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微塑料和多种抗生素胁迫下土壤环境因子的响应特性*

韩冰¹ 李华南^{2,3} 王梓静² 劳旭婷² 冯辰² 金雨萱² 赵薇²
苑子仪² 金信成⁴ 张明哲² 孙世梅^{1**}

(1. 吉林建筑大学 应急科学与工程学院, 长春, 130118; 2. 吉林建筑大学 松辽流域水环境教育部重点实验室, 长春, 130118;
3. 吉林省东昇创联建筑工程有限公司, 通化, 134002; 4. 吉林省通化市城市投资集团工程有限公司, 通化, 134001)

摘要 为了深入探究微塑料 (microplastics, MPs)、抗生素胁迫下土壤环境因子产生的响应特性, 以聚乙烯 (polyethylene, PE)、四环素 (tetracycline, TC)、环丙沙星 (ciprofloxacin, CIP) 为研究对象, 通过单独、联合施用到土壤中 4 周后, 开展了土壤的理化性质、酶活性、抗生素残留、抗生素抗性菌 (antibiotics resistant bacteria, ARB) 抗性、抗生素抗性基因 (antibiotic resistance genes, ARGs)、微生物群落的多样性等方面研究. 结果显示, 施用 MPs 与抗生素的容重比对照组分别增大了 12.3%、16.9%、21.8%. 有机质含量由 39.96 g·kg⁻¹ 变化为 53.21 g·kg⁻¹, 与对照组相比分别增大了 9.16%、12.39%、14.09%、18.47%、32.03%、33.16%、36.04%. 阳离子交换量由对照组的 44.36 cmol·kg⁻¹ 显著变化为 62.45 cmol·kg⁻¹, 与对照组相比分别增大了 24.06%、30.09%、33.97%、36.49%、47.14%、50.93%、56.1%. 实验各组 pH 值在 7.58 — 8.12 之间变化. 实验各组的过氧化氢酶含量分别为 1.653、1.559、1.421、1.486、1.376、1.545、1.524、1.453 IU·g⁻¹; 脲酶含量分别为 89.56、78.32、64.65、66.79、57.27、72.31、71.26、61.56 IU·g⁻¹; 蔗糖酶含量分别为 158.69、149.61、134.56、131.87、123.65、137.26、136.83、126.34 IU·g⁻¹. MPs-TC-CIP 组的过氧化氢酶活性、脲酶活性、蔗糖酶活性分别下降 12.1%、32.3%、26.7%. MPs-TC、MPs-CIP 组与 TC、CIP 实验组相比, 抗生素残留量有所降低, 分别为 187.1%、189.3%; MPs-TC-CIP 实验组的抗生素残留则低于单一施用 CIP、TC 组, 分别为 182.6%、178.7%. 筛选的 TC 抗性菌 (TC resistant bacteria, TCRB) 和 CIP 抗性菌 (CIP resistant bacteria, CIPRB) 的抗性增加 2 倍以上. MPs-TC-CIP 组与对照组相比, *tet W*、*tet O* 的相对丰度比值分别为 1.82、1.78; *qnr A*、*qnr S* 的相对丰度比值分别为 1.68、1.71. 各组中相对丰度较高菌群依次为变形菌 (*Proteobacteria*), 放线菌 (*Actinobacteria*), 酸杆菌 (*Acidobacteria*), 芽单胞菌 (*Gemmatimonadetes*), 厚壁菌 (*Fimicutes*). 施用抗生素、MPs 后 *Proteobacteria*、*Actinobacteria* 相对丰度增加、*myxococcus* 相对丰度减少. 研究结果表明, 施用 MPs 能促进抗生素的富集; 在共同胁迫下, 对土壤理化特性和生物特性影响更为显著, 进一步探明了 MPs 对抗生素残留、ARGs 传播、微生物群落演化特征等潜在影响, 以期为消减污染物的危害、构建综合智能修复体系提供依据.

关键词 微塑料, 抗生素, 抗性菌, 酶活性, 微生物群落多样性.

