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

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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
FID Flame Ionization Detector
IC Ion Chromatograph
LC Liquid Chromatograph
MS Mass Spectrometry
eeq Electron Equivalents
SMD Supplemental Method Details

ABSTRACT

Chlordecone ($C_{10}Cl_{10}O$) is a bishomocubane molecule, that has been used as pesticide, in many countries in Europe, America, and Africa, from the 1960's to 1990's. In the French West Indies, the historic use of chlordecone to control banana weevil infestations has resulted in pollution of large land areas. Although currently banned, chlordecone persists because it adsorbs strongly to soil and its complex structure is stable, particularly under aerobic conditions. A leaching model established that CLD pollution will last in French west indies soils several decades to half a millennium depending on soil type. However, abiotic chemical transformation catalyzed by reduced vitamin B12 has been shown to break down chlordecone by opening the cage structure to produce C9 polychloroindenes, and more recently these C9 polychloroindenes were also observed as products of anaerobic microbiological transformation by *Citrobacter*. To assess the potential for bioremediation, the anaerobic biotransformation of chlordecone by microbes native to soils from the French West Indies was investigated. Anaerobic microcosms were constructed from chlordecone impacted Guadeloupe soil and sludge to mimic natural attenuation and eletron donor-stimulated reductive dechlorination. 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 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. These products were not observed in sterile incubations. Chlordecone concentrations decreased in active microcosms as concentrations of products increased, indicating that anaerobic dechlorination processes have occurred. An crude estimation of partitioning coefficients between soil and water showed that carboxylated intermediates sorb poorly, and as a consequence may be flushed away while polychlorinated indenes sorb strongly to soil. Microbial community analysis in microcosms showed enrichment of anaerobic fermenting and acetogenic microbes possibly involved in anaerobic chlordecone biotransformation. It thus should be possible to stimuilate anaerobic dechlorination through donor amendment to contaminated soils, particularly as some metabolites (in particular

70 pentachloroindene) were already detected in field samples as a result of intrinsic processes.
71 Extensive dechlorination in the microcosms, with evidence for up to 9 Cl atoms removed from
72 the parent molecule is game-changing, giving hope to the possibility of using bioremediation to
73 reduce the impact of CLD contamination.
74
75 **INTRODUCTION**
76 Chlordecone (CLD) was used to control insect pests (mainly the banana weevil) in banana
77 plantations in the Caribbean, particularly in Guadeloupe and Martinique from 1971 to 1993,
78 despite being banned in the United States since 1974 [1, 2]. This pesticide was also used in the
79 USA, as well as in some countries of Africa (Cameroon, Ivory Coast), latin America (Panama,
80 Honduras, Equator, Nicaragua) and Asia [3-5]. Chlordecone ($C_{10}Cl_{10}O$) is a bis-homocubane,
81 comprising a cage structure with 10 chlorine atoms and a ketone functionality. It was
82 commercialized under the brand names Kepone® and Curlone® and was spread at the foot of
83 each banana plant as a solution delivering 1.5 grams of chlordecone per plant. With 800 banana
84 plants per hectare and 2.5 applications per year, the resulting dosage was a significant 3 kg
85 chlordecone per hectare per year. As a consequence of such intensive application, 8–9% of the
86 cultivated areas of Guadeloupe have CLD concentrations higher than 1 mg/kg in topsoil, and
87 some banana fields have concentrations higher than 9 mg/kg [2]. CLD adsorbs strongly to soils
88 rich in organic matter, and its partitioning coefficient (log K_{oc}) has been reported in the range of
89 3.3-3.41 [1, 6]. CLD is only slightly soluble in water (2.7 mg/L at pH 7 and 25°C [7] and tends to
90 bioaccumulate in fatty tissues of living organisms [8, 9]. CLD is also neurotoxic, immunotoxic,
91 hepatotoxic and spermatotoxic to most living organisms [10, 11]. In 2009, CLD was added to the
92 list of persistent organic pollutants in the Stockholm Convention, banning its production and use
93 worldwide. Studies suggest exposure to CLD may be linked to higher occurrence of prostate
94 cancer and to impaired cognitive and motor development in young children [1, 12-14].
95 Watershed modelling predicts that CLD will remain in soils for decades or even centuries
96 because of its stability and affinity for soil organic matter, providing a long term source of
97 contamination to the aquatic environment [2]. Surface water, ground water, sediments and
98 receiving coastal wasters have all been impacted. CLD has also been found to accumulate in
99 animals [15-17] and plants used for food, including tubers and other vegetables grown in soil

100 [18, 19] as well as in marine fauna [9, 20]. Effective methods to decontaminate soil and protect
101 downgradient environments, food and water supply are clearly needed.
102
103 Few researchers have investigated CLD transformation. Early detailed studies by Schrauzer and
104 Katz [21] revealed that reduced vitamin B12 could dechlorinate chlordecone into products
105 including monohydrochlordecone (MHCLD), dihydrochlordecone (DHCLD) and
106 pentachloroindene. Zero valent iron [22] was found to degrade CLD to different products
107 ($C_{10}H_3Cl_9O_2$, $C_{10}H_4Cl_8O_2$, $C_{10}H_5Cl_7O_2$, $C_{10}H_6Cl_6O_2$ and $C_{10}H_7Cl_5O_2$). More recently, Ranguin et
108 al., [23] were also able to show abiotic dechlorination using reduced vitamin B12. The abiotic
109 reduction of CLD with B12, zero-valent iron or sodium sulphide led to formation of
110 hydrochlordecones (HCLDs) and polychloroindenes (PCINs) [24]. Very little data exists on the
111 microbial degradation of CLD. Using Gibbs free energy calculations, Dolfing et al. [25] showed
112 that there is no thermodynamic reason precluding bacterial CLD degradation. Orndorff and
113 Colwell [26] showed that *Pseudomonas aeruginosa strain K03* was able to partially transform
114 CLD to 15% MHCLD and 5% DHCLD, however, the HCLDs detected could have been
115 impurities and not biodegradation products [27]. Under methanogenic conditions, Jablonski et al.
116 [28] reported extensive dechlorination and formation of apolar and polar metabolites in a culture
117 of *Methonosarcina thermophila* at 50ºC. According to this work, 86% of labelled [14C] CLD
118 was dechlorinated. More recently, a low but significant mineralization of chlordecone under
119 aerobic conditions was detected [29]. A fungal strain, *Fusarium oxysporum* MIAE01197, was
120 shown to be able to grow in a liquid culture medium containing CLD as carbon source,
121 however, no degradation products were detected [30]. Very slow, natural transformation of CLD
122 to 5-b-hydrochlordecone was documented in polluted soils indicating the possible natural
123 biotransformation of chlordecone [31]. More recently, the formation of MHCLD, DHCLD,
124 trihydrochlordecone (THCLD) and indene metabolites $C_9Cl_5H_3$ and $C_9Cl_4H_4$ was detected in a
125 mixed bacterial consortium as well as by an isolated *Citrobacter* strain [24, 32], and a third
126 group of metabolites, carboxylated polycholoroindenes (CPCIN) was also reported [24]. The
127 genome of the *Citrobacter* strain in these studies contained no reductive dehalogenase genes and
128 thus transformation was likely cometabolic [32]. A very recent paper by the same team further
129 identified and characterized many chlordecone transformation products [33], using chemical
130 reduction, organic synthesis, and NMR to elucidate isomer structures. Significantly, they further

