Tetracycline and 4-epitetracycline modified the in vitro catabolic activity and structure of a sediment microbial community from a tropical tilapia farm idiosyncratically

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Aquaculture farmers commonly add tetracycline to fish feed or to their ponds to prevent or treat bacterial infections in their crops. To assess the short-term effect of tetracycline (TET) and of one of its reversible epimers, 4-epitetracycline (ETC), on the function and structure of a sediment microbial community from a tropical tilapia farm, we contrasted community-level physiological profiles (CLPP) and phospholipid fatty acid profiles (PLFA) obtained from microcosms exposed for 12 days to 5, 10, 50, or 75 mg kg⁻¹ of these antibiotics. Notwithstanding that the concentration of the antibiotics during the experiment decreased between 13–100% (TET) or 16–61% (ETC), both compounds provoked opposing metabolic responses that did not revert. TET displayed a tendency to inhibit respiration at concentrations < 50 mg kg⁻¹, whereas ETC showed the opposite effect. As revealed by the finding of the fatty acids 11:0 iso 3OH, 16:1w6c, and 18:1w6c, the sediment analyzed was predominantly colonized by Gram-negative bacteria. A marked decrease in fatty acid diversity accompanied the aforementioned metabolic responses, with TET concentrations > 50 mg kg⁻¹ leading to an enrichment of yeast and fungal biomarkers and both antibiotics at concentrations < 10 mg kg⁻¹ selecting for microorganisms with 11:0 iso 3OH. In agreement with CLPP data, differences between the PLFA profiles of control and treated microcosms were more pronounced for TET than for ETC. We conclude that high, yet field-relevant, concentrations of TET and ETC have the potential to modify the composition, and to a lesser extent, the functioning of a sediment microbial community. This study highlights the importance of considering antibiotic degradation products in ecotoxicological research.

Keywords: Tetracycline, 4-epitetracycline, sediment bacteria, CLPP, PLFA.

Introduction

Many antibiotics of clinical relevance, including tetracyclines, are found in aquatic ecosystems from the developed world[1–3] and from tropical countries[4,5] at abnormally high concentrations. In line with these data, a recent comprehensive survey of pharmaceutical and personal care product compounds in Costa Rican surface waters and coastal locations revealed the occurrence of µg L⁻¹ of doxycycline, tetracycline, oxytetracycline, clarithromycin, oxacillin, trimethoprim, ciprofloxacin, sulfamethazine, sulfamethoxazole, clindamycin, lincomycin, norfloxacin, ofloxacin, sulfadimethoxine, sulfathiazole, roxithromycin and penicillin G in areas receiving treated and untreated wastewaters, as well as urban and rural runoff.[6] These emerging pollutants are worrisome not only in view of the worldwide problematic of antibiotic-resistance,[7] but also due to their toxicity to non-target aquatic organisms[8] and key players of biogeochemical processes in sediments.[9]

One of the routes by which antibiotics reach water bodies and sediments is through the use of medicated feed to prevent or treat bacterial infections in aquaculture.[10] This practice is widespread in Latin America[11] and in Asia,[5] a region that concentrates up to 94% of the global aquaculture production.[12] Consistently, antibiotic residues, antibiotic-resistant bacteria, and antibiotic-resistance genes appear in fish and seafood,[13,14] aquaculture farms, and surrounding areas in those countries.[15] Additional risk to humans stems from the use of pond sediments as crop fertilizer.[16,17]

Over 50 percent of the total fishery output in Costa Rica comes from aquaculture farms that grow trout and tilapia
in continental freshwater or shrimp in brackish water ponds through intensive or semi-intensive culture models with partial or full harvesting methods. Only in 2009, more than 20,600 tons of tilapia, ca. 530 tons of trout, and over 5,200 tons of shrimp were produced in this country and almost 6,000 tons of frozen tilapia were exported to the United States of America in 2010.[18]

The tetracyclines are among the most commonly used antibiotics in aquaculture industry.[19] Although fish farmers in Costa Rica deny their use, we have found massive amounts of bacteria resistant to 10 \( \mu g \) mL\(^{-1} \) and 100 \( \mu g \) mL\(^{-1} \) of tetracyclines and a large diversity of mobile \( tet \) genes in sediments and water collected at their ponds (unpublished results), as well as tetracyclines at concentrations that contravene international regulation in fish feed produced locally and marketed as non-medicated.[20]

