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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/g dry weight (dw) sediment (sed). For the dominant phyla, TCS significantly reduced the relative abundances of *Bacteroidetes* and *Firmicutes* at these concentrations, whereas the relative abundances 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 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 the 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 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

64 macroinvertebrates in the microcosms caused significantly higher TCS concentration in the
65 overlying water compared to microcosms without macroinvertebrates (Peng et al., 2018).
66
67 Bacterial communities play important roles in aquatic ecosystems for nutrient re-mineralizing
68 and organic matter decomposition (Burkhardt et al., 2014; Zeng et al., 2014). TCS is toxic to
69 bacteria through inhibiting the enzyme enoyl ACP reductase, an essential component of the
70 bacterial fatty acid biosynthetic pathway (Heath et al., 1998). Since TCS is a broad-spectrum
71 antimicrobial agent and is expected to be retained in the sediment, TCS may negatively affect
72 the sediment bacterial community. Indeed, Drury et al. (2013) added 8 mg/L TCS to the
73 overlying water of an artificial stream and reported reductions in diversity and shifts in
74 taxonomic composition of sediment bacterial communities. However, little is known about the
75 effects of sediment-associated TCS on the sediment bacterial community using more realistic
76 concentrations and including communities, such as benthic macroinvertebrates. Benthic
77 macroinvertebrates, such as Naidid worms (e.g., *Limnodrilus hoffmeisteri*), are broadly
78 distributed in freshwater ecosystems and represent essential links in the aquatic food web (Liu
79 et al., 2014). The bioturbating behaviour (burrowing, particle mixing, irrigation) of benthic
80 macroinvertebrates can influence microbial organic matter mineralization and alter the
81 bacterial community composition (Kristensen, 2000; Zeng et al., 2014). For example, the
82 brittle star *Amphiura filiformis* stimulated the microbial degradation of sediment-associated
83 fluoranthene (Flu) and -pyrene in marine sediments (Granberg et al., 2005; Selck et al., 2005;
84 Granberg and Selck, 2007). In a water-sediment microcosm, the presence of Naidid worms
85 increased the relative abundance of *Betaproteobacteria* and decreased the relative abundance
86 of *Chlorobi* in the surface sediment (Zeng et al., 2014). However, little is known about the
87 interactive effects of hydrophobic organic contaminants and the presence of benthic
88 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 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 a half of the microcosms to create a representative subtropical community. By the end of experiment, there were no deaths of introduced organisms in the unspiked treatments and the 0.8 and 8 μg/g dw sed treatments. However, no macroinvertebrates survived 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), which would confound the interpretation of the microbial observations. In the present study, therefore, we did not include these two treatments of the system with macroinvertebrates.

## 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 $^{\circ}$C) and light controlled room (light intensity: approximately 2200 lux;

photoperiod: 12 h/12 h). In addition to four TCS treatments (T1-T4: 0.8, 8, 80 and 240 μg/g dw), a water control and an acetone control were also included. To examine the interactive effects of sediment-associated TCS and benthic macroinvertebrates on 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), and (ii) without introduced organisms (i.e., only water and sediment). 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 organisms sampling, TCS extraction and analysis, sediment parameters (i.e., ammonia nitrogen ($NH_4$-N), total nitrogen (TN), organic matter (OM) and total phosphorus (TP)) analyses followed methods detailed in Peng et al. (2018).

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 from three replicates of each system-treatment combination.

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 abundances 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 the data set comprising 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.

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

Multivariate Monte Carlo permutation tests were conducted on the OTU table under Redundancy analysis (RDA) option, to examine the individual effects of TCS and macroinvertebrate presence on the sediment bacterial community structure. The relative abundance of OTUs were Arcsin (percentage) transformed in the analyses. Difference in the

bacterial community structure between the water control and acetone control was tested using controls as explanatory variables and macroinvertebrate presence as covariate and constraining the permutation to the covariate. If the bacterial community structure was significantly different between the water control and acetone control, then the water control was excluded in further analyses. The significance of the effects of TCS on the bacterial community structure was tested using treatments of the system without macroinvertebrates as explanatory variables. The significance of the effects of macroinvertebrate presence on the bacterial community structure was tested using macroinvertebrate presence as explanatory variable and treatments (i.e., controls, T1, and T2) as covariates and constraining the permutation to the covariates.

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

To examine the interactive effects of TCS and the presence of macroinvertebrates on the sediment bacterial community, a Monte Carlo permutation test was performed on the OTU table under the RDA option using the interaction between treatments (i.e., acetone control, T1, 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

212 macroinvertebrates. For families showing a significant difference, the same tests were also
213 performed for the acetone control and T1.
214
215 **3. Results**
216 3.1. Sediment bacterial community composition
217 A total of 61 phyla were found in all samples, and phyla with relative abundance > 0.5% are
218 shown in Table S2 and Fig. 1A. *Proteobacteria* (30-34%) was the most abundant phylum in
219 all samples, followed by *Firmicutes* (9.7-23%), *Chloroflexi* (9.6-20%), *Actinobacteria* (6.0-
220 10%), *Acidobacteria* (6.5-7.9%) and *Bacteroidetes* (2.3-5.1%) (Table S2). In the system
221 without macroinvertebrates, there was no significant difference in the relative abundance of
222 *Proteobacteria*, *Actinobacteria* or *Acidobacteria* between treatments. T3 (80 µg/g dw) and T4
223 (240 µg/g dw) had significantly lower relative abundance of *Firmicutes* but significantly
224 higher relative abundance of *Chloroflexi* and *Cyanobacteria* compared to controls, T1 and T2
225 (one-way ANOVA, $p < 0.05$). T4 also had significantly lower relative abundance of
226 *Bacteroidetes* than the acetone control (one-way ANOVA, $p < 0.05$). When analysing the data
227 set comprising controls, T1 and T2 of both systems, there was no significant difference in the
228 relative abundance of *Proteobacteria*, *Chloroflexi*, *Actinobacteria* or *Acidobacteria* between
229 the system with and without macroinvertebrates (two-way ANOVA, $p > 0.05$). The relative
230 abundances of *Firmicutes* and *Bacteroidetes* were significantly lower and higher in the system
231 with compared to without macroinvertebrates, respectively (two-way ANOVA, $p < 0.05$). The
232 relative abundance of *Bacteroidetes* was significantly lower in T2 compared to the controls
233 and T1 (two-way ANOVA, $p < 0.05$). Additionally, there was a significant interactive effect
234 of TCS and macroinvertebrate presence on *Bacteroidetes* (two-way ANOVA, $p < 0.05$).
235

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 the data set comprising controls, T1 and T2 of both systems, 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 the data set comprising controls, T1 and T2 of both systems, 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, $p > 0.05$). However, there was a significant interactive effect of TCS and macroinvertebrate presence on the Pielou's J index (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 the control, T1 and T2 treatments ($p = 0.38$).

