples by two different sample preparation methods, one in which only the liquid concentration
416 was measured (filtered sample) and another in which a mixture of soil and water was analyzed,
417 and quantifying the amount of soil (dry mass) in the sample, we were able to calculate and
418 estimate a distribution coefficient, K_d (l/kg) equal to the ratio of sorbed concentration (mg/kg) to
419 dissolved concentration (mg/l) for most of the analytes (Table 1; raw data in Table S9). This
420 analysis confirmed that CLD and mono- and dihydro- CLD, with K_d values of 130 ± 57, 52 ± 12
421 and 28 ± 6 ml/g (or l/kg), respectively, absorb quite strongly to the Guadeloupe soil used, while
422 the K_d value for pentachloroindene (B5a) was much higher at 5700 ± 220 l/kg, and thus absorb
423 even more strongly. The carboxylated chloroindenes (C compounds) had much lower K_d values
424 ranging from 2 to 11 l/kg, and were found in the aqueous phase. The estimated K_d values
425 correspond well to the retention times by reverse phase LC (Table 1). Our estimated distribution
426 coefficient for chlordecone is in the same range (60-330 l/kg) as one previous report (Fernández-
427 Bayo *et al.,* 2013). A distribution coefficient based on organic content, K_{oc}, of 2,500 l/kg has also
428 been reported (Howard 1991), corresponding to a K_d of 250 l/kg assuming a fraction of organic
429 carbon of 10%. We were not able to find any reports of sorption coefficients for any CLD
430 metabolites.

431

432 **Quantification of the Extent of Transformation in Microcosms.** To quantify the extent of
433 transformation of CLD added to the microcosms over the course of the study, we used data from
434 well-mixed slurry samples from 13 active, 7 poisoned controls, 2 unamended microcosms, and
435 two medium controls (Table 2). The well-mixed slurry samples were analyzed because they
436 capture mass from both the liquid and solid phases, so that we could better compare final
437 measured mass recovered to the total amount of CLD that had been added to the bottles. To
438 attempt a mass balance, MHCLD, DHCLD, and THCLD concentrations were estimated based on
439 the response factor for CLD as no standards were available for these metabolites. Researchers
440 from Genoscope (France) kindly provided us with a small sample of pentachloroindene B1
441 (2,4,5,6,7-pentachloro-1H-indene) that they had managed to chemically purify. As a result, we

442 were able to do a rough estimate of the amount of B5a produced in active microcosms. B4a
443 concentrations were estimated using the response factor for B1. For the carboxylated
444 polychlorinated indenes (C group), we have no proxy for calibration; however, to get a very
445 rough idea of possible concentrations we also used the response factor for B1 for these
446 compounds.
447
448 Using these estimated response factors applied to the areas determined by LC/MS analysis of
449 well-mixed slurry samples, we could calculate total moles recovered per bottle by multiplying
450 concentrations by the total slurry volume. We then compared the CLD and metabolite moles
451 recovered in the bottles after 8 years to the initial amount of CLD added (Table 2; calculations in
452 Table S10). The three different groups of microcosms, poisoned controls, active original
453 microcosms, and active microcosm transfers, did indeed show differences in CLD recovery. In
454 the group of seven poisoned controls, two microcosms (G1 and G3) produced a lot of methane
455 and thus were biologically active, despite having been poisoned. These bottles also exhibited
456 extensive metabolite production, unlike remaining controls (Figure 1 and Table 2). Therefore, for
457 the mole balance analysis, we included those two microcosms into the group of active original
458 microcosms. We were able to recover 63±6% of added CLD in the poisoned controls after 8
459 years, 44±11% in the original microcosms that only receive electron donor in the first 2 years,
460 and only 32±3% of added CLD in the transfers amended regularly with donor and CLD (Table
461 2). The loss of ~37% in the poisoned control group likely results from sorption to glass and
462 stoppers, poor extraction from soils during sample preparation, losses from volatilization, and
463 some minimal losses (<1%) from sample removal. Losses in microbially-active bottles are
464 greater and can be explained by the contribution of biological transformation processes.
465
466 We estimated the total moles recovered as metabolites in the various treatments. Metabolites
467 were not detected in the un-amended slurry microcosms, nor in the medium controls. The
468 inactive poisoned controls had only trace amounts of MHCLD (0.001-0.004 μmoles) and no
469 other metabolites. The active bottles had significantly higher concentrations of metabolites,
470 especially in the transfers that received more CLD and donor. The estimated sum of moles of
471 metabolites ranged from 13% to 98% of the CLD remaining after 8 years in active microcosms.
472 When the sum of all measured metabolites was included in the mole balance, overall recoveries

