设施农业土壤微塑料污染及其对细菌群落多样性的影响

费禹凡^{1,2}, 黄顺寅^{1,2}, 王佳青^{1,2}, 骆永明³, 章海波^{1,2*}

1. 浙江农林大学环境与资源学院, 杭州 311300;

2. 浙江省土壤污染生物修复重点实验室, 杭州 311300;

3. 中国科学院南京土壤研究所, 中国科学院土壤环境与污染修复重点实验室, 南京 210008

* 联系人, E-mail: hbzhang@zafu.edu.cn

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摘要 设施农业土壤普遍使用塑料薄膜覆盖和施加大量有机肥, 可能成为微塑料污染区. 对杭州湾沿岸集中连片设施农业区54个表层土壤样品的调查研究表明, 黑色地膜覆盖土壤中微塑料丰度普遍高于透明棚膜、棚膜与地膜结合覆盖土壤, 微塑料最高丰度可达1560个/kg, 聚乙烯薄膜类微塑料普遍存在. 进一步通过聚乙烯微塑料污染土壤暴露培养29 d的试验结果表明, 微塑料的添加会一定程度抑制土壤细菌群落丰富度的恢复. 16S rRNA高通量测序结果显示, 土壤细菌群落结构的改变不随微塑料暴露量的增加而加剧, 1%暴露量时聚乙烯微塑料对土壤细菌群落结构的影响比5%暴露量时更为显著; 土壤中的微塑料暴露主要使鞘脂单胞菌目(Sphingomonadales)、黄色单胞菌目(Xanthomonadales)、丙酸杆菌目(Propionibacteriales)、Chitinophagales、鞘脂杆菌目(Sphingobacteriales)和黄杆菌目(Flavobacteriales)等相对丰度显著上升, 而未分类的放线菌目(unclassified Actinobacteria)、 β -变形菌目(Beta-proteobacteriales)、粘球菌目(Myxococcales)、芽单胞菌目(Gemmatimonadales)等相对丰度显著下降; KEGG代谢通路预测显示土壤微塑料污染可能会影响异黄酮等次级代谢产物的生物合成, 促进多环芳烃的降解. 未来需进一步从相关功能基因角度探讨微塑料污染对土壤中养分和外源污染物生物转化的影响.

关键词 设施农业土壤, 微塑料, 积累, 微生物群落结构, 功能多样性

微塑料是指环境中粒径<5 mm的塑料颗粒或纤维^[1]. 近年来, 陆地生态系统尤其是农田土壤中的微塑料污染问题受到了广泛关注^[2-6]. 有研究认为, 土壤中每年输入的微塑料量是海洋输入量的4~23倍^[7]. 据估计, 欧洲和北美国家每年通过污泥农用输入到农田土壤中的微塑料量分别高达63000~43000和44000~300000 t^[8]. 除了污泥和有机肥施用输入来源^[8,9], 设施农业中农膜残留也是土壤微塑料的一个重要来源^[4]. 我国是主要的农膜使用国家, 低密度聚乙烯膜的使用量达到70万t/a^[10], 而农膜回收率不足60%^[11]. 近年来, 在国际上也有一些报道长期农膜残留导致土壤中微塑料

污染问题^[12-14]. 同时, 微塑料污染对陆地生态系统的影响在近两年也成为该领域的研究热点. 比如: 土壤微塑料被蚯蚓、跳虫等陆生无脊椎动物摄入后对生物体内酶活的影响^[15,16], 以及对蚯蚓或跳虫肠道微生物群落结构与功能的改变^[17,18]; 植物吸收和积累微塑料的特征^[19]及其对植物生长的影响^[20,21]等. 土壤微生物是陆地生态系统中的主要组成部分, 在有机质分解、养分元素循环和有害物质的降解等方面都起着关键作用. 土壤中微塑料的积累对微生物也有显著影响, 目前有限的研究显示它们对土壤酶活性和微生物的群落结构的改变都有显著作用^[22-24], 而对地下微生物生态系统

