

不同覆盖模式下苹果园根际土壤理化性质及细菌群落特征差异分析

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摘要:【目的】探讨生草覆盖(GC)和稻草覆盖(RSM)两种不同覆盖处理对苹果园根际土壤理化性质和细菌群落结构的影响。【方法】选取经GC和RSM处理的苹果园根际土壤样品,分析两种覆盖处理下土壤的理化性质、酶活性,采用16S rRNA高通量测序技术分析比较不同覆盖处理土壤细菌群落的结构和功能,并通过LEfSe分析和随机森林方法识别核心微生物组和特异性生物标记物,结合RDA冗余分析和Pearson分析探讨关键微生物组丰度与土壤性质的相关性。【结果】GC处理显著提高了土壤中的碱解氮含量和脲酶、碱性磷酸酶活性,而RSM处理则显著提高了土壤含水量和速效钾含量;两种覆盖处理苹果园根际土壤中细菌的优势菌群类别在门和属水平上总体相似,在丰富度上RSM高于GC。此外,识别出5个共有核心微生物组(*RB41*、*MND1*、*Ralstonia*等)及5个特异性生物标记物(*Ellin6067*、*Sphingomonas*、*Nocardioideis*等),其丰度与土壤理化性质存在较强的相关性,其中碱解氮(AN)和速效钾(AK)含量是影响细菌群落分布的主要土壤理化因子。【结论】GC和RSM两种覆盖模式下苹果园根际土壤的理化性质存在显著差异,从而导致细菌群落分布出现差异,RSM处理微生物丰富度高于GC处理,其中AN和AK是主要影响因子。研究结果为西南冷凉高地苹果园的栽培模式优化和土壤质量以及微生物功能提升提供了理论依据。

关键词:苹果园;土壤覆盖;根际土壤;细菌群落;高通量测序

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Analysis of differences in physicochemical properties and bacterial community characteristics of rhizosphere soil in apple orchards under different cover treatments

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Abstract: 【Objective】 Cover cropping is a widely adopted soil management technique used in fruit tree cultivation. By planting specific vegetation to cover the ground, this practice significantly influences nutrient cycling, microbial activity, and biodiversity within the soil ecosystem. Thus, cover cropping plays a critical role in maintaining soil health and enhancing fruit quality. This study explored the impact of the grass cover (GC) and rice straw mulch (RSM) on the physicochemical properties of rhizospheric soil and the structure of bacterial communities in apple orchards. 【Methods】 The soil samples were collected from the apple orchards subjected to both GC and RSM treatments. We compared the physico-

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chemical properties of the soils under each treatment, focusing on key indicators such as alkaline nitrogen (AN), soil moisture (SM), available potassium (AK), soil organic matter (OM), effective phosphorus (AP), pH level, soil bulk density (SBD), and soil porosity (SP). Additionally, enzyme activities were measured, specifically for urease (UA) and alkaline phosphatase (APA). The structure and functionality of the bacterial communities were analyzed using the high-throughput sequencing of 16S rRNA. The core microbiomes and specific biomarkers were identified through LEfSe analysis and random forest methods. Moreover, the redundancy analysis (RDA) and Pearson correlation analysis were performed to explore the relationships between the key microbial abundances and soil physicochemical properties.

【Results】 The significant differences were observed in the physicochemical properties of the soil under different treatments. The GC treatment led to an increase in AN levels, while the RSM treatment enhanced SM and AK. However, no significant differences were detected between the two treatments concerning OM, AP, pH, SBD, and SP. Furthermore, the enzyme activities of UA and APA in the soil with GC treatment were significantly higher than those in the soil with RSM treatment ($p < 0.05$), with increases of 76.81% and 45.24%, respectively. Conversely, there were no significant differences ($p > 0.05$) in the sucrase activity (SA) and catalase activity (CA) between the two types of mulched soils. This suggested that the distinct mulching treatments would have contrasting effects on the soil enzyme activities, with UA and APA exhibiting greater sensitivity to these treatments. The hierarchical clustering analysis based on unweighted UniFrac distances revealed that inter-group differences among the soil samples from the different treatments were greater than intra-group differences. Following the two treatments, the rhizospheric bacterial communities in the apple orchards comprised 38 phyla, 100 classes, 254 orders, 417 families, and 777 genera. At the phylum level, Proteobacteria was the dominant phylum, accounting for 32.41% in the GC sample and 42.37% in the RSM sample. At the genus level, the dominant taxa in the soil with GC treatment were *MND1* (5.41%), *RB41* (5.07%), *Ralstonia* (4.62%), and *Raoultella* (3.42%). In the soil with RSM treatment, they were *MND1* (9.10%), *Ralstonia* (7.11%), *RB41* (2.00%), and *Pseudarthrobacter* (1.94%). Although the dominant bacterial groups at both the phylum and genus levels were largely similar between the soils treated with GC and RSM, the richness of the rhizospheric bacteria was considerably higher in the RSM sample. Moreover, the alpha diversity indices indicated greater bacterial diversity in the RSM soil, although the difference from the GC sample was not statistically significant. Through LEfSe analysis and random forest methods, we identified five core microbial taxa (*RB41*, *MND1*, *Ralstonia*, *Raoultella*, and *Pseudarthrobacter*), as well as five specific biomarkers (*Ellin6067*, *Sphingomonas*, *Nocardioides*, *Subgroup 10*, and *RB41*). The RDA and Pearson analyses revealed strong correlations between these microbial abundances and physicochemical soil properties, with AN and AK emerging as the primary factors that would influence the structure of the rhizosphere bacterial communities.

【Conclusion】 In this study, we discovered significant differences in the physicochemical properties of the rhizospheric soils with GC and RSM treatments in apple orchards. These disparities led to substantial variations in the bacterial community structure. Our findings indicated that GC enhanced the soil alkaline nitrogen content and enzymatic activities, while RSM improved the soil moisture and available potassium levels. The dominant bacterial phyla and genera remained broadly similar in the soils treated with the two treatments, but notable differences in relative abundance were observed, with RSM exhibiting higher bacterial richness than GC. There was a close correlation between the soil physicochemical properties and key bacterial abundances, with AN and AK acting as major influencing factors. Specifically, the bacterial community associated with RSM exhibited a significant positive association with AK levels, while that associated with GC correlated positively

with AN expression. This research would provide a theoretical basis for optimizing cultivation practices and enhancing soil quality and microbial functionality in apple orchards located in the cool highland regions of Southwestern China.