473 after 8 years were more similar regardless of treatment, 63±6%, 60±14%, and 50±13% for
474 controls, originals and transfers, respectively. Given the length of the study and approximations
475 in calibration factors, these results were very reassuring and provided confidence in the
476 measurements.
477
478 **Microbial Community Analysis qPCR and Sequencing Analysis.** While DNA samples were
479 collected and analyzed at various times throughout the 8 years, preliminary analyses have not
480 revealed any clear trends to date. A snap shot of the microbial community and abundance after
481 77 months is shown in Figure 4 (raw data are provided in Tables S11 and S12). The microbial
482 community of the 6 transfers reveals an abundance of fermentative and syntrophic anaerobes,
483 consistent with the electron donor mix (acetone and ethanol) provided. Microcosms GT33 and
484 GT4 produced little to no methane, and contain few or no methanogens. GT4 is the most
485 extensively transferred microcosm and no longer contains soil. It also has the lowest bacterial
486 cell numbers inferred from qPCR of 16S rRNA copies per ml. No particular trends are
487 discernable at this time. Perhaps, as concluded by Chaussonnerie *et al.,* (2016), the microbial
488 transformation is cometabolic, and really only dependent on sufficient availability of reduced
489 vitamin B12. Further studies are clearly warranted.
490
491 **Analysis of Field Samples from 2018.** We wondered if metabolites identified in the microcosms
492 could also be detected in field samples, therefore we returned to Guadeloupe and analyzed 9
493 more samples. (Table 3; raw data in Table S13). Anticipating quite low concentrations in the
494 field samples, we decided to sample larger 5 and 20 ml volumes and perform a liquid-liquid
495 extraction with a concentration step (method 5 and 6, SMD 2) in addition to our already
496 established 1 ml slurry sample preparation method (method 4, Supplemental Method Details 2).
497 Analysis of 6 soil samples from banana plantations in Guadeloupe revealed CLD concentrations
498 in the range 120 to 1000 ng/g soil, or 0.12 to 1.0 mg/kg. These values are pretty typical of
499 Guadeloupe soils: a recent survey (Crabit *et al.,* 2016) reported CLD concentrations in mg/kg in
500 soil of 0.03 (minimum), 2.00 (median), 3.39 (mean), and 24.2 (maximum). MHCLD was
501 detected in all soil samples and ranged from 1 to 8% of CLD based on area from LC/MS
502 analysis. Most soil samples also showed DHCLD, but area counts were 10 to 100 times lower
503 than those of MHCLD. Pentachloroindene (B5a) concentrations were estimated based on