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** 通信联系人 **Corresponding author**, Tel: 0431-84566408, E-mail: sunshimei1966@jlju.edu.cn

Response characteristics of soil environmental factors under the stress of microplastics and antibiotics

HAN Bing¹ LI Huanan^{2,3} WANG Zijing² LAO Xuting² FENG Chen²
 JIN Yuxuan² ZHAO Wei² YUAN Ziyi² JIN Xincheng⁴
 ZHANG Mingzhe² SUN Shimei^{1**}

(1. School of Emergency Science and Engineering, Jilin Jianzhu University, Changchun, 130118, China; 2. Key Laboratory of Songliao Aquatic Environment, Ministry of Education, Jilin Jianzhu University, Changchun, 130118, China; 3. Jilin Province Dongsheng Chuanglian Construction Engineering Co., Ltd., Tonghua, 134002, China; 4. Jilin Province Tonghua City Investment Group Engineering Co., Ltd., Tonghua, 134001, China)

Abstract In order to investigate the effects of microplastics and antibiotics on the response characteristics of soil environmental factors, MPs, Tetracycline (TC) and Ciprofloxacin (CIP) were applied into soil for 4 weeks separately or jointly. In this experiment, a large number of studies were carried out, such as the physicochemical of soil, enzyme activity, antibiotic residues, resistance of antibiotics resistant bacteria (ARB), the antibiotic resistance genes (ARGs) and the diversity of microbial community. As a results, the unit weight of soil were increased 12.3%, 16.9% and 21.8%, respectively. Compared with the control group, the content of organic matter were changed from 39.96 g·kg⁻¹ to 53.21 g·kg⁻¹ by PE and antibiotic. They were increased 9.16%, 12.39%, 14.09%, 18.47%, 32.03%, 33.16% and 36.04%, respectively. So the cation exchange capacity was significantly changed from 44.36 cmol·kg⁻¹ to 62.45 cmol·kg⁻¹, and they were increased 24.06%, 30.09%, 33.97%, 36.49%, 47.14%, 50.93% and 56.1%, respectively. The pH of each group varied from 7.58 to 8.12. The catalase of each group were 1.653, 1.559, 1.421, 1.486, 1.376, 1.545, 1.524, 1.453 IU·g⁻¹, respectively. The urease activity of soil was 89.56, 78.32, 64.65 IU·g⁻¹, 66.79, 1.57.27, 72.3, 71.26 and 61.56 IU·g⁻¹, respectively. The sucrase activities were 158.69, 149.61, 134.56, 131.87, 123.65, 137.26, 136.83 and 126.34 IU·g⁻¹, respectively. The activity of catalase, urease and sucrase with MPs and two antibiotics were reduced 12.1%, 32.3% and 26.7%, respectively. Compared with the control group, the residues of antibiotics were 187.1% and 189.3% by MPs-TC and MPs-CIP, respectively. Additionally, they were lower than the control group, 182.6% and 178.7% by MPs-TC-CIP, respectively. The relative abundance ratios of *tet* W and *tet* O were 1.82 and 1.78, respectively. And the relative abundance ratios of *qnr* A and *qnr* S were 1.68 and 1.71, respectively. The relative abundance of microbial community were followed by *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Gemmatimonadetes* and *Fimicutes*. After the application of antibiotics and MPs, the relative abundance of *Proteobacteria* and *Actinobacteria* were increased, while the relative abundance of *Myxococcus* were decreased. The results showed that MPs could promote the enrichment of antibiotics. Under co-stress with MPs-TC-CIP, the effect of physical, chemical and biological characteristics on soil were more significant than the MPs, TC, and CIP alone or mixed. Furtherly, the potential effects of MPs on were explored, such as antibiotic residues, transmission of ARGs and the microbial community diversity, which were laid a foundation for reducing the harm of pollutants and building a comprehensive intelligent remediation system.

Keywords microplastics, antibiotics, antibiotics resistant bacteria, enzyme activity, microbial diversity.