Key words: Apple orchard; Soil mulching; Rhizosphere soil; Bacterial community; High-throughput sequencing

苹果是世界上最重要的果树之一,具有丰富的营养价值和广泛的市场需求^[1]。土壤覆盖是果树栽培中常用的土壤管理方式,对果树生长和果实品质有重要影响^[2],不仅可以改变土壤的温度、湿度、光照、气体交换等物理特性,还可以提高土壤的有机质含量、养分含量、pH、酶活性等化学属性^[3],从而影响根际土壤微生物的数量、结构和功能^[4]。根际土壤是植物根系和土壤之间的关键交互界面,也是土壤微生物活动最频繁的区域^[5]。这些微生物在维持土壤肥力、促进植物生长、抑制土传病害、调节土壤环境等方面扮演着重要角色^[6]。果树的健康生长、抗逆能力以及果实品质与土壤的理化性质和微生物群落结构密切相关^[7]。因此,研究不同土壤覆盖模式对苹果根际土壤性质及细菌群落特征的影响,对揭示苹果根际微生态过程的机制、优化苹果园管理措施,以及提升苹果品质和栽培效益具有重要的理论和实践意义。

目前,在冷凉高地苹果园中常用的土壤覆盖方式有生草覆盖(GC)和稻草覆盖(RSM)。GC通常被认为能够提高土壤有机质含量和保水性,通过提升土壤中有机质含量,从而促进那些参与有机物分解和养分循环的有益微生物生长^[8-9]。而RSM由于稻草秸秆分解速率较慢,不仅可以在较长时间内维持土壤结构和保持水分,还可以抑制杂草和减少水分蒸发^[10]。同时由于稻草较高的碳氮比特性使得RSM更有利于适应低氮环境的微生物生长,从而在根际土壤形成一个与GC截然不同的微生物群落^[11]。尽管如此,目前关于这两种覆盖模式对苹果园根际土壤性质及细菌群落特征差异的研究较少,鉴于此,笔者在云南省昭通市昭阳区一个建园1年的苹果园中开展田间试验,对比研究不同覆盖模式下的苹果园根际土壤的理化性质、细菌群落组成和功能方面的差异,以期苹果园栽培技术的优化提供科学依据。

1 材料和方法

1.1 试验地概况

试验地位于昭通市昭阳区永丰镇元龙村田源农

业科技有限公司基地,海拔1 946.4 m,全年日照时数1 782.54 h,年均降水量619.81 mm,年均气温12.27 °C,日平均气温 ≥ 10 °C有效积温1 587.67 °C,平均无霜期222.45 d。基地于2018年12月开始整地,2019年3月完成苗木定植,树苗为1年生大苗,采用现代矮砧格架栽培,株行距1.5 m \times 4.0 m,起垄栽培,自由纺锤形树形,供试砧穗组合为烟富3号/M9T337。试验地土壤基础肥力为有机质含量(w,后同)8.6 g \cdot kg⁻¹、有效磷含量11.6 mg \cdot kg⁻¹、碱解氮含量8 mg \cdot kg⁻¹、速效钾含量104 mg \cdot kg⁻¹、pH 7.72。

1.2 试验设计

试验于2019—2020年进行,2019年4月在基地选取2 hm²地块,均分,分别进行生草覆盖(GC)和稻草覆盖(RSM),每个处理设3个重复。GC处理(图1-A)于2019年3月定植完成后撒播草种,草种在春季天气回暖后陆续长出。RSM处理(图1-B)于2019年4月下旬完成覆盖,稻草编织成1.2 m \times 1 m长宽规格草席,草席沿树行覆盖整个垄面,垄底宽1.3 m,垄顶宽1 m,垄高约30 cm。两种处理的田间栽培管理措施完全一致。

1.3 土壤样品采集

于2020年10月25日(秋季施基肥前)进行土壤样品采集,每个地块随机选取3个点,根际土壤采样方法参考Riley和Barber的抖落法^[12],样品去除杂质后,将同一地块的3个土样按四分法混匀后分成两份。一份存入无菌冻存管后,置于干冰中速冻并送样,测定土壤细菌群落结构;另一份风干、研磨、过筛后用于测定土壤理化性质和酶活性。

1.4 样品测定项目及方法

1.4.1 土壤理化指标和酶活性测定 土壤理化指标和酶活性测定由云南三标农林科技有限公司代理完成。参照鲍士旦^[13]的方法测定土壤理化指标,包括土壤容重、比重、水分含量、pH及有机质、碱解氮、有效磷、速效钾含量等。参照关松荫^[14]的方法测定土壤蔗糖酶、脲酶、碱性磷酸酶、过氧化氢酶等的活性。



A. 生草覆盖;B. 稻草覆盖。

A. Grass cover; B. Rice straw mulching.

图1 不同土壤覆盖处理的苹果园

Fig. 1 Apple orchard with different soil mulching treatments

1.4.2 土壤细菌群落结构分析 土壤微生物群落结构的 *16S rRNA* 基因 V4 区域通过高通量扩增子测序进行分析,由成都罗宁生物科技有限公司代理完成。利用 Zymo Research 的 D4301 型试剂盒从土壤样本中提取基因组 DNA,然后通过 0.8% 的琼脂糖电泳法验证 DNA 的完整性。核酸浓度则通过 Tecan F200 设备进行测定。样本中的 16S rRNA V4 区域使用 Applied Biosystems® PCR System 9700 进行 PCR 扩增,扩增使用具有条形码的特异性引物 515F (5'-GTGYCAGCMGCCGCGGTAA-3') 和 806R (5'-GGACTACHVGGGTWTCTAAT-3')。为确保可靠性,每个样本进行 3 次 PCR 重复试验,以获取足够的 PCR 产物,然后通过 2% 的琼脂糖凝胶进行电泳检测;使用 Qubit@ 2.0 Fluorometer (Thermo Scientific) 定量。构建相应的文库,并采用 PE250 测序模式进行高通量测序。利用 Barcode 技术从原始读取 (raw reads) 中分离出各个样本的序列,并移除 Barcode 序列。然后通过 QIIME 软件进行数据质量控制,筛选出高质量的有效数据。采用 UPARSE 算法,并设定 97% 的同一性阈值,对序列进行操作分类单元 (OTU) 聚类,选取每个 OTU 中出现频次最高的序列作为该 OTU 的代表序列。最后进行 OTU 代表序列的功能注释,以确定他们的分类地位^[15]。

1.4.3 数据处理 利用软件 Microsoft Excel 2016、Graphpad prism 9 和 IBM SPSS Statistics 22.0 对试验数据进行初步处理、方差分析、Pearson 相关性分析等;利用软件 CANOCO 5.0 进行冗余分析 (redundancy analysis, RDA);在生科云 (<https://www.bioinformaticscloud.tech>) 和 Omicshare 平台 (<https://www.omicshare.com/tools>)

