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基于高通量测序技术的阳光玫瑰不同砧木根际微生物多样性研究

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摘 要:【目的】探究阳光玫瑰不同砧木和自根苗根际土壤微生物的群落多样性,以期为筛选适宜砧木提供参考。**【方法】**提取3年生阳光玫瑰嫁接和自根苗木的根际土壤微生物总DNA,用IlluminaMiseq高通量测序技术分析了1103P、5BB、Beta和自根苗土壤根际微生物群落多样性。**【结果】**1103P显著提高了根际土壤细菌群落的丰度和多样性,Beta显著提高了真菌群落的丰度和多样性,降低了细菌群落的多样性。4个样品细菌OTU归类到44门133纲345目536科1056属,优势细菌门均为变形菌门(Proteobacteria,24.15%~33.57%)、酸杆菌门(Acidobacteriota,14.83%~~22.82%)、放线菌门(Actinobacteriota,7.24%~10.99%),但优势细菌属组成有较大差异,细菌属水平主坐标分析表明,1103P和5BB明显改变了阳光玫瑰根际土壤细菌群落组成,Beta和自根苗细菌群落组成相似度高。4个样品真菌OTU归类到8门33纲82目188科412属,优势真菌门均为子囊菌门(Ascomycota,55.57%~64.86%)、毛霉菌门(Mucoromycota,5.84%~22.24%)和担子菌门(Basidiomycota,12.72%~19.22%),但优势真菌属组成有较大差异,真菌属水平主坐标分析表明,4个样品真菌群落组成差异较大。对细菌群落(门水平)和土壤理化性质的冗余分析结果表明,有机质含量(OM)、交换性镁(Mg^{2+})含量和pH值对根际土壤优势细菌门组成影响较大。**【结论】**砧木能够改变阳光玫瑰根际土壤微生物群落组成和数量,为筛选适宜砧木和改善根际微生物群落组成提供理论依据。

关键词:葡萄砧木;根际微生物;高通量测序

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Analysis of microbial diversity in rhizosphere soil of Shine Muscat grape on different rootstocks using high-throughput sequencing

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Abstract:【Objective**】** In order to provide reference for rootstock screening and cultivating, the community diversity of rhizosphere soil microorganisms of different rootstocks and self-rooted seedlings of Shine Muscat grape was explored. Rhizospheric microorganisms live at the interface between plant roots and soil and interact with plants. Beneficial rhizosphere microorganisms help plants to obtain nutrients and improve plant resistance to abiotic stresses. Harmful rhizosphere microorganisms compete with plants for nutrients in the soil or infect plants through roots, which further inhibits the healthy growth of plants. Plant roots and soil are two main factors affecting microbial community. **【**Methods**】** Three-year-old Shine Muscat grape was used as the test material. There were three kinds of rootstock,

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including 1103P, 5BB and Beta, and self-rooted seedlings served as the control. Soil organic matter was determined by the potassium dichromate volumetric-dilution heat method. pH value was measured by 1∶1 water soil ratio and a pH meter. Alkaline hydrolysis of nitrogen was analyzed using the alkaline hydrolysis diffusion method. The available phosphorus was extracted by $NaHCO_3$ and further determined by the molybdenum antimony sulfate anti colorimetry. Soil available potassium was extracted by ammonium acetate and then determined by the flame spectrophotometry. Exchangeable calcium and magnesium were extracted with 1 mol·L^{-1} NH_4OAc solution, and the content of soluble calcium and magnesium was determined by the atomic absorption spectrophotometer. The total DNA of rhizosphere soil microorganisms was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.A.). The amplification primers of soil bacteria were 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3'), and the amplification primers of soil fungi were ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'). PCR reaction conditions were followed below: pre-denaturation at 95 ℃ for 5 min; denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, and extension at 72 ℃ for 30 s, 25 cycles; and extension at 72 ℃ for 5 min. The amplified products were recovered by AxyPrepDNA gel Recovery Kit (AXYGEN, U.S.A.). The purified PCR products were completely sequenced with high-throughput on Illumina PE250 platform (Shanghai Lingen Biotechnology Co., Ltd.). The Pair-end (PE) reads were spliced according to overlap relationship. Usearch software and gold database were used to remove the chimera by denovo and reference. After distinguishing samples, OTU clustering analysis and species taxonomy analysis were carried out. The RDP classifier Bayesian algorithm was used to classify 97% OTU representative sequences at similar levels (the confidence threshold was set at 0.7). The databases of Silva (for bacteria) and Unite (for fungi) were further employed, and the community composition of each sample was counted. 【Results】There was significant difference among the four samples ($p<0.05$), in which the sample of 1103P had the lowest pH, while 5BB was the highest. The soil alkali-hydrolyzable nitrogen of 5BB and self-rooted seedlings was significantly higher than that of 1103P and Beta. The organic matter content of 1103P was lower than that of 5BB and self-rooted seedlings. High-throughput sequencing analysis showed that 1103P had the most abundant and diversified bacterial community in rhizosphere soil. Beta significantly increased the abundance and diversity of fungal community but reduced the diversity of bacterial community. The bacterial OTUs of the four samples were classified into 44 phyla, 133 classes, 345 orders, 536 families and 1056 genera. The dominant bacteria were Proteobacteria (24.15%–33.57%), Acidobacteria (14.83%–22.82%) and Actinobacteria (7.24%–10.99%). Compared with self-rooted seedlings, rootstocks enhanced the relative abundance ratios of Acidobacteriota, Patescibacteria, and Verrucomicrobiota, but reduced the abundance ratios of Actinobacteriota and Gemmatimonadota, in which Acidobacteriota of 1103P and Beta increased by 53.87% and 40.54%, respectively. The dominant bacteria of 1103P were *Candidatus Udaeobacter*, RB41, and *Nitrospira*. The dominant bacteria of 5BB were *Bryobacter*, *Chujaibacter* and *Sphingomonas*. The dominant bacteria of Beta were *Steroidobacter*, *Candidatus Udaeobacter* and RB41. The dominant bacteria of self-rooted seedlings were *Nitrospira*, *Bryobacter* and *Candidatus Udaeobacter*. Compared with self-rooted seedlings, 1103P and 5BB had a totally different composition of bacterial community, while the composition of Beta and self-rooted bacterial community was similar. The fungal OTUs of four samples were classified into 8 phyla, 33 classes, 82 orders, 188 families and 412 genera. The dominant fungi were Ascomycota (55.57%–64.86%), Mucoromycota (5.84%–22.24%) and Basidiomycota (12.72%–19.22%), which were quite different among these four samples. The dominant fungi of 1103P were *Mortierella*, *Fusarium*, and *Solicoccozyma*. The

