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沼液施用对麦稻茬口期土壤微生物群落结构特征及功 能的影响

乔宇颖, 奚辉, 李娜, 陈喜靖, 沈阿林, 喻曼*

(浙江省农业科学院环境资源与土壤肥料研究所,杭州 310021)

摘要:为揭示麦稻茬口期沼液施用对土壤微生物群落及功能的影响,通过土柱试验,设置麦秆还田后 3 种处理[无氮肥(CM)、常规施肥(SN)和添加沼液(SZ)],采用 Illumina 高通量测序技术分析了土壤淹水后 1 d 和 21 d 土壤细菌及真菌群落的组成、多样性和结构差异,并对其功能进行预测. 微生物多样性分析表明,1 d 时各处理的真菌 α 多样性指数均显著高于 21 d 处理且各处理间无显著差异; 21 d 时各处理细菌 Simpson 指数开始出现差异,SZ-21 处理的细菌 Simpson 指数高于 SN-21 处理,而细菌 Chao1 指数显著低于 SN-21 处理;细菌群落结构分析表明,1 d 时,SN 处理的厚壁菌门、绿弯菌门和放线菌门与其它处理差异较大,而 21d 时,SZ 和 SN 处理的各细菌菌门相对丰度相似;真菌群落结构分析表明,1 d 时,SZ 处理中的子囊菌门和接合菌门相对丰度高于 SN 和 CM 处理; 21 d 时,SN 和 SZ 处理的子囊菌门相对丰度均低于 CM 处理,而接合菌门的相对丰度均高于CM 处理. NMDS 分析表明,21 d 时,SN 和 SZ 处理的细菌和真菌群落组成均有逐渐相似的趋势. PICRUSt 功能分析表明,不同时期和不同处理的土壤细菌群落在功能层上表现相似,FUNGuild 功能预测反映 SZ-21 和 SN-21 处理的真菌功能主要差异体现在腐生营养型和病理营养型上. 综上说明茬口期施沼液在一定程度上可以替代化肥平衡土壤养分和维持土壤生态功能,但仍可能存在真菌致病风险.

关键词: 沼液; 细菌群落; 真菌群落; 麦稻茬口期; 高通量测序; 功能预测

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Effects of Biogas Slurry Application on Soil Microbial Communities Structure and Function During Wheat-rice Stubble Period

QIAO Yu-ying, XI Hui, LI Na, CHEN Xi-jing, SHEN A-lin, YU Man*

(Institute of Environment, Resource, Soil and Fertilizer, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China)

Abstract: To reveal the effects of biogas slurry application on soil microbial community structure and function, a soil column experiment was constructed with three treatments [(no N addition, CM; conventional fertilization, SN; biogas slurry addition, SZ)]. The differences in composition, diversity, and structure of bacterial and fungal communities on day 1 and day 21 after soil flooding were evaluated, and their functions were predicted using Illumina high-throughput sequencing technology. The results of the analysis of α diversity showed that the fungal α-diversity indexes of CM, SN, and SZ treatments on day 1 were significantly higher than those on day 21, and there was no significant difference among the three treatments. However, the bacterial Simpson index differed among the three treatments on day 21, with SZ-21 showing a higher Simpson index but lower Chaol index compared with those of SZ-21. The analysis of bacterial community structure showed that Firmicutes, Chloroflexi, and Actinobacteria in the SN-1 treatment were different from those in the other treatments on day 1, whereas the relative abundance of bacterial phyla in the SZ and SN treatments were similar on day 21. The analysis of fungal community structure showed that the relative abundance of Ascomycota and Zygomycota in the SZ-1 treatment were higher than those in the SN-1 and CM-1 treatments on day 1. The relative abundance of Ascomycota in the SN-21 and SZ-21 treatments were lower, whereas that of Zygomycota were higher compared with that in CM-21. The analysis of NMDS showed that the composition of bacterial and fungal communities in the SN and SZ treatments showed a gradually similar trend. The PICRUSt analysis showed that the function of the soil bacterial community was similar in the CM, SN, and SZ treatments. The FUNGuild function prediction reflected that the main differences in trophic type between the SN-21 and SZ-21 treatments occurred in saprotroph and pathotroph forms. Therefore, biogas slurry addition in the wheat-rice stubbl

Key words: biogas slurry; bacterial communities; fungal communities; wheat-rice stubble period; Illumina high-throughput sequencing; function prediction

沼液是沼气工程的副产物,氨氮含量较高,无序排放会导致水体环境污染,但合理使用则是一种优质的液体有机肥,沼液用于稻田能提高水稻产量和品质,改善土壤理化性质^[1,2],而过量施用或施用时间不当可能会引发新的环境风险^[3-6],因此,在维持作物产量和维护土壤环境安全条件下,明确沼液的施用时间与施用量仍是当前研究的热点之一.

