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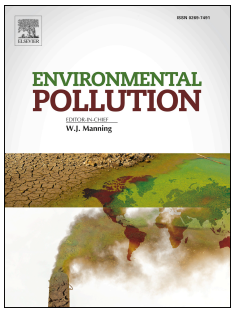

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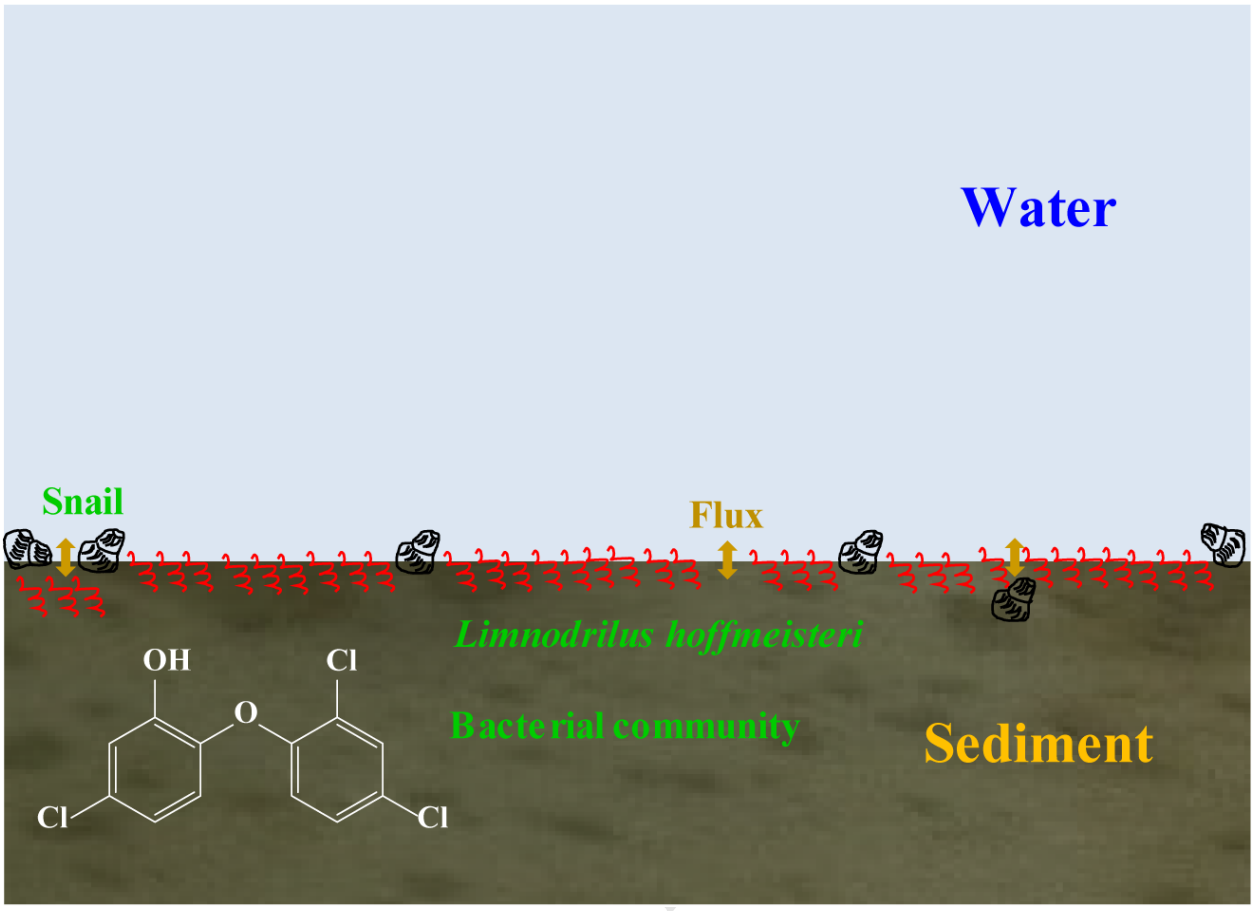

Response of sediment bacterial community to triclosan in subtropical freshwater benthic microcosms

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Abstract The response of sediment bacterial communities in subtropical freshwater benthic microcosms to sediment-associated triclosan (TCS; 28 d exposure) was analyzed using Illumina high-throughput sequencing. This study highlights the interactive effects of TCS and the presence of benthic macroinvertebrates (*Limnodrilus hoffmeisteri* and *Viviparidae bellamya*) on sediment bacterial communities. Our results show that TCS alone significantly altered the taxonomic composition and decreased alpha diversity of sediment bacterial communities at concentrations ≥ 80 µg TCS/g dry weight (dw) sediment (sed). Regarding dominant phyla, TCS significantly reduced the relative abundance of *Bacteroidetes* and *Firmicutes* at these concentrations, whereas the relative abundance of *Chloroflexi* and *Cyanobacteria* increased. In the presence of benthic macroinvertebrates, the sediment bacterial community was affected by 8 µg TCS/g dw sed as well. However, the presence of benthic macroinvertebrates did not cause measurable changes to bacterial community in unspiked (i.e., control) sediment. These results indicate that TCS alone would not alter the sediment bacterial community at environmentally relevant concentrations (up till 8 µg/g dw sed), but may have an effect in combination with the presence of benthic macroinvertebrates. Therefore, we recommend to include benthic macroinvertebrates when assessing the response of sediment bacterial communities during exposure to environmental stress such as organic contaminants.

Keywords Sediment bacterial community; Triclosan; Toxicity; Benthic macroinvertebrates; Microcosm

1. Introduction

Triclosan (2,4,4'-tricloro-2'-hydroxydiphenyl ether, TCS) is an antimicrobial active ingredient used in more than 2000 products, such as soaps, toothpastes, detergents, clothing, toys, carpets, plastics, and paints (FDA, 2016; Halden et al., 2017). Europe banned the use of TCS in human hygiene products in 2015 (ECHA, 2015). Additionally, the U.S. Food and Drug Administration (FDA) has banned the use of TCS in over-the-counter consumer antiseptic wash products (FDA, 2016). However, TCS is still in use in other personal care products and in other parts of the world. Due to the incomplete removal in wastewater treatment plants (WWTPs), TCS has been widely detected in aquatic environments (e.g., Katz et al., 2013; Peng et al., 2017). For example, TCS has been listed among the seven most frequently detected contaminants in streams across the United States (Yueh and Tukey 2016). Moreover, toxicological studies suggest that TCS is toxic to bacteria, algae, crustaceans, fish (especially in early developmental stages), oligochaetes, insects, molluscs and amphibians at environmentally elevated concentrations, with algae as the most sensitive group (Table S1). For example, the lowest toxicity value found for TCS (72 h-EC50 = 0.2 µg/L) is based on the growth inhibition for green alga *Pseudokirchneriella subcapitata* (Yang et al., 2008).

In aquatic environments, TCS is expected to adsorb onto the surface of suspended solids and sediments due to its lipophilic property (log Kow = 4.8) and low aqueous solubility (USEPA, 2010). However, sediment resuspension could occur due to disturbance at the water-sediment interface, e.g. due to the presence of benthic invertebrates (Zhang et al., 2014), which may cause the sediment to become a source of contamination to the overlying water. Indeed, results from the microcosm experiment described in this paper, evaluating the fate and effects of TCS on benthic macroinvertebrates, demonstrated that the presence of benthic macroinvertebrates in the microcosms caused significantly higher TCS concentration in the

overlying water compared to microcosms without macroinvertebrates (Peng et al., 2018). However, as the water was not centrifuged it is not possible to assess if the increased concentration in the overlying water was due to dissolved TCS or TCS associated with re-suspended small-sized sediment particles.

