

Fungal diversity in decaying logs: proportion of ectomycorrhizal fungi revealed by DGGE fingerprinting and pyrosequencing

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Introduction

Wood-decaying fungi have traditionally been explored through sporocarp inventories. Nowadays molecular techniques enable also investigation of cryptic species. In boreal forest soil, ectomycorrhizal (ECM) fungi inhabit various habitats, including decayed wood. We were interested to see the proportion of ECM fungi in the fungal community inhabiting decaying wood. We also wanted to compare DGGE (denaturing gradient gel electrophoresis) fingerprinting to 454-pyrosequencing as a method for fungal community description.

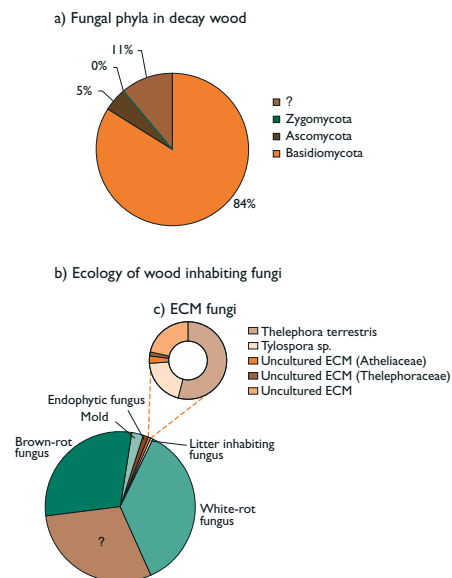


Fig. 1. Decay wood-inhabiting fungi classified by (a) phylum and (b) proposed ecology. (c) ECM fungal species detected. The figures are based on the preliminary pyrosequence data.

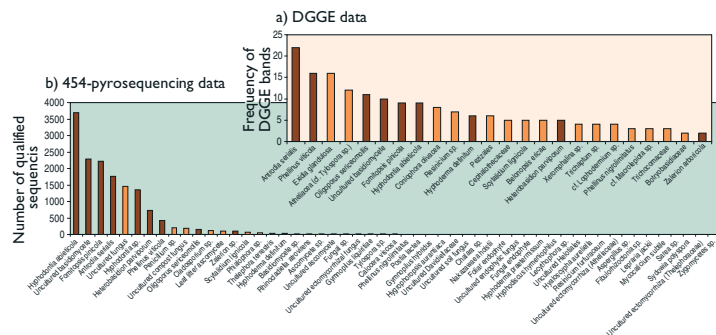
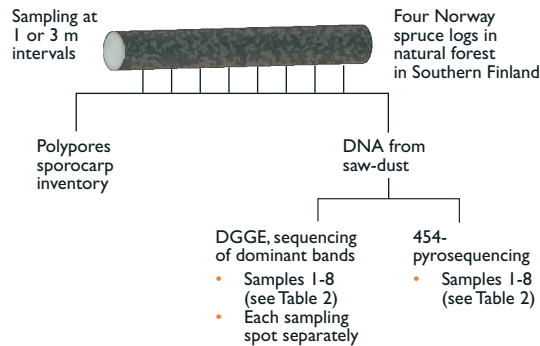


Fig. 3. Decay wood-inhabiting fungi found by (a) DGGE analysis coupled with direct sequencing and (b) 454-pyrosequencing.

Material and Methods



Results and Conclusion

Preliminary results indicate that the proportion of ECM fungi in the sampled decay logs was minor (Fig. 1). Proportion of ECM fungi increased during the wood decomposition (Table 1). Succession and increase in fungal diversity was also noted (Fig. 2). As expected, the pyrosequencing approach gave higher estimates of fungal diversity than DGGE fingerprinting or sporocarp inventory (Table 2). However, DGGE analysis revealed the dominant wood-inhabiting species (Fig. 3).

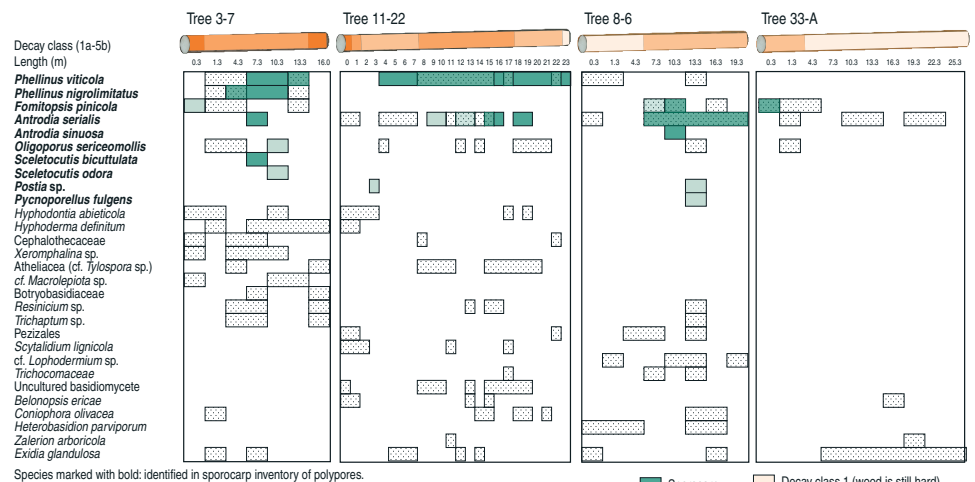


Fig. 2. Horizontal distribution of fungi in the decay logs. Decay class, polypores sporocars and saw-dust DNA samples were analyzed at 1- or 3-m intervals. DNA method used for fungal identification was PCR-DGGE and sequencing.

Table 1. Distribution of ECM fungi between the logs, which presented different decay classes. Result is based on the preliminary pyrosequence data.

	3-7	11-22	8-6	33-A
Thelephora terrestris	9%	55%	36%	0%
Uncultured ECM	8%	77%	0%	15%
Tylospora sp.	100%	0%	0%	0%
Uncultured ECM (Atheliaceae)	100%	0%	0%	0%
Uncultured ECM (Thelephoraceae)	0%	0%	0%	100%
Tot. ECM fungi	30%	46%	20%	5%
Average decay class	3.3	2.5	1.6	1.2

Table 2. Fungal diversity found through sporocarp inventory (polypores sp.), PCR-DGGE and 454-pyrosequencing in eight investigated samples.

Sample	Tree	Composition	Polypores sp.*	DGGE bands	454-identified species
1	33A	a spot: 1.3 m	0	3	7
2	3-7	a spot: 1.3 m	0	2	7
3	11-22	bulked: 1 m intervals	3	13	36
4	11-22	bulked: 3 m intervals	3	11	27
5	8-6	bulked: 3 m intervals	5	13	26
6	3-7	bulked: 3 m intervals	7	9	36
7	33A	bulked: 3 m intervals	1	10	44
8	All	bulked: all	10	17	33

* Dead sporocarps are included to the species numbers

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