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的影响,可能会进一步影响植物生长或植物群落结构的改变^[21,25]。

我国的设施农业以塑料大棚覆盖为主,部分也同时覆盖地膜提高温度。但过去使用的地膜厚度普遍低于发达国家要求的 $>0.02\text{ mm}$,有些地区甚至 $<0.005\text{ mm}$ ^[2,11]。这导致地膜极易碎片化而残留在土壤中形成微塑料污染。因此,本研究希望通过调查设施农田土壤中微塑料的残留特征,了解不同覆膜类型对土壤微塑料残留的影响;由于低密度聚乙烯膜是设施农田使用的主要地膜类型,因此我们希望通过考察不同含量低密度聚乙烯型微塑料污染对土壤微生物群落结构和功能的影响,揭示设施农田土壤中微塑料污染的潜在微生物生态效应,为设施农田土壤微塑料污染的生态风险评估提供理论依据。

1 材料与方法

(i) 供试土壤样品采集。微塑料污染的调查土壤样品采集自杭州湾沿岸的宁绍平原、嘉兴和上海南郊等相对集中的设施农业种植区。据统计,该地区设施农业面积约为4.7万公顷($1\text{公顷}=1\times 10^4\text{ m}^2$),每年有近3万t塑料薄膜用于蔬菜和瓜果种植,种植历史最长的已近30 a^[26]。土壤样品采集过程中,按照设施农田覆膜类型分为黑色地膜覆盖、透明棚膜覆盖、透明棚膜+地膜覆盖3种类型,其中棚膜厚度一般为 $0.08\sim 0.12\text{ mm}$,地膜厚度一般为 $0.008\sim 0.015\text{ mm}$ 。同时采集非设施农田土壤(主要为水田和玉米地)为对照样品,合计共采集54个土壤样品。每个样点的土壤样品采集方法为:随机选择3个大棚/田块($50\text{ m}\times 6\text{ m}$)用不锈钢铲采集6个土壤子样,充分混匀后形成一个复合样品装入玻璃瓶中。所有土壤样品运回实验室后,在 25°C 下风干、去除肉眼可见的根和凋落物,过 5 mm 筛不锈钢网, $>5\text{ mm}$ 部分的塑料碎片收集至样品盒保存,经傅里叶转换红外光谱鉴定后确定为塑料的进行数量和颗粒大小统计。将过筛后的风干土样装入500 mL的宽口玻璃瓶中密封,于常温保存。

(ii) 土壤微塑料的分离与显微镜识别。土壤微塑料的分离方法按照本课题组设计的连续流动——气浮分离装置^[27],并且已在其他类似研究中使用^[14,28]。将 50.0 g 风干过筛后的土壤放入1 L高型烧杯中,用 0.5 mol L^{-1} 六偏磷酸钠溶液超声分散,然后将烧杯放入5 L的大玻璃烧杯中用饱和氯化钠溶液($\rho=1.2\text{ g cm}^{-3}$)以 1.0 L/min 的速度注入气浮。收集含有微塑料和其他低

密度物质的溢流液,并将其转入 $50\text{ }\mu\text{m}$ 的振动筛上过滤。反复浮选3次后合并筛上物质,在碘化钠饱和溶液($\rho=1.6\text{ g cm}^{-3}$)中静置48 h后用 $20\text{ }\mu\text{m}$ 的玻璃纤维膜过滤收集溶液中的浮游物质;再次搅动碘化钠溶液中的沉淀物并静置分离48 h后过滤收集浮游物质,重复3次后将所有玻璃纤维膜上的浮游物质用超纯水清洗到含有过氧化氢(30%, v/v)的玻璃容器中加热反应72 h以去除有机物质。最后,将烧杯中的混合物用玻璃纤维膜过滤,将滤膜储存在样品盒中常温保存。