Bacterial communities play important roles in aquatic ecosystems for nutrient re-mineralizing and organic matter decomposition (Burkhardt et al., 2014; Zeng et al., 2014). TCS is toxic to bacteria through inhibiting the enzyme enoyl ACP reductase, an essential component of the bacterial fatty acid biosynthetic pathway (Heath et al., 1998). Since TCS is a broad-spectrum antimicrobial agent and is expected to be retained in the sediment, TCS may negatively affect the sediment bacterial community. Indeed, Drury et al. (2013) added 8 mg/L TCS to the overlying water of an artificial stream and reported reductions in diversity and shifts in taxonomic composition of sediment bacterial communities. However, little is known about the effects of sediment-associated TCS on the sediment bacterial community using more realistic concentrations and including communities, such as benthic macroinvertebrates. Benthic macroinvertebrates, such as Naidid worms (e.g., *Limnodrilus hoffmeisteri*), are broadly distributed in freshwater ecosystems and represent essential links in the aquatic food web (Liu et al., 2014). The bioturbating behaviour (burrowing, particle mixing, irrigation) of benthic macroinvertebrates can influence microbial organic matter mineralization and alter the bacterial community composition (Kristensen, 2000; Zeng et al., 2014). For example, the brittle star *Amphiura filiformis* stimulated the microbial degradation of sediment-associated fluoranthene (Flu) and -pyrene in marine sediments (Granberg et al., 2005; Selck et al., 2005; Granberg and Selck, 2007). In a water-sediment microcosm, the presence of Naidid worms increased the relative abundance of *Betaproteobacteria* and decreased the relative abundance of *Chlorobi* in the surface sediment (Zeng et al., 2014). However, little is known about the

interactive effects of hydrophobic organic contaminants and the presence of benthic macroinvertebrates on the bacterial community structure and abundance in the sediment.

Using microcosms with or without benthic macroinvertebrates, we assessed the effects of TCS and the presence of benthic macroinvertebrates on sediment bacterial community structure. This study is part of a larger project also assessing the fate and effects of sediment-associated TCS on benthic macroinvertebrates (Peng et al., 2018). The objectives of the present study were i) to examine the response of the sediment bacterial community after exposure to TCS for 28 days, and ii) to determine whether there was an interactive effect of TCS and the presence of benthic macroinvertebrates on the sediment bacterial community. To do this, we spiked wet sediment with TCS at concentrations of 0.8, 8, 80 and 240 μg/g dry weight (dw) sediment (sed), and added a sediment-dwelling worm, *Limnodrilus hoffmeisteri*, a snail, *Viviparidae bellamya*, an insect midge larvae, *Orthocladiinae*, and pelagic species (algae and *Daphnia magna*) to half of the microcosms to create a representative subtropical community.

2. Material and methods

2.1. Microcosm experiment

The microcosm experiment was the same as reported by Peng et al. (2018). Briefly, experimental exposures (28 days) were conducted in indoor rectangular glass microcosms (length and width 30 cm; depth 20 cm; sediment depth 4 cm; water depth 14 cm) placed in a temperature (27 ± 1 °C) and light controlled room (light intensity: approximately 2200 lux; photoperiod: 12 h/12 h). In addition to the four TCS treatments (T1-T4: 0.8, 8, 80 and 240 μg/g dw), a water control and an acetone control were also included. All TCS treatments and the acetone control had the same volume of acetone. To examine the interactive effects of

sediment-associated TCS and benthic macroinvertebrates on the sediment bacterial community, 4 replicates of two types of systems were constructed, namely, (i) with introduced organisms (i.e., 40 *Orthocladiinae*, 240 *Limnodrilus hoffmeisteri*, 6 *Viviparidae bellamya*, 30 *Daphnia magna*, and algae) (n = 4 microcosms with organisms), and (ii) without introduced organisms (i.e., only water and sediment) (n = 4 microcosms without organisms). Accordingly, the effects of TCS on the sediment bacterial community can be examined through exposure in microcosms without introduced organisms, and the effects of benthic macroinvertebrates and its interaction with TCS exposure on the sediment bacterial community can be further assessed by comparing the system containing benthic macroinvertebrates with the system not containing. Details on organisms culturing and traits of benthic macroinvertebrates have been reported in Peng et al. (2018). The introduced organism sampling, TCS extraction and analysis, sediment parameters (i.e., ammonia nitrogen (NH_4-N), total nitrogen (TN), organic matter (OM) and total phosphorus (TP)) were analysed following methods detailed in Peng et al. (2018). TCS was analysed by LC-MS/MS using TCS-$^{13}C_{12}$ as internal standard. Additionally, spiking- and recovery tests were performed to account for matrix effects (see detailed description in Peng et al., 2018).

By the end of the experiment (day 28), all worms and snails survived in the controls (i.e., unspiked sediment) and the two lowest TCS treatments (0.8 and 8 µg/g dw sed) while all worms and snails died in the highest TCS treatment (240 µg/g dw sed) and more than 85% worms died in the second highest TCS treatment (80 µg/g dw sed). Thus, in the present study we did not include the two highest TCS treatments with macroinvertebrates as animal mortality inevitably will confound the interpretation of the microbial observations (i.e., decomposition may impact nitrogen levels and microbial community structure).

2.2. DNA extraction and bacteria community analysis

The effects of TCS on the sediment bacterial community structure and composition were evaluated using deep 16S rRNA sequencing. DNA was isolated from sediment samples using PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. The concentration and purity of DNA extractions were monitored by gel electrophoresis in 2% agarose gels. The isolated DNA was stored at -80 ˚C until use. DNA was diluted to 10 ng/μL with sterile water before sequencing. To compensate for heterogeneity, DNA extraction was performed on three replicates of each system-treatment combination (i.e., samples from the 3 out of 4 microcosms).

The bacterial 16S rRNA genes were amplified at V4 and V5 regions with the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCCTTTGAGTTT-3') (Biddle et al., 2008). The PCR mixture was comprised of 15 μL Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of each primer, 10 ng template DNA and 2 μL H_2O. PCR conditions were 98 ˚C for 1 min for initial denaturation, followed by 30 cycles of 10 seconds at 98 ˚C, 30 seconds at 50 ˚C, 30 seconds at 72 ˚C and a final extension for 5 min at 72 ˚C. The 400-450 bp PCR products were selected by gel electrophoresis and were further purified with GeneJET Gel Extraction Kit (Thermo Scientific). With the TruSeq® DNA PCR-Free Sample Preparation Kit sequencing libraries were constructed, added with index codes, and examined using Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. On the Illumina HiSeq 2500 platform, libraries were sequenced using v2 chemistry to generate 250 bp paired-end reads.

