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### ENVIRONMENTAL SCIENCE

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2000~2020 平尺律
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## 牛粪还田对土壤微生物群落特征的影响

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摘要: 畜禽粪便还田对土壤质量产生了深刻影响,而土壤微生物是反映土壤质量的敏感指标. 基于稀释平板计数和高通量测 序技术,研究了牛粪还田区土壤微生物群落特征及其影响因素.结果表明,与对照点相比,牛粪还田区土壤微生物群落β多样 性发生了显著变化;牛粪还田增加了变形杆菌门(Proteobacteria)的相对丰度,而减少了放线菌门(Actinobacteria)和芽单胞菌 门(Gemmatimonadetes)的相对丰度; 牛粪还田显著改变了土壤微生物群落中的鞘氨醇单胞菌属(Sphingomonas)、PLTA13 属、 MSB-4B10 属、盐单胞菌属(Halomonas)、CCD24 属、Gaiella 属、节杆菌属(Arthrobacter)、芽孢杆菌属(Bacillus)和 Entotheonellaceae 属的相对丰度;影响牛粪还田区土壤微生物群落组成的重要因素是土壤含水量和放线菌数量;牛粪还田增 加了假单胞菌属(Pseudomonas)、梭状芽孢杆菌属(Clostridium)和链球菌属(Streptococcus)等病原菌的相对丰度,对土壤质量存 在一定的生物污染风险. 研究结果可为深入认识牛粪还田对土壤质量的影响提供数据支撑.

关键词:牛粪还田; 高通量测序; 土壤微生物群落; 土壤性质; 生物污染

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### Effects of Cow Manure Application on Soil Microbial Community in Farmland

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Abstract: Although livestock manure serves as an effective source of nutrients for agricultural purposes, it also causes an obvious risk of contamination of heavy metals and pathogens in soil. Soil microorganisms are always considered a sensitive indicator for the change in soil quality. However, knowledge of the effects of cow manure application on soil microbial community is still scarce. Therefore, the characteristics of the soil microbial community and its influencing factors in the farmland with cow manure application were studied using dilution plate counting and high-throughput sequencing. The results showed that the  $\beta$  diversity of the soil microbial community exhibited a significant difference between the cow manure application site and control site. The application of cow manure increased the relative abundance of Proteobacteria but decreased the relative abundances of Actinobacteria and Gemmatimonadetes. Furthermore, the cow manure application significantly changed the relative abundances of Sphingomonas, PLTA13, MSB-4B10, Halomonas, CCD24, Gaiella, Arthrobacter, Bacillus, and Entotheonellaceae. Both the content of soil water and actinomycete numbers were the important influencing factors of soil microbial community composition in the farmland with cow manure application. The cow manure application increased the relative abundance of pathogenic bacteria including Pseudomonas, Clostridium, and Streptococcus, which may have resulted in potential risks of pathogenic contamination for soil quality. The findings of this study are useful for understanding the effects of cow manure application on soil quality in farmland.

Key words; cow manure returning to farmland; high-throughput sequencing; soil microbial community; soil properties; biological pollution

畜牧业作为农业农村经济的重要支柱,为保障 畜产品市场有效供给和促进农民增收做出了重要贡 献. 近年来,随着集约化养殖场的发展,畜禽粪便的 排放量日益增长. 畜禽粪便中含有大量的碳、氮、 磷和钾等营养元素,是农业可持续发展的宝贵资源. Chen 等[1] 研究发现有机粪肥是植物可用养分和有 机物的宝贵来源,有助于提高土壤肥力. 尽管畜禽粪 便还田在改良土壤结构、提高作物产量和品质等方 面具有十分明显的优势,但由于畜禽粪便酸碱度较 低和含有一定数量的病原微生物、抗性基因和重金 属物质,会对土壤质量、农产品和地下水安全产生 深刻影响[2~4]. 文献[5]指出应加强畜禽粪便还田 区的日常监测,及时掌握有害物质含量,严防还田环 境风险. 因此,深入认识畜禽粪便还田对土壤质量的 影响具有重要意义.

