



研究报告

长期不同施肥措施下岩溶水稻土可培养细菌群落变化及其主要影响因素

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摘要:【背景】施肥是目前提高作物产量的较优策略,不同的施肥措施在不同程度上影响土壤肥力和微生物群落结构。【目的】探究岩溶水稻土理化性质变化与细菌群落变化的对应关系,进而反映不同施肥措施对土壤可培养细菌群落的影响。最后选出最优施肥方案,为后续的合理施肥工作提供依据。【方法】对岩溶水稻土进行不施肥、常规施肥、常规施肥加绿肥3种施肥处理,通过对土壤理化性质、可培养细菌群落丰度及多样性变化的研究,探究在不同施肥措施下对岩溶水稻土细菌群落的影响。【结果】对比不施肥处理,常规施肥处理下土壤 pH 值和有机碳含量下降,结合大量研究结果证明,无机肥或氮肥的长期过量施加使土壤 pH 值下降,常规施肥加绿肥有利于有机碳的积累。分离纯化共得到 164 株菌,分别来自 *Actinobacteria*、*Bacteroidetes*、*Firmicutes* 和 *Proteobacteria*。属水平上常规施肥配施绿肥较常规施肥组优势菌属 *Sphingomonas*、*Lysobacter* 的相对丰度增加。细菌群落多样性增加,出现 *Paenibacillus*、*Streptomyces* 和 *Pseudomonas* 等特有功能菌属。优势菌属 *Sphingopyxis*、*Lysobacter*、*Paenibacillus*、*Bosea*、*Streptomyces*、*Pseudomonas* 和 *Bacillus* 与 TN 存在显著正相关,在常规施肥加绿肥处理土壤中增加。【结论】常规施肥加绿肥处理下,固氮、溶磷等功能菌丰度增加,增加土壤肥力,保持土壤养分的可利用性,对作物的增产起重要作用。岩溶水稻土常规施肥配施绿肥处理的效果优于不施肥和常规施肥处理。

关键词: 施肥措施,可培养细菌,群落特征,功能菌

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Variation of cultivable bacterial community structure and the main influencing factors in karst paddy soil under different fertilization regimes

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Abstract: [Background] Fertilization is the better strategy to improve crop yield. Fertilization measures can affect soil fertility and microbial community structure. [Objective] The inter-relationship between physicochemical properties and soil culturable bacterial communities will be explored. An optimal fertilization plan will be selected, and the result will provide the basic data for future work. [Methods] Three fertilization treatments of no fertilization, conventional fertilization, conventional fertilization and green manure were carried out on karst paddy soil. Based on the study of soil physical and chemical properties, abundance and diversity of cultivable bacterial communities, the effect of different fertilization measures on karst paddy soil was discussed about influence bacterial community. [Results] Compared with no fertilization treatment, the soil pH value and organic carbon content decreased along conventional fertilization treatment. Combined with a large number of research results, it proved that the long-term excessive application of inorganic fertilizer or nitrogen fertilizer decreased the soil pH value. The accumulation of organic carbon appeared in conventional fertilization plus green manure. A total of 164 strains of bacteria were obtained by separation and purification, which were from *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. At the genus level, the abundance of conventional fertilization combined with green manure was higher than that of the conventional fertilization group of dominant bacteria of *Sphingomonas* and *Lysobacter*. The diversity of bacterial communities has increased with the emergence of specific functional bacterial genera such as *Paenibacillus*, *Streptomyces* and *Pseudomonas*. The dominant bacteria *Sphingopyxis*, *Lysobacter*, *Paenibacillus*, *Bosea*, *Streptomyces*, *Pseudomonas* and *Bacillus* were significantly positively correlated with TN, and they were enriched in soil treated with conventional fertilization and green manure. [Conclusion] Under the treatment of conventional fertilization and green manure, the abundance of functional bacteria relating to nitrogen fixation and phosphorus increased which could increase soil fertility and have an important role in increasing crop production. The effect of conventional fertilization combined with green manure treatment on karst paddy soil is better than those with no fertilizer and conventional fertilization treatment.

Keywords: Fertilization measures, Culturable bacteria, Bacterial community structure, Functional bacteria

我国岩溶地貌主要分布在贵州、广西、云南三省,面积约 55 万 km², 占我国土地面积的 1/3^[1]。岩溶区碳酸盐岩成土速率慢、土层厚度薄,因此存

在特殊的脆弱性^[2-3]。水稻在我国农业粮食生产中起重要作用,如何在岩溶地区高效种植水稻以满足当地粮食需求是目前需要攻克的难题^[4]。

为了增加土壤肥力, 从而最终实现作物产量提高, 施肥是当前最有效的措施之一^[5]。土壤细菌不仅参与了土壤 C、N 和 P 等元素循环及腐殖质形成的生化过程^[6], 而且是土壤肥力的重要指标, 能够迅速反馈土壤质量的变化^[7-8]。研究表明, 不同施肥措施在不同程度上影响土壤肥力, 进一步影响微生物群落丰度和多样性, 从而影响作物产量^[9-12]。Geisseler 等^[13]在研究施肥对水稻系统土壤微生物的影响中, 发现有机肥的施加对土壤理化性质和细菌群落的影响比无机肥更明显。

石灰性土壤为岩溶区代表性土壤, 前人对石灰红壤性水稻土与细菌之间的关系进行了深入研究^[14-15]。然而不同施肥措施下棕色石灰性土壤细菌群落、细菌与土壤因子的关系尚未涉及。因此, 研究不同施肥措施对棕色石灰性土壤和细菌群落的影响, 探究细菌群落与土壤理化性质之间的关系, 有助于优化岩溶水稻土壤的施肥方案, 进而为岩溶地区作物增产提供理论依据。为此, 本文通过研究岩溶(棕色石灰土)水稻土壤细菌群落结构在不施肥、常规施肥、常规施肥加绿肥 3 种施肥措施下的变化, 探究岩溶土壤理化性质与细菌群落之间的关系, 进而反映不同施肥条件与土壤可培养细菌群落的对应关系, 以期选出最优施肥方案, 为后续合理施肥提供依据。