### 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; $p = 0.002$). Accordingly, T2 of the system with macroinvertebrates was placed separately from the remaining groups on the first axis which captured 17% of the total variation in the bacterial community structure (Fig. 2). T1 of the system without macroinvertebrates was separated

from other groups on the second axis, which captured 6.7% of the total variation (Fig. 2). There were 52 OTUs showing an $r^2 \geq 0.65$ on both axes, and most of these OTUs had either higher or lower relative abundance in the T2 of the system with macroinvertebrates compared to the other system and treatments.

Comparing the 39 most dominant families (> 0.5%) between the two systems of T2, the relative abundances of *Burkholderiaceae*, *Caulobacteraceae* and *Holophagaceae* were significantly higher in the system with than without macroinvertebrates (independent t tests, $p < 0.05$; Fig. 3). For the acetone control and T1, there was no significant difference in the relative abundance of *Burkholderiaceae* or *Caulobacteraceae* between the two systems, however the relative abundance of *Holophagaceae* was significantly higher in the system without than with macroinvertebrates ($p < 0.05$; Fig. 3).

## 4. Discussion

We quantified sediment bacterial community structures in microcosms mimicking subtropical shallow freshwater benthic ecosystems exposed to TCS using Illumina high-throughput sequencing. We found that sediment-associated TCS at concentrations ≥ 80 µg/g dw sed alone significantly altered the sediment bacterial community structure and reduced the richness of sediment bacterial communities. In the presence of benthic macroinvertebrates, 8 µg TCS/g dw sed also induced significant alteration to the sediment bacterial community. However, benthic macroinvertebrates at the density used in the current experiment had no effect on the bacterial community in the unspiked sediment. These results demonstrate a significant interactive effect of 8 µg TCS/g dw sed and the presence of benthic macroinvertebrates on the sediment bacterial community.

311 4.1 Individual effects of TCS on the sediment bacterial community

312 In the system without macroinvertebrates, TCS at concentrations ≥ 80 µg/g dw sed
313 significantly altered the sediment bacterial community structure and reduced the richness of
314 sediment bacterial communities (Table 1). This is comparable to the findings of McNamara et
315 al. (2014), who demonstrated that anaerobic bacterial community structure altered following
316 exposure to TCS at concentrations higher than 50 µg/g in bio-solids. However, 8 µg TCS/g
317 dw sed alone did not significantly influence the richness, evenness or structure of the bacterial
318 community in the sediment after a 28 days exposure under the conditions of the current study
319 (Table 1). Unlike our findings, TCS significantly decreased the bacterial community diversity
320 in the artificial stream sediment after 14 and 34 days exposure at concentration of 5.7 and 8.1
321 µg/g dw sed (Drury et al., 2013). The discrepancy between the two studies could be attributed
322 to the different spiking approaches: the sediment was directly spiked with TCS in the current
323 study, whereas Drury et al. (2013) added the TCS to the water phase to reach a concentration
324 of 8 mg/L, producing a TCS sediment concentration of 0.0018 µg/g dw sed at the beginning
325 of the experiment. Therefore, there may have been a difference in how strongly TCS was
326 bound to the sediment particles and herewith in the bioavailability of TCS to benthic bacteria
327 between the present study and Drury et al. (2013). However, little information is known
328 regarding the relation between spiking method and bioavailability (both for bacteria and
329 invertebrates) of hydrophobic organic contaminants. Additionally, because the exposure ran
330 for 28 days, bacteria might have shown a short-term response to TCS at 0.8 and 8 µg/g dw
331 followed by a rapid recovery. Indeed, TCS at 1.8 µg/L altered bacterial community and
332 affected algal-cyanobacterial abundance and diversity, but recovery and adaptation of the
333 biofilm community were also observed during a 8 weeks exposure period (Lawrence et al.,
334 2015). In parallel with alterations in the sediment bacterial community, TCS at concentrations
335 ≥ 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. Unfortunately, we did not analyse microbial functions which would assist in explaining such difference. Therefore, 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).

386

387 4.3 Interactive effects of TCS and presence of benthic macroinvertebrates on the sediment
388 bacterial community

389 There was a significant interactive effect of 8 µg TCS/g dw sed and macroinvertebrate
390 presence on the sediment bacterial community structure (Fig. 2). This may be associated with
391 the difference in TCS bioavailability due to the disturbance of the water-sediment interface
392 caused by the presence of benthic macroinvertebrates (Cuny et al., 2007; Selck et al., 2005).
393 Due to their feeding strategy which includes ingestion of sediment particles, *L. hoffmeisteri*
394 can be exposed to sediment-associated TCS from the gut, which may result in TCS
395 dissolution and solubilisation in the worm gut (Gilbert et al., 2001; Cuny et al., 2007).
396 Therefore, in addition to potentially increasing bioaccumulation of TCS from the gut into
397 worm tissue, the TCS passage through the worm gut may stimulate the TCS bioavailability to
398 sediment bacterial communities (both in the gut and in the defecated fecal matter). Similar to
399 our findings, a previous study reported that after a 45-d incubation the bioturbation by *N.*
400 *diversicolor* significantly altered the bacterial community structure in oil contaminated coastal
401 sediments, whereas there was no visible changes in the uncontaminated sediment (Cuny et al.,
402 2007).