土壤微生物是土壤肥力的重要组成部分,在维 持土壤生态功能中扮演着重要角色,同时是评价土 壤质量和生产力的重要指标[6~8]. 土壤微生物是陆 地生态系统物质循环的驱动者,也是土壤肥力形成 和持续发展的基础. 土壤微生物群落的结构和功能 可以反映土壤质量状况,也可以反映不断变化的环 境条件[9]. 土壤微生物数量和群落结构的变化能够 直接或间接影响着土壤肥力和土地生产力[10~15]. 因 此,开展畜禽粪便还田区土壤微生物群落特征研究, 能够揭示土壤质量对畜禽粪便还田的响应机制,为 建立科学的还田制度和实现土壤的可持续利用提供 科学依据.

畜禽粪便还田对土壤物理、化学和生物性质产

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生了深刻影响. Jiao 等<sup>[16]</sup>研究发现,施用猪粪能有效地增加土壤氮、磷和有机质等养分含量. 路杨等<sup>[17]</sup>研究发现,猪粪和秸秆还田能够降低耕层土壤容重,增加田间持水量和总孔隙度. Liu 等<sup>[18]</sup>研究发现,施用鸡粪能够提高土壤酶活性,降低土壤中铅的浓度,促进植物生长. 目前,有关畜禽粪便的研究主要集中在其资源化利用<sup>[19-21]</sup>、还田条件下土壤中重金属<sup>[22-24]</sup>、抗生素<sup>[25,26]</sup>和病原菌等污染风险评价方面<sup>[27,28]</sup>,且粪便以猪粪<sup>[29,30]</sup>和鸡粪<sup>[31,32]</sup>为主,而有关牛粪还田对土壤微生物群落特征的影响研究报道相对较少. 因此,本文以牛粪还田区土壤为研究对象,通过野外调查和室内分析,基于稀释平板计数和高通量测序技术,探讨了牛粪还田区土壤微生物群落特征及其影响因素,以期为深入认识牛粪还田对土壤质量的影响提供数据支撑.

### 1 材料与方法

### 1.1 研究区概况

研究区位于河南省焦作市武陟县(34°56′~35°10′N,113°10′~113°39′E),地处河南省西北部和焦作市东南部,黄河和沁河的交汇处.武陟县属暖温带大陆性季风气候,四季分明,夏季高温多雨,冬季寒冷干燥,多年平均气温为14.4℃,多年平均降水量为575.1 mm,无霜期为211 d.土壤类型以黄河泛滥冲积所形成的砂土和砂壤土为主<sup>[33]</sup>,种植制度为冬小麦-夏玉米一年两熟.农业耕作土壤主要为潮土,畜牧业产值占农业总产值的一半以上.

#### 1.2 样品采集及分析方法

#### 1.2.1 土壤样品采集

以牛粪还田区(NR-M)和对照点(NR-C)为研究区,牛粪还田区(NR-M)施肥方式为表土撒施,施入量约为1.9 kg·m<sup>-2</sup>. 在研究区农田内随机布设3个采样点,每个采样点采取3个重复,采集层位为0~20 cm. 在去除土壤样品中的石子和细根等杂物后,置于无菌自封袋中. 每个样品被分成3部分:一部分样品置于液氮中保存,以待土壤微生物群落组成的测定;一部分样品置于冰箱冷冻保存,以待土壤微生物数量的测定. 同时,在研究区内采集牛粪,采取3个重复,混匀,装入无菌EP管中,放置液氮中保存,以待牛粪微生物群落组成的测定.

### 1.2.2 土壤理化性质和微生物数量的测定

土壤 pH、含水量和有机质的测试方法分别为电位法、烘干法和重铬酸钾容量法-稀释热法<sup>[34]</sup>,土壤总磷、氨氮和硝氮的测试方法分别为钼锑抗比色法、靛酚蓝比色法和酚二磺酸比色法<sup>[35]</sup>.

土壤细菌数量(牛肉膏蛋白胨琼脂培养基)、真菌数量(马丁-孟加拉红培养基)和放线菌数量(改良高氏一号培养基)的测定均采用平板表面涂布计数法<sup>[36]</sup>.

### 1.2.3 微生物群落组成的测定

微生物群落组成的测定是由上海派森诺生物科 技有限公司完成.