The produced paired-end reads were assigned to samples according to their unique barcodes, truncated through cutting off the barcode and primer sequence, and merged using Flash

(Magoč and Salzberg 2011). Merged sequences with low quality score (< 27) and/or with short length (< 250 bp) were removed via filtering using the QIIME software package (V1.7.0, Quantitative Insights Into Microbial Ecology) (Caporaso et al., 2010). Then, chimera sequences were removed from resultant reads using UCHIME algorithm through comparison with the Gold database (http://drive5.com/uchime/uchime_download.html). The resultant high-quality sequences with ≥ 97% similarity were clustered into operational taxonomic units (OTUs) using Uparse software (Edgar, 2013). Each representative sequence of OTU was annotated taxonomic information using RDP classifier algorithm (Version 2.2) (Wang et al., 2007) through comparison with the GreenGene Database using a confidence threshold of 70% (DeSantis et al., 2006).

2.3. Statistical analysis

2.3.1 Bacterial community composition

Bacterial community composition: alpha diversity parameters (i.e., observed OTU number, Chao1, Pielou's J index and Good's coverage estimator) were analysed using in-house Perl scripts in the QIIME software package. Differences in alpha diversity indices and relative abundance of the six most abundant phyla/families between treatments or systems were tested using Social Sciences v23.0 software. The significance level was set to 0.05. The normality of these data or residuals was tested with Shapiro-Wilk test while the variance homogeneity was tested using Levene's test. To examine the effects of TCS, a one-way ANOVA or Kruskal-Wallis test was performed on these data of the system without macroinvertebrates. To examine the effects of macroinvertebrates and its interaction with TCS, a two-way ANOVA (factors: treatment and the presence of benthic macroinvertebrates) was performed on these data of controls, T1 and T2 of both systems. If there was a significant main effect in the ANOVA test, post hoc paired comparisons were performed using Tukey's test.

190

191 2.3.2 Individual effects of TCS and macroinvertebrate presence on sediment bacterial
192 community structure

193 Multivariate Monte Carlo permutation tests were conducted on the OTU table under
194 Redundancy analysis (RDA) option, to examine the individual effects of TCS and
195 macroinvertebrate presence on the sediment bacterial community structure. The relative
196 abundance of OTUs in percentages were Arcsin transformed in the analyses. Differences in
197 the bacterial community structure between the water control and acetone control were tested
198 using controls as explanatory variables and macroinvertebrate presence as covariate and
199 constraining the permutation to the covariate. If the bacterial community structure was
200 significantly different between the water control and acetone control, then the water control
201 was excluded in further analyses. The significance of the effects of TCS on the bacterial
202 community structure was tested using treatments of the system without macroinvertebrates as
203 explanatory variables. The significance of the effects of macroinvertebrate presence on the
204 bacterial community structure was tested using macroinvertebrate presence as explanatory
205 variable and treatments (i.e., controls, T1, and T2) as covariates and constraining the
206 permutation to the covariates.

207

208 2.3.3 Interactive effects of TCS and the presence of macroinvertebrates on bacterial
209 community

210 To examine the interactive effects of TCS and the presence of macroinvertebrates on the
211 sediment bacterial community, a Monte Carlo permutation test was performed on the OTU
212 table under the RDA option using the interaction between treatments (i.e., acetone control, T1,
213 and T2) and systems (i.e., with and without macroinvertebrates) as explanatory variables. All

RDA analyses were performed with CANOCO Software package, version 5 (Ter Braak and Šmilauer, 2012).

Because there was a significant interactive effect of 8 µg TCS/g dw sed and the presence of macroinvertebrates on the sediment bacterial community structure, an independent-samples t test or Mann-Whitney U test was further performed to test the difference in the relative abundance of the dominant families (> 0.5%) of T2 between the system with and without macroinvertebrates. For families showing a significant difference, the same tests were also performed for the acetone control and T1.

3. Results

3.1. Sediment bacterial community composition

A total of 61 phyla were found in all samples, and phyla with relative abundance > 0.5% are shown in Table S2 and Fig. 1A. *Proteobacteria* (30-34%) was the most abundant phylum in all samples, followed by *Firmicutes* (9.7-23%), *Chloroflexi* (9.6-20%), *Actinobacteria* (6.0-10%), *Acidobacteria* (6.5-7.9%) and *Bacteroidetes* (2.3-5.1%) (Table S2). In the system without macroinvertebrates, there was no significant difference in the relative abundance of *Proteobacteria*, *Actinobacteria* or *Acidobacteria* between treatments. T3 (80 µg/g dw) and T4 (240 µg/g dw) had significantly lower relative abundance of *Firmicutes* but significantly higher relative abundance of *Chloroflexi* and *Cyanobacteria* compared to controls, T1 and T2 (one-way ANOVA, $p < 0.05$). T4 also had significantly lower relative abundance of *Bacteroidetes* than the acetone control (one-way ANOVA, $p < 0.05$). When analysing both systems (i.e., controls, T1 and T2), there was no significant difference in the relative abundance of *Proteobacteria*, *Chloroflexi*, *Actinobacteria* or *Acidobacteria* between the system with and without macroinvertebrates (two-way ANOVA, $p > 0.05$). The relative

abundance of *Firmicutes* and *Bacteroidetes* were significantly lower and higher in the system with compared to without macroinvertebrates, respectively (two-way ANOVA, $p < 0.05$). The relative abundance of *Bacteroidetes* was significantly lower in T2 compared to the controls and T1 (two-way ANOVA, $p < 0.05$). Additionally, there was a significant interactive effect of TCS and macroinvertebrate presence on *Bacteroidetes* (two-way ANOVA, $p < 0.05$).

A total of 334 families were found in all samples, and families with relative abundance > 0.5% are provided in Table S3. The six most abundant families were *Anaerolineaceae* (4.6-12%; *Chloroflexi*), *Rhodocyclaceae* (3.7-6.3%; *Proteobacteria*), *Bacillaceae* (2.1-4.8%; *Firmicutes*), *Clostridiaceae 1* (2.3-4.2%; *Proteobacteria*), *Comamonadaceae* (3.3-3.9%; *Proteobacteria*) and *Nitrosomonadaceae* (2.1-2.6%; *Proteobacteria*) (Table S3 and Fig. 1B). In the system without macroinvertebrates, there was no significant difference in the relative abundance of *Comamonadaceae* and *Nitrosomonadaceae* between treatments. T3 and T4 had significantly higher relative abundance of *Anaerolineaceae* and *Rhodocyclaceae*, and a significantly lower relative abundance of *Clostridiaceae 1* compared to controls, T1 and T2 (one-way ANOVA, $p < 0.05$). T4 also had significantly lower relative abundance of *Bacillaceae* than all other treatments (one-way ANOVA, $p < 0.05$). When analysing both systems (i.e., controls, T1 and T2), there was no significant difference in the relative abundance of these six families between the system with and without macroinvertebrates or treatments (two-way ANOVA, $p > 0.05$). Additionally, there was no significant interactive effect of TCS and macroinvertebrate presence on these six families (two-way ANOVA, $p > 0.05$).

3.2. Comparison of alpha diversity

The results of alpha biodiversity of sediment bacterial community are presented in Table 1. The estimated Good's coverage of the datasets was higher than 92% in all treatments and

controls, and the Pielou's J index was in the range of 0.84-0.87 across samples. In the system without macroinvertebrates, the Pielou's J index was similar between treatments, whereas the observed OTU numbers (3838-4345) and Chao1 index (5098-6127) were significantly lower at T3 and T4 than controls, T1 and T2 (one-way ANOVA, $p < 0.05$). When analysing both systems (i.e., controls, T1 and T2), there was no significant difference in the observed OTU numbers, Chao1 index or Pielou's J index between the system with and without macroinvertebrates or treatments (two-way ANOVA, $p > 0.05$).

