

不同落叶果树根际微生物群落代谢与组成的差异性研究

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摘 要:【目的】从根际微生物群落代谢与组成方面探讨不同落叶果树根际差异性。【方法】利用 Biolog ECO 板方法与 Illumina Miseq sequencing 技术, 以未种植果树区域土壤为对照, 研究苹果、梨及葡萄等 8 种落叶果树根际微生物群落的代谢与组成。【结果】8 种果树根际微生物的代谢活性与功能多样性指数(除 Pielou 均匀度指数 E 外)均显著高于对照。比较 8 种果树根际微生物群落的碳源利用特征情况发现, 杏树根际微生物群落对酚酸类物质(PC, phenolic compounds)的利用比率显著下降, 对碳水化合物(CH, carbohydrate)的利用比率显著上升, 而对照土壤微生物群落对氨基酸类(AA, amino acids)碳源利用比率最高。细菌与真菌群落纲水平相对丰度表明, 8 种果树根际细菌与真菌群落相对丰度存在明显差异, 其中苹果与核桃 γ -变形菌纲相对丰度较其他果树显著上升; 而与细菌群落相比, 8 种果树根际真菌群落在纲水平上差异更为明显。基于纲水平优势菌与微生物群落代谢之间的相关性分析表明, 微生物群落代谢与 Betaproteobacteria、Alphaproteobacteria 及 Sphingobacteriia 呈显著正相关, 而与 Gemmatimonadetes、Sordariomycetes、Glomeromycetes 及 Chytridiomycetes 均呈显著负相关。另外, RDA 分析表明, 微生物功能多样性(除 E 外)与细菌 α 多样性呈正相关, 而与真菌 α 多样性呈负相关。【结论】8 种果树微生物群落碳代谢存在显著差异。8 种果树根际细菌与真菌群落均存在显著差异, 而真菌群落较细菌差异更为显著。根际微生物群落代谢与细菌群落呈正相关, 而与真菌群落呈负相关。

关键词: 落叶果树; 根际土壤微生物群落; Biolog Eco-板; Illumina Miseq sequencing 技术; 相关分析

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Differences in metabolism and composition of microbial communities in rhizosphere soils with different deciduous fruit trees

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Abstract: 【Objective】Rhizosphere effect driven by plant root exudates can affect the rhizosphere environment, including changes in soil pH, soil physical and chemical characteristics, and microbial communities. As the second genome of plants, rhizospheric microbial community plays a vital role in plant growth, and its composition and metabolism determine the intensity and direction of the rhizospheric nutrient cycle. The main ways for microbe to promote nutrient uptake by plants are mineralization of organic matter and activation of effective states of mineral elements, which can significantly affect fruit yield and quality. However, little is known about the differences between rhizospheric microbial communities in different fruit trees, especially in deciduous fruit trees. Therefore, it is necessary to study the

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composition and metabolism of the soil microbial communities, and to explore the differences of microbial communities in the rhizospheres of different fruit trees. **【Methods】**The experiment was conducted at Xinxiang Integrated Test Base, Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, located in Henan province of China. Eight deciduous fruit trees including apple, pear, apricot, cherry, peach, grape, walnut, and pomegranate, were planted in March 2012. The plots without fruit trees were used as controls (CK). Soil samples were collected from 10-20 cm soil layer in the rhizospheres of eight deciduous fruit trees in May 2017, placed in a sterile bag, and stored at $-80\text{ }^{\circ}\text{C}$. The Biolog EcoPlate method and Illumina Miseq sequencing technology were used to measure the metabolism and composition of different fruit trees rhizospheric microbial communities. **【Results】**The metabolic activity and functional diversity indexes (except Pielou index E) of the microbial communities in the eight fruit trees rhizosphere were significantly higher than those of the CK. The rhizospheric microbial communities of apple, grape, pomegranate and cherry had higher metabolism, while peach, pear and apricot tree had lower metabolism; the E index of peach was higher than those of the others, and the functional diversity indexes (Simpson D, Shannon-Wiener H, McIntosh U and S) of microbial community function of pomegranate, cherry and apple rhizosphere were higher than those of the others. The characteristics of carbon source utilization showed that the utilization ratio of phenolic compounds (PC) of the apricot rhizospheric microbial community decreased significantly, while the utilization ratio of carbohydrate (CH) significantly increased. And the soil microbial community of CK had the highest percentage of amino acids (AA) carbon source utilization. In addition, the percentage of CK's utilization of amino acid was significantly higher than those of the fruit trees. The relative abundances of bacterial and fungal communities at the level of the genus varied with 8 fruit trees rhizospheres; the relative abundance of *γ -proteobacteria* of apple and walnut were significantly higher than those of the other fruit trees. In addition, compared with the bacterial communities, the fungal communities in the rhizosphere of the eight fruit trees were more significantly different. Differences in the bacterial community levels at the family level showed that the relative abundance of Blastocatellaceae in the rhizosphere of apricot trees was significantly higher than those of the other fruit trees (except pomegranate), the lowest was found in apple. The Gemmatimonadaceae relative abundance of the peach trees was significantly higher than those of other fruit trees, and the lowest was found in the pomegranate trees. The relative abundance of Agariceae in walnut rhizosphere was significantly higher than those of the other tree species, while the lowest was found in pomegranate rhizosphere. The relative abundance of Mortierellaceae in apricot and pomegranate rhizospheric soils was relatively higher than those of walnut, cherry, peach and apple. The relative abundance of Nectriaceae and Pleosporaceae in the control sample was significantly higher than those of the rhizospheres of the eight deciduous fruit trees. Correlation analysis between the predominant bacteria at the class level and microbial community metabolism revealed that Betaproteobacteria, Alphaproteobacteria, and Sphingobacteriia were significantly positively related to the microbial community metabolism, while Gemmatimonadetes, Sordariomycetes, Glomeromycetes, and Chytridiomycetes were significantly negatively related to the microbial community metabolism. In addition, RDA analysis showed that the microbial functional diversity (except E) was positively correlated with the bacterial alpha diversity and negatively correlated with the fungal alpha diversity. **【Conclusion】**There were significant differences in the metabolism and functional diversity of the microbial communities of the eight northern deciduous fruit trees. Also, there were significant differences between the bacterial and fungal communities in the rhizospheres of the eight fruit trees, while the differences of the fungal communities were more significant than those of the bacterial communities. The rhizosphere microbial community

metabolism was positively correlated with the bacterial community and negatively correlated with the fungal community.