微生物组总 DNA 提取:首先采用 Mag-bind Soil DNA Kit(Omega) 试剂盒提取样品中的微生物 DNA, 之后采用 NanoDrop NC-2000 分光光度计(Thermo Scientific,美国),在 260 nm 和 280 nm 处分别测定 DNA 的吸光值及 DNA 的浓度,并用 1% 的琼脂糖凝胶电泳检测 DNA 的质量. 调整 DNA 溶液浓度,DNA工作液保存于 4℃,储存液保存于 -20℃.

PCR 扩增: 土壤样本对于 16S rRNA 基因可变区 V5-V7 进行扩增,使用细菌特异性引物 799F(5′-AACMCGATTACATACCCKG-3′)和 1193R(5′-ACGTCATCCCCACCTTCC-3′)进行 PCR 扩增. 牛粪样本对于 16S rRNA 基因可变区 V3-V4 进行扩增,使用细菌特异性引物 338F(5′-ACTCCTACGCGAGGCAGCAGCAGAGCA-3′)和 806R(5′-GGACTACHVGGGTWTCTAAT-3′)进行 PCR 扩增. 扩增后所有样本进行凝胶回收纯化,针对目标条带进行割胶回收,得到纯化的样本;然后进行各样本定量,利用 FLX800T酶标仪(BioTek,美国)对各个样本定量;最后采用标准的 Illumina TruSeq DNA 文库制备实验流程构建所需的上机文库,进行高通量测序分析.

### 1.3 数据处理与统计分析

所有数据分析运用 SPSS 26.0 软件和派森诺基 因云平台(https://www.genescloud.cn/home)进行 处理. 在 Illumina MiSeq 测序平台得到下机数据,利 用 QIIME2 平台(2019.4) 中的 DADA2 插件<sup>[37]</sup>, 对 原始序列数据进行引物切割、质量过滤、去噪和合 并,并去除嵌合体,得到特征序列 ASVs,对比 SILVA 数据库(Release 132)进行分类学注释[38],根据最低 样本序列量的95%进行抽平. 使用 Venn diagrams 包 绘制韦恩图[39]. 利用非度量多维尺度(NMDS)分 析,基于 Bray-Curtis 距离算法,衡量不同分组样本间 群落组成的差异性,采用相似性分析(Anosim)进行 检验[40,41]. 通过 QIIME2 平台(2019.4) 对样本组间 的门水平和属水平分类单元丰度进行统计比较[42]. 通过独立样本 T 检验法分析不同分组样本门/属水 平下土壤微生物组成的差异性,通过相关性分析研 究土壤微生物群落组成与土壤性质之间的关系,T 检验和相关性分析均采用 SPSS 26.0 软件完成.

### 2 结果与分析

### 2.1 牛粪还田区土壤理化性质含量与微生物数量 牛粪还田区(NR-M)与对照点(NR-C)土壤理 化性质特征和微生物数量如表1和表2所示.由表

1 可知,与对照点相比,牛粪的施用提高了土壤氨氮 (AN)、总磷(TP)和有机质(OM)的含量,分别高出了 0.04、0.11 和 0.05 倍.由表 2 可知,与对照点相比,牛粪的施用增加了土壤细菌、真菌和放线菌的数量,分别高出了 1.44、4.09 和 0.34 倍.

表 1 牛粪还田区与对照点的土壤理化性质特征1)

Table 1 Characteristics of soil physicochemical properties in cow manure application site and control site

项目	рН	ω(WC) /%	ω(NN) /mg•kg <sup>-1</sup>	ω(AN) /mg•kg <sup>-1</sup>	ω(TP) /g•kg <sup>-1</sup>	ω(OM) /%
NR-M	$8.33 \pm 0.13$	$20.48 \pm 1.34$	17. 32 $\pm$ 5. 88	12. 94 ± 1. 96	$11.59 \pm 1.74$	$3.64 \pm 0.53$
NR-C	$8.47 \pm 0.06$	$20.84 \pm 2.06$	$21.25 \pm 5.16$	$12.41 \pm 3.98$	10. $46 \pm 2.02$	$3.46 \pm 0.92$

