Recurrent micropollutant exposure leads to divergent degradation abilities and community succession in a freshwater microbiome

Running title: Ecologically disturbing effects of trace micropollutants on a freshwater microbiome

Dandan Izabel-Shen[1#], Shuang Li[2,3,4#], Tingwei Luo[5], Jianjun Wang[6], Yan Li[2,3], Qian Sun[2,3], Chang-Ping Yu[2,7], Anyi Hu[2,3*]

1. Department of Ecology, Environment and Plant Sciences, Stockholm University, 106 91, Stockholm, Sweden
2. CAS Key Laboratory of Urban Pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
3. University of Chinese Academy of Sciences, Beijing 100049, China
4. Department of Environmental Microbiology, UFZ, Helmholtz Centre for Environmental Research, Leipzig, Germany
5. Institute of Marine Microbes and Ecospheres, State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, China
6. State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, 210008, China
7. Graduate Institute of Environmental Engineering, National Taiwan University, Taipei 106, Taiwan

#The authors contributed equally to this work.

*Correspondence to:
Dr. Anyi Hu, ayhu@iue.ac.cn
CAS Key Laboratory of Urban Pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China, Telephone & Fax: 86-592-6190582.

Keywords: Micropollutant removal; preexposure history; community turnover; stochastic assembly; deterministic assembly;

Abstract

The high-volume release of micropollutants into natural surface waters has raised concern due to their environmental accumulation. Persisting micropollutants can impact multiple generations of organisms via press disturbances, but their microbially-mediated degradation and the influence on community assembly remain understudied. Here, freshwater microbial communities in microcosms were treated with four common micropollutants, alone or in combination, and then transferred in stages to fresh medium containing the same level of micropollutants to mimic the recurrent exposure of microbes under environmentally relevant conditions. Our results showed that the degradation of micropollutants was closely linked to the community succession, and that recurrent exposure to micropollutants enhanced the degradation capacity. The partitioning analysis of ecological processes revealed that community assembly was dominated by stochastic processes during early exposure, via random community changes, and by deterministic processes later in the exposure. Analyzing individual taxa

abundances over time revealed two distinct bacterial responses, in which a larger proportion of sensitive than tolerant taxa was present in the disturbed communities, and the abundances of the most sensitive taxa were significantly associated with micropollutant degradation. This study clearly showed that microbial communities are generally vulnerable to persisting micropollutants in aquatic environments, which has important implications for pollution management, especially regarding microbial dynamics and ecosystem functioning in micropollutant removal.

Introduction

The rapid exploitation and widespread use of emerging organic chemicals have resulted in environmental damage and human health issues during the last several decades [1, 2]. These organic chemicals include pharmaceuticals and personal care products, hormones, and industrial chemicals, and are typically classified as micropollutants, because of their occurrence at a low to very low concentration of μg/L and ng/L in the receiving environments [3, 4]. In aquatic systems, for instance, the majority of micropollutants originate from direct and indirect inputs of organic contaminants that are released via the effluents of wastewater treatments plants [1, 5–7]. The micropollutants gradually accumulate at sites of their release and reach concentrations high enough to exert disruptive effects on aquatic organisms such as fish, invertebrates, and microbes [8–13]. Many studies have assessed the eco-toxicity of micropollutants to humans and animals [e.g., 14–17]. However, little is known about the mechanisms by which micropollutants impact the turnover of aquatic microbial communities [6, 18, 19], especially those of freshwater bacteria [20, 21], and the role of microbes on trace micropollutant degradation.

The responses of microbial assemblages to micropollutants can be classified as: subsidy responses, in which, via biodegradation, the microorganisms are able to utilize the micropollutant compounds as carbon sources, and stress responses, in which the growth and survival of microorganisms are inhibited [22, 23]. Among the compounds eliciting subsidy responses are bisphenol A (BPA) and its substituted derivatives (e.g., bisphenol S, BPS) [24], as their relatively simple chemical structure and low toxicity allow both to be utilized as carbon sources by microbial isolates [23, 25]. Stress responses are elicited by pharmaceutical and personal care micropollutants such as triclosan (TCS) and its substituted derivatives (e.g., triclocarban, TCC) [26], because

both inhibit the growth of several microbial taxa, consistent with their resemblance to antimicrobials [22, 27]. Both BPA and TCS in the field strongly influence microbial diversity, especially that of the central species and module communities comprising the microbial co-occurrence network [28].

Microbial responses to chemical pollutants can be modified by the exposure legacy of the community. For example, microbial communities with a history of containment exposure may differ from contaminant-naïve communities in the rate and extent of chemical degradation [29–31]. They may also differ in their plasticity [18] upon subsequent exposure to similar contaminants. However, the ecological drivers of community succession following repeated micropollutant exposure are not well-understood. Studies of community dynamics over multiple generations and a time period relevant for community stability could provide insights into the ecological traits that determine taxon sensitivity and tolerance, and therefore the ability of microbial community members to degrade micropollutants.

Press disturbances in natural environments can impact multiple generations of organisms [32] by altering the affected ecosystems beyond the possibility of recovery or by causing a shift to a community with a new stable state [33]. Following a stress event such as recurrent micropollutant exposure, communities rely on reassembly from the local seedbanks such that succession eventually results in a new steady state. During the early stages of community dynamics, no-longer-dormant local species undergo a secondary succession characterized by new abiotic and biotic interactions. The emergence of and resuscitation from dormancy is considered as stochastic processes that lead to divergent communities [34–36]. When abiotic and biotic conditions allow a stabilization of the initial communities, community formation at a later phase of a press disturbance is expected to converge, through the succession of microbial species

101 [37]. In addition, the plasticity of microbial taxa in response to recurrent micropollutant
102 exposure has been shown to increase over multiple generations [18], with later-
103 assembled communities shifting to a new stable state that differs from the one before
104 the exposure.

105 In this study, a press disturbance was simulated in a microcosm experiment in
106 which a reservoir bacterial community was transferred into fresh medium containing
107 trace concentrations of typical micropollutants that were refreshed with recurrent
108 micropollutant exposure every 5 days. Several different micropollutants are typically
109 present simultaneously within aquatic environments, but whether their interactions lead
110 to effects on microbial communities that differ from those of single micropollutants is
111 unclear [12]. Thus, we also experimentally manipulated single and multiple
112 micropollutants to determine their impacts on taxon coexistence, the regularity of
113 community assembly, and micropollutant degradation ability. We hypothesized, that
114 because BPA elicits a subsidy response and TCS a stress response, the removal rate of
115 BPA would be higher than that of TCS, with larger differences in the removal rates by
116 microbial communities exposed to these micropollutants individually rather than in
117 combination with other micropollutants. Accordingly, the community composition
118 assembled at the initial and late phases of exposure would differ substantially, with
119 convergent dynamics more apparent towards the end of the press disturbance. Finally,
120 we predicted that community dynamics would greatly differ following exposure to BPA
121 vs. TCS whereas the dynamics of communities exposed to a mixture of micropollutants
122 would resemble those of either the BPA- or the TCS-exposed assembly, depending on
123 the number and abundance of the shared taxa.

Materials and Methods

Water collection

We selected the bacterial species pools from Shidou Reservoir, which is one of largest sources of drinking water in Xiamen, China (Hu et al., 2018) and is therefore under governmental protection from pollution originating from household drainages and a nearby dam (Fig. S1). Unlike other contaminated aquatic environments [22, 38, 39], the concentrations of BPA, BPS, TCS, and TCC in Shidou Reservoir (China) are very low (< 1 ng/L), such that the reservoir's waters provide an ideal system to investigate the influence of persistent micropollutants on an aquatic microbiome. Surface water (100 L) from Shidou Reservoir (24˚41'39"N, 118˚0'37"E) was collected at 0.5 m depth on June 1, 2017 (Fig. S1). Temperature, pH, conductivity, dissolved oxygen (DO), and dissolved oxygen saturation, measured *in situ* using a HACH HQ40d multi-parameter meter (HACH, Loveland, CO, USA), were 29.0°C, 8.90, 54.4 μs/cm, 8.84 mg/L and 116.7%, respectively. The concentrations of BPA, BPS, TCS, and TCC in the reservoir water were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (ABI 3200QTRAP, Framingham, MA, USA) with solid-phase extraction and all were < 1 ng/L at the time of sampling (details of the measurements are provided below). The collected water was transported from the field to the laboratory within 2 h after sampling.

Experimental setup

BPA, BPS, TCS, and TCC were dissolved in acetone at concentrations of 800, 800, 400, and 400 μg/L, respectively, and stored as stocks in glass bottles (HPLC grade). The micropollutants were previously shown to be chemically stable when dissolved in an organic solvent [39, 40]. From the 100 L of collected water, 15 L were filtered through

148 a 1.2-μm polycarbonate filter (Millipore, Ireland), to minimize top-down regulation by
149 planktonic grazers, and then used as the initial inoculum. The remaining unfiltered
150 water was filtered through a 0.22-μm polycarbonate filter (Millipore, Cork, Ireland) to
151 remove microorganisms and then stored in the dark for later use in the preparation of
152 incubation medium. Two treatments consisting of single micropollutants (BPA and TCS)
153 and two treatments consisting of micropollutant mixtures (MI = BPA + TCS) and (MII
154 = BPA + BPS + TCS + TCC) were established.

