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紫云英还田与化肥减量配施对稻田土壤细菌群落组成和功能的影响

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摘要: 紫云英是豫南稻区土壤培肥的重要有机肥资源, 探究紫云英还田与化肥减量配施对稻田土壤理化性质和细菌群落特性的影响, 旨在为该区域的土壤培肥和化肥减量提供依据。开展连续 12 a 田间定位试验, 设置了 6 个施肥处理 (空白对照, CK; 单施化肥, F100; 80% 化肥和 22.5 t·hm⁻² 紫云英还田量配施, MV1F80; 80% 化肥和 45 t·hm⁻² 紫云英还田量配施, MV2F80; 60% 化肥和 22.5 t·hm⁻² 紫云英还田量配施, MV1F60; 60% 化肥和 45 t·hm⁻² 紫云英还田量配施, MV2F60)。通过采用高通量测序方法比较不同施肥处理对土壤细菌群落多样性、组成和结构特性的影响; 采用 FAPROTAX 功能预测的方法分析不同施肥处理间功能类群的丰度差异, 并结合土壤理化性质, 探究改变土壤细菌群落结构与功能特性的关键土壤环境因子。结果表明, 紫云英还田与化肥减量配施降低了土壤容重, 提高了土壤有机碳 (SOC)、全氮 (TN)、全磷 (TP) 和全钾 (TK) 含量, 较 CK 提高的范围分别为 12.7%~35.5%、38.2%~65.7%、66.7%~95.2% 和 20.3%~31.6%。紫云英还田与化肥减量配施处理较 F100 处理降低了细菌 Sobs 指数和 Shannon 多样性指数, 且 Sobs 指数和 Shannon 多样性指数均与容重 (BD) 呈显著正相关 ($P < 0.05$), 而与 SOC 和 TN 呈显著负相关 ($P < 0.05$)。MV1F80 和 MV2F60 处理的厚壁菌门 (Firmicutes) 的相对丰度较 F100 处理分别提高了 82.2% 和 67.4% ($P < 0.05$), 而酸杆菌门 (Acidobacteria) 的相对丰度分别减少了 32.6% 和 40.5% ($P < 0.05$), 仅 MV2F60 处理放线菌门 (Actinobacteria) 的相对丰度提高了 30.0% ($P < 0.05$)。RDA 分析表明, 土壤 SOC、TN 和 TK 是显著影响细菌群落结构变化的主要土壤环境因子 ($P < 0.05$)。紫云英还田与化肥减量配施处理还提高了化能异养类、固氮类、发酵类和尿素分解类功能类群丰度, 降低了动物寄生或共生类、人类所有病原体 and 人类病原体肺炎类功能类群丰度, 其中 MV1F80 和 MV2F60 处理效果较好。综上所述, 长期的紫云英还田与化肥减量配施改善了土壤理化性质, 进而改变了土壤细菌群落结构与功能特性, 有助于稻田土壤肥力的提升、微生态系统的稳定和健康, 进而保障区域农业的绿色可持续发展。

关键词: 化肥减量; 紫云英还田; 土壤理化性质; 细菌群落; 功能预测

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Effects of Chinese Milk Vetch Returning Incorporated with Chemical Fertilizer Reduction on the Composition and Function of Soil Bacterial Communities in Paddy Fields

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Abstract: Chinese milk vetch (*Astragalus sinicus* L.) is an important organic nutrient resource in the southern Henan rice-growing area. Thus, the effects of Chinese milk vetch (MV) returning incorporated with reduced chemical fertilizer on the physicochemical properties and bacterial community characteristics in paddy soil were studied. These results can provide a certain theoretical basis for the improvement of soil fertility and reduction of chemical fertilizer in this area. A field experiment was conducted for 12 consecutive years, involving six fertilization treatments (blank control, CK; 100% chemical fertilizer, F100; 80% chemical fertilizer + 22.5 t·hm⁻² MV, MV1F80; 80% chemical fertilizer + 45 t·hm⁻² MV, MV2F80; 60% chemical fertilizer + 22.5 t·hm⁻² MV, MV1F60; and 60% chemical fertilizer + 45 t·hm⁻² MV, MV2F60). The high-throughput sequencing method was used to compare the effects of different fertilization treatments on soil bacterial community diversity, composition, and structural characteristics. The FAPROTAX function prediction method was used to analyze the abundance differences of functional groups between different fertilization treatments. Additionally, combined with soil physicochemical properties and bacterial community characteristics, we explored the key soil environmental factors that changed the structure and functional characteristics of the soil bacterial community. Compared with that under CK, the soil bulk density (BD) under the MV returning incorporated with reduced chemical fertilizer treatment was decreased, whereas soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), and total potassium (TK) were increased by 12.7%-35.5%, 38.2%-65.7%, 66.7%-95.2%, and 20.3%-31.6%, respectively. Compared with that under the F100 treatment, the Sobs index and Shannon diversity