dominant fungi of 5BB were *Botryotrichum*, *Fusarium*, and *Tausonia*. The dominant fungi of Beta were *Fusarium*, *Mortierella*, and *Tausonia*. The dominant fungi of self-rooted seedlings were *Doratomyces*, *Mortierella*, and *Tausonia*. The fungal OTU principal coordinates analysis indicated that there were significant differences in the composition of fungal communities among these four samples. The redundant analysis between bacterial community (phylum) and soil physical and chemical properties implied that the organic matter (OM) content, exchangeable magnesium (Mg^{2+}) content, and pH value had a great impact on the dominant bacteria in rhizosphere soil.【Conclusion】Rootstocks changed the composition and quantity of rhizosphere soil microbial community of Shine Muscat, which favorably provides a theoretical basis for screening suitable rootstocks and improving the composition of rhizosphere microbial community.

Key words: Grape rootstock; Rhizosphere microorganism; High-throughput sequencing

根际微生物是指生活在植物根系和土壤的交界面并与植物相互作用的微生物，其组成由微生物、植物根系和环境的相互作用决定[1-2]，不同植物、土壤类型可形成特定的微生物群落，通过分泌植物生长激素促进根系生长并提高根系对营养物质和水分的吸收能力[3-5]，富集的有益微生物可产生抑菌的抗菌化合物或激活植物免疫系统达到抑菌效果[6-8]，富集的病原微生物将引起连作障碍并与植物竞争养分等[9-11]，进而影响植物的生长发育[12-13]。因此，研究根际微生物多样性和组成对植物的生长发育、抗逆性和病害生物防治有重要意义[14]。

19世纪中叶，科技人员将欧洲种嫁接到美洲种上以防治葡萄根瘤蚜，筛选新的砧木基因型及研究砧穗互作机制成为现代种植的研究方向[15]。关于砧木研究多集中于根系吸收水分和矿质元素能力、对土壤病害和逆境胁迫等的抗性不同，进而影响植株光合能力、树势、抗逆性和果实品质[16-19]等方面，而对砧木根际微生物研究较少。Marasco等[15]利用不同砧木161.49、420A等嫁接葡萄品种巴贝拉，发现砧木显著影响根际微生物群落组成和多样性；王静等[20]研究樱桃砧木根际微生物时发现，适宜的樱桃砧木有利于樱桃树土壤生态环境；而徐龙晓等[21]研究认为，相比砧木差异，土壤质地对苹果根际微生物碳源利用类型影响更大。由于大部分土壤微生物不能培养，传统的微生物分析方法无法准确揭示微生物多样性，随着高通量测序技术的发展，利用基因测序技术极大地方便了根际微生物群落结构的检测。笔者在本研究中利用16S rRNA高通量测序技术分析常用葡萄砧木1103P、5BB和Beta嫁接和自根苗阳光玫瑰根际微生物，揭示微生物群落结构和多样性，以期为阳光玫瑰筛选适宜砧木、改善土壤管理提供参考。

