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环境科学

ENVIRONMENTAL SCIENCE

ISSN 0250-3301 CODEN HCKHDV

HUANJING KEXUE

■ 主办 中国科学院生态环境研究中心

■ 出版 科学出版社



2019

Vol.40 No.7

第40卷 第7期

目次

《大气污染防治行动计划》实施的环境健康效果评估	武卫玲, 薛文博, 王燕丽, 雷宇, 冯涛, 蔡泽林 (2961)
基于达标约束的南京市环境空气质量情景模拟	谢放尖, 史之浩, 李婧祎, 郑新梅, 胡建林, 刘春蕾, 杨峰 (2967)
郑州市 PM _{2.5} 中水溶性离子特征及来源分析	杨留明, 王申博, 郝祺, 韩士杰, 李晨, 赵庆炎, 燕启社, 张瑞芹 (2977)
能见度与 PM _{2.5} 浓度关系及其分布特征	王继康, 张恒德, 桂海林, 饶晓琴, 张碧辉 (2985)
基于扩散模式反演的橡胶轮胎制造行业 VOCs 排放特征	白红祥, 魏巍, 王雅婷, 任云婷, 臧佳欣, 程水源 (2994)
太原市环境空气中挥发性芳香烃碳同位素组成及来源	李颖慧, 闫雨龙, 李志生, 郝爱胜, 李如梅, 杨帆, 彭林 (3001)
办公楼聚集区空气中 PBDEs 谱分布与污染特征	王琳, 邓雅佳, 廖晓勇, 曹红英, 焦杏春 (3008)
南北水调东线中游枢纽湖泊有色可溶性有机物来源组成特征	张柳青, 彭凯, 周蕾, 石玉, 李元鹏, 周永强, 龚志军, 张运林, 杨艳 (3018)
黄土丘陵区小流域不同水体氢氧同位素特征	张荷惠子, 于坤霞, 李占斌, 李鹏, 赵宾华, 柯浩成, 蒋凯鑫 (3030)
基于碳氮同位素的澜沧江水库 TOC 来源差异性分析	王从锋, 胡子龙, 杨正健, 丹勇, 赵萍, 郝文超, 聂小芬, 聂睿, 徐刘得, 刘德富 (3039)
李家河水库污染物来源及水体分层对水质的影响	徐进, 黄廷林, 李凯, 杨尚业, 温成成, 林子深, 李衍庆, 刘雪晴 (3049)
水库淹没带土壤有机氯农药分布特征及风险评价	王霞, 张青琢, 赵高峰, 王晓燕 (3058)
银川市湿地表层水中多环芳烃的分布、来源及生态风险评价	田大年, 党丽慧, 丁润梅, 蔡倩, 张鹏举, 汪岭, 杨慧芳 (3068)
人类活动影响下的垂直气候带岩溶泉地球化学特征对比: 以重庆金佛山水房泉、碧潭泉为例	谢国文, 杨平恒, 盛婷, 邓书金, 洪爱花 (3078)
漓江冲洪积扇地下水化学特征及控制因素分析	唐金平, 张强, 胡漾, 张宇, 聂保伟 (3089)
三峡库区蓄水期和非蓄水期附石藻类群落变化及其影响因子分析	付君珂, 刘黎, 贺新宇, 张红波, 董聪聪, 杨燕君, 施军琼, 吴忠兴 (3099)
降雨对香溪河库湾主要藻种原位生长的影响	翁传松, 刘德富, 张佳磊, 龚川, 沈旭舟 (3108)
绿狐尾藻分解及其氮磷释放特征	童雄, 罗沛, 刘锋, 黄敏, 陈哲, 肖润林, 吴金水 (3118)
微藻固定化条件优化及其污水氨氮去除潜力分析	刘祥, 王婧瑶, 吴娟娟, 彭飞, 王凯军 (3126)
钛酸盐纳米管对水中氨氮的吸附特性	张政, 冯长生, 张晓瑞, 郑建奎, 蒋彩云, 李攀杰, 王玉萍 (3135)
基于缺陷重构的类芬顿光催化剂在降解染料废水中的应用	唐清文, 安晓强, 兰华春, 刘会娟, 李海翔 (3146)
N 原子杂化石墨烯高效活化过一硫酸盐降解 RBK5 染料废水	于永波, 黄湾, 董正玉, 吴丽颖, 张倩, 洪俊明 (3154)
Cu ²⁺ 对以 NO ₃ ⁻ 为电子受体反硝化过程的影响	买文可, 彭永臻, 吉建涛 (3162)
CANON 中试反应器启动及性能优化	孙庆花, 吴迪, 周家中, 郑志佳 (3169)
好氧段碳源浓度对同步去除和富集磷酸盐生物膜的影响	徐林建, 潘杨, 章豪, 冯鑫, 魏攀龙, 尤星怡 (3179)
有机物特性对 AAO 系统污泥沉降性能的影响	刘小博, 袁林江, 陈希, 薛欢婷 (3186)
不同污泥浓度下缺氧 FNA 对硝化菌活性的影响	吕心涛, 周桐, 田夏迪, 谷鹏超, 杨岸明, 王佳伟, 张树军 (3195)
ANAMMOX 菌铁自养反硝化工艺的稳定性	张文静, 黄勇, 毕贞, 胡羽婷, 董石语 (3201)
在线 NaClO 反洗对倒置 A ² O-MBR 系统微生物群落的影响	王旭东, 高森, 王莹莹, 杨永哲, 刚家斌, 符国力, 王磊 (3208)
基于有机物释放和经济性的污泥预处理方法评价	袁悦, 谭学军, 郑舍予 (3216)
北京市清水与再生水协同利用优化模型	张田媛, 谭倩, 王淑萍 (3223)
N-亚硝胺在不同处理工艺污水处理厂中的分布及其去除	柳王荣, 赵建亮, 杨愿愿, 姚理, 刘有胜, 应光国 (3233)
北京市生活污水中曲马多和芬太尼的赋存	周子雷, 杜鹏, 白雅, 韩胜, 黄红梅, 徐泽琼, 李喜青 (3242)
贵阳市污水处理厂中典型抗生素的污染水平及生态风险	杨钊, 李江, 张圣虎, 向福亮, 唐涛涛, 杨娅红 (3249)
紫色土丘陵区畜禽养殖场土壤中抗生素抗性基因分布特征	程建华, 唐翔宇, 刘琛 (3257)
四环素对污泥蚯蚓粪中微生物种群和抗性基因的影响	陈景阳, 夏慧, 黄魁, 吴颖 (3263)
热水解污泥厌氧消化过程中肠球菌抗生素抗性基因与毒力基因的转归	李慧莉, 裴媛玫, 李姗, 何芙蓉, 杨月婷, 魏源送, 佟娟 (3270)
鸡粪与中药渣共堆肥对抗生素抗性基因的影响	武晋萍, 陈建文, 刘勇, 张红, 李君剑 (3276)
宁武亚高山湖泊细菌群落的时空格局及驱动机制	王雪, 刘晋仙, 柴宝峰, 罗正明, 赵鹏宇, 暴家兵 (3285)
石家庄市春季景观水体 nirS 型反硝化细菌群落特征分析	张艺冉, 李再兴, 孙悦, 霍然, 孟文茹, 王凌霄, 申邵恒, 杨子程, 周石磊 (3295)
磷差异性调控水稻根际 nirK/nirS 型反硝化菌组成与丰度	湛钰, 高丹丹, 盛荣, 魏文学, 秦红灵, 张文钊, 侯海军, 汤亚芳 (3304)
会仙岩溶湿地、稻田与旱地土壤细菌群落结构特征比较	贾远航, 靳振江, 袁武, 程跃扬, 邱江梅, 梁锦桃, 潘复静, 刘德深 (3313)
湘中典型稻田系统 Cd 平衡分析	蒋凯, 邓潇, 周航, 龙坚, 李欣阳, 董霞, 王树兵, 刘文辉, 侯红波, 彭佩钦, 廖柏寒 (3324)
改良剂对根际土壤-水稻系统中镉运移的影响	李义纯, 王艳红, 唐明灯, 巫彬芳, 李林峰, 艾绍英, 凌志祥 (3331)
重金属固定植物促生细菌的筛选及其阻控小麦富集重金属效应	韩辉, 王晓宇, 蔡红, 姚伦广, 蔡倩迪, 王逸雪, 陈兆进 (3339)
不同品种辣椒镉亚细胞分布和化学形态特征差异	彭秋, 李桃, 徐卫红, 焦璐琛, 邓继宝 (3347)
添加磷对水稻-油菜轮作土壤 N ₂ O 排放影响	孙正, 苏荣琳, 徐鹏, 吴宏涛, 胡金丽, 赵劲松, 胡荣桂 (3355)
渭北旱原土地利用方式对土壤团聚体稳定性及其有机碳的影响	刘杰, 马艳婷, 王宪玲, Sompouviset Thongsouk, 李利敏, 秦亚旭, 赵志远, 郑伟, 翟丙年 (3361)
不同土地利用类型的土壤中多环芳烃的纵向迁移特征	鲁垠涛, 向鑫鑫, 张士超, 刘明丽, 王静, 李爽, 姚宏, 孙绍斌 (3369)
西湖景区土壤中邻苯二甲酸酯污染水平、来源分析和空间分布特征	廖健, 邓超, 陈怡, 周文钊, 林春绵, 张辉 (3378)
生物炭对壤土土壤容重和团聚体的影响	李倩倩, 许晨阳, 耿增超, 张久成, 陈树兰, 王慧玲, 张妍, 负方悦, 杨林, 董胜虎 (3388)
《环境科学》征订启事 (3194)	《环境科学》征稿简则 (3222)
信息 (3256, 3284, 3323)	