分别对土壤细菌数据进行韦恩图分析 (Venn diagram)、主成分分析、LEfSe 分析和相关性分析。

2 结果与分析

2.1 土壤理化性质

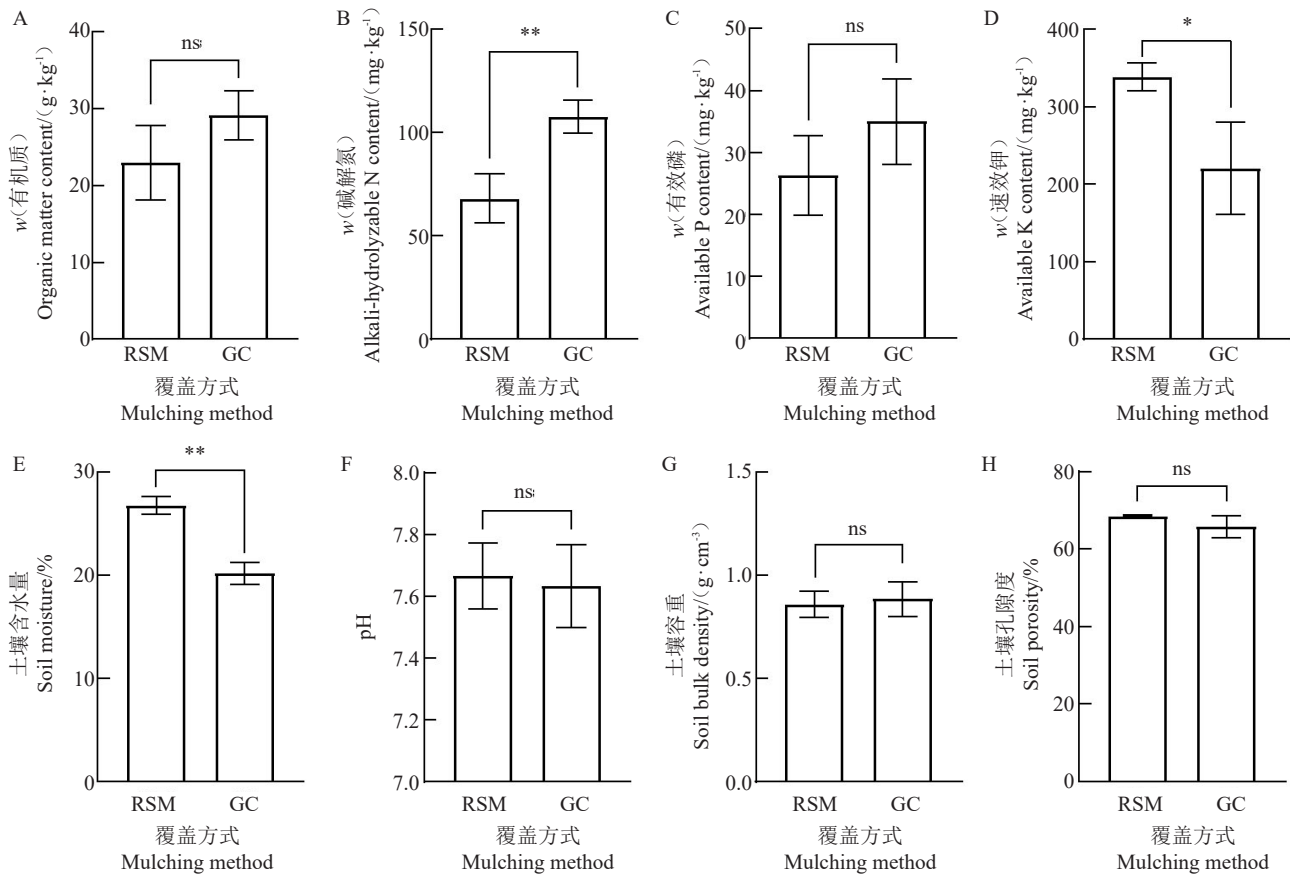
不同覆盖处理苹果园土壤的理化性质见图 2。生草覆盖 (GC) 处理土壤中碱解氮 (AN) 含量显著高于稻草覆盖 (RSM);稻草覆盖处理土壤含水量 (SM) 和速效钾 (AK) 含量显著高于生草覆盖。两种覆盖处理对土壤有机质 (OM) 含量、有效磷 (AP) 含量、pH、土壤容重 (SBD) 和土壤孔隙度 (SP) 均无显著影响。

2.2 土壤酶活性

土壤酶活性是指土壤中的酶对底物的催化作用,反映了土壤的生物活性和肥力水平^[16]。不同覆盖处理对土壤酶活性的影响见图 3。GC 土壤中脲酶 (UA) 和碱性磷酸酶 (APA) 活性均显著高于 RSM 土壤,分别显著提高了 76.81% 和 45.24%,而两种不同覆盖的土壤中,蔗糖酶 (SA) 和过氧化氢酶 (CA) 活性无显著差异。表明不同覆盖对土壤酶活性影响存在差异,其中脲酶和碱性磷酸酶表现较为敏感。

2.3 土壤细菌群落组成特征

利用高通量测序技术对 RSM 处理和 GC 处理的苹果园根际土壤样本中细菌 16S rRNA V4 区域进行了分析。在数据清洗和质量控制之后,获得了 18 122~34 142 条用于研究分析的序列数据。通过聚类算法,在 6 个样本中识别出 13061 个操作分类单元 (OTUs) (表 1),其中,RSM 处理的土壤样本的 OTUs 范围为 3416~4455,GC 处理的土壤样本的 OTUs 范



*表示不同处理间差异显著($p < 0.05$); **表示不同处理间差异极显著($p < 0.01$); ns表示不同处理间没有显著差异($p > 0.05$)。下同。

* indicates significant difference between different treatments ($p < 0.05$); ** indicates extremely significant difference between different treatments ($p < 0.01$); ns indicates no significant difference between different treatments ($p > 0.05$). The same below.

图2 不同覆盖处理土壤理化性质比较

Fig. 2 Effects of different soil cover patterns on the soil physicochemical properties in apple orchards

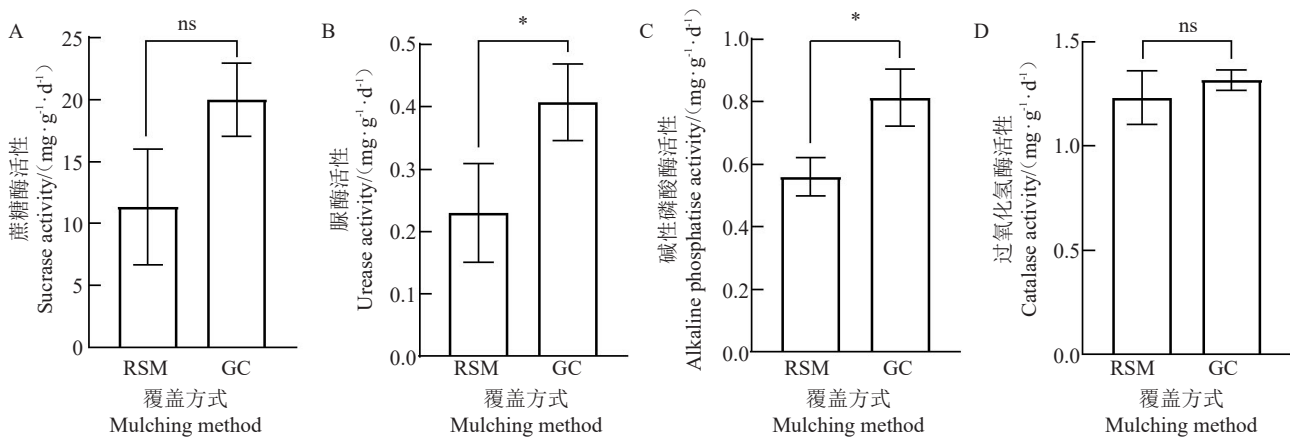


图3 不同覆盖处理土壤酶活性比较

Fig. 3 Effects of two different mulching modes on soil enzyme activity in apple orchards

围为2784-4276。基于未加权UniFrac距离的非度量多维尺度分析(NMDS)结果(图4-A),清晰地区分了RSM和GC土壤处理下的细菌群落。使用未加权UniFrac距离进行的层次聚类(图4-B)将两种