504 response factor for B1, and ranged from 0.5 to 24 ng/g of solid. B5b metabolites were also
505 detected in the soil samples, but area counts were generally lower (up to 30 times) than those of
506 B5a. Two of the 3 activated carbon sludge samples showed quite high CLD concentrations,
507 between 6,000 and 8,500 ng/g soil. MHCLD concentrations were also significant, between 4 and
508 10% of CLD based on area counts. No PCIN was detected in the activated carbon sludge
509 samples. We did not detect CPCIN metabolites in any of the field samples. Due to their
510 hydrophilicity, the CPCIN metabolites would likely not be captured using the liquid-liquid
511 extraction/concentration method (methods 5 and 6) used, and we did not have an alternative
512 method for concentration of these compounds. We could also hypothesise that due to their
513 hydrophilic nature and low sorption, these compounds may have been washed out from the soils
514 by rain. These field data confirm that anaerobic ring opening and dechlorinating processes do
515 occur *in situ* in Guadeloupe soils. A more extensive analysis is thus warranted to determine
516 locations for highest intrinsic activity on the islands and if rates could be accelerated by inducing
517 anaerobic conditions, such as through organic amendment. The mechanism of CLD
518 biotransformation also needs further investigation.
519
520 **ASSOCIATED CONTENT**
521 **Supporting Information.** Method details for field sampling and for microcosm setup including
522 recipe for artificial groundwater, analysis procedure and sample preparation methods for GC-
523 FID, IC, pH and LC-MS analysis, and DNA extraction, amplicon sequencing, and qPCR analysis
524 procedures. Figures showing microcosm transfers and their origins, history of microcosms (CLD
525 added, donor added, methane produced), methane production in active CLD amended MCs
526 during the first 1.5 years of monitoring, TCE and its dechlorination products in a CLD and TCE
527 amended MC (G15) during the first 1.5 years of monitoring, LC/MS scan analysis of CLD (CLD
528 hydrate, hemi-acetal and other observed CLD adducts), and chromatograms and spectra for each
529 of the 19 observed metabolites. Tables showing Guadeloupe field sample details, treatment table
530 for Guadeloupe microcosm study, details of feeding stock preparation, details of CLD and donor
531 amendments, details of GC-FID and IC analysis and pH measurements, results of all LC/MS
532 analysis, normalized LC/MS area counts in GT20, sorption calculations and plots, mass balance
533 calculations, sequencing analysis and qPCR analysis, and concentration calculations for CLD
534 and its degradation products in Guadeloupe field samples.

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624
625

626 **TABLES FOLLOW**

627 **Then FIGURES with LEGENDS for review**

Table 1: Estimated sorption coefficients for CLD and some dechlorinated metabolites in Guadeloupe soils

Compound Name	ID	Measured Kd* (ml/g)	Kd from literature (ml/g) **	MW (g/mol)	LC/MS retention time
Pentachloroindene	B5a	5700 ± 220	-	288.39	12.19 min
Tetrachloroindene	B4a	NA	-	253.94	11.44 min
Chlordecone	**CLD**	**130 ± 57**	**~ 60-330**	**490.64**	**9.86 min**
Monohydrochlorodecone	MHCLD A9a	52 ± 12	-	456.19	9.3 min
Dihydrochlorodecone	DHCLD A8a	28 ± 6	-	421.75	8.88 min
Carboxylated tetrachloroindene	C4a-b	5.6 ± 1.0	-	297.95	5.02 - 5.46 min
Carboxylated trichloroindene	C3a-e	11 ± 2.0	-	263.5	3.03 - 6.69 min
Carboxylated dichloroindene	C2a-d	9.8 ± 4.4	-	229.06	2.02 - 5.25 min
Carboxylated chloroindene	C1a-b	2.1 ± 1.0	-	194.61	2.5 - 2.95 min

*See Table S9 for calculations and plots. Kd values are slope ± standard error of the slope (n=4)
** Only one reference found: 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 2: Extent of transformation of CLD in microcosms and transfers (µmol per bottle) after 8 years of incubation. Concentrations of metabolites are estimates. Samples were taken March 7th 2019 (1 ml slurry samples; sample preparation method 4). Raw data and calculations are shown in Table S10.

