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氟喹诺酮对垂直流人工湿地性能及微生物群落的影响

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摘要: 为研究多种氟喹诺酮对人工湿地净化能力和微生物群落的影响, 在人工湿地进水中添加两个月氧氟沙星、诺氟沙星和环丙沙星, 监测水质变化和微生物群落变化. 结果表明, 进水中添加抗生素后, COD 去除率逐渐降低, 最低达到 70.94%, 但其可随着时间的延长逐步恢复. TP 去除率在添加抗生素后也出现下降并有较大波动. 氨氮去除率维持稳定状态. 可见, 氟喹诺酮对人工湿地的 COD 和 TP 净化能力有较大影响, 对氨氮去除无显著影响. 微生物群落方面, 抗生素添加前后的 Shannon 指数和 Shannoneven 指数无显著变化, 但 Chao1 指数显著增加. 对比前和后两组的群落构成发现, Proteobacteria 的相对丰度明显减小(从 44.90% 降低至 34.12%), 但其仍是优势种群, Firmicutes 的相对丰度明显增加(从 2.55% 增加至 10.55%). 纲水平上, β -Proteobacteria 相对丰度从 17.03% 降低至 8.36%, Clostridia、Bacilli、Bacteroidia 相对丰度分别从 0.50%、1.85%、0.10% 增加至 4.21%、4.64%、2.56%. 在属的分类水平上, *Dechloromonas* 和 *Pseudarthrobacter* 的相对丰度明显下降, 分别从 8.56%、5.10% 下降至 3.16%、1.53%, *Trichococcus*、*Tessaracoccus* 和 *Desulfovibrio* 的相对丰度则分别从 0.66%、0.03%、0.02% 增加到了 3.84%、3.83%、2.06%. 因此, 氟喹诺酮使人工湿地中的微生物群落发生了转变.

关键词: 氟喹诺酮; 人工湿地; 净化效果; 多样性; 群落构成

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Effect of Fluoroquinolones on Performance and Microbial Community of a Vertical Flow Constructed Wetland

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Abstract: In order to investigate the effect of multiple fluoroquinolones on the performance and microbial community of a constructed wetland, ofloxacin, norfloxacin, and ciprofloxacin were added to the influent of the vertical flow constructed wetland for two months. Results indicated that COD removal rate gradually decreased after adding antibiotics, with the minimum removal rate of 70.94%, followed by gradual recovery. TP removal rate also decreased, with some fluctuations, while ammonia removal remained stable. Fluoroquinolones thus have an important effect on COD and TP removal from the constructed wetland, but there is no evident effect on ammonia removal. Based on results of the Shannon index and Shannoneven index, there were no significant changes in the microbial community, while the Chao1 index increasing significantly. Comparing community composition before and after antibiotic addition, the relative abundance of Proteobacteria decreased from 44.90% to 34.12%, still maintaining its predominance, while Firmicutes increased from 2.55% to 10.55%. At the class level, β -Proteobacteria declined from 17.03% to 8.36%, while the relative abundance of Clostridia, Bacilli, and Bacteroidia increased from 0.50%, 1.85%, and 0.10% to 4.21%, 4.64%, and 2.56%, respectively. The genera *Dechloromonas* and *Pseudarthrobacter* decreased from 8.56% and 5.10% to 3.16% and 1.53%, respectively, while *Trichococcus*, *Tessaracoccus*, and *Desulfovibrio* increased from 0.66%, 0.03%, and 0.02% to 3.84%, 3.83%, and 2.06%, respectively. The microbial community of the constructed wetland thus changed under the pressure of multiple fluoroquinolones.

Key words: fluoroquinolones; constructed wetland; performance; diversity; community composition

抗生素是一类在低浓度下就能选择性抑制某些生物生命活动的微生物次级代谢产物及其化学合成或半合成的衍生物(卫生学大辞典). 基于其良好的抑菌效果及生长促进作用, 该类物质在医疗、畜牧和水产养殖等行业得到了广泛使用^[1]. 但大量甚至过量使用及低代谢率也导致了其在环境中较高的检出率^[2~4]. 虽然环境中该类物质的检出浓度一般为

ng ~ μ g 级别, 但由此诱导产生的抗生素抗性基因及伴随的基因水平转移很可能使包括致病菌在内的其

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他微生物获得抗生素抗性, 最终对人体健康和生态安全造成威胁^[5,6]. 研究显示, 抗生素的大量输入还可使环境中原有的微生物群落发生转变^[7], 而微生物群落是生物地球化学循环的重要组成部分, 其结构和功能的改变很可能对原有物质循环过程产生影响^[8]. 氟喹诺酮是应用十分广泛的一类广谱抗生素, 通过抑制细菌体内的 DNA 复制达到抑菌目的^[9], 对革兰氏阳性菌和革兰氏阴性菌均有较好抑制效果. 良好的稳定性使其在沉积物、水生植物、河流湖泊、土壤中均呈现较高的检出率和浓度^[10-12].