滤膜上物质在立体显微镜(SMZ 18)下用25~100倍的放大范围进行观察和鉴定,最小可识别的颗粒大小在 $20\text{ }\mu\text{m}$ 左右。基于一定的规则挑选出微塑料颗粒和纤维,并根据形貌特征分类存放,一些挑选出的疑似微塑料颗粒采用单独存放。挑选出的微塑料颗粒采用Nano measurer程序测量其粒径大小并进行计数。

(iii) 傅里叶转换红外光谱鉴定。显微傅里叶转换红外光谱仪(Thermo Nicolet iN10 MX)的鉴定参考文献^[29]使用的方法。采用透射模式下对收集在滤膜上的微塑料进行扫描,波长范围为 $4000\sim 400\text{ cm}^{-1}$,采集时间为3 s,扫描32次。所有光谱都在自动基线校正模式下通过OMNIC软件(Version 9.1, Thermo Nicolet Corporation)处理后,再利用数据库进行相似性比对,选择拟合度在80%以上的物质作为识别聚合物的成分。

(iv) 微塑料污染的室内培养试验。室内培养试验的供试土壤采自杭州附近的未覆膜农田土壤,其中土壤的基本性质为: pH为6.6、土壤有机质含量为 48.1 g kg^{-1} 、总氮含量为 3.2 g kg^{-1} 。培养试验在人工气候箱(PRX-600B)中进行。供试土壤在风干、去除石块与残根后过 2 mm 筛混匀,加入去离子水至60%田间持水量后在人工气候箱中预培养1周。然后,将平均粒径为 $678\text{ }\mu\text{m}$ 的低密度聚乙烯型(LDPE)不规则颗粒加入至含 600 g 土壤的烧杯中,采用不锈钢勺子分多次搅拌、混匀。根据加入微塑料的量分为以下3个处理:(1) CK, 0%(w/w),土壤中不加入微塑料;(2) PE1,土壤中含1%(w/w)的低密度聚乙烯微塑料;(3) PE5,土壤中含5%(w/w)的低密度聚乙烯微塑料,每个处理设置3个重复。试验设置的微塑料量参考了目前文献报道的微塑料含量以及过去报道试验中的含量。

人工气候箱的培养条件恒定在 27°C 和80%的相对湿度,模拟日光照射,每12 h交替光照和黑暗环境,土壤湿度保持最大持水量的60%,试验过程中每天补充水分。从微塑料加入24 h后取第一次土壤样品开始,分别

在第15、29天取土壤样品。取样时,在每个培养容器中取3个重复样进行分析,每个样品量约为1 g,用于土壤DNA提取和测序分析。

(v) 土壤高通量测序. 采用FastDNA Spin Kit(MP Biomedicals)对土壤样品提取DNA. 通过引物338F(5'-ACTCCTACGGGAGGCAGCA-3')和806R(5'-GGAC-TACHVGGGTWTCTAAT-3')进行PCR扩增细菌16S的V3~V4区. 用Axygen DNA凝胶回收提取试剂盒对PCR产物进行纯化,然后用Quant-iT PicoGreen dsDNA定量检测试剂盒进行定量. 用Illumina MiSeq平台对纯化的扩增子进行高通量测序。

(vi) 数据统计与分析. 根据97%的相似度水平下将优化序列聚类为用于物种分类的操作分类单位(operational taxonomic unit, OTU), 根据细菌16S rRNA Green-genes为参考数据库(Release 13.8, <http://greengenes.secondgenome.com/>)获取OTU表. 统计每个OTU的相对丰度信息,并用Origin 9软件制作不同门水平的微生物群落结构图. 使用QIIME2程序计算细菌群落的丰富度指数(Chao1)、多样性指数(Shannon和Simpson指数)以及覆盖度(Goods coverage). 根据OTU表,采用PICRUST2对获取的16S rRNA基因序列在KEGG数据库(<https://www.kegg.jp/>)中进行细菌群落的代谢通路预测^[30]. 不同处理之间的多重比较采用单因素方差分析中的Turkey统计检验法,以0.05水平统计差异的显著性。