	Sample	Total CLD aded to date	CLD remaining after 8 yrs	CLD recovery	MHCLD, DHCLD and THCLD* (Estimated)	PCINs (B-comp)** (Estimated)	CPCINs (C-comp)** (Estimated)	Sum of all products (Estimated)	Sum all after 8 years (incl. CLD) (Estimated)	Products/CLD remaining (Estimated)	Total recovery after 8 years (Estimated)
		(µmol)	(µmol)	%	(µmol)	(µmol)	(µmol)	(µmol)	(µmol)	%	%
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	4.0	98%	ND	ND	ND	0.0	4.0	0%	98%
	Medium2	8.15	1.7	21%	ND	ND	ND	0.0	1.7	0%	21%
	G28_poisoned	1.63	1.1	69%	0.002	ND	ND	0.002	1.1	0%	69%
	G30_poisoned	1.63	0.96	59%	0.001	ND	ND	0.001	0.97	0%	59%
	G41_poisoned	1.63	1.1	65%	0.004	ND	ND	0.004	1.1	0%	65%
	G50_poisoned	1.63	0.90	55%	0.002	ND	ND	0.002	0.90	0%	55%
	G59_poisoned	1.63	1.1	65%	0.001	ND	ND	0.001	1.1	0%	65%
AVERAGE poisoned contr.		***1.63***	***1.0***	***63%***	***0.002***	***ND***	***ND***	***0.002***	***1.0***	***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	0.83	51%	0.092	0.030	0.10	0.22	1.0	27%	64%
	G3_poisoned#	1.63	0.83	51%	0.079	0.061	0.08	0.22	1.1	26%	64%
	G4	3.26	1.4	42%	0.14	0.047	0.11	0.30	1.7	22%	51%
	G19	3.26	1.7	53%	0.45	0.13	0.24	0.81	2.6	47%	78%
	G31	3.26	1.7	53%	0.13	0.045	0.05	0.22	1.9	13%	60%
	G45	3.26	0.82	25%	0.24	0.031	0.05	0.32	1.1	39%	35%
	G54	3.26	1.2	36%	0.51	0.038	0.10	0.64	1.8	55%	56%
	G63	3.26	1.8	55%	0.034	0.011	0.77	0.82	2.6	45%	81%
	G14 +TCE	3.26	1.0	32%	0.62	0.095	0.20	0.91	2.0	87%	60%
AVERAGE originals		***2.90***	***1.4***	***44%***	***0.30***	***0.06***	***0.22***	***0.57***	***2.0***	***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	5.6	31%	2.4	0.69	1.1	4.2	9.8	75%	54%
	GT3	15.8	5.7	36%	4.1	0.11	1.4	5.6	11	98%	71%
	GT5	23.6	7.3	31%	1.3	0.51	1.1	2.9	10	40%	43%
	GT20	25.7	8.4	33%	2.7	1.0	1.1	4.8	13	58%	52%
	GT33	19.6	6.1	31%	1.5	0.23	1.1	2.9	9.0	48%	46%
	GT4	22.4	6.0	27%	0.48	0.16	0.63	1.3	7.2	21%	32%
AVERAGE transfers		***20.8***	***6.5***	***31%***	***2.1***	***0.45***	***1.1***	***3.6***	***10***	***57%***	***50%***
Standard Dev (n-6)		***3.72***	***1.1***	***3%***	***1.3***	***0.35***	***0.24***	***1.6***	***2.0***	***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 3: Chlordecone and dechlorinated metabolites detected in field samples 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 S13. CLD, MHCLD and DHCLD are quantified/reported as CLD hydrate, MHCLD hydrate and DHCLD hydrate.

	Chlordecone		**Hydrochlordecones**		**Polychloroindenes**		
	CLD		**MHCLD (A9a)**	**DHCLD (A8a)**	**Pentachloroindene (B5a)**		**Tetrachloroindene (B4a)**
	area/g solids	ng/g solids	area/g solids	area/g solids	area/g solids	ng/g solids*	area/g solids
AgSoil1	1.4E+07	120	8.7E+05	2.1E+04	6.3E+03	0.48	4.1E+03
AgSoil2	2.7E+07	130	1.9E+06	1.6E+04	3.0E+04	2.3	5.9E+03
AgSoil3	1.9E+08	1000	5.3E+06	8.0E+04	3.1E+04	2.3	9.9E+02
AgSoil4	6.2E+07	270	5.1E+06	8.8E+04	3.1E+05	24	4.2E+04
AgSoil5	5.7E+07	130	4.2E+06	2.2E+04	1.1E+05	8.8	2.6E+04
AgSoil6	3.5E+07	430	5.0E+05	0.0E+00	1.5E+04	1.1	1.0E+04
AC-1	1.5E+07	24	1.5E+06	7.0E+05	ND	ND	ND
AC-2	3.6E+09	6000	2.8E+08	1.8E+07	ND	ND	ND
AC-3	5.1E+09	8400	1.9E+08	5.8E+06	ND	ND	ND