Key words: Deciduous fruit trees; Rhizosphere microbial community; Biolog Eco-plate; Illumina Miseq sequencing technology; Correlation analysis

自1994年开始,我国成为世界上最大的水果生产与消费国^[1]。随着近年来果树产业种植规模的不断扩大,水果产业占据国民生产总值的比重也日益增大。而落叶果树主要分布在长江以北,多以落叶乔木为主,如苹果、梨、枣、核桃、桃、葡萄、樱桃、杏、李、柿、山楂等^[2-3]。据农业农村部种植业管理司统计,2016年全国水果种植面积前5名中,落叶果树占3/5,而从产量上看,苹果与梨分别占水果产量的24.2%与10.3%。

土壤微生物群落结构与代谢作为土壤微生物生态学的基础内容,对其深入研究能够丰富土壤微生物生态学理论^[4]。根际,受植物根系分泌物的影响且围绕在根的狭窄土壤区域^[5-6],是土壤与植物生态系统物质交换的活跃界面^[7]。根际效应影响根际环境,包括土壤pH、土壤理化特性以及微生物群落的变化^[8]。而根际微生物群落,号称植物的第二基因组对植物生长起着至关重要的作用^[9],其组成与代谢决定了根际养分循环的强度与方向。根据对植物生长的影响,根际微生物可以分为有益菌、致病菌以及中性菌^[10]。而利用Illumina Miseq sequencing技术测定根际微生物群落中的主要细菌与真菌组成^[11-13],能够快速分析果树根际微生物群落存在的有益菌与致病菌^[14-15]。Biolog ECO板技术以碳源利用情况为基础,能够快速反映微生物群落水平的生理代谢概况(CLLP, the community-level physiological profile),已用于果树根际微生物功能多样性的评价^[16-18]。

笔者以苹果、梨、桃、葡萄等8种北方常见落叶果树为研究对象,进而解析果树根际微生物群落的组成与代谢的差异性,确定8种落叶果树根际微生物群落关键微生物与碳代谢特征,丰富落叶果树根际微生态环境特性信息,进而为落叶果树土壤管理提供一定的理论依据与技术支撑。

1 材料和方法

1.1 试验地基本情况

试验在河南省新乡市中国农业科学院郑州果树

研究所新乡综合试验基地(35°7'40"N, 113°45'57"E)进行。该地区属于黄河流域,年平均降水量约为573.4 mm,表层土壤pH为8.05,有机质含量(w , 后同)1.63%,有效磷含量 $125.89 \text{ mg} \cdot \text{kg}^{-1}$,有效钾含量 $202.6 \text{ mg} \cdot \text{kg}^{-1}$,铵态氮含量 $1.39 \text{ mg} \cdot \text{kg}^{-1}$,硝态氮含量 $5.62 \text{ mg} \cdot \text{kg}^{-1}$,可交换Ca和镁含量分别为 3.76 、 $0.32 \text{ g} \cdot \text{kg}^{-1}$,有效Fe含量为 $9.75 \text{ mg} \cdot \text{kg}^{-1}$ 。

1.2 试验设计

2012年3月,种植了8种落叶果树:苹果(品种:‘华硕’;砧木:八棱海棠),梨(品种:‘晚秋黄梨’;砧木:杜梨),杏(品种:‘凯特’,自根砧木),樱桃(品种:‘龙冠’;砧木:ZY-1),桃(品种:‘中油4’;砧木:山桃),葡萄(品种:‘巨峰’,自根砧木),核桃(品种:‘中核1’,自根砧木)和石榴(品种:‘突尼斯’,自根砧木)。以未种植果树的小区为对照(CK)。每个小区(面积 36 m^2)种植9株树(间距 $3 \text{ m} \times 3 \text{ m}$)。相邻小区设置空白区域,随机区组设计,3次重复。每年9月,所有区域施用复合肥 $375 \text{ kg} \cdot \text{hm}^{-2}$ (N:P:K质量比为15:15:15)和有机肥 $9000 \text{ kg} \cdot \text{hm}^{-2}$ 。

2017年5月,每个小区随机选择3株树以抖落法^[19]采集了果树10~20 cm土层根际土壤样品,并将3个土样混合,过2 mm孔径的无菌筛网,−80 °C储存。

1.3 测定项目与方法

1.3.1 微生物碳代谢分析方法 采用Biolog Eco板测定落叶果树根际土壤微生物碳代谢。取相当于1 g烘干土新鲜土壤置于装有99 mL 0.85%灭菌生理盐水的锥形瓶中, $200 \text{ r} \cdot \text{min}^{-1}$ 振荡20 min(28 °C),4 °C下静置30 min,然后每孔加入150 μL 加样于Biolog-Eco微孔板中。25 °C下培养192 h,每24 h用Biolog自动读取仪读数1次。选取培养96 h的数据进行分析^[20-21]。

土壤微生物群落利用碳源的整体能力,用平均吸光值AWCD表示,其计算公式为:

$$AWCD = \sum(C_i - R)/n。$$

式中: C_i 为所测定的31个碳源孔的吸光值; R 为对照孔的吸光值; n 为培养基碳源总数(本研究中

为31)。

McIntosh 指数(U)是基于群落物种多维空间距离的多样性指数,可利用第 i 孔的相对吸光值(n_i)计算得到,公式为:

$$U = \sqrt{\sum n_i^2}。$$

丰富度指数是指被利用碳源的总数,为每孔中(C-R)的值大于0.25的孔个数^[11]。

Shannon-Wiener 指数:用于评估丰富度,其中 P_i 为第 i 孔的相对吸光值与整个平板相对吸光值总和的比率。

$$H' = -\sum (P_i \cdot \ln P_i) ;$$

式中: P_i 为有培养基的孔和对照孔的光密度值差与整板总差的比值,即

$$P_i = \frac{(C_i - R)}{\sum (C_i - R)}。$$

Simpson 指数(D)又称优势度指数,是对多样性方面的集中性度量, D 称为Gini指数(1921年Gini提出)。

$$D = 1 - \sum P_i^2。$$

Pielou 均匀度指数(E)是群落实测多样性与最大多样性的比率。Pielou 均匀度指数计算公式为:

$$E = \frac{H'}{\ln S}。$$

式中: S 为丰富度指数,代表被利用的碳源总数。

1.3.2 微生物结构多样性分析方法 采用 TI-ANamp Soil DNA Kit 107 试剂盒提取土壤样品总DNA,以 A260/A280 与琼脂糖凝胶电泳进行DNA纯度与完整性检测,然后扩增细菌16S rRNA的V3-V4区域与真菌ITS区域,细菌引物为B341F (5'-CCTACGGGNGGCWGCAG-3')与B785R (5'-GACTACHVGGGTATCTAATCC-3'),真菌引物为ITS3F (5'-GATGAAGAACGYAGYRAA-3')与ITS4R (5'-TCCTCCGCYYATTGATATGC-3'),应用 Illumina Miseq sequencing 技术测定根际土壤微生物组成^[22]。

1.4 数据分析

采用 Microsoft Excel 2007 进行数据处理与作图;SPSS 17.0 进行 Duncan 单因素方差分析与相关性分析,以 $p < 0.05$ 作为显著性的标准;分别选取细菌与真菌群落相对丰度最高的8种细菌科(8种果树平均相对丰度 ≥ 2.5)与真菌科(8种果树平均相对丰度 ≥ 0.95)进行分析,并用 Sigma Plot 12.5 作图;采用

Canoco 4.5 进行冗余分析 (RDA, redundancy analysis)。

2 结果与分析

2.1 不同落叶果树根际土壤微生物群落碳代谢多样性

如图1所示,在24 h内,土壤微生物代谢均较为缓慢,随后增强。不同落叶果树根际微生物群落的代谢活力不同,但均较对照显著提高。在8种落叶果树中,苹果、葡萄、石榴与樱桃根际微生物群落的代谢活力一直处于较高水平,而桃、梨以及杏树代谢活力则较低。

如表1所示,8种落叶果树根际土壤微生物群落

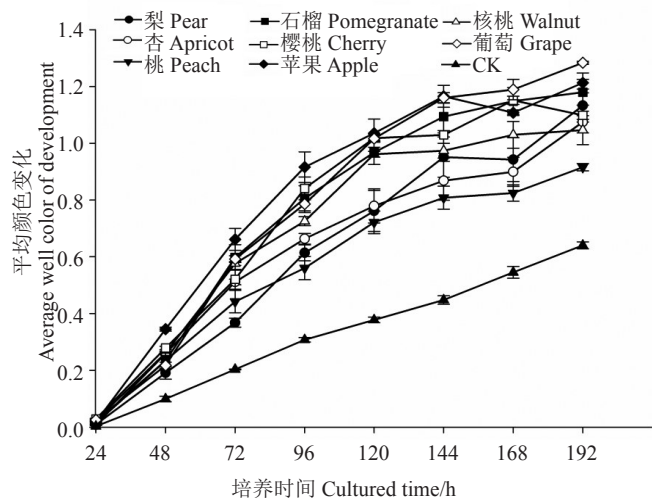


图1 不同落叶果树根际土壤微生物 AWCD 随培养时间的变化

Fig. 1 Changes of average well color development during incubation of soil microbial community in different deciduous fruit trees rhizosphere

功能多样性指数 D 、 H 、 U 及 S 显著高于对照,这表明果树能够显著改变土壤微生物功能多样性。比较不同果树微生物功能多样性指数发现,桃树均匀度指数 E 最高,且显著高于樱桃。而石榴、樱桃及苹果功能多样性指数 D 、 H 、 U 及 S 显著高于杏与桃。因此,在8种果树中,石榴、樱桃以及苹果根际微生物群落功能多样性指数与代谢活力均处于高水平,而杏与桃则较低。

如图2所示,8种果树根际土壤微生物群落对6种碳源利用情况不同。其中,与其他果树相比,杏树根际微生物群落对酚酸类物质PC的利用比率显著下降,而对碳水化合物CH的利用比率显著上升。

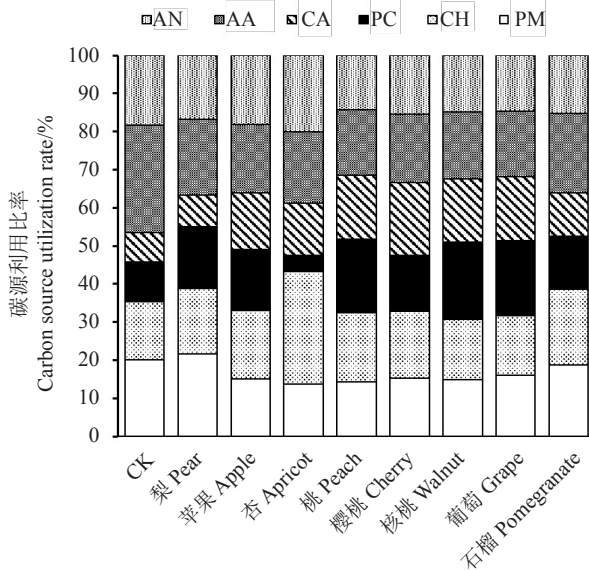
表 1 不同落叶果树根际微生物群落功能多样性指数

Table 1 Functional diversity indices of microbial community soil microbial community in different deciduous fruit trees rhizosphere