人工湿地是一种模拟自然系统并经过人工强化的净水系统, 因成本低、管理容易而获得了广泛的关注和应用, 其在抗生素去除方面同样表现良好. Liu 等^[13]分别采用火山岩和沸石作为基质的人工湿地去除兽用抗生素和抗性基因. 结果显示, 环丙沙星的去除率达到 78% 以上, 且以沸石作为基质的湿地表现更佳. Huang 等^[14]发现垂直流人工湿地可以去除养猪废水中 69%~99% 的土霉素、四环素和金霉素且土壤中抗生素的含量与总氮和氨氮的去除效果存在负相关关系. 但人工湿地对抗生素的去除效果还会受到湿地基质类型、植物种类、pH、溶解氧和温度等环境因素的影响^[15]. 目前, 大多研究关注了湿地对抗生素及抗性基因的去、散播和影响因素等^[16-19], 对湿地中微生物群落在氟喹诺酮压力下的变化关注不足.

目前, 关于抗生素对微生物群落的影响已有不少研究, 但大多研究采用的是单一抗生素或不同种类抗生素的混合物^[20-23], 针对同类多种抗生素的研究较为欠缺. 氟喹诺酮具有良好的吸附性, 但其混合物在吸附过程中会产生竞争效应从而减小单个氟喹诺酮药物的 K_d 值^[24], 进而影响到单个氟喹诺酮药物的吸附性. 因此, 氟喹诺酮混合物对微生物产生的影响可能与单个氟喹诺酮药物存在差异. 本实验选择环境中检出率较高的 3 种氟喹诺酮: 氧氟沙星 (ofloxacin, OFL)、诺氟沙星 (norfloxacin, NOR) 和环丙沙星 (ciprofloxacin, CIP) 的混合物作为添加物, 研究了人工湿地净化能力及微生物群落在其压力下的变化, 以揭示该类抗生素对人工湿地的潜在影响.

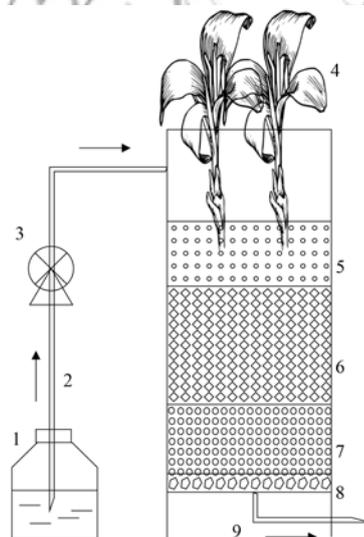
1 材料与方法

1.1 实验装置及运行

本实验中采用由 PVC 制成的圆柱形人工湿地实验装置 (如图 1), 装置直径为 40 cm, 高为 80

cm. 柱体自下而上依次填充卵石 (高约 3 cm)、陶粒 (粒径为 5~8 mm 和 3~6 mm 约 1:1 混合; 高 12 cm)、沸石 (粒径为 3~6 mm; 高 30 cm)、土壤与蛭石的混合物 (体积比约 1:1 混合, 高 15 cm). 装置最上层种植两株美人蕉, 外围有铝箔包裹.