四环素对污泥蚯蚓粪中微生物种群和抗性基因的影响

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摘要: 城市污泥中高含量的四环素可能会致使污泥蚯蚓堆肥产物中携带高丰度的抗性基因, 降低其利用价值. 本实验采用赤子爱胜蚓处理添加不同含量四环素(100、500和1 000 mg·kg⁻¹)的新鲜污泥, 以不添加为对照组, 分析微生物种群和四环素抗性基因(*tetC*、*tetG*、*tetM*、*tetW*和*tetX*)及整合子基因(*intI1*)在不同四环素选择性压力下的变化, 揭示四环素含量对污泥蚯蚓堆肥中微生物种群和抗性基因的影响. 结果表明, 添加四环素降低了污泥蚯蚓堆肥产物中变形菌门的丰度但增加了拟杆菌门的丰度, 且四环素的含量与细菌的 Shannon 和 Pielou 指数有明显的负相关关系. 同时, 添加四环素致使 *intI1* 增加了 4.25 倍; 四环素类抗性基因增加了 4.7~186.9 倍, 其中 *tetM* 基因丰度与四环素含量呈现出显著的正相关关系. 因此初始污泥中较高含量的四环素会改变污泥蚯蚓粪的微生物种群结构, 增加抗生素抗性基因的丰度和传播风险.

关键词: 四环素; 蚯蚓堆肥; 微生物种群; 抗性基因; 污泥

中图分类号: X171.5 文献标识码: A 文章编号: 0250-3301(2019)07-3263-07 DOI: 10.13227/j.hjkk.201810117

Effects of Tetracycline on Microbial Communities and Antibiotic Resistance Genes of Vermicompost from Dewatered Sludge

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Abstract: The high content of tetracycline in municipal sludge may result in vermicomposting products carrying a high abundance of antibiotic resistance genes, thus lowering the utilization value of the vermicompost. Hence, this study aimed to reveal the underlying effects of tetracycline concentrations on the resistance genes involved in vermicomposting systems for sludge recycling. For this purpose, fresh sludge substrates with different concentrations of tetracycline (100, 500, and 1000 mg·kg⁻¹) were vermicomposted for 60 days using *Eisenia foetida*. In parallel, sludge treatment without the addition of the tetracycline was used as a control. During the experiment, changes in bacterial communities, tetracycline resistance genes (*tetC*, *tetG*, *tetM*, *tetW*, and *tetX*), and class 1 integron (*intI1*) were detected using high-throughput sequencing and qualitative PCR, respectively. The results showed that the addition of tetracycline reduced the abundance of Proteobacteria but increased the abundance of Bacteroidetes in the sludge vermicompost. Furthermore, the concentrations of tetracycline had a significant negative correlation with Shannon and Pielou indexes of bacterial diversity. In addition, tetracycline increased *intI1* genes and tetracycline resistance genes in the sludge by 4.25 times and 4.7~186.9 times, respectively. Moreover, there was a significantly positive correlation between the abundance of *tetM* genes and tetracycline concentration. This study suggests that higher concentration of tetracycline in sludge can modify the microbial community structure of vermicompost, enhancing the abundance of antibiotic resistance genes and their associated dissemination risks.