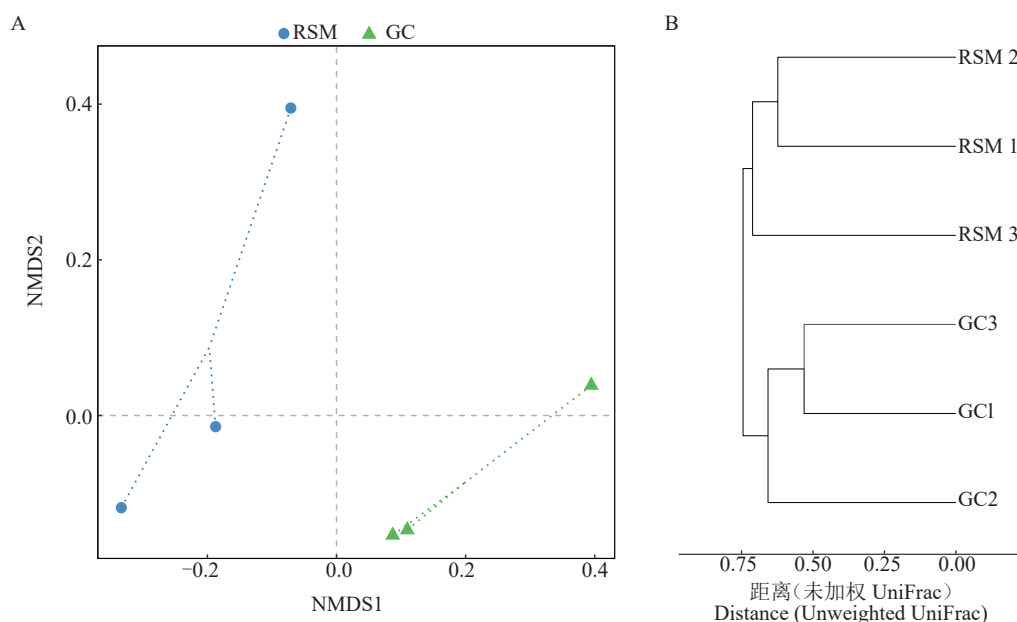
处理后土壤样本的细菌群落分成两个分支,表明土壤样本组间差异大于组内差异。

两种不同覆盖处理后,苹果园根际土壤细菌群落主要由38门、100纲、254目、417科和777属组

表1 物种样本信息和可操作分类单元(OTU)结果

Table 1 Species sample information and operational taxonomic units (OTU) results

样地号 Sample ID	原始序列数 Raw tags	过滤后序列数 Clean tags	有效序列数 Effective tags	平均长度 Average length/nt	可操作分类单元 OTU
RSM1	35 447	34 833	33 624	297	3516
RSM2	32 767	32 334	31 331	296	3416
RSM3	31 389	30 999	30 041	297	4455
GC1	35 338	34 698	33 806	295	2784
GC2	30 281	29 702	28 941	297	4276
GC3	30 685	30 172	29 043	296	3023



A. 基于未加权 UniFrac 的 NMDS; B. 基于未加权 UniFrac 距离的多样性聚类树。

A. NMDS based on the unweighted UniFrac; B. Based on unweighted unifrac distance diversity clustering tree.

图4 RSM 和 GC 处理土壤微生物的聚类信息

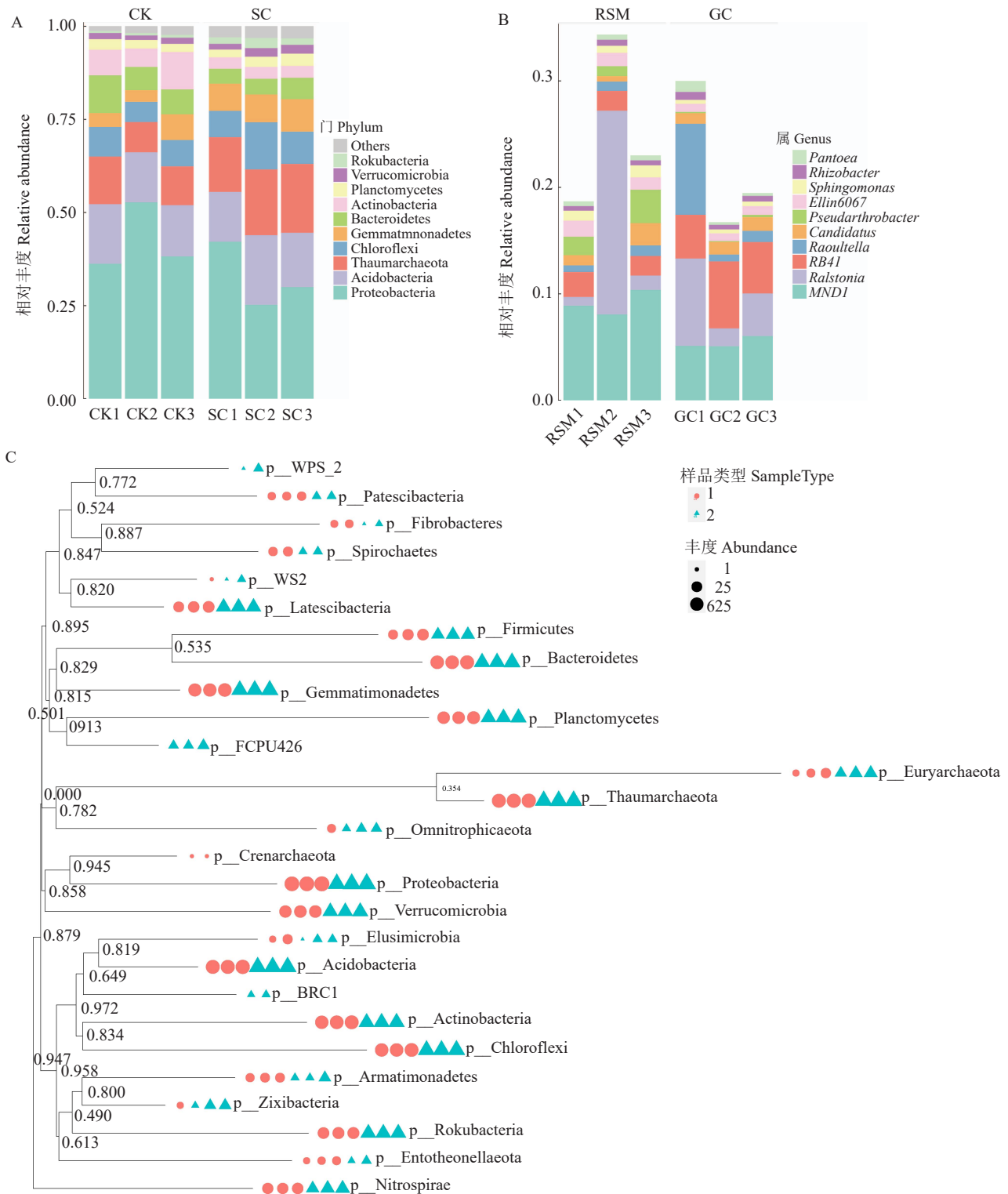
Fig. 4 Clustering information of soil microorganisms of RSM and GC

成。在 GC 处理的土壤样本中, 占优势地位的菌门包括变形菌门 Proteobacteria (32.41%)、奇古菌门 Thaumarchaeota (16.95%)、酸杆菌门 Acidobacteria (15.55%)、绿弯菌门 Chloroflexi (9.46%)、芽单胞菌门 Gemmatimonadetes (7.81%)、拟杆菌门 Bacteroidetes (4.68%)、放线菌门 Actinobacteria (3.11%) 及浮霉菌门 Planctomycetes (2.70%) (图 5-A)。在属级别上, GC 土壤中的优势菌属包括 *MNDI* (5.41%)、*RB41* (5.07%)、*Ralstonia* (4.62%) 和 *Raoultella* (3.42%) (图 5-B 和 5-E)。在 RSM 处理的土壤样本中, 优势菌门同样以变形菌门 Proteobacteria 为主 (42.37%), 其次是酸杆菌门 Acidobacteria (14.38%)、奇古菌门 Thaumarchaeota (10.44%)、拟杆菌门 Bacteroidetes (7.69%)、放线菌门 Actinobacteria