实验进水采用人工配水, 每 10.0 L 水中含有 4.40 g 葡萄糖, 0.77 g 氯化铵, 0.24 g 磷酸二氢钾, 0.14 g 二水氯化钙, 0.20 g 七水硫酸镁, 0.45 g 六水三氯化铁, 4.00 g 碳酸氢钠, 由蠕动泵实现连续进水, 每日进水量为 9.2 L, 水力停留时间约为 39 h. 装置前期运行 3 个月以构建稳定的系统及微生物体系, 之后开始在进水中添加抗生素, 使 3 种抗生素在进水中的浓度分别为氧氟沙星 $100 \mu\text{g}\cdot\text{L}^{-1}$ 、诺氟沙星 $50 \mu\text{g}\cdot\text{L}^{-1}$ 、环丙沙星 $50 \mu\text{g}\cdot\text{L}^{-1}$. 氧氟沙星 (纯度 99%)、诺氟沙星 (纯度 99%)、环丙沙星 (纯度 $\geq 98\%$) 均购买于上海源叶生物科技有限公司, 配水所用其他试剂均为分析纯. 抗生素储备液用含有 0.2% 乙酸的去离子水于棕色瓶中分别配置并存于冰箱中, 每月更新一次. 进水中添加抗生素的时间为 2017 年 4~6 月.



1. 进水桶; 2. 进水管; 3. 蠕动泵; 4. 美人蕉; 5. 土蛭混合层;
6. 沸石层; 7. 陶粒层; 8. 卵石层; 9. 出水管;
箭头所示为水流方向

图 1 人工湿地实验装置示意

Fig. 1 Constructed wetland used in this experiment

1.2 水质指标测定

本实验中, 进出水的 COD、氨氮、TP 浓度每 3 d 测定一次, COD 浓度测定采用快速消解分光光度法, 氨氮浓度采用纳氏试剂分光光度法, TP 浓度采用钼酸铵分光光度法. 出水抗生素浓度每周测定一次, 参考文献^[25]采用 UPLC-MS/MS 完成.

1.3 16S rRNA 测序分析

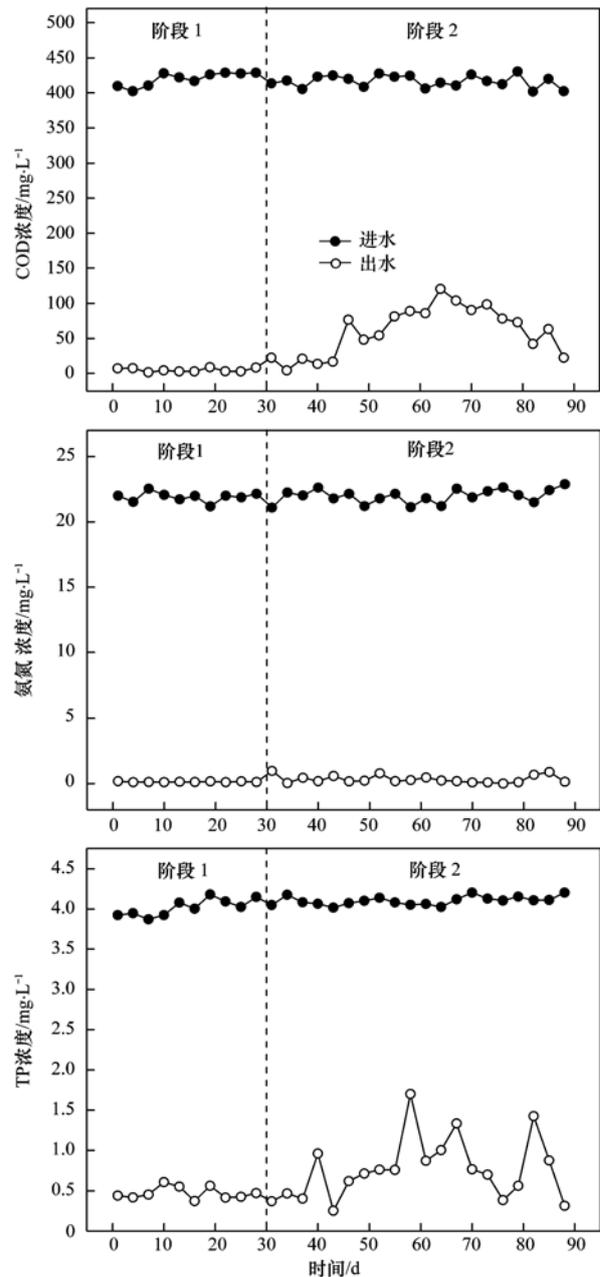
在未添加抗生素前(C组)和添加抗生素两个月后(T组)分别取3份最上层基质进行16S rRNA测序分析,每次取样间隔1d,取样深度为表面下4~5 cm.样品中的DNA提取采用Fast DNA SPIN Kit For Soil(MP Biotechnology)试剂盒完成.采用引物338F和806R对V3-V4可变区进行PCR扩增,反应条件为95℃预变性3 min,95℃变性0.5 min,55℃退火0.5 min,72℃延伸0.5 min,循环27次,并在72℃最终延伸5 min.扩增产物用2%琼脂糖凝胶电泳进行质量检测,然后在Illumina MiSeq平台完成测序分析.测序由美吉生物医药公司完成.测序结果按照97%的相似度进行OTU分类,采用RDP classifier进行分类学统计,然后计算每组样本的Shannon指数、Shannoneven指数和Chao1指数.采用非度量多维尺度分析(non-metric multidimensional scaling, NMDS)判断两组样本整体差异.显著性水平为 $P < 0.05$.