土壤微生物是土壤养分元素循环的主要驱动力,其群落结构和生态功能的变化可用于评估土壤肥力水平高低和生态系统的稳定性,比土壤理化性

质能更有效和快速响应土壤质量的变化^[7~10]. 尤其是随着高通量测序技术的普及,国内外研究者已采用该方法深入研究了沼液还田后土壤微生物群落组成和分布特征.有研究发现,沼液还田能显著改变土壤微生物群落结构,增加细菌、真菌和放线菌的数

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作者简介: 乔宇颖(1994~),女,硕士研究生,主要研究方向为土壤

微生物与土壤生态, E-mail: qiaoyy18@ nwafu. edu. cn

* 通信作者,E-mail;yuman@zaas.ac.cn

量[11~14]. 但是,现有研究对沼液还田与土壤微生物 群落组成和多样性的关系暂无完全一致的定论,且 关注土壤微生物生态功能的研究相对较少,如 Tang 等[13]研究发现沼液添加对土壤细菌和真菌的影响 存在差异, 沼液处理下的土壤细菌群落结构组成更 复杂: Yu 等[15]的研究结果显示, 沼液提高了根际 土壤中的真菌群落多样性,但不能提高细菌多样性; Xu 等[16]认为中等剂量的沼液施用可以提高土壤细 菌多样性. PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states)和 FUNGuild 分别是针对细菌和真菌菌群功能和代谢 的预测软件(数据库),可以通过高通量测序获得的 基因序列预测对应的细菌和真菌代谢功能和生态功 能,目前不同生态系统下关于微生物群落功能预测 已有不少应用[17~22],将该方法用于预测沼液还田后 土壤微生物功能特征有助于进一步深刻理解沼液还 田土壤环境生态安全.

麦稻茬口期(麦秆还田后水稻移栽前)是长江 中下游稻麦轮作过程中的一个特殊时期,短期内大 量秸秆还田的腐解产物和土壤氮素的快速消耗极易 导致土壤生态环境不稳定,造成下茬作物根系发育 受阻, 茬口期内的水肥管理对改善水稻苗期土壤环 境至关重要[23~26].有研究者尝试在麦稻茬口期施用 沼液替代化肥,并发现沼液在短期施用条件下能促 进水稻幼苗生长,提高土壤细菌丰富度及多样性和 土壤质量[27,28]. 但是,已有研究缺乏对土壤真菌微 生物群落结构分析及整个群落的功能预测. 因此,本 文通过土柱试验模拟麦稻轮作茬口期,运用 Illumina 高通量测序技术研究麦秆还田下茬口期内土壤微生 物群落组成的变化,除分析土壤微生物组成和多样 性之外,还完善其细菌和真菌功能预测,探讨沼液施 用短期内对土壤微生态的响应机制,以期为调控和 优化麦稻茬口期内的沼液利用提供理论基础.

1 材料与方法

1.1 试验概况与试验设计

本试验供试土壤采自浙江安吉桐杭镇,粉砂质水稻土. 采集时间为 2018 年 5 月(小麦收获后),采集深度为 0 ~ 20 cm,自然风干,去除根系残叶和石子,过 2 mm 筛后备用. 土壤基本理化性质为:pH 值7. 10, ω (全氮)0. 25 g·kg⁻¹, ω (全磷)0. 68 g·kg⁻¹, ω (全钾)25. 10 g·kg⁻¹, ω (有机质)4. 50 g·kg⁻¹. 供试沼液取自嘉兴科皇牧业有限公司沼液膜浓缩设备陶瓷膜出水,原液为畜禽养殖废弃物厌氧发酵后液相产物,基本理化性质为:pH 值7.59, ω (全氮)1. 28 g·L⁻¹, ω (全磷)0. 07 g·L⁻¹, ω (全钾)0. 74

g·L⁻¹,EC 值 9. 25 mS·cm⁻¹. 还田麦秆风干后粉碎成 2 cm 的碎段. 试验共设 3 个处理: 无氮肥处理(CM)、常规施肥处理(SN)、沼液处理(SZ),每个处理设 3 个重复. 麦秆添加量为 2 g·kg⁻¹. SN 和 SZ处理氮投入水平为 0. 033 g·kg⁻¹,其中 SN 处理中的氮以尿素(46%)形式投入,SZ 处理的氮以沼液形式投入. 为避免沼液中磷钾养分的干扰,CM 和 SN 处理中对应添加过磷酸钙(12%)和氯化钾(60%),投入量为过磷酸钙 0. 017 g·kg⁻¹ 和氯化钾 0. 032 g·kg⁻¹.

土柱尺寸为内径 30 cm×高 40 cm,麦秆粉碎后与土壤充分混匀后装入土柱装置.土壤填充深度为20 cm,分层填装,填装前根据土壤容重1.4 g·cm⁻³,将对应土层土壤浇水至最大田间持水量,填装后用平板轻轻压实.所有处理均先填装土壤,然后将肥料或沼液均匀洒在土层表面,最后进行淹水处理,以上步骤均在同一天完成.土壤保持淹水(3~5 cm)状态,每隔2 d补充一次.