2 结果与讨论

2.1 不同覆膜类型设施农业土壤中微塑料的丰度差异

根据调查结果,该地区设施农田土壤中微塑料丰度在20~1560个/kg,平均为443个/kg,包括薄膜、碎片和纤维3种类型. 不同类型的微塑料在聚合物组成上有差异,其中,薄膜类主要为聚乙烯,碎片类以聚丙烯、聚乙烯和聚酯为主,纤维类以尼龙和聚酯为主。

按照设施农田的覆膜类型对研究区土壤中微塑料和>5 mm的塑料碎片进行比较分析,结果如图1所示. 未覆膜的普通大田种植土壤中的微塑料和>5 mm的塑料碎片的平均丰度均低于覆膜的设施农田土壤. 也有报道显示大棚菜地或果园土壤中微塑料丰度要显著高于普通林地或一般农田^[13,14,31],这表明目前这种塑料大棚或地膜覆盖的设施农田种植方式可能会带来更多的

图1 (网络版彩色)不同覆膜类型设施农业土壤中微塑料(a)和>5 mm塑料碎片(b)的丰度比较;多重比较统计(Turkey test)显示黑色地膜覆盖土壤中微塑料丰度要显著($P<0.05$)高于普通大田土壤、透明棚膜+地膜覆盖土壤

Figure 1 (Color online) Comparison of microplastics (a) and mesoplastics (b) abundances among the soils of protected agriculture with different plastic cover types. The results of multiple comparisons with Turkey test showed that the microplastics abundance in the soils covered with black mulch had a significant ($P<0.05$) higher than that in soils of conventional farmlands and farmlands with transparent shed plus mulching

土壤微塑料污染。

通过比较设施农田不同覆膜方式下土壤微塑料的积累情况,也发现微塑料的总量和不同类型微塑料的含量都存在明显区别(图1)。其中,较为突出的是黑色地膜覆盖的土壤,其微塑料和>5 mm塑料碎片的含量都高于其他类型,总体趋势是黑色地膜>透明棚膜>透明棚膜+地膜. 这可能是由于黑色地膜在无棚膜阻挡下直接受光照射,并且对光反射少,容易在光照下破碎化为塑料碎片甚至微塑料. 有研究表明,紫外光照是野外塑料垃圾进一步降解为微塑料的主要原因^[32]. 而透明棚膜在使用一段时间后基本都会回收,在土壤中残留较少。

本研究发现的主要微塑料类型是薄膜类、碎片类和纤维类. 其中,土壤中的纤维类微塑料一般认为主要来源于污泥和灌溉^[8,13]. 通过比较薄膜和碎片这两类塑

料颗粒的粒径可以发现, 碎片类塑料颗粒的粒径要明显小于薄膜类塑料颗粒, 其中碎片类96%左右均为微塑料(<5 mm), 且不同覆膜类型的土壤区别不大(图2)。这表明碎片类塑料颗粒来源较为一致, 可能主要为有机肥大量施用带入的。研究表明, 设施农业土壤中有机肥的施用量是一般大田的10倍以上^[33], 而有机肥中普遍含有微塑料, 特别是以碎片类微塑料为主^[9]。不同覆膜类型土壤中薄膜类塑料颗粒的粒径大小总体上要大于碎片类, 其中, 微塑料占66%~90%。透明棚膜土壤中薄膜类微塑料的颗粒最小, 而普通大田的薄膜类微塑料颗粒粒径相对较大(图2)。由于棚膜基本被回收, 因此由棚膜覆盖土壤中的薄膜类微塑料可能主要来自其他输入, 比如灌溉等。