1 材料与方法

1.1 试验材料

试验土壤样品采自广西桂林国家级耕地质量监测点, 该站点建于 1987 年, 土壤类型为棕色石灰土母质潜育水稻土, 并对土壤进行连续 20 年的不施肥、常规施肥和常规施肥配施绿肥处理。早稻和晚稻常规施肥量为 $N:P_2O_5:K_2O=160:82.5:180 \text{ kg/hm}^2$, 常规施肥加绿肥处理方式具体为紫云英与水稻轮作, 紫云英还田量约为 $22\ 500 \text{ kg/hm}^2$ 。于 2019 年 6 月在该站点相应处理点进行采样, 每个施肥措施区用“S”形多点采样法将土壤表层(0–15 cm)土壤混合, 装入无菌装样袋立即低温运回实验室, 去除动

植物残体后一部分土壤用于土壤细菌培养, 一部分土壤自然风干后用于土壤理化性质测定。

1.2 主要试剂和仪器

PCR 所用引物、核酸染色剂、PCR 反应体系混合试剂, 生工生物工程(上海)股份有限公司; Chelex 100, 西格玛奥德里奇(上海)贸易有限公司; 培养基使用 R2A 与 SC 培养基, 具体配方参考任坤等的研究方法^[16]。紫外可见分光光度计、原子吸收分光光度计, 北京普析通用仪器有限责任公司; PCR 仪, Bio-Rad 公司。

1.3 土壤理化性质的测定

土壤 pH 值采用电位计法测定; 速效钾(available potassium, AK)的测定采用醋酸铵-火焰光度计法; 碱解氮(available nitrogen, AN)的测定采用碱解扩散法; 有效磷(available phosphorus, AP)的测定采用碳酸氢钠浸提为钼锑抗比色法; 土壤有机碳(soil organic carbon, SOC)的测定采用重铬酸钾容量法-稀释热法; 全氮(total nitrogen, TN)的测定采用硫酸钾、硫酸铜和硒粉作催化剂, 加入浓硫酸消煮, 定氮仪自动分析法。交换性钙(exchangeable calcium, E-Ca)、镁(exchangeable magnesium, E-Mg)的测定采用乙酸铵溶液作交换剂, 浸出交换性钙、镁后使用原子吸收分光光度法测定。具体分析步骤见土壤农业化学分析方法^[17]。

1.4 可培养细菌的培养纯化

试验采用两种培养基, 分别为 R2A 和 SC。研究表明 R2A 培养基适合大部分细菌生长, 而且可培养出某些特有细菌, 用 SC 培养基可以观察是否能培养出难培养菌种^[18]。根据培养结果进一步选择合适的培养基进行后续培养。根据不同施肥措施与不同培养基组合, 分别命名为不施肥 R2A、不施肥 SC、常规施肥 R2A、常规施肥 SC、常规施肥加绿肥 R2A、常规施肥加绿肥 SC。

无菌条件下在不施肥组土样中称取相当于干土 1.44 g 于 25 mL 锥形瓶, 常规施肥和常规施肥加绿肥两组同理分别称取相当于干土 1.60 g 、 1.43 g

于锥形瓶中,置于 28 °C 培养箱中风干后,向锥形瓶加入 20 mL 无菌水,适当振速振荡 1 h 后,制作样品梯度稀释液(10^{-1} 、 10^{-2} 、 10^{-3} 和 10^{-4})。分别吸取 100 μ L 稀释梯度为 10^{-3} 、 10^{-4} 样液加入两种培养基平板上涂布,设 2 组重复,将所得平板倒置于 28 °C 培养箱中培养。观察平板菌生长状况,10–14 d 后对细菌进行鉴别统计。

无菌环境下使用尖头竹签挑取单菌落,将挑选的菌落采用平板划线法^[19]转接至 R2A 培养基上,倒置于 28 °C 培养箱培养,并观察菌种生长情况、纯化程度,若非纯菌则对其继续进行纯化,直到得到纯种菌落,收集转至 20%甘油管中,置于–80 °C 冰箱保存备用。

1.5 细菌 DNA 提取及 PCR 扩增

无菌环境下取 50 μ L Chelex 100 溶液置于装有菌株的 PCR 管中,搅拌均匀后在 PCR 仪上进行 Chelex 程序裂解,Chelex 程序参数:99 °C 25 min。随后室温下使用微型离心机 1 000 r/min 离心 1 min 至分层,上清液即为所提取的 DNA。获取到的菌株 DNA 进行 16S rRNA 基因序列扩增、测序。扩增使用正向引物 27F (5'-AGAGT TTGATCCTGGCT-3') 和反向引物 1492R (5'-GGTTACCTTGTACGACTT-3')。PCR 反应体系(25 μ L):反应体系混合液 12.5 μ L,正、反向引物浓度为 0.4 μ mol/L,样品 DNA 提取液 1 μ L,双蒸水补足。PCR 反应条件:95 °C 5 min;94 °C 30 s,52 °C 30 s,72 °C 1.5 min,35 个循环;72 °C 10 min;12 °C 保存。