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index of the bacterial community under the MV returning incorporated with reduced chemical fertilizer were decreased, and the Sobs index and Shannon diversity index were significantly positively correlated with BD ($P < 0.05$) but significantly negatively correlated with SOC and TN ($P < 0.05$). Compared with that under the F100 treatment, the relative abundances of Firmicutes under the MV1F80 and MV2F60 treatments were significantly increased by 82.2% and 67.4% ($P < 0.05$), but the relative abundances of Acidobacteria were significantly reduced by 32.6% and 40.5% ($P < 0.05$), respectively. The relative abundance of Actinobacteria under the MV2F60 treatment was significantly increased by 30.0% ($P < 0.05$) compared with that under the F100 treatment. According to RDA analysis, soil SOC, TN, and TK were the main soil environmental factors that significantly affected bacterial community ($P < 0.05$). Compared with that under CK and the F100 treatment, the abundance of functional groups of chemoheterotrophy, nitrogen fixation, fermentation, and ureolysis under the MV returning incorporated with reduced chemical fertilizer treatment were improved, whereas the abundance of functional groups of animal parasites or symbionts, all human pathogens, and human pathogen pneumonia were reduced, particularly under MV1F80 and MV2F60. To summarize, the long-term MV returning to the field incorporated with reduced chemical fertilizer improved the soil physical and chemical properties, thus changing the structure and functional characteristics of the soil bacterial communities, contributing to the improvement in the soil fertility, stability, and health of microecosystems in paddy fields, thus ensuring the green and sustainable development of regional agriculture.

Key words: fertilizer reduction; Chinese milk vetch returning; soil physicochemical properties; bacterial community; function prediction

随着人口的不断增长,预计 2050 年全球粮食生产需要在 2005 年的基础上增加 60%~110% 才能满足需求^[1]. 施用化肥是提高作物产量不可或缺的农业措施^[2],然而长期大量的化肥投入不仅降低了资源利用效率,还会带来负面影响,如土壤质量退化和环境污染等一系列生态环境问题,进而影响作物生产系统的可持续性^[3]. 而有机无机配施技术模式已被大量研究证明,不仅可以减少化肥的投入,还比单施化肥模式有更好的增产效应^[4,5],然而有机无机配施技术模式也存在一定的不确定性,源于有机肥(如牛粪、猪粪等)可能包含抗生素和重金属等,增加二次污染的风险^[6,7]. 因此,绿肥作为一种清洁有机肥源得到了广泛的关注. 在我国南方稻田生产体系中,种植和翻压豆科绿肥(如紫云英, *Astragalus sinicus* L.) 已成为主要可持续生产模式,不仅可以为水稻生长提供充足养分,减少化肥投入,降低环境风险,还可以培肥土壤,改善土壤理化性质,同时影响土壤微生物学特性^[8~10].

土壤微生物参与了多种农业生态过程,如营养循环和有机物料腐解等,是衡量土壤肥力和土壤健康程度的重要指标^[11]. 土壤细菌是微生物中丰度最高的群体,同时也是养分活化的驱动者,其群落组成和功能关系到土壤肥力和农业可持续性^[12]. 有研究指出,施肥能够提高土壤肥力,改变细菌的丰富度和功能多样性,而不合理的施肥可能降低某些功能基因丰度^[13,14]. 绿肥还田可以为细菌的生长提供充足的新鲜有机质和氮钾养分资源,有助于营养循环和土壤肥力的提升^[15]. Stark 等^[16]研究发现绿肥还田提高了微生物的生物量和活性,进而改变了土壤微生物群落组成. 一个 31 a 不同轮作体系的试验发现,种植冬绿肥改变了微生物群落结构,提高了有益细菌丰度^[17]. Tao 等^[18]研究发现紫云英还田较蚕豆和野豌豆还田显著增加了酸杆菌门(Acidobacteria)和疣微菌门(Verrucomicrobia)的相对丰度,进而提高了玉米产量.

前人关于绿肥还田的研究主要集中在土壤培

肥、资源利用效率和作物产量方面^[8,15],针对微生物群落特性也有一部分研究^[17,18],不过主要集中在不同绿肥轮作体系中探究种植和翻压绿肥对细菌群落特性的影响,而紫云英还田与化肥减量配施对细菌群落特性的影响和驱动细菌群落结构与功能变化的主控因子的研究较少,且紫云英还田与化肥减量配施对细菌群落功能特性的影响也尚缺少研究. 另外,不同的土壤类型、作物类型和施肥措施等都会影响土壤微生物学特性^[19]. 因此,本文基于 12 a 的长期定位试验,通过高通量测序方法,探究细菌群落组成和功能对长期紫云英还田与化肥减量配施的响应差异及其与土壤理化性质的关系,以期为进一步优化施肥提供理论依据,助推我国南方稻田绿色可持续生产.

1 材料与方法

1.1 研究地点

试验区位于河南省信阳市农业科学院试验园区(北纬 32°06'10", 东经 114°03'27"),该区属于亚热带向暖温带过渡区,年均降雨 900~1 400 mm,年均气温 15℃. 田间试验始于 2009 年,试验土壤为黄棕壤性潜育型水稻土,基础土壤(0~20 cm)特性为:土壤有机碳 ω (SOC) 14.7 g·kg⁻¹, 碱解氮 ω (AN) 81.5 mg·kg⁻¹, 有效磷 ω (AP) 10.5 mg·kg⁻¹, 速效钾 ω (AK) 58.2 mg·kg⁻¹.

1.2 试验设计与管理

供试材料:本试验紫云英选用信紫 1 号,在水稻收获后原地种植,于次年 4 月上旬紫云英盛花期翻压还田(根据不同小区试验要求翻压),多余部分移出小区,少的补充,其中紫云英鲜草含水量 90%,干草中含氮 37.5 g·kg⁻¹、含磷 3.4 g·kg⁻¹和含钾 35.0 g·kg⁻¹. 供试水稻品种为扬两优 013,于每年 5 月下旬划行移栽,移栽密度为 16.7 cm×20 cm,每穴 2 棵基本苗.