2.2 土壤聚乙烯微塑料积累对微生物结构多样性的影响

表1是土壤中加入聚乙烯微塑料后对细菌群落丰富度、多样性和覆盖度的影响。在培养期内, 供试土壤细菌群落的丰富度(Chao1指数)先下降后上升; 在多样性方面, Simpson指数基本没有变化, Shannon指数略有下降; 覆盖度(Goods coverage)略有上升。不同处理之间, Chao1指数在微塑料加入1 d后与对照呈现显著差异($P<0.01$), 但到培养后期则未见显著差异。Shannon指数和覆盖度指数在培养初期和末期与对照相比较, 均有显著性差异($P<0.01$)。从培养初期到末期的 α 多样性

图2 (网络版彩色)不同覆膜类型设施农田土壤中薄膜类(a)和碎片类塑料(b)的粒径大小分布的累积频率曲线图

Figure 2 (Color online) The cumulative frequency distribution of the diameter size of films (a) and fragments (b) observed in the soils of protected agriculture with different plastic cover types

指数变幅来看, 微塑料加入的处理(PE1和PE5)均要大于对照。其中, 培养29 d后, 对照处理中Chao1指数平均变化幅度为24.1%, 在培养后期群落丰富度的恢复较

表1 聚乙烯微塑料对土壤中细菌群落 α 多样性的影响^{a)}

Table 1 Effect of polyethylene microplastics on the alpha diversity of bacterial community in the test soil

α 多样性指数	培养时间(d)	对照(CK)	PE1处理	PE5处理
Chao1	1	9507.0 $\pm$ 759.2	11799.6 $\pm$ 883.9*	10614.8 $\pm$ 1002.2*
	15	4511.7 $\pm$ 92.2	6421.3 $\pm$ 180.4	5803.1 $\pm$ 448.4
	29	7215.1 $\pm$ 830.6	6596.8 $\pm$ 432.7	5882.3 $\pm$ 436.4
Simpson	1	0.996 $\pm$ 0.0004	0.997 $\pm$ 0.0003	0.997 $\pm$ 0.0005
	15	0.997 $\pm$ 0.0004	0.997 $\pm$ 0.0003	0.997 $\pm$ 0.0004
	29	0.996 $\pm$ 0.0012	0.997 $\pm$ 0.0004	0.996 $\pm$ 0.0018
Shannon	1	10.47 $\pm$ 0.13	10.76 $\pm$ 0.13	10.62 $\pm$ 0.10*
	15	10.22 $\pm$ 0.20	10.45 $\pm$ 0.11	10.35 $\pm$ 0.10
	29	10.24 $\pm$ 0.23	10.29 $\pm$ 0.13*	10.16 $\pm$ 0.28
Goods coverage	1	0.957 $\pm$ 0.006	0.945 $\pm$ 0.006	0.950 $\pm$ 0.007*
	15	0.972 $\pm$ 0.009	0.961 $\pm$ 0.004	0.966 $\pm$ 0.004
	29	0.984 $\pm$ 0.003	0.987 $\pm$ 0.003*	0.989 $\pm$ 0.003

a) *表示相对于对照处理的差异显著性水平($P<0.05$); PE1和PE5分别代表添加1%和5%低密度聚乙烯微塑料含量的土壤培养处理

快;而PE1和PE5处理中Chao1指数的平均变化幅度分别为44.1%和44.6%,显示微塑料对细菌群落丰富度的恢复有较强的抑制作用。但是,Huang等人^[34]研究显示,在 0.076 g kg^{-1} 的低剂量聚乙烯微塑料浓度下,在培养后期,微塑料对土壤中细菌群落丰富度具有显著的促进作用,但对细菌群落多样性没有影响。这可能反映了不同剂量水平微塑料暴露对土壤细菌群落的影响。