Key words: tetracycline; vermicomposting; microbial community; resistance genes; sludge

抗生素被广泛使用在医疗和养殖业中^[1,2]. 据统计, 2013 年我国抗生素的使用量达到了 16.2 万 t^[3], 但约有 75% 的抗生素未被有效吸收, 会以原药的形式代谢到环境中^[4]. 环境中残留大量的抗生素导致了诸多携带抗性基因的耐药菌出现, 对人类的健康带来了潜在的危害^[5]. 抗生素及其抗性基因经市政管道流入污水处理厂, 最终汇集在城市污泥中^[6]. 已有检测表明脱水污泥中存在多种高丰度的抗性基因, 主要有四环素类、喹诺酮类、磺胺类等^[7-9]. 抗性基因作为一种新型污染物, 其潜在的环境风险比抗生素本身更大^[10]. 相关学者对污泥中抗性基因的消减进行了研究. 结果表明无论是厌氧消化还是高温堆肥, 对抗性基因的消减均是选择性的, 仅有少部分抗性基因可以被完全去除^[11-13]. 因此, 污泥资源化同时降低抗性基因风险已成为亟

待解决的课题.

蚯蚓堆肥是利用蚯蚓和微生物的协同作用将污泥中的有机质分解转化的过程^[14], 其主要包括蚯蚓肠道的消化过程和微生物的分解过程^[15]. 与其他方法相比, 蚯蚓能显著提升污泥中有效态的氮磷钾等元素的含量以及多元降解菌和农业益生菌群等功能化菌群的多样性^[16-19], 因此蚯蚓堆肥被认为是一种有竞争力的污泥资源化方法. 但问题是由于污泥中存在每日变化的高含量抗生素, 可能会影响功能性菌群, 进而影响污泥蚯蚓堆肥产物的性质.

收稿日期: 2018-10-18; 修订日期: 2019-01-18

基金项目: 国家自然科学基金项目(51868036); 甘肃省自然科学基金项目(17JR5RA104); 兰州交通大学校青年基金项目(2018030)

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并且,污泥中抗生素含量对抗性基因有一定的选择性压力^[20, 21],也会增加污泥蚯蚓粪抗性基因的传播风险.基于此,研究四环素含量对污泥蚯蚓粪中微生物及其抗性基因的影响,对增加污泥蚯蚓粪的利用价值和控制抗性基因的传播是至关重要的.然而,对此研究目前还鲜见报道.

本实验以城市污泥中含量较多的四环素作为供试抗生素^[22],以不添加四环素为对照组,添加不同含量四环素为实验组,比较四环素含量对蚯蚓堆肥产物中微生物种群结构和抗性基因丰度的差异,以期为提高污泥蚯蚓粪的利用价值和控制四环素抗性基因及其传播提供科学依据.

1 材料与方法

1.1 供试材料

本实验采用经过新鲜污泥驯化的赤子爱胜蚓(*Eisenia foetida*)作为堆肥蚓种,单体重约为0.4 g.反应器为底部打孔的矩形塑料盒(46 cm × 17 cm × 13 cm).污泥取自兰州市安宁七里河污水处理厂脱泥车间的新鲜脱水污泥.

1.2 实验方法

所得新鲜污泥利用5 mm网孔的铁丝网进行造粒.然后将0、400、2 000和4 000 mg的四环素粉末(分析纯,99.9%;Ruibio, Germany)分别加入到4 kg造粒污泥中.将四环素和污泥充分混匀后,对其再次造粒,作为蚯蚓的供试基质.然后在各反应器中接种100条蚯蚓,开始蚯蚓堆肥实验,其投加密度参照文献^[18]的实验进行.本实验中,将4组不同四环素含量为0、100、500和1 000 mg·kg⁻¹分别标记成T0、T100、T500和T1000,每组均设3个平行反应器.为保持水分和湿度,所有反应器用带孔的保鲜膜覆盖,一周喷洒一次自来水并翻堆一次.60 d后,取出蚯蚓和蚯蚓处理后的样品.每个反应器样品分两组保存,一组干燥后研磨,然后过60目筛,置于4℃冰箱保存;另一组置于-20℃冰箱中冷冻保存.

1.3 测试方法

1.3.1 理化指标

将风干样品与去离子水混匀(干样:水=1:50; kg·L⁻¹),磁力搅拌30 min后,测定pH值(PHS-3C, 雷磁).将风干样品与超纯水混匀(干样:水=1:50; kg·L⁻¹),将上述混合液稀释10倍后,过0.45 μm滤膜,用TOC仪(Shimadzu, Japan)测定样品的溶解性有机碳.总碳和总氮采用元素分析仪(Yanaco CHN CORDER MT-6, Japan)进行测定.

1.3.2 DNA提取和高通量荧光定量PCR

将每组的平行样品混合后,利用DNeasy® PowerSoil® Kit(QIAGEN, Germany)试剂盒提取总DNA,然后用1%的琼脂糖电泳进行浓度检测.