试验设计:本试验采用随机区组试验设计,包括 6 个处理:①不施肥对照(CK);②100% 化肥

(F100); ③紫云英还田 $22.5 \text{ t} \cdot \text{hm}^{-2}$ + 80% 化肥 (MV1F80); ④紫云英还田 $45 \text{ t} \cdot \text{hm}^{-2}$ + 80% 化肥 (MV2F80); ⑤紫云英还田 $22.5 \text{ t} \cdot \text{hm}^{-2}$ + 60% 化肥 (MV1F60); ⑥紫云英还田 $45 \text{ t} \cdot \text{hm}^{-2}$ + 60% 化肥 (MV2F60). 每个处理包括 3 次重复, 共计 18 个小区. 每个小区面积为 6.66 m^2 ($3.33 \text{ m} \times 2.0 \text{ m}$), 小区间筑田埂并覆膜防止串水串肥, 埂宽 30 cm.

肥料管理: 施肥类型为尿素 (46% N)、过磷酸钙 (12% P_2O_5) 和氯化钾 (60% K_2O). 100% 化肥为氮肥 $165 \text{ kg} \cdot \text{hm}^{-2}$ (以 N 计)、磷肥 $112.5 \text{ kg} \cdot \text{hm}^{-2}$ (以 P_2O_5 计) 和钾肥 $112.5 \text{ kg} \cdot \text{hm}^{-2}$ (以 K_2O 计), 80% 和 60% 化肥分别减少氮和钾的用量, 磷的用量不减少, 其中氮肥分 3 次施肥, 基肥占 50%, 分蘖肥占 30%, 孕穗肥占 20%, 磷钾全部基施, 其他田间管理和当地管理方式一致.

1.3 测定项目与方法

样品采集: 于 2020 年 9 月水稻收获前, 采集表层土壤 (0 ~ 20 cm) 样品, 混匀后分成 3 份, 放入保温箱带回实验室, 一份土壤样品风干, 用于测试土壤养分含量; 一份土壤样品保留鲜土, 用于测试土壤无机氮; 另一份样品即刻保存于 -80°C , 用于细菌群落特性分析.

土壤理化性质测定^[20,21]: 土壤容重采用环刀法测定; 土壤 SOC 和 TN 采用碳氮元素分析仪 (Elementar, Langensfeld, Germany) 进行测试; 土壤无机氮 (Nmin) 经 $1 \text{ mol} \cdot \text{L}^{-1}$ KCl 溶液浸提后采用连续流动分析仪 (AA3, SEAL Analytical, Germany) 测定; 全磷 (TP) 采用 $\text{H}_2\text{SO}_4\text{-HClO}_4$ 消煮和钼锑抗比色法测定; 有效磷 (AP) 采用 $0.5 \text{ mol} \cdot \text{L}^{-1}$ NaHCO_3 浸提-钼锑抗比色法测定; 全钾 (TK) 和速效钾 (AK) 分别采用 NaOH 熔融-火焰光度法和 $1 \text{ mol} \cdot \text{L}^{-1}$ NH_4OAc 浸提-火焰光度法.

土壤样品微生物的 DNA 抽提和 PCR 扩增: 根据 E. Z. N. A.® soil DNA kit (Omega Bio-tek, Norcross, GA, U. S.) 说明书进行微生物群落总 DNA 抽提, 使用 1% 的琼脂糖凝胶电泳检测 DNA 的

提取质量, 使用 NanoDrop2000 测定 DNA 浓度和纯度; 使用 338F (5'-ACTCTACGGGAGGCAGCAG-3') 和 806R (5'-GGACTACHVGGGTWTCTAAT-3') 对 16S rRNA 基因 V3-V4 可变区进行 PCR 扩增. 每个样本 3 个重复.

Illumina MiSeq 测序: 将同一样本的 PCR 产物混合后使用 2% 琼脂糖凝胶回收 PCR 产物, 利用 AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) 进行回收产物纯化, 2% 琼脂糖凝胶电泳检测, 并用 Quantus™ Fluorometer (Promega, USA) 对回收产物进行检测定量. 通过 Illumina 公司的 MiSeq PE300 平台进行测序, 由上海美吉生物医药科技有限公司提供技术支持.

1.4 数据统计与分析

采用 Microsoft Excel 2019 软件整理试验数据. 采用 SAS 软件进行单因素方差分析 (one-way ANOVA) 和 Duncan 多重比较法分析不同施肥处理间土壤理化性质、群落 α 多样性 (丰富度指数: Sobs; 多样性指数: Shannon; 均匀度指数: Shannoneven; 覆盖度指数: Coverage)、群落组成和功能的差异显著性 ($P < 0.05$), 采用 SPSS 20.0 软件进行各个指标的斯皮尔曼 (Spearman) 相关性分析; 通过美吉生物云平台研究土壤理化性质对细菌群落结构的影响 (冗余分析 RDA); 采用 FAPROTAX 数据库进行细菌功能预测. 采用 Origin 2021 和 Sigmaplot 12.5 软件进行数据作图.