表 1 不同施肥措施下土壤理化性质

Table 1 Physical and chemical properties of soil under different fertilization

理化参数	不施肥	常规施肥	常规施肥加绿肥
Physical and chemical parameters	No fertilization	Conventional fertilization	Conventional fertilization and green manure
pH	7.26	6.72	7.36
土壤有机碳 Soil organic carbon (g/kg)	30.33	21.66	37.21
有效磷 Available phosphorus (mg/kg)	2.80	15.22	28.88
全氮 Total nitrogen (mg/kg)	1 886.55	1 927.05	3 733.23
碱解氮 Available nitrogen (mg/kg)	160.40	131.55	189.95
速效钾 Available potassium (mg/kg)	95.99	127.94	166.99
交换性钙 Exchangeable calcium (cmol/kg)	28.85	10.23	18.65
交换性镁 Exchangeable magnesium (cmol/kg)	1.45	1.05	1.51

PCR 扩增结束后用 0.8% (质量体积比)琼脂糖凝胶对 PCR 产物进行凝胶电泳检测,预计目的条带为 1 500 bp 左右^[20],将 PCR 产物送至生工生物工程(上海)股份有限公司广州分公司进行测序。

1.6 数据处理

测序得到的 16S rRNA 基因序列使用 Mothur 软件进行去重复^[21]和 OTU 聚类,从每个 OTU 中选择一条作为代表序列,在 NCBI 中进行 BLAST 比对。使用 MEGA 5.0^[22]构建 NJ 系统发育进化树,将建树参数中的 No. of Bootstrap Replications 值改为 1 000,其余参数默认。基于 OTU 聚类分析结果,使用 Pearson 相关系数方法对土壤理化性质与丰度大于 1%的优势 OTU 结合进行相关性分析,并用 R 语言绘制相关性热图,对不同措施下可培养细菌进行 α 多样性分析。

2 结果与分析

2.1 不同施肥措施下土壤理化性质的测定

通过不施肥、常规施肥和常规施肥加绿肥处理下的土壤理化性质测定结果如表 1 所示。常规施肥处理下土壤 pH 为 6.72,有机碳含量为 21.66 g/kg,碱解氮含量为 131.55 mg/kg,上述指标及交换性钙、镁含量均低于不施肥和常规施肥加绿肥两组土壤测定值。同时,有效磷、全氮和速效钾含量随不施肥、常规施肥和常规施肥加绿肥处理方式呈现递增趋势,其中常规施肥加绿肥组增长幅度最大,该组全氮含量为 3 733.23 mg/kg、有效磷含量为 28.88 mg/kg,而不施肥土壤有效磷含量只有 2.80 mg/kg。

2.2 可培养细菌丰度

培养完全结束后, 比较可培养菌株数量得出: 常规施肥加绿肥>不施肥>常规施肥; 使用培养法最大值计算得到可培养细菌丰度。3 组所得最大值都来自 R2A 培养基, 其中不施肥组最大值为 1.20×10^4 CFU/g, 与常规施肥加绿肥组最大值 1.29×10^4 CFU/g 相近, 而且均大于常规施肥组最大值 7.60×10^3 CFU/g, 丰度最小值为 3.00×10^2 CFU/g, 出现在 SC 培养基下不施肥组(表 2)。

2.3 可培养细菌系统发育

通过对不同措施下土壤细菌纯化培养共获得 164 株菌, 其中不施肥组得到 65 株菌, 常肥组得到 29 株菌, 常规施肥加绿肥组得到 70 株菌。使用 Mothur 软件去重复后得到 79 个 OTU, 不施肥组分

属 48 个 OTU, 常规施肥组分属 17 个 OTU, 常规施肥加绿肥组分属 39 个 OTU (表 3)。此次培养得出细菌有变形菌门、放线菌门、拟杆菌门和厚壁菌门, 其中变形菌门菌株数占总菌株数比最高, 达 73.17%。根据系统发育进化树显示(图 1), 本次可培养细菌分别属于 *Actinobacteria*、*Sphingobacteriia*、*Flavobacteriia*、*Bacilli*、*Alphaproteobacteria*、*Betaproteobacteria*、*Gammaproteobacteria* 和 *Chitinophagia*。结合表 4 来看, 纲水平上常规施肥加绿肥组丰度最高, 变形菌门 *Alphaproteobacteria* 在不施肥组和常规施肥加绿肥组占比最大, 分别为 49.2%和 45.7%, 常规施肥组中占比最大的则为变形菌门 *Gammaproteobacteria* (44.8%)。另外, 常规施肥加绿肥组培养出 *Sphingobacteriia*, 其他两组均无。

表 2 可培养细菌丰度表

Table 2 Abundance table of culturable bacteria (CFU/g)