1.2 土壤样品采集

分别于土壤淹水后 1 d 和 21 d 采集土壤,使用 铲子 5 点混合取样,取样深度为 $0 \sim 20$ cm,样品采集后放入 -20 % 冰箱保存,用于 DNA 提取以及后续的生物信息学分析.

1.3 样品总 DNA 提取

采用试剂盒(E. Z. N. A. ® soil DNA kit, OMEGA)按其操作手册提取土壤微生物基因组DNA. 所提取的土壤总 DNA 的浓度和纯度使用Nanodrop 2000UV-Vis 分光光度计(Thermo Scientific, U. S. A.)检测.

1.4 高通量测序

细菌采用引物 338F(5'-ACTCCTACGGGAGGC AGCAG-3') 和 806R (5'-GGACTACHVGGGTWTC TAAT-3') 扩增其 16S rRNA 基因的 V3-V4 高变区序 列. 真菌采用引物 ITS1F (5'-CGTAGGTGAACCTG CGGAGATC- 3') 和 ITS2R (5'-GTTCAGCGGGTG-ATCCTGCCTG -3') 扩增其 ITS rRNA 区序列. 二者 PCR 扩增程序分别为:95℃ 预变性 3 min,95℃ 变性 30 s, 55℃退火 30 s, 72℃延伸 45 s, 72℃修复延伸 10 min, 27 个循环; 95℃预变性 3 min, 95℃变性 30 s, 55℃退火 30 s, 72℃延伸 45 s, 72℃修复延伸 10 min, 35 个循环. 338F 和 806R 引物的 PCR 正式试 验采用 TranGen AP221-02: TransStart Fastpfu DNA Polymerase, 20 µL 反应体系. ITS1F 和 ITS2R 引物 的 PCR 正式试验采用 TaKaRa rTag DNA Polymerase, 20 μL 反应体系. 利用上海美吉生物医 药科技有限公司的 MiSeq PE300 测序仪 (Illumina Inc, San Diego, CA, USA)完成高通量测序.

1.5 数据分析

1.5.1 生物学信息分析

利用 QIIME (Quantitative Insights into Microbial Ecology) 软件对原始序列进行质量过滤,得到高质量序列. 利用 UPARSE (version 7.0.1090 http://drive5.com/uparse/)软件将 97% 相似水平下的高质量序列归为多个 OTU (operational taxonomic units). 采用 RDP classifier 贝叶斯算法对 97% 相似水平的OTU代表序列进行分类学分析,置信度阈值为 0.7,细菌种类的分类基于 Silva (Release138 http://www.arb-silva.de)数据库,真菌种类的分类基于 Unite (Release 8.0 http://unite.ut.ee/index.php)的真菌ITS 数据库.按照最低测序深度对样本进行随机重抽样,借助 Mothur(v.1.30.2)软件分析细菌和真菌群落的 Alpha 多样性(Shannon 多样性指数、Simpson 多样性指数和 Chao1 指数).

1.5.2 PICRUSt 功能预测分析

采用 PICRUSt 软件进行细菌功能预测分析,利用 QIIME 获得的 colsed OTU Table 与 COG 和 KEGG 数据库进行比对,获得功能预测信息,具体分析步骤基于 在线分析 平台(http://picrust. github. io/picrust)^[29].

1.5.3 真菌功能类群 FUNGuild 分析

利用 FUNGuild v1.0 工具对真菌的营养类型及 其功能类群分类. FUNGuild 是一个基于目前已发表 的文献或权威网站的数据库,通过数据库的比对分 析,可以得到"极可能"(highly probable)、"很可能" (probable)和"可能"(possible)这3个置信度等级. 为了避免过度解读真菌功能类群,只保留置信度 "极可能"(highly probable)和"很可能"(probable) 这2个等级,无法识别的真菌群落被标记为"未定 义"(undefined)^[30].

1.5.4 统计学分析

运用 IBM SPSS Statistics 25 软件进行单因素方差分析细菌和真菌群落的 Alpha 多样性指数;运用 Origin 2019b 软件绘制土壤细菌和真菌群落结构的 相对丰度图以及真菌群落的功能预测占比图;采用基于 Bray-Curits 距离矩阵的非度量多维尺度分析 (non-metric multidimensional scaling, NMDS)确定细菌和真菌群落的结构差异;利用 R 语言 vegan 软件包进行 ANOSIM 相似性分析,以判断组间差异是否显著大于组内差异;运用 GraphPad Prism 8.0.1 软件绘制细菌预测基因丰度热图;运用 STAMP (v2.1.3)进行真菌功能差异显著分析及绘图.

