How does fungal diversity change based on woody debris type? A case study in Northern Spain

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Studies on lignicolous fungal communities in southern Europe are scarce and most of them focused on coarse woody debris (CWD). Fine (FWD) and very fine woody debris (VFWD) are rarely studied. For this study, all woody debris of 90 beechwood plots (in Navarre, Northern Spain) was checked to search for lignicolous macromycetes. Although most species were found on CWD, small sized woody debris being especially diverse on Ascomycota species, and big sized woody debris on Basidiomycota ones. The CAP analysis revealed that fungal communities from different diameter woody debris classes were dissimilar and that fungal composition changed gradually, from smaller diameter woody debris towards coarser ones, and vice versa. Formed groups were also different in fungal species richness, VFWD and CWD groups having a higher number of habitual and frequent species. Additionally, fungal composition changed according to beechwood types; thus faithful species for each woody debris type in different beechwood types were found. Finally, the structure and composition of fungal community changed in different decay stages and the CAP analysis showed three main groups: communities associated with weakly, moderately and strongly decayed wood.

**Key words:** decay stage, diameter, Fagus sylvatica, lignicolous fungal community, macromycetes, Navarre

INTRODUCTION

Dead wood is an essential component in forest ecosystems, maintaining carbon store in long term and providing the nutrients and habitat to a large number of organisms, including birds, mammals, arthropods, nematodes, bryophytes, lichens, fungi and bacteria. Fungi are a crucial part of this biodiversity (Siitonen, 2001), and they play a key role for the diversity of organisms associated with dead wood. The enzymatic and physical actions of wood-inhabiting fungi chemically and structurally change the wood resulting in numerous habitats and food resources (Niemelä et al., 1995; Renvall, 1995) that are irreplaceable for most of the other organisms living in dead wood (Boddy, 2001).

Nevertheless, organisms associated with wood represent an important but vulnerable group (Heilmann-Clausen, Christensen, 2004). Man has severely manipulated the European beech forest landscape for centuries (Peterken, 1996) causing a simplification of biological and structural complexity in most present-day beech forests. In this way, the regular extraction of any woody debris in traditional forest management has caused a large decrease of this habitat (Harmon et al., 1986). In addition, forest fragmentation has imposed further difficulties for dispersal of dead wood dependent organisms (Lonsdale et al., 2008). The combination of these reasons has caused most wood dependent organisms to have decreased or become extinct locally and regionally (ECCB 1995; Siitonen, 2001).
At the regional scale, climatic conditions, soil type, tree species composition, continuity and management history have been identified as important variables influencing fungal species diversity and community structure (Boddy, 1992; Sippola, Renvall, 1999; Küffer, Senn-Irlet, 2005a; Heilmann-Clausen et al., 2005). At the local scale, wood inhabiting fungal composition is conditioned by dead wood quality, which is mostly influenced by three features: size of dead woody debris, degree of decomposition and host tree species (Küffer, 2008). Most of the works that have studied wood inhabiting fungal diversity have focused on large logs and trunks (Bader et al., 1995; Heilmann-Clausen, Christensen, 2003), although recently the emphasis has been laid on smaller woody debris (Heilmann-Clausen, Christensen, 2004; Nordén et al., 2004; Küffer, Senn-Irlet, 2005b). Results have shown that branches and twigs may be particularly rich in species where there is little other substrate available, such as in managed forests (Küffer, Senn-Irlet, 2005b), and that ascomycetes are especially highly dependent on fine woody debris (Nordén et al., 2004).

As dead wood undergoes physical and chemical changes during the decomposition process, a successional pathway is established, firstly colonising pioneer species, later being replaced by others. Some studies have revealed that decay stage is the most important variable for understanding fungal community at the local scale (Renvall, 1995; Höiland, Bendiksen, 1996; Heilmann-Clausen, 2001).

In Northern and Central Europe several studies about the effect of wood inhabiting fungi on the biodiversity of forest ecosystems have been carried out, however, in Southern Europe there are still areas where lignicolous fungal diversity is not fully known (Tellería, 2002) and studies of lignicolous fungal communities are scarce, such as the Iberian Peninsula (Heilmann-Clausen, Walleyn, 2007). It is also noteworthy that there are few studies that have dealt with all the macromycetes fruiting in dead wood, especially not taking into account those that grow on very fine woody debris and resupinate fungi due to their laborious collection and identification, despite being the main agents of wood decay (Swift, 1982).

