The impact of land use changes on soil fungal community and structure

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Abstract] To understand the impact of land use changes on the composition and structure of fungal communities from Miyaluo county in subalpine forest area of western Sichuan, the molecular diversity of 18S rDNA genes from soil obtained at a 20-year-old spruce plantation []Picea likiangensis var baloufianan[] and cropland sites were examined using a PCR-based cloning approach. DNA was directly extracted from the soil microorganisms and amplified the 18S rDNA gene fragment using PCR by the specific primers of EF-4[ 5’-GGAAGGG [A/G] TGTATTATTAG-3’ and Fung-5[ 5’-GTAAA AGTCCTGTGGTCG-3’. For the gene fragment[] diverse PCR products were characterized by cloning[] restriction fragment length polymorphism [RFLP] analysis and sequencing. A total of 238 clones and 56 operational taxonomic units []OTUs] which were digested by the restriction enzymes MspI and RsaI were obtained from all samples. The 20-year-old spruce plantation and cropland sites were received 137 and 101 clones and 37 and 19 OTUs respectively. There were different significant dominant groups of clones occurring in both samples and shared 6 OTUs. 20-year-old spruce plantation and

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cropland sites had one and two significant dominant groups which account for 20.4% \( \text{and 25.7%} \) and 21.8% \( \text{of all clones} \) respectively. There also were some secondary dominant groups of clones which account for 7.3% \( \text{and 8.9%} \) of all clones. Fourteen 18S rDNA clones were sequenced and their nucleotide identity was from 86% \( \text{to} \) 99%. Compared the known sequences with the deposited in the data bank \( \text{www.ncbi.nlm.nih.gov} \) their level of nucleotide identity was from 92% \( \text{to} \) 100%. The phylogenetic tree was constructed by the Clustal W and Mega softwares. 14 sequences could be subdivided into 3 clusters in the phylogenetic tree. The clone sequences of 20-year-old spruce plantation site were completely clustered into the first and the third clusters and the clone sequences of cropland site only distributed in the second cluster. Therefore both 20-year-old spruce plantation and cropland sites had the high fungal diversity and land use changes significant influenced the fungal community and structure.

**Key Words** land use changes \( \text{fungi} \) community and structure 18S rDNA PCR-RFLP

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1.1  
1.2  
1.3  
1.4  
1.5  

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均匀混合后取少量装入灭菌的封口聚乙烯袋,带回实验室保存备用。

1. **方法**

### 1.1 DNA 提取

Richard [21] 和张于光等 [22] 的方法提取土壤微生物总 DNA。将溶解的 DNA 在 20 μmol/L dNTPs、0.0 mmol/L Mg²⁺ 和 550bp 的 PCR 产物中 PCR 后,在 × PCR Buffer 200μmol/L dNTPs、0 mmol/L Mg²⁺、25 μmol/L、2 μl 混合后于 94 °C 3 min/94 °C 1 min/52 °C 45 s/72 °C 1 min 40 s。琼脂糖电泳回收试剂盒 ( Bio Basic Inc. ) 后用 1.5% Agarose 进行电泳回收。

### 1.2 18S rDNA 与 PCR

18S rDNA EF-4 [23] 5’-GGAAGGG A/G TGTATTATTAG-3’ 与 Fung-5r [24] 5’-GTAAAAGCTCTGGTCC-3’ 的引物,于 18S rDNA 的 5’ 和 3’ 端扩增。将 PCR 产物加入 200μmol/L dNTPs、0 mmol/L Mg²⁺、25 μmol/L、2 μl 混合后于 94 °C 3 min/94 °C 1 min/52 °C 45 s/72 °C 1 min 40 s。琼脂糖电泳回收试剂盒 ( Bio Basic Inc. ) 后用 1.5% Agarose 进行电泳回收。

### 1.3 PCR RFLP

将 PCR 产物通过 PCR 进行 RFLP 分析,通过 GeneGenius 软件分析酶切结果,共获得不同的 9=C 7345 产物 10 个。

### 1.4 18S rDNA 与 RFLP

利用 18S rDNA 的 5’ 和 3’ 端扩增,将 PCR 产物加入 200μmol/L dNTPs、0 mmol/L Mg²⁺、25 μmol/L、2 μl 混合后于 94 °C 3 min/94 °C 1 min/52 °C 45 s/72 °C 1 min 40 s。琼脂糖电泳回收试剂盒 ( Bio Basic Inc. ) 后用 1.5% Agarose 进行电泳回收。

<table>
<thead>
<tr>
<th>样品编号</th>
<th>获得克隆数</th>
<th>群落多样性指数</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG=1</td>
<td>919</td>
<td>1.37</td>
</tr>
<tr>
<td>RGB</td>
<td>919</td>
<td>1.37</td>
</tr>
</tbody>
</table>

### 1.5 核苷酸序列登录号

将测序得到的序列提交 GenBank,数据库登录号为 JQ834804-JQ834808, JQ834815-JQ834836。

### 1.6 2 项目二

#### 2.1 18S rDNA 与 RFLP

利用 18S rDNA EF-4 的 5’-GGAAGGG A/G TGTATTATTAG-3’ 与 Fung-5r 5’-GTAAAAGCTCTGGTCC-3’ 的引物,于 18S rDNA 的 5’ 和 3’ 端扩增,获得 238 个 OTUs。将 PCR 产物加入 200μmol/L dNTPs、0 mmol/L Mg²⁺、25 μmol/L、2 μl 混合后于 94 °C 3 min/94 °C 1 min/52 °C 45 s/72 °C 1 min 40 s。琼脂糖电泳回收试剂盒 ( Bio Basic Inc. ) 后用 1.5% Agarose 进行电泳回收。