(7.26%)、绿弯菌门 Chloroflexi (6.80%)、芽单胞菌门 Gemmatimonadetes (4.61%) 和浮霉菌门 Planctomycetes (2.47%) (图 5-A); 在属级别上, RSM 土壤的优势菌属是 *MNDI* (9.10%)、*Ralstonia* (7.11%)、*RB41* (2.00%) 和 *Pseudarthrobacter* (1.94%) (图 5-B、D)。

图 5-C 展示了在门水平上细菌群落的系统发育关系, 其中圆形和三角形的大小反映了不同菌群的丰度。图 5-D~E 分别展示了 RSM 和 GC 处理的土壤中丰度前十的菌属及其所属的菌门, 结果表明, 两种不同覆盖模式处理后, 苹果园土壤中细菌的优势菌群类别在门和属水平上总体相似, 但其丰度存在差异。

Alpha 多样性作为评估特定区域或生态系统内物种丰富度的指标, 通过 Chao1 丰富度估计量、香农-威纳多样性指数、辛普森多样性指数和 Faith's



A. 门水平的丰度直方图; B. 属水平的丰度直方图; C. 门水平的系统发育树; D. RSM 处理前 10 个细菌属及其对应门水平物种的饼图; E. GC 处理前 10 个细菌属及其对应门水平物种的饼图; F. 样本内多样性 (α -多样性); G. RSM 和 GC 处理后土壤核心微生物丰度的热图。

A. Histograms of phyla abundances; B. Histograms of genus abundances; C. Phylogenetic tree at phylum level; D. Pie chart of the top 10 bacterial genera and their corresponding phylum-level species under RSM treatment; E. Pie chart of the top 10 microbial genera and their corresponding phylum-level species under GC treatment; F. Within-sample diversity (α -diversity); G. Heatmap of core microbial abundance in soil after RSM and GC treatments.

图 5 不同覆盖处理土壤细菌群落组成的分类

Fig. 5 Classification of the soil bacterial community composition under two different mulching modes

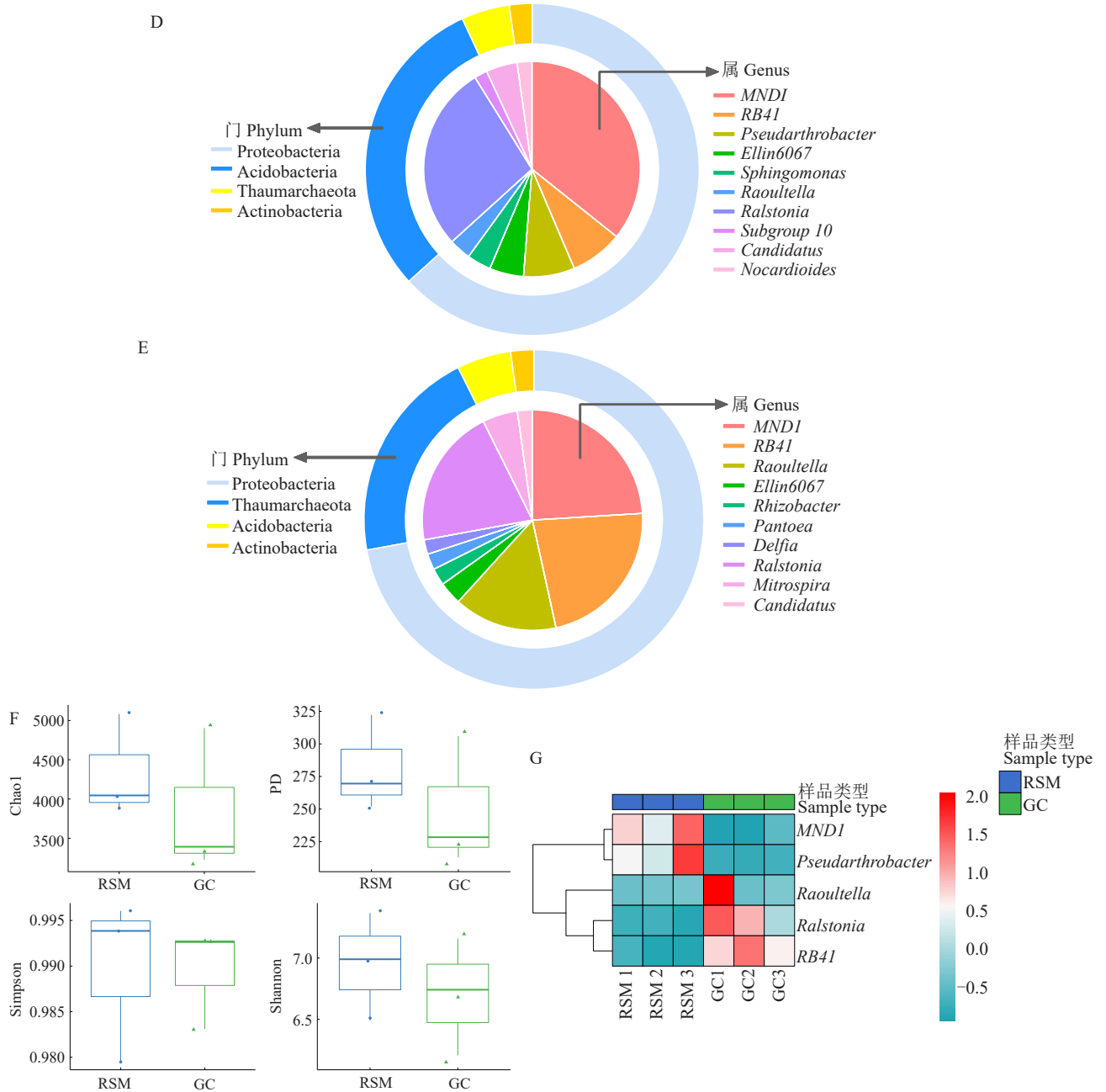


图5 (续) Fig.5 (Continued)

Phylogenetic Diversity 等指标来度量^[17]。图 5-F 显示,尽管 RSM 处理的土壤表现出较高的细菌多样性,但与 GC 处理相比,差异并不显著。

通过 OTU 分析确定 RSM 和 GC 处理土壤的核心微生物组(属水平)主要包括 *RB41*、*MNDI*、*Ralstonia*、*Raoultella* 和 *Pseudarthrobacter*。核心微生物组的丰度热图(图 5-G)显示,RSM 和 GC 处理土壤中 *MNDI* 和 *RB41* 的丰度较高。

2.4 土壤细菌群落差异分析

LEfSe (linear discriminant analysis effect size)