<sup>1)</sup> WC表示含水量,NN表示硝氮,AN表示氨氮,TP表示总磷,OM表示有机质

表 2 牛粪还田区与对照点的土壤微生物数量/cfu·g-1

Table 2 Quantity of soil microorganisms in cow manure application site and control site/cfu·g<sup>-1</sup>

项目	细菌	真菌	放线菌
NR-M	$(3.96 \pm 1.43) \times 10^6$	$(52.85 \pm 70.91) \times 10^3$	$(5.09 \pm 2.10) \times 10^5$
NR-C	$(1.62 \pm 0.75) \times 10^6$	$(10.37 \pm 10.38) \times 10^3$	$(3.79 \pm 1.56) \times 10^5$

### 2.2 牛粪还田区土壤微生物 ASVs 数目

牛粪还田区(NR-M)与对照点(NR-C)土壤微生物 ASVs 数目如图 1 所示. 从中可知,牛粪还田区与对照点土壤微生物 ASVs 数共计29 014个,其中共有 ASVs 数为3 590个,约占总数的 12.37%.牛粪还田区特有的土壤微生物 ASVs 数为12 930个,约占44.56%;对照点特有的土壤微生物 ASVs 数为12 494个,约占43.06%.

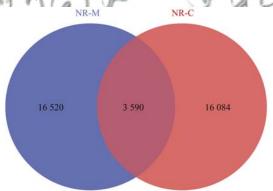


图 1 牛粪还田区和对照点土壤微生物 ASVs 数目

Fig. 1 Number of soil microbial ASVs in cow manure application site and control site

#### 2.3 牛粪还田区土壤微生物群落β多样性

基于 ASV 水平的牛粪还田区(NR-M)与对照点(NR-C)土壤微生物群落 β 多样性的 NMDS 分析结果如图 2 所示. 从中可知, NMDS 的 stress 系数小于0.2,表明图中反映的样本差异结果是非常可靠的. 牛粪还田区的 9 个土壤样品均分布在第一和第三象限,对照点 9 个土壤样品均分布在第二和第四象限,表明不同研究区之间土壤微生物群落结构发生了变化. Anosim 结果表明(表 3),不同研究区之间土壤

微生物群落结构具有显著性差异,进一步验证了 NMDS 的分析结果. 由此可见,牛粪还田导致土壤微 生物群落结构发生了显著变化.

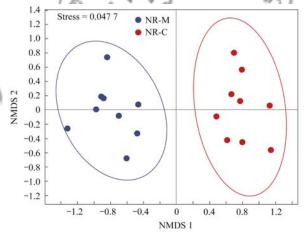


图 2 ASV 水平下的土壤微生物群落 β 多样性的 NMDS 分析 Fig. 2 NMDS analysis of soil microbial community β diversity at ASV level

#### 2.4 牛粪还田区土壤微生物群落组成

牛粪还田区(NR-M)与对照点(NR-C)土壤微生物群落在门和属分类水平下的相对丰度和差异性分析分别见图 3 和图 4 所示. 由图 3(a)可知,在门水平下,牛粪还田区与对照点土壤微生物群落均以变形杆菌门(Proteobacteria)为主,其次是放线菌门(Actinobacteria)、酸杆菌门(Acidobacteria)和绿弯菌门(Chloroflexi),它们占相对丰度的 70%以上. 与对照点相比,牛粪还田区土壤微生物群落组成中变形杆菌门(Proteobacteria)的相对丰度提高了 4.4%,而放线菌门(Actinobacteria)和 芽单胞菌门(Gemmatimonadetes)的相对丰度分别降低了 4.6%

和1.7%. 由图3(b) 可知,在属水平下,牛粪还田区与对照点土壤微生物群落均以 Subgroup\_6 属、MND1属、Rokubacteriales属、鞘氨醇单胞菌属(Sphingomonas)和 Gaiella属的相对丰度较高. 与对

照点相比,牛粪还田区土壤微生物群落组成中 PLTA13 属的相对丰度提高了 0.98%,而鞘氨醇单胞 菌属(Sphingomonas)和 Gaiella 属的相对丰度分别降 低了 1.14% 和 1%.

