Culturable fungi associated with wood decay of Picea abies in subalpine forest soils: a field-mesocosm case study

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Fungi are the principal wood decomposers in forest ecosystems and their activity provides wood necromass to other living organisms. However, the wood decay mechanisms and the associated microbial community are largely unknown, especially in Alpine areas. In this study, the culturable fraction of fungal communities associated with the decomposition of Norway spruce (Picea abies [L.] Karst) deadwood in subalpine forest soils were determined using microbiological methods coupled with molecular identification. Fungal communities were evaluated using in-field mesocosms after one year of exposition of P. abies wood blocks along an altitudinal gradient ranging from 1200 up to 2000 m a.s.l. comprising eight subalpine sites, four of them located at north- and other four at south-facing slopes. Although many saprotrophic species were isolated from the wood blocks, several white-rot species as the pathogenic fungi Armillaria cepistipes and Heterobasidion annosum, along with soft-rot fungi such as Lecythophora sp. were identified. Our results further indicated that the wood-inhabiting fungal community was mainly influenced by topographic features and by the chemical properties of the wood blocks, providing first insights into the effect of different slope exposure on the deadwood mycobiont in the subalpine forest ecosystem.

Keywords: Wood-inhabiting Fungi, Basidiomycota, Subalpine Forest, Wood Decomposition, Norway Spruce, Slope Exposure

Introduction

In forest ecosystems, a wide variety of wood-decaying fungi are saproxylic species that depend on deadwood during some stages of their life cycle (Speight 1989). The most important wood-decaying species belong to Basidiomycota and have generally been classified into two main groups, white-rot and brown-rot fungi, based on the cell wall component degraded (Riley et al. 2014, Pramod et al. 2015). Another important form of wood decay known as soft-rot includes several species belonging to mainly Ascomycota, which typically attack wood with a higher moisture content and preservative-treated wood (Rayner & Boddy 1988). The rate of wood decomposition is influenced by the chemical properties and the cell structures of the wood, the nature and the abundance of the wood-decomposing organisms, as well as by different ecological and climatic factors (Rajala et al. 2012, Hiscox et al. 2016). In fact, the influence of slope exposure on wood decay dynamics has recently been demonstrated in subalpine environments (Fravolini et al. 2016, Petritto et al. 2016a, Petritto et al. 2016b, Gómez-Bardón et al. 2017) and in subtropical and tropical forests (Geml et al. 2014, Parahong et al. 2017). However, it is so far unknown how exposure and, in general, climate affect the wood-inhabiting microbiota. In this context, we performed a study on the culturable wood-inhabiting fungi (WIF) in-field mesocosm experiment with the purpose of evaluating the fungal community colonizing Norway spruce (Picea abies [L.] Karst) deadwood after one year of exposition to natural conditions in subalpine forests.

Material and methods

The investigation area is located in Val di Rabbi in the south Alpine belt in northern Italy between a rather warm Insubrien and a cold Alpine climate. Eight sites along an altitudinal gradient ranging from 1200 up to 2000 m a.s.l. were investigated (Tab. S1 and Fig. S1 in the Supplementary material); four sites were positioned at north-facing slopes (N-4) and other four at south-facing (S6-9) slopes (Egli et al. 2006, Bardelli et al. 2017). At each study site a field experiment using soil mesocosms was set up as described in Fravolini et al. (2016). Mesocosms (PVC tubes having diameter = 10.2 cm and height = 25 cm) were installed into the natural soil in August 2012, that is one year prior to the addition of the wood blocks of P. abies, at a distance of > 1 m
from large trees and > 0.5 m from the adjacent mesocosms, leaving at the surface a border of about 1 cm. Wood blocks from the same P. abies tree and with a uniform size (2 × 5 × 5 cm) were placed on the soil surface in each of the mesocosm tubes.

Three replicate mesocosms were installed in each of the eight study sites (Fig. S2 in the Supplementary material). P. abies wood samples and soil samples (0-5 cm depth) were collected from each mesocosm in June 2014, placed in polyethylene bags and transported on ice to the laboratory for their physico-chemical characterization (Tab. S2 in the Supplementary material). Wood and soil physico-chemical properties were assessed as described by Fravolini et al. (2016) and Bardelli et al. (2017), respectively.