3.3 Individual effects of TCS and benthic macroinvertebrate presence

There was a significant difference in the sediment bacterial community composition at the OTU level between the water control and acetone control (Monte Carlo permutation test; $p = 0.022$). In the system without macroinvertebrates, there was no significant difference in the bacterial community structure between the acetone control and the two lowest TCS treatments (i.e., T1 and T2). However, the bacterial community structure of the 80 and 240 µg TCS/g dw sed treatments were significantly different from that of the acetone control ($p = 0.008$ and 0.002, respectively).

The results of the Monte Carlo permutation test show that there was no significant difference in the sediment bacterial community composition at the OTU level between the two systems for the data set including only controls ($p = 0.44$) or the data set comprising controls, T1 and T2 ($p = 0.38$).

3.4 Interactive effects of TCS and benthic macroinvertebrate presence

There was a significant interactive effect of 8 µg TCS/g dw sed and macroinvertebrate presence on the bacterial community structure (Monte Carlo permutation test; $p = 0.002$).

289 Accordingly, T2 of the system with macroinvertebrates was placed separately from the
290 remaining groups on the first axis which captured 17% of the total variation in the bacterial
291 community structure (Fig. 2). T1 of the system without macroinvertebrates was separated
292 from the other groups on the second axis which captured 6.7% of the total variation (Fig. 2).
293
294 Comparing the 39 most dominant families (> 0.5%) between the two systems of T2, the
295 relative abundance of *Burkholderiaceae*, *Caulobacteraceae* and *Holophagaceae* were
296 significantly higher in the system with than without macroinvertebrates (independent t tests, p
297 < 0.05; Fig. 3). For the acetone control and T1, there was no significant difference in the
298 relative abundance of *Burkholderiaceae* or *Caulobacteraceae* between the two systems,
299 however the relative abundance of *Holophagaceae* was significantly lower in the system with
300 than without macroinvertebrates ($p < 0.05$; Fig. 3).
301
302 **4. Discussion**
303 We quantified sediment bacterial community structures in microcosms mimicking subtropical
304 shallow freshwater benthic ecosystems exposed to TCS using Illumina high-throughput
305 sequencing. We found that sediment-associated TCS at concentrations ≥ 80 µg/g dw sed alone
306 significantly altered the sediment bacterial community structure and reduced the richness of
307 sediment bacterial communities. In the presence of benthic macroinvertebrates, 8 µg TCS/g
308 dw sed also induced significant alteration to the sediment bacterial community. However,
309 benthic macroinvertebrates at the density used in the current experiment had no effect on the
310 bacterial community in the unspiked sediment. These results demonstrate a significant
311 interactive effect of 8 µg TCS/g dw sed and the presence of benthic macroinvertebrates on the
312 sediment bacterial community.
313

4.1 Individual effects of TCS on the sediment bacterial community

In the system without macroinvertebrates, TCS at concentrations ≥ 80 µg/g dw sed significantly altered the sediment bacterial community structure and reduced the richness of sediment bacterial communities (Table 1). This is comparable to the findings of McNamara et al. (2014), who demonstrated that anaerobic bacterial community structure altered following exposure to TCS at concentrations higher than 50 µg/g in bio-solids. However, 8 µg TCS/g dw sed alone did not significantly influence the richness, evenness or structure of the bacterial community in the sediment after a 28 days exposure under the conditions of the current study (Table 1). Unlike our findings, TCS significantly decreased the bacterial community diversity in the artificial stream sediment after 14 and 34 days exposure at concentration of 5.7 and 8.1 µg/g dw sed (Drury et al., 2013). The discrepancy between the two studies could be attributed to the different spiking approaches: the sediment was directly spiked with TCS in the current study, whereas Drury et al. (2013) added the TCS to the water phase to reach a concentration of 8 mg/L, producing a TCS sediment concentration of 0.0018 µg/g dw sed at the beginning of the experiment. Therefore, there may have been a difference in how strongly TCS was bound to the sediment particles and herewith in the bioavailability of TCS to benthic bacteria between the present study and Drury et al. (2013). However, little information is known regarding the relation between spiking method and bioavailability (both for bacteria and invertebrates) of hydrophobic organic contaminants. Additionally, because the exposure ran for 28 days, bacteria might have shown a short-term response to TCS at 0.8 and 8 µg/g dw followed by a rapid recovery. Indeed, TCS at 1.8 µg/L altered bacterial community and affected algal-cyanobacterial abundance and diversity, but recovery and adaptation of the biofilm community were also observed during an eight weeks exposure period (Lawrence et al., 2015). In parallel with alterations in the sediment bacterial community, TCS at concentrations ≥ 80 µg/g dw sed significantly enhanced sediment NH_4-N levels (Peng et al.,

2018). This is likely to be associated with the effects of TCS on nitrifying and denitrifying taxa of the bacterial community in the sediment. For example, Waller and Kookana (2009) found that TCS at concentration ≥ 50 µg/g dw affected the nitrogen cycle in clay soil. We did not analyse microbial functions, but since this information would assist in explaining such differences, we recommend to analyse microbial functions in combination with microbial community composition in future studies.

Additionally, TCS at concentrations ≥ 80 µg/g dw alone also significantly affected the relative abundance of several dominant bacterial taxa. For example, 80 and 240 µg TCS/g dw sed significantly increased the relative abundance of *Chloroflexi* (Table S2 and Fig. 1A). This could be attributed to the capacity of some bacteria belonging to *Chloroflexi* to dechlorinate organochlorines (Krzmarzick et al. 2012). Likewise, during a 618 days incubation, TCS exposure resulted in a 20-fold increase in the abundance of *Dehalococcoides*-like *Chloroflexi* 16S rRNA genes (determined by qPCR) in anaerobic soil at environmentally relevant concentrations compared with a 5-fold increase in abundance under the absence of TCS (McNamara and Krzmarzick, 2013). Since *Chloroflexi* are important for sediment carbon cycling and organohalide respiration (Hug et al., 2013), they may contribute to the slow dissipation of TCS, an organochlorine, as observed in the microcosms (Peng et al., 2018). Similar to *Chloroflexi*, TCS at these concentrations also increased the relative abundance of *Cyanobacteria* (Table S2 and Fig. 1A), which is in agreement with the findings from previous laboratory studies (Drury et al., 2013; Lawrence et al., 2015). However, during the same period, these treatments inhibited the growth of pelagic algae (Peng et al., 2018). These findings confirmed the conclusion that some *cyanobacteria* are more tolerant to TCS exposure than other algae or are able to adapt (Lawrence et al., 2009; 2015; Drury et al., 2013). Unlike *Chloroflexi* and *Cyanobacteria*, TCS significantly reduced the relative

abundance of *Firmicutes* at 80 and 240 µg/g dw sed (Table S2 and Fig. 1A). Likewise, a previous study found that the relative abundance of *Firmicutes* was negatively correlated with TCS concentration in the effluent of an urban wastewater (Novo et al., 2013). Based on these findings, *Firmicutes* were more sensitive to sediment-associated TCS than *Chloroflexi* and *Cyanobacteria*.