近年来, 在环境中不断增加的塑料制品经不断碎裂、分化为粒径微小、难降解的微塑料(MPs), 广泛分布于土壤、水环境、大气^[1-2]. Feng、Akdogan 等认为, MPs 比表面积大、附着位点多, 对土壤酸碱度、持水量、孔隙度、湿度、团聚结构、生物量、酶活性等均产生较为严重的不良作用, 对植物、动物的

生长产生毒性作用^[3-4]。胡晓婧认为,施用聚苯乙烯后土壤可溶性有机碳、氮、磷增加,微生物代谢通路会做出响应,种植的大豆干重降低、根直径减小^[5]。此外,国外学者发现微塑料引起土壤的容重、团聚体、持水性、微生物群落多样性等理化特性和生物学性质出现较大的变化,影响某些降解功能基因表达,加快了土壤中氮循环、碳循环,增强了对多种污染物吸附性,调控能力受到一定的影响,损害了生态环境健康^[6]。此外,作为新兴污染物的抗生素对生态环境、人类健康带来的潜在危害也是当下需要特别重点关注和彻底解除^[7]。抗生素在预防和治疗人类和动物疾病的同时,也残留在污水处理厂终端、养殖场、灌溉沟渠、垃圾处理厂等。其中,聚乙烯(PE)是无味、无毒、耐侵蚀、电绝缘性高、应用性广的塑料产品。四环素(TC)、环丙沙星(CIP)是目前在治疗多种疾病中应用较广、较多的药物,但是也可能影响酶活性、传递抗性基因,危害生命安全^[8-9]。进入土壤中的抗生素影响呼吸、矿化、硝化作用,并通过基因突变、基因转移、土著菌的变性,对生态环境、人类的造成不可逆转危害,迫切需要加大力度进行综合处理^[10-11]。已报道的研究表明,MPs和抗生素的复合污染产生的生态效应更为复杂、危害更为严重。周曙屹等认为MPs改变ARGs的丰度、分布^[12]。目前有关MPs与抗生素的复合污染研究多集中于水培试验所获得研究结论,而对土壤的联合污染研究较少^[13],分子水平的研究更为罕见,亟需从分子水平上阐明微塑料—抗生素在土壤富集、吸附、降解、响应效应^[14]。

基于项目组前期研究工作的基础上,选择在土壤中普遍存在且检出率较高的PE、TC、CIP为研究对象,开展复合污染对土壤的理化特性、酶活性、抗生素残留、四环素抗性菌(TC resistant bacteria, TCRB)抗性、环丙沙星抗性菌(CIP resistant bacteria, CIPRB)抗性、抗生素抗性基因(antibiotic resistance genes, ARGs)的研究,进一步探明了MPs对抗生素残留、ARGs传播、微生物群落演化特征等潜在影响,以期为消减污染物的危害、构建综合智能修复体系提供依据。

1 材料与方法(Materials and methods)

1.1 试剂

青霉素(PNC)、利福平(RFP)、TC、磺胺甲噁唑(SMZ)、氯霉素(CHL)、CIP、庆大霉素(CN)等购自中国药品监督所。MPs购自上海阳励机电科技股份有限公司。DNA提取试剂盒购自TIANGEN公司。

1.2 实验设计

在2020年8月,采集北方某城市郊区农田剔除残渣的土壤,风干、研磨、过筛,按照每份500g,装于15cm×20cm×30cm花盆内。选择PE(粒径为204.6 μm、比表面积为2.11 m²·g⁻¹的粉末)、分别将实验各组添加物浓度配制为1.0 g·kg⁻¹,按照表1分组设计投加到土壤,4周后,进行土壤的理化特性、抗生素的残留量、ARB的抗性、ARGs的丰度、微生物群落多样性的检测^[15]。具体实验设计见表1。

表1 实验分组设计

Table 1 Experimental group design

组别 Group	添加物 Additives	备注 Note
CK	未添加	
M1	PE	
M2	TC	
M3	CIP	将PE、TC、CIP配制浓度为1.0 g·kg ⁻¹ ,投加到土壤4周,每组随机分离10株抗性菌,进行土壤的理化特性、抗生素残留、ARB、ARGs、微生物群落等检测。
M4	TC+CIP	
M5	PE+TC	
M6	PE+CIP	
M7	PE+TC+CIP	

1.3 土壤特性的检测、分析

按照文献[16]的方法,将微塑料、抗生素胁迫的土壤样品处理后,采用环刀法进行土壤称重后,计算土壤容重;分别使用氯化钡-硫酸强迫交换法、酸度计、重铬酸钾容量-稀释热法,依次测定土壤的阳离子交换量、pH、有机质含量,每个样品测定3次。