403

404 There was also a significant interactive effect of 8 µg TCS/g dw sed and macroinvertebrate
405 presence on a few dominant families, including *Burkholderiaceae*, *Caulobacteraceae* and
406 *Holophagaceae*, as their relative abundances were significantly higher due to the presence of
407 benthic macroinvertebrates in the 8 µg/g dw treatment but not in the acetone control or 0.8
408 µg/g dw treatment (Fig. 3). It is possible that these positive interactive effects were related to
409 the involvement of these bacteria in the TCS degradation process. Indeed, *Cupriavidus* (a
410 genus of *Burkholderiaceae*), *Brevundimonas* (a genus of *Caulobacteraceae*), and *Geothrix* (a

411 genus of *Holophagaceae*) are associated with the biodegradation of aromatic compounds (e.g.
412 p-xylene), diclofop-methyl (a chlorinated pesticide) and TCS, respectively (Bacosa et al.,
413 2012; Zhang et al., 2018; Wang et al., 2018). Therefore, *Cupriavidus* and *Brevundimonas* may
414 be capable of degrading TCS as well and thereby stimulate their growth by using TCS as a
415 carbon source. Additionally, since *Cupriavidus* exist in the gut of *Eisenia fetida* (an
416 earthworm) (Ma et al., 2017), bacteria of the above three families may exist in the guts of
417 macroinvertebrates as well and further promote TCS degradation in macroinvertebrates,
418 which could also produce elevated levels of bacteria in the sediment following excretion.
419 Indeed, the presence of benthic macroinvertebrates slightly accelerated TCS dissipation in the
420 system (Peng et al. 2018). However, further studies are required to elucidate such
421 relationships.
422
423 In summary, our results indicate that sediment-associated TCS (both in absence and presence
424 of benthic macroinvertebrates) would not impact the sediment bacterial communities at
425 environmentally relevant concentrations (Table S4). However, when TCS concentration
426 reached 80 µg/g dw, TCS alone significantly altered the taxonomic composition and reduced
427 the alpha diversity of sediment bacterial communities. Additionally, benthic
428 macroinvertebrate presence interacted with TCS to increase the TCS toxicity to the sediment
429 bacterial community, resulting in a significant alteration to the sediment bacterial community
430 structure when TCS concentration reached 8 µg/g dw sed (~ 5 fold-reported maximum, 1.33
431 µg/g dw: Zhao et al., 2010). These results suggest the importance of considering the
432 interaction between hydrophobic organic compounds and the presence of benthic
433 macroinvertebrates when assessing effects of sediment-associated chemicals on sediment
434 bacterial communities.
435

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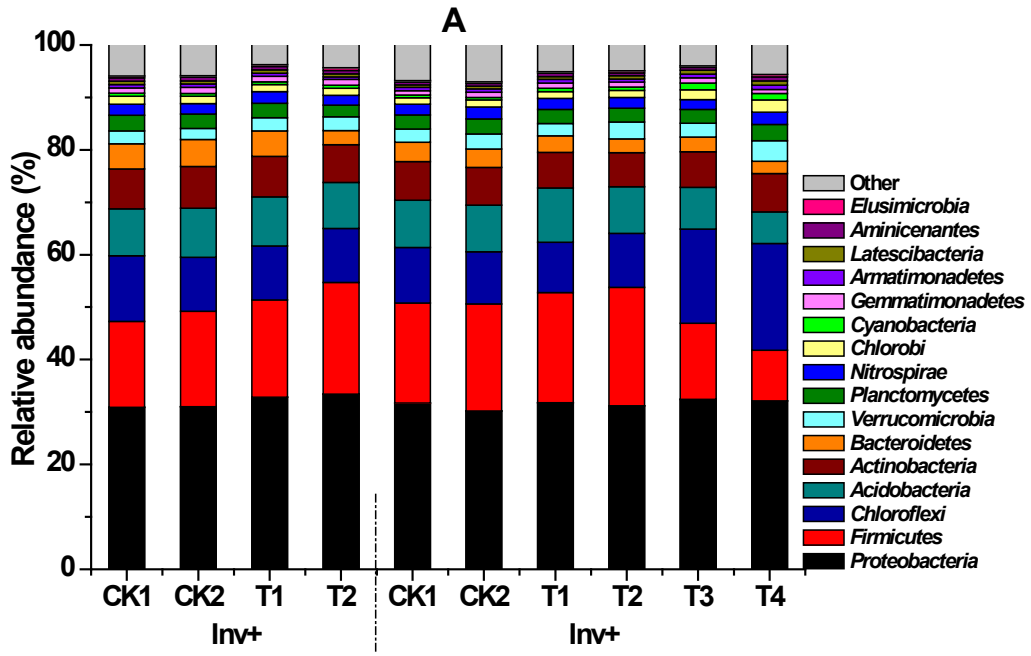
**Figure captions:**

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

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

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

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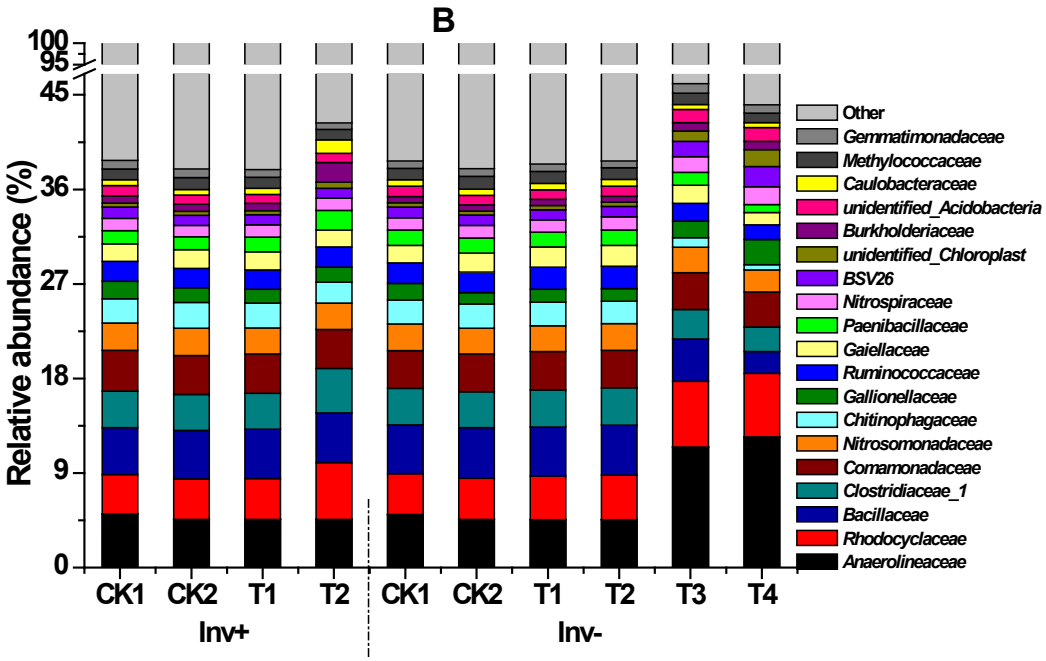



591

592 Fig. 1 The relative abundance (%) of the dominant bacterial phyla (> 0.5%; A) and families (>
593 1%; B). Inv+ and Inv- represent microcosms with and without benthic macroinvertebrates,
594 respectively. CK1 and CK2 indicate water control and acetone control, respectively. T1-T4
595 indicate TCS treatments with concentrations of 0.8, 8, 80 and 240 μg/g dw sed, respectively.
596 Three replicates were evaluated for each system-treatment combination.