4.2 Individual effects of benthic macroinvertebrates on the sediment bacterial community

The presence of benthic macroinvertebrates alone did not induce measurable changes to the structure of bacterial community in the unspiked sediment, but significantly altered the relative abundance of a few bacteria, such as *Firmicutes* and *Bacteroidetes* (Table S2). This is likely related to biological activities, such as worm bioturbation, that may alter the oxygen concentration in the sediment and across the sediment-water interface (Mermillod-Blondin et al., 2005; Zhang et al., 2010). For example, the bulk-deposit feeder *L. hoffmeisteri* used in our study ingest sediment at depth and defecate at the sediment surface using a conveyor-belt feeding strategy (Reible et al., 1996). Therefore, *L. hoffmeisteri* can transport anoxic sediment to the sediment surface and increase the penetration of oxygen into the sediment column via irrigation of their burrows with oxygen-rich overlying water. Similar stimulating effects of macrofaunal bioturbation on the oxygenation of deeper anoxic sediments has been reported for sediments inhabited by the polychaete *Nereis diversicolor* and the brittle star *A. filiformis* (Granberg et al., 2005; Selck et al., 2005). Additionally, deposit-feeding organisms may use microbes as a food source and thereby depress the abundance of microbes (Tachet et al., 2000). Our results are partly in line with a previous study, which found that the presence of benthic macroinvertebrates (i.e., *Corbicula fluminea*, tubificid worms, and *Chironomidae* larvae) altered the dominant bacterial groups in sediments due to bioturbation by benthic macroinvertebrates (Zeng et al., 2014). Although an earlier study found that the bioturbation

of *L. hoffmeisteri* increased nitrogen release from sediments to the overlying water (Wu et al., 2011), here we did not find similar results. In that study authors used a density of 10000-20000 ind./m^2 of *L. hoffmeisteri* whereas in the present study we used a much lower density (i.e., 2667 ind./m^2). We speculated that the lower density in our study is the course for the lack of finding a significant release of nitrogen from the sediment to the overlying water compared to the microcosms without macroinvertebrates in our study.

4.3 Interactive effects of TCS and presence of benthic macroinvertebrates on the sediment bacterial community

There was a significant interactive effect of 8 µg TCS/g dw sed and macroinvertebrate presence on the sediment bacterial community structure (Fig. 2). This may be associated with the difference in TCS bioavailability due to the disturbance of the water-sediment interface caused by the presence of benthic macroinvertebrates (Cuny et al., 2007; Selck et al., 2005). Due to their feeding strategy which includes ingestion of sediment particles, *L. hoffmeisteri* can be exposed to sediment-associated TCS from the gut, which may result in TCS dissolution and solubilisation in the worm gut (Gilbert et al., 2001; Cuny et al., 2007). Therefore, in addition to potentially increasing bioaccumulation of TCS from the gut into worm tissue, the TCS passage through the worm gut may stimulate the TCS bioavailability to sediment bacterial communities (both in the gut and in the defecated fecal matter). Similar to our findings, a previous study reported that after 45-d incubation the bioturbation by *N. diversicolor* significantly altered the bacterial community structure in oil contaminated coastal sediments, whereas there was no visible changes in the uncontaminated sediment (Cuny et al., 2007).

There was also a significant interactive effect of 8 µg TCS/g dw sed and macroinvertebrate presence on a few dominant families, including *Burkholderiaceae*, *Caulobacteraceae* and *Holophagaceae*, as their relative abundance were significantly higher due to the presence of benthic macroinvertebrates in the 8 µg/g dw treatment but not in the acetone control or 0.8 µg/g dw treatment (Fig. 3). It is possible that these positive interactive effects were related to the involvement of these bacteria in the TCS degradation process. Indeed, *Cupriavidus* (a genus of *Burkholderiaceae*), *Brevundimonas* (a genus of *Caulobacteraceae*), and *Geothrix* (a genus of *Holophagaceae*) are associated with the biodegradation of aromatic compounds (e.g., p-xylene), diclofop-methyl (a chlorinated pesticide) and TCS, respectively (Bacosa et al., 2012; Zhang et al., 2018; Wang et al., 2018). Therefore, *Cupriavidus* and *Brevundimonas* may be capable of degrading TCS as well and thereby stimulate their growth by using TCS as a carbon source. Additionally, since *Cupriavidus* exist in the gut of *Eisenia fetida* (an earthworm) (Ma et al., 2017), bacteria of the above three families may exist in the guts of macroinvertebrates as well and further promote TCS degradation in macroinvertebrates, which could also produce elevated levels of bacteria in the sediment following excretion. Indeed, the presence of benthic macroinvertebrates slightly accelerated TCS dissipation in the system (Peng et al. 2018). However, further studies are required to elucidate such relationships.

In summary, our results indicate that sediment-associated TCS (both in absence and presence of benthic macroinvertebrates) would not impact the sediment bacterial communities at environmentally relevant concentrations (Table S4). However, when TCS concentration reached 80 µg/g dw, TCS alone significantly altered the taxonomic composition and reduced the alpha diversity of sediment bacterial communities. Additionally, benthic macroinvertebrate presence interacted with TCS to increase the TCS activity to the sediment

bacterial community, resulting in a significant alteration to the sediment bacterial community structure when TCS concentration reached 8 µg/g dw sed (~ 5 fold-reported maximum, 1.33 µg/g dw: Zhao et al., 2010). These results suggest the importance of considering the interaction between hydrophobic organic compounds and the presence of benthic macroinvertebrates when assessing effects of sediment-associated chemicals on sediment bacterial communities.

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References

Bacosa, H.P., Suto, K., Inoue, C., 2012. Bacterial community dynamics during the preferential degradation of aromatic hydrocarbons by a microbial consortium. Int. Biodeter. Biodegr. 74, 109-115.

Biddle, J.F., Fitz-Gibbon, S., Schuster, S.C., Brenchley, J.E., House, C.H., 2008. Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. PNAS. 105, 10583-10588.

Burkhardt, B.G., Watkins-Brandt, K.S., Defforey, D., Paytan, A., White, A.E., 2014. Remineralization of phytoplankton-derived organic matter by natural populations of heterotrophic bacteria. Mar. Chem. 163, 1-9.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. methods 7, 335-336.

Cuny, P., Miralles, G., Cornet-Barthaux, V., Acquaviva, M., Stora, G., Grossi, V., Gilbert, F., 2007. Influence of bioturbation by the polychaete Nereis diversicolor on the structure of bacterial communities in oil contaminated coastal sediments. Mar. Pollut. Bull. 54, 452-459.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microb. 72, 5069-5072.

Drury, B., Scott, J., Rosi-Marshall, E.J., Kelly, J.J., 2013. Triclosan exposure increases triclosan resistance and influences taxonomic composition of sediment bacterial communities. Environ. Sci. Technol. 47, 8923-8930.

ECHA (European Chemicals Agency). 2015. Biocidal Products Committee (BPC): Opinion on the Application for Approval of the Active Substance: Triclosan Product-type: 1. ECHA/BPC/066/2015. Helsinki, Finland. https://echa.europa.eu/documents/10162/efc985e4-8802-4ebb-8245-29708747a358 [accessed 10 July 2018].

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. methods 10, 996.

FDA (U.S. Food and Drug Administration). 2016. 21 CFR Part 310 Safety and Effectiveness of Consumer Antiseptics. Topical Antimicrobial Drug Products for Over-the-Counter Human Use. Final Rule. Fed Reg 81:61106-61130.

Gilbert, F., Stora, G., Desrosiers, G., Deflandre, B., Bertrand, J.-C., Poggiale, J.-C., Gagne, J.-P., 2001. Alteration and release of aliphatic compounds by the polychaete Nereis virens (Sars) experimentally fed with hydrocarbons. J. Exp. Mar. Biol. Ecol. 256, 199-213.