树种 Species	均匀度指数 Pielou substrate evenness (<i>E</i>)	优势度指数 Simpson index (<i>D</i>)	多样性指数 Shannon's diversity index (<i>H'</i>)	均一性指数 McIntosh index (<i>U</i>)	丰富度指数 substrate richness (<i>S</i>)
CK	1.029±0.024 a	0.914±0.005 d	2.609±0.06 e	2.771±1.6 d	12.667±7.313 e
梨 Pear	1.016±0.012 ab	0.95±0.001 b	3.106±0.004 c	4.272±2.467 c	21.333±12.317 c
苹果 Apple	0.996±0.001 ab	0.96±0 a	3.281±0.003 a	5.664±3.27 a	27.000±15.589 a
杏 Apricot	1.001±0.01 ab	0.949±0.001 b	3.107±0.007 c	4.63±2.673 bc	22.333±12.895 bc
桃 Peach	1.025±0.008 a	0.94±0.003 c	2.962±0.049 d	4.262±2.461 c	18.000±10.393 d
樱桃 Cherry	0.986±0.005 b	0.958±0.001 a	3.249±0.021 ab	5.326±3.075 ab	27.000±15.589 a
核桃 Walnut	1.000±0.014 ab	0.955±0.001 ab	3.186±0.021 bc	4.775±2.757 bc	24.333±14.049 b
葡萄 Grape	1.004±0.006 ab	0.955±0.001 ab	3.191±0.013 bc	5.193±2.998 ab	24.000±13.857 b
石榴 Pomegranate	0.997±0.007 ab	0.959±0 a	3.285±0.002 a	5.042±2.911 ab	27.000±15.589 a

注:同一列数据后英文小写字母不同表示处理间某指标差异达 0.05 显著水平。下同。

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



PM, CH, PC, CA, AA, AN 分别代表聚合物类、碳水化合物类、酚类、羧酸类、氨基酸类以及胺类化合物。

PM. Polymers; CH. Carbohydrate; PC. Phenolic compounds; CA. Carboxylic acids; AA. Amino acids; AN. Amines.

图 2 不同落叶果树根际土壤微生物对碳源的利用特征
Fig. 2 Soil microbial utilization of different carbon sources in different deciduous fruit trees rhizosphere

在 6 种碳源中,CK 对氨基酸类碳源 AA 利用比率最高,显著高于果树根际土壤。

2.2 不同落叶果树根际土壤微生物群落结构多样性

如图 3 所示,8 种果树根际土壤细菌群落(纲水平上)相对丰度(图 3-A)存在差异,其中与另外 6 种果树相比,苹果与核桃 Gammaproteobacteria (γ -变形菌)纲相对丰度显著上升。与细菌群落相比,8 种果

树根际真菌群落在纲水平上的差异更为显著(图 3-B)。

如图 4 所示,选取 8 个相对丰度最高的细菌科研究 8 种落叶果树根际细菌群落。其中杏根际 *Blastocatellaceae* 相对丰度显著高于其他果树(除石榴外),而苹果显著低于其他果树。*Sphingomonadaceae* 相对丰度:苹果最高,而 CK 最低。*Gemmatimonadaceae* 相对丰度:桃显著高于其他果树,而石榴最低。*Ambiguous* 相对丰度:梨与 CK 显著高于其他果树,而石榴显著低于其他果树。梨、苹果、杏、桃以及樱桃根际 *Nitrosomonadaceae* 相对丰度显著高于核桃、葡萄以及石榴。石榴根际的 *Chitinophagaceae* 与 *Cytophagaceae* 相对丰度最高,显著高于其他果树。苹果根际 *Xanthomonadaceae* 相对丰度最高,而桃树最低。综上,不同落叶果树根际细菌基于科水平的相对丰度存在显著差别。

如图 5 所示,选取 8 个相对丰度最高的真菌科研究 8 种落叶果树根际真菌群落的差异性。*Agariceae* 相对丰度:核桃根际最高,显著高于其他树种,而石榴最低。杏与石榴 *Mortierellaceae* 相对丰度最高,且显著高于核桃、樱桃、桃树及苹果。CK 的 *Nectriaceae* 与 *Pleosporaceae* 相对丰度最高,且显著高于果树根际。桃根际土壤中 *Pluteaceae* 与 *Leptosphaeriaceae* 相对丰度显著高于其他果树。葡萄根际 *Phyllachoraceae* 相对丰度最高,显著高于石榴、核桃、樱桃、杏、苹果以及梨。杏与桃根际 *Glomeraceae* 相对丰度显著高于其他果树。比较图 4 与图 5 可知,与细菌群落相比,真菌群落与树种之间的关系更为紧密。