供试土壤中的细菌群落总体上以变形菌门(Proteobacteria)和放线菌门(Actinobacteria)为主,两者相对丰度累计占70%以上。土壤在培养过程中,优势细菌群落均表现为变形菌门相对丰度下降、而放线菌门相对丰度上升的趋势(图3)。但是,添加微塑料处理的变形菌门相对丰度下降的幅度与放线菌门相对丰度上升的幅度均略低于对照处理,尤其是1%的微塑料添加量处理(PE1)更为明显。在29 d培养结束后,PE1处理土壤中的变形菌门相对丰度要高于对照,而放线菌门相对丰度则低于对照。从图4可以进一步看出,PE1处理与对照相比,变形菌门中显著上调的OTU数要多于显著下调的OTU数。其中,显著下调的细菌种群主要集中在 β -变形菌目(Betaproteobacteriales)和粘球菌目(Myxococcales),而显著上调则主要集中在鞘脂单胞菌目(Sphingomonadales)、黄色单胞菌目(Xanthomonadales)、未分类的 α -变形菌目(unclassified Alphaproteobacteria)和 γ -变形菌目(unclassified Gammaproteobacteria)。放线菌门中,PE1处理土壤中显著上调和下调的OTU数相差不大,但前者主要聚集在丙酸杆菌目(Propionibacteriales),

而后者主要聚集在未分类的放线菌目(unclassified Actinobacteria)。

微塑料处理土壤(PE1)中,拟杆菌门(Bacteroidetes)和芽单胞菌门(Gemmatimonadetes)的相对丰度与对照相比也出现明显变化,其中相对丰度前者略有上升,后者略有下降(图3)。从图4进一步看出,拟杆菌门中,显著上调的OTU数要明显多于显著下调的OTU数,其中下调的是Chitinophagales,上调包括Chitinophagales、鞘脂杆菌目(Sphingobacteriales)和黄杆菌目(Flavobacteriales)。芽单胞菌门的芽单胞菌目(Gemmatimonadales)均表现显著下调。

由于目前对土壤中微塑料污染下的微生物生态效应研究还非常有限,通过对比有限的几篇报道发现,土壤中添加PE类微塑料后细菌群落的变化并不一致。Huang等人^[34]研究显示,土壤中加入低剂量(0.076 g kg^{-1})PE膜($2\text{ mm}\times 2\text{ mm}$)后培养一段时间,放线菌门丰度显著下降,变形菌门丰度显著上升,与我们的研究结果接近;但他们认为,土壤中放线菌门相对丰度降低是由于一些可降解PE类的放线菌富集在微塑料上导致的^[34,35]。本研究中,由于微塑料颗粒较小,未将其分离出土壤后分析表面微生物群落,因此,无法判断是否在微塑料表面富集了放线菌。也有报道在种植小麦的盆栽土壤中加入1% PE微塑料后2个月内,发现根际土壤中变形菌门的相对丰度降低,而放线菌门的丰度增加,但4个月后这种差异又逐渐变小^[36]。但Ren等人^[37]发现在土壤中加入5%的PE微塑料($<150\text{ }\mu\text{m}$)培养30 d后,微

图3 不同含量水平的聚乙烯微塑料对土壤中细菌门水平上的群落相对丰度影响

Figure 3 Effects of different addition levels of polyethylene microplastics on the relative abundance of soil bacterial phylum

图 4 基于metagenomeSeq差异OTU的曼哈顿图. 用于指示培养29 d后, PE1处理土壤中细菌群落在目水平上的影响, 与对照相比, 显著上调的OTU(a)和显著下调的OTU(b); 每个圆点或圈代表1个OTU, 圈的大小代表其相对丰度, 虚线以上为具有显著差异的OTU(用彩色圆点或圆环标志), 对有显著差异的OTU点数排在前15的目, 添加灰度背景, 并在顶部标注其名称

Figure 4 Manhattan plots based on the metagenomeSeq analysis showing OTU with significant difference between PE1 treatment and control in bacterial order after 29 d soil incubation. Upregulation (a) and downregulation (b) are presented, each dot or circle on the plot denotes one OTU and its size represents the abundance of the OTU. The color dots or circles located above the dash line means the OTU with significant difference between the PE1 and control and the top 15 orders with significant difference are shown with grey background and annotated with their names on the top