2 结果与分析

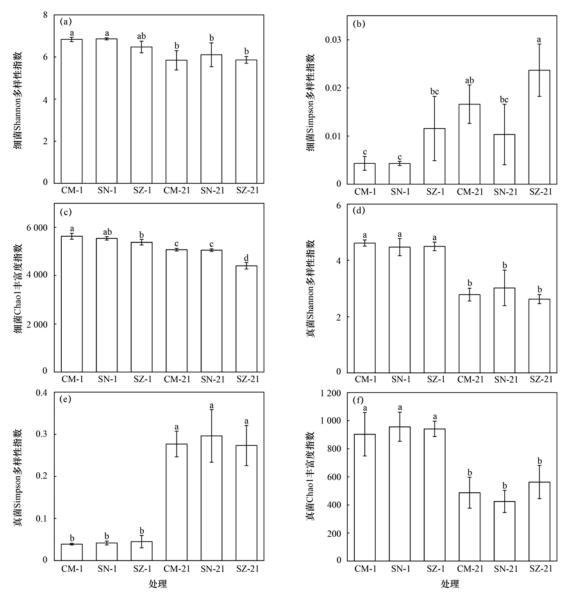
2.1 沼液施用对茬口期土壤微生物群落多样性的影响

本文运用 Shannon 指数、Simpson 指数和 Chaol 指数分别反映麦稻茬口期的微生物群落多样性、群落优势物种多样性和群落物种丰富度(图 1). Shannon 指数越高,则物种群落的多样性越高; Simpson 指数越低,则优势物种多样性越高; Chaol 指数越高,则丰富度越高. 结果显示,1 d 时各处理的真菌 α 多样性指数均显著高于 21 d 处理且各处理间无显著差异,表明各处理真菌群落变化主要受淹水影响,对施氮方式响应不明显; 21 d 时各处理细菌 Simpson 指数开始出现差异,SZ-21 处理的细菌 Simpson 指数开始出现差异,SZ-21 处理的细菌 Simpson 指数高于 SN-21 处理,而细菌 Chaol 指数显著低于 SN-21 处理,说明 21 d 后,沼液处理的细菌丰富度较氮肥处理有所下降,但优势物种多样性有所上升.

2.2 沼液施用对茬口期土壤细菌和真菌群落结构 的影响

对测序结果进行比对,得到3 277 024 条有效16S rRNA 基因序列,基于97%的相似性阈值进行聚类,得到8 231个 OTU (operational taxonomic units).聚类结果表明,细菌菌群分属 48 门、130 纲、258 目、487 科、988 属和2 135种.

门和属水平上细菌群落的物种组成如图 2 所 示,在门和属水平上分别检测到 10 种和 25 种相对 丰度大于1%的细菌门和属. 从图 2(a)可以看出, 茬口期秸秆还田和施肥导致土壤细菌群落在短期内 发生了明显的变化.1 d时,门水平上不同处理间丰 度比例差异较大的为厚壁菌门、绿弯菌门和放线菌 门,SN-1 处理厚壁菌门的占比仅有 4.76%,远低于 SZ-1 和 CM-1 处理,而放线菌门和绿弯菌门的占比 明显高于 SZ-1 和 CM-1 处理. 各处理细菌群落主要 包括变形菌门(Proteobacteria, 30.90%~33.75%)、 酸杆菌门(Acidobacteria, 14.63%~18.03%)、放线 菌门(Actinobacteria, 12.37%~17.71%)、厚壁菌门 (Firmicutes, 4.76% ~ 20.02%) 和绿弯菌门 (Chloroflexi, 7.69%~11.40%), 其次为芽单胞菌门 (Gemmatimonadetes, 3.00% ~ 4.62%)、拟杆菌门 (Bcateroidetes, 1.96% ~ 4.94%)、硝化螺旋菌门 (Nitrospirae, 1.64% ~ 2.26%)、蓝藻菌门 (Cyanobacteria, 0.94% ~ 1.29%) 和疣微菌门 (Verrucomicrobia, 1.16%~1.24%). 21 d 时, 门水 平上各处理细菌群落占比发生变化,厚壁菌门和放 线菌门在3个处理间差别最为明显,SN-21处理和



(a)、(b)和(c)为细菌,(d)、(e)和(f)为真菌;不同小写字母表示处理间差异显著(P<0.05)

图1 不同处理土壤细菌和真菌的 α 多样性

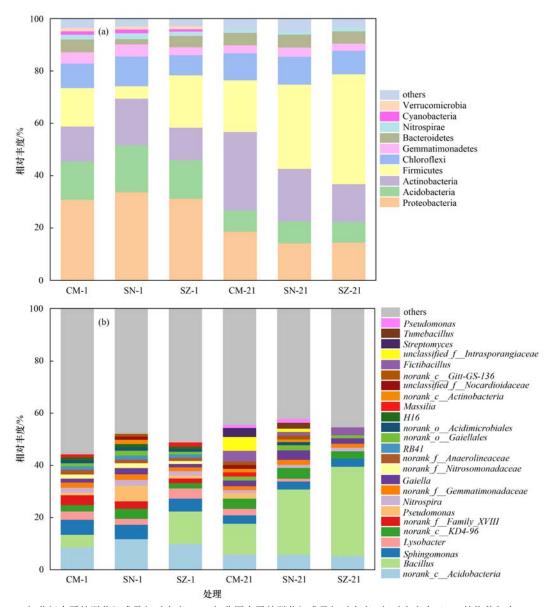
Fig. 1 The α diversity of soil bacterial and fungal communities