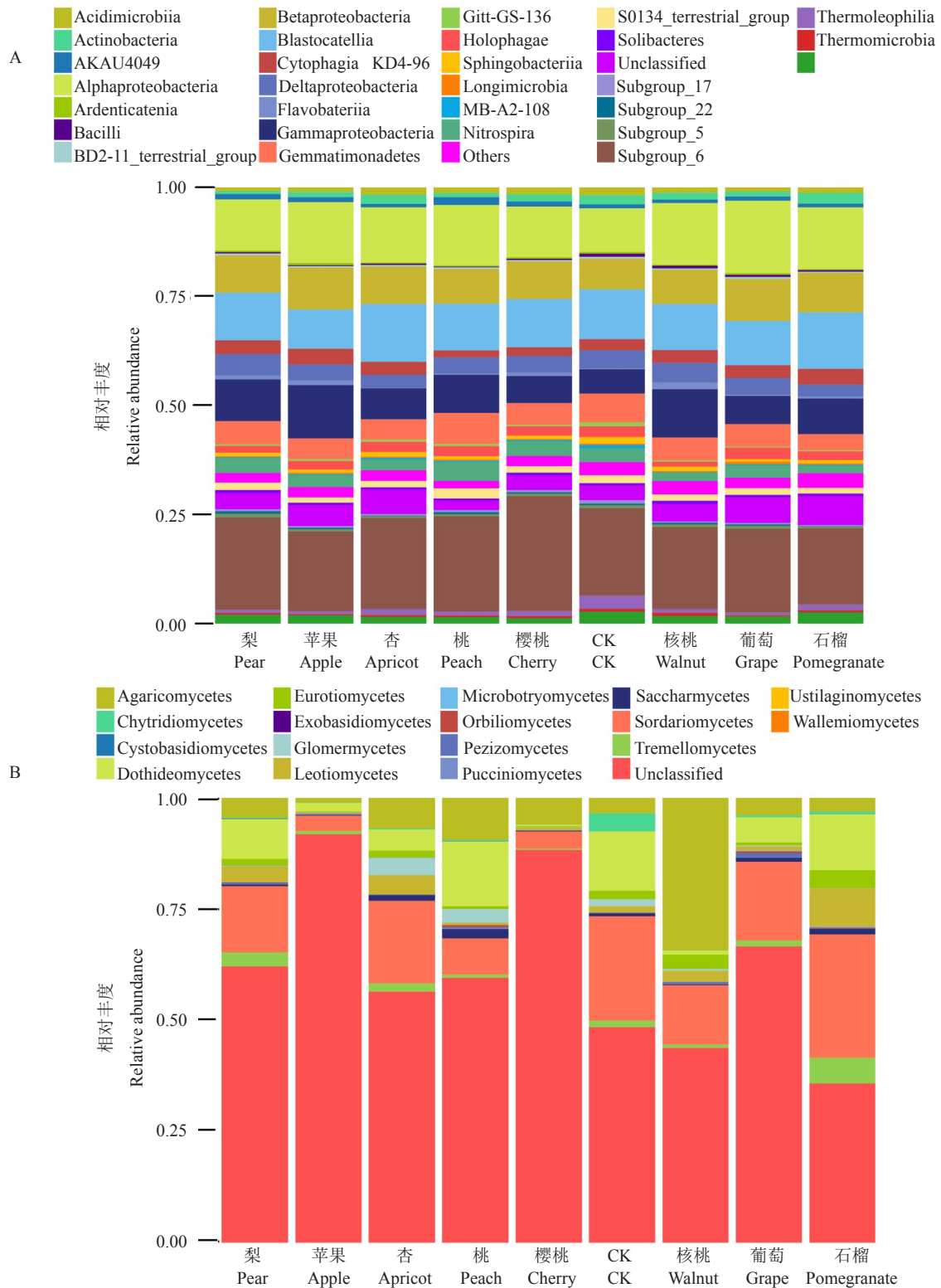


图 3 基于纲水平上的根际细菌(A)与真菌(B)群落相对丰度

Fig. 3 The relative abundance of rhizosphere bacterial (A) and fungal (B) communities based on the level of class

如图 6 所示,微生物碳代谢(除 E 外)与细菌 α 多样性指数所指方向成锐角,即微生物碳代谢与细菌 α 多样性指数呈正相关,而与真菌 α 多样性指数所指方向成钝角,即微生物碳代谢与真菌 α 多样性指数

呈负相关。其中,真菌 α 多样性指数 Shannon、Simpson 与 SPEC AX1 的相关性为 0.6794 与 0.626,与微生物碳代谢指数(除 E 与 AN 外)均为显著负相关。

如表 2 所示,基于纲水平的优势菌(包括 10 个细

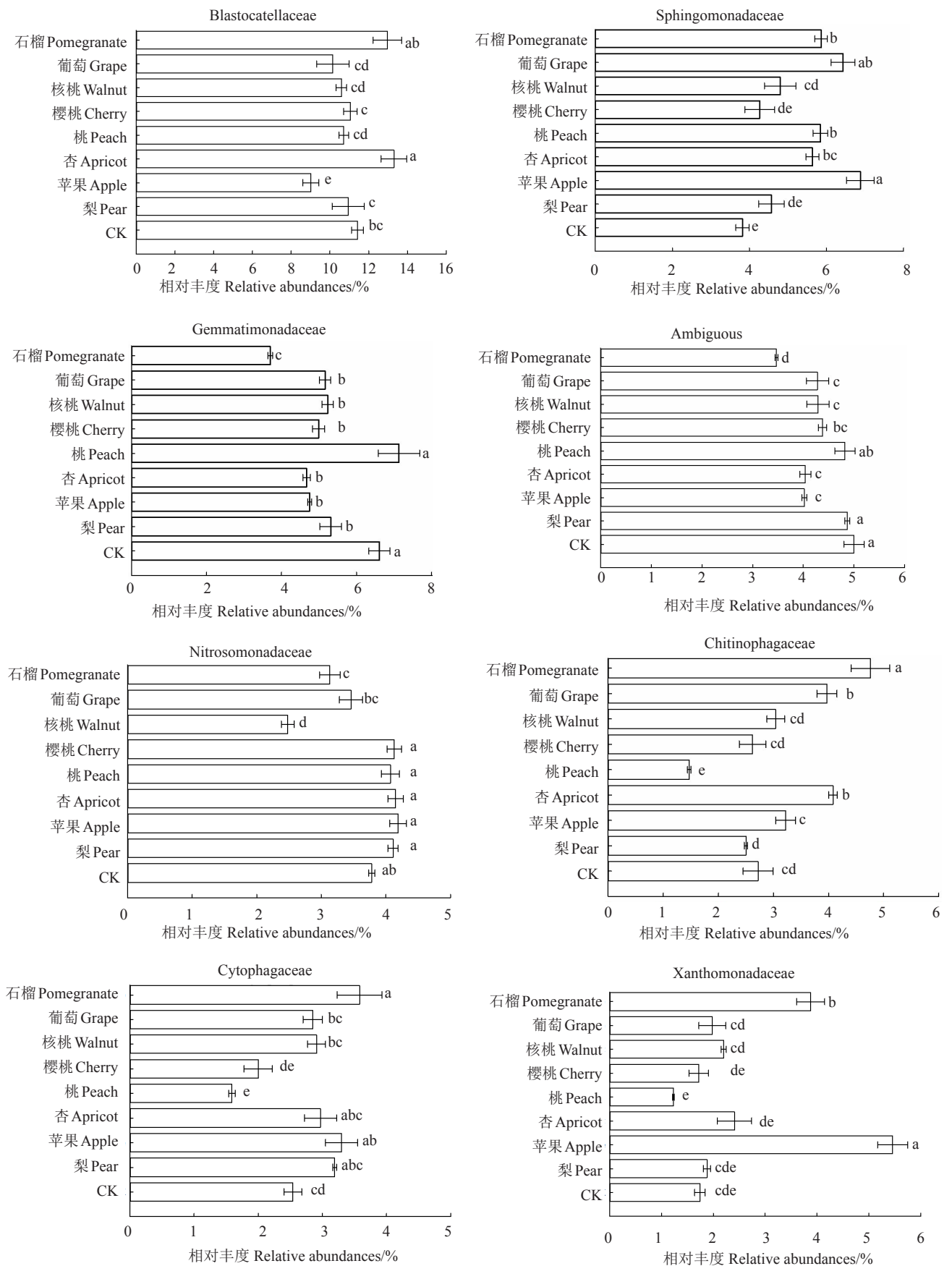


图 4 基于科水平上的根际细菌群落相对丰度

Fig. 4 The relative abundance of rhizosphere bacterial communities based on the level of family

