

苹果叶内生菌对山田胶锈菌侵染的时间响应

李云帆¹, 陶思齐^{1,2}, 梁英梅³

(1. 北京林业大学森林培育与保护教育部重点实验室北京 100083; 2. 国家林业和草原局黑龙江三江平原湿地生态观测研究站 黑龙江双鸭山 518000; 3. 北京林业大学博物馆 北京 100083)

摘要:【目的】本研究通过探究山田胶锈菌侵染苹果叶片后不同发病阶段苹果叶片内生细菌和真菌群落结构差异,为植物内生菌群资源的开发和利用提供了有价值的见解,有助于提高苹果锈病的管理策略,为理解寄主内生菌在病原体与寄主相互作用中发挥的功能奠定了基础。【方法】利用 Miseq 高通量测序细菌 16S 核糖体 RNA 和真菌 ITS 核糖体 RNA 技术,探索了接种山田胶锈菌后 9 个不同阶段苹果叶片内生微生物群落的变化,同时相应时间段的未接种叶片作为试验对照组也做了相同处理以及分析,并且运用 PICRUST2 平台预测了特定发病时间点微生物群落发挥的不同代谢功能。【方法】利用 Miseq 高通量测序细菌 16S 核糖体 RNA 和真菌 ITS 核糖体 RNA 技术,探索了接种山田胶锈菌后 9 个不同阶段苹果叶片内生微生物群落的变化,同时相应时间段的未接种叶片作为试验对照组也做了相同处理以及分析,并且运用 picrust2 平台预测了特定发病时间点微生物群落发挥的不同代谢功能。【结果】随着苹果锈病病程的不断发展,除肉眼可见的叶片形成褪绿斑、形成性孢子器、产生蜜露、形成锈孢子器之外,叶内生微生物组也发生了改变,甚至在肉眼无法识别病症的发病早期,叶内生微生物群落已然发生着巨大的波动。其中在接种和未接种锈菌的叶片中,高丰度微生物的组成结构是相似的。对于细菌内生菌而言, Proteobacteria、Firmicutes、Actinobacteriota、Bacteroidota、Acidobacteriota 所占比例较高,而真菌群落中高度丰富的类群主要为子囊菌门(Ascomycota)和担子菌门(Basidiomycota)。进一步做多样性分析显示,接种锈菌叶片中微生物群落的 α 多样性先增加,后减少,ASV 数量增加,群落均匀度也有所提高,借助微生物共发生网络分析显示,接种锈菌叶片中细菌群落的复杂性最初降低,随后增加,真菌群落复杂性变化不明显。在微生物群落的功能预测方面,我们发现苹果叶内生细菌群落在病原锈菌发展到性孢子时期时,遗传信息处理、代谢和环境信息处理途径中表现出更高的活性。内生真菌也在性孢子期和锈孢子期显著富集了大量的代谢途径,表现出异常活跃的生命代谢过程。【结论】锈菌病原物的入侵破坏了微生物群落的多样性、聚集和网络,显著影响了它们的生态功能。其中寄主内生细菌群落的聚集受到锈病的深刻影响,而真菌群落主要受发育期的影响,其群落变化从一种新的角度诠释了寄主面对病原物入侵后的免疫调节作用和病原菌的致病作用,增强了我们对植物微生物组的组装、共发生和功能的疾病诱导改变的理解。**关键词:** 叶内生菌; 微生物组; 植物病原菌; 锈菌侵染; 植物-微生物互作

Time-Course Responses of Apple Leaf Endophytes to the Infection of *Gymnosporangium yamadae*

Li Yunfan¹ Tao Siqi^{1,2} Liang Yingmei³

(1. The Key Laboratory for Silviculture and Conservation of Ministry of Education, Beijing Forestry University, Beijing 100083; 2. Ecological Observation and Research Station of Heilongjiang Sanjiang Plain Wetlands, National Forestry and Grassland Administration, Shuangyashan 518000; 3. Museum of Beijing Forestry University, Beijing Forestry University, Beijing 100083)

Abstract: 【Objective】 By exploring the differences in the community structure of endophytic bacteria and fungi in different stages of apple leaf infection by *Gymnosporangium yamadae*, this study offers valuable insights for the development and exploitation of plant endophytic resources, thereby contributing to enhanced strategies for managing apple rust, establishing a foundation for

comprehending the role of host endophytes in the interaction between pathogens and hosts.

【Method】 This study scrutinizes alternations in endophytic microbial communities within apple leaves at nine different stages after infection with *G. yamadae* through MiSeq high-throughput sequencing of the bacterial 16S rRNA amplicons and fungal ITS rRNA amplicons, at the same time, the uninfected leaves in the corresponding time period are also treated and analyzed, additionally, employing the PICRUSt2 platform, this study provides preliminary insights into the functions of microbial communities at specific disease timepoints. **【Result】** During the occurrence and development of rust disease, in addition to the visible leaves forming faded spots, spermogonia, nectar, and aecia, the endophytic microbiome has also changed. Even in the early stage of the disease that cannot be recognized by the naked eye, the endophytic microbial community has undergone huge fluctuations. In the leaves of infected and uninfected rust, the composition and structure of high-abundance microorganisms are similar. For endophytic bacteria, Proteobacteria, Firmicutes, Actinobacteriota, Bacteroidota and Acidobacteriota accounts for a relatively high proportion. Ascomycota and Basidiomycota are the most abundant groups in the fungal community. Further diversity analysis shows that a discernible pattern characterized by an initial increase and subsequent decrease in the alpha diversity of microbial communities in infected leaves, as the number of ASVs increased, the uniformity of the community also increased. A microbial co-occurrence network analysis reveals that the complexity of the bacterial community in infected leaves diminished initially and then rebounded. In terms of functional prediction of microbial communities, we find during the spermogonial stage, endophytic bacteria particularly exhibited heightened activity in genetic information processing, metabolism, and environmental information processing pathways. Endophytic fungi also significantly enriched a large number of metabolic pathways during the spermogonial stage and aecial stage, exhibiting abnormally active life activities. **【Conclusion】** Our research provides compelling evidence that the pathogen invasion disrupts the microbial community's diversity, aggregation, and networks, significantly impacting their ecological functions. The aggregation of the host endophytic bacterial community is profoundly affected by rust disease, while the fungal community is mainly affected by the development stage. The changes of the community explains the immune regulation of the host in the face of pathogen invasion and the pathogenicity of pathogenic fungi from a new perspective, enhancing our understanding of disease-induced alterations in the assembly, co-occurrence, and functionality of plant microbiomes.

Key words: endophyte; microbiome; plant pathogen; rust infection; plant-microorganism interaction