1　材料和方法

1.1　供试材料

土壤样品采自山东省招远市大户庄园葡萄园，暖温带大陆性季风气候，年平均气温11.5 ℃，年降水量607 mm，年日照数2503 h。砧木品种有1103P、5BB和Beta，自根苗为对照。以4个葡萄园地块3年生阳光玫瑰为试材，每个地块666.7 m^2，南北行向，篱架栽培，单干单臂树形+V形叶幕，所有葡萄地块统一常规管理。葡萄转色期测定4个地块土壤理化性质和微生物，无显著差异，葡萄采收期将取样器消毒灭菌，每个葡萄地块选取生长良好的阳光玫瑰植株5株，按照五点取样法采集根际土壤，混合均匀，设置3次生物学重复。采样时去除地表杂质，以树干为中心，挖取离地表约30 cm行内土壤，抖落挖出的土壤根系上附着的大块土壤，收集根系上附着的粉状或粒状土壤，经低温保存送回实验室，过2 mm无菌筛网去除杂质，分为两组，一组保存于无菌管中放置在−80 ℃冰箱用于微生物检测，另一组自然风干用于土壤理化性质测定。

1.2　土壤理化性质测定

土壤有机质采用重铬酸钾容量法−稀释热法测定；土壤pH值用水土质量比1∶1，pH计测定；碱解氮含量采用碱解扩散法进行分析；有效磷含量采用$NaHCO_3$浸提并利用硫酸钼锑抗比色法进行测定；通过醋酸铵浸提土壤速效钾，并利用火焰光度法测定其含量；采用1 mol·L^{-1} NH_4OAc溶液浸提交换性钙镁，并运用原子吸收分光光度计测定浸提液中的钙镁含量。

1.3　土壤DNA提取与PCR扩增测序

用E.Z.N.A.® Soil DNA Kit（Omega Bio- tek,

Norcross, GA, U.S.A.)试剂盒提取土壤总DNA，利用1%的琼脂糖凝胶进行电泳检测。土壤细菌扩增引物为341F(5'-CCTAYGGGRBGCASCAG-3')和806R(5'-GGACTACNNGGGTATCTAAT-3')，土壤真菌扩增引物为ITS1F(5'-CTTGGTCATTTAGAGGAAGTAA-3')和ITS2R(5'-GCTGCGTTCTTCATCGATGC-3')，PCR反应程序：95 ℃预变性5 min；95 ℃变性30 s，55 ℃退火30 s，72 ℃延伸30 s，共25个循环；最后72 ℃延伸5 min。PCR扩增产物经AxyPrepDNA凝胶回收试剂盒(AXYGEN，美国)纯化回收后用于高通量测序。

1.4 序列处理分析

Illumina PE250测序技术委托上海凌恩生物科技有限公司，按照公司质控规定进行：根据barcode得到样品的有效序列；过滤read尾部20以下质量值的碱基，设置10 bp窗口，若窗口内平均质量值低于20，自窗口截去后端碱基，过滤质控后50 bp以下read；根据PE reads的overlap关系，拼接成对reads成一条序列，最小overlap长度10 bp；拼接序列的overlap区允许最大错配比率0.2，筛除不符合的序列；区分样品时根据序列首尾两端barcode和引物，调整序列方向，barcode允许错配数为0，最大引物错配数为2；用Usearch软件和gold数据库，采用denovo和reference结合的方式去除嵌合体，区分样本后进行OTU聚类分析和物种分类学分析，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)，比对数据库为Silva(细菌)和Unite(真菌)，统计各样本的群落组成。

1.5 数据分析

应用SPSS 19.0对土壤样品理化性质、微生物多样性指数统计和微生物门属丰度进行显著性分析($p<0.05$)；应用上海凌恩生物科技有限公司云平台R语言包进行主坐标分析作图(Principal Coordinates Analysis，PCoA)和组间差异检验(Adonis，ANOSIM)。由于样品数少(12个)而物种数目(细菌1056属，真菌412属)很大，比较各样品微生物组成差异时，在细菌和真菌属水平上做PCoA分析，并进行组间差异检验，以检验组间的差异是否显著大于组内差异。

2 结果与分析

2.1 土壤理化性质

不同砧木和自根苗阳光玫瑰根际土壤理化性质检测结果如表1所示，4个样品pH值存在显著差异($p<0.05$)，其中1103P最低，5BB最高；5BB和自根苗土壤碱解氮(N)含量显著高于1103P和Beta；1103P有机质含量显著低于5BB和自根苗的根际土壤样品；Beta有效磷(P)含量显著低于其他3个样品；自根苗根际土壤速效钾(K)含量显著高于砧木；自根苗交换性钙(Ca)含量和砧木无显著差异，5BB显著低于Beta；交换性镁(Mg)含量4个样品无显著差异。