2 结果与讨论

2.1 人工湿地运行效果

系统进出水COD、氨氮、TP的浓度变化如图2所示.整个实验过程中,氨氮去除率始终大于95.40%,未受到抗生素的明显影响.COD和TP的去除效果变化较为明显.未添加抗生素阶段,COD去除率大于97.88%;抗生素添加初期,出水COD浓度未呈现较大变化,但从添加后的第16d开始,出水COD浓度开始逐渐上升,直至第34d达到最大值 $120.4 \text{ mg} \cdot \text{L}^{-1}$,去除率为70.94%.之后,出水COD浓度逐渐下降直至实验末期.对于TP,未添加抗生素期间,TP去除率大于84.52%;添加抗生素后,出水TP浓度呈现上升趋势并伴随较大幅度波动.综上,氟喹诺酮对人工湿地的净化性能产生了负面影响且主要体现在COD和TP去除方面.本实验中,除有4周的出水中含有抗生素检出外,其余出水中抗生素的浓度均低于检出限,因此,垂直流人工湿地对氟喹诺酮具有良好的去除效果.

类似结果已有报道.Amorim等^[26]研究氧氟沙星、诺氟沙星和环丙沙星对好氧颗粒污泥SBR系统的影响时发现当进水中添加抗生素后,出水COD浓度和TP浓度呈现上升趋势,而氨氮浓度未受明显影响.SBR添加氧氟沙星后,COD、氨氮和TP的去除效果与本实验相似^[27].Zheng等^[28]发现当诺氟沙星浓度为 $0 \sim 6 \text{ mg} \cdot \text{L}^{-1}$ 时,SBR对COD和氨氮



阶段1:未添加抗生素;阶段2:添加抗生素

图2 进出水COD、氨氮、TP浓度曲线

Fig. 2 Concentrations of COD, ammonia and TP in influent and effluent

的去除效果保持稳定,当诺氟沙星浓度上升至 $35 \text{ mg} \cdot \text{L}^{-1}$ 时,COD和氨氮去除率由90.7%和94.1%下降至76.5%和73.6%.可见,氟喹诺酮会对工艺出水水质产生消极影响.氟喹诺酮具有良好的吸附性,可通过静电作用力、氢键、疏水力等被有机质、无机矿物颗粒强烈吸附^[29],在人工湿地^[13]、污水处理厂^[30],吸附都是该类抗生素的重要去除途径.本实验中,土蛭混合层和其中的有机质均具备良好的吸附性,因此系统展现了良好的抗生素净化能力.同

时, 基质对氟喹诺酮的强烈吸附也对微生物产生了一定的保护作用^[31], 避免了抗生素添加初期出水水质的剧烈变化. 随着添加时间的延长, 系统中抗生素的积累量逐渐上升, 其负面影响也逐渐显现^[14], 与之伴随的便是出水 COD 浓度和 TP 浓度的上升. 尽管氟喹诺酮是一类广谱抗生素, 但微生物亦可通过外排泵机制、改变细胞内的抗生素敏感部位、使抗生素失活等方式增加自身对抗生素的耐受性和适应性^[32]. 值得注意的是, 某些抗生素还可以作为微生物代谢的碳源^[33]. 另外, 微生物群落还可通过调整内部结构逐步适应抗生素的存在^[34]. 这些原因都可能使系统的净化性能在抗生素添加后期逐步恢复. 因此, 虽然氟喹诺酮会对人工湿地的净化能力产生影响, 但其可以随着时间的延长逐步恢复.