本实验选择两种外排泵基因(*tetC*、*tetG*)、两种核糖体保护基因(*tetM*、*tetW*)、一种酶失活基因(*tetX*)及一种整合子基因(*intI1*)进行检测.利用高通量荧光定量PCR进行相对定量检测(Applied Biosystems, ViiA® 7, USA; 上海启因生物技术有限公司).使用的引物与Huang等^[23]一致,采用FastStart Universal SYBR Green Master(ROX, Roche, USA)作为荧光染料.两步qPCR反应条件为:95℃预变性10 min; 40个循环包括95℃变性30 s, 60℃延伸30 s.

1.3.3 高通量测序

采用带有Barcode信息的引物341F(5'-CCTAYGGGRBGCASCAG-3')和806R(5'-GGACTACNNGGTATCTAAT-3')对细菌16S rDNA的V3~V4区进行扩增.高保真酶Phusion® High-Fidelity PCR Master Mix(New England Biolabs)被用于PCR反应.所得到的PCR产物用2%的琼脂糖电泳进行检测.然后使用GeneJET胶回收试剂盒(Thermo Scientific, USA)纯化.利用Ion Plus Fragment Library Kit 48 rxns建库试剂盒(ThermoFisher, USA)建立测序文库.利用Qubit® 2.0 Fluorometer(Thermo Scientific, USA)对其进行检测.合格后在Life Ion S5™测序平台(诺禾致源生物信息科技有限公司)上进行测序.对于结果中低质量部分采用Cutadapt软件进行剪切,然后将序列与UCHIME Algorithm和数据库Gold database进行对比,得出最终的基因序列.

1.4 统计方法

采用Statistica 10统计软件对pH、溶解性有机碳、总碳、总氮、抗性基因丰度在各组间的差异进行one way ANOVA分析(Hsd-test),本实验显著性水平为0.05.

1.5 物种多样性计算

本实验以科水平来对生物多样性进行分析,使用公式(1)计算Shannon指数,公式(2)计算Pielou指数^[24].

$$P_i = \frac{n_i}{N}, \quad H' = - \sum_{i=1}^S (P_i \times \log_2 P_i) \quad (1)$$

$$H_{\max} = \log_2 S, \quad J = \frac{H'}{H_{\max}} \quad (2)$$

式中, S 为群落中所有物种数目; N 为群落中所有个体数目; n_i 为群落中第*i*个物种的个体数量; H' 为香农指数, J 为Pielou指数.

2 结果与讨论

2.1 四环素含量对理化性质的影响

由表 1 可知,与新鲜污泥相比,各组蚯蚓处理产物中 pH 均显著降低($P<0.05$),其中 T0 组的 pH 值最低.与 T0 相比,T100、T500 和 T1000 组 pH 值均略有增加,最大值出现在 T100 组.这一结果说明添加四环素对污泥蚯蚓粪中的 pH 有一定的影响.实验后,各组污泥蚯蚓粪中总碳显著降低了 55.7%~58.8% ($P<0.05$).与 T0 相比,T100 组总碳含量略有降低,而 T500 和 T1000 组略有升高,并且添加四环素与无添加之间无显著性差异($P>0.05$).同时溶解性有机碳也呈现出与总碳相近的变化趋势.对总氮而言,与新鲜污泥相比,

添加四环素的污泥蚯蚓堆肥产物中显著下降了 76.3%~77.9%,最低值出现在 T100 组.并且添加四环素组与无添加组相比均无显著差异($P>0.05$),说明四环素的添加对总氮含量无显著影响($P>0.05$).本研究中四环素的添加对碳、氮含量变化都无显著性影响,可推测其对有机质的降解亦无显著影响.王桂珍等^[25]利用鸡粪进行高温堆肥后,抗生素对有机物的降解无明显的影响.但是郭梦婷等^[26]利用猪粪进行高温堆肥后发现高含量($150\text{ mg}\cdot\text{kg}^{-1}$)的抗生素会抑制微生物对氮的分解.而本研究中 $1\,000\text{ mg}\cdot\text{kg}^{-1}$ 的四环素对总氮仍无影响,可能是由于蚯蚓的存在能够将部分含量的抗生素吸收或是分解,致使其对微生物的影响减弱.

表 1 蚯蚓堆肥前后污泥理化性质的变化¹⁾/ $\text{mg}\cdot\text{g}^{-1}$

Table 1 Changes in physicochemical properties of sludge before and after vermicomposting/ $\text{mg}\cdot\text{g}^{-1}$				
项目	pH	溶解性有机碳	总碳	总氮
IS	$7.40\pm0.01\text{a}$	$0.46\pm0.01\text{a}$	$193.92\pm0.32\text{a}$	$48.51\pm0.28\text{a}$
T0	$6.27\pm0.01\text{b}$	$0.21\pm0.01\text{b}$	$80.43\pm1.68\text{bc}$	$11.51\pm0.24\text{b}$
T100	$6.98\pm0.02\text{c}$	$0.23\pm0.01\text{b}$	$79.87\pm0.01\text{c}$	$10.68\pm0.26\text{b}$
T500	$6.38\pm0.05\text{de}$	$0.26\pm0.00\text{c}$	$83.29\pm1.59\text{bc}$	$11.32\pm0.09\text{b}$
T1000	$6.37\pm0.05\text{be}$	$0.26\pm0.01\text{c}$	$85.95\pm0.37\text{b}$	$11.46\pm0.00\text{b}$

1) IS 代表新鲜污泥, T0、T100、T500 和 T1000 分别代表污泥中四环素含量为 0、100、500 和 $1\,000\text{ mg}\cdot\text{kg}^{-1}$; 同一指标的两个处理组之间存在相同字母表明其两两之间不具有显著差异($P>0.05$), 同行字母之间没有比较意义, 下同

另外, pH 最高值, 总碳和总氮的最低值都出现在 T100 组, 这一原因可能与蚯蚓的生物量有关. 因为在实验过程中发现 T100 组中蚯蚓的生长状态最好. 相关资料表明低含量四环素可作为动物助长剂^[27]; 因此 $100\text{ mg}\cdot\text{kg}^{-1}$ 四环素含量促进了蚯蚓生长, 进而激发了微生物的活性和数量, 加速了碳氮的分解.