This study aims to answer the following questions: (1) How diverse and dissimilar are the fungal communities in different-sized woody debris? (2) Is it possible to assess the changes in the fungal community throughout the wood decaying process?

**MATERIALS AND METHODS**

**Study area**

The area of study was located in the north of the province of Navarre (Northern Spain, see Fig. 1). In the territory the beechwood is well represented and corresponds to the south-western limit of the natural distribution area of European beech. According to the biogeographical areas and substrate, the principal beechwood types were selected:

![Fig. 1. Map of the Iberian Peninsula and the location of 90 sampled plots in Navarre. The numbers correspond to the three principal beechwood types: 1 - Carici sylvaticae – Fago sylvaticae S.; 2 - Saxifrago hirsutae – Fago sylvaticae S.; 3 - Scillo – Fago sylvaticae S.](image-url)
1) basophilous and ombrophilous beechwoods in the Cantabrian-Atlantic Province with calcareous soil (*Carici sylvatica* – *Fago sylvatica* S.); 2) acidophilous and ombrophilous ones in the Cantabrian-Atlantic Province with acidic soil (*Saxifrago hirsutae* – *Fago sylvatica* S.); 3) Pyrenean basophilous and ombrophilous beechwoods in the Pyrenean Province with calcareous soil (*Scillo – Fago sylvatica* S.) (Loidi, Báscones, 1995). Climatic conditions and vegetation vary considerably among the classified forest stands (Table 1). Pyrenean ones (beechwood type 3) have a more mountainous character than others, growing at higher altitude and with lower temperatures. In the Cantabrian-Atlantic region two beechwood types can be differentiated according to the substratum and rainfall characteristics: on the one hand, beechwoods with basophilous substratum with low precipitation (beechwood type 1), and, on the other hand, those growing on acidophilous substratum and with higher precipitation (beechwood type 2). All stands are dominated by beech, but in some cases other tree species also occur (Table 1).

### DATA COLLECTION

According to three different beechwood types, a stratified random sampling scheme was used for data collection. In each beechwood type, 30 rectangular plots of 100 m² were randomly located (Fig. 1). To ensure the presence of all woody debris types, the randomization was restricted by the presence of at least one big log. For each plot all woody debris were checked for fungal identification and all lignicolous macromycetes were identified (fungi with sporocarps larger than 1 mm), registering their frequency for different woody debris types according to the diameter, decay stage and host species. When microscopic identification was necessary the material was removed for further identification in the laboratory.

According to the diameter, the following classification of woody debris has been used: 1) Very Fine Woody Debris (VFWD): branches and twigs with ≤ 5 cm diameter; 2) Fine Woody Debris (FWD): logs with diameter between 5 and 10 cm; 3) Coarse Woody Debris (CWD): logs or snags with diameter ≥ 10 cm.

Decay stages were defined following a modified classification of Renvall (1995): (I) wood hard, trunk or branch is a solid piece, pushed knife penetrates only a few mm into the wood; (II) wood fairly hard, bark usually present but not firmly attached, pushed knife penetrates 1–2 cm into the wood; (III) wood fairly soft, small areas of sapwood already decomposed and without bark where knife penetrates easily; (IV) wood soft, wood pieces extensively decayed and usually larger sections of the wood completely decomposed, the knife penetrates through the wood easily; (V) wood very soft, almost completely decomposed