Goodman, M., Naiman, D.Q., LaKind, J.S., 2018. Systematic review of the literature on triclosan and health outcomes in humans. Crit. Rev. Toxicol. 48, 1-51.

Granberg, M.E., Hansen, R., Selck, H., 2005. Relative importance of macrofaunal burrows for the microbial mineralization of pyrene in marine sediments: impact of macrofaunal species and organic matter quality. Mar. Ecol. Prog. Ser. 288, 59-74.

Granberg, M.E., Selck, H., 2007. Effects of sediment organic matter quality on bioaccumulation, degradation, and distribution of pyrene in two macrofaunal species and their surrounding sediment. Mar. Environ. Res. 64, 313-335.

Halden, R.U., Lindeman, A.E., Aiello, A.E., Andrews, D., Arnold, W.A., Fair, P., Fuoco, R.E., Geer, L.A., Johnson, P.I., Lohmann, R., 2017. The Florence Statement on Triclosan and Triclocarban. Environ. Health Persp. 125, 064501.

Heath, R.J., Yu, Y.-T., Shapiro, M.A., Olson, E., Rock, C.O., 1998. Broad spectrum antimicrobial biocides target the FabI component of fatty acid synthesis. J. Biol. Chem. 273, 30316-30320.

Hug, L.A., Castelle, C.J., Wrighton, K.C., Thomas, B.C., Sharon, I., Frischkorn, K.R., Williams, K.H., Tringe, S.G., Banfield, J.F., 2013. Community genomic analyses constrain the distribution of metabolic traits across the Chloroflexi phylum and indicate roles in sediment carbon cycling. Microbiome 1, 22.

Katz, D.R., Cantwell, M.G., Sullivan, J.C., Perron, M.M., Burgess, R.M., Ho, K.T., Charpentier, M.A., 2013. Factors regulating the accumulation and spatial distribution of the emerging contaminant triclosan in the sediments of an urbanized estuary: Greenwich Bay, Rhode Island, USA. Sci. Total Environ. 443, 123-133.

Kristensen, E., 2000. Life at Interfaces and Under Extreme Conditions. Springer, pp. 1-24.

Krzmarzick, M.J., Crary, B.B., Harding, J.J., Oyerinde, O.O., Leri, A.C., Myneni, S.C., Novak, P.J., 2012. Natural niche for organohalide-respiring Chloroflexi. Appl. Environ. Microb. 78, 393-401.

Lawrence, J., Zhu, B., Swerhone, G., Roy, J., Wassenaar, L., Topp, E., Korber, D., 2009. Comparative microscale analysis of the effects of triclosan and triclocarban on the structure and function of river biofilm communities. Sci. Total Environ. 407, 3307-3316.

Lawrence, J., Topp, E., Waiser, M., Tumber, V., Roy, J., Swerhone, G., Leavitt, P., Paule, A., Korber, D., 2015. Resilience and recovery: The effect of triclosan exposure timing during development, on the structure and function of river biofilm communities. Aquat. Toxicol. 161, 253-266.

Liu, Y., Zhang, J., Zhao, L., Zhang, X., Xie, S., 2014. Spatial distribution of bacterial communities in high-altitude freshwater wetland sediment. Limnology 15, 249-256.

Ma, L., Xie, Y., Han, Z., Giesy, J.P., Zhang, X., 2017. Responses of earthworms and microbial communities in their guts to Triclosan. Chemosphere 168, 1194-1202.

Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957-2963.

Martínez-Paz, P., Morales, M., Urien, J., Morcillo, G., Martínez-Guitarte, J.L., 2017. Endocrine-related genes are altered by antibacterial agent triclosan in Chironomus riparius aquatic larvae. Ecotoxicol. Environ. Saf. 140, 185-190.

McNamara, P.J., Krzmarzick, M.J., 2013. Triclosan enriches for Dehalococcoides-like Chloroflexi in anaerobic soil at environmentally relevant concentrations. FEMS Microbiol. Lett. 344, 48-52.

McNamara, P.J., LaPara, T.M., Novak, P.J., 2014. The impacts of triclosan on anaerobic community structures, function, and antimicrobial resistance. Environ. Sci. Technol. 48, 7393-7400.

Mermillod-Blondin, F., Nogaro, G., Datry, T., Malard, F., Gibert, J., 2005. Do tubificid worms influence the fate of organic matter and pollutants in stormwater sediments? Environ. Pollut. 134, 57-69.

Reible, D., Popov, V., Valsaraj, K., Thibodeaux, L., Lin, F., Dikshit, M., Todaro, M., Fleeger, J., 1996. Contaminant fluxes from sediment due to tubificid oligochaete bioturbation. Water Res. 30, 704-714.

Peng, F.-J., Diepens, N.J., Pan, C.-G., Bracewell, S.A., Ying, G.-G., Salvito, D., Selck, H., Van den Brink, P.J., 2018. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117-125.

Peng, F.-J., Pan, C.-G., Zhang, M., Zhang, N.-S., Windfeld, R., Salvito, D., Selck, H., Van den Brink, P.J., Ying, G.-G., 2017. Occurrence and ecological risk assessment of emerging organic chemicals in urban rivers: Guangzhou as a case study in China. Sci. Total Environ. 589, 46-55.

Selck, H., Granberg, M.E., Forbes, V.E., 2005. Impact of sediment organic matter quality on the fate and effects of fluoranthene in the infaunal brittle star Amphiura filiformis. Mar. Environ. Res. 59, 19-45.

Suriya, J., Shekar, M.C., Nathani, N.M., Suganya, T., Bharathiraja, S., Krishnan, M., 2017. Assessment of bacterial community composition in response to uranium levels in sediment samples of sacred Cauvery River. Appl. Microbiol. Biot. 101, 831-841.

Tachet H, Richoux P, Bournaud M, Usseglio-Polatera P. Invertebrates déau douce; systematique, biologie, ecologie. CNRS Editions, Paris, France. 2000.

Ter Braak, C., Smilauer, P., 2012. Canoco 5, Windows release (5.00). Software for Mutivariate Data Exploration, Testing, and Summarization. Biometris, Plant Research International, Wageningen.

559 USEPA, 2010. US EPA-Pesticides-Fact Sheet for Triclosan
560 https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-
561 054901_1-Mar-10.pdf#page=1&zoom=auto,-13,798
562 Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid
563 assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microb.
564 73, 5261-5267.
565 Wang, L., Liu, Y., Wang, C., Zhao, X., Mahadeva, G.D., Wu, Y., Ma, J., Zhao, F., 2018.
566 Anoxic biodegradation of triclosan and the removal of its antimicrobial effect in
567 microbial fuel cells. J. Hazard. Mater. 344, 669-678.
568 Wu, F., Chen, J., Yan, Y., Hu, X., Tan, W., Sun, S., 2011. The influence of Limnodrilus
569 hoffmeisteri bioturbation on nitrogen release from sediments in the East Lake Dongting.
570 J. Lak. Sci. 23, 731-737.
571 Yang, L.-H., Ying, G.-G., Su, H.-C., Stauber, J.L., Adams, M.S., Binet, M.T., 2008. Growth
572 inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater
573 microalgae *Pseudokirchneriella subcapitata*. Environ. Toxicol. Chem. 27, 1201-1208.
574 Yueh, M.F., Tukey, R.H., 2016. Triclosan: a widespread environmental toxicant with many
575 biological effects. Annu. Rev. Pharmacol. Toxicol. 56, 251-272.
576 Zeng, J., Zhao, D.-Y., Liu, P., Yu, Z.-B., Huang, R., Wu, Q.L., 2014. Effects of benthic
577 macroinvertebrates bioturbation on the bacterial community composition in lake
578 sediments. Can. J. Microbiol. 60, 517-524.
579 Zhang, L., Gu, X., Fan, C., Shang, J., Shen, Q., Wang, Z., Shen, J., 2010. Impact of different
580 benthic animals on phosphorus dynamics across the sediment-water interface. J. Environ.
581 Sci. 22, 1674-1682.