表 3 基于 Anosim 分析获得的组间差异<sup>1)</sup>

		Table 3	Differences between	een groups based on Anos	im analysis		
项目	组1	组2	样本数量	置换检验次数	R	P	$\overline{q}$
参数	NR-M	NR-C	18	999	0. 9334	0. 001	0. 001

1) P < 0.05, 表示组间差异显著

由图 4(a) 可知,在门分类水平下,芽单胞菌门 (Gemmatimonadetes)和内生菌门(Entotheonellaeota)的相对丰度在牛粪还田区与对照点之间存在显著性差异(P < 0.05).由图 4(b) 可知,在属水平下,变形杆菌门中的鞘氨醇单胞菌属(Sphingomonas)、PLTA13 属、MSB-4B10属、盐单胞菌属(Halomonas)和 CCD24 属,放线菌门中的 Gaiella 属和节杆菌属(Arthrobacter),厚壁菌门中的芽孢杆菌属(Bacillus),内生菌门中的 Entotheonellaceae 属的相对丰度在牛粪还田区与对照点土壤之间存在显著性差异(P < 0.05).

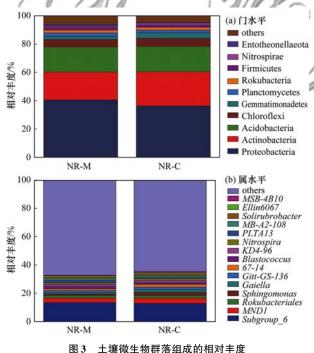


Fig. 3 Relative abundance of soil microbial community composition

2.5 土壤微生物群落组成与土壤性质之间的相关性 牛粪还田区(NR-M)土壤微生物群落组成与土 壤性质之间的相关系数如表 4 所示. 从中可知, MND1属、芽球菌属(Blastococcus)、硝化螺菌属 (Nitrospira)、芽孢杆菌属(Bacillus)和酸杆菌属 (Acidibacter)的相对丰度与含水量之间呈现显著相

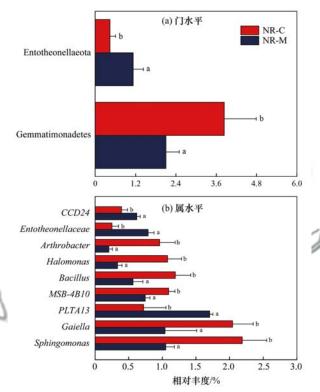


图 4 牛粪还田区与对照点土壤微生物群落组成差异

Fig. 4 Differences in soil microbial community composition between cow manure application site and control site

关性(P<0.05), Gitt-GS-13 属和 MB-A2-10 属的相对丰度与含水量之间呈现极显著相关性(P<0.01). 芽球菌属(Blastococcus)的相对丰度与放线菌数量之间呈现显著相关性(P<0.05), MND1 属、Rokubacteriales 属、硝化螺菌属(Nitrospira)和 MSB-4B10 属的相对丰度与放线菌数量之间呈现极显著相关性(P<0.01). 由此可见,土壤含水量和放线菌数量是影响牛粪还田区土壤微生物群落组成的关键因子.

### 2.6 牛粪微生物与土壤微生物之间的比较

属水平下牛粪微生物与牛粪还田区(NR-M)和对照点(NR-C)土壤微生物之间的比较如表 5 所示.从中可知,与对照点相比,牛粪还田区土壤微生物中侏囊菌属(Nannocystis)、Planifilum属、假单胞菌属(Pseudomonas)、短波单胞菌属(Brevundimonas)、假

黄单胞菌属(Pseudoxanthomonas)和梭状芽孢杆菌属(Clostridium)的相对丰度较高,并且新增出现了土芽孢杆菌属(Geobacillus)、赖氨酸芽胞杆菌属

(Lysinibacillus)、链球菌属(Streptococcus)、甲基暖菌属(Methylocaldum) 和藤黄单孢菌属(Luteimonas).而上述菌属均在牛粪中被检测出.