131 detected and quantified some of these same transformation products in soil samples from
132 Martinique.
133
134 The objective of our work was to evaluate anaerobic microbial transformation of CLD in batch
135 bottle microcosms constructed with soil from Guadeloupe and with simulated groundwater that
136 mimic natural attenuation and electron donor-simulated reductive dechlorination. An LC-
137 Orbitrap MS method was eventually developed for small volume samples, where we found that
138 the choice of extractive co-solvent was critical to the detection of both polar and non-polar
139 metabolites. The isotopic fingerprint of compounds with multiple chlorines enabled
140 identification of metabolites. Microcosms were maintained under strictly anaerobic conditions,
141 and we were able to document slow but extensive dechlorination of CLD to PCIN and CPCIN
142 metabolites with up to 9 chlorine atoms removed. A crude estimation of partitioning coefficients
143 between soil and water was possible to provide first insights into the fate and transport of these
144 CLD metabolites. With a suitable detection method in hand, we then analyzed several field
145 samples from Guadeloupe. In these samples, MHCLD, DHCLD and pentachloroindene
146 metabolites were convincingly detected, indicating that these dechlorinating reactions can also
147 proceed *in situ*, most likely where anaerobic field conditions can be found.
148
149 **MATERIALS AND METHODS**
150 **Collection of Field Samples.** Field samples were collected twice, first in the fall of 2010 for
151 microcosm setup, and second in the spring of 2018 to analyze CLD and potential degradation
152 products by LC/MS using a refined protocol. In 2010, samples were collected from seven
153 locations in the south of Basse-Terre island, Guadeloupe (Table S1A): three andosol samples
154 from agricultural areas used for banana production (soil); three fluvisol samples from river banks
155 near a banana production area (soil and water); and one sludge sample from an anaerobic
156 digester at a sugar cane distillery plant. In 2018, nine different locations were sampled (Table
157 S1B): six from CLD-impacted agricultural areas (as soil and water slurries); and three activated
158 carbon sludge samples from a water treatment plant that handles chlordecone-contaminated
159 water (sampling details in Supplemental Method Details (SMD) 1A).
160

161 **Chemicals.** CLD (neat) and CLD standard (analytic standard 1mg/mL in MeOH) were
162 purchased from Accustandard (New Haven, USA). Hexanes and acetone (Fisher), water and
163 methanol (Caledon Laboratory Chemicals), and ethanol (Commercial Alcohols, Brampton,
164 Canada) were all of HPLC grade. TCE was purchased from Sigma and had a purity of 99.5%. A
165 pentachloroindene standard (2,4,5,6,7-pentachloro-1H-indene) referred to as B1, was kindly
166 provided by researchers at Genoscope (Évry, France).
167
168 **Microcosm Setup and Enrichment.** In December 2010, a microcosm study consisting of 13
169 different conditions each in triplicate for a total of 39 microcosms was initiated (Table S2A).
170 Microcosms contained soil and water samples from Guadeloupe and were augmented with
171 artificial groundwater (recipe in SMD 1B) as there was not enough field water. One set of
172 microcosms contained sludge from an anaerobic digester instead of soil. The microcosms were
173 prepared in 160 ml serum glass bottles (Fisher Scientific) sealed with blue butyl stoppers (Bellco
174 Glass Inc.) with 22.5 ml soil and 80 ml water. Rezasurin (1 mg/l) was added to one microcosm
175 from each triplicate set as an indicator of anaerobic conditions. At setup, 15 microcosms (4
176 different soils and one sludge sample, all in triplicate) were poisoned by adding mercuric
177 chloride (0.05%) and sodium azide (0.02%). The 15 poisoned control microcosms and 18 of the
178 24 active microcosms were amended with CLD at a target concentration of 10 mg/l (which is
179 above the solubility of ~2 mg/L [1, 8], therefore a separate phase of CLD was expected). Three
180 other active microcosms were amended with both CLD and trichloroethene (TCE) at target
181 concentrations of 10 mg/l each, and three microcosms were left unamended. TCE was added as a
182 positive control for reductive dechlorination. All microcosms, except from the three unamended
183 bottles, received an initial dose of an electron donor mix of acetone and ethanol, each at ~80
184 mg/L. The ratio of donor (acetone/ethanol) to CLD (acceptor) in terms of electron equivalents
185 (eeq) was around 100:1, assuming 20 eeq/mol CLD for complete dechlorination. This ratio is
186 very high, and higher than typically used for other chlorinated electron acceptors like TCE (5:1)
187 because more acetone and ethanol were needed to dissolve CLD (solid powder) into the feeding
188 stock solution (Table S3). The microcosms were incubated in the dark, unshaken in an anaerobic
189 glovebox (Coy Lab Products, Grass Lake, MI, USA) for about 8 years. During the first 2.5 years,
190 all the active microcosms were re-amended with CLD twice and electron donor five times. Those
191 microcosms that also received TCE were re-amended with TCE twice during this period. Six

192 active microcosms were transferred into a defined pre-reduced anaerobic mineral medium [34] in
193 slightly larger bottles: one after 1 year, one after 1.5 years, and four after 2.5 years (Figure S1
194 and Table S2B). These transfers were made in 250 ml Boston Round glass bottles (VWR) to a
195 total liquid volume of 200 ml and sealed with screw-cap Mininert™ valves (Chromatographic
196 specialties). Subsequently, these transferred bottles (GT5, GT20, GT33, GT4, GT15, GT3) were
197 re-amended with donor and acceptor regularly. All the other microcosms were not maintained
198 after 2.5 years, but some were sampled a few times for comparison with the transfers. The
199 amounts and frequency of amendments to original microcosms and to transfers over the full
200 study are shown in Table S4. Two bottles with medium only (Medium1, Medium2) were
201 prepared in 2016 for use as controls for LC/MS analysis. These were set up in 250 ml Boston
202 Round glass bottles with 200 ml mineral medium (no culture) and amended with CLD feeding
203 stock to CLD concentrations of 10 and 20 mg/l (Table S2C).
204
205 **Microcosm Sampling and Analysis.** For the first 1.5 years, liquid samples (1 ml) from
206 microcosms were sampled regularly and analyzed for methane, ethene, ethane, and chlorinated
207 ethenes by Gas Chromatography with Flame Ionization Detector (GC-FID), but beyond this
208 time, only the transferred microcosms were analyzed routinely.
209
210 Anion analysis (IC) and pH measurements were made a few times over the course of the study.
211 Samples for DNA extraction were taken from the 6 transfers (GT5, GT20, GT33, GT4, GT15,
212 GT3) about 7 years into the study and the microbial community composition was assessed by
213 small subunit (SSU) rRNA gene amplicon sequencing and Quantitative Polymerase Chain
214 Reaction (qPCR) analysis. Method details of GC, IC, pH and microbial community analysis are
215 described in SMD 2.
216
217 When the microcosms were first established, we did not have a good method to measure CLD or
218 its daughter products. Nevertheless, during the first 1.5 years, 1 ml liquid samples (in duplicate)
219 were taken every 1-2 months from each microcosm and archived frozen at -20ºC. Once an
220 appropriate LC/MS method was developed, analyses by LC/MS were performed approximately
221 once per year, and more frequently in the last two years. The transfers (GT5, GT20, GT33, GT4,
222 GT15, GT3) were analyzed most often, and some poisoned controls, medium controls and some