582 Zhang, L., Shang, J., He, W., You, B., Fan, C., 2014. The role of tubificid worms
583 (Limnodrilus hoffmeisteri) in sediment resuspension: a microcosm study. Ann. Limnol-
584 Int. J. Lim. 50, 253-260.
585 Zhang, H., Yu, T., Li, J., Wang, Y.-R., Wang, G.-L., Li, F., Liu, Y., Xiong, M.-H., Ma, Y.-Q.,
586 2018. Two dcm Gene Clusters Essential for the Degradation of Diclofop-methyl in a
587 Microbial Consortium of Rhodococcus sp. JT-3 and Brevundimonas sp. JT-9. J. Agric.
588 Food. Chem. 66, 12217-12226.
589 Zhao, J.-L,, Ying, G.-G., Liu, Y.-S., Chen, F., Yang, J.-F., Wang, L., 2010. Occurrence and
590 risks of triclosan and triclocarban in the Zhujiang River system, South China: from
591 source to the receiving environment. J. Hazard Mater. 179, 215-222.

592 **Figure captions:**

593 **Fig. 1** The relative abundance (%) of the dominant bacterial phyla (> 0.5%; **A**) and families (>
594 1%; **B**).

595

596 **Fig. 2** RDA biplot showing the interactive effects of TCS and the presence of benthic
597 macroinvertebrates on the sediment bacterial community structure.

598

599 **Fig. 3** The relative abundance (%) of dominant bacterial families showing a significant
600 difference between the system with (Inv+, solid bars) and without (inv-, dashed bars)
601 introduced organisms in the 8 µg/g dw sed treatment.

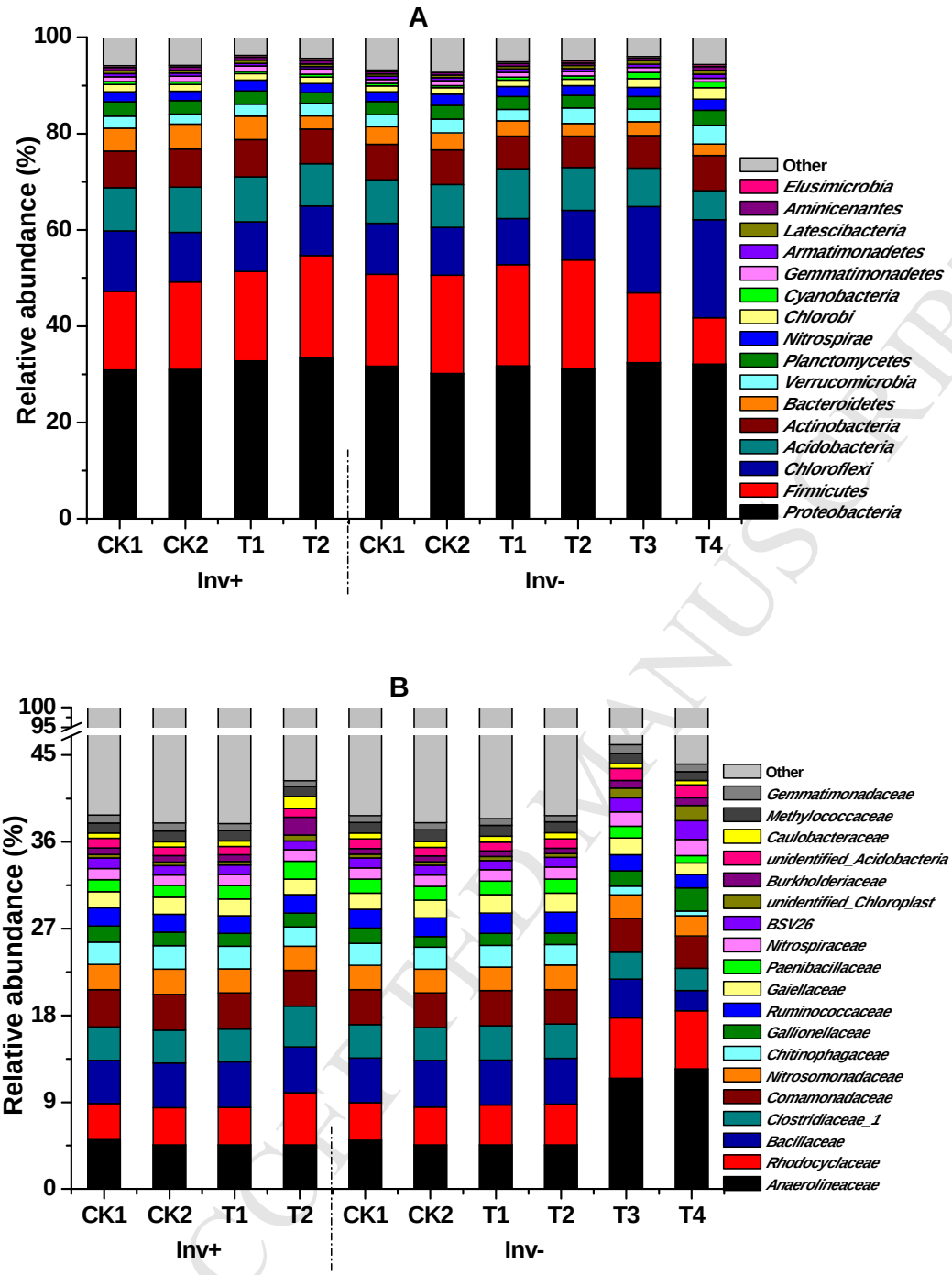


Fig. 1 The relative abundance (%) of the dominant bacterial phyla (> 0.5%; **A**) and families (> 1%; **B**). Inv+ and Inv- represent microcosms with and without benthic macroinvertebrates, respectively. CK1 and CK2 indicate water control and acetone control, respectively. T1-T4 indicate TCS treatments with concentrations of 0.8, 8, 80 and 240 µg/g dw sed, respectively. Three replicates were evaluated for each system-treatment combination.