2.2 微生物群落变化

2.2.1 微生物群落概况

测序后得到各分类水平的微生物数目如图 3 所示, 样本的 Shannon 指数、Shannoneven 指数、Chao1 指数如表 1 所示. 对比两组结果发现, T 组样本的 Chao1 指数显著高于 C 组, 但 Shannon 指数和 Shannoneven 指数变化并不显著 ($P > 0.05$) (表 1). NMDS 分析结果 (如图 4) 显示, 不同组样本点间距离较大, 同组样本点间距离较小, 两组样本点呈现明显分离趋势. 可见, 氟喹诺酮确实使系统中的微生物群落产生了转变.

表 1 α 多样性指数¹⁾

Table 1 The α diversity indices

多样性指数	C-1	C-2	C-3	T-1	T-2	T-3
Shannon	6.227 426	6.094 299	6.146 344	6.256 913	6.318 335	6.201 667
Shannoneven	0.820 823	0.801 681	0.808 156	0.807 324	0.811 053	0.797 184
Chao1	2 358.80	2 443.46	2 347.62	2 789.94	2 769.82	2 813.08

1) C 代表未添加抗生素的组, T 代表添加抗生素的组, 短线后面的数字代表样品序号

2.2.2 门水平微生物群落变化

抗生素添加前后各样本的门水平微生物群落构成如图 5 所示, 其中 C 组的优势种群为 Proteobacteria (44.90%)、Actinobacteria (24.61%)、Chloroflexi (7.37%)、Acidobacteria (7.93%)、Bacteroidetes (4.37%), T 组的优势种群为 Proteobacteria (34.12%)、Actinobacteria (21.82%)、Firmicutes (10.55%)、Acidobacteria (8.31%)、Chloroflexi (7.99%) 和 Bacteroidetes (5.25%). Proteobacteria、Actinobacteria、Chloroflexi、Acidobacteria 与 Bacteroidetes 始终为优

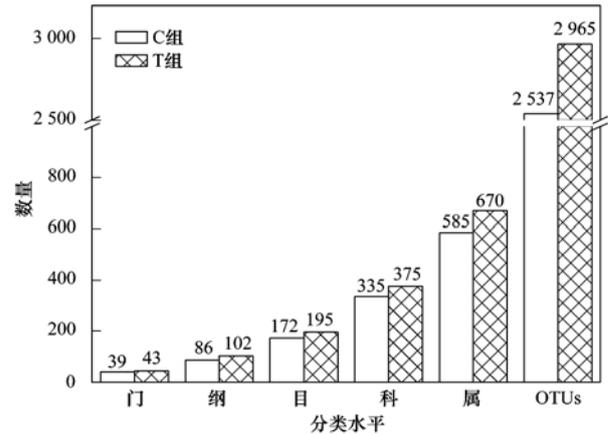


图 3 各分类水平微生物数目

Fig. 3 Species numbers for each classification level

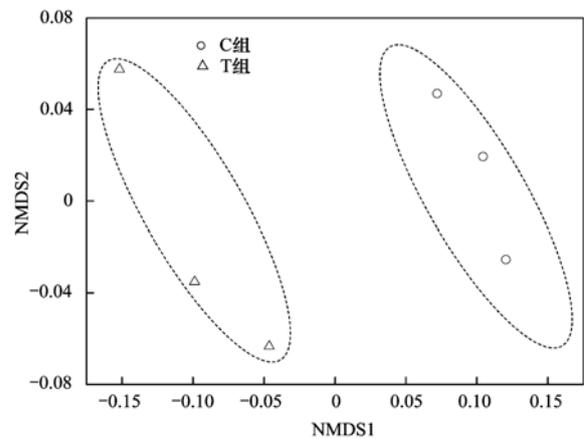
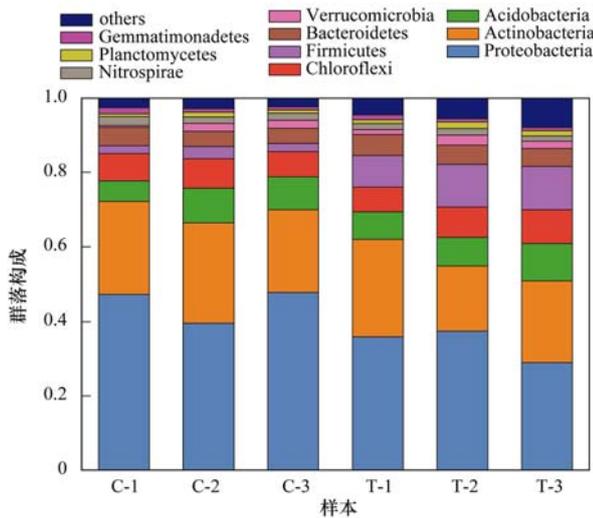


图 4 NMDS 分析

Fig. 4 NMDS analysis

势种群, 其总丰度在 C 组和 T 组中分别占比 89.17%、77.49%, 这与其他研究中的结果类似^[35].