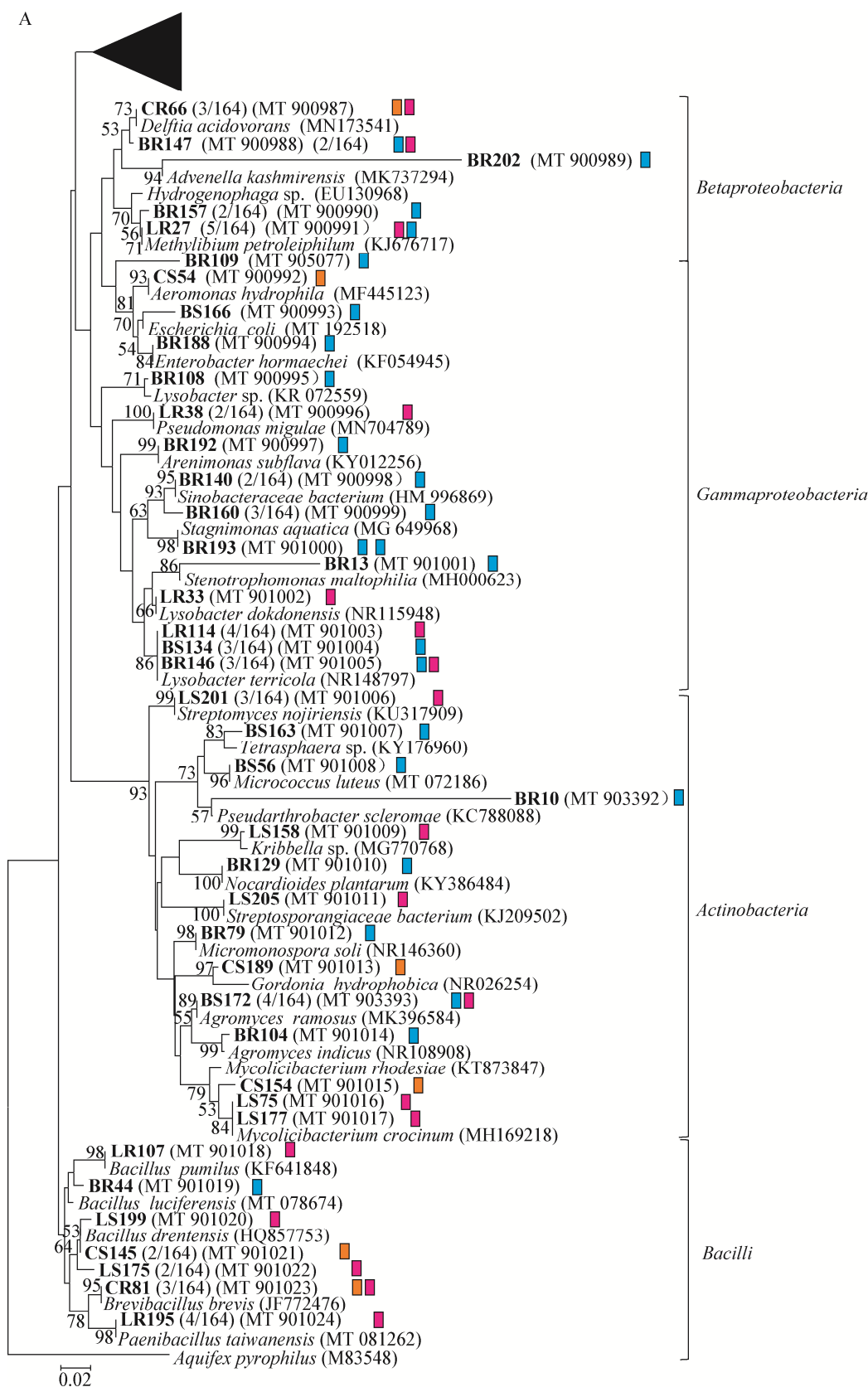
项目		不施肥	常规施肥	常规施肥加绿肥
Item		No fertilization	Conventional fertilization	Conventional fertilization and green manure
培养基	R2A	1.20×10^4	7.60×10^3	1.29×10^4
Culture medium	SC	3.00×10^2	3.35×10^3	8.50×10^2
最大值 Maximum		1.20×10^4	7.60×10^3	1.29×10^4

表 3 不同施肥措施土壤培养细菌的系统发育关系及组成

Table 3 Phylogenetic relationship and composition of soil culturable bacteria under different fertilization

样品	不施肥	常规施肥	常规施肥加绿肥
Sample	No fertilization	Conventional fertilization	Conventional fertilization and green manure
Total strain	65	29	70
Number of OTUs	48	17	39
<i>Actinobacteria</i>	8(12.3%)	2(6.9%)	9(12.9%)
<i>Sphingobacteriia</i>			1(1.4%)
<i>Flavobacteriia</i>	1(1.5%)	4(13.8%)	2(2.9%)
<i>Bacilli</i>	1(1.5%)	4(13.8%)	9(12.9%)
<i>Alphaproteobacteria</i>	32(49.2%)	4(13.8%)	32(45.7%)
<i>Betaproteobacteria</i>	5(7.7%)	2(6.9%)	6(8.6%)
<i>Gammaproteobacteria</i>	17(26.2%)	13(44.8%)	9(12.9%)
<i>Chitinophagia</i>	1(1.5%)		2(2.9%)

Note: The numbers in the brackets are the relative abundance.