1.4 土壤酶活性的检测、分析

按照文献 [17] 的方法,分别通过氨释放、高锰酸钾滴定、葡萄糖含量检测方法,分别进行了脲酶、过氧化氢酶、蔗糖酶等多种酶活性的检测,并按照相关公式进行计算分析。

1.5 抗生素残留量的检测

按照文献 [18] 方法,采集的土壤样品经氢氧化钠-甲醇混合液处理、二氯甲烷萃取、浓缩、梯度洗脱、回收、净化后,分别进行 TC、CIP 的检测。

1.6 抗生素抗性菌的抗性分析

按照文献 [19] 方法,制备普通营养琼脂培养基上,分别添加 $16 \text{ mg}\cdot\text{mL}^{-1}$ 的 TC、 $4 \text{ mg}\cdot\text{mL}^{-1}$ CIP 制备抗生素的培养皿。取适量过滤样品,分别涂布在 TC、CIP 的培养基上,经 $37 \text{ }^{\circ}\text{C}$ 的恒温箱内培养一段时间,挑取单个菌落,继续培养。按照临床实验室标准化协会的规则,每组随机挑取 10 株抗性菌,分别检测 SMZ、TC、PNC、CIP、CHL、CN、RFP 的抗性。

1.7 抗生素抗性基因的检测

收集、处理每组的土壤样品后,按照文献 [20-21] 方法,分别设计引物序列进行 TC 类抗性基因 (TC antibiotic resistance genes, TCARGs) 中选取 *tet W*、*tet O* 目的基因和环丙沙星抗性基因 (CIP antibiotic resistance genes, CIPARGs) 中 *qnr A*、*qnr S* 的目的基因,使用土壤 DNA 试剂盒分别提取 TC、CIP 目的基因后使用 1.5% 的琼脂糖凝胶电泳进行检测。

1.8 土壤微生物群落分析

按照文献 [22] 的方法,设计引物、应用试剂盒进行 PCR 扩增、回收、纯化后用进行抗生素和微塑料复合暴露下对微生物群落生物学信息的研究。

1.9 数据统计与分析

经多种仪器设备检测的数据,使用 Excel、SPSS16.0、Origin 软件,采用样 T 检验、单因素方差、Turkey's 等方法进行数据统计、分析与图表制作。

2 结果与讨论 (Results and discussion)

2.1 土壤特性的检测结果

土壤的酸碱度、矿物质、离子交换量、深度、温度、有机质、持水量等特性对抗生素的降解、转化、吸附、残留、迁移等都产生不同的影响^[23]。抗生素的疏水性受到酸碱度影响,官能团与矿物质形成螯合物影响吸附性能,改变土壤的离子交换量。MPs 可以通过吸附某些污染物、堵塞细胞壁孔隙、抑制营养物质转运,导致生态环境的污染加剧^[24]。本研究中,实验各组的容重依次为 1.022、1.081、1.113、1.098、1.131、1.147、1.194、 $1.245 \text{ g}\cdot\text{cm}^{-3}$;施用 MPs-抗生素的实验组与对照组相比,容重分别为增大了 12.3%、16.9%、21.8%。有机质含量由 $39.96 \text{ g}\cdot\text{kg}^{-1}$ 变化为 $53.21 \text{ g}\cdot\text{kg}^{-1}$,施用 MPs、抗生素的实验组与对照组相比,分别增大了 9.16%、12.39%、14.09%、18.47%、32.03%、33.16%、36.04%。阳离子交换量由对照组的 $44.36 \text{ cmol}\cdot\text{kg}^{-1}$ 显著变化为 $62.45 \text{ cmol}\cdot\text{kg}^{-1}$,施用 MPs、抗生素的实验组与对照组相比,分别增大了 24.06%、30.09%、33.97%、36.49%、47.14%、50.93%、56.1%。实验各组 pH 值在 7.58 — 8.12 之间变化。2 种抗生素和 MPs 协同作用下对土壤的容重、阳离子交换量、有机质含量产生不同的影响,而对酸碱度的变化影响较小,结果见图 1。