2 结果与分析

2.1 土壤理化性质

不同的施肥处理下土壤的理化性质如表 1 所示. 不同的施肥处理显著影响了 SOC、TN、TP 和 TK. 与 CK 相比, MV2F80、MV1F80 和 MV2F60 处理显著提高了 SOC, 增幅分别为 35.5%、26.4% 和 25.5%; 仅 MV2F80 处理的 SOC 较 F100 处理显著提高 19.2%; 而紫云英还田与化肥减量配施处理均

表 1 不同施肥处理对土壤理化特性的影响¹⁾

Table 1 Effect of different fertilization treatments on soil physicochemical properties

处理	BD / $\text{g} \cdot \text{cm}^{-3}$	ω (SOC) / $\text{g} \cdot \text{kg}^{-1}$	ω (TN) / $\text{g} \cdot \text{kg}^{-1}$	ω (TP) / $\text{g} \cdot \text{kg}^{-1}$	ω (TK) / $\text{g} \cdot \text{kg}^{-1}$	ω (Nmin) / $\text{mg} \cdot \text{kg}^{-1}$	ω (AP) / $\text{mg} \cdot \text{kg}^{-1}$	ω (AK) / $\text{mg} \cdot \text{kg}^{-1}$
CK	$1.32 \pm 0.11\text{a}$	$11.0 \pm 1.02\text{c}$	$0.984 \pm 0.22\text{c}$	$0.21 \pm 0.04\text{b}$	$5.66 \pm 0.65\text{b}$	$24.0 \pm 2.54\text{bc}$	$2.81 \pm 0.48\text{a}$	$84.0 \pm 2.89\text{a}$
F100	$1.31 \pm 0.05\text{a}$	$12.5 \pm 1.58\text{bc}$	$1.27 \pm 0.11\text{b}$	$0.39 \pm 0.08\text{a}$	$6.81 \pm 0.44\text{a}$	$31.2 \pm 6.33\text{abc}$	$3.52 \pm 1.22\text{a}$	$87.1 \pm 6.70\text{a}$
MV1F80	$1.30 \pm 0.05\text{a}$	$13.9 \pm 1.16\text{ab}$	$1.36 \pm 0.11\text{b}$	$0.35 \pm 0.01\text{a}$	$6.81 \pm 0.82\text{a}$	$22.9 \pm 3.65\text{c}$	$4.22 \pm 0.16\text{a}$	$89.5 \pm 5.55\text{a}$
MV2F80	$1.18 \pm 0.02\text{ab}$	$14.9 \pm 0.60\text{a}$	$1.43 \pm 0.17\text{ab}$	$0.35 \pm 0.01\text{a}$	$7.02 \pm 0.54\text{a}$	$27.7 \pm 5.02\text{abc}$	$3.54 \pm 1.33\text{a}$	$91.1 \pm 10.3\text{a}$
MV1F60	$1.27 \pm 0.13\text{ab}$	$12.4 \pm 0.53\text{bc}$	$1.45 \pm 0.01\text{ab}$	$0.41 \pm 0.07\text{a}$	$7.45 \pm 0.45\text{a}$	$32.8 \pm 7.79\text{ab}$	$3.09 \pm 0.47\text{a}$	$90.1 \pm 13.9\text{a}$
MV2F60	$1.16 \pm 0.07\text{b}$	$13.8 \pm 0.82\text{ab}$	$1.63 \pm 0.03\text{a}$	$0.37 \pm 0.02\text{a}$	$7.31 \pm 0.33\text{a}$	$35.0 \pm 5.55\text{a}$	$2.93 \pm 0.46\text{a}$	$95.9 \pm 6.23\text{a}$

1) 同列不同小写字母表示处理间差异显著 ($P < 0.05$)

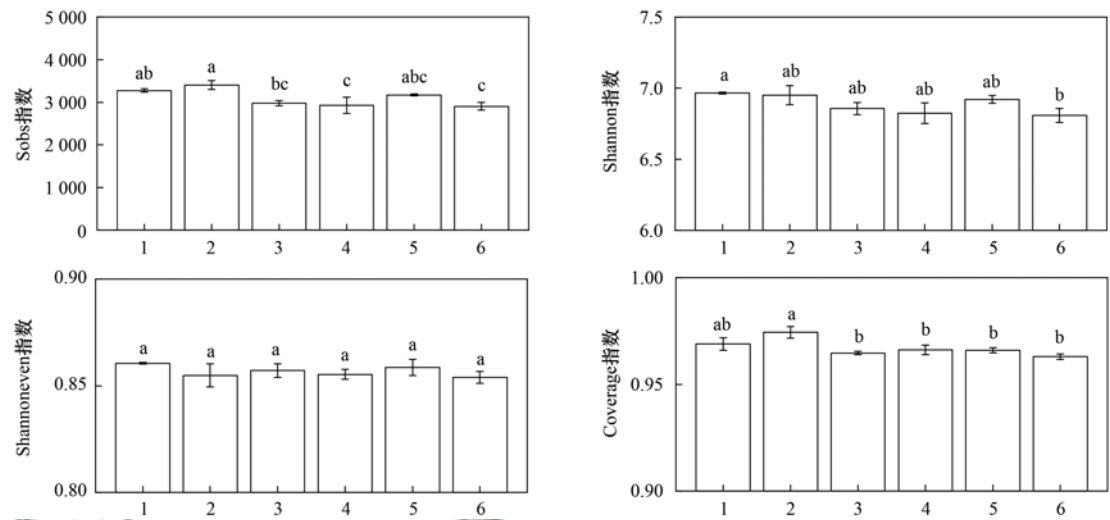
显著提高了 TN,增幅为 38.2%~65.7%,仅 MV2F60 处理的 TN 显著高于 F100 处理,增幅为 28.3%;对于 TP 和 TK 来说,施肥处理均显著高于 CK,但是施肥处理间无显著差异. MV2F60 处理的 BD 较 CK 显著降低了 12.1%,土壤 Nmin 提高了 45.8%,其他处理与 CK 均为显著差异;而对于 AP 和 AK 来说,施肥处理均高于 CK,但是各处理间差异未达到显著水平.