表 4 属水平下牛粪还田区土壤微生物群落组成与土壤性质之间的相关性1)

TP 1.1 4	C 1.1 1. 1	1 . 1 . 1	5. 5.1 1 11		10 00 00 00 1 1 1
Lable 4	Correlation between soil	l microbial commiin	ity composition and soil	properties in cow man	ure application site at the genus level

菌属	pН	含水量	硝氮	氨氮	总磷	有机质	细菌	真菌	放线菌
MNDI	0. 73 *	- 0. 75 *	0.05	-0.55	-0.48	-0.55	-0.21	-0.01	- 0. 89 **
Rokubacteriales	0.48	-0.56	0. 15	-0.26	-0.78 *	-0.76*	-0.07	-0.25	- 0. 86 **
Gitt-GS-13	0. 63	- 0. 83 **	0. 25	-0.81 **	-0.05	-0.28	-0.29	-0.36	-0.33
Blastococcus	-0.46	0.66*	0. 15	0.50	0.35	0.71 *	0.38	0.35	0. 74 *
Nitrospira	0.43	- 0. 73 *	-0.15	-0.53	-0.31	-0.55	-0.13	-0.15	- 0. 82 **
MB-A2-10	0. 76 *	- 0. 83 **	0. 25	-0.83 **	-0.08	-0.18	-0.26	-0.15	-0.45
MSB-4B10	0.58	-0.48	0. 26	-0.26	-0.60	-0.45	0.008	0. 15	- 0. 92 **
Bacillus	0.43	- 0. 68 *	-0.11	-0.56	-0.25	-0.71*	-0.52	-0.63	-0.34
Acidibacter	-0.43	0. 76 *	0.48	0. 80 **	-0.18	0.05	0. 17	-0.01	0. 43
SCI-I-84	-0.60	0.45	0.16	0.41	0	0.51	0.77 *	0.16	0. 18

<sup>1) \*</sup>表示在 0.05 水平上显著相关性, \*\*表示在 0.01 水平上显著相关性

表 5 属水平下牛粪微生物与土壤微生物的比较1)

Table 5 Comparison between cow manure microbes and soil microbes at the genus level

菌属	牛粪	牛粪还田区	对照点
Geobacillus	0. 007 581 ± 0. 003 628	0. 000 021 ± 0. 000 059	ND /
Lysinibacillus	$0.004097 \pm 0.004018$	0. 000 089 ± 0. 000 178	ND
Streptococcus	$0.000026 \pm 0.000052$	$0.000017 \pm 0.000048$	ND
Methylocaldum	$0.001\ 205 \pm 0.001\ 693$	$0.000023 \pm 0.000067$	ND (
Luteimonas	$0.001731 \pm 0.002076$	$0.000016 \pm 0.000047$	ND
Nannocystis	$0.000204 \pm 0.000268$	$0.000388 \pm 0.000305$	$0.000267 \pm 0.000175$
Planifilum	$0.002642 \pm 0.001438$	$0.000\ 101\ \pm 0.000\ 155$	$0.000009 \pm 0.000026$
Pseudomonas	$0.000096 \pm 0.000079$	$0.006702 \pm 0.004371$	$0.004348 \pm 0.004744$
Brevundimonas	$0.000034 \pm 0.000039$	$0.000193 \pm 0.000447$	$0.000133 \pm 0.000137$
Pseudox anthomonas	$0.004384 \pm 0.002635$	$0.000599 \pm 0.000587$	$0.000169 \pm 0.000193$
Clostridium	$0.002117 \pm 0.000914$	$0.000569 \pm 0.000308$	$0.000176 \pm 0.000337$

<sup>1)</sup> ND 表示菌属未检测出

#### 3 讨论

3.1 牛粪还田区土壤微生物群落组成及其影响因素 本研究中,与对照点土壤相比,牛粪还田增加了 变形杆菌门(Proteobacteria)的相对丰度,而减小了 放线菌门(Actinobacteria)和芽单胞菌门 (Gemmatimonadetes)的相对丰度. 表明不同菌门对 有机肥施用的响应存在差异. 粪便施用后,土壤有机 碳和养分含量的增加,为作物生长提供了营养元素, 提高了根系的生理活性,从而影响了与根际效应关 系密切的变形杆菌门,导致其相对丰度的增加[43]. 与之不同的是,刘平静等[4]认为土壤有机质含量与 放线菌门和芽单胞菌门的相对丰度呈负相关关系. 分析其原因可能为该门细菌对有机肥的施用非常敏 感,有机肥的施用会抑制其生长和繁殖,导致其相对 丰度减少.此外,部分菌门(Gemmatimonadetes、 Entotheonellaeota) 和部分菌属(Sphingomonas、 PLTA13 MSB-4B10 Halomonas CCD24 Gaiella Arthrobacter、Bacillus、Entotheonellaceae)的相对丰度在牛粪还田区与对照点土壤之间存在显著性差异 (P < 0.05).由此可见,牛粪还田显著影响了土壤微生物群落组成特征.