223 of the original active microcosms were analyzed occasionally for comparison. Sampling and
224 sample preparation procedures for the LC/MS analysis are described below with further details
225 in SMD 2.
226
227 **Sampling and Sample Preparation for CLD Analysis by LC/MS.** Sampling procedures,
228 sample preparation methods and chromatographic and MS methods for analysis of CLD and
229 dechlorinated products were improved progressively (explained in SMD 2). Eventually, two
230 satisfactory sample preparation methods were developed for the microcosms, one for sampling
231 liquid only (method 3) and one for sampling a soil/water slurry (method 4) (details in SMD 2).
232 Samples were always taken using glass syringes (Hamilton Company, Reno, USA) and stored in
233 glass vials with caps with PTFE lined septa (Agilent) to minimize sorption. All samples and
234 standards were filtered through 0.2 μm Millex PTFE syringe filters (Millipore, Burlington,
235 USA). For the liquid samples (method 3), bottles were shaken and left to settle overnight. The
236 next day 0.75 ml liquid was carefully sampled (avoiding collecting any solids) and was placed
237 into a 2 ml glass vial. The sample was then centrifuged for 5 minutes at 3000 rpm, and 0.5 ml of
238 the supernatant was transferred into a new vial containing 0.5 ml methanol. After mixing, the
239 sample was filtered into a final 2 ml glass sampling vial. For the slurry samples (method 4),
240 bottles were shaken and a 1 ml sample was taken with syringe immediately, before the solids
241 settled to get a representative sample of the soil/water slurry. The slurry sample was added to a
242 vial containing 1 ml methanol, shaken gently for 10 min and left sitting for 30 min. The sample
243 was then centrifuged for 5 minutes at 3000 rpm, and the supernatant was filtered into the final 2
244 ml sampling vial. The addition of methanol to the sample had three purposes; to help extract
245 compounds associated with the solids, to help clean up the sample matrix by inducing
246 precipitation of salts from the sample matrix, and to reduce sorption of compounds to filter
247 membranes. For some of the samples the dry weight of solids was measured after drying the
248 sample in a drying oven at 105°C for several hours to estimate sorbed mass.
249
250 The field samples taken in 2018 were expected to have much lower concentrations of CLD and
251 daughter products than the microcosms, therefore a different extraction and concentration
252 procedure was used. Two different sample volumes (5 ml and 20 ml) were extracted by liquid-
253 liquid extraction using a mix of 15% acetone and 85% hexane (methods 5 & 6). The extracts

254 were evaporated to dryness under a stream of nitrogen and the samples were re-dissolved in 1ml
255 and 0.5ml methanol and filtered. Sample preparation procedures, LC/MS instrumentation and
256 methods were in constant development over the course of the study. Only the final optimized
257 method, used from April 9th 2018, is described in detail here. The history and development of the
258 protocol is explained in SMD 2.
259
260 **Liquid Chromatography Mass Spectrometry (LC/MS) Analysis.** Chromatography was
261 carried out on a ZORBAX Rapid Resolution High Definition Phenyl-Hexyl column (50mm x
262 3.0mm, 1.8um) (Agilent, Santa Clara, USA) equipped with a guard column, using a Thermo
263 Scientific Ultimate 3000 UHPLC (Thermo Fisher Scientific, Waltham, MA). The column
264 temperature was 40ºC and the flow rate was set to 300 $\mu L \cdot min^{-1}$. The eluents used were water
265 (A) and methanol (B), and both eluents contained 5 mM of ammonium acetate. The gradient
266 started at 50% B , followed by a linear gradient to 100% B over 8 min, then a hold at 100% B for
267 4 min, then a return to 50% B over 1min, and finally a re-equilibration under the initial
268 conditions of 50% B for 5 min (total runtime 18 min). Liquid samples (10 µL) were injected
269 using an Ultimate 3000 UHPLC autosampler, with autosampler temperature of 8ºC. Compounds
270 were detected and quantified using a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher
271 Scientific) equipped with a Heated Electrospray Ionization (HESI II) probe, operating in
272 negative ionization mode. Mass spectra were acquired over an m/z range from 80 to 750 with the
273 mass resolution set to 140k, and common setting parameters were as follows; AGC Target: 1E6,
274 max injection time 200 ms, spray voltage 4 kV, capillary temperature 320ºC, sheath gas 15, aux
275 gas 5, spare gas 2, and s-lens RF level 55. Data from the LC/MS were processed through
276 Xcalibur Qual Browser (Thermo Fisher Scientific). Calibration standard solutions of CLD (0.02,
277 0.2, 1.7, 2.6, 3.6 mg/l) were prepared from successive dilutions of a purchased analytical
278 standard (1 mg/mL CLD in MeOH). Similarly, a sample of pentachloroindene B1 (2,4,5,6,7-
279 pentachloro-1H-indene) (from Genoscope, France), stored as a 0.4 mg/ml acetonitrile stock
280 solution, was diluted to make five concentration level (0.02, 0.1, 0.2, 0.5 and 1 mg/l) and used as
281 standards for estimates of CLD metabolite concentrations. The dilutions of CLD and B1 were
282 prepared in a 50/50% mix of methanol and mineral medium. Standard dilutions were made fresh
283 the day before or on the same day as they were run.
284

285 **RESULTS AND DISCUSSION**

286 **Overview of Microcosm Data.** CLD is a challenging molecule to quantify, particularly in small
287 volume samples from a microcosm. It took us several years after microcosm set up to develop a
288 suitable method, and until that time, we periodically amended microcosms with CLD without
289 knowing if it was being degraded or not. We rationalized that dechlorination would be slow and
290 limited by the low solubility of CLD. Therefore, provided that we had good controls, we would
291 eventually be able to identify activity by comparing active microcosms to poisoned and medium
292 controls.
293
294 To provide an overview of how each set of microcosms was treated over the course of about
295 3000 days (~8 years), cumulative CLD and electron donor (acetone and ethanol) amendments
296 were plotted over time (Figure S2; with data in Table S4). We monitored methane production,
297 sulfate consumption and TCE dechlorination (where added), as indications of anaerobic
298 microbial activity. All active microcosms except for those made from distillery sludge samples
299 (G63 series) produced methane during the first 1.5 years (Figure S3). Beyond 1.5 years, we
300 continued to monitor and amend only six of the microcosms. Two (GT4 and GT3) were 20%
301 transfers from original bottles into anaerobic medium, that received CLD and donor (GT4) or
302 CLD+TCE and donor (GT3). Transfer GT4 contained none of the original microcosm soil. Four
303 other transfers were made by pouring the entire contents of the original microcosm into slightly
304 larger bottles sealed with Teflon Mininert caps, topped up with medium and amended with CLD
305 and donor (GT5, GT20 and GT33) or CLD+TCE and donor (GT15) (Figure S1). Transfers GT5,
306 GT20 and GT15 continued to produce methane after being transferred, GT33 stopped producing
307 methane after the first re-amendment of CLD, while GT3 and GT4 did not produce any methane
308 after the transfer (Figure S2). In microcosms that received TCE in addition to CLD, TCE was
309 partially dechlorinated to trans- and cis-DCE, with possibly small amounts of ethane produced
310 (Figure S4), but the 20% transfer (GT3) no longer dechlorinated TCE. No ethene was ever found
311 in any of the microcosms (Table S5). In the transfers that did not produce methane (GT33, GT3
312 and GT4), we observed production of acetate (Table S5) although not enough to explain
313 complete transformation of donor. As a result, pH dropped to below 6 in these microcosms (and
314 was occasionally re-adjusted), which probably subsequently eliminated methanogens (Table S5).
315 Sulfate (~1.2 mM initially) was present in all soil microcosms but was depleted within the first