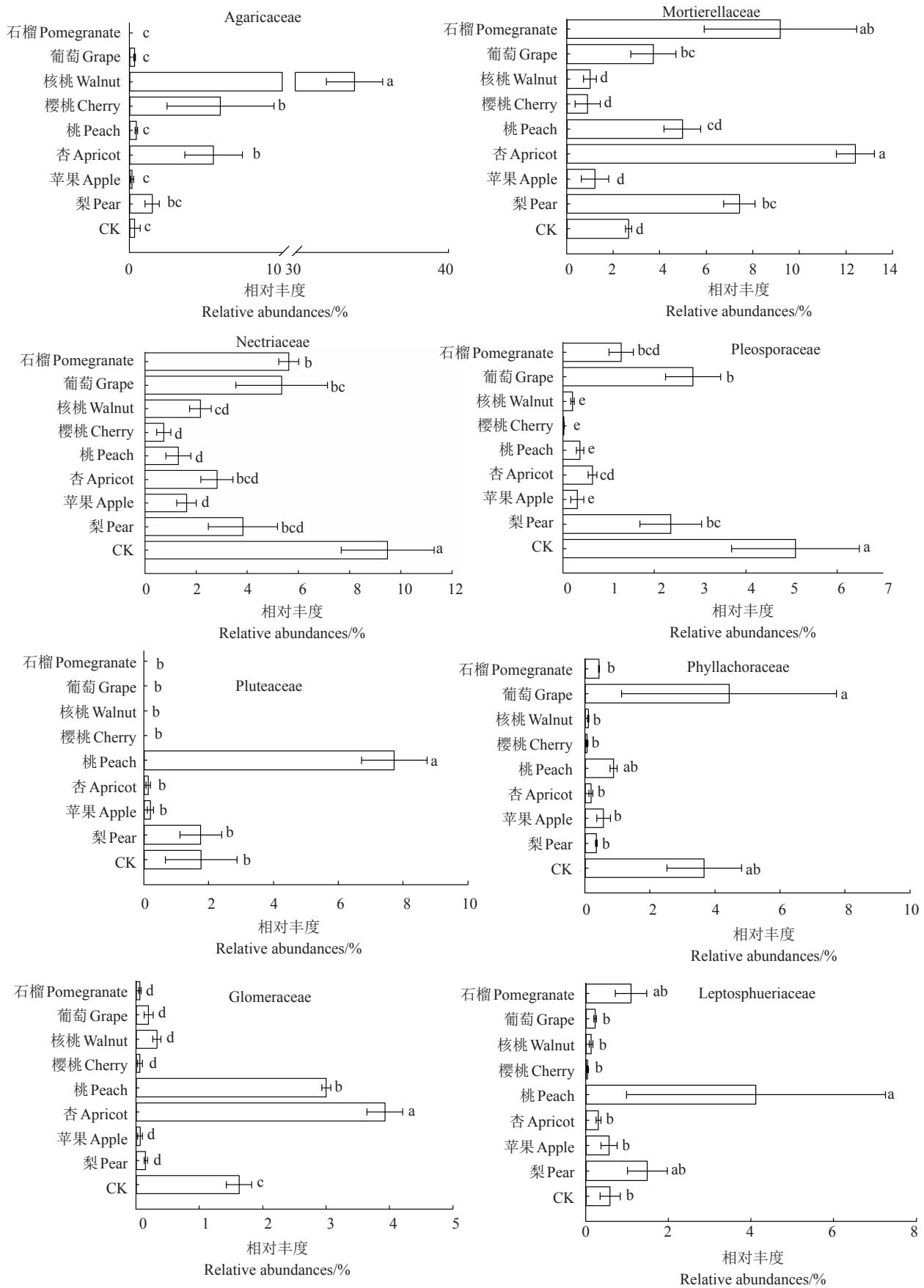
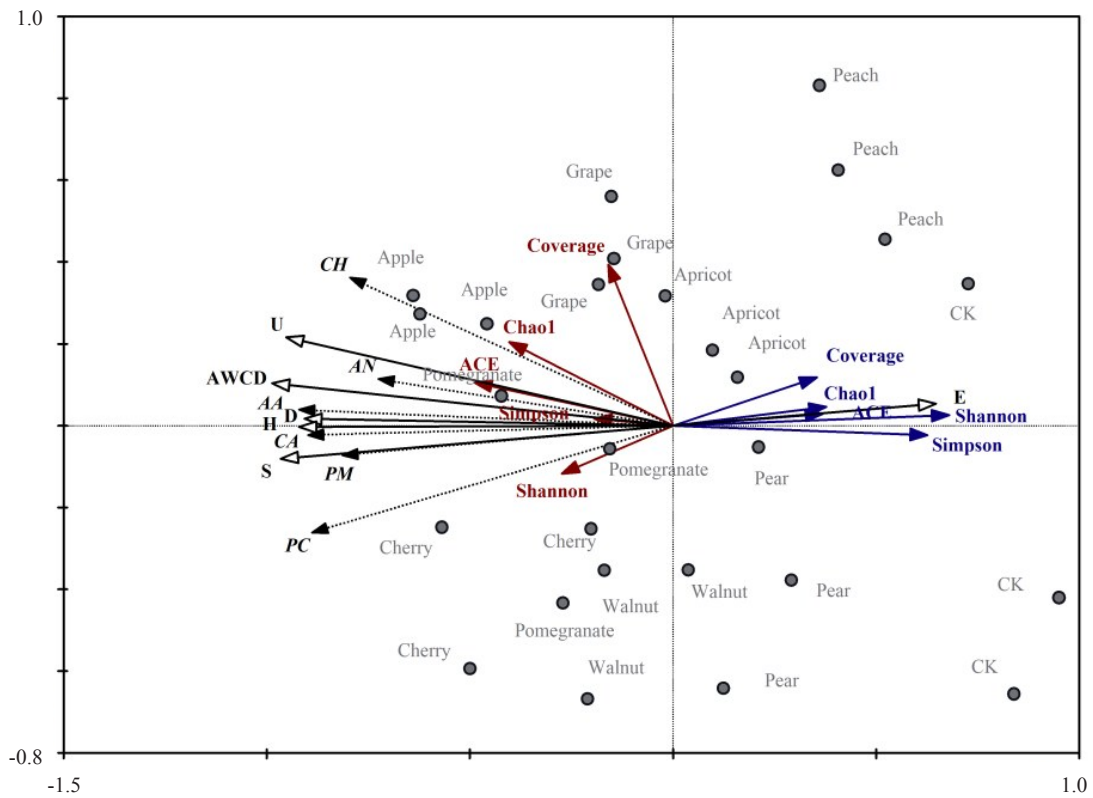