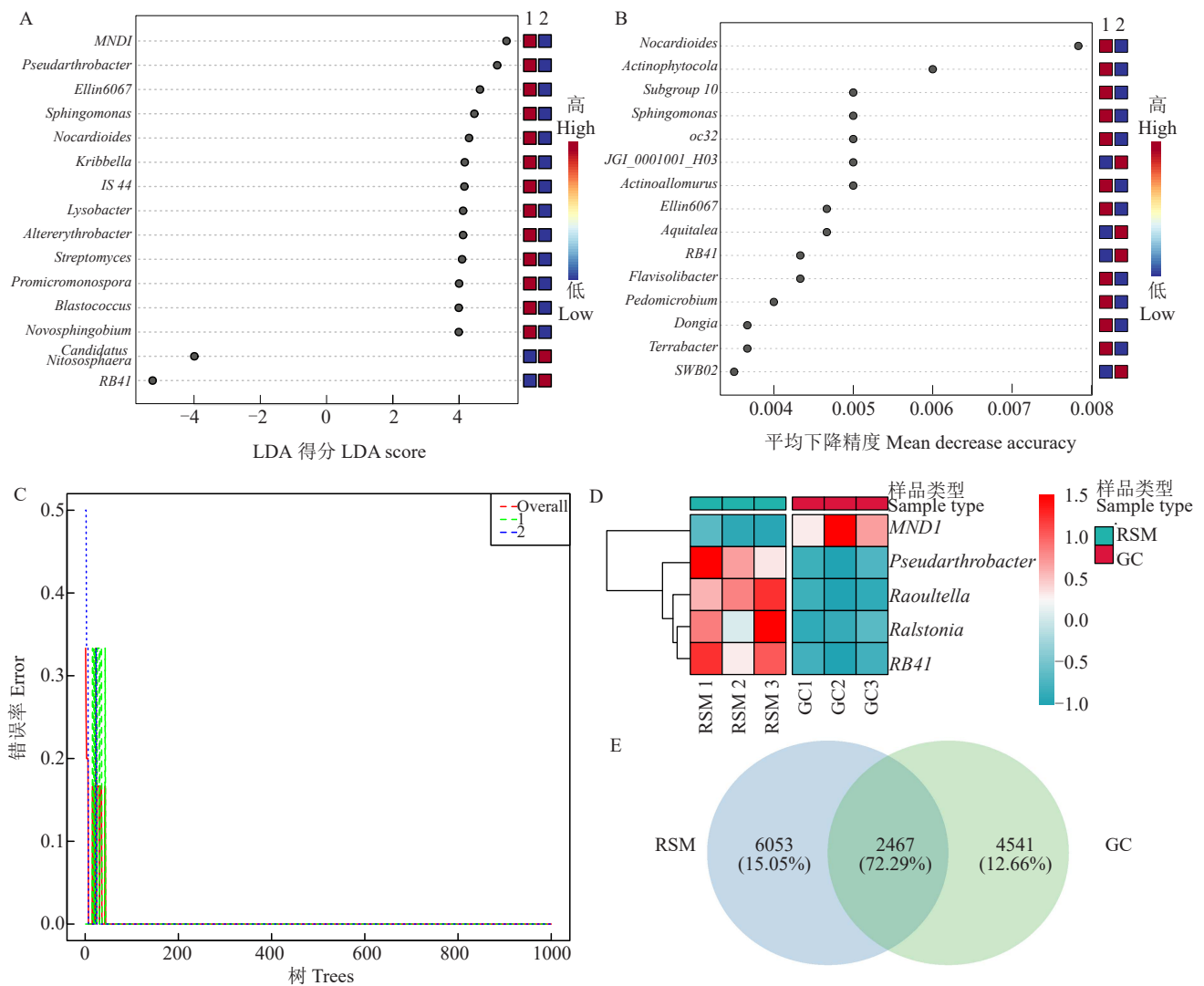
分析能够在分组内进行亚组间的比较,以识别在丰度上组间存在显著差异的生物标志物^[18]。图 6-A 显示了线性判别分析(LDA)得分超过 2 的前 15 个生物标志物。图 6-B 描述了这些生物标志物按贡献大小降序排列的情况。图 6-C 显示,当随机森林分析使用 1000 棵树作为参数设置时,可以达到最低的误差率(误差率=0)。通过结合这两种分析方法,识别出 5 个能区别不同覆盖处理的苹果园土壤样本的生物标志物。5 个生物标志物(biomarkers)的丰度热图显示(图 6-D),*Ellin6067* (Nitrosomonadaceae)、

Sphingomonas、*Nocardioides*、*Subgroup 10* (Thermoanaerobaculaceae) 在 RSM 样本中丰度较高, *RB41* (Pyrinomonadaceae) 在 GC 样本中丰度较高, 这一结果与 LDA 得分值相符。在两种不同覆盖模式的土壤样品中, 共有的操作分类单元(OTUs)为 2467 个, RSM 土壤样品特有 OTUs 数量为 6053 个, GC 土壤样品特有的 OTUs 为 4541 个, 表明 RSM 土壤的微生物丰度高于 GC(图 6-E), 与图 5-F 的结果相符。

2.5 土壤微生物群落代谢特征分析

Tax4Fun 工具被用于预测两种覆盖模式下苹果

园土壤细菌群落的代谢功能。该工具首先利用 SILVA 数据库对 *16S rRNA* 基因测序数据执行聚类及注释, 随后应用 BLASTN 技术构建与 KEGG 数据库中的原核生物分类的关联矩阵; 最终通过这些关联分析预测微生物群落的功能。分析结果归类于六大 KEGG 一级代谢通路, 涵盖环境信息处理、新陈代谢、细胞过程、遗传信息处理、人类疾病以及生物体系统等领域(图 7-A), 其中新陈代谢通路在所有通路中丰度最高, 超过 60%。共有 37 种 KEGG 二级代谢通路被注释, 对这些通路进行 Wilcoxon 秩和检验



A. LEfSe 分析得到属水平的前 15 个生物标志物分组样本类型图形; B. 随机森林方法识别的显著特征; C. 通过 RF 分类得到的累积误差率; D. 两种不同覆盖模式下土壤的生物标志物细菌丰度的热图; E. 两种不同覆盖模式下的土壤中共有/独有 OTUs。

A. Graphical summary at genus level in group sample type of the top 15 biomarkers by LEfSe; B. Significant features identified by Random Forest; C. Cumulative error rates obtained through RF classification; D. Heatmap of biomarker bacterial abundance in soil under two different mulching modes; E. Common/unique OTU in soil under two different mulching modes.