155 Time-serial microcosms comprising seven inoculation batches from batch 1
156 (named hereafter 'B1') through batch 7 ('B7') were established to investigate the
157 microbial community response to a press disturbance of micropollutants resulting from
158 a single short-term event but maintained for 35 days (Fig. 1). Prior to the experimental
159 initiation, the caps of the bottles containing the micropollutants were loosened for > 12
160 h to volatilize the acetone to minimize its influence on the microbial responses. In
161 microcosm B1, 600 ml of the initial inoculum was added to bottles that already
162 contained the micropollutants and nutrients, such that the final concentration of each
163 micropollutant in the treatments was as follows: BPA: 1 μg/L BPA, TCS: 1 μg/L TCS,
164 MI: 1 μg/L BPA + 1 μg/L TCS, and MII: 1 μg/L BPA + 0.1 μg/L BPS + 1 μg/L TCS +
165 0.1 μg/L TCC. The final concentration of each micropollutant in the microcosms closely
166 represented their ambient environmental conditions as reported in literature [22, 41].
167 Further, given the low nutrient concentrations [42], we adjusted the concentrations of
168 nitrogen and phosphorus in the filtered water to 128.6 and 8.04 μM to promote
169 microbial growth by the addition of KNO_3 and KH_2PO_4, respectively [43]. After 5 days
170 of incubation, 60 mL of the B1 inoculum was pipetted from each of the replicates and
171 transferred to a sterile bottle containing 540 mL of 0.22-μm-filtered water and freshly
172 added micropollutants. This newly inoculated microcosm served as batch 2 (named

173 hereafter 'B2'). The micropollutant treatments and controls were amended with 128.6
174 μM N and 8.04 μM P after each inoculation to maintain similar amounts of these
175 nutrients throughout the experiment. The same inoculation procedure was applied at
176 each 5-day interval to initiate microcosms B3–B7, such that the experimental period
177 was 35 days (Fig. 1). Microbial cells were transferred between pairwise replicates, with
178 the remainder used in the downstream analyses described below. An additional set of
179 experimental bottles without micropollutant addition served as the controls. In total,
180 there were five treatments, each with three replicates, for a total of 15 microcosm
181 replicates per batch. All microcosms, i.e., 105 experimental bottles during the 35 days,
182 were incubated in the dark at 28°C with shaking at 100 rpm.

183 Additional experiments were conducted to determine (i) micropollutant removal
184 rates in the absence of microorganisms, to rule out the gradual degradation of the
185 micropollutants in the treatments due to their chemical instability, and (ii) how the
186 ability of the microorganisms to transform micropollutants differed depending on
187 whether they have previous exposures or not. Details on the methods and the results of
188 these two experiments are provided in the Supplemental information.

189 **Micropollutant analysis**

190 For each microcosm batch, ~20 mL of the inoculum was removed from each bottle at
191 0, 2 and 5 days for micropollutant analysis. The four micropollutants BPA, BPS, TCS,
192 and TCC were concentrated and purified by a 12-port Visiprep-DL SPE vacuum
193 manifold (Supelco, Bellefonte, PA, USA) with Oasis HLB cartridges (500 mg, 6 mL;
194 Waters, Millford, MA, USA), and then were measured using LC-MS/MS (ABI
195 3200QTRAP, Framingham, MA, USA), as described in our previous work (Sun et al.
196 2017). Chromatographic separation was performed by using a Kinetex C18 column

197 (100 mm × 4.6 mm, 2.6 μm; Phenomenex, Torrance, CA, USA) from Shimadzu LC
198 system (Shimadzu, Japan).

199 **DNA extraction and 16S rDNA amplicon high-throughput sequencing**

200 The remaining inoculum (~470 mL) of each replicate was filtered onto 0.22-μm
201 polycarbonate filters (Millipore, Ireland), which were stored at −80°C until used for
202 DNA extraction. DNA from the 105 samples was extracted using the FastDNA for soil
203 kit (MP Biomedical, Santa Ana, CA, USA) according to the manufacturer's protocol,
204 except that the beating speed was set to 6.0 m/s for 80 s. The universal primer pair 515F
205 (5'-GTG YCA GCM GCC GCG GTA-3') and 907R (5'-CCG YCA ATT YMT TTR
206 AGT TT-3') was used to amplify the V4-V5 region of the bacterial 16S rRNA genes.
207 The PCR products were purified, mixed in equal amounts, and sequenced on an
208 Illumina MiSeq platform with the 2 × 300 bp paired-end protocol at the Majorbio Bio-
209 Pharm Technology Co., Ltd. (Shanghai, China).

210 **Sequence analysis**

211 The raw sequencing data were processed using the LotuS pipeline [44] with the
212 following criteria: i) average sequence quality > 27, ii) sequence length > 170 bp, iii)
213 no ambiguous bases, iv) no mismatches with the barcodes or primers, and v)
214 homopolymer length < 8 bp. Operational taxonomic units (OTUs) were clustered at a
215 97% similarity using UPARSE [45]. A representative sequence of each OTU was
216 taxonomically quantified using the RDP classifier with the Silva database v132 [46], at
217 a bootstrap cutoff of 80%. All samples were rarified to 36, 000 sequence reads (the
218 smallest library size). The subsampled OTU table contained 1375 OTUs. The α- and β-
219 diversity indices of bacterial communities were calculated from the rarified dataset

220 using QIIME v1.9.1 [47] with the script "core_diversity.py".

221 **Inferring the community growth phase from the16S rRNA gene copy number**

222 The 16S rRNA gene copy number among prokaryotes within a defined period indicates
223 the number of individuals within a community and reflects the growth phase of that
224 community [48]. In this study, the 16S rRNA gene copy number of each OTU found in
225 the samples was estimated using the rrnDB database v5.5 [49] and the weighted mean
226 rRNA gene copy number of all OTUs found in each sample was then calculated as
227 described previously [50]. The abundance-weighted average gene copy number of a
228 community was plotted against the sampling time for each treatment. The similar
229 overarching pattern of the average rRNA gene copy numbers of the bacterial
230 communities in all microcosms over time (Figs. S2A-F) indicated the similar growth
231 trajectories of those communities. In addition, a hierarchical cluster analysis with
232 complete clustering criterion was used to cluster the similarity of average gene copy
233 numbers from all micropollutant-treated microcosms belonging to the same batch type,
234 with an exclusion of the controls (Fig. S2G). Three growth progression phrases across
235 all microcosm batches were identified based on similar average gene copy numbers and
236 the time continuity of the inoculation (Fig. S2G): phase 1 (B1–B2), phase 2 (B3–B4),
237 and phase 3 (B5–B7).

238 **Relative contribution of ecological processes**

239 According to a quantitative ecological framework [51, 52], community assembly can
240 be summarized by five major ecological processes: i) homogenizing selection for a

241 convergent composition between communities, ii) variable selection for a divergent
242 composition between communities, iii) dispersal limitation for a divergent composition
243 between communities due to limited dispersal events, iv) homogenizing dispersal for a
244 convergent composition between communities due to unlimited dispersal, and v)
245 ecological drift (referred to "undominated processes" in [52]). The phylogenetic
246 turnover among the communities was quantified by the weighted β-mean nearest taxon
247 distance (βMNTD). The βNTI was calculated as the difference between the observed
248 βMNTD and the mean of the null distribution (999 randomizations). The criteria
249 determining the fraction of each process were as follows: i) homogenizing and variable
250 selection: higher (> 2) and lower (< −2) βNTI, respectively; ii) dispersal limitation and
251 homogenizing dispersal: non-significant |βNTI| (< 2) but RC_{bray} > 0.95 and < −0.95,
252 respectively. iii) ecological drift: non-significant |βNTI| value (< 2) and non-significant
253 $|RC_{bray}|$ value (< 0.95) [52]. To infer the relative contributions of the different
254 community assembly processes to community turnover over time, for each pair
255 consisting of a micropollutant treatment and its control the βNTI and RC_{bray} values were
256 calculated separately for each of the three growth phrases. By comparing the
257 community assembly process in the treatment vs. the controls, we were able to
258 minimize confounding effects that may have obscured community variation and thus
259 better predict the treatment effect. The same analyses were applied to each pair of
260 communities from the different treatments. The relative contribution was expressed as
261 the portion of pairwise sample assembly that could be attributed to each ecological
262 process.

263 **Ecological grouping of microbial responses to micropollutants**

264 The responses of individual OTUs to micropollutant addition were identified by

265 analyzing the differences in the relative abundance of each OTU during the three
266 growth phases for each treatment, using the 'DESeq2' R package v 1.26.0 [53].
267 Significant values were corrected for multiple tests using the Benjamini-Hochberg
268 correction with an adjusted α value of 0.05. The responses were categorized as: i)
269 sensitive, when relative OTU abundances were significantly higher in phase 1 than in
270 phases 2 and 3; ii) opportunistic, when relative abundances were significantly higher in
271 phase 2 than in phases 1 and 3; iii) tolerant, when relative abundances were significantly
272 higher in phase 3 than in phases 1 and 2. The ecological categories represented a group
273 of bacterial members whose responsiveness to the experimental conditions could be
274 documented collectively. In addition, to visualize the phylogenetic relationships of the
275 OTUs assigned to the ecological groups, representative sequences of those OTUs were
276 used to construct a phylogenetic tree using iTOL v5 [54]. A Spearman correlation test
277 was used to explore the relationships between the OTUs with ecological strategies and
278 the micropollutant removal rates. The *P*-value was corrected using the Benjamini–
279 Hochberg method.

280 **Micropollutant turnover and community analysis**

281 A Kruskal-Wallis test was used to analyze the difference in the removal rates between
282 the single micropollutant treatments (BPA vs. TCS) and between any two
283 micropollutants for the mixture treatments (either MI or MII).

284 Differences in the bacterial communities in all microcosms were explored using
285 non-metric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity
286 metric and by fitting removal rates of micropollutants to the ordination. This allowed
287 us to assess whether the degradation of micropollutants was tied to the microbial

288 succession. A permutational multivariate analysis of variance (PERMANOVA) [55]
289 was conducted to test the effects of micropollutants, inoculation time (as indicated by
290 the microcosm batch), and their interactive effects on the taxonomic composition of the
291 bacterial communities. A pairwise PERMANOVA was performed to test for significant
292 differences in community composition between each treatment pair. The multivariate
293 analyses were performed using the R package 'vegan'[56]. Whether communities with
294 the shared taxa exhibit similar dynamics was assessed in a dissimilarity-overlap
295 analysis [57]. Briefly, the relative abundance of each OTU in each community was
296 calculated to identify sets of OTUs within each pair of communities (i.e., within a
297 treatment over time and between treatments). In the calculation of dissimilarity, only
298 the shared OTUs were considered, and the relative abundance of shared taxa was
299 transformed to add up to 1. The dissimilarity between renormalized vectors X and Y
300 can be calculated using root Jensen–Shannon divergence. Based on the dissimilarity-
301 overlap analysis, 15 pairwise comparisons were obtained in this study. This method and
302 the R script for this analysis were modified from [58].