SZ-21 处理各细菌菌门的相对丰度较为相似,其中,厚壁菌门占比在 SN-21 和 SZ-21 处理高于 CM-21 处理,放线菌门占比在 SN-21 和 SZ-21 处理中低于 CM-21 处理.各处理的主要菌门依然为厚壁菌门 (Firmicutes, 19.73% ~ 41.95%)、放线菌门 (Actinobacteria, 14.46% ~ 29.88%)、变形菌门 (Proteobacteria, 14.24% ~ 18.73%)、绿弯菌门 (Chloroflexi, 8.98% ~ 10.66%)和酸杆菌门 (Acidobacteria, 7.90% ~ 8.36%),但各菌门的相对丰度变化明显.其次为拟杆菌门(Bcateroidetes, 4.73% ~ 4.99%)、芽单胞菌门(Gemmatimonadetes, 2.72% ~ 3.50%)和硝化螺旋菌门(Nitrospirae, 1.13% ~ 1.37%).

图 2(b) 反映了各处理样本细菌在属水平上的变化情况.1 d 时, CM 处理、SN 处理与 SZ 处理在属

水平上的细菌群落开始出现差异,其中,芽孢杆菌属 (Bacillus)在 SZ-1 处理中丰度最高,占比 12.52%,但在 SN-1 处理中低于 1%;假单胞菌属 (Pseudomonas)在 SN-1 处理中丰度最高,占比5.99%,但 CM-1 和 SZ-1 处理中占比仅为 1% 左右.21 d时,不同处理在属水平上的细菌群落结构组成及相对丰度差异更为明显, CM-21 处理的群落结构组成及相对丰度差异更为明显, CM-21 处理的群落结构组成较为复杂(相对丰度大于 1%的菌属有 18 种),而 SZ-21 处理的群落结构组成较为简单(相对丰度大于 1%的菌属有 9 种).另外,芽孢杆菌属(Bacillus)在不同处理间的丰度占比差异最明显,在SZ-21、SN-21 和 CM-21 处理中占比分别为34.18%、25.14%和11.88%.

对测序结果进行比对,得到3 697 192条有效 ITS 基因序列,基于 97% 的相似性阈值进行聚类,得



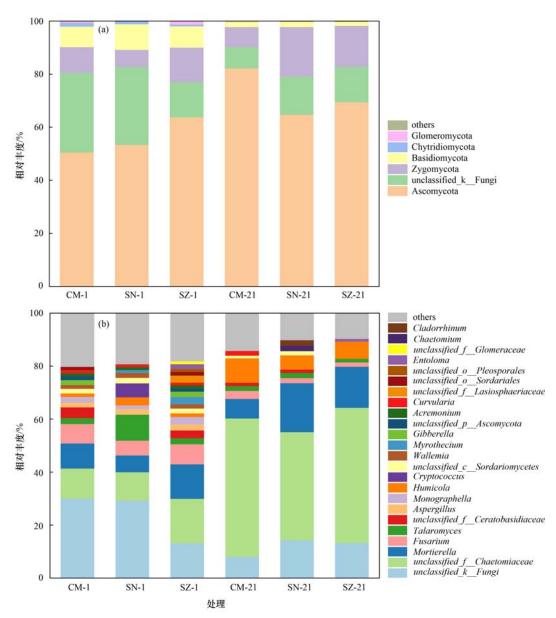
(a)细菌门水平的群落组成及相对丰度; (b)细菌属水平的群落组成及相对丰度; 相对丰度小于1%的物种归为 others **图 2 各处理土壤细菌门和属水平的群落组成及相对丰度**

Fig. 2 Composition and relative abundance of bacterial phylum and genus levels in treatments

到2 684个 OTU. 聚类结果表明, 真菌菌群分属 7 门、29 纲、85 目、194 科、422 属和 717 种.

门和属水平上真菌群落的物种组成如图 3 所示,在门和属水平上分别检测到 6 种和 24 种相对丰度大于 1% 的真菌门和属. 1 d 时, SN-1 处理与 CM-1 处理真菌门水平上的菌群占比相似,而 SZ-1 处理中的子囊菌门和接合菌门相对丰度高于 SN-1 处理和 CM-1 处理. 各处理真菌群落主要包括子囊菌门(Ascomycota, 50.58% ~ 63.82%)、未知菌门(unclassified_k_Fungi, 13.24% ~ 30.02%)、接合菌门(Zygomycota, 6.43% ~ 13.03%)和担子菌门(Basidiomycota, 7.90% ~ 9.66%), 其次为壶菌门(Chytridiomycota, 0.70% ~ 1.38%)和球囊菌门(Glomeromycota, 0.04% ~ 1.13%). 21 d 时, SN-21处理与 SZ-21 处理的群落组成更为相似,两个处理