2.2 土壤中多种酶活性的检测结果

TC 和 CIP 的含有三酰胺、羧酸根等特殊官能团,易形成螯合物,依靠氢键、离子键破坏非酶活性中心,具有较高的迁移性、降解性,多种土壤酶的活性受到不同的影响^[25]。由于抗生素、微塑料进入土壤后对物理化学性质、碳循环、氮循环产生相应影响,进而各种酶的活性出现相应的变化^[26]。单独施用微塑料、抗生素后,酶活性均下降,复合施用后酶活性降低更为显著。实验各组的过氧化氢酶分别为 1.653、1.559、1.421、1.486、1.376、1.545、1.524、 $1.453 \text{ IU}\cdot\text{g}^{-1}$;脲酶分别 89.56、78.32、64.65、66.79、57.27、72.3、71.26、 $61.56 \text{ IU}\cdot\text{g}^{-1}$;蔗糖酶分别为 158.69、149.61、134.56、131.87、123.65、137.26、136.83、 $126.34 \text{ IU}\cdot\text{g}^{-1}$ 。TC 和 CIP 复合施用引起过氧化氢酶活性、脲酶活性、蔗糖酶活性分别下降 16.7%、36.1%、23.1%。MPs 与 2 种抗生素能导致过氧化氢酶活性、脲酶活性、蔗糖酶活性分别下降 12.1%、32.3%、26.7%。结果见图 2。