<table>
<thead>
<tr>
<th>Beechwood type</th>
<th>Size (ha)</th>
<th>Other trees or shrubs</th>
<th>Elevation range (m)</th>
<th>Substratum (Bedrock)</th>
<th>Tave¹ (°C)</th>
<th>Tmin² (°C)</th>
<th>Tmax³ (°C)</th>
<th>Precipitation⁴ (mm)</th>
<th>Ic⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basophilous</strong>&lt;br&gt;(Type 1)</td>
<td>24.7</td>
<td><em>Crataegus monogyna</em>, <em>C. laevigata</em></td>
<td>525–1 460</td>
<td>limestone, dolomite</td>
<td>11</td>
<td>4.5</td>
<td>18.5</td>
<td>1 300–1 600</td>
<td>14</td>
</tr>
<tr>
<td><strong>Acidophilous</strong>&lt;br&gt;(Type 2)</td>
<td>24</td>
<td><em>Ilex aquifolium</em>, <em>Quercus robur</em></td>
<td>225–1 550</td>
<td>sandstone, clay</td>
<td>12</td>
<td>5.5</td>
<td>19</td>
<td>2 000–2 500</td>
<td>13</td>
</tr>
<tr>
<td><strong>Pyrenean</strong>&lt;br&gt;(Type 3)</td>
<td>19</td>
<td><em>Abies alba</em>, <em>Pinus sylvestris</em>, <em>Buxus sempervirens</em></td>
<td>800–1 700</td>
<td>limestone, sandstone</td>
<td>9</td>
<td>2</td>
<td>16.5</td>
<td>1 500</td>
<td>15</td>
</tr>
</tbody>
</table>

¹Mean annual temperature  
²Mean temperature of the coldest month  
³Mean temperature of the warmest month  
⁴Annual precipitation  
⁵Simple continentality index (mean annual temperature range)  
Rivas-Martínez, Rivas-Sáenz (1996–2009)
and disintegrates easily between fingers. The decay stage was obtained for each fungal species, measuring in the area where fruitbodies grew.

The fieldwork was carried out in 2010 during the main period of fruiting production (end of September to early December) with one visit to each plot. A species found on a single piece of wood was considered as one record, regardless of the number of fruitbodies. The frequency of each species was recorded by the number of records in each woody debris piece of each plot.

The collected fruitbodies were identified, mostly following Eriksson, Ryvarden (1973, 1975, 1976); Eriksson et al. (1978, 1981, 1984); Hjortstam et al. (1987, 1988); Jülich, Stalpers (1980); Tellería, Melo (1995); Bernicchia (2005); Breitenbach, Kränzlin (1986); Bernicchia, Gorjón (2010). In addition, for some groups special literature was consulted (e. g. Boidin, 1994; Dueñas, 2005).

DATA ANALYSIS

To analyze and compare fungal communities multivariate analyses were carried out using PRIMER-E and PERMANOVA+ for PRIMER 6 software package (Clarke, Gorley, 2006; Anderson et al., 2008). To observe the similarity of fungal communities Bray-Curtis resemblance index was used (Bray, Curtis, 1957). To determine the response of fungal composition to factors (e. g. woody debris class, beechwood type and wood decay stage) with the analysis of variance, Permanova statistical method was used. To observe the samples grouping in two-dimensional scale, canonical analysis of principal coordinates (CAP) was run. Spearman’s rank coefficient was used to observe correlation between species and formed groups. Permutational tests of homogeneity of multivariate dispersions (PERMDISP) were used to check the homogeneity of groups.

RESULTS

Fungal diversity

In total, 3799 records of 274 species were collected over the period of investigation in all the study plots. The species belong to 143 genera, 25 orders and 2 phyla. The largest part of fungi belongs to the phylum Basidiomycota (90%), whereas the phylum Ascomycota comprises a smaller part (10%). The number of species found in debris of each beechwood type were similar, 180 species in basophilous, 170 species in acidophilous and 169 in Pyrenean beechwoods. Five most common species of the phylum Ascomycota were Diatrype disciformis (Hoffm.) Fr. (344 records), Hypoxylon fragiforme (Pers.) J. Kickx f. (329 records), Biscogniauxia nummularia (Bull.) Kuntze (119 records), and Kretzschmaria deusta (Hoffm.) P. M. D. Martin (72 records); Five most common species of Basidiomycota were Stereum ostrea (Blume, T. Nees) Fr. (152 records), Xenasmatella vaga (Fr.) Stalpers (115 records), Stereum hirsutum (Willd.) Pers. (113 records), Fomes fomentarius (L.) J. J. Kickx (112 records) and Trametes gibbosa (Pers.) Fr. (95 records). On the other hand, 28% of the collected species are rare, with just a single record.