表1 土壤样品理化性质

Table 1 Physical and chemical properties of soil samples

样品 Sample	pH	w(碱解氮) Alkali-hydrolyzale nitrogen content/(mg·kg^{-1})	w(有效磷) Available phosphorous content/(mg·kg^{-1})	w(速效钾) Available potassium content/(g·kg^{-1})	w(有机质) Organic matter content/(g·kg^{-1})	w(交换性钙) Exchangeable Ca content/(g·kg^{-1})	w(交换性镁) Exchangeable Mg^{2+} content/(g·kg^{-1})
1103P	5.83±0.02 a	42.24±2.42 b	54.23±4.74 b	0.20±0.02 a	18.61±4.06 a	0.88±0.10 ab	0.56±0.41 a
5BB	6.55±0.00 d	89.84±4.53 c	54.03±0.70 b	0.34±0.03 b	36.36±0.43 d	0.61±0.03 a	0.11±0.01 a
Beta	6.07±0.01 b	23.35±0.06 a	47.24±2.55 a	0.18±0.01 a	22.44±4.94 ab	1.33±0.04 b	0.43±0.03 a
自根 Self-rooted	6.34±0.20 c	90.04±0.86 c	54.38±2.77 b	0.42±0.01 c	26.40±2.23 bc	1.06±0.48 ab	0.44±0.34 a

注：不同小写字母表示在 $p<0.05$ 差异显著。下同。

Note: Different small letters indicate significant difference at $p<0.05$. The same below.

2.2 根际土壤微生物多样性

4个根际土壤样品中细菌和真菌的α多样性指数见表2：细菌OTU显著高于真菌，随着测序深度的增加，土壤细菌测序覆盖率为96.35%~97.17%。按照97%的相似水平将序列划分成不同的OTU，用chao1算法估计样本中所含OTU数目即为Chao指数，OTU和Chao反映了土壤微生物群落丰度，细菌OTU数和群落丰度指数Chao均为1103P最大；Shannon指数和Simpson指数估算样本中微生物多

表2　阳光玫瑰根际土壤中细菌和真菌α多样性指数统计

Table 2　α diversity index statistics of fungi and bacteria in the rhizosphere soil of Shine Muscat

微生物类群 Microbial community	样品 Samples	97%相似水平 Similarity level of 97%				
		OTU	Chao	Shannon	Simpson	Coverage/%
细菌 Bacteria	1103P	5 316.00±104.65 b	6 332.26±161.74 b	7.55±0.06 b	0.001 6±0.000 4 a	96.35
	5BB	4 724.50±181.73 a	5 828.09±23.25 a	7.35±0.05 a	0.002 2±0.000 1 a	96.82
	Beta	4 748.50±61.52 ab	5 877.14±26.75 a	7.21±0.08 a	0.003 8±0.000 4 b	96.38
	自根 Self-rooted	5 112.00±350.72 ab	6 253.27±257.85 ab	7.37±0.08 ab	0.001 8±0.000 1 a	97.17
真菌 Fungi	1103P	682.00±73.54 a	734.51±51.12 a	4.71±0.22 ab	0.021 9±0.004 6 a	99.83
	5BB	761.50±12.02 a	850.77±8.84 b	4.52±0.09 ab	0.030 9±0.004 4 a	99.73
	Beta	662.00±24.04 a	704.67±22.94 a	4.74±0.04 b	0.021 9±0.003 1 a	99.87
	自根 Self-rooted	704.50±33.23 a	788.31±39.05 ab	4.48±0.26 a	0.036 6±0.021 1 a	99.76

样性，Shannon指数越大，Simpson指数越小，群落多样性指数越高，细菌Shannon指数1103P显著高于其他砧木，Simpson指数1103P略低于其他砧木，细菌群落多样性排序为1103P＞自根＞5BB＞Beta。

4个根际土壤样品真菌测序覆盖率达到99.73%~99.87%，OTU数和丰度指数Chao高低顺序均为5BB＞自根＞1103P＞Beta，不同砧木根际土壤真菌Shannon指数Beta显著高于自根，砧木间无显著差异，4个样品的Simpson指数无显著差异，真菌群落多样性为Beta＞1103P＞5BB＞自根。

2.3　微生物分类学组成分析

2.3.1　*细菌分类学组成分析*　4个根际土壤样品细菌OTU归类到44门133纲345目536科1056属，图1-A为样品细菌在门分类学水平上的主要物种分布，其中，门水平分类选取各砧木相对丰度大于1%的物种，色块长度代表物种相对丰度所占比例。由图1可知，细菌优势门类群主要为变形菌门（Proteobacteria，24.15% ~33.57% ）、酸杆菌门（Acidobacteriota，14.83% ~22.82% ）、放线菌门（Actinobacteriota，7.24%~10.99%），而厚壁菌门（Firmicutes，0.91%~2.55%）和硝化螺旋菌门（Nitrospirota，1.01%~2.85%）相对丰度比例较低。其中，变形菌门、酸杆菌门和放线菌门相对丰度比例最高的分别为5BB、1103P和自根苗。