For the determination of the cultivable fungal decomposers all the wood blocks were surface sterilized by flaming and five small samples were cut out and placed on Petri dishes containing Hagem agar (HA) medium (Vasiliauskas & Sterlístico 1998) with addition of chloramphenicol (0.035 g L⁻¹, Sigma, MO, USA) and streptomycin (0.018 g L⁻¹, Sigma) in three replicates. All the inoculated plates were incubated at room temperature and continuously observed for 2 weeks. Soil samples were analysed by the Dilution Plate Technique: 10 g of soil were diluted with sterile water 1:10 (w/v) and mechanically shaken for 20 min. The suspensions were further diluted 1:10 and 1 mL aliquots of the suspension 10-4 were homogeneously distributed and incubated at room temperature and continuously observed for 2 weeks. Fungal colonies were afterwards sub-cultured and the species in pure culture were identified based on classical morphological features using light microscopy. The fungal morphological identification was further confirmed by sequencing of the ITS2 region (Miller et al. 2016) using the ITS3 (5'-GCATCGATGAAGAACGCA -1) - ITS4 (5'-TCTTCCGCTTATTGATATGC -3') primer pair (White et al. 1990) as described by Maresi et al. (2013). The ITS sequences were compared to those from the NCBI database (http://www.ncbi.nlm.nih.gov) to ascertain closest sequence matches. To visualize the WIF community compositions, we used three dimensional non-metric multidimensional scaling (3D-NMDS) analysis based on the Bray-Curtis dissimilarity index calculated in R (R Core Team 2015). Abiotic factors (including site, altitude and exposure) and chemical properties of the soil and deadwood were fitted to the NMDS ordination plots using the “envfit” function in the “vegan” package of R, and goodness-of-fit statistics (R²) were calculated with P values based on 999 permutations (Oksanen 2013).

Results and discussion

In total 215 fungal isolates were cultured and 52 fungal taxa were morphologically identified and their identification was complemented by molecular analysis based on ITS sequences. The isolates on BLAST analyses showed ≥97%-100% identity to the available sequences in NCBI (Tab. S3 and Fig. S3 in Supplementary material). Overall, Ascomycota was the dominant phylum (32/52 taxa), followed by Zygomycota (11/52), Basidiomycota (8/52) and sterile mycelia. In particular, 42 taxa were isolated from the soil samples in direct contact with the wood blocks; 18 taxa were common to both slopes, while 11 taxa were exclusively isolated from north- and 13 taxa from south-facing sites (Fig. 1). The soil cultivable fungal population was mainly characterized by Ascomycota (26 taxa) and Zygomycota (10 taxa) with a lower proportion of Basidiomycota (5 taxa). Differences in community composition among the different sites were also observed (Fig. 1, Fig. 2a, Fig. 2b), and total nitrogen (TN), electrical conductivity (EC) and altitude correlated significantly with the soil fungal community composition (Tab. 1). From the wood blocks, a total of 24 fungal species were identified, of which 13 belong to Ascomycota, 6 to Zygomycota and 4 to Basidiomycota.
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Tab. 1 - Goodness-of-fit statistics (R²) for factors fitted to the three dimensional non-metric multidimensional scaling (3D-NMDS) ordination of the soil and wood fungal community composition. The significance was based on 999 permutations. (nd): not detected; (EC): electrical conductivity; (TC): total carbon; (TN): total nitrogen; (Ptot): total phosphorous; (Pav): available phosphorous; (*): P < 0.05; (**): P < 0.01; (***): P < 0.001.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Soil (R²)</th>
<th>P</th>
<th>Wood (R²)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure</td>
<td>0.209</td>
<td>0.168</td>
<td>0.358</td>
<td>0.025*</td>
</tr>
<tr>
<td>Altitude</td>
<td>0.305</td>
<td>0.045*</td>
<td>0.184</td>
<td>0.378</td>
</tr>
<tr>
<td>Moisture</td>
<td>0.168</td>
<td>0.284</td>
<td>0.303</td>
<td>0.048*</td>
</tr>
<tr>
<td>Volatile solids</td>
<td>0.199</td>
<td>0.218</td>
<td>0.095</td>
<td>0.585</td>
</tr>
<tr>
<td>pH</td>
<td>0.136</td>
<td>0.389</td>
<td>0.018</td>
<td>0.943</td>
</tr>
<tr>
<td>EC</td>
<td>0.410</td>
<td>0.013*</td>
<td>0.0210</td>
<td>0.924</td>
</tr>
<tr>
<td>TC</td>
<td>0.205</td>
<td>0.206</td>
<td>0.461</td>
<td>0.006**</td>
</tr>
<tr>
<td>TN</td>
<td>0.427</td>
<td>0.009**</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>0.248</td>
<td>0.116</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>0.279</td>
<td>0.077</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ptot</td>
<td>0.089</td>
<td>0.617</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pav</td>
<td>0.086</td>
<td>0.629</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cellulose</td>
<td>-</td>
<td>-</td>
<td>0.182</td>
<td>0.227</td>
</tr>
<tr>
<td>Lignin</td>
<td>-</td>
<td>-</td>
<td>0.095</td>
<td>0.588</td>
</tr>
</tbody>
</table>