2.2 土壤细菌群落多样性

不同施肥处理下土壤细菌群落 α 多样性指数如图 1 所示. 紫云英还田与化肥减量配施处理的 Sobs 指数均低于 CK 和 F100 处理,尤其是 MV2F80 和 MV2F60 处理. 从 Shannon 指数来看,各施肥处理

均低于 CK 处理,尤其是 MV2F60;对于 Shannoneven 指数来说,各处理间均无显著差异. 而各样本的 Coverage 指数均高于 96.1%,说明测序深度能够比较真实地反映土壤样本中细菌群落的多样性.

各处理间的细菌群落 α 多样性与土壤理化特性的 Spearman 相关性如表 2 所示. 细菌 Sobs 指数和 Shannon 指数与 BD 均呈显著正相关($P < 0.05$),与 SOC 呈显著负相关($P < 0.01$ 和 $P < 0.05$);TN 与细菌 Sobs 指数($P < 0.01$)、Shannon 指数($P < 0.05$)和 Shannoneven 指数($P < 0.05$)也呈显著负相关;AK 与细菌 Sobs 指数呈显著负相关($P < 0.05$).



1. CK, 2. F100, 3. MV1F80, 4. MV2F80, 5. MV1F60, 6. MV2F60; 不同小写字母表示处理间差异显著 ($P < 0.05$)

图 1 不同施肥处理对土壤细菌群落 α 多样性的影响

Fig. 1 Effect of different fertilization treatments on alpha diversity of soil bacterial communities

表 2 细菌群落 α 多样性与土壤理化特性之间的 Spearman 相关性¹⁾

Table 2 Spearman correlation between alpha diversity of bacterial communities and soil physicochemical properties

多样性指数	BD	SOC	TN	TP	TK	Nmin	AP	AK
Sobs	0.488 *	-0.622 **	-0.656 **	ns	ns	ns	ns	-0.523 *
Shannon	0.496 *	-0.534 *	-0.568 *	ns	ns	ns	ns	ns
Shannoneven	ns	ns	-0.546 *	ns	ns	ns	ns	ns
Coverage	0.531 *	-0.480 *	ns	ns	ns	ns	ns	-0.492 *

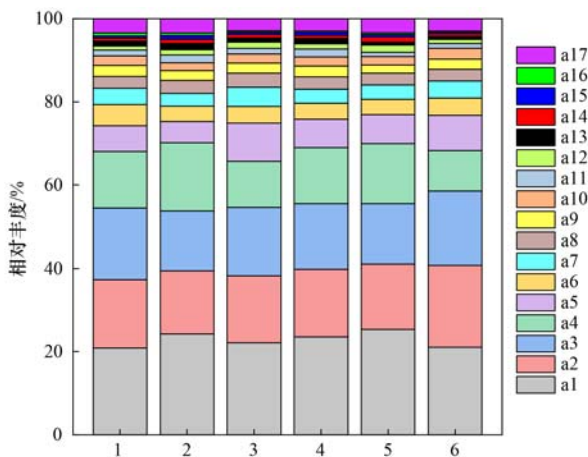
1) ns、* 和 ** 分别表示不相关、在 0.05 水平显著相关和在 0.01 水平显著相关

2.3 土壤细菌群落组成与差异

不同施肥处理下细菌群落结构在门水平的物种组成如图 2 所示. 绿弯菌门 (Chloroflexi)、放线菌门 (Actinobacteria)、变形菌门 (Proteobacteria)、酸杆菌门 (Acidobacteria) 和厚壁菌门 (Firmicutes) 是主要细菌菌群,在 18 个样本中平均相对丰度分别为 22.9%、16.5%、15.9%、13.1%和 7.12%,约占总丰度的 75.5%,另有 11 类菌门相对丰度大于 1.00%,约占总丰度的 21.3%,而相对丰度小于 1.00%的菌门约占 3.20%.

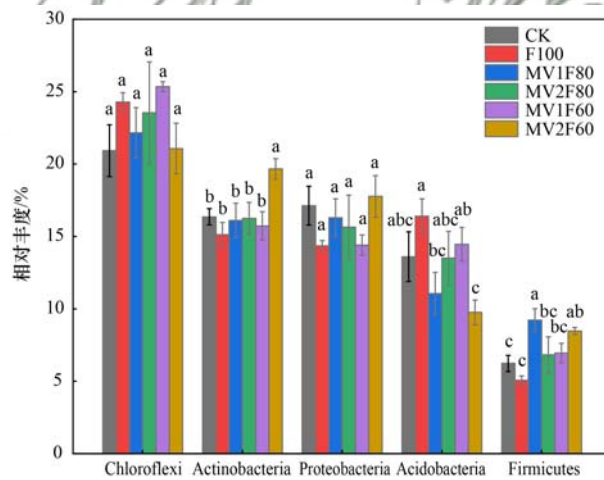
如图 3 所示,在细菌主要菌门中,不同施肥处理

显著影响了厚壁菌门 (Firmicutes) 的相对丰度 ($P < 0.05$),其中 MV1F80 和 MV2F60 的厚壁菌门 (Firmicutes) 的相对丰度较 CK 显著增加了 48.0% 和 35.9% ($P < 0.05$),较 F100 处理显著增加了 82.2% 和 67.4% ($P < 0.05$),其他处理间无显著差异;对于放线菌门 (Actinobacteria) 来说,MV2F60 处理的相对丰度显著高于其他处理 ($P < 0.05$). 与 F100 相比,所有处理均降低了酸杆菌门 (Acidobacteria) 的相对丰度,尤其是 MV1F80 和 MV2F60 显著降低了 32.6% 和 40.5% ($P < 0.05$).