从图 5 中可看出, Proteobacteria 的相对丰度明显减少而 Firmicutes 的相对丰度明显上升. Kim 等^[36]研究恩诺沙星对人体肠道微生物群落影响时发现, 随着恩诺沙星浓度的增加, Proteobacteria 和 Bacteroidetes 的比例逐渐减小, 而 Firmicutes 所占比例逐渐增加. 本实验所用的诺氟沙星和环丙沙星为恩诺沙星的生物降解中间体^[37]. Xiong 等^[38]发现粪肥中的氟喹诺酮会对土壤中的 Proteobacteria 产生



C 代表未添加抗生素的组, T 代表添加抗生素的组, 短线后面的数字代表样品序号, 下同

图 5 门水平的群落构成

Fig. 5 Community composition at phylum level

抑制, 且施用了含氟喹诺酮肥料的土壤中 Firmicutes 的丰度要高于对照组. Yan 等^[39]发现氧氟沙星、磺胺甲噁唑可使人工湿地中 Firmicutes 的相对丰度随抗生素浓度逐渐增加. 可见, 氟喹诺酮对 Proteobacteria 有抑制作用而对 Firmicutes 有促进作用. 在众多水处理工艺中, Proteobacteria 均是发挥重要功能的优势种群, 与系统净化能力密切相关, 因此本实验中 COD 和 TP 净化效果的下降很可能与 Proteobacteria 相对丰度的明显下降有关.

2.2.3 纲水平微生物群落变化

C 组和 T 组各样本纲水平的微生物群落构成如图 6 所示, 其中 C 组的优势种群是 Actinobacteria (24.61%)、 β -Proteobacteria (17.03%)、 α -Proteobacteria (11.69%)、 δ -Proteobacteria (8.84%)、Acidobacteria (7.93%)、 γ -Proteobacteria (7.05%), T 组的优势种群是 Actinobacteria (21.82%)、 α -Proteobacteria (12.53%)、 β -Proteobacteria (8.36%)、Acidobacteria (8.31%)、 δ -Proteobacteria (7.52%) 和 γ -Proteobacteria (5.65%). 比较两组的构成发现, 相对丰度大于 5% 的优势种群中, β -Proteobacteria 和 γ -Proteobacteria 的相对丰度变化较大(如图 6). 有研究显示, 废水中的四环素类、磺胺类和喹诺酮类抗生素与 β -Proteobacteria 和 γ -Proteobacteria 之间存在负相关关系^[40]. 另外, 当同样具有苯环结构的三氯生进入到水-沉积物系统中时, β -Proteobacteria 的相对丰度也发生了大幅下降^[41]. 可见, β -

Proteobacteria 对该类物质的出现比较敏感. β -Proteobacteria 是 Proteobacteria 的重要组成部分, 其中含有大量可以实现有机物降解、脱氮除磷等功能的微生物, 因此其丰度的减小可能与出水 COD 浓度和 TP 浓度上升之间存在相关关系. 与 β -Proteobacteria 的变化趋势相反, Clostridia、Bacilli、Bacteroidia 的相对丰度呈现了比较明显的增加(如图 6), 分别从 C 组的 0.50%、1.85%、0.10% 增加到了 T 组的 4.21%、4.64% 和 2.56%. 有报道称, Clostridia 和 Bacilli 在盘尼西林和土霉素污染环境中呈现出很高的丰度^[7], 且四环素类和磺胺类对 Clostridia 有选择性优势^[42]. 因此, Clostridia 和 Bacilli 可能与抗生素污染环境之间存在潜在联系^[43]. Liao 等^[44]还发现 Bacteroidia 中含有能够降解环丙沙星的菌种. 可见, Clostridia、Bacilli 和 Bacteroidia 对氟喹诺酮具有较强适应性. 综上, 氟喹诺酮对 Clostridia、Bacilli 和 Bacteroidia 具有选择性优势, 对 β -Proteobacteria 具有选择性抑制作用.