的子囊菌门的相对丰度均低于 CM 处理,而接合菌门的相对丰度均高于 CM 处理. 各处理的主要真菌群 落变为子囊菌门(Ascomycota, 64.75%~82.28%)、接合菌门(Zygomycota, 7.41%~18.74%)、未知菌门(unclassified_k_Fungi,8.13%~14.39%)、担子菌门(Basidiomycota, 1.54%~2.04%). 各处理真菌在属水平上的变化如图 3(b)所示, 1 d 时, 3 个处理在属水平上的真菌群落结构就呈现差异,SZ-1处理中如rclassified_f_Chaetomiaceae、被孢霉属(Mortierella)和镰刀菌属(Fusarium)的相对丰度明显高于其它处理;SN-1处理中篮状菌属(Talaromyces)和隐球菌属(Cryptococcus)相对丰度较高,而CM-1处理和SZ-1处理中的相对丰度均小于1%.21d时,3个处理的真菌群落组成较1d时更简单,SN-21和SZ-21处理



(a) 真菌门水平的群落组成及相对丰度; (b) 真菌属水平的群落组成及相对丰度; 相对丰度小于 1% 的物种归为 others 图 3 各处理土壤真菌门水平和属水平的群落组成

Fig. 3 Composition and relative abundance of fungal phylum and genus levels in treatments

中主要菌属占比接近,其中 $unclassified_f_$ Chaetomiaceae 和被孢霉属的相对丰度均高于CM-21处理,而镰刀菌属和腐质霉属的相对丰度均低于CM-21处理.

2.3 沼液施用对茬口期土壤细菌和真菌群落 β 多样性的影响

为进一步揭示各处理在1 d 和21 d 时的种群结构变化,采用 NMDS 和 ANOSIM 方法结合,分析了细菌和真菌群落的β多样性(OTU 水平).结果如图4 所示,两个图的 stress 均小于 0.1,表明排序结果较好.对细菌群落的 NMDS 分析表明[图 4(a)],1 d 和21 d 时,3 个处理的细菌群落完全分离,分别集中在 NMDS axis1 轴的左右两侧.1 d 时,CM、SN 和 SZ 处理的细菌群落组成明显不相同,并且 SN 和 SZ 处

理的细菌群落分布在 CM 处理的两侧,但在 21 d 时,SN 处理和 SZ 处理的细菌群落组成有相似的趋势.与细菌群落相同的是,各处理下的真菌群落组成在 1 d 和 21 d 时也存在明显的不同,同样分布在 NMDS axis1 轴的左右两侧[图 4(b)]. 21 d 时,SN 处理和 SZ 处理的真菌群落组成相似度明显升高.

2.4 土壤细菌群落功能预测分析

基于 KEGG 数据库(Kyoto encyclopedia of genes and genomes) 预测的结果表明(图 5),在一级功能层共获得 6 类生物代谢通路功能分析:代谢(metabolism)、遗传信息处理(genetic information processing)、环境信息处理(environmental information processing)、细胞过程(cellular processes)、有机系统(organismal systems)和人类疾

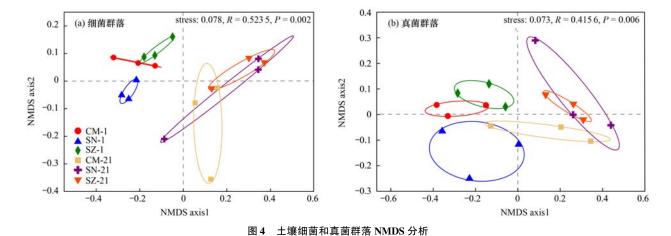


Fig. 4 NMDS analysis of soil bacterial and fungal communities

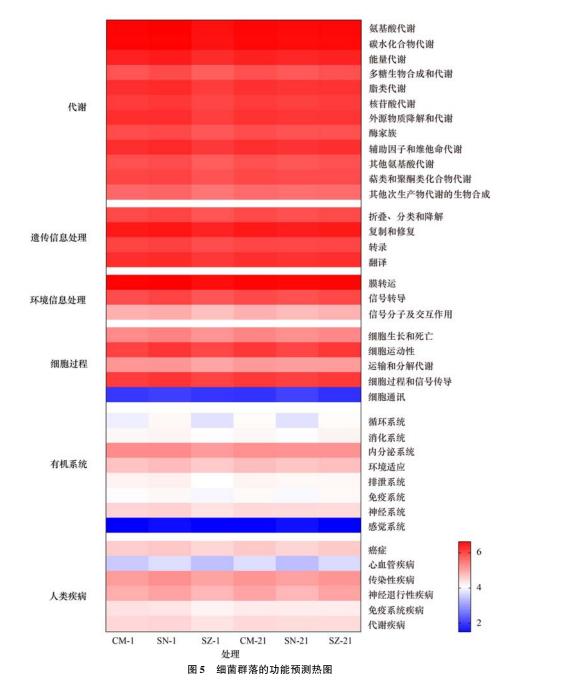


Fig. 5 Function prediction of bacterial community

病(human diseases). 其中代谢、遗传信息处理和环境信息处理为各处理的主要功能组成. 不同时期和不同处理的土壤细菌群落在各功能层上表现相似.