1. CK, 2. F100, 3. MV1F80, 4. MV2F80, 5. MV1F60, 6. MV2F60; a1. Chloroflexi, a2. Actinobacteria, a3. Proteobacteria, a4. Acidobacteria, a5. Firmicutes, a6. Myxococcota, a7. Myxococcota, a8. Nitrospirae, a9. Gemmatimonadetes, a10. Bacteroidota, a11. MBNT15, a12. Planctomycetota, a13. Methylomirabilota, a14. Verrucomicrobia, a15. Latescibacteria, a16. Cyanobacteria, a17. others; 将所有样本中相对丰度均小于 1% 的物种归为 others

图 2 不同施肥处理对土壤细菌群落组成的影响
Fig. 2 Effect of different fertilization treatments on soil bacterial community compositions



不同小写字母表示处理间差异显著 ($P < 0.05$)

图 3 不同施肥处理对土壤细菌群落主要菌门丰度的影响
Fig. 3 Effect of different fertilization treatments on abundance of dominant phyla in soil bacteria

表 3 不同施肥处理对土壤细菌群落功能的影响¹⁾

Table 3 Effect of different fertilization treatments on soil bacterial community function

功能类群	CK	F100	MV1F80	MV2F80	MV1F60	MV2F60	P 值
化能异养类	27.2 ± 1.39bc	26.0 ± 2.45c	29.6 ± 1.25ab	28.2 ± 1.22abc	28.9 ± 0.45ab	30.0 ± 0.88a	0.037 7
固氮类	5.87 ± 0.96b	5.19 ± 0.62b	9.43 ± 2.12a	7.44 ± 1.02ab	8.32 ± 1.32a	9.31 ± 0.78a	0.010 7
发酵类	4.29 ± 0.26c	4.24 ± 0.24c	8.60 ± 1.35a	6.15 ± 1.23b	7.30 ± 1.32ab	7.93 ± 1.66a	0.000 4
动物寄生或共生类	4.38 ± 0.47a	4.56 ± 0.35a	3.34 ± 0.25c	4.20 ± 0.80ab	3.52 ± 0.50bc	3.34 ± 0.21c	0.022 7
人类所有病原体	4.37 ± 0.47a	4.55 ± 0.36a	3.34 ± 0.25c	4.20 ± 0.80ab	3.49 ± 0.50bc	3.33 ± 0.21c	0.020 6
人类病原体肺炎	4.08 ± 0.38a	4.23 ± 0.40a	3.12 ± 0.26b	3.86 ± 0.76ab	3.22 ± 0.35b	3.15 ± 0.19b	0.020 5
硫酸盐呼吸类	1.15 ± 0.34a	0.865 ± 0.05ab	0.796 ± 0.20bc	0.774 ± 0.06bc	0.819 ± 0.14bc	0.543 ± 0.06c	0.030 6
尿素分解类	0.406 ± 0.12cd	0.387 ± 0.03d	1.15 ± 0.36a	0.761 ± 0.08bc	0.902 ± 0.09ab	1.12 ± 0.31ab	0.001 7

1) 同行不同小写字母表示处理间差异显著 ($P < 0.05$)

细菌群落结构与土壤理化性质的冗余分析 (RDA) 如图 4 所示. 结果表明, RDA1 和 RDA2 分别解释了土壤细菌群落变异的 29.0% 和 8.75%, 前两轴共解释了 37.8% 的细菌群落总变异, 其中, SOC ($R^2 = 0.450 2$, $P = 0.015$)、TN ($R^2 = 0.390 8$, $P = 0.016$) 和 TK ($R^2 = 0.406 5$, $P = 0.013$) 是影响细菌群落的主要土壤环境因子.

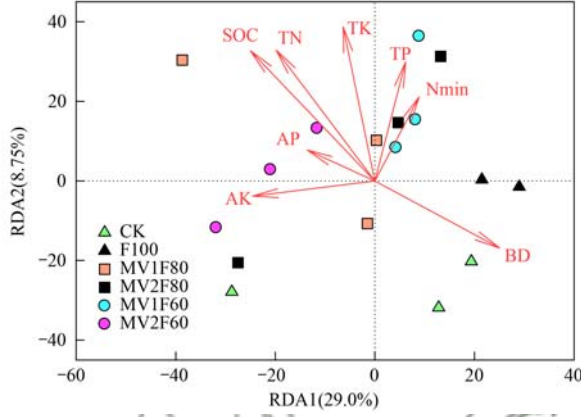


图 4 土壤细菌群落与土壤化学特性间的冗余分析
Fig. 4 Redundancy analysis (RDA) of soil bacterial community and soil physicochemical properties

2.4 土壤细菌功能预测

本研究采用 FAPROTAX 对土壤细菌群落进行功能预测和注释, 共获得 50 种功能分组. 其中, 相对丰度大于 1% 的功能类型有 22 个, 共占总丰度的 94.5%~96.0%, 如表 3 所示. 将该 22 种功能类型进行单因素方差分析, 筛选出在处理间有显著差异的 8 项功能类群. MV2F60 处理的化能异养类种群相对丰度显著高于 CK 和 F100 ($P < 0.05$), 其他处理间无显著差异; 4 个紫云英还田与化肥减量配施处理的发酵作用种群相对丰度均显著高于 CK 和 F100 ($P < 0.05$); 与 CK 和 F100 相比, MV1F80、MV1F60 和 MV2F60 显著提高了固氮作用和尿素分解种群的相对丰度 ($P < 0.05$), 降低了动物寄生或共生类和人类病原体类的种群相对丰度 ($P < 0.05$); 与 CK 相比, 4 个紫云英还田与化肥减量配施处理显著降低了硫酸盐呼吸的种群相对丰度 ($P < 0.05$).