316 year and was not added to transfers (Table S5). We suspect that iron(III) carried over from soil
317 may have also served as an acceptor in some bottles. High chloride background (>12 mM) was
318 present in the microcosms, precluding using chloride increase as a proxy for dechlorination. In
319 summary, we established that all of the active (non-poisoned) microcosms were microbially
320 active initially as well as after 8 years of incubation, despite repeated additions of chlordecone
321 and donor (acetone or ethanol), but that there were significant differences in their extent of
322 methanogenesis depending on particular treatment history.
323
324 **Development of an LC/MS Method for Chlordecone and metabolites.** To enable a time-
325 course analysis and mass balance in microcosms, we needed to develop a CLD analysis method
326 suitable for repeated sampling in microcosms using small sample volumes. CLD analyses are
327 challenging for many reasons, including strong sorption of CLD to soil (and to rubber stoppers),
328 low water solubility of CLD and certain metabolites, lack of authentic standards for degradation
329 products, poor/variable or inconsistent ionization, as well as matrix and solvent effects. We
330 hoped that the high sensitivity and mass accuracy of Orbitrap MS technology would facilitate
331 identification of compounds despite lack of standards. The various early trials and final method
332 are described in SMD 2. Moriwaki and Hasegawa [35] were first to report use of LC/MS for
333 CLD; they used a water/methanol gradient and negative ionization mode, although only
334 standards in methanol were tested. Durimel *et al.,* [36] also detected CLD by LC/MS using a
335 water/acetonitrile gradient, also in negative mode. Cimetiere et al*.,* [37] included formic acid (pH
336 2) to the eluent and observed adduct (M+HCOOH+OH) formation. These authors recommended
337 addition of a co-solvent (acetonitrile, methanol) to avoid adsorption of CLD to filters and to
338 desorb CLD from suspended matter (acetonitrile). They concluded that the presence of salts in
339 sample matrixes weaken the CLD MS signal, which made us realize the importance of preparing
340 standards in the same matrix as the samples.
341
342 Based on these prior studies, standards were prepared in 50% medium and 50% methanol.
343 Methanol was added to samples before filtration to decrease sorption and to precipitate salts.
344 Methanol addition proved later to be a good solvent to mix with water to recover compounds
345 from soil particles. As first described by Harless et al., [38] and observed by others [24, 39],
346 CLD actually exists as a gem-diol (hydrate) in water or a hemiacetal in methanol and can also

347 form various adducts in the presence of compounds often used in LC eluents, such as acetate and
348 formate. In this study chromatographic separation, ionization and signal intensity were
349 maximized when ammonium acetate was included in the water and methanol eluents. We
350 observed many CLD adducts (hydrate, formate, acetate) and hemiacetal in our analyses (Figure
351 S5), that required careful data interpretation. Hydrochlordecones (MHCLD, DHCLD and
352 THCLD) also showed similar adduct formation. In this study we chose to calibrate and report the
353 hydrate forms of CLD, MHCLD, DHCLD and THCLD. All of the LC/MS data collected during
354 this study are compiled in Table S7. We initially only sampled the liquid phase (centrifuged and
355 filtered) to avoid variability related to sorption to solids in the microcosms. However, sorption to
356 soil in the microcosm was very strong, precluding a satisfactory mass balance when considering
357 only liquid phase samples. We eventually had to adapt the method to include and quantify CLD
358 on solids. Despite all these challenges, dechlorination products were clearly observed, as
359 described in the next section.
360
361 **Detection of CLD Metabolites using best method.** A series of metabolites were observed in all
362 the active original microcosms and transfers over the course of the study (Figure 1 with data in
363 Table S7). Despite not having standards, the accuracy of the Orbitrap MS enabled identification
364 of metabolites by matching first or highest m/z values of the mass spectrum to the presumed
365 structure, and further evaluating the full characteristic isotope patterns for these multi-chlorinated
366 transformation products (details below). Table S6 lists all observed metabolites with their
367 measured and theoretical masses (and IDs), and Figure S6 illustrates the characteristic mass
368 spectra for these metabolites (observed and theoretical). The identified metabolites were assigned
369 to three different groups: group A - hydrochlordecones (HCLD); group B - polychloroindenes
370 (PCIN); and group C - carboxylated polychloroindenes (CPCIN) to be consistent with previous
371 studies detailing the masses and NMR structures of CLD metabolites [24, 32, 40]. In this study, a
372 total of 19 different dechlorination products were detected by LC/MS in the microcosms.
373 Multiple isomers of certain metabolites were found, having exactly the same mass but varying
374 retention time. Retention times were also consistent with the relative polarity of each compound.
375 In this study we relied on exact masses, unique isotope distribution patterns, and retention times
376 to support the indentity of metabolites classes, namely hydrochlordecones, polychloroindenes,
377 and carboxylated polychloroindenes with varying numbers of chlorine substituents. The