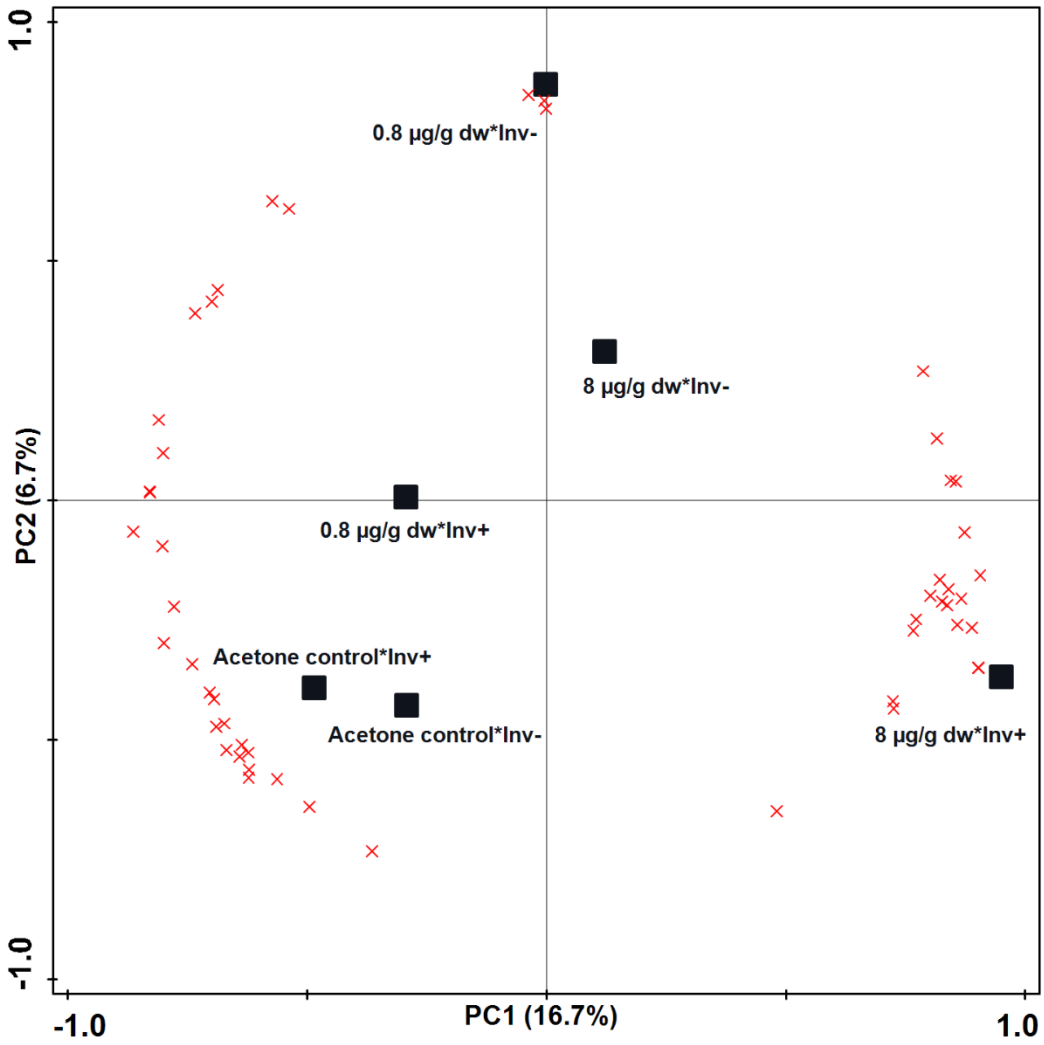
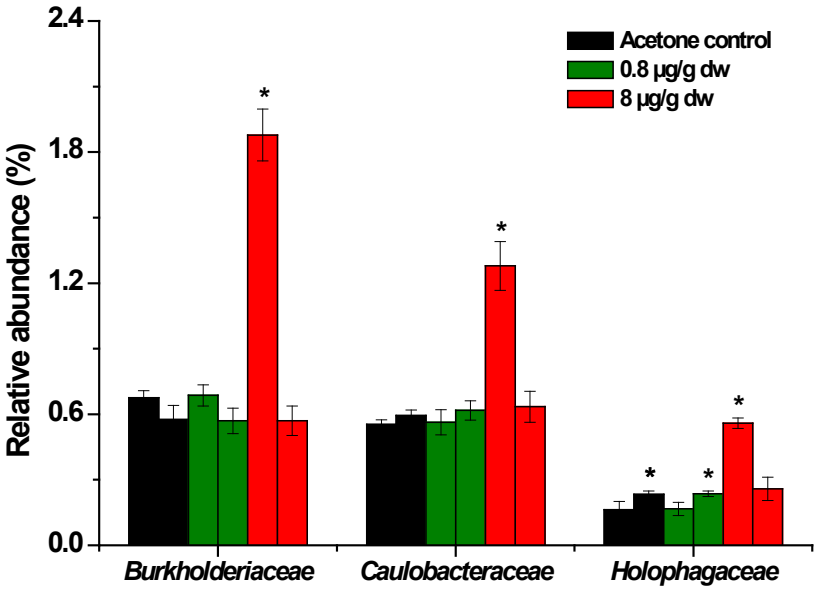



Fig. 2 RDA biplot showing the interactive effects of TCS and the presence of benthic macroinvertebrates on the sediment bacterial community structure. Explanatory variables explain 37.8% of the total variation in OTU composition. Only OTUs with $R^2 \geq 0.65$ on both axes are shown in the diagrams, which produces 52 OTUs in the graph. Square and x symbols represent environmental variables and OTUs, respectively. See Table S8 for OTU interpretation. 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.




606

607 Fig. 3 The relative abundance (%) of dominant bacterial families showing a significant
608 difference between the system with (Inv+, left) and without (inv-, right) introduced organisms
609 in the 8 µg/g dw sed treatment. Error bar represents standard error of the mean (n = 3). For the
610 same family, columns with the same colour on the left and right represent microcosms with
611 and without introduced organisms, respectively. * symbols represent systems that had
612 significantly higher relative abundance of *Burkholderiaceae*, *Caulobacteraceae* or
613 *Holophagaceae* than their corresponding systems ($p < 0.05$).