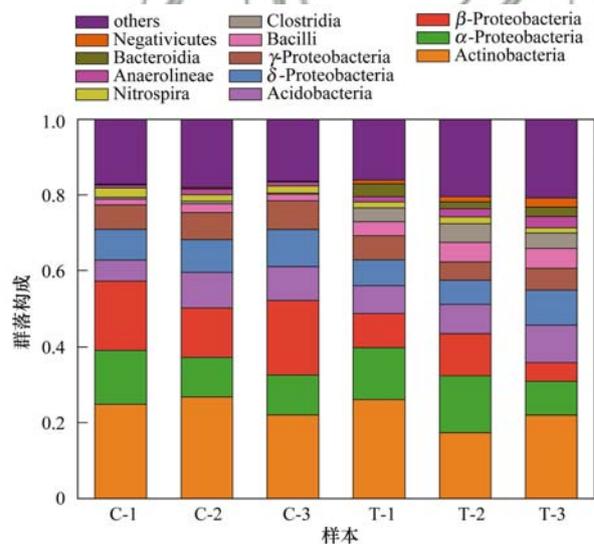


图 6 纲水平的群落构成

Fig. 6 Community composition at class level

2.2.4 属水平微生物群落变化

本实验中, C 组和 T 组分别检测到 585 个属和 670 个属, 其中 551 个属为两组共有, 其中 42.65%、15.79%、9.44%、7.26%、5.26% 的属分别包含于 Proteobacteria、Actinobacteria、Bacteroidetes、Firmicutes、Chloroflexi. 在属的分类水平上, C 组的优势种群为 *Dechloromonas* (8.56%)、*Pseudarthrobacter* (5.10%)、*Pseudomonas* (3.37%)、*Skermanella* (2.28%)、*Nitrospira* (2.03%), T 组的优势种群为 *Trichococcus* (3.84%)、*Tessaracoccus*

(3.83%)、*Dechloromonas* (3.16%)、*Pseudomonas* (3.15%)、*Desulfovibrio* (2.06%)。两组样本中丰度前 15 位属的 Heatmap 图如图 7 所示,从中可看出 *Dechloromonas* (属于 Proteobacteria)、*Pseudarthrobacter* (属于 Actinobacteria) 的丰度下降明显,而 *Trichococcus* (属于 Firmicutes)、*Tessaracoccus* (属于 Actinobacteria)、*Desulfovibrio* (属于 Proteobacteria) 的丰度则出现明显上升,因此群落中不同种类的微生物在氟喹诺酮压力下呈现了不同的丰度变化趋势。其中,*Dechloromonas* 可以还原高氯酸盐,是生物水处理系统中常见的聚磷菌^[45],因此该属丰度的减小可能与出水 TP 浓度上升有关。另一个丰度减小的属 *Pseudarthrobacter* 中的部分菌种(如 *Pseudarthrobacter sulfonivorans* strain Ar51)可在低温下降解原油和多苯化合物^[46],但本实验期间气温较高,可能抑制了其对复杂有机物的适应能力。*Trichococcus* 具有良好的抗生素适应性,*Trichococcus flocculiformi* 更是在各类抗生素环境中广泛存在的一类微生物^[7],因此该属在氟喹诺酮的选择性压力下呈现了更高的丰度。*Tessaracoccus* 是一类兼性厌氧(除 *T. lubricantis* 外)的革兰氏阳性菌,在活性污泥、海底沉积物、被油污染的含盐土中均有被分离出来的菌株^[47],因此,该属对环境良好的适应能力使其未受到抗生素的负面影响。*Desulfovibrio* 可以将有机物或者分子氢的氧化与硫酸盐还原过程联系起来生成硫化氢并从中获得能量,是严格厌氧菌。有报道显示,包括 *Desulfovibrio* 在内的多种硫酸盐还原菌都可以受到环丙沙星的促进作用^[7],这与本实验中的结果类似。*Nitrosomonas* 和 *Nitrospira* 是重要的氨氧化菌,其丰度在前后两组未出现较大变化,这与出水氨氮浓度一直保持稳定状态的结果相一致。