consistency of the results with previous reports [24, 33] and highly characteristic chlorine isotope pattern leave little doubt to the structure of the compounds, other than the actual position of substituents on the rings. Only purification of each compound and NMR could resolve such structural details, and these are beyond the scope of this work, and not necessary to evaluate extent of dechlorination. An example chromatogram from one of the samples, bottle GT20 sampled June 29th 2018, is shown in Figure 2. Chlordecone and 17 of the 19 observed dechlorination products detected in this study are shown, including the non-polar B compounds (polychloroindenes, PCINs) showing longer retention times than CLD, and the more polar C compounds (carboxylated polychloroindenes, CPCINs) with much shorter retention times (Figure 2). Compounds with retention times 9.3, 8.88, and 8.05 min and mass to charge ratios m/z 468.7264, m/z 434.7661 and m/z 400.8042 respectively, could be attributed to the hydrate forms of MHCLD (A9a) $[C_{10}Cl_9O_2H_2]^-$, DHCLD (A8a) $[C_{10}Cl_8O_2H_3]^-$ and THCLD (A7a) $[C_{10}Cl_7O_2H_4]^-$ obtained by the loss of 1, 2 and 3 chlorine atoms from CLD (Figure 2 and Table S6). Three metabolites, less polar than CLD and with longer retention times at 12.19, 11.44 and 10.34 min, respectively, exhibiting mass to charge ratios of m/z 284.8616, m/z 250.9006 and m/z 216.9382 were identified as pentachloroindene (B5a) $[C_9Cl_5H_2]^-$, tetrachloroindene (B4a) $[C_9Cl_4H_3]^-$ and trichloroindene (B3a) $[C_9Cl_3H_4]^-$ (Figure 2 and Table S6). Thirteen (13) metabolites more polar that CLD were assigned to group C, and observed at retention times ranging from 2.02 to 6.69 min (Figure 2 and Table S6). Compounds C4a and C4b, detected respectively at retention times of 5.47 and 5.02 min, and with a corresponding m/z of 294.8899 could be attributed to two isomers of a carboxylated tetrachloroindene compound $[C_{10}Cl_4O_2H_3]^-$ obtained by the loss of 6 chlorine atoms. At retention times between 3.03 and 6.69 min, 5 isomers (C3a-C3e) with a corresponding m/z of 260.9287 were detected and attributed to a carboxylated trichloroindene compound $[C_{10}Cl_3O_2H_4]^-$ obtained by the loss of 7 chlorine atoms. Four isomers (C2a-C2d) obtained by the loss of 8 chlorine atoms were detected at retention times between 2.02 and 5.25 min and had m/z of 226.9677, assigned to be a carboxylated dichloroindene $[C_{10}Cl_2O_2H_5]^-$. Two last isomers (C1a and C1b) with retention times at 2.5 and 2.95 min and m/z of 193.0064 were classified as carboxylated monochloroindene $[C_{10}ClO_2H_6]^-$, obtained by the loss of 9 chlorine atoms from CLD.

408 In summary, CLD metabolites observed in the microcosms could be classified into 3 families of
409 compounds: group A compounds including mono-, di- and trihydrochlordecone derivatives with
410 proposed neutral formula [$C_{10}Cl_{10-n}OH_n$, n= 1,2,3]; group B non-polar "open cage" structures
411 including three polychloroindene compounds with proposed neutral formula [$C_9Cl_{5-n}H_{3+n}$,
412 n=0,1,2]; and group C polar "open cage" structures consisting of carboxylated polychloroindene
413 derivatives with neutral formula [$C_{10}Cl_{4-n}O_2H_{4+n}$, n=0-3].
414
415 Due to a number of challenges with and changes in sample preparation and LC/MS analysis over
416 time, it was difficult to get an accurate picture of concentration changes of CLD over time.
417 Therefore, when we finally settled on a good method, we re-analyzed some frozen archived
418 samples to get a comparable set of data. Results from a selection of samples from GT20 with
419 comparable sample preparation (liquid phase only, no soil) and analytical methods are shown in
420 Figure 3 (raw data in Table S8). Results show that none of the monitored metabolites were
421 detected in the sample taken two weeks into the experiment, but by 8 months into the study, we
422 observed MHCLD, DHCLD, and two CPCINs with loss of 8 and 9 chlorines. Later sampling
423 time points showed increasing concentrations of MHCLD, DHCLD, and four CPCINs with loss
424 of 6 to 9 chlorines. Pentachloroindene (B5a) only showed up in the last sampling point, however
425 it was found to sorb strongly to soil, and we would therefore not expect to see it in these liquid
426 phase samples analyzed here. Also, because we kept adding more CLD to the bottles over time,
427 we could not use aqueous CLD concentration changes as a measure of degradation rate. Despite
428 not seeing a clear decrease in CLD concentrations in the liquid phase, highly dechlorinated
429 metabolites with up to 9 chlorine removed were observed in the active bottles but not in the
430 controls indicating that biological processes were involved in the dechlorination of CLD into
431 HCLD, PCIN and CPCIN metabolites.
432
433 **Effect of Sorption.** Strong sorption of CLD and non-polar PCID metabolites to soil particles
434 made it difficult to evaluate the fate of CLD in the bottles by sampling the aqueous phase only.
435 We therefore changed our approach to also extract soil in our samples. By analyzing the same
436 samples by two different sample preparation methods, one in which only the liquid concentration
437 was measured (filtered sample) and another in which a mixture of soil and water was analyzed,
438 and quantifying the amount of soil (dry mass) in the sample, we were able to calculate and

439 estimate a distribution coefficient, K_d (l/kg) equal to the ratio of sorbed concentration (mg/kg) to
440 dissolved concentration (mg/l) for most of the analytes (Table 1; raw data in Table S9). This
441 analysis confirmed that CLD and mono- and dihydro- CLD, with K_d values of 130 ± 57, 52 ± 12
442 and 28 ± 6 ml/g (or l/kg), respectively, absorb quite strongly to the Guadeloupe soil used, while
443 the K_d value for pentachloroindene (B5a) was much higher at 5700 ± 220 l/kg, and thus absorb
444 even more strongly. The carboxylated chloroindenes (C compounds) had much lower K_d values
445 ranging from 2 to 11 l/kg, and were found in the aqueous phase. The estimated K_d values
446 correspond well to the retention times by reverse phase LC (Table 1). Our estimated distribution
447 coefficient for chlordecone is in the same range (60-330 l/kg) as one previous report [41]. A
448 distribution coefficient based on organic content, K_{oc}, of 2,500 l/kg has also been reported [8],
449 corresponding to a K_d of 250 l/kg assuming a fraction of organic carbon of 10%. We were not
450 able to find any reports of sorption coefficients for any CLD metabolites.
451
452 **Quantification of the Extent of Transformation in Microcosms and mass balance**
453 **calculations.** To quantify the extent of transformation of CLD added to the microcosms over the
454 course of the study, we used data from well-mixed slurry samples from 13 active, 7 poisoned
455 controls, 2 unamended microcosms, and two medium controls (Table 2). The well-mixed slurry
456 samples were analyzed because they capture mass from both the liquid and solid phases, so that
457 we could better compare final measured mass recovered to the total amount of CLD that had
458 been added to the bottles. To attempt a mass balance, MHCLD, DHCLD, and THCLD
459 concentrations were estimated based on the response factor for CLD as no standards were
460 available for these metabolites. Researchers from Genoscope (France) kindly provided us with a
461 small sample of pentachloroindene B1 (2,4,5,6,7-pentachloro-1H-indene) that they had managed
462 to chemically purify. As a result, we were able to do a rough estimate of the amount of B5a
463 produced in active microcosms. B4a concentrations were estimated using the response factor for
464 B1. For the carboxylated polychlorinated indenes (C group), we have no proxy for calibration;
465 however, to get a very rough idea of possible concentrations we also used the response factor for
466 B1 for these compounds.
467
468 Using these estimated response factors applied to the areas determined by LC/MS analysis of
469 well-mixed slurry samples, we could calculate total moles recovered per bottle by multiplying