塑料处理土壤中变形菌门取代放线菌门成为占主导的优势菌,而对照土壤中仍然以放线菌占主导.此外,近年来的一些研究也表明,微塑料对土壤生物的影响可能是通过改变土壤物理环境起作用,比如改变土壤的保水性、容重等,并且微塑料对土壤物理性质的改变不完全受到暴露量的影响,微塑料的形态、粒径等也会起决定作用^[38].与此同时,土壤微生物群落可能对不同物理性质改变的适应性的不同而发生变化^[39].但目前这方面的研究还非常有限,需要结合更多的微塑料和土壤类型来探讨其对土壤微生物群落结构的影响.

2.3 土壤聚乙烯微塑料积累对微生物功能多样性预测

KEGG数据库的核心为生物代谢通路分析数据库(KEGG Pathway Database, <http://www.genome.jp/kegg/pathway.html>),它将代谢通路分为六大类,包括代谢、遗传信息处理、环境信息处理、细胞进程、生物体系统和人类疾病^[30].图5是根据获得的代谢通路丰度数据进行差异分析后得到的PE1处理与对照之间具有显著差异的代谢通路,其中除了上皮细胞细菌入侵属于细胞进程通路外,其余6条通路均与代谢相关,并且主要是次级代谢产物的生物合成通路,如异黄酮、克拉维酸等.预测结果显示,土壤中聚乙烯微塑料积累后导致的代谢通路下调的途径包括上皮细胞细菌入侵、异黄酮合成、II型聚酮骨架的生物合成、类倍半萜烯生物合成、GPI锚定蛋白生物合成、克拉维酸生物合成、萘降解.

合成;而GPI锚定蛋白生物合成、克拉维酸生物合成以及萘降解代谢途径则为上调通路.其中,与类倍半萜生物合成相关的黏细菌(*Myxococcus xanthus*)属于粘球菌目^[40],该细菌也是变形菌门中受微塑料积累下调比较集中的种群(图4).异黄酮可以促进豆科作物的根与根瘤菌的结合,诱导根瘤的形成,提高豆科植物固氮的能力^[41].因此如果异黄酮的生物合成受到微塑料积累而导致显著下调可能会对作物固氮带来影响.在上调通路中,萘降解途径是差异倍数最大的一个,这可能与PE1处理土壤中变形菌门中鞘脂单胞菌目和黄色单胞菌目的普遍上调有关(图4).在这两类细菌中,已发现有许多与多环芳烃等碳氢化合物降解有关菌株^[42].因此,土壤中微塑料的积累是否会影响对土壤多环芳烃的降解也值得关注.

3 结论

杭州湾沿岸设施农业土壤中主要微塑料类型包括碎片类、薄膜类和纤维类,其中聚乙烯型薄膜类微塑料污染普遍,并且在黑色地膜覆盖种植的土壤中微塑料的总丰度和5 mm以上塑料碎片的丰度均要高于其他类型种植土壤.土壤中聚乙烯型微塑料短期暴露可以改变细菌的群落结构组成,但与微塑料添加量不成正比关系.聚乙烯型微塑料积累会使土壤中鞘脂单胞菌目、黄色单胞菌目、丙酸杆菌目、Chitinophagales、鞘脂杆菌目和黄杆菌目等相对丰度显著上升,而未分

图5 (网络版彩色)土壤中1%的聚乙烯微塑料暴露量对KEGG代谢通路的影响预测

Figure 5 (Color online) Prediction of KEGG metabolism pathways after exposure of 1% polyethylene microplastics in the test soil