303 To better understand community turnover over time, we quantified the
304 compositional variation between the micropollutant-treated and non-treated
305 communities (i.e., control) at each time point using the Bray-Curtis dissimilarity metric.
306 The rate of community turnover over time was evaluated with a linear regression model
307 (see the Supplemental methods for details). A Wilcoxon test was then applied to test for
308 significant differences in community turnover between microcosm B1 (day 5) and the

309 other microcosm batches from later time points (B2–B7), using the 'ggsignif' package
310 [59]. The data obtained in this study were visualized using the R package 'ggplot2'.

311 **Results**

312 **Transformation of trace micropollutants**

313 In the microcosms treated with single micropollutant, a higher proportion of BPA (96.84
314 ± 7.90%) than TCS (21.83 ± 24.11%) was removed (Kruskal-Wallis test, $P < 0.05$; Fig.
315 2A) whereas the removal of the two micropollutants from the MI microcosms did not
316 significantly differ ($P > 0.05$) (17.86 ± 15.17% for BPA and 25.11 ± 18.88 % for TCS;
317 Fig. 2B). In the MII microcosms, the percentage of removed BPA (65.83 ± 6.54%) was
318 highest ($P < 0.05$), followed by the TCS (13.76 ± 16.76%), BPS (5.93 ± 8.97%), and
319 TCC (11.97 ± 14.08%), but the differences were not significant (Fig. 2C).

320 **Community succession following micropollutant exposure**

321 The bacterial communities in the microcosms became taxonomically distinct both over
322 time and by micropollutant type (Fig. S3). The variation in the community composition
323 among all microcosm batches correlated with differences in the removal rates of BPA,
324 with higher association in its treatments alone, but was also correlated with TCA, with
325 higher association in TCA single and mixed treatments (Fig. S3). The inoculation time
326 (PERMANOVA, $R^2 = 0.149$), micropollutant addition ($R^2 = 0.11$), and their interaction
327 ($R^2 = 0.055$) significantly influenced the community composition (all $P < 0.001$) (Table
328 S1). Irrespective of the inoculation time, the composition of the TCS, MI, and MII
329 communities differed significantly from that of the controls (pairwise PERMANOVA,
330 $P < 0.01$; Table S1) but not from each other. Conversely, the BPA communities
331 significantly differed from the other three treatments but not from the controls.

332 The dissimilarity in the micropollutant-treated vs. control communities
333 significantly increased over time, as each inoculation was replenished with
334 micropollutants (linear regression, all $P < 0.001$, Fig. S4). Community dissimilarity was
335 more pronounced at late (B6 or B7) than at early (B1 or B2) microcosm batches.
336 Furthermore, the communities of the first inoculation (B1) impacted the response of
337 some communities inoculated at later time points (Fig. 3A). The impact of the B1
338 community composition on later communities persisted across B2–B6 in the BPA-
339 treated microcosms and B2–B5 in the MII-treated microcosms, as evidenced by their
340 community resemblances (Wilcoxon test, $P > 0.05$; Fig. 3A). In both BPA and MII
341 communities, the correlation between dissimilarity and overlap (i.e., the number of
342 shared OTUs between the community pair) was negative ($P < 0.05$ Fig. 3B). This result
343 was not unexpected since the dispersal of microbial cells from one condition to another
344 similar environment may drive community dynamics towards convergence [60, 61].
345 However, in the TCS and MI treatments, an impact of the initial communities on later
346 ones occurred only in B2, which is supported by the fact that the B3–B7 communities
347 were more dissimilar to B1 ($P < 0.05$; Fig. 3A). The negative correlation of the
348 dissimilarity and overlap analysis was nonsignificant for the TCS and MI communities
349 such that the high overlap did not result in a low dissimilarity (Fig. 3B). This suggests
350 that there were differences in the dynamics of the shared taxa in those TCS and MI
351 communities over time compared to the communities in the other treatments.

352 Analyses of each pair of communities from the different treatments did not reveal
353 a significant correlation between dissimilarity and overlap in the community pairs of
354 BPA vs. TCS, BPA vs. MI, and TCS vs. MII (Wilcoxon test, $P > 0.05$, Fig. 4). However,
355 similar population dynamics were determined for the communities assembled in the
356 BPA vs. MII and TCS vs. MI pairs, as indicated by the significant negative correlation

357 between high overlap and low dissimilarity ($P < 0.05$, Fig. 4).

358 **OTUs with distinct responses to micropollutant addition**

359 In all treatments, a greater number of sensitive OTUs (41–64), a moderate number of
360 tolerant OTUs (6–15), and fewer opportunistic OTUs (0–3) were identified across
361 phases 1 (B1−B2), 2 (B3−B4), and 3(B5−B7) (Table 1 and Fig. 5). Regardless of the
362 micropollutant type, members of Actinobacteria, Planctomycetes, and Verrucomicrobia
363 primarily consisted of sensitive OTUs (Table 1; Fig. 5). Those of Alphaproteobacteria,
364 Gammaproteobacteria and Bacteroidetes contained more sensitive than tolerant OTUs.
365 The majority of the tolerant OTUs were unique to one treatment, with only two OTUs
366 tolerating micropollutant addition in all treatments. By contrast, many sensitive OTUs
367 (51) were shared between treatments, and at least between two treatments (Table S3).
368 Among the overlapping, 20 OTUs found in all treatments, most were members of
369 Actinobacteria (Table S3; Fig. 5).

370 Interestingly, the relative abundances of 17 of the 64 sensitive OTUs in the BPA
371 microcosms correlated negatively with the removal rate of BPA (Spearman correlation,
372 $P < 0.01$, Table S2), whereas for the two tolerant OTUs a positive correlation between
373 the two parameters was determined (Table S2). Among the 47 sensitive OTUs in the
374 TCS microcosms, 28 correlated positively with the removal rate of TCS (Table S2).

375 **Partition of ecological processes on community variation**

376 Overall, homogenizing selection played a minor role in influencing community
377 variation across phases for each community pair (micropollutant-treated and control),
378 as it accounted for less than 8.33% of the ecological processes (Fig. 6). Instead, variable
379 selection (13.89–61.73%) and ecological drift (30.86–80.56%) dominated the

380 ecological processes across the three phases. These two processes exhibited
381 compensatory contributions, showing increasing variable selection but decreasing
382 ecological drift along the phases (Fig. 6). In the case of dispersal limitation, its relative
383 proportion was greater at phase 1 than the other phases for all treatments, especially
384 BPA and MII.

385 The relative proportion of ecological processes attributable to dispersal limitation
386 decreased across all three phases and was consistent for all between-treatment pairwise
387 comparisons. The three other processes exhibited a compensatory relationship (Fig. S5).
388 Specifically, variable selection and ecological drift prevailed at phases 2 and 3 and
389 replaced one another in comparisons of BPA and each of the other three treatments.
390 However, in comparisons of TCS vs MI or MII, ecological drift was over-represented
391 in all phases (> 50%), while variable selection accounted for a small fraction of the
392 ecological processes (Fig. S5). The trend for MI vs. MII was similar whereas there was
393 no clear trend for the process of homogenizing selection across any of the phases in all
394 comparisons.

395 **Discussion**

396 In the present study, the effects of persistent micropollutants on microbial community
397 dynamics were experimentally investigated to gain insights into community assembly
398 mechanisms. Our first hypothesis, that differences in the removal rates of BPA vs. TCS
399 in environments exposed to single vs. mixed micropollutants was not supported by the
400 results obtained for the MI and MII treatments. The energy required by the microbial
401 communities to counteract the opposing responses to BPA and TCS in the MI treatment
402 [23, 25] likely accounted for the profound inhibition of BPA degradation (Fig. 2B). In
403 MII, a significantly larger fraction of BPA than of the three other micropollutants was

404 removed, consistent with the synergistic effects of BPS and TCC on the microbially
405 mediated degradation of BPA. We further demonstrated that degradation was a
406 biological rather than a chemical process and that bacterial taxa were responsible for
407 those transformations, which are supported by the chemical stability and negligible
408 transformation of the four micropollutants in the bacteria-free microcosms (Fig. S6).
409 We also found that pre-exposure to micropollutants can enhance the degradation
410 capacity and dissipation rate by microbes later confronted with those or similar
411 chemicals, which is consistent with previous literature [18, 31]. This was supported by
412 the fact that the taxa pre-exposed to low concentrations of BPA and TCS fully degraded
413 higher concentrations of those micropollutants during subsequent exposures and
414 degradation occurred at a faster rate (Fig. S7), but only when BPA and TCS were
415 provided alone and not in combination. Our results therefore revealed that microbial
416 communities can transform each of the four micropollutants surveyed, which is
417 especially true for BPA.

418 The larger proportion of sensitive than tolerant taxa in all treatments suggested that
419 the microbial community was not robust to the persistence of micropollutants in its
420 surroundings. Despite the strong effect of TCS on microbial dynamics in the MI and
421 MII treatments, in the latter BPA was removed at a much higher rate than any of the
422 other micropollutants. It is important to consider that the compositional response to a
423 disturbance is often slower than the functional response [62]. Furthermore, most of the
424 sensitive taxa tended to correlate negatively or positively with the removal of BPA or
425 TCS, respectively (Table S2). Those taxa may have affected micropollutant degradation
426 either directly or indirectly, such as by providing enzymatic repertoires to other
427 community members that enabled degradation, including interspecies interactions [63,
428 64]. Actinobacteria, Alphaproteobacteria, and Bacteroidetes made up the majority of

429 the sensitive taxa; their associations with TCS transformation were previously reported
430 [65, 66].