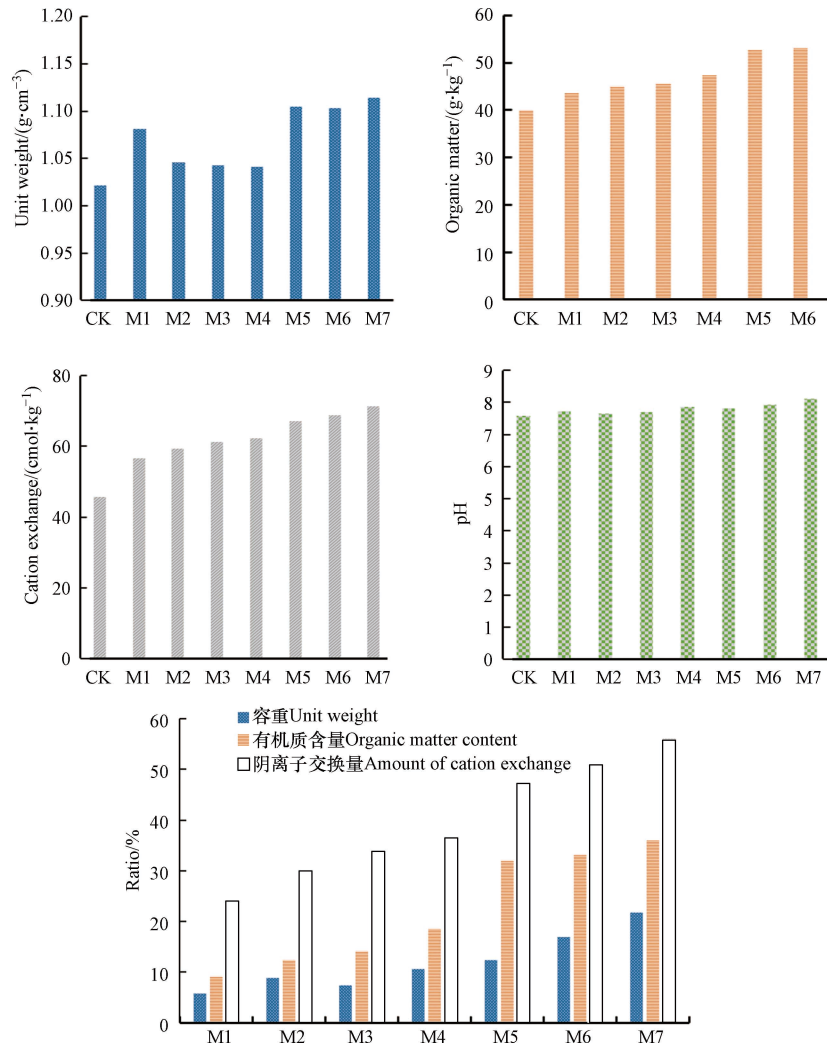


图 1 土壤的特性检查结果

Fig.1 Test results of soil characteristics

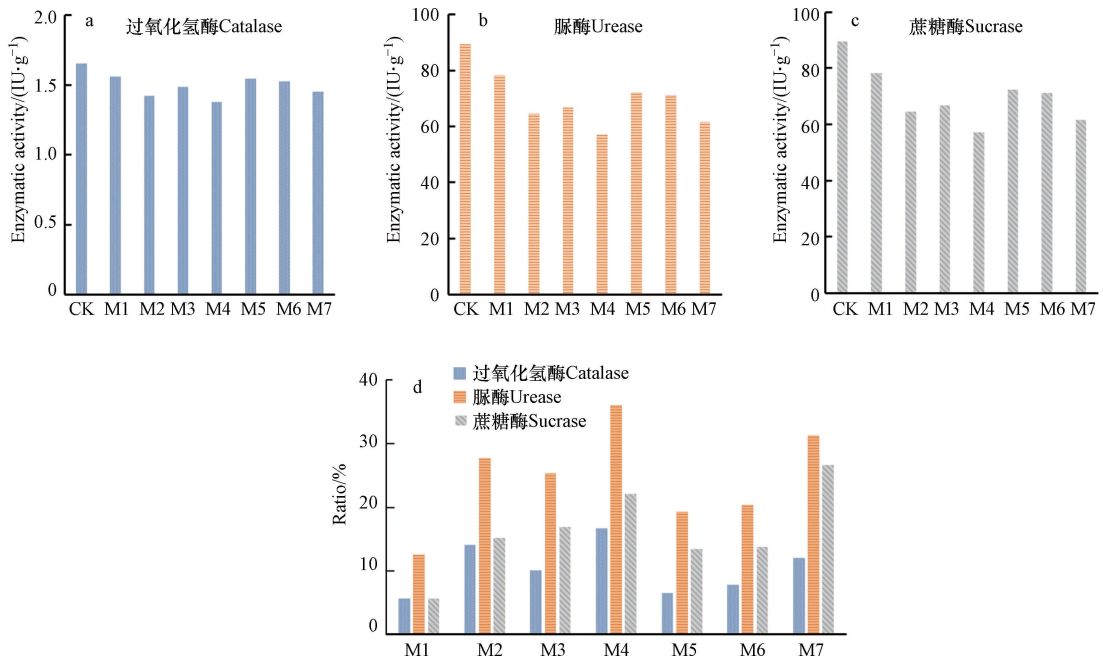


图 2 土壤中三种酶活性的检测结果

(a)过氧化氢酶 (b)脲酶 (c)蔗糖酶 (d)降低比率

Fig.2 Three kinds of enzyme activities in soils

(a) Catalase (b) Urease (c) Sucrase (d) Reduction ratio of three enzymes

2.3 土壤中抗生素残留量的测定结果

由图 3 可见, 单一施用 MPs 实验组的 TC、CIP 含量分别为 93.8%、95.2%, 表明少量的抗生素被 MPs 吸附, 致使土壤残留的抗生素比例略有降低. 而单一施用 TC、CIP 实验组的残留量分别为 216.2%、202.3%; MPs-TC 与 MPs-CIP 组添加抗生素的残留量分别为 187.1%、189.3%; MPs-TC-CIP 实验组的抗生素残留则低于单一施用 CIP、TC 组, 分别为 182.6%、178.7%; TC、CIP 混合使用中抗生素残留量较高, 分别为 198.1%、191.5%. 研究表明抗生素和 MPs 的浓度、种类、暴露时间、种类等因素均能产生不同的影响^[27]. 由于 PE 表面存在明显的褶皱和孔洞、比表面积大, 吸附能力强, 与抗生素间通过微孔填充、范德华力、静电力等作用, 对 TC、CIP 的化学形态结构产生一定的影响, 降低抗生素的残留. 因此, 以对照组为参考值, 计算与之检出比率更能清晰掌握 MPs 对抗生素吸附情况^[28]. MPs 成为抗生素的吸附载体, 在一定时间内降低抗生素残留, 但是长时间复合毒性效应机制需要进行综合研究.