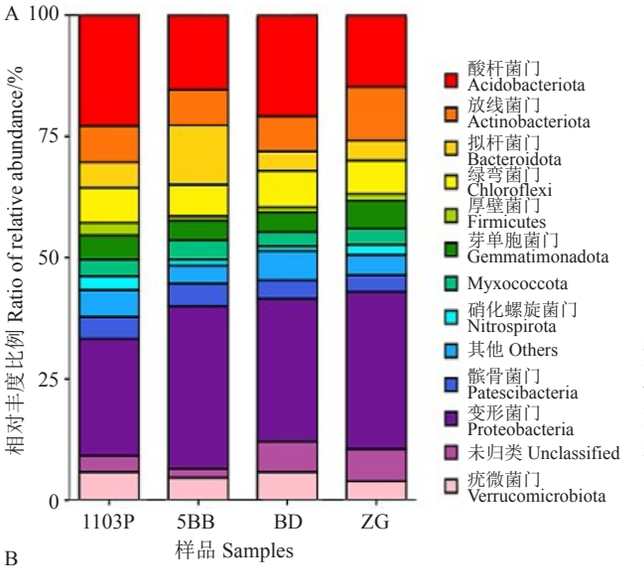
与自根苗相比，砧木增加了酸杆菌门、髌骨菌门（Patescibacteria）和疣微菌门（Verrucomicrobiota）的相对丰度比例，降低了放线菌门和芽单胞菌门（Gemmatimonadota）丰度比例，其中1103P和Beta的酸杆菌门分别比自根增加53.87%和40.54%，不同砧木之间细菌门组成和相对丰度也不一样，5BB的拟杆菌门（Bacteroidota，12.27%）相对丰度比例显著高于其他组（3.98%~5.23%）（图1-A）。

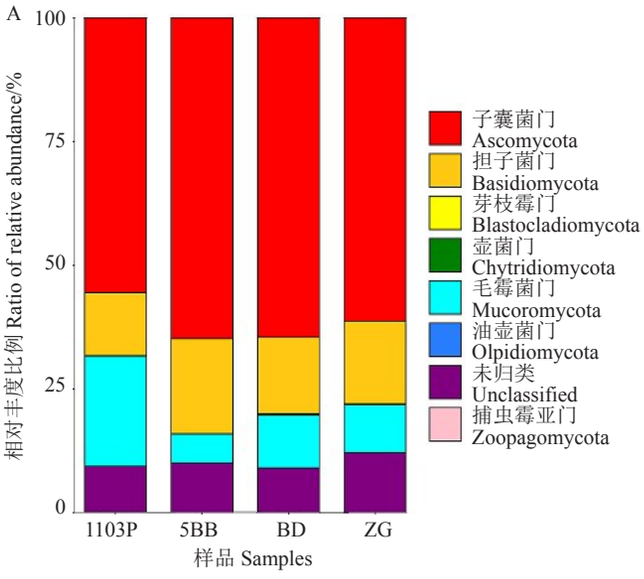
图1-B展示4个根际土壤样品中细菌在属分类学水平上相对丰度比例排名前15的物种。除暂未命名的细菌外，相对丰度较高的细菌为RB41（0.65%~5.29%）、*Candidatus Udaeobacter*（1.28%~3.12%）、苔藓杆菌属（*Bryobacter*，2.03%~2.15%）、硝化螺菌属（*Nitrospira*，1.01%~2.85%）和芽单胞菌属（*Gemmatimonas*，0.80%~1.99%）。其中，1103P的优势细菌属为*Candidatus Udaeobacter*、RB41和硝化螺菌属，5BB的优势细菌属为苔藓杆菌属、邱贾伊杆菌属（*Chujaibacter*）和鞘氨醇单胞菌属（*Sphingomonas*），Beta的优势细菌属为立体杆菌属（*Steroidobacter*）、*Candidatus Udaeobacter*和RB41，自根的优势细菌属为硝化螺菌属、苔藓杆菌属（*Bryobacter*）和假丝酵母属。