2.2 四环素含量对微生物种群的影响

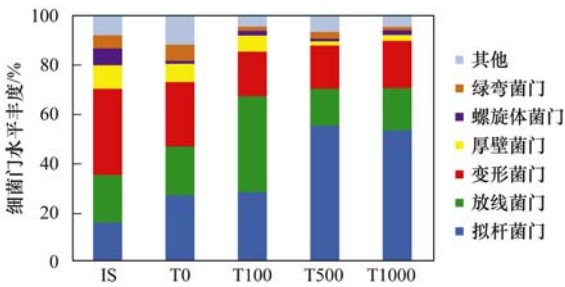
Shannon 指数表示微生物种群多样性的高低, Pielou 指数代表微生物种群均匀度的大小. 由表 2 可知, 与 T0 组相比, 随着加入四环素含量的升高, 微生物种群的多样性和均匀度都呈现出较强的负相关关系. 这说明四环素对微生物的多样性和均匀度均有显著的抑制作用. 人畜粪便好氧堆肥的过程中也发现随着四环素含量的增加, 生物多样性指数呈现下降的趋势^[28].

图 1 为蚯蚓堆肥前后细菌门水平的变化, 新鲜污泥中优势种群主要有变形菌门(35%)、放线菌门(19%)、拟杆菌门(16%)和厚壁菌门(10%). 污泥蚯蚓粪 T0 组中优势种群为拟杆菌门(27%)、变形菌门(26%)和放线菌门(20%). 与 T0 组相比, 添加四环素组中的拟杆菌门均有增加, 在 T500 组表现出最大的增加. 有学者在高温堆肥中也发现添

表 2 新鲜污泥及堆肥产物中微生物 Shannon 指数和 Pielou 指数

Table 2 Microbial indexes of Shannon and Pielou in fresh sludge and vermicomposting products		
项目	Shannon 指数	Pielou 指数
IS	4.96	0.79
T0	4.87	0.77
T100	3.98	0.63
T500	3.30	0.53
T1000	3.25	0.52

加四环素含量为 $500\text{ mg}\cdot\text{kg}^{-1}$ 时也含有大量的拟杆菌门^[28]. 拟杆菌群是大分子有机物的主要降解



IS 代表新鲜污泥, T0、T100、T500 和 T1000 分别代表污泥中四环素含量为 0、100、500 和 $1\,000\text{ mg}\cdot\text{kg}^{-1}$, 下同

图 1 蚯蚓堆肥前后污泥中细菌种群门水平结构变化

Fig. 1 Changes in the bacterial community structure of sludge before and after vermicomposting at the phylum level

者^[29],四环素组中高丰度的拟杆菌群暗示其处理中可能存在诸多大分子有机物未被降解.对放线菌门而言,其丰度与 T0 组相比,在 T100 组升高了 1.97 倍,但是在 T500 和 T1000 组分别减小了 0.77 倍和 0.87 倍.这可能是因为蚯蚓在 T100 组的生长状态较好,蚯蚓的排泄物中放线菌的含量较高所导致的^[30].Huang 等^[31]检测到蔬菜蚯蚓堆肥产物中放线菌的数量达到了 10^{10} copies·g⁻¹.另外,变形菌门在 T0、T100、T500 和 T1000 的丰度分别为 26%、18%、18% 和 19%,说明添加四环素会抑制变形菌门丰度,且这种抑制与其剂量无关.

图 2 表示蚯蚓堆肥前后微生物种群属水平的变

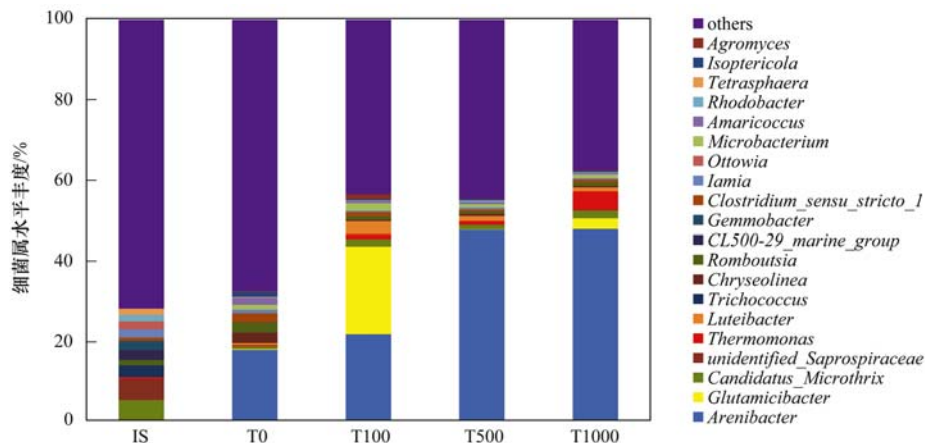


图 2 蚯蚓堆肥前后细菌种群属水平结构变化

Fig. 2 Changes in the bacterial community structure of sludge before and after vermicomposting at the genus level

2.3 四环素含量对抗性基因的影响

由图 3(a)可知,与新鲜污泥相比,T0 组的 *intI1* 基因丰度显著降低了 0.7 倍 ($P < 0.05$).与 T0 组相比,添加抗生素的处理组中 *intI1* 基因丰度显著增高了 5.1 ~ 5.7 倍 ($P < 0.05$),但 T100、T500 和 T1000 这 3 组之间无显著性差异 ($P > 0.05$).表明尽管添加了较低的四环素含量,但仍会使污泥蚯蚓粪中 *intI1* 基因的丰度增加.*intI1* 基因是整合子基因,是抗性基因传播的载体.在添加四环素后,四环素对细菌产生选择性压力,使 *intI1* 基因丰度增加,从而导致细菌中抗性基因的丰度增加^[35].在蚯蚓堆肥后 *intI1* 基因丰度的显著降低,证明了蚯蚓堆肥可以抑制整合子的丰度,控制抗性基因的传播.这与之前的研究结果一致^[23, 36].