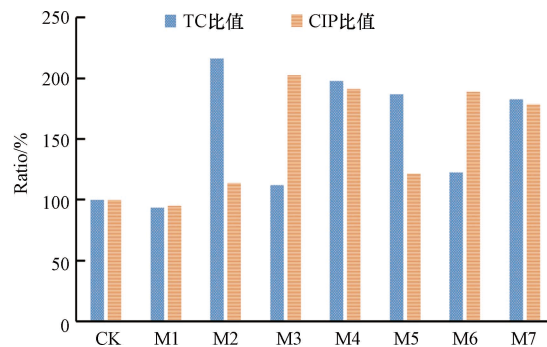


图 3 土壤中抗生素残留量比值变化的检测结果
Fig.3 Detection results antibiotic residue ratio in soil

2.4 土壤中 ARB 抗性的检测结果

前期研究表明, 土壤种微生物在长期、高含量的抗生素选择压力逐渐产生适应性、获得抗性, 诱导抗性菌的生成^[29]. 由于大多抗生素都有较宽的抗菌谱, 可抑制多种细菌和真菌, 市土壤微生物获得抗性, 并传播. 因此, 分析抗性菌的抗生素抗性有利于探究抗生素在土壤吸附、降解, 及微生物特性变化的效应机制^[30]. 本研究中, 开展了 TCARB、CIPARB 对 PEN、CIP、TC、CHL、RFP、CN 等抗生素的抗性分析, 对照组分离培养的抗性菌株中只有对 PEN、CIP、SMZ 产生抗性, 对其他抗生素未产生抗性; 施用抗生素的实验组中获得了多重抗生素抗性, 施用 MPs 实验组吸附了少量抗生素, 抗生素抗性略有降低; 而施用了抗生 MPs 实验组的 ARB 抗性的检出比例明显高于未施用抗生素、MPs 的对照组. 结果见图 4.

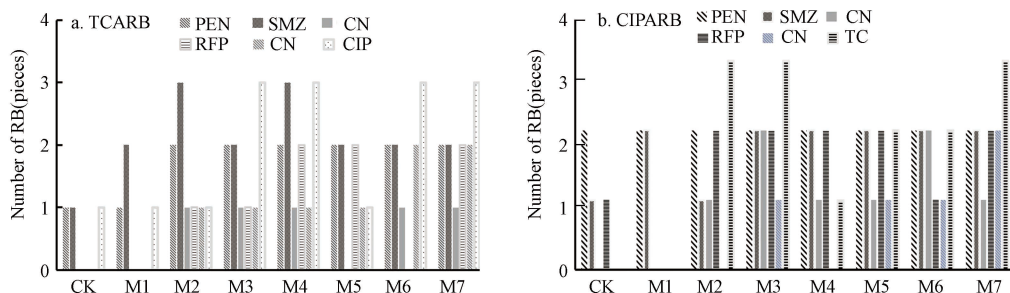


图 4 土壤抗生素抗性菌抗性的检测结果
(a)TCARB (b)CIPARB

Fig.4 Detection results of ARB resistance in soil

2.5 土壤中 ARGs 的测定结果

本研究中, 分别施用抗生素实验组相应的 ARGs 丰度比高, 只施用 MPs 实验组低于对照组的丰度比值. 值得注意的是, 施用 MPs、TC、CIP 混合暴露后与对照组的 *tet W*、*tet O* 丰度比值分别为 1.82、1.78; 与 *qnr A*、*qnr S* 丰度比值分别为 1.68、1.71. 结果见图 5. 多种抗生素进入生态环境中, 土著微生

物通过改变细胞壁的通透性、作用靶位等多种途径获得抗生素产生抗性,产生 ARB,通过水平转移、垂向转移等方式多重种属的菌株间扩散 ARGs^[31].在一定程度上因抗生素长时间暴露引发 ARGs 持续在较高的水平传播^[32].因此,通过抗性基因丰度的检测有助于分析抗生素残对土著微生物变化特性和生态环境造成的威胁.