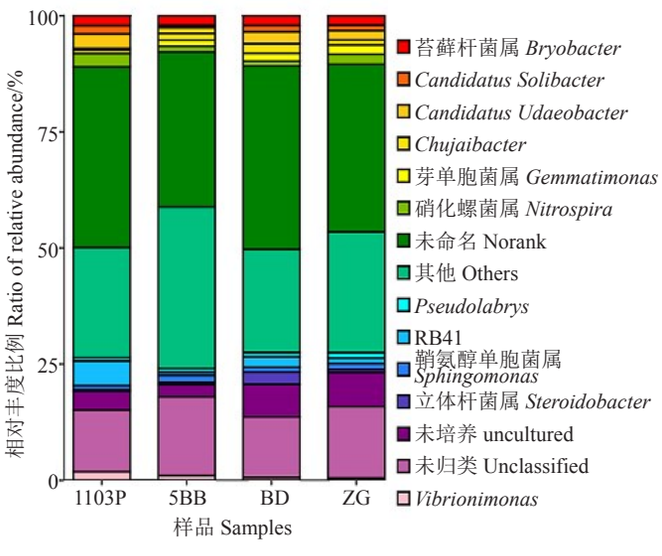
根际细菌OTU组间差异检验*R*值为0.955（$p<0.05$），表明组间差异大于组内差异。4个根际土壤样品细菌属水平PCoA分析结果表明，在两个坐标轴上Beta和自根苗根际细菌属均不能较好地区分，说明Beta和自根根际土壤细菌群落属水平组成相似性高，1103P和5BB根际细菌属能较好地区分自根苗，说明1103P和5BB改变了阳光玫瑰根际土壤细菌群落组成（图1-C）。

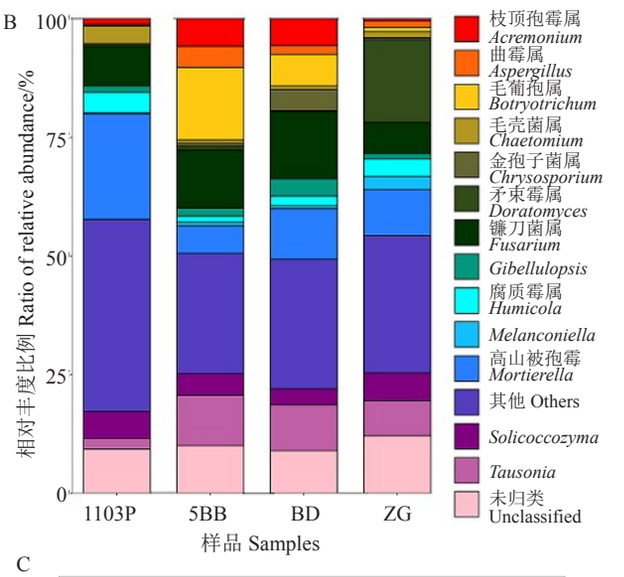
2.3.2　*真菌分类学组成分析*　4个根际土壤样品真菌OTU归类到8门33纲82目188科412属，门分类学水平上的真菌物种分布如图2-A所示，真菌优势菌群为子囊菌门（Ascomycota，55.57%~64.86%）、毛霉菌门（Mucoromycota，5.84%~22.24%）和担子菌门（Basidiomycota，12.72%~19.22%），相对丰度比例平均占89.72%。其中，1103P的子囊菌门和担子菌门（Basidiomycota）相对丰度比例均低于其他3组样品，毛霉菌门显著高于其他3组样品。

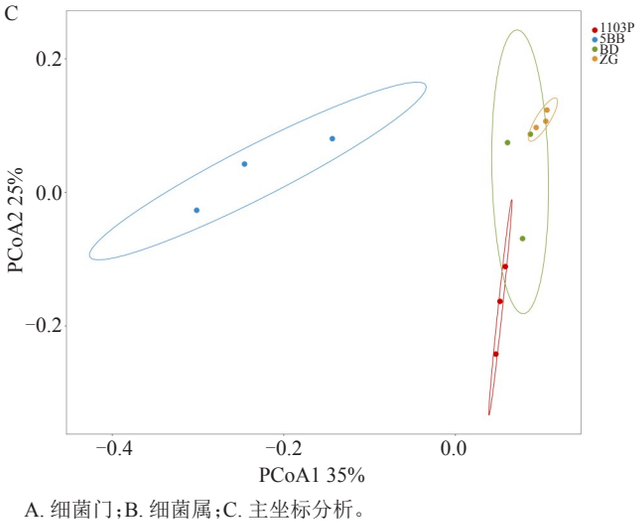
图2-B为土壤样品真菌在属分类学水平上相对

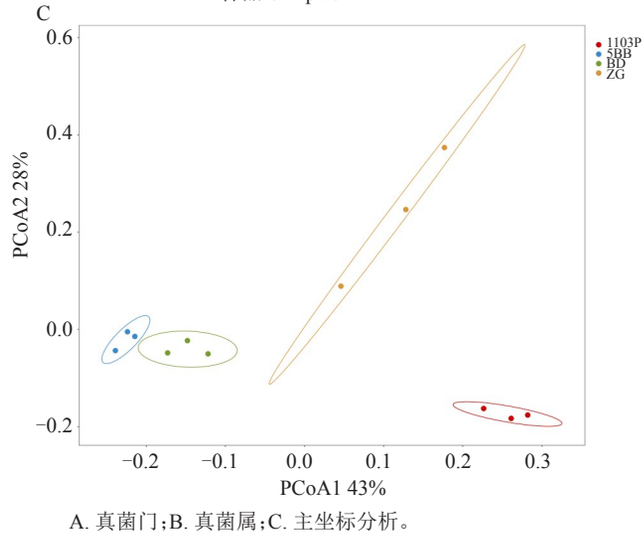












A. 细菌门；B. 细菌属；C. 主坐标分析。

A. Phylum of bacteria; B. Genus of bacteria; C. Principal coordinates analysis.

图 1 细菌的水平分布柱状图和主坐标分析

Fig. 1 Horizontal distribution histogram of bacteria and principal coordinates analysis