431 There was less support for the second hypothesis, that communities assembled at
432 the early stage of micropollutant exposure would be compositionally distinct from those
433 that formed at the later stage, when community dynamics converge. Our findings
434 generally corroborated previous studies showing that the persistence of micropollutants
435 even at low concentrations can influence microbial community membership and
436 abundance over time [28, 31, 67]. Another study found that microbial communities in
437 identical habitats share a large number of taxa and exhibit convergent abundance
438 dynamics over time [58]. This was the case for the communities assembled in the BPA
439 and MII treatments, as indicated by the significantly negative dissimilarity-overlap
440 relationships (Fig. 3B). The dynamics of the communities within those environments
441 were driven by a set of shared taxa that interacted similarly with both the respective
442 environments and other community members. However, the less pronounced negative
443 dissimilarity-overlap relationships in the case of the TCS and MI treatments indicated
444 that those communities had not reached equilibrium or were characterized by multiple
445 stable states [57]. Within those treatments, the microbial taxa shared among different
446 inoculation batches interacted differently with the same environment over time. Hence,
447 microbial communities assembled in identical habitats can perceive those habitats
448 differently such that their feedback on the surroundings and other, coexisting members
449 will differ as well, as noted elsewhere [58, 68].

450 The third hypothesis, that the dynamics of microbial communities exposed to
451 mixed micropollutants would resemble those of the single micropollutant treatments,
452 was supported in the case of TCS vs. MI and MII. Community dynamics in those
453 treatments showed a consistent negative dissimilarity-overlap correlation, suggesting

454 that TCS overpowered the other micropollutants in the mixed treatments. This
455 explanation was supported by the significant correlation between the changes in 16S
456 rRNA gene copy numbers (Fig. S2) and the resemblance of the communities in the TCS,
457 MI, or MII treatments (Fig. S3).

458 Despite evidence of regularity in the community dynamics across treatments, as
459 revealed by the dissimilarity-overlap analysis, there was a gradual transition from
460 stochastic to deterministic processes towards the later stage of micropollutant exposure.
461 Thus, in all treatments ecological drift played a major role during phases 1 and 2 of
462 community growth progression (Fig. 6). This can be explained by the strong ecological
463 drift and thus the random changes in abundances within microbial communities
464 confined to microcosms, where dispersal is limited and locally dormant cells are
465 awakened to colonize empty niches in the environments upon disturbances [37, 69, 70].
466 However, the increasing contribution of variable selection to community assembly over
467 time suggested that the high phylogenetic turnover of the communities was driven by
468 heterogenous abiotic and biotic conditions [52, 71]. The increasing effects of the
469 micropollutants on the communities over time led to higher species selection and an
470 elevated impact on the interactions among those species within the community, such
471 that the drivers of community assembly no longer resembled those of the control and
472 resulted in a high rate of community turnover (Fig. 3A; Fig. S4). Our findings suggest
473 that the repeated introduction of a micropollutant enhances succession in microbial
474 communities [72].

475 We also considered the potential role of priority effects on community assembly,
476 independent of the ecological process model used in our study. Priority effects emerge
477 when early colonizers exhaust or modify available resources, thus influencing the
478 establishment success of later colonizers [73]. A strong priority effect in the case of
479 TCS and MI was evidenced by the significant difference in the composition of the
480 initially inoculated vs. any of the later inoculated communities (Fig. 3A). Hence, once
481 they were re-inoculated, taxa that had thrived in the B1 batch modified the
482 environments of later batches, such that some taxa (presumably activated from a seed
483 bank, given that no source of new taxa was externally introduced into the systems) were
484 less or more favorable in later batches. However, for the BPA and MII treatments, the
485 effects occurred at later stage of the press disturbance, suggesting the increasing
486 strength of priority effects with increasing establishment time, as noted elsewhere [74].
487 Overall, although our microcosms represent an ideal conditions which is removed
488 from *in situ* conditions, our experimental findings are valuable in at least three aspects,
489 that is : i) the prediction of microbial responses to persistent micropollutants, ii) the
490 harnessing of ecological principles and microbiomes in pollution management [75, 76,
491 77], and (iii) the development of a conceptual model to be used to predict when and
492 how stochastic and deterministic processes contribute to community assembly after the
493 exposure of persistent micropollutants. Our results suggest that strong ecological drift
494 drives random community changes at the early stage of the exposure. Prolonged
495 ecological drift, variable selection resulting from the effects of micropollutants, and the
496 abiotic and biotic conditions experienced differently by different microbes then

497 determine community succession at the mid-stage of the exposure. Additionally, the
498 high environmental heterogeneity together with the low densities of the communities
499 permitted the emergence of priority effects, in which the early establishment of taxa in
500 micropollutant-containing environments allowed a faster community succession. This
501 was supported by the decrease in the average gene copy number at the intermediate
502 phase (Fig. S2), when community assembly was driven by various ecological drivers
503 [78]. Finally, strong variable selection accompanied by increased priority effects likely
504 set the communities on distinct trajectories, with multiple equilibria at the late stage of
505 the exposure. In fact, more advanced community succession and multiple equilibria
506 characterized the end of the press disturbance, and both played a larger role in the TCS
507 and MII (and to a lesser extent in the BPA) communities than in the MI community
508 (Fig. 7).

509 In summary, our experimental design and ecological approaches showed that, while
510 microbial communities are generally vulnerable to micropollutant persistence, pre-
511 exposure to micropollutants may enhance the microbial degradation capacity. This
512 work can aid in microbe-pollution management by providing insights into
513 micropollutant removal as well as microbial assembly mechanisms and dynamics.
514 From the perspectives of microbiome engineering, this study demonstrates the
515 importance of the microbiome's preexposure history in the design of long-term
516 experiments that include artificial selection to direct the evolution [76, 79] of a target
517 microbiome highly optimized for pollutant removal in an aquatic environment.

518 **Data availability**

519 The sequencing data were deposited in the NCBI SRA database with the Bioproject
520 number PRJNA497759. Our R scripts for statistics, data visualization and computing
521 notes are available on a zenodo archive (https://doi.org/10.5281/zenodo.5242686)
522 of the Github repository (https://github.com/AnyiHu/Impacts-of-micropollutants-on-
523 freshwater-microbiome).

524 **Acknowledgements**

525 We thank Dr. Linwei Wu for her assistance in calculating the copy number of 16S
526 rRNA genes, and Dr. Hongjie Wang for his assistance in the molecular experiments.
527 This work was supported by the National Natural Science Foundation of China
528 (31870475, U1805244 and 31470539), the International Partnership Program of
529 Chinese Academy of Sciences (132C35KYSB20200012), and the Natural Science
530 Foundation of Fujian, China (2019I0030).

531 **Author contributions**

532 AH and CPY conceived and designed the study. LS and TL performed the experiments
533 and measurement analyses. YL and QS provided the assistance for the LC-MS/MS
534 analysis. DIS, LS, JW and AH performed the data analyses and wrote the manuscript.
535 All authors discussed the results and commented on the manuscript.

536 **Competing Interests**

537 The authors declare no competing interests.

References

1. Schwarzenbach RP, Escher BI, Fenner K, Hofstetter TB, Johnson CA, von Gunten U, et al. The Challenge of Micropollutants in Aquatic Systems. Science. 2006; 313:1072–1077.
2. Deblonde T, Cossu-Leguille C, Hartemann P. Emerging pollutants in wastewater: A review of the literature. Int J Hyg Envir Heal. 2011; 214:442–448.
3. Luo YL, Guo WS, Ngo HH, Nghiem LD, Hai FI, Zhang J, et al. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci Total Environ. 2014; 473:619–641.
4. Wang, Z.; Zhang, X. H.; Huang, Y.; Wang, H., Comprehensive evaluation of pharmaceuticals and personal care products (PPCPs) in typical highly urbanized regions across China. Environ Pollut. 2015; 204:223–232.
5. Eggen RIL, Hollender J, Joss A, Schärer M, Stamm C. Reducing the discharge of micropollutants in the aquatic environment: The benefits of upgrading wastewater treatment plants. Environ Sci Technol. 2014; 48:7683–7689.
6. Vila-costa M. Anthropogenic dissolved organic carbon and marine microbiomes. ISME J. 2020; 14:2646–2648.
7. da Silva GCX, Medeiros de Abreu CH, Ward ND, Belúcio LP, Brito DC, Cunha HFA, et al. Environmental Impacts of Dam Reservoir Filling in the East Amazon. Front Water. 2020; 2:11.
8. Kuroda K, Murakami M, Oguma K, Muramatsu Y, Takada H, Takizawa S. Assessment of groundwater pollution in Tokyo using PPCPs as sewage markers. Environ Sci Technol. 2012; 46:1455–1464.
9. Yang X, Chen F, Meng F, Xie Y, Chen H, Young K, et al. Occurrence and fate of PPCPs and correlations with water quality parameters in urban riverine waters of the Pearl River Delta, South China. ESPR. 2013; 20:5864–5875.
10. Liu WR, Zhao JL, Liu YS, Chen ZF, Yang YY, Zhang QQ, et al.Biocides in the Yangtze River of China: spatiotemporal distribution, mass load and risk assessment. Environ Pollut. 2015; 200:53–63.
11. Roberts J, Kumar A, Du J, Hepplewhite C, Ellis DJ. Christy AG, et al. Pharmaceuticals and personal care products (PPCPs) in Australia's largest inland sewage treatment plant, and its contribution to a major Australian river during high and low flow. Sci Total Environ. 2016; 541:1625–1637.
12. Rodea-Palomares I, Gonzalez-Pleiter M, Gonzalo S, Rosal R, Leganes F, Sabater S, et al. Hidden drivers of low-dose pharmaceutical pollutant mixtures revealed by the novel GSA-QHTS screening method. Sci Adv. 2016; 2:e1601272.
13. Sun Q, Li Y, Li M, Ashfaq M, Lv M, Wang H, et al. PPCPs in Jiulong River estuary (China): Spatiotemporal distributions, fate, and their use as chemical markers of wastewater. Chemosphere. 2016; 150:596–604.
14. Adeoya-Osiguwa SA, Markoulaki S. Pocock V, Milligan SR, Fraser LR.17β-Estradiol and environmental estrogens significantly affect mammalian sperm function. Hum Reprod. 2003; 18:100–107.
15. Tatarazako N, Ishibashi H. Teshima K, Kishi K, Arizono K. Effects of triclosan on various aquatic organisms. Environ Sci. 2004; 11:133–140.
16. MacLusky NJ, Hajszan T, Leranth C. The environmental estrogen bisphenol a inhibits

estradiol-induced hippocampal synaptogenesis. Environ Health Persp. 2005; 113:675–679.