*estimated values based on analysis of B1 standards run in 2019
ND: Not detected

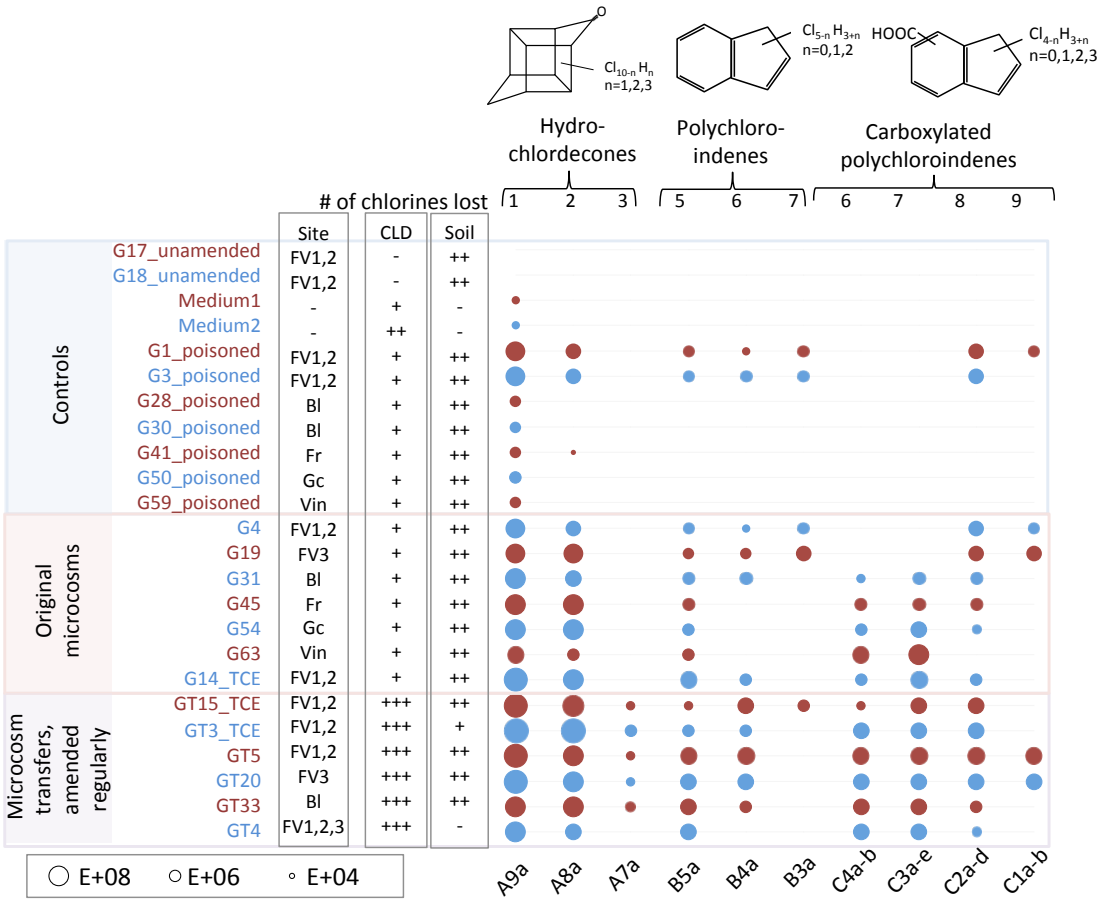


Figure 1: Dechlorinated metabolites observed in anaerobic microcosms constructed from Guadeloupe soil and water. The size of each circle is proportional to the area count from LC/MS analysis of slurry samples performed March 7th 2019 (see Table S7 for raw data).