Tab. 2 - PERMANOVA table showing differences in community composition (based on a Bray-Curtis dissimilarity matrix) among altitude and exposure, for the soil and wood fungal communities. (•): P < 0.05; (**): P < 0.01; (***): P < 0.001.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Soil fungi (R²)</th>
<th>P</th>
<th>Wood fungi (R²)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>altitude</td>
<td>0.18</td>
<td>0.001***</td>
<td>0.07</td>
<td>0.05*</td>
</tr>
<tr>
<td>exposure</td>
<td>0.06</td>
<td>0.05*</td>
<td>0.2</td>
<td>0.001***</td>
</tr>
<tr>
<td>altitude : exposure</td>
<td>0.11</td>
<td>0.01**</td>
<td>0.12</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

deadwood using culture-independent molecular techniques on forest plots of the German Biodiversity Exploratories in southwestern Germany (Hoppe et al. 2016). Furthermore, these species represent necrotrophic and saprotrophic fungi including some of the most detrimental pathogens in conifer forests, which are capable of degrading wood, infecting the roots and stems and causing root white-rot (Keč & Solheim 2011, Gori et al. 2013, Pramod et al. 2015). Lecytophora sp. and Humicola sp. were isolated from the wood blocks at site N1 and from soil at site S6, respectively, and species affiliated with these genera have already been associated with soft-rot decay of treated wood (Bugos et al. 1988). Interestingly, we found that three WIF taxa affiliated with the Trichoderma genus (T. viridescens, T. atroviride, T. citrinoviride) were also isolated in a recent study on wood decay fungi across European forest ecosystems (Blaszczyk et al. 2016). Although they are frequently isolated in decaying wood and plant material (Longa et al. 2009), Trichoderma spp. are weak decomposers of non-decayed wood, but the delignification performed by white-rot fungi improves the accessibility of the woody material (Fukasawa et al. 2011). The basidiomycete Coprinellus radians was isolated from the wood blocks at sites S7 and S8 and from the soil samples at N2 and S8 and it has been hypothesized that Coprinus species might have abilities of white-rot fungi on pre-decayed wood (Badalyan et al. 2011). Moreover, several saprotrophic ascomycetes species found in this study like Aspergillus sp., Penicillium sp., Cadosporium sp. and Epicoccum sp., along with members of the phylum Zygomycetes may influence the decay process in large woody debris, but in general they play a subordinate role as direct agents of wood decay (Stenèl et al. 2008) because they primarily utilize compounds derived from the action of wood degraders (Lindahl & Olsson 2004).

In summary, the study of cultured fungi associated with the early stage of P. abies deadwood decay in subalpine forest soils resulted in a large number of species mainly belonging to the phyla Ascomycota and Zygomycota, which are generally saprotrophic with low impact as direct wood decay agents. Most of the isolates could be cold-adapted as they belong to species that are reported in periglacial soil at about 2500 m a.s.l. (Rodolli et al. 2016). In fact, methods based on culturing are known to
favor rapidly-growing fungi (like saprotrrophs), and on the contrary, obliged plant pathogens or mutualistic biotrophic fungi such as ecto-mycorrhizal species are notoriously difficult or impossible to isolate (Bonito et al. 2016). However, several white-rot species affiliated with Basidiomycota – that have been previously associated with the decay process of deadwood in forest ecosystems – were isolated and identified with the cultivation method used in the present study. Some of these species were only isolated from wood blocks at particular sites, indicating that, besides deadwood chemical properties, altitude and exposure are important drivers for WIF community composition in subalpine forest ecosystems. A further step in the identification of the fungal taxa associated with deadwood decay processes would consist in a taxonomical characterization of the fungal community of the studied wood blocks using high-throughput next generation sequencing technologies.

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References


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Supplementary Material

Tab. S1 - Characteristics of the eight study sites at north- and south-facing slopes (N1-4 and S6-9, respectively) in Val di Rabbi (Trentino, Italy).

Tab. S2 - Physico-chemical properties of soil and wood samples collected in July 2014 in the in-field mesocosm experiment.

Tab. S3 - Fungal taxa isolated from soil (So) and wood (W) along an altitudinal gradient in subalpine forest in Val di Rabbi (Trentino, Italy).

Fig. S1 - Overview of the study area (Trentino, Italy) with major vegetation units.

Fig. S2 - Example of the destructive in-field mesocosm sampling (wood and soil).

Fig. S3 - Number of fungal taxa identified for each site for the wood (a) and soil (b) samples (n=3) along an altitudinal gradient in a subalpine forest in Val di Rabbi (Trentino, Italy).

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