图 6 两种不同覆盖处理土壤细菌群落差异特征

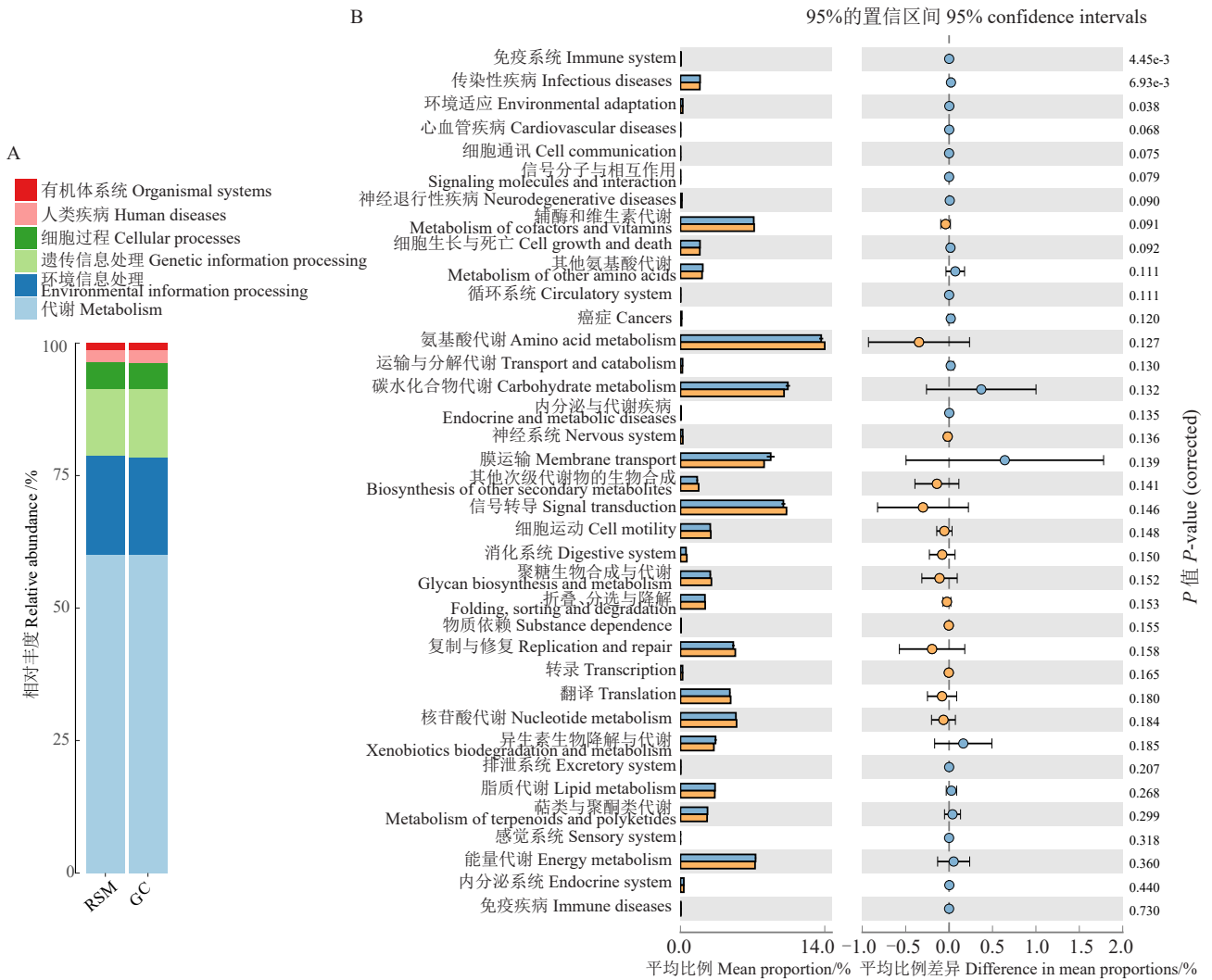
Fig. 6 Differential features of soil bacterial community under two different mulching modes

以评估不同覆盖处理下土壤样本间的丰度差异。图7-B展示了基于组间差异的分析结果,特别是在新陈代谢通路中,氨基酸代谢(amino acid metabolism, ko00270)和碳水化合物代谢(carbohydrate metabolism, ko00051)在RSM和GC处理的土壤微生物样本中相对丰度较高,均超过10%。此外,这些样本在环境信息处理的信号转导(signal transduction, ko02020)和膜运输(membrane transport, ko02010)路径中的相对丰度也较高,均超过8%。但两种不同覆盖处理的苹果园土壤微生物样本在注释的免疫系统(immune system)、传染病(infectious diseases)、环境适应(environmental adaptation)的相对丰度呈显

著差异($p < 0.05$),且RSM处理的样本中3条代谢通路相关基因的丰度均高于GC处理。

2.6 相关性分析

在两种不同覆盖模式的苹果园土壤中,共发现3个差异显著的理化指标和2个差异显著的酶活性,基于土壤性质与细菌群落的相关性分析,将这些数据与9个土壤关键细菌群落的相对丰度进行相关性分析,以分析不同覆盖模式对苹果园土壤细菌群落组成特征差异的生态机制。初步通过CANOCO 5软件进行了DCA(物种-样本)分析,结果发现第一轴的梯度长度为1.42(小于4),因此选用RDA模型来筛选和分析数据。结果如图8所示,RDA的前两

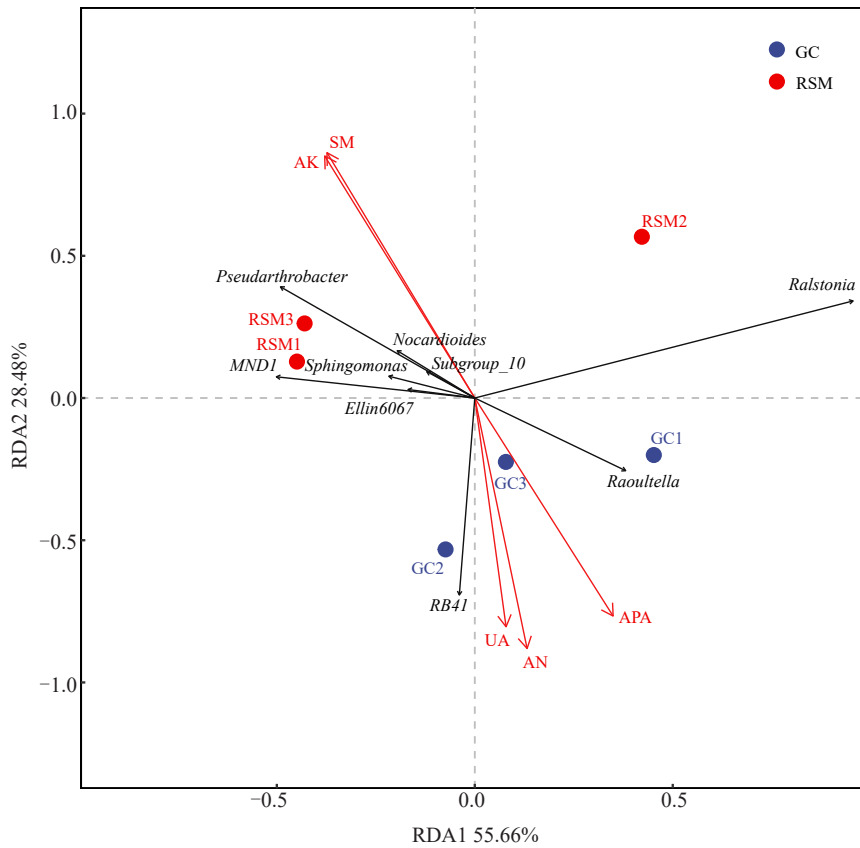


A. KEGG 一级代谢通路富集柱状图; B. KEGG 二级代谢通路相对丰度及方差(差异显著性)分析。

A. KEGG primary metabolic pathway enrichment histogram; B. Analysis of the relative abundance and variance (difference significance) of KEGG secondary metabolic pathways.

图7 不同覆盖处理土壤中细菌群落的功能分析

Fig. 7 Functional analysis of bacterial communities in soil under different mulching treatments



黑线表示土壤主要细菌属,红线表示土壤主要环境因子。AN, 碱解氮; AK, 速效钾; SM, 土壤含水量; APA, 碱性磷酸酶; UA, 脲酶。下同。

The black line indicates the main bacterial genera in the soil, and the red line indicates the main environmental factors in the soil. AN, alkali hydrolyzed nitrogen; AK, quick available potassium; SM, soil water content; APA, alkaline phosphatase; UA, urease. The same below.