将上述 8 项差异功能与土壤部分理化特性 (BD、SOC 和 TN)、细菌 α 多样性指数和主要菌门相对丰度指标进行相关分析(表 4)。化能异养类和发酵类功能与 BD 均呈显著负相关(r 为 -0.484 和 -0.525),而 SOC 与固氮类和发酵类功能均呈显著正相关(r 为 0.492 和 0.560);化能异养类、固氮类和发酵类功能均与 TN 呈显著正相关(r 为 $0.493 \sim 0.586$),而与细菌 Sobs 指数呈显著负相关(r 为 $-0.498 \sim -0.593$);化能异养类功能与放线菌门、变形菌门和厚壁菌门呈显著正

相关(r 为 $0.470 \sim 0.713$),而与酸杆菌门呈极显著负相关($r = -0.666$);动物寄生或共生类、人类所有病原体和人类病原体肺炎类功能与放线菌门呈显著负相关(r 为 $-0.486 \sim -0.515$),与厚壁菌门呈极显著负相关(r 为 $-0.787 \sim -0.835$),而与酸杆菌门呈显著正相关(r 为 $0.540 \sim 0.587$);另外,发酵类功能与酸杆菌门呈显著负相关($r = -0.503$),固氮类、发酵类和尿素分解类功能与厚壁菌门均呈极显著正相关(r 为 $0.612 \sim 0.839$)。

表 4 土壤细菌群落功能与土壤理化特性和主要菌门相对丰度之间的关系¹⁾

功能类群	BD	SOC	TN	Chloroflexi	Actinobacteria	Proteobacteria	Acidobacteria	Firmicutes
化能异养类	-0.484 *	ns	0.493 *	ns	0.639 **	0.470 *	-0.666 **	0.713 **
固氮类	ns	0.492 *	0.541 *	ns	ns	ns	ns	0.818 **
发酵类	-0.525 *	0.560 *	0.586 *	ns	ns	ns	-0.503 *	0.839 **
动物寄生或共生类	ns	ns	ns	ns	-0.498 *	ns	0.540 *	-0.787 **
人类所有病原体	ns	ns	ns	ns	-0.515 *	ns	0.556 *	-0.798 **
人类病原体肺炎	ns	ns	ns	ns	-0.486 *	ns	0.587 *	-0.835 **
硫酸盐呼吸类	ns	ns	-0.692 **	ns	ns	ns	ns	ns
尿素分解类	ns	ns	0.563 *	ns	ns	ns	ns	0.612 **

1) ns、* 和 ** 分别表示不相关、在 0.05 水平显著相关和在 0.01 水平显著相关

3 讨论

3.1 紫云英还田与化肥减量配施对土壤理化性质的影响

本研究发现,紫云英还田与化肥减量配施处理降低了土壤容重,其中在 MV2F60 处理下达到显著水平,这与 Zhou 等^[8]的研究结果类似。其原因可能是较高的紫云英还田量可以改善土壤孔隙结构,提高大团聚体含量^[22,23]。另外,高的紫云英还田量与化肥减量配施均显著提高了土壤有机碳,这主要是因为外源有机物料的添加激发了土壤微生物的活性,促进了土壤中碳的积累^[24,25],这与 Chen 等^[26]的研究结果一致。与 CK 相比,紫云英还田与化肥减量配施处理均显著增加了土壤全氮、全磷和全钾,这可能是由于豆科绿肥作物的固氮作用和活化土壤磷钾的生物学特性,致使紫云英秸秆本身富含氮磷钾养分^[27,28],而化肥的投入促进了作物生长发育,提高了秸秆和根系的还田量,进一步提高了养分的投入量^[29]。此外,紫云英还田与化肥减量配施处理中较低的化肥用量处理的土壤总氮较高,且在 MV2F60 处理显著高于单施化肥处理,这可能因为过多的化肥氮的投入降低土壤碳氮比,同时为异养微生物提供了充足氮源,促进了有机氮的矿化分解,增加氮素损失^[30,31]。

3.2 紫云英还田与化肥减量配施对土壤细菌群落

多样性和群落结构的影响

不同的施肥措施会影响土壤细菌群落的 α 多样性^[32]。本研究发现,与单施化肥相比,紫云英还田与化肥减量配施降低了细菌群落的 Sobs 指数和 Shannon 指数,紫云英还田量越高,效果越明显,其中以 MV2F60 处理效果最为明显,这与 Tao 等^[18]和 Caban 等^[33]的报道一致。此外,紫云英还田与化肥减量配施处理显著影响了土壤细菌的 β 多样性,改变了细菌的群落结构,这与 Ren 等^[34]关于有机肥部分替代化肥对土壤细菌群落特性影响的研究结果一致。这可能是由于长期紫云英还田与化肥减量配施改变了土壤细菌群落组成,促使一些特定的细菌类群增长,增强了细菌群落之间对营养物质的竞争,从而抑制了其他细菌类群的生长^[17]。