**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 |

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

Supplementary Material

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

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**Contents:**

**Table S1** Summary of the aquatic eco-toxicity data for TCS.

**Table S2** The average relative abundance of phyla in the sediment (> 0.5%).

**Table S3** The average relative abundance of families in the sediment (> 0.5%).

**Table S4** TCS concentrations in surface water and sediment.

**References**

**Table S1** Summary of the aquatic eco-toxicity data for TCS.

| Species | Trophic group | Duration, Effect, Endpoint | Value (μg/L) | Reference |
|---|---|---|---|---|
| *Anabaena flos-aqua* | Algae | 96 h, Biomass, $EC_{50}$ | 0.97 | (Orvos et al., 2002) |
| *Dunaliella tertiolecta* | Algae | 96 h, Cell density, NOEC | 3.55 | (DeLorenzo and Fleming, 2008) |
| *Navicula pelliculosa* | Algae | 96 h, Biomass, $EC_{50}$ | 19.1 | (Orvos et al., 2002) |
| *Pseudokirchneriella subcapitata* | Algae | 72 h, Growth inhibition, $IC_{50}$ | 0.53 | (Yang et al., 2008) |
| *Pseudokirchneriella subcapitata* | Algae | 72 h, Growth inhibition, NOEC | 0.2 | (Yang et al., 2008) |
| *Pseudokirchneriella subcapitata* | Algae | 72 h, Growth inhibition, LOEC | 0.4 | (Yang et al., 2008) |
| *Pseudokirchneriella subcapitata* | Algae | 96 h, Growth, NOEC | 8.3 | (Harada et al., 2008) |
| *Pseudokirchneriella subcapitata* | Algae | 72 h, Growth inhibition, $EC_{50}$ | 5.1 | (Tamura et al., 2013) |
| *Pseudokirchneriella subcapitata* | Algae | 72 h, Growth inhibition, NOEC | 0.53 | (Tamura et al., 2013) |
| *Scenedesmus subspicatus* | Algae | 72 h, Growth rate, $EC_{50}$ | 2.8 | (Orvos et al., 2002) |
| *Scenedesmus subspicatus* | Algae | 72 h, Growth rate, NOEC | 0.5 | (Orvos et al., 2002) |
| *Scenedesmus subspicatus* | Algae | 96 h, Biomass, $EC_{50}$ | 1.4 | (Orvos et al., 2002) |
| *Scenedesmus subspicatus* | Algae | 96 h, Biomass, NOEC | 0.69 | (Orvos et al., 2002) |
| *Selenastrum capricornutum* | Algae | 96 h, Biomass, $EC_{50}$ | 4.46 | (Orvos et al., 2002) |
| *Skeletonema costatum* | Algae | 96 h, Biomass, $EC_{50}$ | >66.0 | (Orvos et al., 2002) |
| *Anabaena flos-aquae* | Bacterial | 96 h, Growth, $EC_{50}$ | 1.0 | (Orvos et al., 2002) |
| *Anabaena flos-aquae* | Bacterial | 96 h, Biomass, $EC_{50}$ | 1.6 | (Orvos et al., 2002) |
| *Vibrio. fischeri* | Bacterial | 15 min, Microtox, $EC_{50}$ | 280 | (Farré et al., 2008) |
| *Ceriodaphnia dubia* | Crustacean | 7 d, Survival, NOEC | 50 | (Orvos et al., 2002) |
| *Ceriodaphnia dubia* | Crustacean | 7 d, Survival, LOEC | 339 | (Orvos et al., 2002) |
| *Ceriodaphnia dubia* | Crustacean | 7 d, Reproduction, NOEC | 6 | (Orvos et al., 2002) |
| *Ceriodaphnia dubia* | Crustacean | 7 d, Reproduction, NOEC | 182 | (Orvos et al., 2002) |
| *Ceriodaphnia dubia* | Crustacean | 8 d, Reproduction, NOEC | 30 | (Tamura et al., 2013) |
| *Daphnia magna* | Crustacean | 21 d, Reproduction, NOEC | 40 | (Orvos et al., 2002) |
| *Daphnia magna* | Crustacean | 21 d, Reproduction, LOEC | 200 | (Orvos et al., 2002) |
| *Daphnia magna* | Crustacean | 21 d, Survival, NOEC | 200 | (Orvos et al., 2002) |
| *Daphnia magna* | Crustacean | 48 h, Mobility, $EC_{50}$ | 390 | (Orvos et al., 2002) |
| *Daphnia magna* | Crustacean | 48 h, Immobilization, $EC_{50}$ | 180 | (Tamura et al., 2013) |
| *Daphnia magna* | Crustacean | 48 h, Mobility, $EC_{50}$ | 338 | (Wang et al., 2013) |
| *Daphnia magna* | Crustacean | 21 d, Reproduction, $EC_{10}$ | 45 | (Wang et al., 2013) |
| *Hyalella azteca* | Crustacean | 10 d, Survival, $LC_{50}$ | 200 | (Dussault et al., 2008) |
| *Hyalella azteca* | Crustacean | 10 d, Growth, $EC_{50}$ | 250 | (Dussault et al., 2008) |
| *Neocaridina denticulata sinensis* | Crustacean | 96 h, Mortality, $LC_{50}$ | 772 | (Wang et al., 2013) |
| *Thamnocephalus platyurus* | Crustacean | 24 h, Mortality, $LC_{50}$ | 470 | (Kim et al., 2009a) |
| *Lemna gibba* | Duckweed | 7 d, Biomass, $EC_{50}$ | >62.5 | (Orvos et al., 2002) |
| *Danio rerio* | Fish | 9 d, hatching, Survival, NOEC | 26 | (Tamura et al., 2013) |
| *Lepomis macrochirus* | Fish | 48 h, Mortality, $LC_{50}$ | 410 | (Orvos et al., 2002) |
| *Lepomis macrochirus* | Fish | 96 h, Mortality, $LC_{50}$ | 370 | (Orvos et al., 2002) |
| *Oncorhynchus mykiss* | Fish | 35 d, Survival, NOEC | 34.1 | (Orvos et al., 2002) |
| *Oncorhynchus mykiss* | Fish | 35 d, Survival, LOEC | 71.3 | (Orvos et al., 2002) |
| *Oryzias latipes* | Fish | 96 h, Mortality, $LC_{50}$ | 600 | (Kim et al., 2009a) |
| *Oryzias latipes* | Fish | 96 h, Larvae mortality, $LC_{50}$ | 602 | (Ishibashi et al., 2004) |
| *Oryzias latipes* | Fish | 96 h, Embryos mortality, $LC_{50}$ | 399 | (Ishibashi et al., 2004) |
| *Oryzias latipes* | Fish | 96 h, Mortality, $LC_{50}$ | 210 | (Tamura et al., 2013) |
| *Pimephales promelas* | Fish | 24 h, Mortality, $LC_{50}$ | 360 | (Orvos et al., 2002) |
| *Pimephales promelas* | Fish | 48 h, Mortality, $LC_{50}$ | 270 | (Orvos et al., 2002) |
| *Pimephales promelas* | Fish | 72 h, Mortality, $LC_{50}$ | 270 | (Orvos et al., 2002) |
| *Pimephales promelas* | Fish | 96 h, Mortality, $LC_{50}$ | 260 | (Orvos et al., 2002) |
| *Chironomus riparius* | Insect | 10 d, Survival, $LC_{50}$ | 400 | (Dussault et al., 2008) |
| *Chironomus riparius* | Insect | 10 d, Growth, $EC_{50}$ | 280 | (Dussault et al., 2008) |
| *Chironomus plumosus* | Insect | 96 h, Mortality, $LC_{50}$ | 2890 | (Wang et al., 2013) |
| *Potamopyrgus antipodarum* | Mollusca | 28 d, Reproduction, NOEC | 0.17 | (Geiß et al., 2016) |
| *Limnodrilus hoffmeisteri* | Oligochaeta | 96 h, Mortality, $LC_{50}$ | 2046 | (Wang et al., 2013) |
| *Tubifex tubifex* | Oligochaeta | 96 h, Mortality, $LC_{50}$ | 259 | (Khatikarn et al., 2016) |