与新鲜污泥相比,T0 组外排泵类基因 *tetC* 和 *tetG* 的丰度略有升高,但是增加量不具有显著性差异 ($P > 0.05$).T0 组中核糖体保护基因 *tetM* 和 *tetW* 的丰度都有一定的下降,钝化酶基因 *tetX* 丰度显著降低 ($P < 0.05$),这表明蚯蚓堆肥对污泥中抗性基因丰度的消减具有一定的选择性.值得注意的是,蚯蚓堆肥对相同机制的抗性基因产生的影响是类似

的.新鲜污泥中优势菌属为 *Candidatus_Microthrix* (5%) 和 *unidentified_Saprospiraceae* (5%).蚯蚓处理后,添加四环素实验组的蚯蚓粪中 *Arenibacter* 的丰度比 T0 组增加了 1.26 ~ 2.66 倍.*Arenibacter* 是一种可以降解多环芳烃的细菌^[32].高丰度的 *Arenibacter* 出现在高含量四环素组可能和四环素降解的过程中产生大量的多环芳烃类物质有关.*Glutamicibacter* 是属于放线菌门中的一类微生物^[33],是一种农业益生菌,其大量存在,对于污泥蚯蚓堆肥产物的农用起到良好的促进作用^[34].*Glutamicibacter* 在 T100 组的丰度极高可能是由于蚯蚓的活性较好所致.

的,这与韦蓓等^[37]的研究结果相似.与 T0 相比,添加四环素组抗性基因的相对丰度都有所升高 (T100 组的 *tetW* 除外),其中 *tetC*、*tetG* 和 *tetM* 这 3 种基因均在 T1000 组呈现出最高丰度.这一结果进一步证实高四环素含量增加了其抗性基因的选择性压力,导致抗性基因的丰度增加^[21].另外本研究还发现 *tetM* 的基因丰度与四环素含量呈现出极强的线性关系 ($r = 0.998$).虽然高温堆肥也会使抗性基因 *tetM* 的丰度升高,但这种选择性压力下的线性关系在高温堆肥中并没有出现^[20, 21, 38].另外 *tetX* 基因丰度在 T100 组呈现出最大值,这可能与蚯蚓肠道自身携带的抗性基因有关.江承亮等^[39]利用猪粪蝇蛆堆肥发现其最终产物中的抗性基因多样性和蝇蛆肠道有密切的关系.

已有研究表明,抗性基因的多样性和微生物种群以及环境因子均有密不可分的关系^[40].污泥中抗生素含量不仅能够改变微生物的生存环境,而且也会直接对微生物的生长和繁殖产生抑制作用,致使微生物的种群结构发生变化,进而影响抗性基因的多样性^[41].在蚯蚓堆肥体系中,蚯蚓也会直接和间接地改变污泥微环境,影响微生物种群结构^[18].

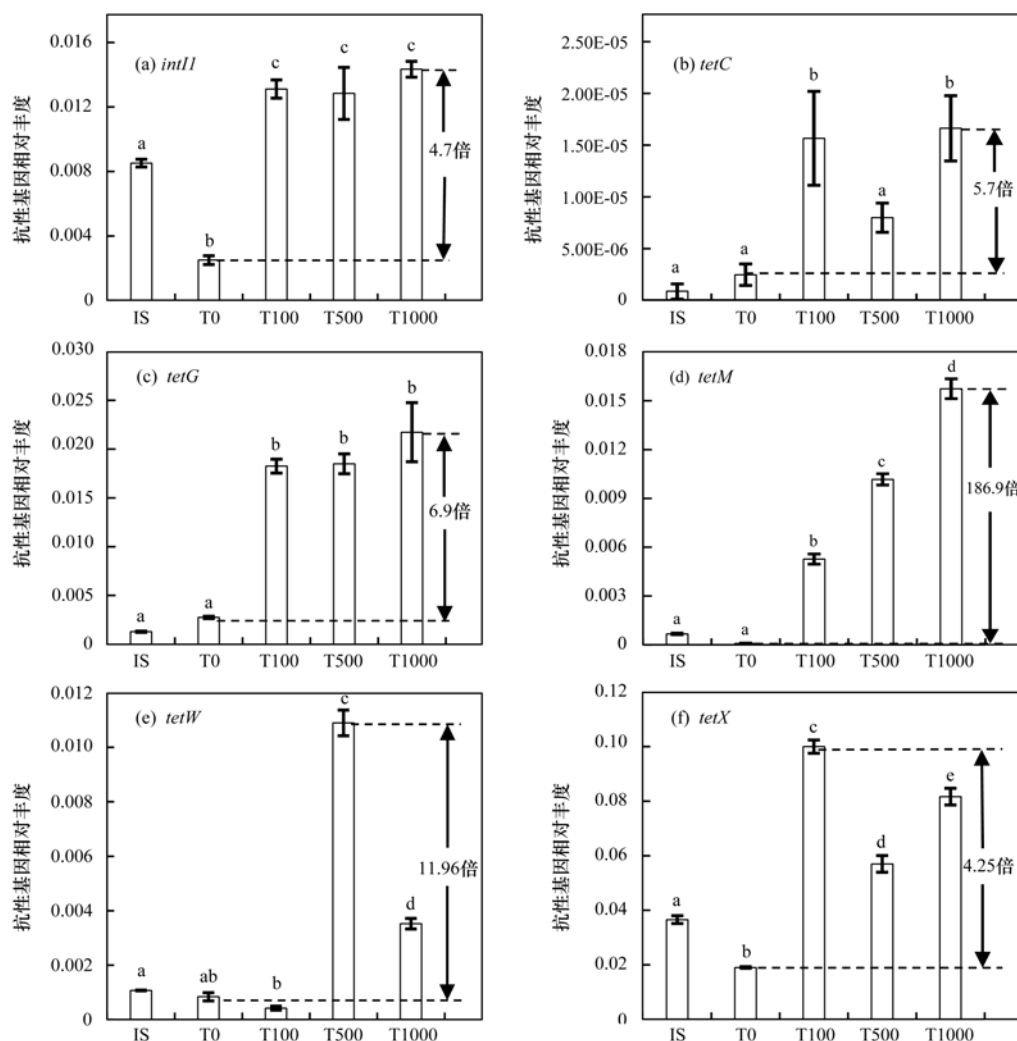


图3 蚯蚓堆肥前后污泥中 *intII* 基因与四环素抗性基因的相对丰度

Fig. 3 Changes in the relative abundances of *intII* genes and antibiotic resistant genes of sludge before and after vermicomposting