### 1.7 比较分析

利用 M-C 和 Shannon-Weaver 指数比较分析。M-C 指数为 20.4%, Shannon-Weaver 指数为 25.7%。
2.2 18S rDNA

<table>
<thead>
<tr>
<th>Clone No.</th>
<th>GenBank No.</th>
<th>Sequence comparison</th>
<th>Percent similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-80-06</td>
<td>DQ834836</td>
<td>556bp</td>
<td>99%</td>
</tr>
<tr>
<td>M-80-10</td>
<td>DQ834837</td>
<td>541bp</td>
<td>96%</td>
</tr>
<tr>
<td>M-80-20</td>
<td>DQ834838</td>
<td>549bp</td>
<td>98%</td>
</tr>
<tr>
<td>M-80-53</td>
<td>DQ834839</td>
<td>541bp</td>
<td>96%</td>
</tr>
<tr>
<td>M-80-64</td>
<td>DQ834840</td>
<td>518bp</td>
<td>92%</td>
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<td>M-80-66</td>
<td>DQ834841</td>
<td>556bp</td>
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<td>DQ834842</td>
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<tr>
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<td>DQ834843</td>
<td>557bp</td>
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<td>M-C-01</td>
<td>DQ834804</td>
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<td>M-C-12</td>
<td>DQ834805</td>
<td>548bp</td>
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<tr>
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<td>DQ834806</td>
<td>542bp</td>
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</tr>
<tr>
<td>M-C-32</td>
<td>DQ834807</td>
<td>548bp</td>
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</tr>
<tr>
<td>M-C-35</td>
<td>DQ834808</td>
<td>537bp</td>
<td>96%</td>
</tr>
<tr>
<td>M-C-58</td>
<td>DQ834815</td>
<td>545bp</td>
<td>97%</td>
</tr>
</tbody>
</table>

* The nucleotides of sequence comparison with the nearest similarity with the known sequence in Genbank.*

Table 2 Characters of 18S rDNA sequence in different soil samples
根据遗传距离建立的基因系统发育树

树用邻接法构建, 各枝上的数字是重抽样分析的支持百分比

讨论

土壤中只有极少一部分微生物是可以被培养的, 绝大多数因为无法培养而不为人所知。基于土壤微生物群落总分子方法避免了传统分离培养方法的缺点, 因此, 被广泛应用于土壤微生物群落结构、功能以及动态监测研究。另外, 随着测序以及数据库的建立和发展, 也促进了以为基础的分子生物学方法用于研究环境样品中微生物种群的多样性和特异性, 以及环境和种群进化的关系。

序列因其保守性强, 并含有可变区, 受环境因素影响较小, 常常被用于比较不同真菌菌株间的系统发育, 因此, 基因的研究和分析使真核生物之间的进化关系和特定环境下微生物种群的鉴定成为可能, 对微生物生态和微生物种群结构的研究提供了便利。

本研究应用和测序分析对川西亚高山米亚罗林区、菜地土壤的真菌基因的多样性和系统发育进行了比较。结果表明, 两类不同的土地利用类型具有明显不同的真菌克隆数、数和多样性指数, 在具有相对较高的数和多样性指数, 并且, 两个样地间具有不同地优势种群。因此, 表明具有更为丰富的真菌多样性, 土地利用变化明显地影响了土壤真菌的群落结果。已有的研究也大都表明土地利用覆盖变化对土壤真菌多样性具有明显的影响, 等同时应用、和测序研究了澳大利亚天然林和与之相邻地松树人工林土壤真菌多样性, 结果表明两者的真菌群落结构具有明显差异。等应用核糖体基因间隔区自动分析（）研究不同地理起源、植被覆盖、具有理化差异地土壤真菌群落, 发现不同地点土壤真菌图谱条带的数量、亮度及群落优势结构截然不同, 拉姆托热带地区土壤真菌主要条带在, 而其它地区在之间。等通过图谱分析了盐沼泽地子囊菌群落腐生真菌腐生早期和晚期群落组学报卷。

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影响土壤有机质的含量，而土壤有机质的增减也能改变土壤真菌的数量和组成。

从系统发育树上可以看，不同样地具有不同的优势种群，所有序列在系统发育树上都明显的分属于不同的簇，表明两种土地利用类成，指出其具有重要地空间同种性。

目前对土地利用变化影响土壤有机碳分解的机制还不完全清楚，土地利用变化可能通过影响土壤微生物应该说明的是，表中的数值并不是样地中真菌数量的极限值，因为工作量、时间和经费等的局限性，从并没有全部被用于克隆，且获得的克隆也没有全部用于

真菌参与土壤有机质的分解和腐殖质合成，直接

DNA

RFLP

References


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图1 18S rDNA 克隆文库中的OTUs 多样性和所占比例
Fig.1 Distribution of OTUs and clones in clone libraries for 18S rDNA