Hypogeous ectomycorrhizal fungi on Abies spp. roots and in small mammal diet in a Sierra Nevada mixed-conifer forest

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Objective:
- Quantitate species richness and relative abundance of ectomycorrhizal fungi on roots that
  1) form hypogeous or secotioid sporocarps
  2) are most commonly dispersed by small mammals

Introduction
Hypogeous fungi are important components of the food web in western coniferous forests (e.g. Maser et al. 1978). They are also very species-rich yielding as many as 65-80 species across a single hectare of mixed-conifer forest (North 2002). Their importance is further supported by studies in the Pacific Northwest which show that hypogeous sporocarp production can be much greater and is more temporally stable (North et al. 1997, Smith et al. 2002) than epigeous sporocarp production.

While they are abundant and important based on the sporocarp record, hypogeous fruiting taxa are noticeably absent in studies of ectomycorrhizal (ECM) root communities made up no more than 9% of the ECM species composition (Kenny et al. in press) and 15% of the ECM root biomass (Stendell et al. 1999). In this study we attempt to resolve this discrepancy to gain better insight into the ECM carbon-capturing potential (root biomass vs sporocarp record) and the potential biases inherent in our approaches to ECM root community analysis.

Study site - Teakettle Experimental Forest
- Located in the southern Sierra Nevada (California, USA)
- Old growth mixed-conifer
- Abies spp. dominated

Conditions in this forest should increase the importance of hypogeous fungi
- Summers are hot and dry
- A heavy litter layer has resulted from fire suppression and forms barriers to sporocarp emergence (image on left)
- Mammal mycorrhizal activity is high

Methods
The ECM root community centered around Abies spp. was characterized across 3 years (Izzo, unpublished). 104 species groups were identified by sequence analysis

78 sporocarps across 28 genera (17 new to the database) were chosen from the North (2002) study and lab herbarium samples directed at genera which were underrepresented in GenBank by ITS sequence

Scat samples from Glanocorys subrubens and Tamias speciosus were collected from Teakettle Forest across the same 3 years as the root study and the mammal mycorrhizal community characterized by spore typing (Meyer 2003). Fifteen scat samples were chosen across mammal species, year, location, and season to maximize potential to detect unique species

All samples were compared by sequence analysis (direct sequencing of PCR product) of the internally transcribed spacer region (ITS)

Results of sporocarp-root matching
- 21 root species identified as hypogeous species

<table>
<thead>
<tr>
<th>ECM sequence type</th>
<th>Root match (accession number if from this study)</th>
<th>% similarity by overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizopogon sp.</td>
<td>96% (AF377085)</td>
<td>99%/224</td>
</tr>
<tr>
<td>Geopora sp.</td>
<td>98% (AF230890)</td>
<td>99%/241</td>
</tr>
<tr>
<td>Russula sp.</td>
<td>96% (AF062930)</td>
<td>99%/241</td>
</tr>
</tbody>
</table>

Results of scat-root matching
- Successful PCR amplification of 55/58 scat samples
- Only 3 matches to root species
- Full characterization limited by direct sequence approach and primer bias

Conclusions
1) Species which fruit in hypogeous/secotioid manner are more prevalent on ECM roots than previously seen
- Methodological bias is certainly one reason why this important group has been missed. Directed effort to sample hypogeous fruiting for ECM community studies and addition of more sequences which allow species-level designations will allow us more insight into the contribution of this important group of fungi across more forest types.
- Teakettle forest conditions may be important factors in prominence of hypogeous abundance but a more direct comparative study is needed

2) Direct molecular analysis of fungi in small mammal scat is feasible and allows new views into fungal fructifying and dispersal ecology.