A. 真菌门；B. 真菌属；C. 主坐标分析。

A. Phylum of fungi; B. Genus of fungi; C. Principal coordinates analysis.

图 2 真菌的水平分布柱状图和主坐标分析

Fig. 2 Horizontal distribution histogram of fungi and principal coordinates analysis

丰度比例排名前15的物种。优势真菌为高山被孢霉（*Mortierella*，5.84%~22.19%）、镰刀菌属（*Fusarium*，6.57% ~14.22% ）和 *Tausonia* 属（2.29% ~10.58%）。不同处理之间优势真菌属组成不同，1103P的优势真菌属为高山被孢霉、镰刀菌属和*Solicoccozyma*；5BB的优势真菌属为毛葡孢属（*Botryotrichum*）、镰刀菌属和 *Tausonia* 属；Beta的优势真菌属为镰刀菌属、高山被孢霉和 *Tausonia* 属；自根的优势真菌属为矛束霉属（*Doratomyces*）、高山被孢霉和 *Tausonia* 属。

根际真菌OTU组间差异检验 R 值为0.857（$p<0.05$），表明组间差异大于组内差异。4个根际土壤样品真菌OTU主坐标分析如图2-C所示，自根苗和1103P根际真菌群落在第一主成分正半轴，5BB和Beta根际真菌群落在负半轴，4个样品根际真菌均能较好区分，即不同砧木根际土壤真菌群落分布差异较大。

2.4　土壤细菌群落与土壤理化性质的冗余分析

通过对4个根际土壤样品细菌群落结构与土壤理化性质的冗余分析，发现有机质含量、交换性镁含量和pH值对根际土壤优势细菌的影响较大。变形菌门和拟杆菌门的丰度变化与有机质含量、pH值及碱解氮、有效磷和速效钾含量呈正相关，与交换性镁、交换性钙含量呈负相关；酸杆菌门和放线菌门丰度变化趋势与有机质含量、pH值和碱解氮含量呈负相关，与交换性镁和交换性钙含量呈正相关（图3）。

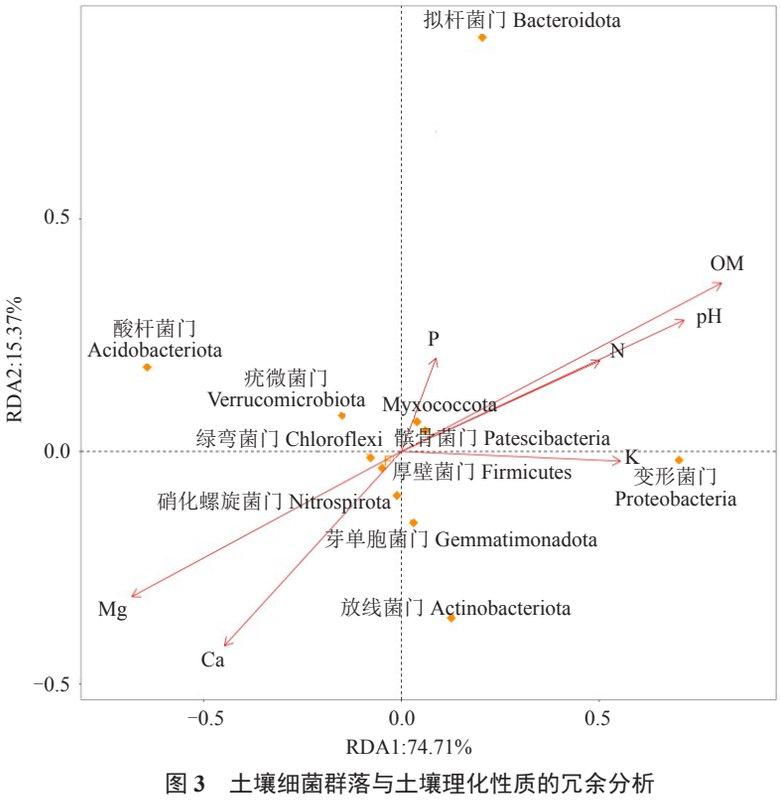


图3　土壤细菌群落与土壤理化性质的冗余分析

Fig. 3　Redundancy analysis between bacterial communities and soil chemical properties