图 5 基于科水平上的根际真菌群落相对丰度

Fig. 5 The relative abundance of rhizosphere fungal communities based on the level of family



红色为细菌 α 多样性指数, 蓝色为真菌 α 多样性指数。

Red represents the bacterial alpha diversity index, and blue represents the fungal alpha diversity index.

图 6 根际微生物代谢与 α 多样性之间的冗余分析

Fig. 6 Redundancy analysis (RDA) of microbial metabolism and alpha diversity

表 2 根际微生物群落结构与功能多样性之间的相关性

Table 2 Pearson correlation coefficients between microbial class relative abundances and functional diversity

		PM	CH	PC	CA	AA	AN	AWCD
细菌 Bacteria	Alphaproteobacteria	0.372	0.698**	0.219	0.404*	0.524**	0.416*	0.557**
	Betaproteobacteria	0.792**	0.706**	0.533**	0.572**	0.849**	0.635**	0.768**
	Deltaproteobacteria	0.029	-0.29	-0.031	-0.436*	-0.212	-0.302	-0.256
	Gammaproteobacteria	0.275	0.361	0.395*	0.383*	0.307	0.331	0.386*
	Gemmatimonadetes	-0.596**	-0.412*	-0.673**	-0.708**	-0.724**	-0.586**	-0.679**
	Blastocatellia	-0.225	-0.153	-0.192	-0.048	-0.278	-0.164	-0.188
	Sphingobacteriia	0.467*	0.334	0.345	0.445*	0.588**	0.422*	0.441*
	Nitrospira	-0.281	-0.182	-0.481*	-0.545**	-0.437*	-0.317	-0.381*
	Cytophagia	0.474*	0.113	0.435*	0.336	0.482*	0.357	0.337
	Actinobacteria	-0.246	-0.242	0.03	0.231	-0.083	0.007	-0.089
真菌 Fungi	Sordariomycetes	-0.286	-0.405*	-0.376	-0.257	-0.25	-0.276	-0.385*
	Agaricomycetes	-0.174	0.116	0.067	0.068	-0.121	-0.046	-0.006
	Dothideomycetes	-0.535**	-0.352	-0.741**	-0.533**	-0.571**	-0.346	-0.590**
	Leotiomycetes	0.04	0.018	0.034	0.222	0.085	0.156	0.075
	Tremellomycetes	0.097	-0.007	-0.064	0.091	0.087	0.123	0.023
	Eurotiomycetes	-0.118	-0.158	0.001	0.091	-0.1	-0.116	-0.132
	Glomeromycetes	-0.595**	-0.216	-0.543**	-0.395*	-0.612**	-0.202	-0.492**
	Saccharomycetes	-0.359	0.082	-0.491**	-0.142	-0.348	-0.021	-0.218
	Chytridiomycetes	-0.239	-0.457*	-0.184	-0.277	-0.291	-0.393*	-0.413*
	Pezizomycetes	0.234	0.186	0.088	-0.154	0.259	-0.066	0.087

注: **. 在 0.01 水平(双侧)上显著相关。*. 在 0.05 水平(双侧)上显著相关。

Note: **. Significantly off at 0.01 level. *. Significantly correlated at the 0.05 level.

菌纲与 10 个真菌纲)与根际微生物群落代谢之间的相关性分析表明,根际微生物群落代谢与 Betaproteobacteria 呈显著正相关,而与 Gemmatimonadetes 则均呈显著负相关。Alphaproteobacteria 与碳水化合物、羧酸、氨基酸、胺类利用率以及 AWCD 呈显著正相关。Sphingobacteriia 与聚合物、羧酸类、氨基酸、胺类以及 AWCD 呈显著正相关。而优势真菌与群落代谢之间的显著性相关均表现为负相关。其中 Sordariomycetes 与碳水化合物类利用率、AWCD 呈显著相关。Dothideomycetes 与聚合物、酚酸类、羧酸类、氨基酸类以及 AWCD 呈显著负相关。Glomeromycetes 与聚合物、酚酸类、羧酸类、氨基酸类利用率以及 AWCD 呈显著负相关。Saccharomycetes 与酚酸类利用率呈显著负相关。Chytridiomycetes 与碳水化合物、胺类以及 AWCD 呈显著负相关。