图 8 土壤主要细菌(属水平)与环境因子 RDA 分析

Fig. 8 RDA analysis of soil main bacteria (genus level) and environmental factors

个轴解释了细菌群落 84.14%的变异,其中,第一排序轴的解释变量为 55.66%,第二排序轴的解释变量为 28.48%,表明环境因子在较大程度上可以解释土壤细菌群落的差异,其中 AN($r^2=0.8930, p=0.047$)和 AK($r^2=0.8580, p=0.049$)对土壤细菌群落组成具有显著影响。RSM 组的土壤样品性质与 SM 和 AK 呈正相关,与 AN、APA 和 UA 呈负相关,而 GC 组的土壤样品特性与 AN、APA 和 UA 呈正相关,与 SM 和 AK 呈负相关。

Pearson 相关性分析结果(图 9)与 RDA 结果相呼应, *Nocardioiodes*、*MND1*、*Ellin6067* 和 *Subgroup 10* 丰度与 AK 和 SM 含量呈显著正相关,其中 *Ellin6067* 和 *Subgroup 10* 丰度还与 AN 和 APA 含量呈显著负相关; *Sphingomonas* 丰度与 AK 含量呈显著正相关; *RB41* 丰度与 AK 和 SM 含量呈显著负相关,与 AN 和 APA 含量呈显著正相关。

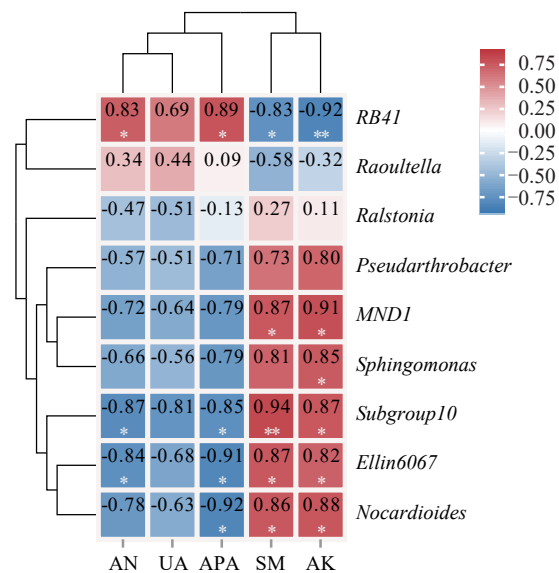


图 9 关键属与环境因子间的 Pearson 相关性分析热图

Fig. 9 Heatmap of Pearson correlation analysis between key genera and environmental factors

3 讨 论

笔者探讨了GC和RSM处理对苹果园根际土壤理化性质和细菌群落结构的影响。GC处理显著提高了土壤中的AN含量和UA、APA活性,而RSM处理则显著提高了SM和AK含量。两种覆盖方式下土壤细菌的优势菌群在分类学上相似,RSM处理下的微生物相对丰度高于GC处理。这一结论再次证实不同覆盖方式会影响土壤理化性质,从而间接调控根际细菌群落的结构和功能。

3.1 不同覆盖处理影响苹果园根际土壤的理化性质

RSM处理显著提高了土壤中SM和AK含量。由于稻草覆盖后隔断了土壤表面与大气间直接的水分联系,抑制了土壤中水分的蒸发,因此提高了土壤含水量^[19]。同时水稻秸秆中K含量较高,一般在1.5%~2.5%之间,而且其钾素主要以交换态和水溶态存在,通过秸秆还田可以增加根际土壤的钾素含量和供应量,从而提高植物的钾素含量和钾素利用效率^[20]。GC处理的土壤AN含量及UA、APA酶活性较高,该研究结果与Li等^[21]的报道一致。果园生草后,一方面草本植物的根系可以增加土壤有机质的输入,优化土壤的团粒结构和增强其通透性,提高土壤微生物的活性和土壤中酶活性(如脲酶可以催化脲分解为氨和二氧化碳),从而提高土壤碱解氮的含量^[22];另一方面草本植物根际固氮菌还可以与苹果树形成共生固氮的关系,利用空气中的氮气,为土壤提供氮源^[23]。

3.2 不同覆盖处理影响土壤细菌群落的组成和丰度

由于土壤细菌群落对土壤环境因素敏感,覆盖作物可能通过改变土壤的理化性质来影响土壤细菌群落^[24]。研究发现两种覆盖处理后,苹果园根际土壤中细菌的优势菌群类别在门和属水平上总体相似,细菌群落多样性差异不显著,但相对丰度存在显著差异,该结果与Xie等^[25]的研究一致。两种不同覆盖处理的土壤核心微生物组(属水平)为*RB41*、*MND1*、*Ralstonia*、*Raoultella*、*Pseudarthrobacter*。LEfSe分析RSM和GC处理土壤细菌在丰度上有显著差异的生物标志物有*RB41*、*Ellin6067*、*Sphingomonas*、*Nocardioideis*、*Subgroup 10*。不同覆盖物对土壤细菌的筛选作用也可能与土壤细菌的适应性、竞争力、协同性等因素有关^[26]。RSM覆盖土壤中*Sphingomonas*相对丰度较高可能是稻草携带所

致,*Sphingomonas*可作为水稻的内生菌,提高水稻抗病性,通过分泌胞外信号小分子干扰病原菌的毒力因子生物合成通路^[27]。同时,水稻秸秆覆盖后土壤与空气之间的通透性变弱,有利于厌氧细菌的生长,可能导致*Subgroup 10*的相对丰度提高,因为*Subgroup 10*是一种利用纤维素和木质素等多糖作为碳源的厌氧细菌^[28]。

不同覆盖影响土壤根际细菌的组成和丰度^[29],导致土壤细菌的代谢功能产生差异^[30]。RSM处理的土壤细菌群落在免疫系统、传染病、环境适应等方面的功能基因丰度增加,可能是由于土壤中*MND1*和*Raoultella*的相对丰度较高。*MND1*和*Raoultella*可以分泌抗生素,抑制一些病原菌的生长,增强植物的抗病性,以及土壤细菌的抵抗力和适应性^[31]。

3.3 不同覆盖处理土壤细菌群落与土壤理化性质的相关性

土壤理化性质与土壤微生物群落结构和植物生长密切相关^[24]。在本研究中,AN和AK含量是影响不同覆盖处理土壤细菌群落结构的主要环境因子,RSM处理的土壤细菌群落与土壤AK含量呈显著正相关,GC处理的土壤细菌群落与AN含量呈显著正相关,这可能与不同处理土壤细菌群落中优势菌属的特性有关。GC处理的土壤样品中*RB41*相对丰度较高,*RB41*是一种常见的、丰富的土壤细菌,能够利用土壤中的碳源和营养物质,参与土壤碳循环,与布拉氏菌和链霉菌一起占了土壤中一半以上的碳利用量^[32],*RB41*丰度与脲酶活性呈正相关,可能通过影响土壤碳循环,从而间接提高土壤碱解氮的含量^[33]。RSM处理的土壤样品中*MND1*和*Ellin6067*的相对丰度较高,*MND1*可以利用水稻秸秆中的纤维素作为碳源进行发酵^[34],而*Ellin6067*作为氨氧化细菌可以将氨氮转化为亚硝酸盐,参与土壤氮循环,从而降低土壤碱解氮的含量^[35]。因此,推测不同覆盖处理后细菌群落的组成影响了土壤中AN的含量。

4 结 论

不同覆盖处理对苹果园根际土壤的理化性质和酶活性具有显著影响,从而改变了土壤细菌群落的组成和功能。本研究结果表明,RSM处理土壤的SM和AK含量较高,而GC处理的土壤AN含量和UA、APA活性较高。RSM与GC处理的苹果园土壤中根际细菌的优势菌群类别在门和属水平上总体相

似,但RSM处理的细菌丰度高于GC处理。RSM处理的土壤细菌群落与AK含量呈显著正相关,GC处理的土壤细菌群落与AN含量呈显著正相关。因此,在西南冷凉高地苹果栽培过程中,可以根据土壤基础肥力状况合理选择覆盖方式,以调节根际土壤的理化性质和细菌群落结构,促进土壤健康和果树生长。

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