17. Rosi-Marshall EJ, Kincaid DW, Bechtold HA, Royer TV, Rojas M, Kelly JJ. Pharmaceuticals suppress algal growth and microbial respiration and alter bacterial communities in stream biofilms. Ecol Appl. 2013; 23:583–593.

18. Cerro-Gálvez E, Dachs J, Lundin D, Fernández-Pinos MC, Sebastián M, Vila-Costa M. Responses of Coastal Marine Microbiomes Exposed to Anthropogenic Dissolved Organic Carbon. Environ Sci Technol. 2021; 55:9609–9621.

19. Martinez-Varela A, Cerro-Gálvez E, Auladell A, Sharma S, Moran MA, Kiene RP, et al. Bacterial responses to background organic pollutants in the northeast subarctic Pacific Ocean. (In Press, Environ Microbiol). https://doi.org/10.1111/1462-2920.15646

20. Adyari B, Shen D, Li S, Zhang L, Rashid A, Sun Q, et al. Strong impact of micropollutants on prokaryotic communities at the horizontal but not vertical scales in a subtropical reservoir, China. Sci Total Environ. 2020; 721:137767.

21. Tlili A, Corcoll N, Arrhenius Å, Backhaus T, Hollender J, Creusot N, et al. Tolerance patterns in stream biofilms link complex chemical pollution to ecological impacts. Environ Sci Technol. 2020; 54:10745–10753.

22. Chalew TEA, Halden RU. Environmental Exposure of Aquatic and Terrestrial Biota to Triclosan and Triclocarban. J Am Water Resour Assoc. 2009; 45:4–13.

23. Zhang WW, Yin K, Chen LX. Bacteria-mediated bisphenol A degradation. Appl Microbiol Biotechnol. 2013; 97:5681–5689.

24. Staples CA, Dome PB, Klecka GM, Oblock ST, Harris LR. A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere. 1998; 36:2149-2173.

25. Choi YJ, Lee LS. Aerobic Soil Biodegradation of Bisphenol (BPA) Alternatives Bisphenol S and Bisphenol AF Compared to BPA. Environ Sci Technol. 2017; 51:13698–13704.

26. McMurry LM, Oethinger M, Levy SB. Triclosan targets lipid synthesis. Nature. 1998; 394:531–532.

27. Cabana H, Jiwan JLH, Rozenberg R, Elisashvili V, Penninckx M, Agathos SN, et al. Elimination of endocrine disrupting chemicals nonylphenol and bisphenol A and personal care product ingredient triclosan using enzyme preparation from the white rot fungus Coriolopsis polyzona. Chemosphere. 2007; 67:770–778.

28. Hu A, Ju F, Hou L, Li J, Yang X, Wang H, et al. Strong impact of anthropogenic contamination on the co-occurrence patterns of a riverine microbial community. Environ Microbiol. 2017; 19;4993–5009.

29. Boyd TJ, Smith DC, Apple JK, Hamdan LJ, Osburn CL, Montgomery MT. Evaluating PAH biodegradation relative to total bacterial carbon demand in coastal ecosystems: Are PAHs truly recalcitrant? Microbial Ecology Research Trends. 2008. 1–38 p.

30. Okere UV, Cabrerizo A, Dachs J, Ogbonnaya UO, Jones KC, Semple KT. Effects of pre-exposure on the indigenous biodegradation of ^{14}C-phenanthrene in Antarctic soils. Int Biodeterior Biodegrad. 2017; 125:189–99.

31. Coll C, Bier R, Li Z, Langenheder S, Gorokhova E, Sobek A. Association between Aquatic Micropollutant Dissipation and River Sediment Bacterial Communities. Environ Sci Technol. 2020; 54:14380–14392.

32. Bender EA, Case TJ, Gilpin ME. Perturbation experiments in community ecology : theory and practice. Ecology. 1984; 65:1–13.

33. Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, et al. Fundamentals of microbial community resistance and resilience. Front Microbiol. 2012;3:1–19.
34. Buerger S, Spoering A, Gavrish E, Leslin C, Ling L, Epstein SS. Microbial scout hypothesis, stochastic exit from dormancy, and the nature of slow growers. Appl Environ Microbiol. 2012; 78:3221–3228.
35. Lee S, Sorensen JW, Grady KL, Tobin TC, Shade A. Divergent extremes but convergent recovery of bacterial and archaeal soil communities to an ongoing subterranean coal mine fire. ISME J. 2017; 11:1447–1459.
36. Lennon JT, den Hollander F, Wilke-Berenguer M, Blatch J. Principles of seed banks and the emergence of complexity from dormancy. Nat Commun. 2021; 12:4807.
37. Philippot L, Griffiths BS, Langenheder S. Microbial Community Resilience across Ecosystems and Multiple Disturbances. Microbiol Mol Biol Rev. 2021; 85:e00026-20.
38. Im J, Loffler FE. Fate of Bisphenol A in Terrestrial and Aquatic Environments. Environ Sci Technol. 2016; 50;8403–8416.
39. Sun Q, Li M, Ma C, Chen X, Xie X, Yu CP. Seasonal and spatial variations of PPCP occurrence, removal and mass loading in three wastewater treatment plants located in different urbanization areas in Xiamen, China. Environ Pollut. 2016; 208:371–33
40. Sun Q, Wang Y, Li Y, Ashfaq M, Dai L, Xie X, et al. Fate and mass balance of bisphenol analogues in wastewater treatment plants in Xiamen City, China. Environ Pollution. 2017; 225:542–549.
41. Yamazaki E, Yamashita N, Taniyasu S, Lam J, Lam PKS. Moon HB, et al. Bisphenol A and other bisphenol analogues including BPS and BPF in surface water samples from Japan, China, Korea and India. Ecotoxicol Environ Saf. 2015; 122:565–572.
42. Hu A, Li S, Zhang L, Wang H, Yang J, Luo Z, et al. Prokaryotic footprints in urban water ecosystems: A case study of urban landscape ponds in a coastal city, China. Environ Pollut. 2018; 242:1729–1739.
43. Wang J, Pan F, Soininen J, Heino J, Shen J. Nutrient enrichment modifies temperature-biodiversity relationships in large-scale field experiments. Nat Commun. 2016; 7:13960.
44. Hildebrand F, Tito RY, Voigt AY, Bork P,Raes J. LotuS: an efficient and user-friendly OTU processing pipeline. Microbiome. 2014; 2:30.
45. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013; 10:996–998.
46. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: Improved data processing and web- based tools. Nucleic Acids Res. 2013; 41:D590– D596.
47. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al, QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7:335–336.
48. Roller BRK, Stoddard SF, Schmidt TM. Exploiting rRNA Operon Copy Number to Investigate Bacterial Reproductive Strategies. Nat Microbiol. 2017; 1:16160.
49. Klappenbach JA, Saxman PR, Cole JR, Schmidt TM. rrndb: the ribosomal RNA operon copy number database. Nucleic Acids Res. 2001; 29:181–184.
50. Wu LW, Yang YF, Chen S, Zhao MX, Zhu ZW, Yang SH, et al. Long-term successional dynamics of microbial association networks in anaerobic digestion processes. Water Res.

2016; 104:1–10.
51. Stegen JC, Lin X, Fredrickson JK, Chen X, Kennedy DW, Murray CJ, et al. Quantifying community assembly processes and identifying features that impose them. ISME J. 2013; 7:2069–2079.
52. Stegen JC, Lin X, Fredrickson JK, Konopka AE. Estimating and mapping ecological processes influencing microbial community assembly. Front Microbiol. 2015; 6:370.
53. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014; 15:1–21.
54. Letunic I, Bork P. Interactive tree of life (iTOL) v4: Recent updates and new developments. Nucleic Acids Res. 2019; 47:W256–W259.
55. Anderson MJ. Permutation tests for univariate or multivarite analysis of variance and regression. Can J Fish Aquat Sci. 2001; 58:626–639.
56. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. Vegan: community ecology package. 2018, R package version 1.17-4. Available from: https://cran.r-project.org/package=vegan
57. Bashan A, Gibson TE, Friedman J, Carey VJ, Weiss ST, Hohmann EL, et al. Universality of Human Microbial Dynamics. Nature. 2016; 534:259–262.
58. Vila JCC, Liu YY, Sanchez A. Dissimilarity–Overlap analysis of replicate enrichment communities. ISME J. 2020; 14:2505–2513.
59. Ahlmann-Eltze C, Patil I. ggsignif: Significance Brackets for “ggplot2”. R package version 0.6.1. 2021;(123). Available from: https://cran.r-project.org/package=ggsignif
60. Lindström ES, Östman Ö. The importance of dispersal for bacterial community composition and functioning. PLoS One. 2011; 6:e25883.
61. Shen D, Langenheder S, Jürgens K. Dispersal modifies the diversity and composition of active bacterial communities in response to a salinity Disturbance. Front Microbiol. 2018; 9:2188.
62. Berga M, Székely AJ, Langenheder S. Effects of disturbance intensity and frequency on bacterial community composition and function. PLoS One. 2012; 7(5).
63. Freilich S, Zarecki R, Eilam O, Segal ES, Henry CS, Kupiec M, et al. Competitive and cooperative metabolic interactions in bacterial communities. Nat Commun. 2011; 2:589.
64. Pacheco AR, Moel M, Segrè D. Costless metabolic secretions as drivers of interspecies interactions in microbial ecosystems. Nat Commun. 2019; 10:103.
65. Lee DG, Zhao F, Rezenom YH, Russell DH, Chu KH. Biodegradation of triclosan by a wastewater microorganism. Water Res. 2012; 46:4226–4234.
66. Martin EK, Kemal S, Henrik NR, Alexander E, Natalia C, Henrik JC, et al. Triclosan changes community composition and selects for specific bacterial taxa in marine periphyton biofilms in low nanomolar concentrations. Ecotoxicology. 2020; 29:1083–1094.
67. Wolff D, Krah D, Dötsch A, Ghattas AK, Wick A, Ternes TA. Insights into the variability of microbial community composition and micropollutant degradation in diverse biological wastewater treatment systems. Water Res. 2018; 143:313–324.
68. Bajić D, Vila JCC, Blount ZD, Sánchez A. On the deformability of an empirical fitness landscape by microbial evolution. Proc Natl Acad Sci U S A. 2018;115:11286–11291.
69. Nemergut DR, Schmidt SK, Fukami T, O’Neill SP, Bilinski TM, Stanish LF, et al. Patterns and processes of microbial community assembly. Microbiol Mol Biol Rev. 2013; 77:342–