3 讨 论

3.1 不同落叶果树根际微生物群落代谢差异

植物自身有塑造根际特有微生物群落的特性,而这些微生物群落的特异性必然引起根际微生物群落碳源利用的差异。研究发现不同植被土壤微生物群落代谢与功能多样性存在明显差异^[23-26],这些与本研究结果均保持一致,即不同树种根际微生物群落的代谢特征存在差异性。8 种果树根际微生物群落 AWCD 与功能多样性指标显著高于对照,这表明随着落叶果树种植土壤微生物群落碳代谢水平随之升高。这可能跟果树的种植(尤其是根际效应)能够改善土壤微生物群落的营养环境有关^[23]。研究发现杏根际土壤微生物群落碳源利用特征显示出明显差异,其中酚酸类物质的利用率显著下降,而碳水化合物的则显著上升。孙浩元等^[21]在杏根系分泌物主要成分时发现,杏根系存在大量的 p-xylene 和 dimethyl-benzene,可能是这些分泌物含量与种类的差别造成了杏根际碳源利用特征的差异性。而未种植果树的土壤对氨基酸类的利用率最高,表明对照土壤微生物群落嗜氨基酸类物质,这与我们之前的研究保持一致^[27]。另外,杏与桃树根际微生物活性与功能多样性均低于其他果树,这可能归结为:杏与桃树根际含有丰富的苦杏仁苷^[28-29],而苦杏仁苷在土壤酶活性与微生物降解下形成氢氰酸与甲苯醛^[30],进而导致杏与桃树根际微生物代谢活性与功能多样

性指数下降^[31-32]。

3.2 不同果树根际微生物群落组成差异

根际微生物作为植物第二基因组,对植物生长与健康发挥着重要作用,因此其群落组成成为近来研究的热点之一^[9]。在研究树种与根际微生物群落之间的关系时发现,树种可以通过影响土壤 pH、总氮及总碳等,进而对土壤微生物群落的密度和群落组成^[33-34]发挥作用。根际微生物群落分析表明,8 种果树根际真菌群落结构的变化较细菌群落更为显著。此外,不同落叶果树根际土壤中微生物群落的组成表明,真菌群落比细菌群落具有更多独特的分类群和更少的多样性^[22]。而 Urbanová 等^[35]发现,森林生态系统中的真菌群落多样性显著小于细菌群落,且表现为更为明显的专一性趋势。综上,8 种落叶果树对微生物群落的影响主要体现在真菌群落。

为了阐明树种进化与根际微生物群落的关系,笔者进行了 8 种果树根际微生物群落聚类分析,结果表明虽然每种果树根际微生物群落与其他果树相区分,而 8 种果树根际微生物群落相似性与果树进化分类无明显关系。Vieira 等^[36]发现植物特有微生物的种类相对来说极少,而这些特有微生物的存在与根际特定的分泌物有关,而土壤的质地如水分含量、土壤类型则显著影响植物特性与植物根际分泌物的组成。因此,笔者猜测是果树根际分泌物的种类与含量而不是树种的生物学分类决定了果树根际微生物的相对丰度^[24-27],进而造成群落的差异性。

Blastocatellaceae 中大多数菌生活的范围极广,能够在营养水平较低和干旱的条件下生存,同时适应的 pH 与温度范围也很广泛^[37-39]。Blastocatellaceae 在 8 种果树根际微生物群落中是最为优势的细菌科,其中杏根际 Blastocatellaceae 的相对丰度显著高于苹果根际,可能与土壤营养水平相关,而杏根际土壤有机质含量显著高于苹果^[22]。Gemmatimonadaceae 是目前已发现的土壤最主要的细菌科之一^[40],Cline 等^[41]研究发现 Gemmatimonadaceae 相对丰度随着 pH(5.4~6.4)的上升而升高,且在弃耕地相对丰度比原始森林高。本研究发现在 8 种果树根际的 Gemmatimonadaceae 与土壤 pH(6.9~8.1)呈显著正相关,与 Cline 等^[41]的结果保持一致。而 Wang 等^[42]研究发现在其研究的土壤中 Gemmatimonadaceae 与 pH(5.75~8.23)则不相关。而发生这种现象的原因可以归结于 Gemmatimonadaceae 偏爱生存在中性土

壤中^[43],而Wang等^[42]的土壤pH跨度较大,因此出现与本研究不一致结果。另外,Liu等^[44]认为Gemmatimonadaceae相对丰度高可能有益于大气环境,因此该菌在将来研究果树根际微生物环境安全中可能发挥重要作用。8种果树根际Sphingomonadaceae显著高于对照,这可能是由于其能够利用果树根际产生的多种不易降解的碳源^[45]。研究表明,Nectriaceae科中包含多种草本与木本植物的致病菌,例如*Dactylonectria macrodidyma*能够导致葡萄、鳄梨及橄榄等果树的病害^[46],而*Gliocephalotrichum*则是导致一些热带水果储藏变质的重要病菌之一^[47]。在本研究中对照土壤中Nectriaceae拥有最高的相对丰度,种植后果树有显著下降,这可能是因为果树的种植改变了原有的土壤微生物群落的结构。而在分析8种果树根际Nectriaceae相对丰度时发现,葡萄根际的Nectriaceae相对丰度显著高于苹果、桃、樱桃以及核桃,这可能与葡萄对Nectriaceae的敏感性有关。研究发现Nectriaceae对葡萄的危害严重,能够导致幼苗的枯枝病、根腐病以及黑脚病^[48]。

3.3 不同落叶果树根际微生物群落组成与代谢之间的关系

细菌纲与微生物群落碳代谢密切相关,Alphaproteobacteria、Betaproteobacteria以及Sphingobacteriia与微生物群落碳源利用率均呈正相关,而Gemmatimonadetes的则均呈显著负相关。另外,真菌群落中与微生物碳代谢有相关关系的纲均为显著负相关。原因可能归为以下几方面:一方面真菌群落在碳代谢过程中起消极作用,造成真菌数量增加而碳代谢活力下降的趋势;另一方面跟Eco-板自身的特性有关,可能是Eco-板中的碳源培养环境不适应真菌群落的培养;再一方面也可能是与碳源代谢呈正相关的细菌纲抑制这些真菌之间的生长。

4 结 论

8种落叶果树的种植能够显著影响根际微生物群落的代谢与功能多样性指数,其中石榴、樱桃及苹果根际微生物群落代谢水平与功能多样性指数均较高,而杏树与桃树则处于低水平。8种果树能够显著影响根际微生物群落的组成,且与细菌群落组成的差异相比,8种果树在真菌群落组成的差异上更为显著。微生物群落功能多样性与结构多样性之间的相关性分析与RDA分析表明,8种果树根际微生

物细菌群落与代谢呈正相关,而真菌群落与群落代谢呈负相关。

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