470 concentrations by the total slurry volume. We then compared the CLD and metabolite moles
471 recovered in the bottles after 8 years to the initial amount of CLD added (Table 2; calculations in
472 Table S10). The three different groups of microcosms, poisoned controls, active original
473 microcosms, and active microcosm transfers, did indeed show differences in CLD recovery. In
474 the group of seven poisoned controls, two microcosms (G1 and G3) produced a lot of methane
475 and thus were biologically active, despite having been poisoned. These bottles also exhibited
476 extensive metabolite production, unlike remaining controls (Figure 1 and Table 2). Therefore, for
477 the mole balance analysis, we included those two microcosms into the group of active original
478 microcosms. We were able to recover 63±6% of added CLD in the poisoned controls after 8
479 years, 44±11% in the original microcosms that only receive electron donor in the first 2 years,
480 and only 31±3% of added CLD in the transfers amended regularly with donor and CLD (Table
481 2). The loss of ~37% in the poisoned control group likely results from sorption to glass and
482 stoppers, poor extraction from soils during sample preparation, losses from volatilization, and
483 some minimal losses (<1%) from sample removal. Losses in microbially-active bottles are
484 greater and can be explained by the contribution of biological transformation processes.
485
486 We estimated the total moles recovered as metabolites in the various treatments. Metabolites
487 were not detected in the un-amended slurry microcosms, nor in the medium controls. The
488 inactive poisoned controls had only trace amounts of MHCLD (0.001-0.004 μmoles) and no
489 other metabolites. The active bottles had significantly higher concentrations of metabolites,
490 especially in the transfers that received more CLD and donor. The estimated sum of moles of
491 metabolites ranged from 13% to 98% of the CLD remaining after 8 years in active microcosms.
492 When the sum of all measured metabolites was included in the mole balance, overall recoveries
493 after 8 years were more similar regardless of treatment, 63±6%, 60±14%, and 50±13% for
494 controls, originals and transfers, respectively. Given the length of the study and approximations
495 in calibration factors, these results were very reassuring and provided confidence in the
496 measurements.
497
498 **Microbial Community Analysis qPCR and Sequencing Analysis.** While DNA samples were
499 collected and analyzed at various times throughout the 8 years, preliminary analyses have not
500 revealed any clear trends to date. A snap shot of the microbial community and abundance after

501 77 months is shown in Figure 4 (raw data are provided in Tables S11 and S12). The microbial
502 community of the 6 transfers reveals an abundance of fermentative and syntrophic anaerobes,
503 consistent with the electron donor mix (acetone and ethanol) provided. Microcosms GT33 and
504 GT4 produced little to no methane, and contain few or no methanogens. GT4 is the most
505 extensively transferred microcosm and no longer contains soil. It also has the lowest bacterial
506 cell numbers inferred from qPCR of 16S rRNA copies per ml. No particular trends are
507 discernable at this time. Perhaps, as concluded by Chaussonnerie et al., [32], the microbial
508 transformation is cometabolic, and really only dependent on sufficient availability of reduced
509 vitamin B12. Further studies are clearly warranted.
510
511 **Analysis of Field Samples from 2018.** We wondered if metabolites identified in the microcosms
512 could also be detected in field samples, therefore we collected fresh soil sampless from the same
513 locations in Guadeloupe that were previously sampled for the microcosm study and 9 samples
514 were analyzed. (Table 3; raw data in Table S13). Anticipating quite low concentrations in the
515 field samples, we decided to sample larger 5 and 20 ml volumes and perform a liquid-liquid
516 extraction with a concentration step (method 5 and 6, SMD 2) in addition to our already
517 established 1 ml slurry sample preparation method (method 4, SMD 2). Analysis of 6 soil
518 samples from banana plantations in Guadeloupe revealed CLD concentrations in the range 120 to
519 1000 ng/g soil, or 0.12 to 1.0 mg/kg. These values are pretty typical of Guadeloupe soils: a
520 recent survey [42] reported CLD concentrations in mg/kg in soil of 0.03 (minimum), 2.00
521 (median), 3.39 (mean), and 24.2 (maximum). MHCLD was detected in all soil samples and
522 ranged from 1 to 8% of CLD based on area from LC/MS analysis. Most soil samples also
523 showed DHCLD, but area counts were 10 to 100 times lower than those of MHCLD.
524 Pentachloroindene (B5a) concentrations were estimated based on response factor for B1, and
525 ranged from 0.5 to 24 ng/g of solid. B4a metabolites were also detected in the soil samples, but
526 area counts were generally lower (up to 30 times) than those of B5a. Two of the 3 activated
527 carbon sludge samples showed quite high CLD concentrations, between 6,000 and 8,500 ng/g.
528 MHCLD concentrations were also significant, between 4 and 10% of CLD based on area counts.
529 No PCIN was detected in the activated carbon sludge samples. We did not detect CPCIN
530 metabolites in any of the field samples. Due to their hydrophilicity, the CPCIN metabolites
531 would likely not be captured using the liquid-liquid extraction/concentration method (methods 5

532 and 6) used, and we did not have an alternative method for concentration of these compounds.
533 We could also hypothesise that due to their hydrophilic nature and low sorption, these
534 compounds may have been washed out from the soils by rain. Regardless, these field data
535 confirm that anaerobic ring opening and dechlorinating processes do occur *in situ* in Guadeloupe
536 soils. A more extensive analysis is thus warranted to determine locations for highest intrinsic
537 activity on the islands and if rates could be accelerated by inducing anaerobic conditions, such as
538 through organic amendment. The mechanism of CLD biotransformation also needs further
539 investigation.
540

541 **CONCLUSIONS**

542 We have provided convincing LC/MS evidence for extensive dechlorination of CLD by
543 indigenous microorganisms in chlordecone polluted soils. At least 19 different metabolites were
544 detected as CLD concentrations progressively decreased over long-term microcosm incubations.
545 Metabolites included hydrochlordecones, and the open-cage polychlorinated indenes and
546 polychlorinated carboxylated indenes. Evidence for up to 9 Cl removed from the parent
547 chlordecone molecule was found. Carboxylated intermediates were found to sorb poorly to soil.
548 They may be flushed away while the polychlorinated indenes stick strongly to the soil. Further
549 experiments are warranted to determine how to increase dechlorination rates and to further study
550 the fate of these new CLD metabolites. The good news is that less chlorinated open-cage
551 structures are more likely to be biodegradable by a wider variety of microbes under both aerobic
552 and anaerobic conditions; this is the first glimpse of hope that anaerobic bioremediation may be a
553 viable approach for chlordecone.
554

555 **ASSOCIATED CONTENT**

556 **Supporting Information.** Suporting Method Details (SMD) for field sampling and for
557 microcosm setup including recipe for artificial groundwater, analysis procedure and sample
558 preparation methods for GC-FID, IC, pH and LC/MS analysis, and DNA extraction, amplicon
559 sequencing, and qPCR analysis procedures. Figures showing microcosm transfers and their
560 origins, history of microcosms (CLD added, donor added, methane produced), methane
561 production in active CLD amended MCs during the first 1.5 years of monitoring, TCE and its
562 dechlorination products in a CLD and TCE amended MC (G15) during the first 1.5 years of

563 monitoring, LC/MS scan analysis of CLD (CLD hydrate, hemi-acetal and other observed CLD
564 adducts), and chromatograms and spectra for each of the 19 observed metabolites. Tables
565 showing Guadeloupe field sample details, treatment table for Guadeloupe microcosm study,
566 details of feeding stock preparation, details of CLD and donor amendments, details of GC-FID
567 and IC analysis and pH measurements, list of dechlorinated metabolites identified in microcosm
568 samples, results of all LC/MS analysis, normalized LC/MS area counts in GT20, sorption
569 calculations and plots, mass balance calculations, sequencing analysis and qPCR analysis, and
570 concentration calculations for CLD and its degradation products in Guadeloupe field samples.
571