56.
70. Zhou J, Ning D. Stochastic community assembly : does It matter in microbial ecology. Microbiol Mol Biol Rev. 2017; 81:1–32.
71. Vellend M. Conceptual synthesis in community ecology. Q Rev Biol. 2010; 85:183–206.
72. Jiao S, Zhang Z, Yang F, Lin Y, Chen W, Wei G. Temporal dynamics of microbial communities in microcosms in response to pollutants. Mol Ecol. 2017; 26:923–936.
73. Fukami T. Historical contingency in community assembly: integrating Niches, species pools, and priority effects. Annu Rev Ecol Evol Syst. 2015; 46:1–23.
74. Svoboda P, Lindström ES, Osman OA, Langenheder S. Dispersal timing determines the importance of priority effects in bacterial communities.ISME J.2017; 12:644–646.
75. Bernstein HC. Reconciling Ecological and Engineering Design Principles for Building Microbiomes. mSystems. 2019; 4:1–5.
76. Borchert E, Hammerschmidt K, Hentschel U, Deines P. Enhancing Microbial Pollutant Degradation by Integrating Eco-Evolutionary Principles with Environmental Biotechnology. (In Press). https://doi.org/10.1016/j.tim.2021.03.002
77. Rocca JD, Muscarella ME, Peralta AL, Izabel-Shen D, Simonin M. Guided by microbes: Applying community coalescence principles for predictive microbiome engineering. mSystems. 2021; 6:e00538-21.
78. Nemergut DR, Knelman JE, Ferrenberg S, Bilinski T, Melbourne B, Jiang L, et al. Decreases in average bacterial community rRNA operon copy number during succession. ISME J. 2016; 10:1147–1156.
79. Change C, Vila JCC, Bender M, Li R, Mankowski MC, Bassette M, et al. Engineering complex communities by directed evolution. Nat Ecol Evol. 2021; 5:1011-1023.

738 **Table 1.** Number of OTUs assigned to the ecological categories opportunistic, sensitive,
739 and tolerant in the four micropollutant treatments: 'BPA' microcosms containing
740 bisphenol A, 'TCS' microcosms containing triclosan, 'MI' microcosms containing a
741 mixture of bisphenol A and triclosan, 'MII' microcosms containing a mixture of
742 bisphenol A, triclosan, bisphenol S and triclocarban.

	Number of OTUs in each ecological category		
Treatment	Opportunistic	Sensitive	Tolerant
BPA	1	64	6
TCS	1	47	15
MI	3	51	12
MII	0	41	10

743

744 **Figure 1.** Experimental setup. In microcosm batch 1 (B1), 600 ml of the initial
745 inoculum was added to bottles containing the micropollutants. Starting from the B1, 60
746 mL (10%) of the inoculum was pipetted from each of the replicates and transferred to
747 fresh medium containing 540 mL of 0.22-µm-filtered water and freshly added
748 micropollutants every 5 days. This procedure was carried out in the seven batches of
749 microcosms from batch 1 (B1) through batch 7 (B7). Samples for bacterial community
750 characterization and micropollutant removal were collected prior to the next inoculation.
751 Treatments are color-coded: 'Control' is (without micropollutant addition, grey), 'BPA'
752 (microcosms added with bisphenol A, red), 'TCS' (microcosms added with triclosan,
753 blue), 'MI' (microcosms added with a mixture containing bisphenol A and triclosan,
754 purple), 'MII' (microcosms added with a mixture containing bisphenol A and triclosan,
755 bisphenol S and triclocarban, yellow).

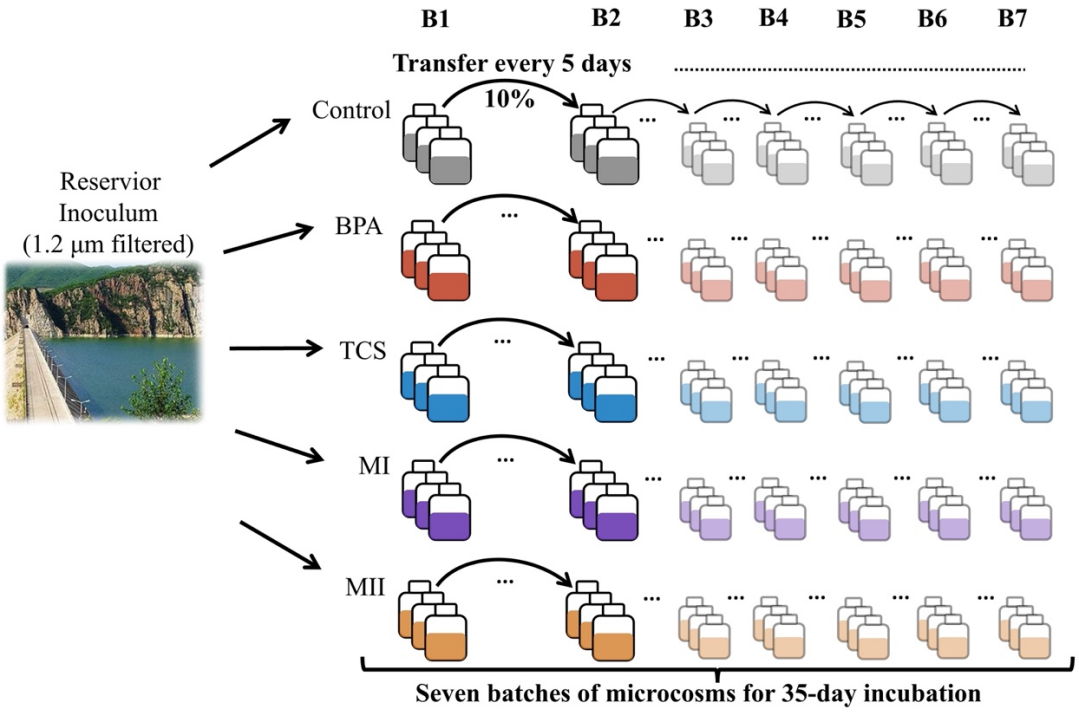


Figure 2. Percent removal of bisphenol A (BPA), triclosan (TCS), bisphenol S (BPS), and triclocarbon (TCC) from the microcosms. (A) Removal in the single-micropollutant (BPA and TCA) treatments; (B) removal in the mixed-micropollutant (MI and MII) treatments. Removal was defined as the decrease in the concentration of the chemical relative to the total concentration within each batch of microcosms (differing by 5-day intervals). The open circles represent the removal percentage obtained from each microcosm batch, and the black circles represent the outliner. Tukey's *post-hoc* test was used to determine the taxonomic level at which the differences occurred. The corresponding homogeneous groups were determined at significance level $P < 0.05$ and are indicated by a and b.