已有研究表明,土壤性质对土壤微生物丰度及 群落多样性具有显著影响<sup>[45,46]</sup>.本研究发现,影响 牛粪还田区土壤微生物群落组成的重要因素是土壤 含水量和放线菌数量. Sheik 等<sup>[47]</sup>研究表明,土壤含 水量是影响微生物群落组成的重要因子,这与本文 的研究结果是一致的. 这可能是由于土壤含水量在 调节微生物碳氮循环方面发挥重要作用,土壤微生 物群落对土壤含水量的变化较为敏感<sup>[48]</sup>. 另外,土 壤放线菌在有机质的分解中占主导地位,参与有机 质分解和腐殖质形成分解过程. 牛粪施入增加了土 壤中有机质含量,促进了放线菌的繁殖以及分解作 用,从而导致其对土壤微生物群落有较强的影响作 用<sup>[49,50]</sup>.

本研究中,牛粪还田区土壤的优势菌属为

Subgroup\_6 属、MND1 属、Rokubacteriales 属、鞘氨醇单胞菌属(Sphingomonas)和 Gaiella 属. 彭玉娇等<sup>[51]</sup>研究表明,在猪粪还田土壤中,Bryobacter 属、Chujaibacter 属和乳杆菌属(Lactobacillus)为优势菌属;在鸡粪还田土壤中,Chujaibacter 属、Romboutsia属和 Acidipila 属为优势菌属. 谭骏等<sup>[52]</sup>研究表明,在土壤中施入蚯蚓粪后,芽孢杆菌属(Bacillus)、鞘氨醇单胞菌属(Sphingomonas)和 Vicinamibacterales属为主要优势菌属. 由此可见,不同粪便类型是影响土壤微生物群落组成的重要因素. 分析其原因应为,畜禽粪便还田会影响土壤微生物的分解代谢,不同粪便由于成分不同,其影响存在差异<sup>[53]</sup>. 牛粪还田区土壤微生物对碳水化合物、氨基酸、脂类、醇类和有机酸等的分解代谢能力相对较低,进而影响土壤微生物数量和群落结构使其产生差异<sup>[12]</sup>.

#### 3.2 牛粪施用对土壤病原菌的影响

畜禽粪便施入土壤后,除了能够为土壤提供大量的营养元素外,它含有的微生物也会进入到土壤环境中.本研究发现,对照点和还田区土壤中共检测出913个菌属,牛粪中共检测出228个菌属,三者共有15个菌属.表明牛粪中的微生物进入土壤后,其中大部分微生物是不能在土壤中存活的.这是因为牛粪微生物主要由肠道菌群组成,由于土壤和肠道的环境条件不同,同时它们与土著微生物相比,在土壤环境中的竞争力不强,导致大部分牛粪微生物无法在土壤中生存[54].

值得注意的是,牛粪施用增加了假单胞菌属 (Pseudomonas)的相对丰度,这与 Kang 等[55]的研究 结果是一致的. 假单胞菌属为革兰氏阴性杆菌,能够 引起人和动物的类鼻疽病,导致败血症或休克. 另 外,牛粪施用也增加了梭状芽孢杆菌属 (Clostridium)和链球菌属(Streptococcus)的相对丰 度. 梭状芽孢杆菌属(Clostridium)为革兰氏阳性梭 状杆菌属,主要存在于土壤、动物肠道和腐败物中, 能够引起破伤风等疾病. 链球菌属(Streptococcus)是 常见的化脓性球菌、广泛存在于动物粪便以及自然 界、引起各种化脓性炎症、猩红热、丹毒、新生儿败血 症、脑膜炎、产褥热以及链球菌变态反应性疾病等. 由于牛肠道中的革兰氏阳性菌以梭菌和杆菌为主, 在牛粪被还田后,革兰氏阳性梭状芽孢杆菌能够在 土壤中继续存活[56],从而导致其在土壤中的相对丰 度增加. 因此,牛粪还田引发的潜在生物污染风险值 得引起人们足够的重视.