因此在蚯蚓堆肥体系中,如何准确揭示蚯蚓、宿主微生物、抗性基因及其环境因子之间的关系,仍有待进一步研究。

3 结论

(1) 初始污泥中高含量的四环素抑制蚯蚓堆肥产物中微生物种群的多样性和均匀度,改变微生物菌群结构。

(2) 初始污泥中高含量的四环素增加蚯蚓堆肥产物中抗性基因传播的风险。

(3) 蚯蚓堆肥前宜控制初始污泥中四环素含量。

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CONTENTS

Health Benefit Evaluation for Air Pollution Prevention and Control Action Plan in China	WU Wei-ling, XUE Wen-bo, WANG Yan-li, <i>et al.</i> (2961)
Scenario Simulation Study Constrained by the Ambient Air Quality Standards in Nanjing	XIE Fang-jian, SHI Zhi-hao, LI Jing-yi, <i>et al.</i> (2967)
Characteristics and Source Analysis of Water-Soluble Ions in PM _{2.5} in Zhengzhou	YANG Liu-ming, WANG Shen-bo, HAO Qi, <i>et al.</i> (2977)
Relationship Between Atmospheric Visibility and PM _{2.5} Concentrations and Distributions	WANG Ji-kang, ZHANG Heng-de, GUI Hai-lin, <i>et al.</i> (2985)
Characteristics of VOCs Emitted from the Rubber Tire Manufacturing Industry Based on the Inverse-Dispersion Calculation Method	BAI Hong-xiang, WEI Wei, WANG Ya-ting, <i>et al.</i> (2994)
Stable Carbon Isotope Compositions and Source Apportionments of Volatile Aromatic Compounds in the Urban Atmosphere of Taiyuan, China	LI Ying-hui, YAN Yu-long, LI Zhi-sheng, <i>et al.</i> (3001)
Spectral Distribution and Pollution Characteristics of Polybrominated Diphenyl Ethers in the Air of an Office Building Clustered Area	WANG Lin, DENG Ya-jia, LIAO Xiao-yong, <i>et al.</i> (3008)
Characterizing Chromophoric Dissolved Organic Matter in Key Lakes in the Middle Reaches of the East Route of the South-North Water Diversion Project	ZHANG Liu-qing, PENG Kai, ZHOU Lei, <i>et al.</i> (3018)
Characteristics of Hydrogen and Oxygen Isotopes in Different Water Bodies in Hilly and Gully Regions of the Loess Plateau	ZHANG He-hui-zi, YU Kun-xia, LI Zhan-bin, <i>et al.</i> (3030)
Analysis of Total Organic Carbon Source Differences Between New and Old Cascade Reservoirs using Carbon and Nitrogen Isotopes	WANG Cong-feng, HU Zi-long, YANG Zheng-jian, <i>et al.</i> (3039)
Pollution Sources and the Stratification Effects on Water Quality in Lijiahe Reservoir	XU Jin, HUANG Ting-lin, LI Kai, <i>et al.</i> (3049)
Distribution and Potential Risk of Organochlorine Pesticides in the Soil of a Submerged Area Around Miyun Reservoir	WANG Xia, ZHANG Qing-zhuo, ZHAO Gao-feng, <i>et al.</i> (3058)
Distribution, Sources, and Ecological Risk Assessment of Polycyclic Aromatic Hydrocarbons in the Surface Waters of the Yinchuan Wetlands	TIAN Da-nian, DANG Li-hui, DING Run-mei, <i>et al.</i> (3068)
Comparison of the Geochemical Characteristics of Karst Springs of a Vertically Zoned Climate Region under Human Activity: A Case of Shuifang Spring and Bitan Spring in the Jinfo Mountain Area, Chongqing	XIE Guo-wen, YANG Ping-heng, SHENG Ting, <i>et al.</i> (3078)
Groundwater Chemical Characteristics and Analysis of Their Controlling Factors in an Alluvial Fan of Jianjiang River	TANG Jin-ping, ZHANG Qiang, HU Yang, <i>et al.</i> (3089)
Dynamics of Epilithic Algae Communities and Their Relationship with Environmental Factors During Storage and Non-storage Periods in the Three Gorges Reservoir	FU Jun-ke, LIU Li, HE Xin-yu, <i>et al.</i> (3099)
Influence of Rainfall on the <i>in situ</i> Growth of Dominant Algae Species in Xiangxi River	WENG Chuan-song, LIU De-fu, ZHANG Jia-lei, <i>et al.</i> (3108)
Decomposition of <i>Myriophyllum aquaticum</i> and the Associated Release of Nitrogen and Phosphorus	TONG Xiong, LUO Pei, LIU Feng, <i>et al.</i> (3118)
Optimization of the Parameters for Microalgae Immobilization and Analysis of Its Recovery Potential for Ammonia Nitrogen in Wastewater	LIU Xiang, WANG Jing-yao, WU Juan-juan, <i>et al.</i> (3126)
Ammonium Adsorption Characteristics in Aqueous Solution by Titanate Nanotubes	ZHANG Zheng, FENG Chang-sheng, ZHANG Xiao-nui, <i>et al.</i> (3135)
Application of Fenton-like Photocatalysts Based on Defect Reconstruction in Degradation of Dye Wastewater	TANG Qing-wen, AN Xiao-qiang, LAN Hua-chun, <i>et al.</i> (3146)
Degradation of RBK5 with Peroxymonosulfate Efficiently Activated by N-Doped Graphene	YU Yong-bo, HUANG Wan, DONG Zheng-yu, <i>et al.</i> (3154)
Effect of Cu ²⁺ on Denitrification Using NO ₂ ⁻ as an Electron Acceptor	MAI Wen-ke, PENG Yong-zhen, JI Jian-tao (3162)
Start-up and Performance Optimization of a CANON Pilot Reactor	SUN Qing-hua, WU Di, ZHOU Jia-zhong, <i>et al.</i> (3169)