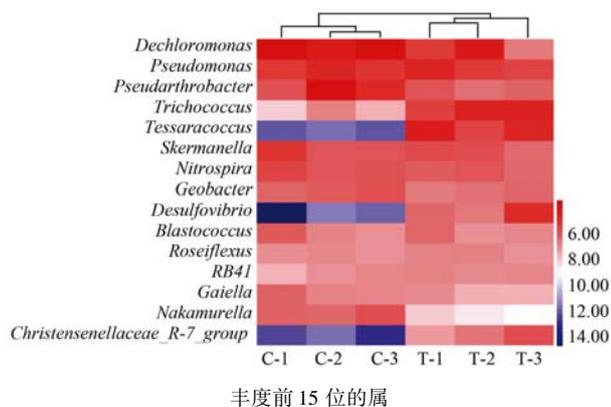


图 7 属水平的 Heatmap 图

Fig. 7 Heatmap graph at genus level

3 结论

(1) 氟喹诺酮会对人工湿地净化性能产生负面影响,主要体现在对 COD 和 TP 的去除方面,但其可以随着时间的延长逐步恢复;同时垂直流人工湿地对氟喹诺酮具有良好的净化效果。

(2) 氟喹诺酮使人工湿地中的微生物群落发生了转变. Shannon 指数和 Shannoneven 指数无显著变化,Chao1 指数显著增加;群落结构也发生了明显转变。

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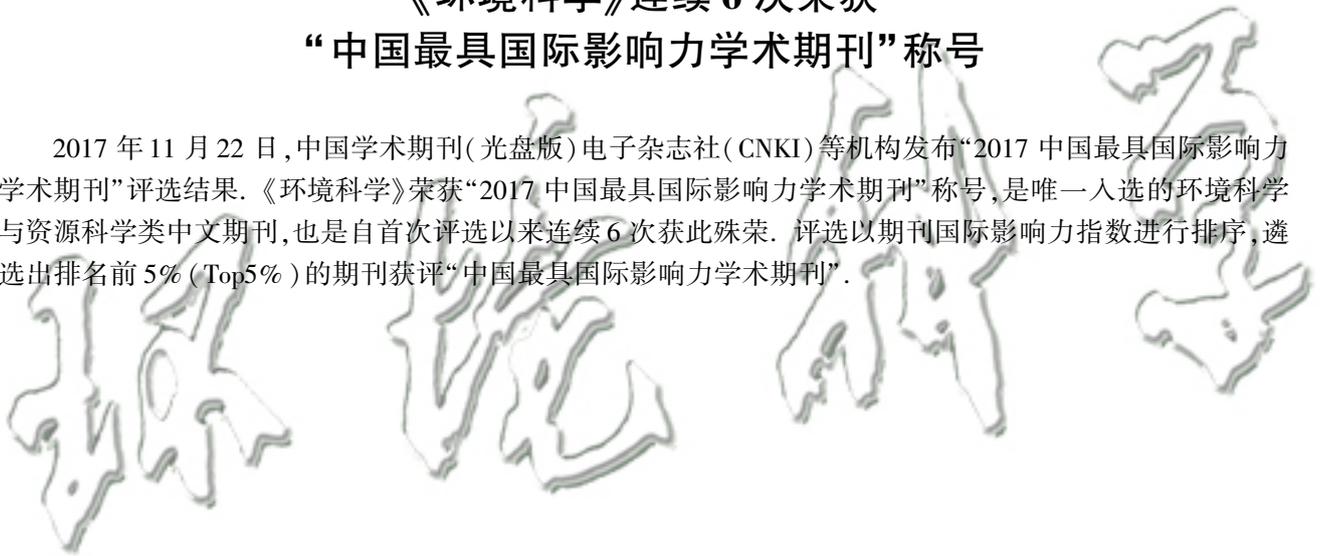
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《环境科学》连续 6 次荣获 “中国最具国际影响力学术期刊”称号

2017 年 11 月 22 日,中国学术期刊(光盘版)电子杂志社(CNKI)等机构发布“2017 中国最具国际影响力学术期刊”评选结果.《环境科学》荣获“2017 中国最具国际影响力学术期刊”称号,是唯一入选的环境科学与资源科学类中文期刊,也是自首次评选以来连续 6 次获此殊荣. 评选以期刊国际影响力指数进行排序,遴选出排名前 5% (Top5%) 的期刊获评“中国最具国际影响力学术期刊”.



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