Based on this information, and bearing in mind that tetracycline [TET; (4S,4aS,5aS,6S,12aR)-4-(dimethylamino)-1,6,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide] can isomerize to 4-epitetracycline [ETC; (4R,4aS,5aS,6S,12aR)-4-(dimethylamino)-1,6,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide] in nature,[21] we performed a microcosm study to compare the short-term effect of TET and ETC on the functioning and structure of a sediment microbial community from a large tilapia farm in the tropics.

More than 20 years ago, Garland and Mills introduced the utility of Biolog Ecoplates\textsuperscript{TM} to reveal community-dependent patterns of carbon source utilization and thereby classify heterotrophic microbial communities upon direct incubation of whole environmental samples.[22] In this established method, microbial respiration of organic substrates transforms a transparent redox indicator into a colored substance and, after data manipulation and transformation, the kinetics of color development are used to compare samples or treatments using multivariate statistics.[23] This so-called Community-Level Physiological Profiling (CLPP) overlooks the role of non-culturable organisms and does not reveal the identity of major contributors to the phenotype, yet it produces large datasets rapidly[24] and has high discriminatory power.[25] Thus, CLPP has extensively been used in the last two decades to assess the effects of various types of pollutants on the function of living microbial assemblages.[26] On the other hand, information regarding the biomass, composition, nutritional status, and metabolic activity of microbial communities can be deduced from the analysis of Phospholipid Fatty Acids (PLFA) and other lipid biomarkers.[27] PLFA are present in all biological membranes, but their abundance is quantitatively and qualitatively unique across taxa. Methods for gas chromatographic determination of PLFA in environmental samples exist[28] and the resulting profiles can be exploited to demonstrate structural shifts in community structure or to identify dominant members of a microbial community by means of ordination methods or through inference from data obtained from pure cultures, respectively[29]. In this study, we used CLPP to appraise community shifts at the functional level and PLFA to infer the overall structural response of the microbial community to the treatments and to clarify whether CLPP changes were due to physiological adaptation or extinction of sensitive species.

Annual aquaculture production has more than tripled within the past 15 years, and by 2015, aquaculture is predicted to account for 39% of the world’s seafood production.[18] This justifies further research on this topic in the tropics, where regulatory guidelines regarding the use of antibiotics are disregarded, systematic programs to monitor antibiotic usage do not exist, and farmers and workers lack education regarding the safe and efficient use of these drugs. Moreover, this study is relevant because the environmental activity of antibiotic degradation products is seldom addressed.

Materials and methods

Sediment description

The sediment analyzed was collected in November 2011 at the bottom of an effluent channel in a tilapia farm of ca. 210 Ha (first 0–20 cm). This farm is located in a dry region that concentrates about 90% of the tilapia production and a large proportion of the area dedicated to shrimp farming in Costa Rica (Guanacaste). Nearly 40 different compounds from 15 families are used in Guanacaste and a recent risk assessment of antibiotics in the region revealed high hazard indicators and hazard quotients > 1 for the tetracyclines (unpublished results). At the moment of collection, overlaying water had a pH = 7.5, 6.0 mg L\(^{-1} \) of dissolved oxygen (26°C), and a conductivity of 129 \( \mu S \) cm\(^{-1} \) (HQd portable meter, Hach). The sediment was composed of 72% clay, 19% sand and 9% silt. Moreover, it contained 7.5% of organic matter (dry weight, LOI). Additional physicochemical characteristics influencing the fate and transport of tetracyclines in sediments appear in Table 1. Oxytetracycline and florfenicol, at concentrations of ng L\(^{-1} \), were sporadically detected in water and sediment samples collected at this channel during an 18-month screening performed between 2008 and 2010 (unpublished results). The sediment was transported on ice to the laboratory and stored at room temperature for 3 days prior to the beginning of the experiments.