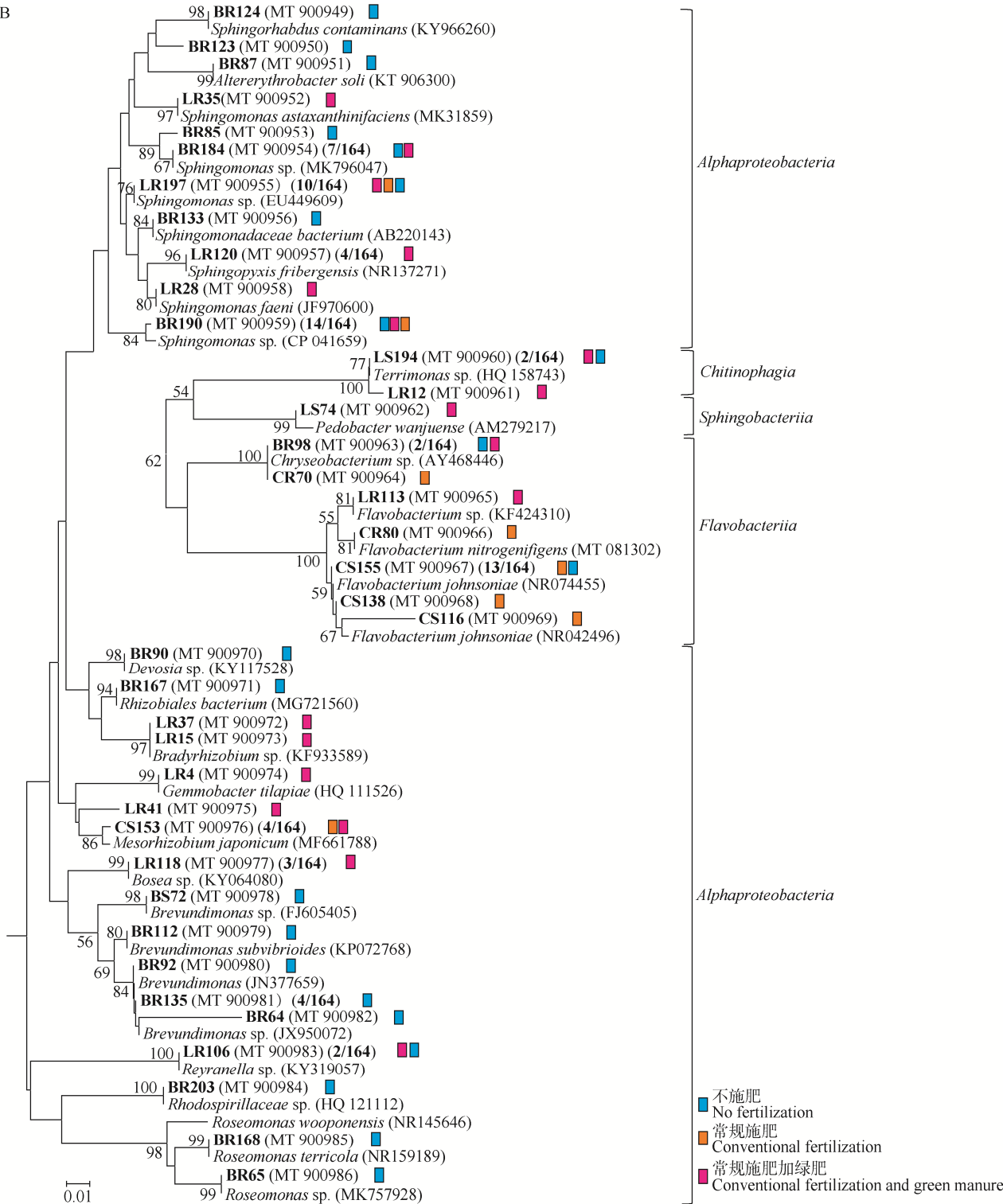


图 1 3 种施肥措施下土壤可培养细菌种群发育进化树

Figure 1 Evolutionary tree of soil culturable bacteria population under three fertilization

Note: The letters and numbers in the first bracket indicate the NCBI registration number, and the other bracket indicates the number of strains obtained by this OTU. If the number is “1”, it will not be marked. The number of branching points indicates bootstrap values based on 1 000 replications are listed as percentages at the branching points. The ruler in Figure A indicates 0.02 substitutions per nucleotide position, the ruler in Figure B indicates 0.01 substitutions per nucleotide position. Figure B is the folded part of the black triangle in Figure A.

2.4 可培养细菌 α 多样性分析

使用 R 语言计算得出不同施肥措施下土壤可培养细菌菌株 16S rRNA 基因在 97% OTU 水平下的 α 多样性指数(表 4)。不施肥处理下 Shannon、Simpson 和 Equitability 指数分别为 3.09、0.95 和 0.97, 常规施肥加绿肥处理下 Shannon、Simpson 和 Equitability 指数分别为 2.95、0.94 和 0.97, 3 组中常规施肥组的 3 个多样性指数最低, 依次为 2.15、0.8 和 0.81。

本次 3 组样品可培养细菌共 164 株分别来自变形菌门、放线菌门、拟杆菌门和厚壁菌门 4 门 8 纲 20 目 31 科 41 属。门水平各门菌株数大小关系为: 变形菌门>放线菌门>厚壁菌门>拟杆菌门。属水平上来看, 占总菌株数比最高的是 *Sphingomonas*, 达 23.8%; 其次为 *Stenotrophomonas*, 占总菌株数 7.9%, 可以看出 *Sphingomonas* 数量明显高于其他类群(图 2)。但对比图 3 可发现, *Sphingomonas* 在不施肥组 (24.2%)和常肥加绿肥组(30%)中占比最大, 高于其他优势类群, 而在常规施肥组占比最大优势类群则为 *Stenotrophomonas* (40.7%)。

从属水平分析不同施肥措施下土壤可培养细菌的群落结构, 结果如图 3 所示, 比较可培养菌株属水平丰度得出: 不施肥>常肥加绿肥>常规施肥。在 3 种施肥措施下, 不施肥组样品分离得到菌株 62 株, 分别属于 4 门 7 纲 14 目 20 科 25 属; 常规施肥组样品分离得到菌株 27 株, 分别属于 4 门 6 纲 8 目 9 科 11 属; 常规施肥加绿肥组样品分离得到菌株 70 株, 分别属于 4 门 8 纲 16 目 17 科 22 属。

Brevundimonas、*Stagnimonas*、*Roseomonas*、*Sphingorhabdus*、*Hydrogenophaga*、*Enterobacter*、

Micromonospora、*Nocardioides*、*Pseudolabrys*、*Devosia*、*Altererythrobacter*、*Arenimonas*、*Micrococcus* 和 *Tetrasphaera* 为不施肥组特有种群, 共 14 个属, 其中分属变形菌门有 10 个, 数量最多, 其余属于放线菌门。常规施肥组中特有种群有 3 个, 分别为 *Gordonia*、*Mesorhizobium* 和 *Xanthomonas*, 分别属于变形菌门(66.7%)、放线菌门(33.3%); 常规施肥加绿肥组发现特有种群 10 个, 分别为 *Paenibacillus*、*Mesorhizobium*、*Streptomyces*、*Bosea*、*Bradyrhizobium*、*Pseudomonas*、*Pedobacter*、*Gemmobacter*、*Kribbella* 和 *Nonomuraea*, 此组特有种群多数来自变形菌门(50%)。属于 *Pseudomonas* 的菌株共分离得到 2 株, 占总菌株量 1.2%, 在系统发育进化树上显示与 *Pseudomonas migulae* 存在 100% 相似度(图 3), 此外, 该组特有种群中 *Paenibacillus* 属于厚壁菌门, *Pedobacter* 属于拟杆菌门, 其他两组均无属于上述两门的特有类群。