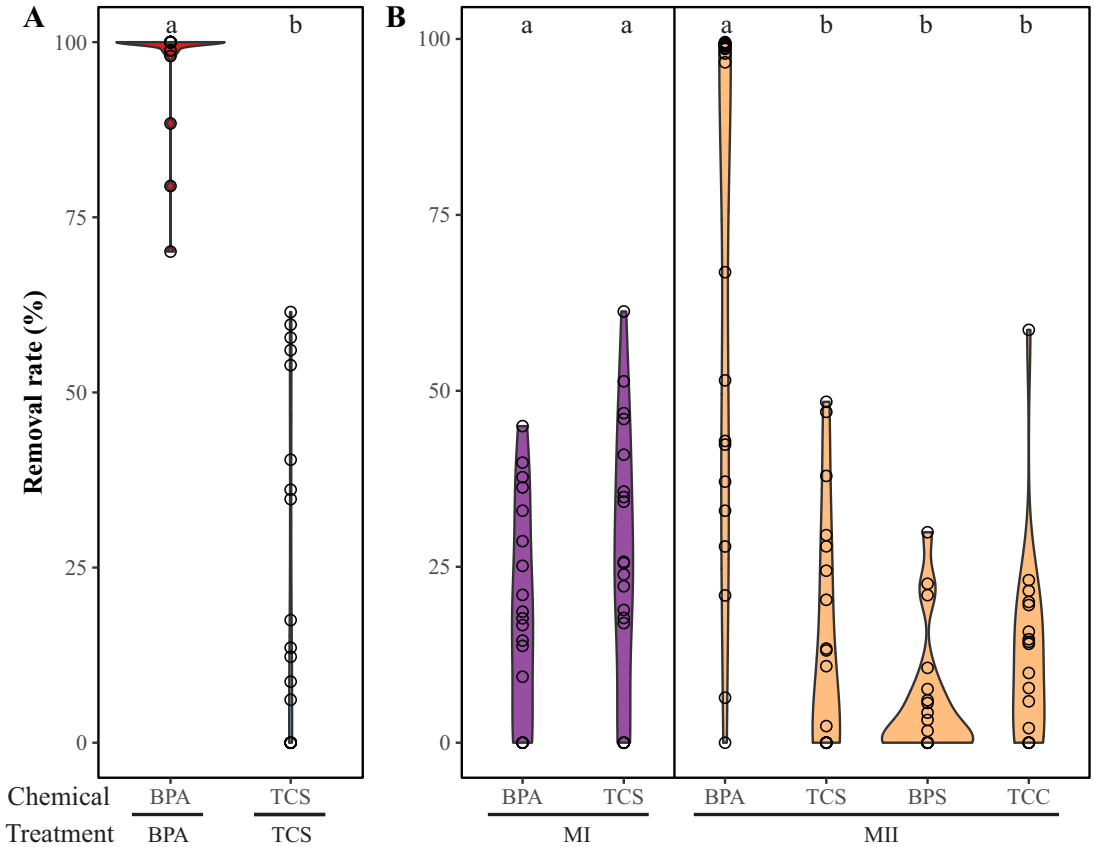


Figure 3. Bacterial community dissimilarity in the micropollutant treatments vs. the controls (i.e., 'Con.') over time (A) and the dynamic regularity within treatments (B). Significant differences in the Bray-Curtis dissimilarity for each pairwise comparison of the treatment and control (B1 microcosms vs. later microcosms in (A) were determined in a Wilcoxon test: ***, $P < 0.001$; **, $0.001 < P < 0.01$; *, $0.01 < P < 0.05$. The dissimilarity-overlap analysis within each treatment is shown in (B); out of total 210 sample pairs, only the sample pairs that had median overlap above cutoff = 0.913 were shown in the plots. The gray lines indicate the linear regression with the slope (m), and the P value (the fraction of bootstrap realizations in which the slope was negative). Gray shaded areas indicate the 95% confidence intervals of the linear regression. Significance level was determined at $P < 0.05$.

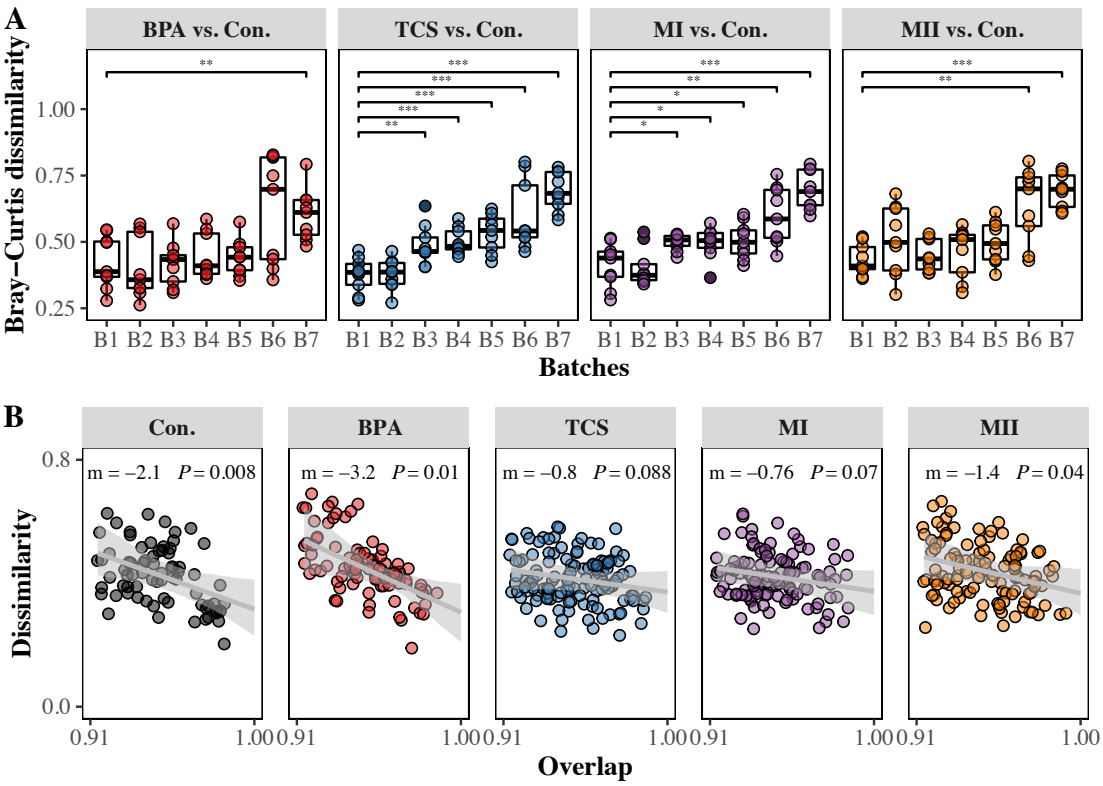
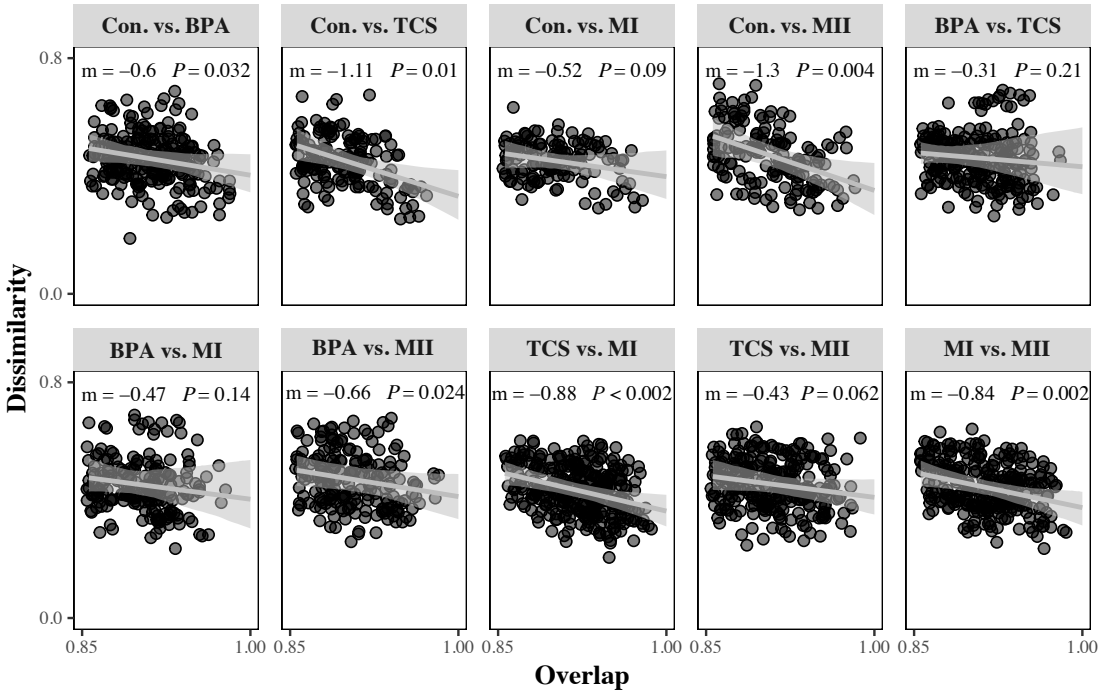
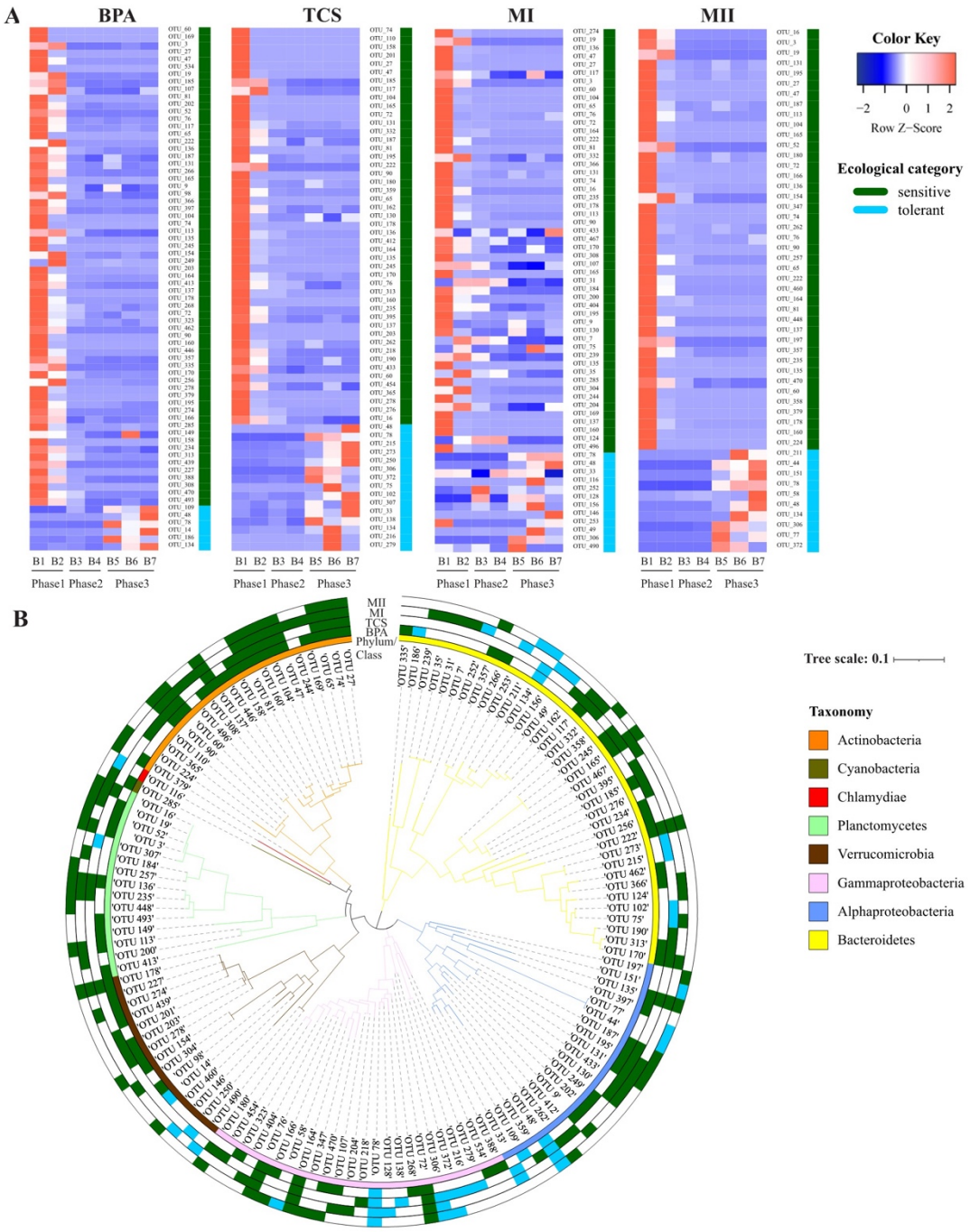