Exposure of the microcosms to TET and ETC

Microcosms containing 100 g of sediment (dry weight) were prepared in plastic recipients. After an equilibration period of 72 h, the microcosms were exposed in triplicate to 5, 10, 50 and 75 mg kg\(^{-1} \) of TET or ETC (Sigma-Aldrich) for 12 days. Similar concentrations have been found in sediments from tilapia farms.[30] Moreover, this range of...
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Table 1. Chemical analysis of the sediment analyzed and properties of tetracycline (TET) and 4-epitetracycline (ETC) that influence their environmental fate and transport.

<table>
<thead>
<tr>
<th>Sediment composition</th>
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</thead>
<tbody>
<tr>
<td>pH</td>
<td>Cu</td>
<td>Mg</td>
<td>K</td>
<td>P</td>
<td>Zn</td>
<td>Cu</td>
<td>Fe</td>
<td>Mn</td>
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</tr>
<tr>
<td>7.40 ± 1.48</td>
<td>25.78 ± 2.58</td>
<td>17.76 ± 1.78</td>
<td>0.27 ± 0.03</td>
<td>15 ± 4</td>
<td>2.2 ± 0.3</td>
<td>9 ± 1</td>
<td>19 ± 4</td>
<td>199 ± 10</td>
<td></td>
</tr>
</tbody>
</table>

| TET and ETC properties |  |  |  |  |  |
|------------------------|---|---|---|---|
| Molar Mass (g mol⁻¹)   | Log S | Solubilityₜₐₜₜ (mg L⁻¹, 25°C) | Log K₂ₜ (P) | Log D₂ₜ (pH 7) |
| 444.1533              | −3.12 | 231 | −1.33 | −0.17 |

concentrations gave rise to detectable CLPP responses in previous investigations of tropical sediments and soils exposed to oxytetracycline.[31] A control microcosm without antibiotics was run in parallel. The microcosms were kept at room temperature (23–24°C) in the dark to prevent Ca²⁺-facilitated photolysis of the antibiotics[32] and their humidity was maintained at approximately 90% to limit confounding effects due to drying. Since tetracyclines are bacteriostatic, 500 µL of a solution containing 100 µM arginine, threonine, citric acid, glucose, 4-hydroxy benzoic acid, ascorbic acid, ethanolamine, tryptophan, putrescine, fructose, and lysine were added to the microcosms on a daily basis to stimulate bacterial growth. The structures of TET and ETC appear in Figure 1.

**Determination of CLPP**

CLPP were obtained at day 0 and day 12 using plates containing carboxylic acids (n = 21), amines (n = 6), aminoacids (n = 18), carbohydrates (n = 30), phenolic compounds (n = 6), and polymers (n = 12) (Biolog Ecoplates™). Arginine, threonine, glucose, 4-hydroxy benzoic acid, ascorbic acid, ethanolamine, tryptophan, putrescine, fructose, and lysine were included in the EcoPlates. Bacteria in 10 g of sediment from each microcosm were extracted in 0.1 g/100 mL pyrophosphate by manual agitation for 2 min and 5 pulses of 10 s each of mild sonication.[33] Soil particles were separated by centrifugation at 500 g for 15 min and the supernatants containing bacteria were recovered. After serial dilution in 10 mM phosphate buffer pH = 7 containing 9 g l⁻¹ NaCl (3⁻⁴ to 3⁻⁶), 100 µL of these bacterial suspensions was added to the wells of three plates. Color development was followed for 12 days and absorbance values were transformed into area under the curve (AUC) values through curve integration. Dilutions with similar AUC were used to compare antibiotic treatments.

**HPLC detection of TET and ETC**

The concentration of TET and ETC in the microcosms was determined with a modification of a HPLC method for detection of these compounds in animal feed at days