**Table S2** The average relative abundance of phyla in the sediment (> 0.5%).

| Phyla | Int CK1 | Int CK2 | Int T1 | Int T2 | CK1 | CK2 | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Proteobacteria* | 31.1±2.19 | 31.0±0.73 | 32.8±3.18 | 33.4±1.13 | 31.7±2.90 | 30.2±2.51 | 31.8±3.64 | 31.2±3.11 | 32.4±1.21 | 32.1±1.38 |
| *Firmicutes* | 15.8±1.35 | 18.2±2.55 | 19.2±1.76 | 21.3±2.01 | 21.1±2.01 | 20.4±1.91 | 21.0±4.30 | 22.6±1.94 | 14.5±1.68 | 9.67±0.77 |
| *Chloroflexi* | 13.0±1.38 | 10.3±1.35 | 10.3±0.60 | 10.3±1.02 | 10.9±2.19 | 9.97±1.24 | 9.56±1.93 | 10.3±1.74 | 18.0±1.57 | 20.4±0.40 |
| *Acidobacteria* | 7.60±0.98 | 7.94±1.65 | 7.71±0.80 | 7.21±0.89 | 7.01±0.62 | 7.18±0.46 | 6.75±0.86 | 6.48±0.69 | 6.77±0.87 | 7.33±0.28 |
| *Actinobacteria* | 10.78±1.53 | 9.39±1.50 | 9.36±1.34 | 8.78±0.49 | 9.37±1.93 | 8.91±0.76 | 10.38±2.22 | 8.88±0.52 | 7.96±1.69 | 6.00±0.90 |
| *Bacteroidetes* | 4.72±0.30 | 5.12±0.29 | 4.82±0.59 | 2.71±0.24 | 2.76±0.68 | 3.48±0.37 | 3.18±0.27 | 2.65±0.20 | 2.83±0.29 | 2.30±0.19 |
| *Verrucomicrobia* | 2.08±0.26 | 2.12±0.30 | 2.53±0.71 | 2.61±0.28 | 1.82±0.79 | 2.92±0.52 | 2.32±0.26 | 3.20±1.14 | 2.63±0.73 | 3.95±0.10 |
| *Planctomycetes* | 3.22±0.42 | 2.78±0.23 | 2.77±0.43 | 2.23±0.34 | 2.49±0.54 | 2.82±0.39 | 2.68±0.47 | 2.63±0.93 | 2.60±0.33 | 3.10±0.07 |
| *Nitrospirae* | 2.43±0.36 | 1.97±0.20 | 2.22±0.40 | 1.86±0.16 | 2.21±0.15 | 2.29±0.31 | 2.12±0.30 | 2.07±0.54 | 1.92±0.54 | 2.34±0.04 |
| *Chlorobi* | 1.90±0.36 | 1.43±0.13 | 1.37±0.08 | 1.37±0.17 | 1.53±0.26 | 1.34±0.13 | 1.31±0.19 | 1.32±0.27 | 1.86±0.28 | 2.34±0.04 |
| *Cyanobacteria* | 0.24±0.01 | 0.49±0.03 | 0.52±0.07 | 0.59±0.15 | 0.33±0.03 | 0.49±0.04 | 0.62±0.08 | 0.63±0.01 | 1.30±0.35 | 1.75±0.23 |
| *Gemmatimonadetes* | 1.08±0.08 | 1.21±0.19 | 1.06±0.17 | 1.11±0.20 | 0.80±0.15 | 0.89±0.03 | 0.99±0.12 | 0.95±0.06 | 0.95±0.08 | 0.77±0.04 |
| *Armatimonadetes* | 1.08±0.13 | 0.64±0.06 | 0.56±0.06 | 0.46±0.07 | 1.04±0.16 | 0.65±0.05 | 0.70±0.11 | 0.61±0.08 | 0.72±0.07 | 0.78±0.07 |
| *Latescibacteria* | 0.85±0.05 | 0.58±0.09 | 0.64±0.13 | 0.58±0.13 | 0.56±0.04 | 0.53±0.08 | 0.52±0.07 | 0.62±0.10 | 0.78±0.11 | 0.83±0.09 |
| *Aminicenantes* | 0.70±0.05 | 0.66±0.08 | 0.65±0.02 | 0.61±0.17 | 0.52±0.07 | 0.53±0.08 | 0.66±0.09 | 0.55±0.06 | 0.48±0.09 | 0.74±0.05 |
| *Elusimicrobia* | 0.60±0.08 | 0.30±0.06 | 0.33±0.08 | 0.53±0.12 | 0.62±0.01 | 0.30±0.01 | 0.32±0.09 | 0.41±0.05 | 0.35±0.05 | 0.47±0.09 |

Int means microcosms with benthic macroinvertebrates.
CK1 and CK2 represent water control and acetone control, respectively.
T1-T4 represent treatments with TCS spiked concentrations of 0.8, 8, 80 and 240 μg/g dw, respectively.
Three replicates were measured for each system-treatment combination.