Figure 4. The Dissimilarity-Overlap analysis of pairwise treatments and in the treatments vs. controls (i.e., 'Con.'). Out of total 441 sample pairs, only the dark gray points that had median overlap above cutoff = 0.854 were shown in the plots. The gray lines indicate the linear regression with the slope (m) and the *P* value (the fraction of bootstrap realizations in which the slope was negative). Gray shaded areas indicate the 95% confidence intervals. Significance level was detected at $P < 0.05$.



783 **Figure 5.** Changes in the relative abundances (rows of heatmap) of the OTUs assigned
784 to the ecological categories tolerant and sensitive across the three growth phases
785 (columns of heatmap) (A) and their phylogenetic distribution (B). In (A), color
786 gradients of heatmap represent the relative abundances of individual by column with
787 warm colors (toward red) indicating high abundance and cold colors (toward blue
788 indicating low abundance within the sample. Column labels are batch IDs and the
789 grouping of growth phases, and row labels the OTU IDs. Tolerant and sensitive OTUs
790 were denoted in green and blue colors, respectively. In (B), the innermost circle
791 indicates the taxonomy (phylum/class) of the OTUs. The outer circles indicate the
792 ecological group of OTUs in each treatment group (BPA, TCS, MI, MII from inside to
793 outside, respectively).


A
BPA
TCS
MI
MII
Color Key
−2 0 1 2
Row Z−Score
Ecological category
sensitive
tolerant
B1 B2 B3 B4 B5 B6 B7
Phase1 Phase2 Phase3
B
MII
MI
TCS
BPA
Phylum/
Class
Tree scale: 0.1
Taxonomy
Actinobacteria
Cyanobacteria
Chlamydiae
Planctomycetes
Verrucomicrobia
Gammaproteobacteria
Alphaproteobacteria
Bacteroidetes

794

Figure 6. Relative proportion of the ecological processes (homogeneous selection, variable selection dispersal limitation, and ecological drift) contributing to the communities assembled in the micropollutant treatments (BPA, TCS, MI or MII) vs. the controls (i.e., 'Con.'). Each of the three phases (corresponding to the early, mid, and late stages of the press disturbance) was classified according to the similarity in the average 16S rRNA gene copy numbers as an indicator of community growth progression. Three growth phrases across microcosm batches were: phase 1 (B1–B2), phase 2 (B3–B4), and phase 3 (B5–B7), as shown in Fig. S2G.

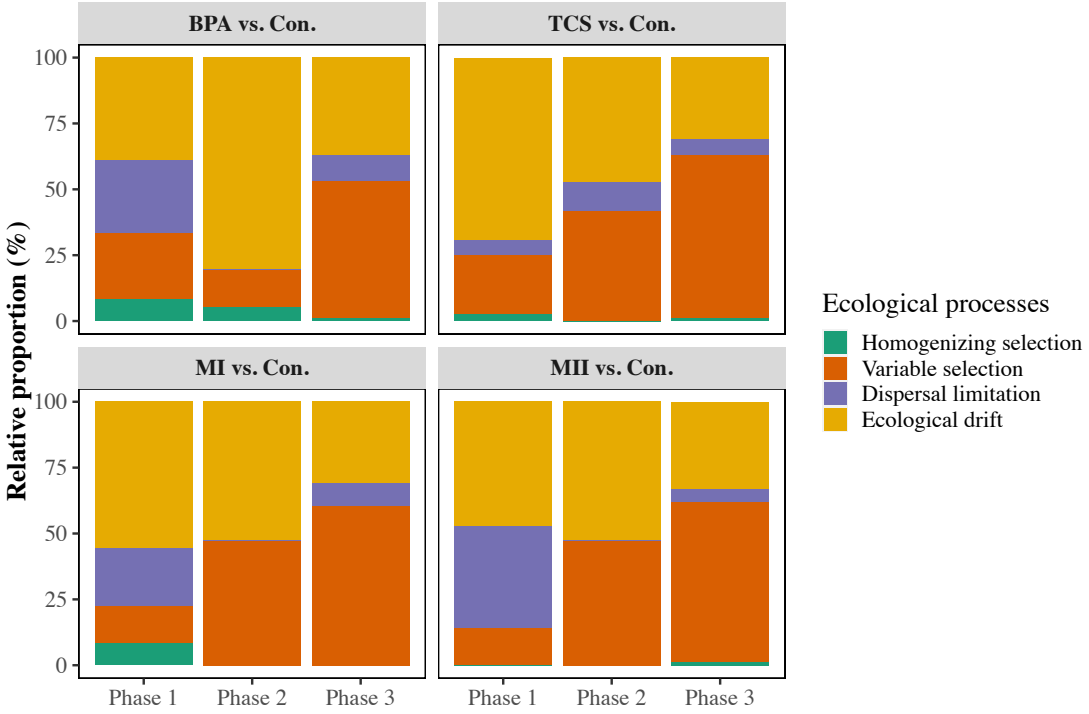
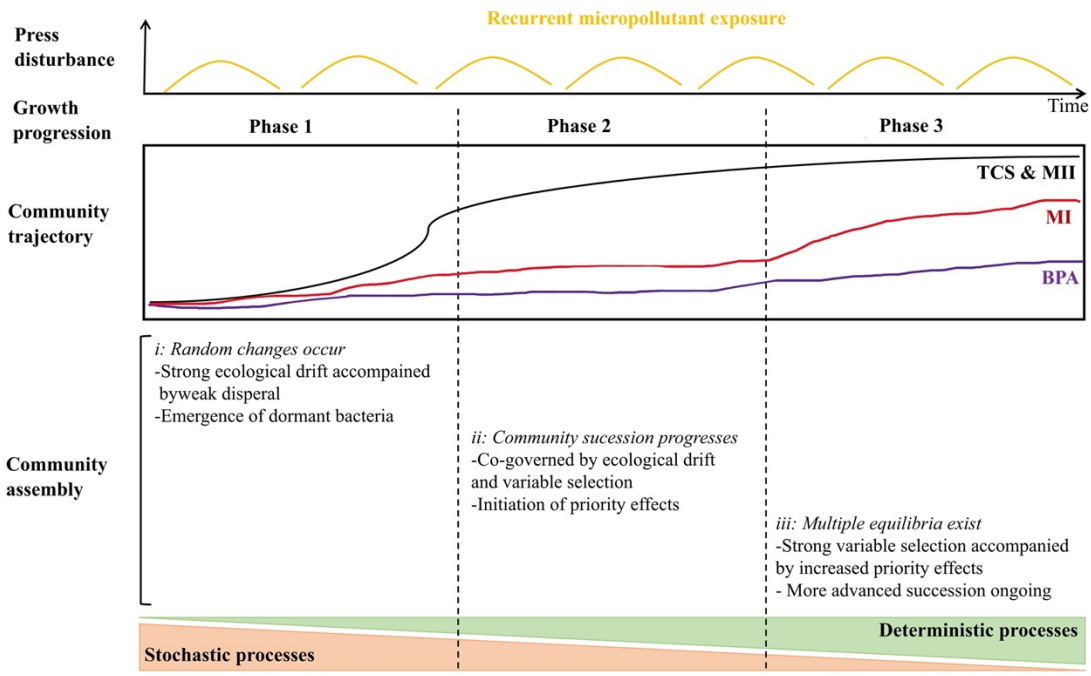



Figure 7 Conceptual model of microbial community assembly processes following a press disturbance (e.g., recurrent micropollutant exposure). Considering the time continuity of the batch inoculation, the three phases of community growth progression (1, 2, and 3) also correspond to the early, mid, and late stages of the 35-day disturbance. This conceptual model illustrates when and how stochastic/deterministic processes contribute to the dynamics of microbial communities repeatedly exposed to micropollutants. Changes in community trajectory over time in the TCS or the MII treatment are indicated by the black line, MI treatment by the red line, and the BPA treatment by the purple line. Overall, stochastic and deterministic processes act in concert during community dynamics: stochastic assembly dominates at the early stage of a press disturbance/community development, and deterministic assembly at the later stage. Major community assembly mechanisms are delineated for each phase. Community succession accelerates at phase 2 such that multiple equilibria occur at phase 3. Note that community succession and multiple equilibria are not exclusive, but community turnover is high towards the end of the disturbance.