![Downloaded by [Ryerson University] at 16:22 05 March 2013](image-url)
To extract the analytes, 15 g of lyophilized sediment was passed through a 300 µm sieve and homogenized in 20 mL of a methanolic solution containing citric acid (0.5 g/100 mL) and nitric acid (10 mL/100 mL) using two 15-min pulses of sonication. After centrifugation for 10 min at 1000 g, the supernatants were recovered in a new tube and the pellets were re-extracted with 10 mL of the same methanolic solution. The resulting extracts were cleaned-up using Oasis HLB solid phase extraction columns (33 µm, 60 mg, 3 mL; Waters) that were preconditioned with 3 mL of methanol, 3 mL of 1 M HCl, and 3 mL of water. After loading, the columns were washed with 3 mL of 20 mM oxalic acid (pH 4.0) and 2 mL of water and then dried for 10 min using vacuum. Analytes were eluted with 2 mL of methanol. Instrumental analysis was performed with an Agilent 1200 series HPLC equipped with a quaternary pump (QuatPump G1311A), an autosampler (Infinity 1260 G1329A), a fluorescence detector (FLD G1312A), a Zorbax SB C18 column (3 µm, 250 mm × 4.6 mm), and a Pinnacle PCX Delta post-column derivatization system (Pickering Laboratories). A mobile phase composed of acetonitrile (A) and 20 mM trifluoroacetic acid (B) was used at a flow of 0.7 mL min⁻¹ according to the following gradient: 0–10 min: 15% A + 85% B, 10–15 min: 60% A + 40% B. Fluorescent derivatives of TET and ETC were generated with 0.15 mL min⁻¹ of 100 mM magnesium acetate in dimethyl sulfoxide at 30 °C (λ ex = 399 nm; λ em = 473 nm). HPLC-grade acetonitrile and methanol, as well as ACS-grade nitric acid, were purchased from J.T. Baker Reagent Chemicals. ACS-grade oxalic acid dihydrate, citric acid, hydrochloric acid, and trifluoroacetic acid were obtained from Sigma-Aldrich. Water with a specific resistance of 18.2 MΩ·cm at 25 °C was purified with a Millipore Milli-Q Advantage A10 system. All analyses were done in triplicate. This method has limits of detection and of quantitation of 49 µg kg⁻¹ and 145 µg kg⁻¹ for TET and of 98 µg kg⁻¹ and 178 µg kg⁻¹ for ETC, respectively. Moreover, its recoveries for oxytetracycline at the 0.5 and 5 mg kg⁻¹ spike levels ranges from 74 to 117% (10 replicates, unpublished data).

**PLFA analyses**

Five g of freeze-dried and sieved sediment was mixed with the chloroform-methanol-phosphate buffer system of Bligh,[34] as modified by White and collaborators.[35] Thereafter, PLFA were separated from neutral and glycolipid fatty acids using SampliQ® Silica and Amino (NH₂) solid phase extraction columns (Agilent Technologies). After mild alkaline methanalysis, PLFA were identified and quantified using the Eukarya method and Eukarya library of a GC-FID based Sherlock® Microbial Identification System (MIDI, Newark, DE, USA). Fatty acids naming was as follows: <number of carbon atoms> : <number of double bonds> w <position of double bonds from the methyl end>. Thus, 16:1w7c denotes a fatty acid containing 16 carbon atoms and one double bond in the cis conformation seven carbons away from the CH₃ end. The prefixes i, a, and cy refer to iso-, anteiso- and cyclopropyl-fatty acids. Total peak areas were normalized and fatty acid abundance was expressed in mass percentages.

**Statistical analyses**

Principal component analyses (PCA) of CLPP and PLFA data were performed to identify the combination of variables that best distinguished the treatments from the control.

**Results**

The average catabolic profiles of the control and the antibiotic treatments differed (Fig. 2). Moreover, TET and ETC gave rise to opposing average metabolic responses.

**Fig. 3.** Average color development across 12 days for sediment bacteria exposed to increasing concentrations of A) 4-epitetracycline (ETC) or B) tetracycline (TET) in a microcosm experiment. Mean of three replicates ± SE.
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This discrimination was strongly influenced by the respiration of amino acids, polymers, amines and carbohydrates assayed, although the two major components only explained about 70% of the variance in the dataset (Fig. 2). CLPP profiles of microcosms exposed to 10 mg kg\(^{-1}\) of TET or ETC were more similar to the CLPP profile of the control than to CLPP profiles of microcosms exposed to 5 mg kg\(^{-1}\) of the antibiotics (Fig. 2). Exposure to 50 mg kg\(^{-1}\) or 75 mg kg\(^{-1}\) of the antibiotics led to the strongest metabolic alterations, with responses to TET being more divergent than responses to ETC (Fig. 2).