**Table S3** The average relative abundance of families in the sediment (> 0.5%).

| Family | IntCK1 | IntCK2 | IntT1 | IntT2 | CK1 | CK2 | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Anaerolineaceae* | 5.11±0.16 | 4.59±0.17 | 4.58±0.16 | 4.60±0.18 | 5.05±0.36 | 4.59±0.30 | 4.55±0.24 | 4.55±0.14 | 11.5±1.13 | 12.4±1.07 |
| *Rhodocyclaceae* | 3.73±0.38 | 3.84±0.29 | 3.88±0.28 | 5.40±0.29 | 3.88±0.68 | 3.90±0.77 | 4.16±0.54 | 4.24±0.68 | 6.25±0.85 | 6.04±0.11 |
| *Bacillaceae* | 4.49±0.17 | 4.63±0.46 | 4.68±0.63 | 4.73±0.25 | 4.66±0.73 | 4.83±0.39 | 4.68±0.51 | 4.76±0.34 | 4.04±0.22 | 2.07±0.17 |
| *Clostridiaceae_1* | 3.46±0.28 | 3.40±0.71 | 3.42±0.40 | 4.22±0.40 | 3.46±0.25 | 3.42±0.48 | 3.53±0.48 | 3.58±0.30 | 2.81±0.41 | 2.34±0.31 |
| *Comamonadaceae* | 3.89±0.44 | 3.72±0.60 | 3.76±0.39 | 3.71±0.53 | 3.59±0.52 | 3.57±0.35 | 3.65±0.10 | 3.56±0.15 | 3.5±0.93 | 3.33±0.38 |
| *Nitrosomonadaceae* | 2.59±0.29 | 2.58±0.31 | 2.47±0.41 | 2.52±0.21 | 2.55±0.11 | 2.46±0.41 | 2.46±0.14 | 2.54±0.33 | 2.44±0.41 | 2.10±0.11 |
| *Chitinophagaceae* | 2.31±0.26 | 2.47±0.22 | 2.38±0.39 | 1.98±0.11 | 2.28±0.14 | 2.30±0.39 | 2.24±0.23 | 2.12±0.14 | 0.88±0.17 | 0.52±0.02 |
| *Gallionellaceae* | 1.68±0.05 | 1.39±0.19 | 1.31±0.11 | 1.44±0.15 | 1.57±0.20 | 1.08±0.05 | 1.22±0.13 | 1.20±0.05 | 1.58±0.11 | 2.39±0.12 |
| *Gaiellaceae* | 1.66±0.30 | 1.80±0.23 | 1.72±0.59 | 1.62±0.20 | 1.68±0.37 | 1.81±0.09 | 1.92±0.29 | 1.97±0.19 | 1.71±0.14 | 1.16±0.09 |
| *Ruminococcaceae* | 1.89±0.13 | 1.84±0.42 | 1.83±0.31 | 1.92±0.15 | 1.96±0.30 | 1.98±0.20 | 2.11±0.19 | 2.14±0.08 | 1.71±0.25 | 1.41±0.05 |
| *unidentified_Chloroplast* | 0.38±0.10 | 0.38±0.04 | 0.38±0.05 | 0.59±0.23 | 0.41±0.07 | 0.39±0.13 | 0.42±0.11 | 0.39±0.03 | 0.98±0.16 | 1.61±0.05 |
| *Paenibacillaceae* | 1.35±0.24 | 1.21±0.24 | 1.41±0.22 | 1.87±0.20 | 1.45±0.07 | 1.41±0.12 | 1.42±0.11 | 1.48±0.21 | 1.23±0.20 | 0.75±0.08 |
| *Burkholderiaceae* | 0.67±0.02 | 0.68±0.03 | 0.69±0.05 | 1.88±0.12 | 0.60±0.05 | 0.58±0.06 | 0.57±0.06 | 0.57±0.07 | 0.80±0.06 | 0.79±0.13 |
| *Nitrospiraceae* | 1.17±0.12 | 1.08±0.12 | 1.18±0.13 | 1.17±0.25 | 1.18±0.25 | 1.21±0.19 | 1.15±0.07 | 1.24±0.27 | 1.49±0.52 | 1.70±0.02 |
| *BSV26* | 1.07±0.05 | 0.96±0.10 | 0.95±0.07 | 0.92±0.06 | 1.00±0.18 | 0.99±0.20 | 0.97±0.08 | 1.01±0.08 | 1.47±0.26 | 1.92±0.15 |
| *unidentified_Acidobacteria* | 1.02±0.05 | 0.87±0.09 | 0.87±0.06 | 0.86±0.08 | 0.99±0.19 | 0.89±0.06 | 0.90±0.08 | 0.96±0.10 | 1.25±0.28 | 1.33±0.03 |
| *Methylococcaceae* | 1.04±0.12 | 1.13±0.10 | 1.08±0.09 | 1.00±0.10 | 1.09±0.09 | 1.22±0.21 | 1.14±0.11 | 1.11±0.08 | 1.07±0.04 | 0.90±0.17 |
| *Caulobacteraceae* | 0.53±0.04 | 0.55±0.02 | 0.56±0.06 | 1.28±0.11 | 0.60±0.09 | 0.59±0.03 | 0.62±0.04 | 0.63±0.07 | 0.48±0.06 | 0.46±0.04 |
| *Opitutaceae* | 0.63±0.08 | 0.63±0.08 | 0.62±0.05 | 0.64±0.06 | 0.63±0.09 | 0.65±0.04 | 0.63±0.04 | 0.62±0.06 | 0.58±0.04 | 0.64±0.10 |
| *Coriobacteriaceae* | 0.89±0.07 | 0.90±0.14 | 0.98±0.08 | 0.84±0.11 | 0.87±0.14 | 0.85±0.03 | 0.89±0.08 | 0.94±0.11 | 0.70±0.17 | 0.64±0.05 |
| *Gemmatimonadaceae* | 0.80±0.04 | 0.81±0.07 | 0.73±0.06 | 0.63±0.11 | 0.71±0.07 | 0.73±0.03 | 0.70±0.08 | 0.66±0.02 | 0.90±0.07 | 0.80±0.03 |
| *Peptostreptococcaceae* | 0.67±0.03 | 0.67±0.07 | 0.68±0.03 | 0.69±0.02 | 0.81±0.18 | 0.83±0.05 | 0.84±0.08 | 0.85±0.13 | 0.61±0.04 | 0.47±0.10 |
| *Planctomycetaceae* | 0.72±0.06 | 0.76±0.12 | 0.76±0.21 | 0.67±0.05 | 0.73±0.14 | 0.75±0.12 | 0.77±0.14 | 0.78±0.18 | 0.73±0.04 | 0.75±0.03 |
| *Methylophilaceae* | 0.67±0.08 | 0.68±0.10 | 0.63±0.10 | 0.53±0.06 | 0.67±0.05 | 0.66±0.01 | 0.66±0.06 | 0.66±0.07 | 0.49±0.15 | 0.55±0.10 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Veillonellaceae* | 0.65±0.08 | 0.66±0.07 | 0.68±0.04 | 0.67±0.04 | 0.66±0.08 | 0.66±0.10 | 0.67±0.07 | 0.68±0.06 | 0.55±0.04 | 0.43±0.02 |
| *env.OPS_17* | 0.60±0.03 | 0.61±0.05 | 0.62±0.06 | 0.60±0.06 | 0.59±0.03 | 0.61±0.08 | 0.60±0.04 | 0.60±0.03 | 0.59±0.03 | 0.59±0.03 |
| *Peptococcaceae* | 0.62±0.10 | 0.63±0.11 | 0.61±0.12 | 0.70±0.11 | 0.63±0.06 | 0.65±0.05 | 0.64±0.08 | 0.66±0.09 | 0.60±0.02 | 0.41±0.03 |
| *Syntrophaceae* | 0.50±0.04 | 0.47±0.01 | 0.48±0.02 | 0.37±0.05 | 0.49±0.08 | 0.48±0.04 | 0.48±0.01 | 0.50±0.08 | 0.45±0.01 | 0.53±0.08 |
| *Rhodospirillaceae* | 0.54±0.07 | 0.53±0.01 | 0.50±0.03 | 0.48±0.10 | 0.55±0.06 | 0.53±0.08 | 0.53±0.06 | 0.49±0.03 | 0.46±0.08 | 0.42±0.04 |
| *Cytophagaceae* | 0.54±0.07 | 0.52±0.09 | 0.51±0.05 | 0.52±0.05 | 0.53±0.11 | 0.52±0.07 | 0.53±0.11 | 0.54±0.09 | 0.31±0.03 | 0.18±0.01 |
| *Xanthomonadaceae* | 0.53±0.07 | 0.55±0.11 | 0.52±0.10 | 0.55±0.03 | 0.51±0.07 | 0.56±0.11 | 0.57±0.02 | 0.55±0.07 | 0.23±0.02 | 0.12±0.01 |
| *Acidobacteriaceae_.Subgroup_1* | 0.49±0.14 | 0.40±0.03 | 0.38±0.02 | 0.38±0.05 | 0.47±0.13 | 0.38±0.05 | 0.39±0.09 | 0.42±0.03 | 0.61±0.02 | 0.67±0.03 |
| *Micromonosporaceae* | 0.43±0.11 | 0.43±0.07 | 0.43±0.08 | 0.63±0.15 | 0.44±0.05 | 0.47±0.04 | 0.45±0.05 | 0.45±0.05 | 0.38±0.06 | 0.21±0.01 |
| *Planococcaceae* | 0.47±0.14 | 0.50±0.10 | 0.47±0.13 | 0.50±0.06 | 0.46±0.07 | 0.50±0.08 | 0.51±0.11 | 0.52±0.10 | 0.39±0.05 | 0.21±0.02 |
| *SJA-149* | 0.48±0.07 | 0.46±0.10 | 0.43±0.04 | 0.30±0.07 | 0.47±0.03 | 0.42±0.07 | 0.43±0.03 | 0.46±0.10 | 0.67±0.14 | 0.71±0.04 |
| *Alicyclobacillaceae* | 0.49±0.14 | 0.48±0.11 | 0.52±0.10 | 0.66±0.07 | 0.50±0.13 | 0.49±0.09 | 0.49±0.09 | 0.49±0.13 | 0.41±0.10 | 0.26±0.08 |
| *Erysipelotrichaceae* | 0.42±0.05 | 0.43±0.14 | 0.41±0.07 | 0.54±0.18 | 0.43±0.10 | 0.44±0.07 | 0.44±0.03 | 0.45±0.03 | 0.38±0.06 | 0.24±0.02 |
| *Haliangiaceae* | 0.48±0.11 | 0.54±0.12 | 0.54±0.01 | 0.46±0.03 | 0.47±0.09 | 0.54±0.08 | 0.51±0.04 | 0.44±0.04 | 0.41±0.01 | 0.35±0.02 |
| *Holophagaceae* | 0.17±0.03 | 0.16±0.04 | 0.17±0.03 | 0.56±0.02 | 0.22±0.01 | 0.23±0.02 | 0.24±0.01 | 0.26±0.05 | 0.21±0.02 | 0.09±0.01 |