Fig. 2 shows the distribution of species richness and number overlap in three woody debris classes. As shown in this figure, the number of species changed with woody debris size, and the largest part of the species (201 species) appeared on CWD, while on FWD were found 164, and on VFWD 157. Moreover, CWD being the most diverse woody debris type in the number of species, it also had the majority of exclusive species (70). The number of species that different woody debris classes shared also varied and debris types that share the highest number of species were CWD and FWD, while debris with lower species in common were CWD and VFWD.
Although most lignicolous species were found on CWD, not all the fungal groups had shown the same trend (Fig. 3). In case of Ascomycota, 50% of species were found exclusively on VFWD, 20% exclusively on FWD and 30% exclusively on CWD, whereas Basidiomycota had shown an inverse distribution among each woody debris class (Fig. 3).

The greatest part of fungi (69%) was found in the earliest decay stages (I and II), whereas in advanced decayed wood the number of species found was lower, 48% in III decay stage and 37% in IV and V decay stages.

**Fig. 3.** The proportion of the number of species found exclusively on each woody debris class (VFWD, FWD and CWD) for Basidiomycota and Ascomycota

**Fungal community**

The differences in species composition among different woody debris classes of sampled plots are precisely visualized in the CAP analysis (Fig. 4). The canonical analysis of principal coordinates revealed that fungal communities from different woody debris classes were statistically different (Permanova test, \( p = 0.0001 \)) and that fungal composition changed gradually, from smaller diameter woody debris towards coarser ones, and vice versa (axis \( x \)). The correlation between axis \( x \) and fungal composition was 0.69. This analysis also demonstrated that fungal communities were different in faithful species, VFWD and CWD having a higher number of constant and frequent species (axis \( y \)), like *Diatrype disciformis* (Hoffm.) Fr. and *Bisporella citrina* (Batsch) Korf, S. E. Carp. on VFWD and *Fomes fomentarius* (L.) J. J. Kickx and *Kretzschmaria deusta* (Hoffm.) P. M. D. Martin on CWD (species with at least 0.5 Spearman’s correlation value), whilst FWD was characterized by the absence of such faithful species (Fig. 4, Table 2).

Permanova test showed that there was an interaction between beechwood type and woody debris class (\( p = 0.0051 \)). In this way fungal communities
of each woody debris class in each beechwood type were different. In the analysis of fungal composition for each forest type it has been observed that these were dissimilar, with one exception, fungal communities of CWD, in Pyrenean and basophilous beechwoods (Pair Wise test). In Fig. 5 the spatial ordination of beechwood plots for each woody debris class is represented. The most associated species of acidophilous beechwoods were *Trametes versicolor* (L.) Lloyd on all woody debris classes, *Hyphoderma setigerum* (Fr.) Donk on FWD, and *Trichaptum biforme* (Fr.) Ryvarden on FWD and

<table>
<thead>
<tr>
<th>CWD</th>
<th>Spearman's correlation</th>
<th>VFWD</th>
<th>Spearman's correlation</th>
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</thead>
<tbody>
<tr>
<td><em>Fomes fomentarius</em></td>
<td>0.75</td>
<td><em>Diatrype disciformis</em></td>
<td>0.8</td>
</tr>
<tr>
<td><em>Kretzschmaria deusta</em></td>
<td>0.6</td>
<td><em>Bisporella citrina</em></td>
<td>0.5</td>
</tr>
<tr>
<td><em>Fomitopsis pinicola</em></td>
<td>0.4</td>
<td><em>Gloeocystidiellum porosum</em></td>
<td>0.4</td>
</tr>
<tr>
<td><em>Oudemansiella mucida</em></td>
<td>0.35</td>
<td><em>Exidia thuretiana</em></td>
<td>0.4</td>
</tr>
<tr>
<td><em>Asccoryne cylichniun</em></td>
<td>0.25</td>
<td><em>Peniophora cinnerea</em></td>
<td>0.3</td>
</tr>
<tr>
<td><em>Ganoderma pfefferi</em></td>
<td>0.25</td>
<td><em>Athelopsis glaucina</em></td>
<td>0.25</td>
</tr>
<tr>
<td><em>Trichaptum biforme</em></td>
<td>0.25</td>
<td><em>Skeletocutis nivea</em></td>
<td>0.25</td>
</tr>
</tbody>
</table>