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582

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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

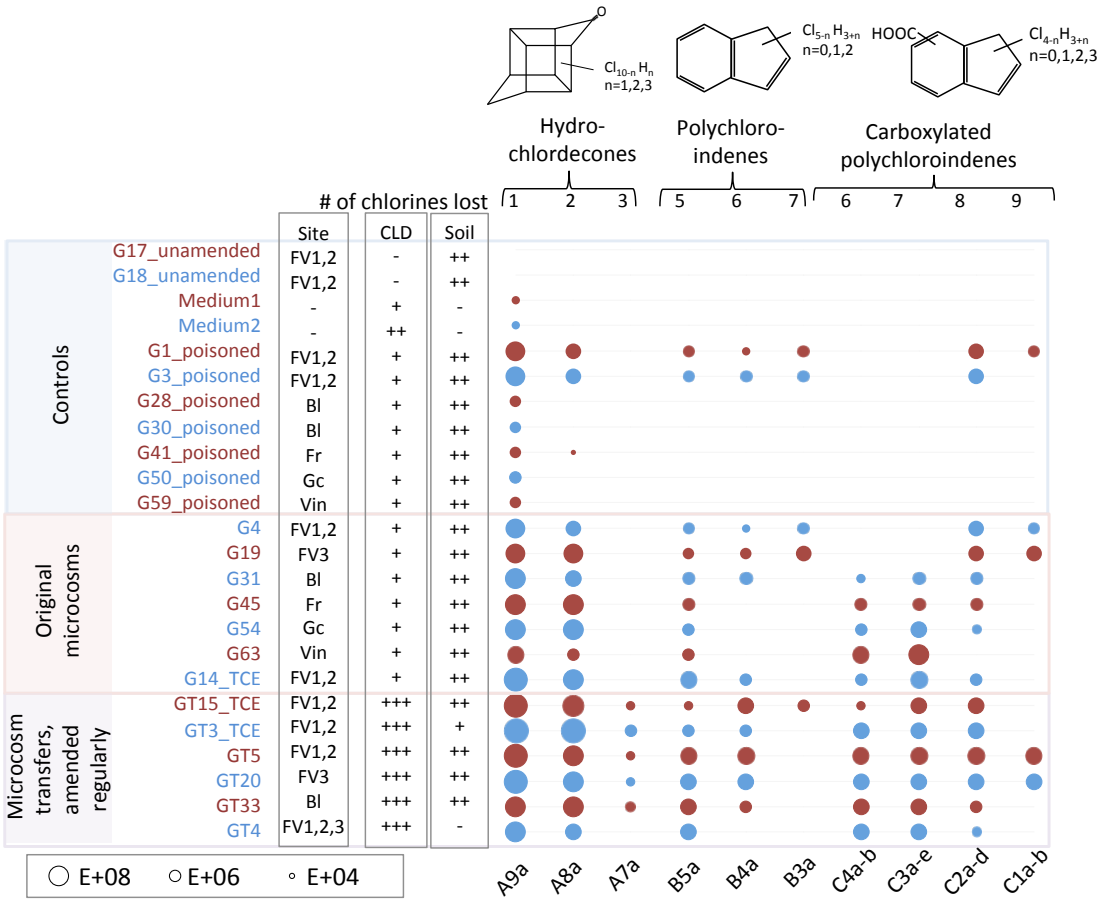


Figure 1: Dechlorinated metabolites observed in anaerobic microcosms constructed from Guadeloupe soil and water. The size of each circle is proportional to the area count from LC/MS analysis of slurry samples performed March 7th 2019 (day 3038) (see Table S7 for raw data).

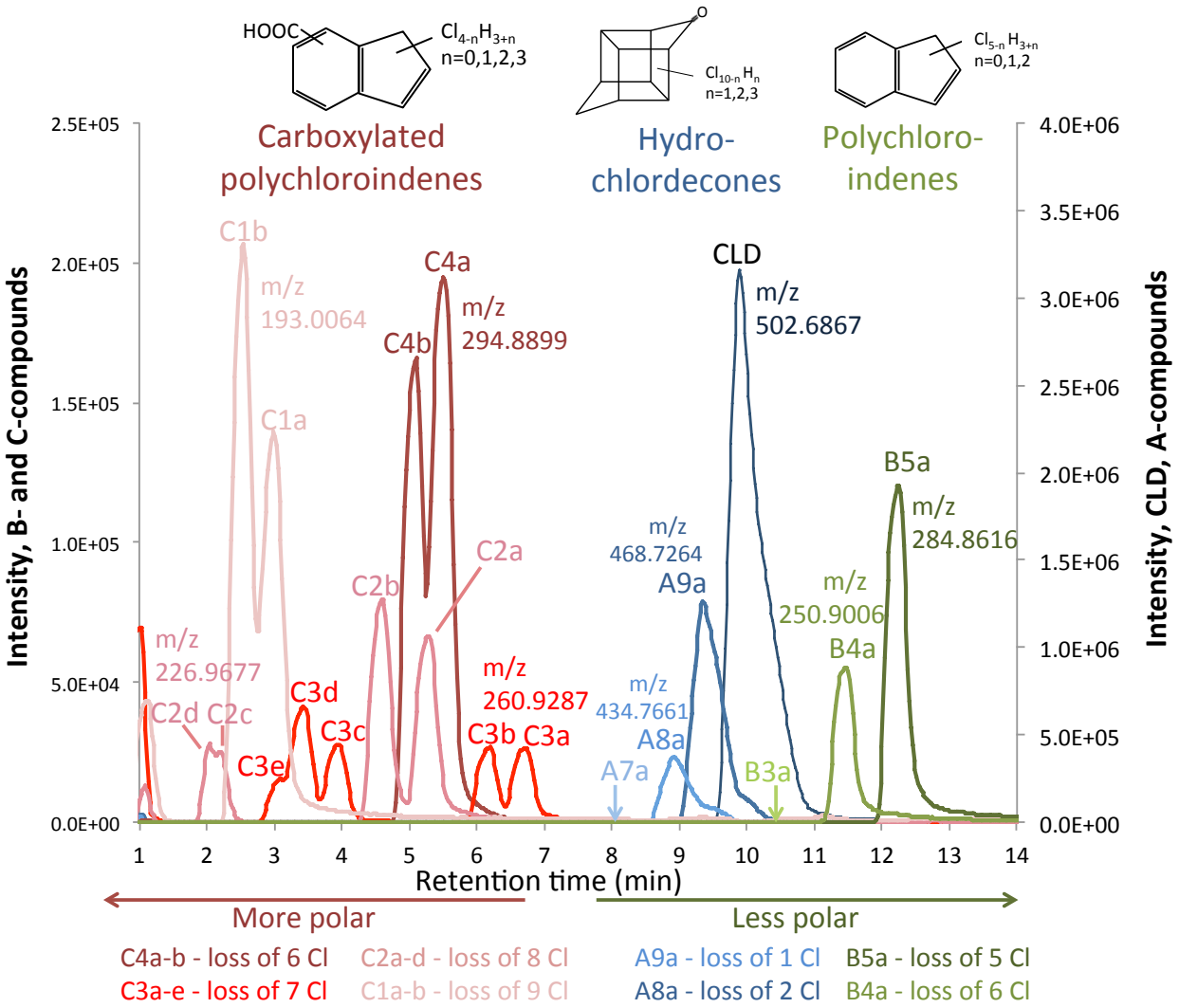


Figure 2: Chromatograms of chlordecone and its dechlorinated metabolites in sample GT20 June 29th 2018 (slurry sample). Results are from LC/MS, equipped with ESI, in negative mode. Observed m/z values (monoisotopic) for the different compounds were; 502.6879 (CLD, $[C_{10}Cl_{10}O_2H]^-$), 468.7264 (A9a, $[C_{10}Cl_9O_2H_2]^-$), 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.