2.5 土壤理化性质和占优势可培养细菌群落的关系

土壤理化性质和优势 OTU (>1%)的关系如图 4 所示。OTU3 (*Stenotrophomonas maltophilia*)与 pH、交换性镁(E-Mg)显著负相关, 与有效磷(AP)、速效钾(AK)相关性不显著; OTU (7、14、18、13、17、22 和 27, *Sphingopyxis*、*Lysobacter*、*Paenibacillus*、*Bosea*、*Streptomyces*、*Pseudomonas* 和 *Bacillus*)与全氮(TN)显著正相关, 与碱解氮(AN)和有效磷(AP)正相关; OTU10 (*Lysobacter*)与有机碳(SOC)显著相关, 与 AN 存在极显著正相关关系, 与 pH、TN 和 E-Mg 正相关; OTU (9 和 11, *Brevibacillus* 和 *Delftia*)与 E-Ca 显著负相关。

表 4 不同施肥措施下土壤可培养细菌菌株 α 多样性指数
Table 4 Alpha diversity index of soil culturable bacteria strains under different fertilization (at 97% OTU level)

施肥措施	菌株数(个)	Shannon index	Simpson index	Equitability index
Fertilization measures	Number of bacteria			
不施肥 No fertilization	65	3.09	0.95	0.97
常规施肥 Conventional fertilization	29	2.15	0.80	0.81
常规施肥加绿肥	70	2.95	0.94	0.97
Conventional fertilization and green manure				

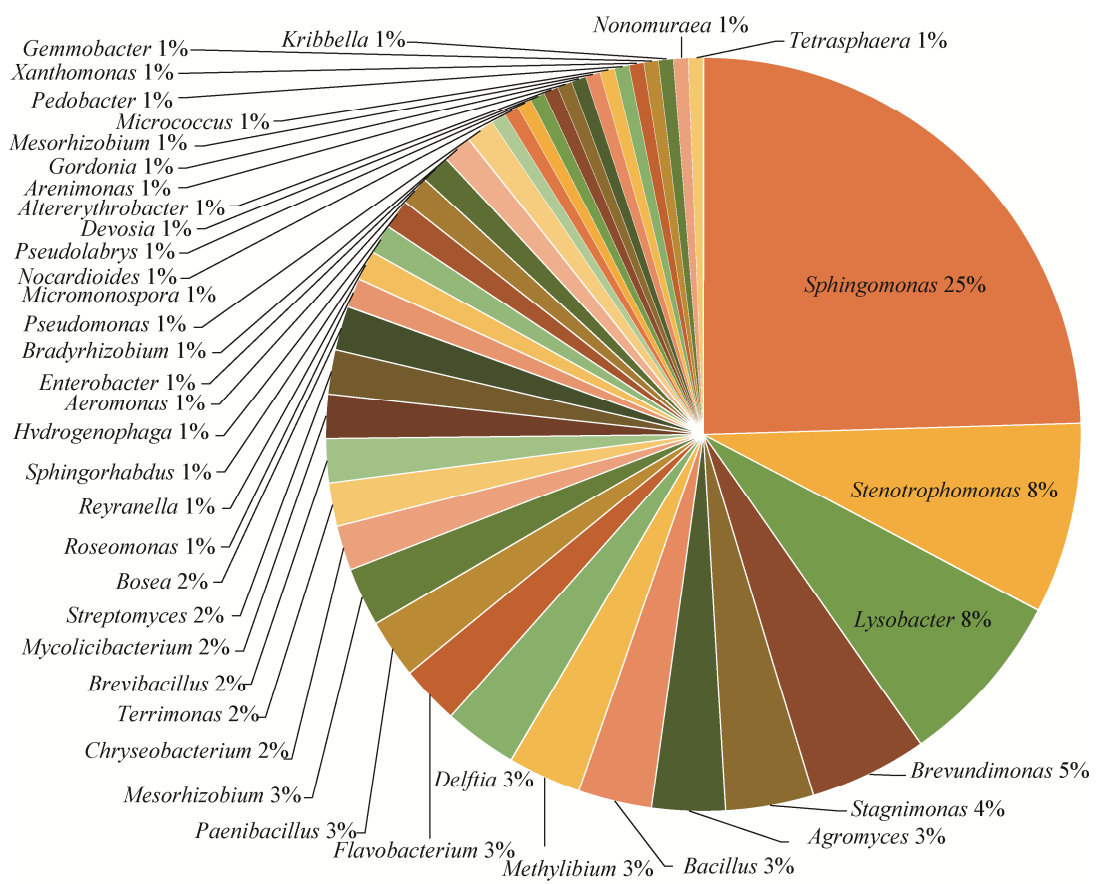


图 2 获得总可培养细菌属水平分布情况
Figure 2 Obtain the horizontal distribution of total culturable bacteria