When average AUC are compared, TET displayed a dose-dependent tendency to inhibit microbial respiration at concentrations <50 mg kg\(^{-1}\). At 75 mg kg\(^{-1}\), by contrast, TET seemed to stimulate respiration (Fig. 3). On the other hand, ETC-treated microcosms exhibited similar or slightly higher average AUC than the unexposed control (Fig. 3).

**Fig. 4.** Microbial respiration of different types of carbon sources across 12 days for sediment bacteria exposed to increasing concentrations of A) 4-epitetracycline (ETC) or B) tetracycline (TET) in a microcosm experiment. Mean of three replicates ± SE.
metabolic responses to TET and ETC were particularly evident through analysis of CLPP obtained with carbohydrates and polymers (Fig. 4).

The sediment used to prepare the microcosms had μg kg⁻¹ of TET and no detectable ETC at the moment of collection. Treated microcosms had between 84–97% or 83–157% of the nominal concentrations of TET and ETC at the beginning of the experiment, respectively. These concentrations decreased between 13–100% (TET) or 16–61% (ETC) within 12 days and the losses were inversely related to the amount of antibiotics added (Table 2). At day 12, ETC was not detected in any of the microcosm exposed to TET. Signals of an unidentified tetracycline were seen in microcosms exposed to 50 and 70 mg kg⁻¹ of TET (0.92 to 1.99 mg kg⁻¹).

With regard to the PLFA analyses, control profiles were more similar to ETC profiles than to TET profiles (Fig. 5). More fatty acids were detected in the control (n = 12) than in the sediments that received TET (4 ≤ n ≤ 8) or ETC (3 ≤ n ≤ 11). The control contained high amounts of a hydroxylated fatty acid and of monounsaturated fatty acids of 16 and 18 carbon atoms, as well as low amounts of iso and anteiso fatty acids, and polyunsaturated fatty acids (Table 3). The communities exposed to the highest concentration of TET tested exhibited low but detectable amounts of 18:2 w6c and/or 18:3 w6c, while those challenged with <10 mg kg⁻¹ of TET and ETC displayed elevated relative abundances of 11:0 iso 3OH (Fig. 5 and Table 3).

Discussion

While TET displayed a tendency to inhibit respiration at concentrations <50 mg kg⁻¹, ETC seemed to do the opposite. Although puzzling at first glance, this difference coincides with the fact that small modifications in the backbone of the tetracyclines lead to profound changes in their effects on inflammation, proteolysis, angiogenesis, apoptosis, ionophoresis, bone metabolism and protein synthesis. Moreover, this discrepancy stimulates current controversies on the lethal and non-lethal events triggered by antibiotics upon binding to classical and unrecognized targets.

Thiele-Bruhn and Beck did not detect changes in the microbial basal respiration of two topsoil samples exposed to <1000 mg kg⁻¹ oxytetracycline. By contrast, Vaclavík et al. measured respirations 1.3–1.7 times above background levels for soil bacteria exposed for 35 days to 60 and 600 mg kg⁻¹ of TET, chlortetracycline, isochlortetracycline, and oxytetracycline in a biodegradation test. In their study, respiration effects were generally dose-independent and the tetracyclines served as carbon substrates. Furthermore, Liu and coworkers observed little variations on the microbial respiration of soils that received 300 mg kg⁻¹ dry weight of tetracyclines. In our experiments, high yet field-relevant concentrations of TET and ETC caused weak but detectable variations in the in vitro microbial respiration of various types of carbon sources.