Int means microcosms with benthic macroinvertebrates.
CK1 and CK2 represent water control and acetone control, respectively.
T1-T4 represent treatments with TCS spiked concentrations of 0.8, 8, 80 and 240 μg/g dw, respectively.
Three replicates were measured for each system-treatment combination.

**Table S4** TCS concentrations in surface water and sediment.

| Region | Surface waters (μg/L) | Sediment (μg/g dw) | Reference |
|---|---|---|---|
| China | n.d-0.478 | n.d-1.329 | 1,2,3 |
| Korea | n.d-0.082 | | 4,5,6 |
| Australia | 0.014-0.075 | | 7 |
| Greece | 0.003-0.098 | | 8 |
| Germany | <0.003-0.01 | | 9 |
| Romania | n.d-0.0643 | | 10 |
| U.K. | n.d-0.095 | | 11 |
| Span | n.d-0.285 | n.d-0.388 | 12,13,14 |
| USA | 0.0005-0.0283 | <0.0005-0.4 | 15,16,17,18 |

n.d, not detected.

[1] Zhao et al., 2009; [2] Zhao et al., 2010; [3] Chen et al., 2014; [4] Kim et al., 2007; [5] Kim et al., 2009b; [6] Yoon et al., 2010; [7] Kookana et al., 2011; [8] Stasinakis et al., 2012; [9] Bester et al., 2005; [10] Moldovan, 2006; [11] Kasprzyk-Hordern et al., 2008; [12] Kantiani et al., 2008; [13] Villaverde-de-Sáa et al., 2010; [14] Gorga etal., 2015; [15] Wilson et al., 2009; [16] Kumar et al., 2010; [17] Katz et al., 2013; [18] Gautam et al., 2014;

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