类放线菌目、 β -变形菌目、粘球菌、芽单胞菌目等相对丰度显著下降。同时, 聚乙烯微塑料的积累还可能影响土壤中异黄酮、多环芳烃等生物代谢通路。未来研

究可进一步结合微塑料和土壤类型从养分循环与污染物降解相关的功能基因变化的角度探讨微塑料积累对土壤微生物功能多样性的影响及其机制。

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Summary for “设施农业土壤微塑料污染及其对细菌群落多样性的影响”

Microplastics contamination in the protected agricultural soils and its effects on bacterial community diversity

Yufan Fei^{1,2}, Shunyin Huang^{1,2}, Jiaqing Wang^{1,2}, Yongming Luo³ & Haibo Zhang^{1,2*}

¹ School of Environment and Resources, Zhejiang Agriculture and Forestry University, Hangzhou 311300, China;

² Key Laboratory of Soil Contamination Bioremediation of Zhejiang Province, Hangzhou 311300, China;

³ Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

* Corresponding author, E-mail: hbzhang@zafu.edu.cn

Microplastics (MPs) are defined as plastic particles or fibers of size <5 mm and have been of concern in terrestrial ecosystems for several years. Protected agricultural soils have been assumed to be potentially contaminated with MPs because of the use of large amounts of plastic films and annual compost inputs. However, there is little published information on MP contamination of protected agricultural soils and its ecological effects on soil biota remain unknown. Here, a total of 54 surface (0–10 cm depth) soils were sampled from intensively managed protected agricultural soils around Hangzhou Bay, east China. MPs were extracted from the soil samples using continuous air flotation followed by density separation. Stereomicroscopy and micro-Fourier transform infrared spectroscopy (μ -FTIR) were used for identification after the extracted MPs were pre-sorted based on their shapes. In addition, a 29-d soil incubation experiment was conducted to observe the changes in microbial community diversity due to MP contamination. Soils were spiked with 1% and 5% (w/w) low density polyethylene (LDPE) MPs (average size 678 μ m) and sampled at an intervals of 1, 15 and 29 d in an incubation experiment. The soil bacterial community in these samples was analyzed using high-throughput sequencing technology. A field survey of the protected agricultural soils indicates that soil MP abundance ranged from 20 to 1560 pieces kg^{-1} with an average of 443 pieces kg^{-1} . Fragments dominated the MP types which comprised films, fragments and fibers, likely originating from compost applications to the soils. LDPE MPs occurred mainly in MP films and were widely distributed in soil samples from mulched soils. Soil MP was also influenced by the type of plastic mulching film used. Relatively high MP abundance was found in soils covered with black mulching film compared with other types of film. Soil MP contamination was found to affect soil microorganisms in the incubation experiment. The Chao1 index (which indicates the richness of the bacterial community) decreased initially and then increased in both PE-spiked and PE-free soils during incubation for 29 d. However, larger decreases and smaller increases in Chao1 were observed in PE-spiked than PE-free soils, indicating that the presence of PE in the soil might inhibit the recovery of the bacterial community richness. The 16S rRNA high-throughput sequencing results indicate that changes in bacterial community diversity were not proportional to the PE microplastic contents of the soils. Larger changes occurred in soils spiked with 1% PE than in those spiked with 5% PE. After addition of PE MPs a significant ($P < 0.05$) increase in the relative abundance of the bacterial orders Sphingomonadales, Xanthomonadales, Propionibacteriales, Chitinophagales, Sphingobacteriales and Flavobacteriales was observed but the relative abundance of the orders unclassified Actinobacteria, Betaproteobacteriales, Myxococcales and Gemmatimonadales decreased significantly ($P < 0.05$). Functional profiling of the bacterial communities was predicted using KEGG pathways. A total of seven KEGG pathways showed significant difference between the 1% PE spiked and PE-free soils. For example, the biosynthesis of secondary metabolites such as isoflavonoids will be impacted and naphthalene degradation will be promoted after addition of PE microplastics. Further studies are planned on the transformation of soil nutrients and xenobiotic pollutants by the selection of functional genes.

protected agricultural soil, microplastics, accumulation, microbial community structure, functional diversity

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