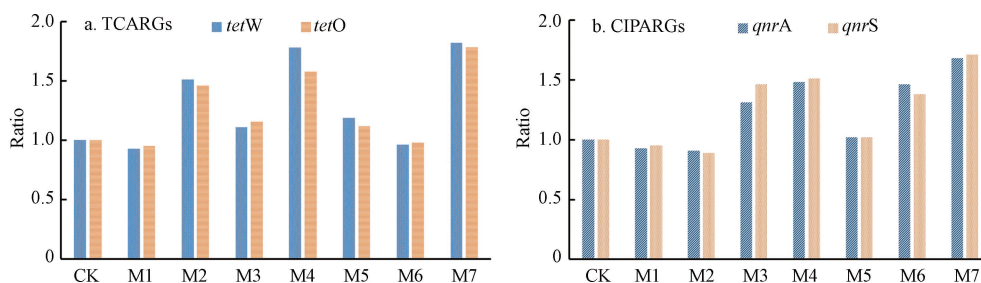


图5 土壤中 ARGs 的绝对丰度比值的结果
(a)TC (b)CIP

Fig.5 Detection results of absolute abundance ratio of ARGs in soils

2.6 MPs 和抗生素胁迫下微生物群落的检测结果

由图6可见,微生物群落相对丰度最高为变形菌门(*Proteobacteria*)超过40%;其次为相对丰度较高的放线菌(*Actinobacteria*).单独施用 MPs 后 *Proteobacteria* 和 *Actinobacteria* 相应地增多,而其他种类群落则降低;单独施用 TC、CIP 后硝化螺旋菌(*Nitrospirae*)和奇古菌(*Thaumarchaeot*)的相对丰度略有增加,导致其他菌群丰度下降.施用抗生素、MPs 后 *Proteobacteria*、*Actinobacteria*、粘多菌(*Myxococcus*)的相对丰度出现变化,对土壤的微生物群落的多样性影响更为复杂.由于抗生素的吸附能力、降解能力与环境因素、土著微生物生态位存在相互响应机制,彼此互为相应的影响^[33].脱霞霞认为长期覆膜的土壤富集有益微生物菌群,抗生素改变土著微生物的氮循环、磷循环效能,甚至会破坏系统功能的稳定性,改变微生物群落结构的多样性,对土壤造成一定威胁^[34].

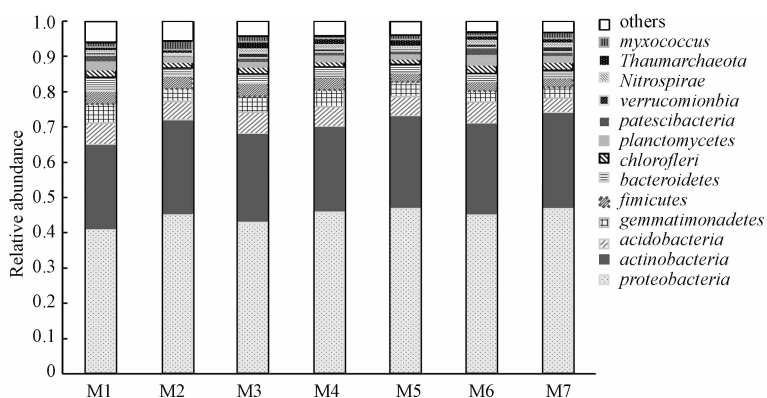


图6 微塑料和抗生素胁迫下微生物群落的检测结果

Fig.6 Combined effects of microplastics and antibiotic on microbial community in soils

3 结论(Conclusion)

抗生素、MPs 持久性地赋存于土壤中,促使土壤 ARGs 相对丰度发生改变,极有可能生态平衡和人体健康构成潜在威胁.本研究中,通过 PE、TC、CIP 胁迫下,开展了土壤理化特性、酶活性、抗生素残留量、ARGs、微生物结构特性的研究和数据分析,得出以下结论.

- (1) MPs 与抗生素胁迫对土壤理化特性产生综合影响,酶活性变化更为显著.
- (2) MPs 与抗生素胁迫下土壤环境中抗生素残留量增多,诱导 ARB 的抗性增加,ARGs 相对丰度增高.
- (3) MPs 与抗生素胁迫下土壤微生物群落多样性出现降低的趋势,PE-TC-CIP 共同胁迫下比单一

污染毒性效应更为显著。

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