That the reversible epimer 4-epitetracycline is active elaborates results by Halling-Sørensen et al., who determined comparable minimum inhibitory concentrations of ETC and TET for activate sludge- and tetracycline-sensitive soil bacteria in growth inhibition experiments using μg mL⁻¹ of these substances in solid culture media. It is likely that the modest activity observed for ETC is to some degree indirect, as it partially reconverts to TET in the presence of chelating metals under alkaline conditions. Calcium, magnesium, iron, and manganese were present in the sediment analyzed, possibly as FeS and FeOH oxides, or as Mn³⁺ oxyhydroxides and Mn⁴⁺ oxides, and the pH of overlying water at the moment of its collection was slightly alkaline. Rather subtle functional effects accompanied the marked loss in fatty acid diversity and, consequently, the more evident structural shifts observed in the antibiotic treatments. Thus, it is likely that the sediment community analyzed had a high degree of metabolic redundancy and that the alterations observed were due to selection of resistant species rather than to transient metabolic adaptations, as would have been indicated by enrichments in the relative abundance of cyclo and trans fatty acids. In this regard, conjugative transfer of tet genes is a recurring mechanism by which bacteria in fish farms and related environments become resistant to tetracyclines. Alternatively, bacteria can develop antibiotic tolerance through cooperative survival strategies such as crowd-sourcing, antibiotic-dependent growth, induction of cellular quiescence in persisters, mutations altering the citric acid cycle, aerobic respiration and the synthesis of defensive mechanisms towards oxidative stress.
CLPP profiles of microcosms receiving 10 mg kg$^{-1}$ were more closely related to the profile of the control than to CLPP profiles of microcosms treated with 5, 50 and 75 mg kg$^{-1}$ of the antibiotics. Therefore, our results harmonize with the hormetic dose-response relationship that we observed in the past in tropical soil bacteria exposed to oxytetracycline.$^{[31]}$ Furthermore, they confirm that antibiotics play different roles in nature according to their concentration.$^{[38,52]}$

TET and ETC dissipated in our microcosms in a concentration-dependent manner, with TET degrading faster than ETC across the whole range of concentrations tested. This confirms that ETC was less active than TET. Additionally, the fast degradation kinetics strongly suggests that the metabolic and structural alterations detected were secondary to early events, that the resilience of the sediment community was low, and that its recovery to basal levels was slow. These notions are further supported by the fact that the effects recorded for TET and ETC possibly represent underestimations due to their anticipated low bioavailability in a sediment rich in clay and divalent and trivalent metal ions.$^{[53,54]}$ Under these conditions, the negatively charged phenolic diketone moiety in the BCD ring of the tetracyclines chelates Ca$^{2+}$ and Mg$^{2+}$ from clay minerals strongly.$^{[55]}$

Using standard first-order degradation kinetics, approximations of the half-life of TET and ETC and of their decay constants in our system come close to 61 and 47 d and 0.011–0.015, respectively. These estimates surpass previous figures that place the half-life of different tetracyclines in

![Fig. 5. Biplot depicting the scores and loadings of a principal component analysis calculated with PLFA data of sediment bacteria exposed to 0, 5, 10, 50 and 75 mg kg$^{-1}$ of tetracycline (T) or of its epimer 4-epitetracycline (ET) for 12 days (color figure available online).](image-url)
Table 3. Phospholipid fatty acids profiles of a microbial community from a tropical aquaculture sediment exposed to increasing concentrations of tetracycline (TET) and of its epimer 4-epitetracycline (ETC) for 12 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mg kg(^{-1}))</th>
<th>11:0 iso 3OH</th>
<th>15:0 iso</th>
<th>15:0 anteiso</th>
<th>15:0</th>
<th>16:0 iso</th>
<th>15:1 G</th>
<th>17:0 iso</th>
<th>18:1 w9c</th>
<th>18:1 w9t</th>
<th>18:0</th>
<th>19:0 cyclo c11–12 cis 9,10 epoxy</th>
<th>18:0</th>
<th>18:3 w6c</th>
<th>18:2 w6c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>22.1 ± 11.5</td>
<td>9.4 ± 0.3</td>
<td>3.4 ± 0.1</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>18.1 ± 0.6</td>
<td>19.0 ± 0.4</td>
<td>2.8 ± 2.8</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>10.9 ± 1.8</td>
<td>&lt;3</td>
<td>5.2 ± 0.0</td>
<td>ND</td>
</tr>
<tr>
<td>TET</td>
<td>5</td>
<td>41.6 ± 4.6</td>
<td>4.0 ± 4.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>11.6 ± 3.3</td>
<td>14.6 ± 3.9</td>
<td>ND</td>
<td>ND</td>
<td>&lt;3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>22.0 ± 22.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>76.9 ± 3.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>7.5 ± 1.1</td>
<td>11.7 ± 3.9</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td></td>
<td>50</td>
<td>33.6 ± 20.9</td>
<td>9.0 ± 2.7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>23.8 ± 7.7</td>
<td>26.5 ± 8.3</td>
<td>ND</td>
<td>ND</td>
<td>7.0 ± 2.2</td>
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<tr>
<td></td>
<td>75</td>
<td>35.9 ± 9.2</td>
<td>7.5 ± 1.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>20.4 ± 2.3</td>
<td>25.6 ± 3.8</td>
<td>ND</td>
<td>ND</td>
<td>&lt;3</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>ETC</td>
<td>5</td>
<td>40.7 ± 13.4</td>
<td>8.1 ± 0.1</td>
<td>ND</td>
<td>ND</td>
<td>&lt;3</td>
<td>15.8 ± 0.8</td>
<td>20.6 ± 0.8</td>
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<td>10</td>
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<td></td>
<td>50</td>
<td>36.2 ± 9.1</td>
<td>5.9 ± 1.2</td>
<td>ND</td>
<td>ND</td>
<td>&lt;3</td>
<td>11.8 ± 1.1</td>
<td>14.0 ± 1.0</td>
<td>ND</td>
<td>ND</td>
<td>&lt;3</td>
<td>4.6 ± 2.3</td>
<td>ND</td>
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<td></td>
<td>75</td>
<td>37.4 ± 15.7</td>
<td>8.5 ± 1.9</td>
<td>ND</td>
<td>ND</td>
<td>&lt;3</td>
<td>16.8 ± 3.8</td>
<td>18.3 ± 4.6</td>
<td>ND</td>
<td>ND</td>
<td>&lt;3</td>
<td>7.1 ± 1.0</td>
<td>&lt;3</td>
<td>&lt;3</td>
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</table>