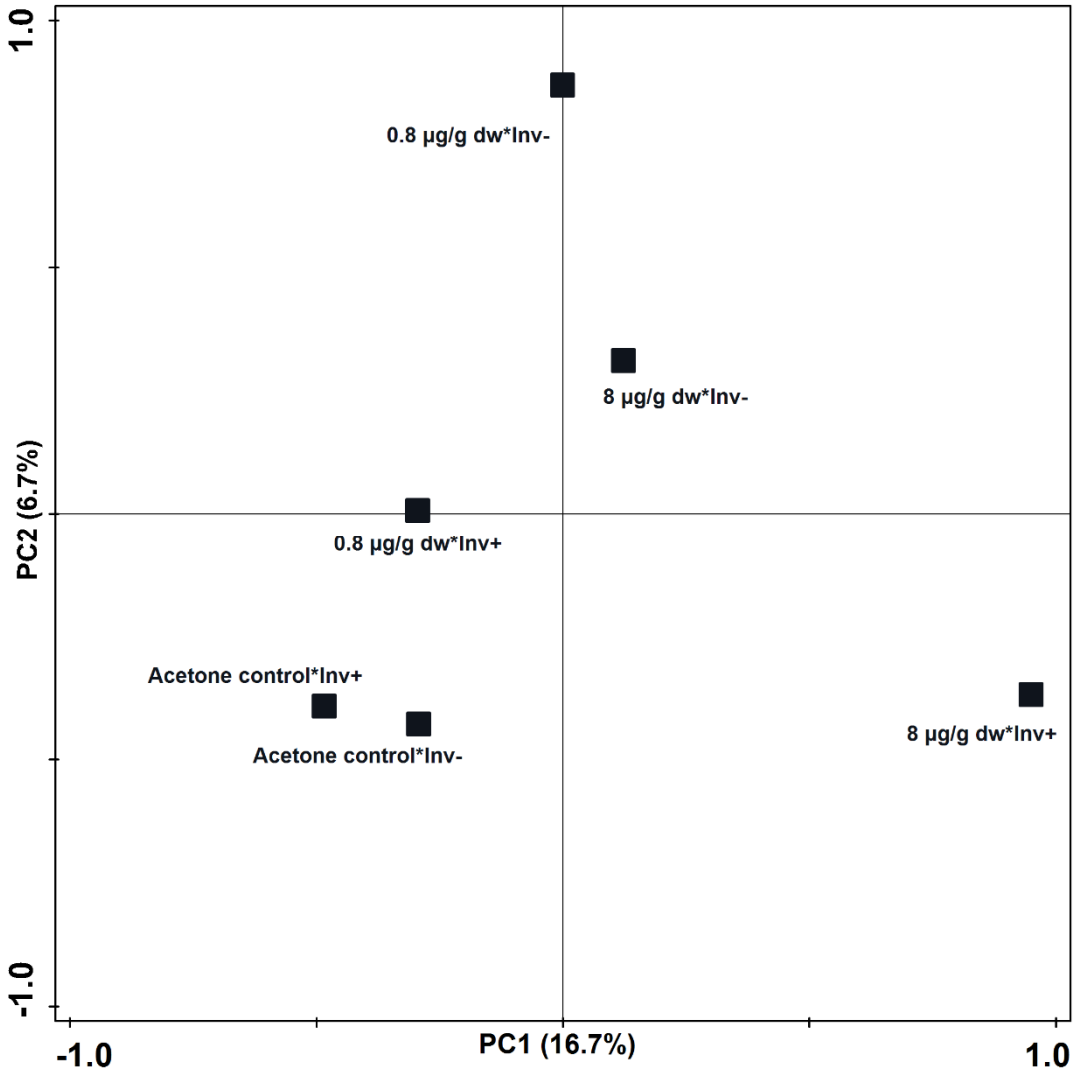
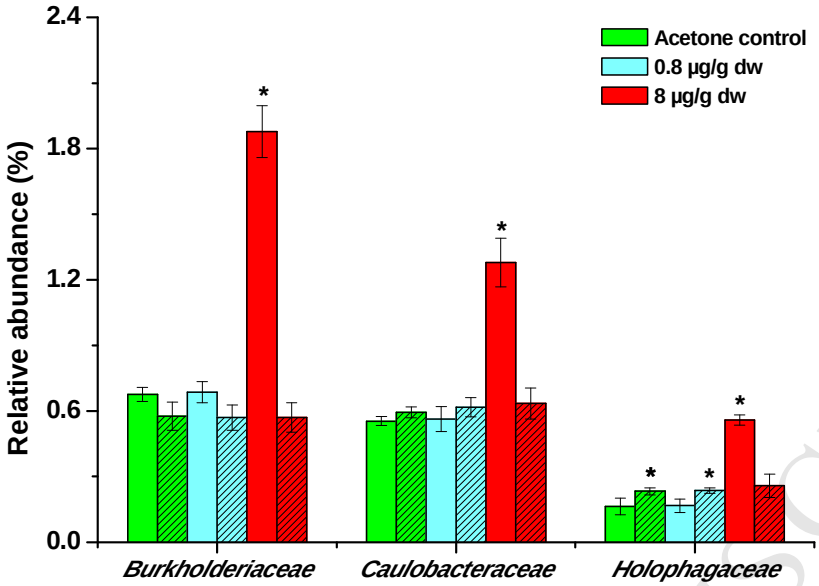


Fig. 2 RDA biplot showing the interactive effects of TCS and the presence of benthic macroinvertebrates on the sediment bacterial community structure. Black square represents environmental variables that explain 37.8% of the total variation in OTU composition. Inv+ and Inv- represent microcosms with and without introduced organisms, respectively. Three replicates were measured for each system-treatment combination. The *p* values were 0.01 and 0.004 for the permutation tests on the first and all axes, respectively.



616

617 **Fig. 3** The relative abundance (%) of dominant bacterial families showing a significant
618 difference between the system with (Inv+, solid bars) and without (inv-, dashed bars)
619 introduced organisms in the 8 µg/g dw sed treatment. Error bar represents standard error of
620 the mean (n = 3). * symbols represent systems that had significantly higher relative
621 abundance of *Burkholderiaceae*, *Caulobacteraceae* or *Holophagaceae* than their
622 corresponding systems ($p < 0.05$).

623 **Table 1** The richness and diversity of sediment bacterial community.

System	Treatment	OTUs	Chao1	Pielou's J	Good's coverage
Inv+	CK1	4274±205	5981±163	0.87±0.00	0.94±0.02
	CK2	4225±176	5967±202	0.86±0.01	0.93±0.01
	T1	4345±146	5960±138	0.87±0.01	0.93±0.01
	T2	3968±278	5774±103	0.84±0.00	0.93±0.01
Inv-	CK1	4185±146	5996±202	0.86±0.01	0.94±0.01
	CK2	4272±178	6085±268	0.87±0.01	0.93±0.01
	T1	4137±111	6127±281	0.86±0.01	0.94±0.02
	T2	4315±87	6006±249	0.86±0.02	0.93±0.01
	T3	3893±97*	5355±83*	0.84±0.01	0.94±0.01
	T4	3838±131*	5098±128*	0.84±0.01	0.94±0.02

624 Three replicates were measured for each system-treatment combination;
625 OTUs, Operational taxonomic units; Chao 1, Chao 1 index; Pielou's J, Pielou's J index;
626 Good's coverage, Good's coverage index;
627 Inv+ and Inv- represent microcosms with and without benthic macroinvertebrates,
628 respectively.
629 CK1 and CK2 indicate water control and acetone control, respectively.
630 T1-T4 indicate treatments with TCS spiked concentrations of 0.8, 8, 80 and 240 μg/g dry
631 weight (dw) sed, respectively.
632 * denotes treatment that is significantly different from the acetone control at the 0.05 level.

Highlights

- 80 µg TCS/g dw alone altered sediment bacterial community composition and structure
- 80 µg TCS/g dw alone decreased alpha diversity of sediment bacterial community
- Benthic macroinvertebrates enhanced TCS activity to sediment bacterial community