2.5 茬口期不同施氮方式的土壤真菌群落功能预测分析

根据 FUNGuild 功能预测可获得样品中真菌的功能分类及在各样品中的丰度信息(图 6),结果显示,真菌群落营养类型可分为 7 类,病理营养型(pathotroph)、病理-腐生营养型(pathotroph-saprotroph)、病理-腐生营养型(pathotroph-saprotroph-symbiotroph)、病理-共生营养型(pathotroph-saprotroph-symbiotroph)、腐生营养型(saprotroph)、腐生营养型(saprotroph)、腐生一类生营养型(saprotroph)、腐生营养型(symbiotroph). CM-1、SN-1 和 SZ-1 处理均以腐生营养型(10.08%、20.21%和 16.10%)和病理营养型(8.39%、5.84%和8.90%)为主;而21 d后CM-21、SN-21 和 SZ-21 处理的病理营养型占比均降低,以腐生营养型(17.22%、10.96%和 10.95%)为主,这表明21 d 时沼液和尿素处理的腐生营养型真菌占比几乎一致.

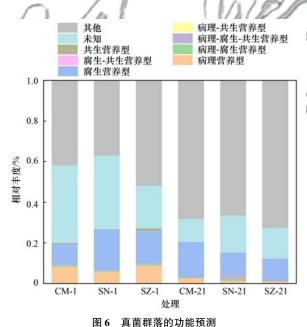


Fig. 6 Function prediction of fungal community

对上述7类营养型进行进一步分类,通过功能类群鉴定,检测出55种功能群,其中20种功能群在SN-1与SZ-1处理间存在显著差异,24种功能群在SN-21与SZ-21处理间存在显著差异(图7).SZ-1处理有9种功能群的占比显著大于SN-1,这9种功能群以腐生营养型为主[粪腐生-木质腐生真菌(dung saprotroph-wood saprotroph)、粪腐生-未定义腐生真菌(dung saprotroph-undefined saprotroph)、叶子腐生真菌(leaf saprotroph)和植物腐生-木质腐生

真菌(plant saprotroph-wood saprotroph)],其次为共 生营养型[丛枝菌根真菌(arbuscular mycorrhizal)和 外生菌根真菌(ectomycorrhizal)],还有病理营养型 「植物病原真菌(plant pathogen)]、病理-腐生营养 型「真菌寄生-木质腐生真菌 (fungal parasite-wood saprotroph)]和病理-腐生-共生营养型[植物内生-地 衣寄生-植物病原-未定义腐生真菌(endophyte-lichen parasite-plant pathogen-undefined saprotroph)]. SZ-21 处理有 12 种功能群的占比显著大于 SN-21,这 12 种功能群以腐生营养型[未定义腐生-木质腐生真菌 (undefined saprotroph-wood saprotroph)、未定义腐生 真菌 (undefined saprotroph)、粪腐生真菌 (dung saprotroph) 和 粪 腐 生-土 壤 腐 生 真 菌 (dung saprotroph-soil saprotroph)]和病理营养型为主[动物 病原菌 (animal pathogen)、植物病原菌 (plant pathogen)、地衣寄生虫(lichen parasite)、真菌寄生 菌(fungal parasite)],其次为共生营养型[外生菌根 真菌(ectomycorrhizal)和植物内生菌(endophyte)], 腐生共生营养型「外生未定义腐生真菌 (ectomycorrhizal-undefined saprotroph)]和病理-共生 营养型「植物内生-植物病原菌(endophyte-plant pathogen)]. 以上结果表明沼液与尿素添加 21 d 后 的真菌功能差异主要发生在腐生营养型和病理营养 型上.

3 讨论

土壤茬口期是稻麦轮作体系的交替时期,茬口 期适当地添加氮素有利于下茬作物的生长,平衡土 壤中的碳氮比,在一定程度上可以缓解秸秆腐解与 植物争氮的情况[26,28]. 尿素属于酰胺态氮肥,一般 经过土壤微生物的作用转化为铵态氮之后,再供作 物吸收. 沼液中的氮素主要以铵态氮的形式存在,一 般情况下沼液中铵态氮占总氮量的70%以上,是一 种缓速兼备的液体有机肥[28]. 茬口期施氮肥后使得 细菌的 Shannon、Simpson 和 Chaol 指数有所降低, 这与先前的研究结果不一致[27],其原因可能是受土 壤水分管理的影响不同. 由于茬口期淹水的土壤环 境条件会加剧微生物群落之间的竞争,影响了细菌 的群落多样性[31],从而造成了细菌多样性的改变. 在秸秆还田的基础上,尿素和沼液的配施使真菌群 落 Shannon、Simpson 和 Chaol 指数显著降低,这与 吴宪等[32]研究的结果一致.