Effects of Aerobic Carbon Sources on Biofilm with Simultaneous Phosphate Removal and Enrichment	XU Lin-jian, PAN Yang, ZHANG Hao, <i>et al.</i> (3179)
Effect of Organic Characteristics on Sludge Settability in an AAO System	LIU Xiao-bo, YUAN Lin-jiang, CHEN Xi, <i>et al.</i> (3186)
Effect of Free Nitrous Acid on the Activity of Nitrifying Bacteria in Different Sludge Concentrations Under Anoxic Conditions	LÜ Xin-tao, ZHOU Tong, TIAN Xia-di, <i>et al.</i> (3195)
Stability of ZVI-dependent Autotrophic Denitrification by ANAMMOX Bacteria	ZHANG Wen-jing, HUANG Yong, BI Zhen, <i>et al.</i> (3201)
Effect of On-line NaClO Backwashing on Microbial Communities in an Inverted A ² O-MBR System	WANG Xu-dong, GAO Miao, WANG Ying-ying, <i>et al.</i> (3208)
Evaluation of Organic Matter Release and Economy for Various Pretreatments of Sewage Sludge	YUAN Yue, TAN Xue-jun, ZHENG She-yu (3216)
Model-based Optimization for the Coordinated Supply of Clear and Reclaimed Water in the Central Districts of Beijing, China	ZHANG Tian-yuan, TAN Qian, WANG Shu-ping (3223)
Occurrence and Removal of N-nitrosamines in the Wastewater Treatment Plants Using Different Treatment Processes	LIU Wang-rong, ZHAO Jiang-liang, YANG Yuan-yuan, <i>et al.</i> (3233)
Occurrence of Tramadol and Fentanyl Use in Domestic Wastewater in Beijing	ZHOU Zi-lei, DU Peng, BAI Ya, <i>et al.</i> (3242)
Pollution Level and Ecological Risk of Typical Antibiotics in Guiyang Wastewater Treatment Plants	YANG Zhao, LI Jiang, ZHANG Sheng-hu, <i>et al.</i> (3249)
Characteristics of Antibiotic Resistance Genes in Various Livestock Feedlot Soils of the Hilly Purple Soil Region	CHENG Jian-hua, TANG Xiang-yu, LIU Chen (3257)
Effects of Tetracycline on Microbial Communities and Antibiotic Resistance Genes of Vermicompost from Dewatered Sludge	CHEN Jing-yang, XIA Hui, HUANG Kui, <i>et al.</i> (3263)
Fate of Antibiotic Resistance Genes and Virulence Genes in Enterococci During Anaerobic Digestion Process of Thermal Hydrolyzed Sludge	LI Hui-li, PEI Yuan-mei, LI Shan, <i>et al.</i> (3270)
Effect of Co-composting of Chicken Manure with Chinese Medicinal Herbal Residues on Antibiotic Resistance Genes	WU Jin-ping, CHEN Jian-wen, LIU Yong, <i>et al.</i> (3276)
Spatio-temporal Patterns of Microbial Communities and Their Driving Mechanisms in Subalpine Lakes, Ningwu, Shanxi	WANG Xue, LIU Jin-xian, CHAI Bao-feng, <i>et al.</i> (3285)
Community Structure Characteristics of <i>nirS</i> Denitrifying Bacteria of Spring Typical Parkland Waterbodies in Shijiazhuang City	ZHANG Yi-ran, LI Zai-xing, SUN Yue, <i>et al.</i> (3295)
Differential Responses of Rhizospheric <i>nirK</i> - and <i>nirS</i> -type Denitrifier Communities to Different Phosphorus Levels in Paddy Soil	ZHAN Yu, GAO Dan-dan, SHENG Rong, <i>et al.</i> (3304)
Comparison of Soil Bacterial Community Structure Between Paddy Fields and Dry Land in the Huixian Karst Wetland, China	JIA Yuan-hang, JIN Zhen-jiang, YUAN Wu, <i>et al.</i> (3313)
Cd Balance Analysis of a Typical Rice Paddy System in Central Hunan	JIANG Kai, DENG Xiao, ZHOU Hang, <i>et al.</i> (3324)
Effects of an Amendment on Cadmium Transportation in the Rhizosphere Soil-Rice System	LI Yi-chun, WANG Yan-hong, TANG Ming-deng, <i>et al.</i> (3331)
Isolation of Heavy Metal Immobilizing and Plant Growth-Promoting Bacteria and Its Effects on Reducing Heavy Metal Accumulation in Wheat	HAN Hui, WANG Xiao-yu, CAI Hong, <i>et al.</i> (3339)
Differences in the Cadmium-Enrichment Capacity and Subcellular Distribution and Chemical Form of Cadmium in Different Varieties of Pepper	PENG Qiu, LI Tao, XU Wei-hong, <i>et al.</i> (3347)
Effect of Phosphorus Addition on N ₂ O Emissions from Rice-Rapeseed Rotation Soils	SUN Zheng, SU Rong-lin, XU Peng, <i>et al.</i> (3355)
Impact of Land Use Type on the Stability and Organic Carbon Content of Soil Aggregates in the Weibei Dryland	LIU Jie, MA Yan-ting, WANG Xian-ling, <i>et al.</i> (3361)
Vertical Distribution Characteristics of PAHs in Soils with Different Land Use Types During Rapid Urbanization	LU Yin-tao, XIANG Xin-xin, ZHANG Shi-chao, <i>et al.</i> (3369)
Pollution Levels, Sources, and Spatial Distribution of Phthalate Esters in Soils of the West Lake Scenic Area	LIAO Jian, DENG Chao, CHEN Yi, <i>et al.</i> (3378)
Impact of Biochar on Soil Bulk Density and Aggregates of Lou Soil	LI Qian-qian, XU Chen-yang, GENG Zeng-chao, <i>et al.</i> (3388)