3　讨　论

Clegg等[22]和邵微等[23]研究均表明，微生物群落多样性的增加有利于提高土壤系统的稳定性和应对生态环境恶化时的缓冲能力，微生物功能多样性与细菌α多样性呈正相关。本研究中，高通量测序结果表明1103P细菌α多样性最高，能够显著增加根际细菌的多样性和丰度，孙茜[24]研究表明，1103P、5BB、SO4、3309C这4种葡萄砧木的抗旱、耐盐和耐碱能力存在显著差异，1103P均为最好，可能是1103P根际细菌多样性的增加提高了根系对环境胁迫的缓冲能力。笔者在本研究中发现细菌多样性显著高于真菌，与前人对森林生态系统、落叶果树和酿酒葡萄的根际微生物研究结果相一致[23,25-26]。本文真菌属水平的PCoA分析结果表明，4个样品真菌组成差异较大，说明砧木对真菌组成影响较大。

根际微生物群落被认为是植物的第二基因组[27]，是根际微生态的重要组成部分，植物根系通过

分泌代谢产物、改变根际环境等，逐渐形成特定的微生物群落[28-31]。已有研究表明酸杆菌门含有可编码纤维素酶和半纤维素酶的基因，可以通过降解木质素和纤维素，提高土壤养分含量[32-33]。前人分别通过分析云南丘北县、香格里拉和河北怀来酿酒葡萄园根际土壤微生物情况，发现在门水平上，不同产区不同品种优势细菌相似，均为变形菌门、酸杆菌门、放线菌门和芽单胞菌门等，但不同产区不同品种优势菌相对丰度不同[26,34-35]，这些报道与本研究的结果相吻合。杨敏等[26]和宋雪洁[35]报道表明放线菌门相对丰度高于酸杆菌门，而本研究表明，酸杆菌门相对丰度比例平均为18.48%，显著高于放线菌门（8.26%），且相对自根苗，砧木均增加根际土壤的酸杆菌门丰度，所以，这些葡萄砧木可通过改善菌群结构进而有效提高土壤的养分含量。在细菌属水平上，本研究表明1103P根际土壤中硝化螺菌属相对丰度相对较高，薛银刚等[36]研究认为部分该类细菌参与土壤氮磷循环，有固氮解磷作用，因此，1103P可能有助于改善果园土壤环境。特别地，Brewer等[37]研究认为*Candidatus Udaeobacter*是一类需氧异养微生物，有许多氨基酸和维生素营养缺陷，通过牺牲代谢的多功能性以提高效率和相对丰度，本研究中证实4个样品的*Candidatus Udaeobacter*相对丰度均较高，特别是1103P根际土壤中此类细菌相对丰度最高，可能是1103P土壤微生物多样性较高引起的。真菌分类学组成比较单一，子囊菌门、毛霉菌门和担子菌门相对丰度比例较高，与Hibbett等[38]研究结果一致，同自根苗相比，1103P显著增加了毛霉菌门的相对丰度比例，毛霉菌门腐生，广泛分布于酒曲、植物残体、腐败有机物、动物粪便和土壤中，可产生蛋白酶、淀粉酶、谷氨酰胺酶等复杂酶系，分解大分子有机物成小分子，可能利于植物根吸收。综上所述，阳光玫瑰不同砧木和自根根际土壤样品门水平上优势菌群较为一致，不同样品优势菌群相对丰度不同，1103P微生物结构可能更有利于根系对土壤营养的吸收。

除了根系分泌物等生物因素，土壤理化情况等非生物因素对根际微生物群落组成也发挥了重要作用。宋雪洁[35]研究表明随着葡萄园有机肥含量增加，土壤细菌多样性增加，放线菌门、浮菌门和拟杆菌门丰度增加，变形菌门、芽单胞菌门和厚壁菌门丰度显著下降。杨敏等[26]研究香格里拉不同葡萄园土壤微生物和土壤理化性质间关系，认为土壤电导率、碱解氮、有机质和速效钾含量是根际细菌群落组成的关键因素，pH和速效磷含量影响不显著。此外，前人多项研究表明酸杆菌门相对丰度与pH值呈负相关[39-41]，本研究结果与之相一致，再次证实了酸杆菌门可能更适合酸性环境生长，而影响土壤微生物群落组成和分布的环境因素不完全一致，可能原因为根际微生物结构的影响因素较多，除了土壤理化性质，根系分泌物[42-43]、重金属、有机污染物[44-45]等也是重要影响因素。因此，在固定产区、管理方式和根系等情况下，可通过改善土壤营养情况调节微生物群落组成和分布。

4 结 论

砧木可以改变根际土壤微生物多样性和组成，其中，1103P和5BB对根际微生物影响较大，土壤理化性质中有机质、交换性镁含量和pH值对根际土壤优势细菌的影响较大。

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