各处理中变形菌门(Proteobacteria)、酸杆菌门(Acidobacteria)、放线菌门(Actinobacteria)、厚壁菌门(Firmicutes)和绿弯菌门(Chloroflexi)是细菌群落的优势菌门(图 1),这与以往学者的研究结果一



(a) SN-1 与 SZ-1 之间的差异; (b) SN-21 与 SZ-21 之间的差异

图 7 预测真菌功能基因在不同样品间的差异

Fig. 7 Variation in predicted functional profiles in different samples

致^[33,34]. 并且有研究指出,这几类微生物菌群在长期施肥的水稻土中也处于优势地位,可能在有机物矿化过程中有着重要贡献^[35]. 21 d 后各处理酸杆菌门(Acidobacteria)的相对丰度均有所降低,可能是其属于贫营养型微生物更适合生活在营养物质较为缺乏的环境中^[36],因此随着土壤中麦秆的腐解和氮

肥的作用,反而不利于酸杆菌门的生长. 放线菌门 (Actinobacteria) 在 SZ 处理下的相对丰度随时间有 所增大,沼液的添加可能激发了土壤中可降解麦秆等有机物的微生物种的生长,提升了土壤微生物的 秸秆分解能力^[37]. 氮肥添加 21 d 后,厚壁菌门 (Firmicutes)丰度明显增加,抑制了病原微生物群,

促进了土壤朝着有益微生物的方向发展,有防治植物病虫害的功能^[38]. 芽孢杆菌属(*Bacillus*)是厚壁菌门中的重要菌属^[38],它的快速增多使其成为茬口期的第一优势菌属,这与厚壁菌门所占比例在 21 d内快速上升的结果一致. 通过 PICRUSt 基因预测热图,也反映出不同处理间的主要功能基因均为与代谢相关功能,说明代谢在茬口期起着极其重要的作用,土壤微生物通过代谢活动加快秸秆的腐解.

茬口期施氮后,相比于土壤细菌群落结构,真菌 群落结构的改变更为剧烈. 通常真菌会率先进入麦 秆残体发挥作用,使其破碎化或腐殖质化^[39],这与 前人的研究结果一致[15,40,41],并且 SN 和 SZ 两个处 理的真菌群落组成有相似发展的趋势. 真菌还可以 与还田麦秆形成共生体,有效获取植物所需的养 分[42],如本研究中,添加沼液处理的外生菌根真菌 占比较尿素处理更高(图7),说明沼液较尿素更容 易被植物体所吸收. 子囊菌门(Ascomycota)和接合 菌门(Zygomycota)是本研究中真菌群落的优势菌门 (图2),这与Bei等[43]研究的结果一致.子囊菌门在 各处理丰度组成中占比最高,这与茬口期真菌的重 要功能类型为腐生营养型的结果一致[44]. 被孢霉属 (Mortierella)的比例在21 d 内升高,且成为仅次于 unclassified_f_Chaetomiaceae 属的优势菌属,这可 能与 Mortierella 是一种与土壤肥力密切相关的腐生 真菌有关[45],它能够产生多种生物活性物质,具有 改善土壤健康状况、有效促进植株的生长的作用, 是土壤中常见的有益微生物,并且可能具有生防功 能[46]. 另外, 茬口期配施尿素和沼液会降低镰刀菌 属的丰度,且配施沼液降低的更为明显,而镰刀菌属 (Fusarium)常被认为是禾本科作物的潜在致病 菌[47],这表明施沼液有降低土壤部分致病风险的可 能. 另外, FUNGuild 功能预测反映出茬口期施氮 21 d后的土壤中真菌以腐生营养型为主要功能营养 型,这是由于土壤处于淹水状态,枯落物的有机质分 解缓慢,为了完成有机质的分解,从而刺激了腐生真 菌的大量生长[31]. 但是, FUNGuild 是基于已有文献 对真菌功能进行判别分析,划分类别不够全面和完 善,还存在较多未知的功能注释,因此仍需进一步研 兖[42].

麦稻茬口期配施尿素和沼液并未造成门水平上 微生物多样性以及细菌和真菌群落的显著差别,这 表明在短时间内微生物群落对不同施肥方式有一定 的缓冲性,这为茬口期施用沼液有机肥替代尿素化 肥提供了可能性,可能达到在减少化肥施用量的同时,保持土壤的基本稳定的目的.

4 结论

- (1) 茬口期土壤淹水降低了土壤微生物多样性,施用沼液相较于施用尿素会降低土壤细菌丰富度,但能提高优势物种多样性.
- (2)淹水初期,施用尿素处理的厚壁菌门、绿弯菌门和放线菌门与其它处理表现出较大差异,受淹水时间和施氮方式影响较大的细菌种属为芽孢杆菌属;真菌种群结构受施氮方式影响较大,施用沼液后被孢霉属(Mortierella)和镰刀菌属(Fusarium)的相对丰度较高,而施用尿素后篮状菌属(Talaromyces)和隐球菌属(Cryptococcus)的相对丰度较高.淹水21d后,施用沼液和尿素处理的土壤微生物种群结构趋于相似.
- (3) 茬口期主要土壤细菌类群代谢功能表现相似. 21d 时施用沼液和尿素处理的腐生营养型功能相似,但病理营养型功能开始出现差异,表明茬口期施沼液在一定程度上可以替代化肥平衡土壤养分和维持土壤生态功能,但仍可能存在真菌致病风险. 参考文献:
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