\(^a\)ND: not detected
sediments between < 1 h and 22 d.\[36,57\] TET and ETC conceivably underwent different degradation pathways, as an unidentified tetracycline was exclusively detected in the microcosms that received the parental antibiotic. This compound was not ETC and it is unlikely to be anhydrotetracycline and epi-anhydrotetracycline, as the pH of overlying water was 7.5 and TET is known to epimerize at the C4 position at pH levels between 3 and 5, or to suffer from dehydration at the C6 position under very strong acidic conditions.\[58\] In the future, a combination of chemical and microbiological methods including NMR, MS, and whole-cell biosensors can be applied to identify this unidentified metabolite as well as heavy metals, pesticides, and other inhibitory substances that could co-exist in tilapia pond sediments and synergize with the tetracyclines.

Gram-negative bacilli classified to the genera Vibrio and Aeromonas are common inhabitants of tilapia ponds on account of their association with fish and fresh and brackish water.\[59\] In agreement with this, the PLFA profile of the control sediment was dominated by typical biomarkers of the Vibrionaceae, such as the combination of 16:lw7, 18:lw9, and 16:0.\[60\] Biomarkers of Gram-positive bacteria,\[61] such as iso and anteiso fatty acids, and polyunsaturated fatty acids characteristic of yeast and fungi\[62,63\] were also found, but in much lower amounts. The amplified average respiration of microcosms that received 75 mg kg\(^{-1}\) of TET is characteristic of stressed organisms,\[64\] although the concomitant increase in the relative abundance of polyunsaturated fatty acids gives us ground to believe that this was due to proliferation of fungi and yeast, which are by definition insensitive to tetracyclines and could have used bacteria killed by TET as a carbon source. On the other hand, both antibiotics at concentrations < 10 mg kg\(^{-1}\) selected for bacteria with 11:0 iso 3OH, which is a common biomarker of bacteria intrinsically resistant to antibiotics and disinfectants allocated to the genus Xanthomonas.\[65,66\] Deeper phylogenetic resolution could have been achieved with more laborious and time-consuming culture-independent techniques based on DNA amplification and sequencing; however, CLPP and PLFA sufficed to demonstrate that the changes in community function did not go hand-in-hand with community structure shifts.

Conclusions

We report functional and structural alterations for a sediment microbial community exposed for 12 days to mg kg\(^{-1}\) of TET and of a TET derivative known to arise in nature. Both antibiotics impacted the sediment microbial community studied idiosyncratically, with TET displaying effects of stronger magnitude than ETC. This result highlights the importance of considering antibiotic derivatives in investigations related to the ecotoxicology of antibiotics.

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