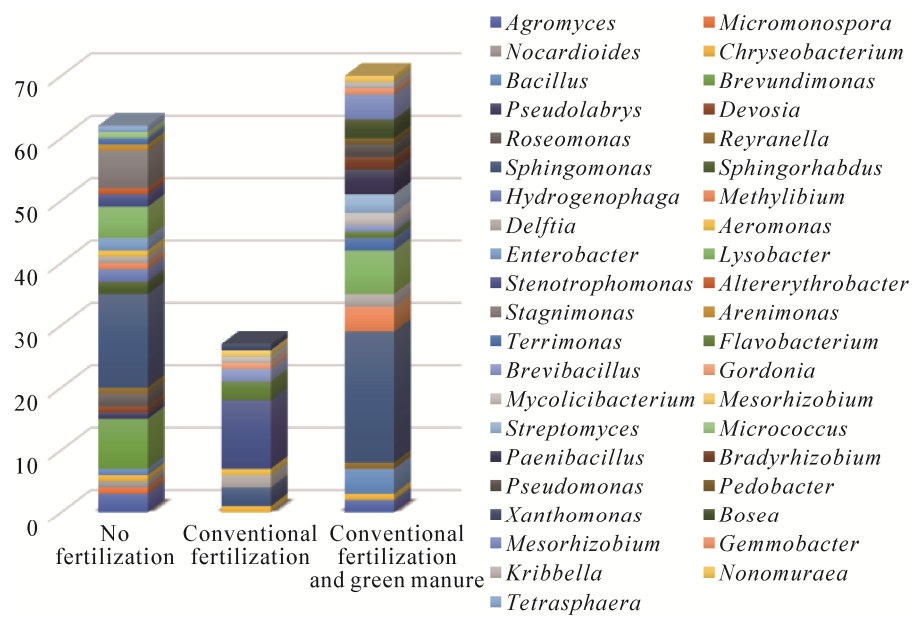


图 3 不同措施下土壤可培养细菌柱状图
Figure 3 Histogram of culturable bacteria in soil under different fertilization

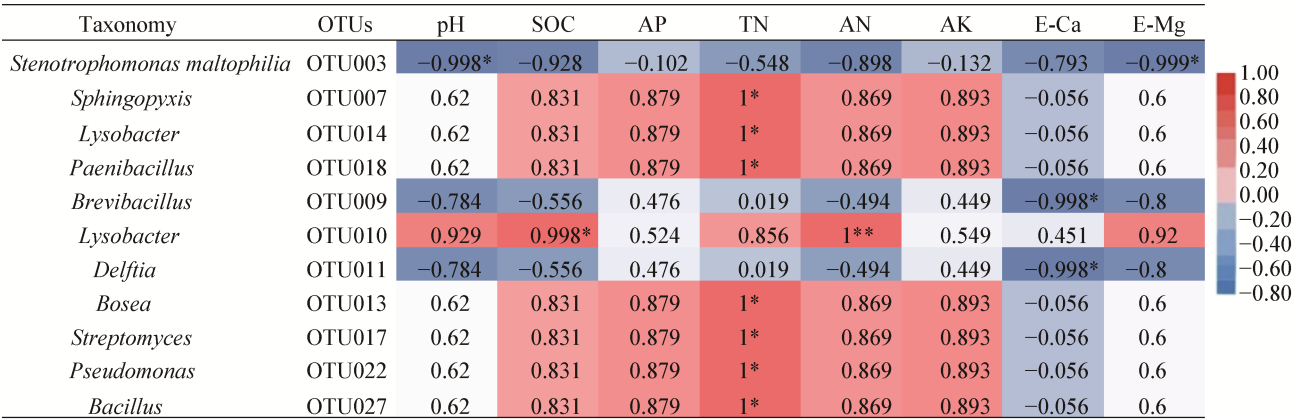


图 4 优势 OTU 与土壤理化性质显著相关关系图

Figure 4 The correlation diagram between dominant OTUs and soil physical and chemical properties

Note: Significance levels are denoted as follows: $P<0.05$ (*) and $P<0.01$ (**).

3 讨论

土壤 pH 值主要受成土母质影响，棕色石灰土母质主要以白云岩和石灰岩为主，偏碱是其显著特征之一^[23]。本研究中常规施肥处理发现土壤 pH 值下降，而常规施肥加绿肥处理土壤 pH 值未受影响(表 1)，说明长期施无机肥或氮肥的过量施加都会造成土壤 pH 值下降^[24]，主要是由于尿素经水解、硝化反应生成的硝酸根与土壤中氢离子结合，导致土壤变酸^[25]，这与丁建莉等^[26]、刘平静等^[27]实验结果一致。本研究表明可培养细菌丰度变化与土壤 pH 值及有机碳含量变化规律一致(表 1、2)，说明土壤 pH 值下降抑制了某些细菌的生长繁殖，导致细菌丰度和多样性降低^[28]。前人研究指出施肥造成土壤 pH 改变时，pH 值为影响土壤细菌群落结构的主导因子^[29]。土壤有机碳为土壤细菌提供碳源，以维持细菌活性^[30]，常规施肥处理下土壤可利用碳源减少，不足以支持细菌的生命活动^[31]，因此可培养细菌丰度下降；部分细菌对生长环境要求低，在低碳源条件下仍能维持生长^[32]。例如 *Lysobacter* 对营养要求低，可利用碳源广泛，如单糖和苹果酸等^[33]，因此能够在寡营养环境下生存。类似的优势菌属 *Sphingomonas* 是异养好氧的革兰氏阴性菌，细胞膜具有鞘脂糖^[34]，耐受极端营养环境^[35]。然而常规施肥组中上述细菌可培养丰度极低，造成了不同施肥