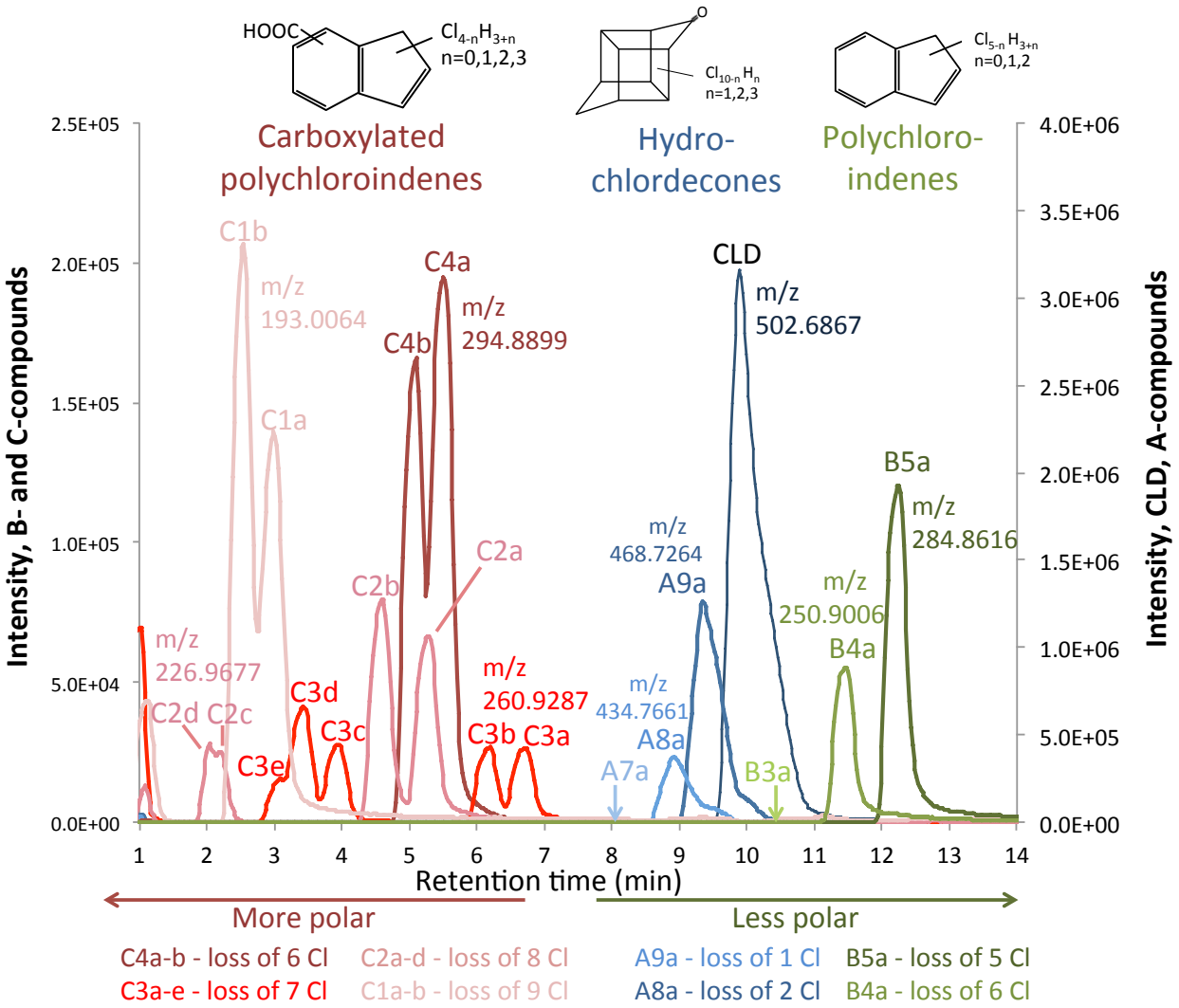


Figure 2: Chromatograms of chlordecone and its dechlorinated metabolites in sample GT20 June 29th 2018 (slurry sample). Results are from LC/MS, equipped with ESI, in negative mode. Observed m/z values (monoisotopic) for the different compounds were; 502.6879 (CLD, $[C_{10}Cl_{10}O_2H]^-$), 468.7264 (A9a, $[C_{10}Cl_9O_2H_2]^-$), 434.7661 (A8a, $[C_{10}Cl_8O_2H_3]^-$), 284.8616 (B5a, $[C_9Cl_5H_2]^-$), 250.9006 (B4a, $[C_9Cl_4H_3]^-$), 294.8899 (C4a-b, $[C_{10}Cl_4O_2H_3]^-$), 260.9287 (C3a-e, $[C_{10}Cl_3O_2H_4]^-$), 226.9677 (C2a-d, $[C_{10}Cl_2O_2H_5]^-$), 193.0064 (C1a-b, $[C_{10}ClO_2H_6]^-$) (see details in Table S6). Metabolites m/z 400.8042 (A7a, $[C_{10}Cl_7O_2H_4]^-$) and 216.9382 (B3a, $[C_{10}Cl_7O_2H_4]^-$) 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 quantified/reported in the forms of CLD hydrate, MHCLD hydrate, DHCLD hydrate and THCLD hydrate.