不同施肥处理中,绿弯菌门、放线菌门、变形菌门、酸杆菌门和厚壁菌门是细菌群落的优势菌门,这与 Gao 等^[10]在多位点、多年的绿肥-水稻轮作试验中得到的细菌优势类群相似。但不同施肥处理间优势类群的相对丰度存在一定差异。与单施化肥相比,紫云英还田与化肥减量配施增加了放线菌门的相对丰度,降低了酸杆菌门的相对丰度,尤其是 MV2F60 处理,这是因为放线菌门作为富营养菌,需要通过分解作物残渣获取碳氮等营养基质而快速生长,紫云英还田可以为放线菌门的生长提供充足的营养基质^[34];酸杆菌门易富集在低肥力土壤上^[12],

而本研究也已证实,紫云英还田与化肥减量配施提高了土壤有机碳和全氮.厚壁菌门可以通过分泌胞外酶,降解有机物料和植物源多糖,并参与各种碳氮代谢功能,有效促进土壤碳氮循环^[35,36].本研究发现,与CK和单施化肥相比,MV1F80和MV2F60处理显著提高了厚壁菌门的相对丰度,而对于MV1F60和MV2F80处理来说,厚壁菌门的相对丰度没有统计学差异,这可能是因为优化的紫云英还田与化肥减量配施比例可以提供合适的养分供应量,进而提高参与土壤碳氮代谢过程的厚壁菌门的相对丰度^[37].

土壤特性是影响细菌群落特性的重要因子^[38].相关性分析结果表明,土壤细菌群落的多样性指数均与土壤容重呈显著正相关,而与有机碳显著负相关,这与Pan等^[39]的研究结果类似.同时也有研究表明,土壤细菌群落的多样性指数与土壤容重呈显著负相关,而与有机碳显著正相关^[34,40].利用RDA分析土壤理化指标对土壤细菌群落结构特征的影响,研究发现,不同的施肥处理下显著影响土壤细菌群落结构的主要土壤环境因子是土壤有机碳、全氮和全钾,这与Zhang等^[17]的报道类似;但在南方双季稻田的研究发现,土壤有机碳、速效磷和pH值是影响土壤细菌群落结构的主要环境因子^[41].研究结果的差异可能与生态区域、轮作方式和取样时间的不同有关.

3.3 紫云英与化肥配施对土壤细菌群落功能的影响

通过FAPROTAX功能预测揭示了土壤细菌群落功能对不同施肥措施的响应特性.紫云英还田与化肥减量配施提高了化能异养类、发酵类、固氮类和尿素分解类功能类群丰度,这可能是由于紫云英作为豆科绿肥作物,不仅可以充分用光、水、热等自然资源,增加碳的固持,还可以通过根瘤菌固定大气中的氮,富集于紫云英秸秆中^[8],而土壤中大多数细菌均可以通过降解有机物料中的碳氮化合物,进而促进自身的生长繁殖,提高与碳氮循环相关的细菌功能类群丰度^[42].相关分析也表明,发酵类和固氮类功能与有机碳显著正相关,化能异养类、发酵类、固氮类和尿素分解类功能与总氮显著正相关,这也证实了前人的研究,化能异养类和发酵类功能与碳循环相关,而固氮类和尿素分解类功能与土壤氮循环相关^[43,44].细菌功能类群丰度与主要细菌门丰度的相关分析表明,化能异养类功能类群丰度与放线菌门、变形菌门和酸杆菌门显著相关,这与Rivett等^[45]的研究结果类似,可能是因为这3种细菌菌门中的部分微生物参与了土壤有机质的矿化,执行了细菌的化能异养类功能种群的功能^[46,47].厚

壁菌门相对丰度与化能异养类、发酵类、固氮类和尿素分解类功能类群丰度呈显著正相关,这可能是由于厚壁菌门中存在一些固氮菌属,可以利用紫云英秸秆中的碳氮等能量来源,促进自身生长,有利于土壤碳氮循环过程的发生^[12].本研究还找到了3种病原菌功能,分别是动物寄生或共生类、人类所有病原体 and 人类病原体肺炎.本研究发现,紫云英还田与化肥减量配施处理均降低了这3种病原菌功能类群丰度,且相关分析表明,这3种病原菌功能类群丰度与厚壁菌门和放线菌门的相对丰度呈极显著负相关,而与酸杆菌门的相对丰度呈极显著正相关.这说明长期的紫云英还田与化肥减量配施可以改善土壤微生态环境,提高有益菌丰度,降低致病菌丰度,有益于土壤健康.

4 结论

紫云英还田与化肥减量配施降低了土壤容重、增加了有机碳和全氮含量,在相同化肥减量的基础上,较高紫云英还田量的效果更显著.土壤肥力特性的改善,促进了土壤细菌群落的变化,其中,有机碳、全氮和全钾是影响细菌群落结构的主要环境因子.此外,紫云英还田与化肥减量配施降低了细菌 α 多样性指数,增加了厚壁菌门的相对丰度,还提高了化能异养类、发酵类、固氮类和尿素分解类功能类群丰度,降低与病原菌相关的功能类群丰度.综合以上研究结果,MV2F60是较为适宜的紫云英还田量与化肥减量的配施组合.

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