处理下可培养细菌丰度差异的原因可能是不同类型菌群自身生存策略不同^[36]。此外，细菌固定 CO₂ 是碳循环的关键环节，其中卡尔文循环是固定 CO₂ 的主要途径。常规施肥加绿肥处理组特有的 *Bradyrhizobium* 和 *Mesorhizobium* 具有核酮糖-1,5-二磷酸羧化酶/加氧酶(RubisCO)^[37]，通过卡尔文循环固定 CO₂，在碳循环中起关键作用^[38]。同时，它们还可利用丰富的多糖类物质作为碳源合成自身有机物质——聚羟基烷酸酯进行异养繁殖^[39]；常规施肥加绿肥处理后，优势菌 *Sphingomonas* 丰度和多样性增加，该菌属主要通过降解木质素实现参与碳循环^[40]，木质素是含芳香化合物最多也是含有机碳最多的混合物，能够在土壤中转化成腐殖质，其降解对碳循环有着重要意义^[41]。研究表明这类固碳菌丰度和多样性的降低是加速土壤有机碳损失的重要原因^[42]，本次研究中固碳细菌与有机碳的互作关系与 Kandeler 等^[43]研究结果一致。施肥增加了土壤有机碳含量，其中有机肥配施化肥的处理效果最明显^[44-46]。单施有机肥或有机肥配施化肥为土壤细菌提供碳源和生长所需营养^[47]，增强土壤细菌活性，促进其对新鲜有机物质的固定^[48]，进而明显提高土壤有机碳贮量^[49]。有机碳贮量增加可为作物提供营养，促进其生长^[50]。因此，在石灰性土壤上进行绿肥配施化肥较常规施肥处理更利于保持土壤 pH 值稳定，

增加细菌多样性和有机碳的累积。

通过对不同施肥措施下细菌群落结构的分析,发现不同处理下的优势类群在门、纲水平上相似。本研究土壤中变形菌门和放线菌门为优势类群,与前人研究结果一致^[51-52]。培养得到的功能菌如固氮菌属 *Bacillus*^[53]、*Paenibacillus*^[54]和 *Pseudomonas*^[55]等及溶磷菌属 *Gordonia* 和 *Enterobacter* 等^[56],与张翔等^[57]研究结果相似。石灰性水稻土碳酸钙含量高,石灰反应强烈,土壤有机碳易于积累,但营养元素供给速率慢^[58]。基于这些特性,提高石灰性水稻土营养元素的供应能力和对水稻种植至关重要。TN 是土壤中各种形态无机氮和有机氮的总量,也是土壤肥力的核心之一^[59],土壤氮素的有效性指标通常是碱解氮。本研究中,优势菌属 *Sphingopyxis*、*Lysobacter*、*Paenibacillus*、*Bosea*、*Streptomyces*、*Pseudomonas* 和 *Bacillus* 与 TN 存在显著正相关,与 AN 正相关(图 4),说明与 N 相关的细菌在该处理的土壤中大量出现,其中具固氮功能的菌属占比较大,表明土壤 N 含量的增加可能是固氮功能菌发挥主导作用,氮素循环过程主要由细菌参与^[60],固氮环节中 *Paenibacillus*^[61]和 *Pseudomonas*^[62]等可将空气中的分子氮转化为有机氮,这在很大程度上增加了土壤的氮素营养,从而促进了作物增产^[63]。然而在氮素循环的其他环节均有功能细菌参与^[64],如 *Micrococcus* 和 *Flavobacterium* 参与氨化作用,将有机氮分解成为氨与氨化合物^[65]。

磷作为作物的必需营养元素,而石灰土中大量碳酸钙与其结合生成难溶的、不易被作物吸收的磷酸钙盐,导致磷素供应能力低^[66]。本实验结果表明,通过与不施肥处理相比,施肥可提高土壤有效 P 含量,不同施肥措施土壤 AP 含量变化(表 1)与溶磷菌丰度与多样性变化具有相同趋势,与罗明等^[67]研究一致。解磷细菌可以将难溶性无机磷转化为可溶性磷,提高土壤有效磷含量并促进作物吸收^[68]。释放葡萄糖酸或柠檬酸等有机酸是细菌溶磷的重要途径之一^[69],可培养的溶磷菌 *Pseudomonas* K3 主要通过分泌苹果酸,同时络合土壤中各种金属离子与

矿物吸附磷发生竞争吸附,从而溶解不溶性磷^[70];此外,也有学者认为 *Pseudomonas* 某些菌种通过呼吸作用或 NH_4^+ 同化作用产生质子^[71],而 *Sphingomonas* 主要通过产磷酸酶溶解土壤无机磷^[72],从而使难溶性无机磷溶解。另外,大部分溶磷菌具有促进作物生长和提高作物产量的能力^[73],如 *Enterobacter* 能够分泌吡啶乙酸促进作物生长,在一定条件下对水稻也存在促生作用^[74]。因此,我们推测在土壤细菌群落中溶磷细菌丰度和多样性是影响土壤 P 含量变化的关键因素之一,并对作物生长和增产起一定作用。

综上所述,营养元素含量作为土壤肥力的重要指标,对作物生长和增产起重要作用。本研究中土壤理化性质的变化与其对应功能菌属的变化保持一致,可见土壤细菌在土壤元素转化中具有重要作用。

4 结论

(1) 长期施无机肥或氮肥的过量施加都会使土壤 pH 值下降, pH 值是影响岩溶水稻土壤可培养细菌丰度的主要原因。

(2) 常规施肥处理下土壤有机碳含量明显降低,土壤细菌可利用碳源减少导致活性下降,固碳速率降低,而常规施肥加绿肥更有利于细菌活性功能的保持和有机碳的积累。

(3) 常规施肥加绿肥处理下,土壤细菌群落多样性和优势菌丰度增加,固碳、固氮和溶磷功能菌大量出现,土壤理化性质的变化与其相应功能菌属的变化具有相同变化趋势,岩溶水稻土进行常规施肥配施绿肥处理所得效果要优于不施肥和常规施肥。

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