通过相关性分析对病原菌与非病原菌之间的关系进行研究(见表 6). 结果发现,假单胞菌属(Pseudomonas)的相对丰度与TRA3-20属的相对丰

度之间呈现显著正相关性(P < 0.05),与 bacteriap25 属的相对丰度之间呈现显著负相关性(P < 0.05). 梭状芽孢杆菌(Clostridium)的相对丰度与PLTA13 属(P < 0.05)、NB1-i属(P < 0.01)、CCD24属(P<0.05), Thauera  $\mathbb{A}(P < 0.01)$ , OM190  $\mathbb{A}(P < 0.01)$ 0.05)和 Azoarcus 属(P < 0.01)的相对丰度之间呈 现显著正相关性, 与 67-14 属 (P < 0.01)、 Arthrobacter  $\mathbb{A}(P < 0.01)$   $\mathbb{A}(P < 0.01)$   $\mathbb{A}(P < 0.01)$ 0.05)的相对丰度之间呈现显著负相关性.链球菌 属(Streptococcus)的相对丰度与 Pirellula 属和 Subgroup\_17 属的相对丰度之间呈现显著负相关性 (P<0.05). 由此可见,病原菌和非病原微生物之间 存在着复杂的相互作用关系,这主要是因为微生物 的代谢物可分泌到环境中,诱导它们相互作用,从而 产生协同、拮抗或中立的作用结果[57].一方面,部 分非病原微生物和病原菌是拮抗关系,能够抑制其 生长;另一方面,部分非病原微生物能够促进病原 菌的生长,因为它们能够利用病原菌的代谢物,这种 共生关系能够增加它们对土壤中营养物质的获 取[58,59].

表 6 属水平下病原菌与非病原菌之间的相关性1)

Table 6 Correlation between pathogenic and non-

pa	ithogenic bacteria a	it the genus iever	1
非病原菌	18	病原菌	8
北州水西	Pseudomonas	Clostridium	Streptococcus
PLTA13	0. 38	0. 52 *	-0.08
NBI- $j$	0.38	0. 65 **	-0.31
TRA3-20	0. 54 *	0.35	-0.24
CCD24	0. 44	0.55 *	-0.04
Thauera	0. 27	0. 67 **	-0.12
OM190	0. 23	0.56*	-0.39
Azoarcus	0. 16	0. 62 **	0
67-14	-0.44	-0.60 **	0. 16
Arthrobacter	-0.19	-0.73 **	0. 01
Pirellula	-0.06	0	- 0. 49 *
Subgroup_7	-0.33	-0.53*	-0.06
bacteriap25	-0.57*	-0.13	0. 12
$Subgroup\_17$	-0.07	-0.34	- 0. 48 *

1) \* 表示在 0.05 水平上显著相关性,\*\* 表示在 0.01 水平上显著相关性

### 4 结论

- (1)与对照点相比,牛粪还田区土壤微生物群落β多样性发生了显著变化.
- (2) 牛粪还田增加了变形杆菌门(Proteobacteria)的相对丰度,而减少了放线菌门(Actinobacteria)和芽单胞菌门(Gemmatimonadetes)的相对丰度.
- (3)牛粪还田显著改变了土壤微生物群落中的 鞘氨醇单胞菌属(Sphingomonas)、PLTA13 属、MSB-

- 4B10 属、盐单胞菌属(Halomonas)、CCD24 属、Gaiella 属、节杆菌属(Arthrobacter)、芽孢杆菌属(Bacillus)和 Entotheonellaceae 属的相对丰度(P < 0.05).
- (4)影响牛粪还田区土壤微生物群落组成的重要因素是土壤含水量和放线菌数量.
- (5)大部分牛粪微生物不能够在土壤中存活, 但牛粪还田增加了假单胞菌属(Pseudomonas)和梭 状芽孢杆菌属(Clostridium)等病原菌的相对丰度, 并且引入了新的病原菌链球菌属(Streptococcus),对 土壤质量存在一定的生物污染.

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# **HUANJING KEXUE**

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