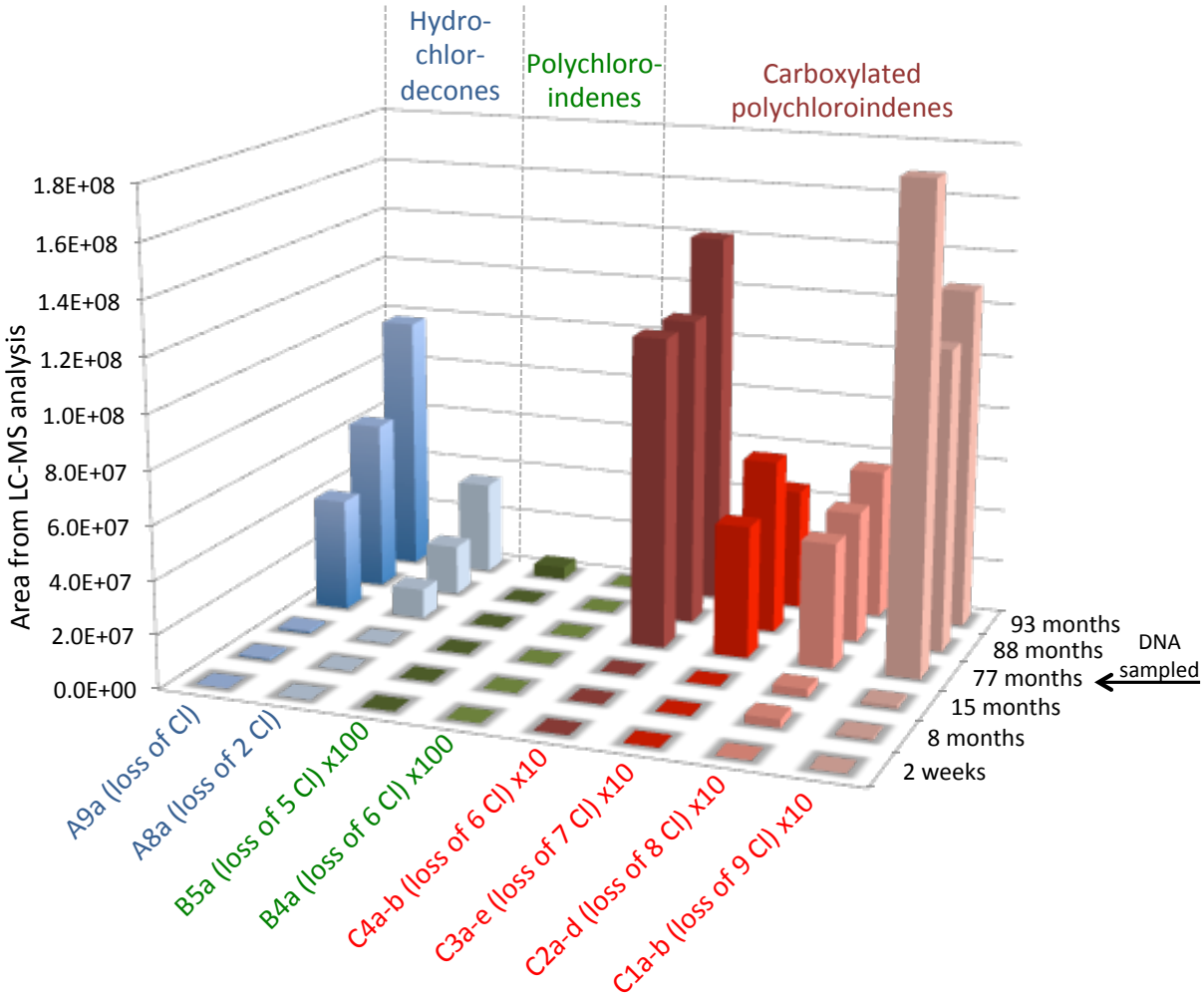
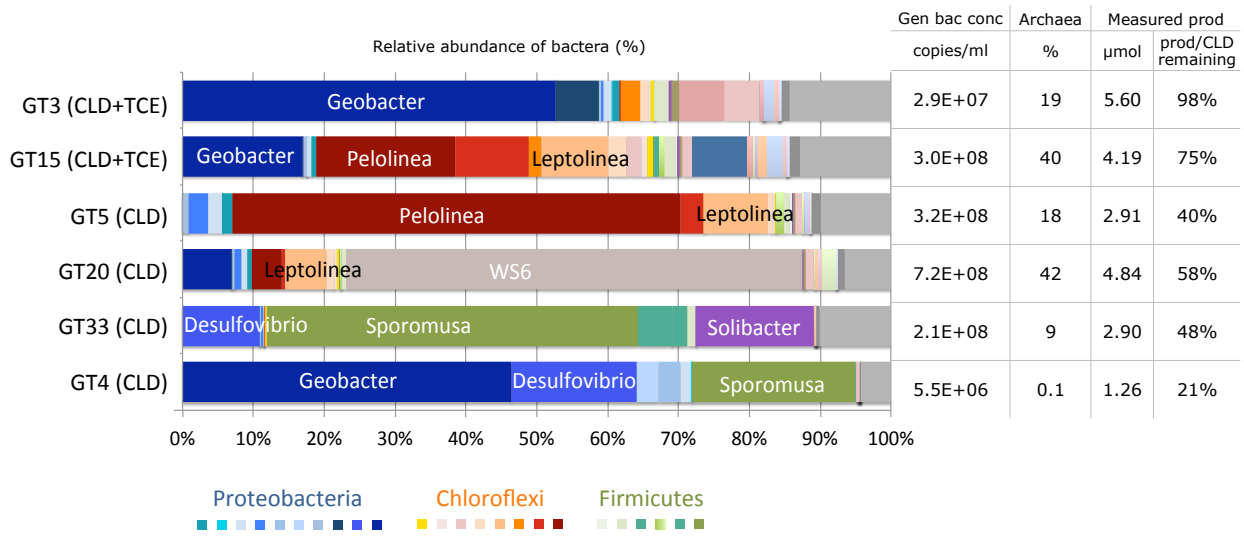


Figure 3: Dechlorinated metabolites from chlordecone over time in one of the anaerobic microcosms, transfer GT20. Only samples that were prepared the same way are included in this graph (sample preparation method 3, liquid phase only, no soil, see supplemental method details). Areas of B compounds were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