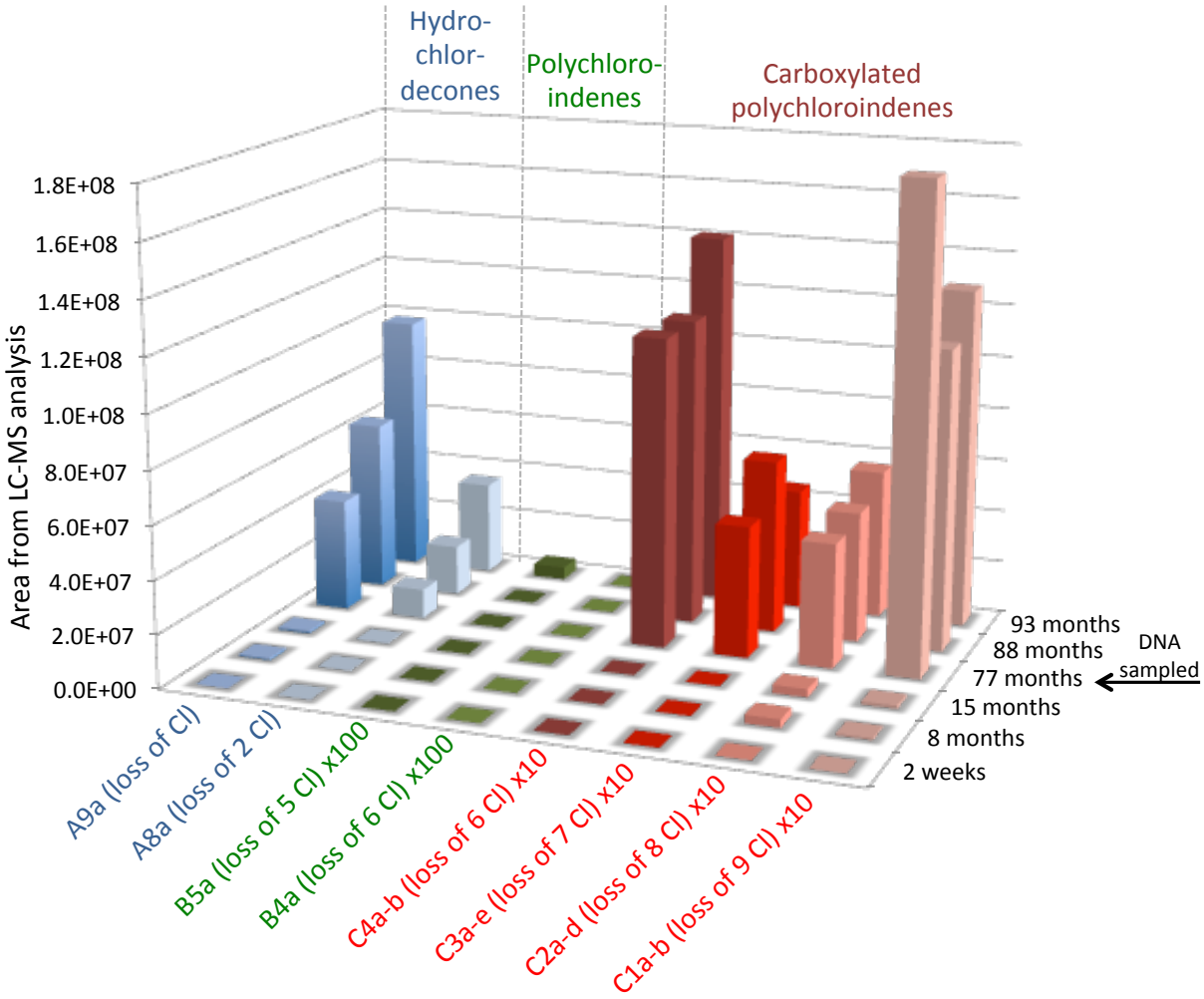
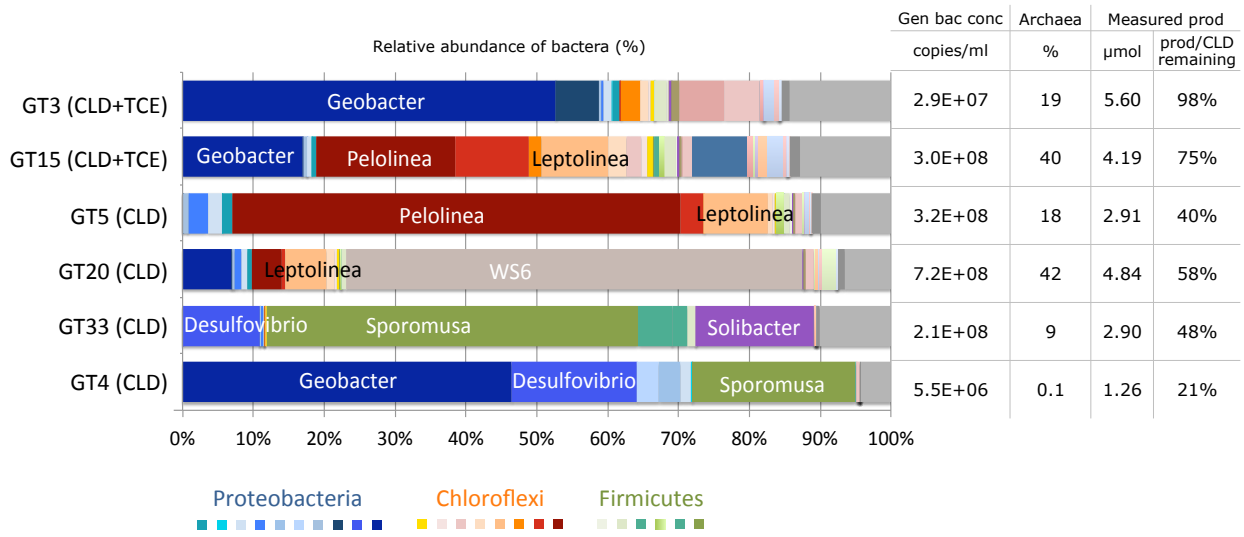


Figure 3: Dechlorinated metabolites from chlordecone over time in one of the anaerobic microcosms, transfer GT20. Only samples that were prepared the same way are included in this graph (sample preparation method 3, liquid phase only, no soil, see supplemental method details). Areas of B compounds were 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 S8).



	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 4: Microbial community in microcosm transfers. DNA was sampled 77 months after microcosm setup. The bar chart to the left shows relative abundance of bacteria obtained through small subunit (SSU) rRNA gene fragment sequencing. Table to the right shows concentration of general bacteria measured by qPCR (copies/ml), relative abundance of archaea (%) from sequencing, 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 S11 and S12.