![Fig. 5](image-url)  
**Fig. 5.** Canonical analysis of principal coordinates (CAP) in three woody debris classes for all beechwood plots. Species with at least 0.4 Spearman’s correlation value are also given.
CWD. For basophilous ones the most associated species were *Exidia thuretiana* (Lév.) Fr. and *Diatrype disciformis* (Hoffm.) Fr. on VFWD. In case of Pyrenean beechwoods, the most associated species was *Royoporus badius* (Pers.) A. B. De on CWD. The species *Xenasmatella vaga* (Fr.) Stalpers was faithful in beechwoods with a more continental character (Pyrenean and basophilous ones) both on VFWD and FWD.

The structure and composition of fungal community was different according to wood decay stages (Fig. 6), and the CAP analysis revealed that there were three statistically different groups (Permanova test, p = 0.0001). Firstly, the fungal communities of the earliest stages of decomposition process (I and II decay stages), followed by communities of intermediate decomposition stages (III decay stage), and finally the communities that grow in strongly decayed wood (IV and V decay stages).

**DISCUSSION**

In this study, fungal diversity has been quantified recording the presence of macromycetes on wood. Although there were certainly mycelia which did not produce fruitbodies, the macromycetes inventory is presently assumed as one of the most appropriate methods in large field surveys, because molecular methods are still significantly skewed (Avis et al., 2010), expensive and not yet available for many species (Ovaskainen et al., 2010); whilst fruitbody inventory provides data suitable for comparison between different woody debris classes (Heilmann-Clausen, Christensen, 2004; Küffer, Senn-Irlet, 2005b).

The properties of dead wood vary depending on woody debris size and decomposing process. On the one hand, woody debris diameter determines the microclimate, decomposition rate, ecological niches diversity and the amount of...
substrate. On the other hand, in the course of decomposition process, woody debris undergoes structural and chemical changes. The effect of these two variables on fungal communities composition is shown in this study by the CAP analyses.

In terms of woody debris diameter, our results have shown that fungal composition changes significantly in three woody debris classes, and that each debris category possesses species which grow exclusively on it. Thereby, fungal communities that grow in VFWD must be specialized to unstable climatic conditions, high decomposition rates, and high surface area and wood volume ratio (i.e. less wood volume per surface area unit). Nevertheless, CWD offers to wood inhabiting fungi a more stable resource and a large amount of substrate. In this way, both VFWD and CWD are characterized by constant and frequent species such as Bisporella citrina and Diatrype disciformis on VFWD, and Fomes fomentarius and Kretzschmaria deusta on CWD (with a high correlation value between debris class and species). The conditions that FWD offers are between those given by VFWD and CWD remains. Thus the fungal community found in this woody debris class, in many cases, overlapped the other two communities and is marked by the absence of faithful species.

Woody debris with diameter less than 10 cm has often been neglected in many earlier studies, and only CWD has been studied, due to CWD volume in managed forests being considerably lower than in unmanaged ones, and also because wood inhabiting organisms diversity per unit is higher on CWD than on thinner ones. However, the interest in understanding mycobiont diversity related to thinner woody debris has recently increased, after VFWD class had been proposed. Although the number of exclusive species decreases in thinner woody debris, it is remarkable that VFWD is especially important for specific groups of fungi, such as Ascomycota, which are adapted to live in branches and twigs (Rayner, Boddy, 1997). Nordén et al. (2004) were the first to point out the difference in frequency between Ascomycota and Basidiomycota on CWD and FWD. The data of this study are in agreement with the results obtained by Nordén et al. in deciduous forests of Central Europe, where the importance of small diameter woody debris for the phylum Ascomycota was revealed.

Several studies have shown that fungal community changes during wood decomposition process (Renvall, 1995; Heilmann-Clausen, Christensen, 2003; Heilmann-Clausen et al., 2005), and some authors hold that decay gradient is the dominant factor structuring communities (Høiland, Ben-diksen, 1996; Lindblad, 1997). As can be seen in Fig. 6, according to wood decay stage three main groups can be distinguished: (1) pioneer species, preferring recently fallen or weakly decomposed wood, (2) intermediate stage species, which replace previous species when easily decomposable components of the wood were consumed, (3) late stage species, the ones that are specialised in degrading more complex components of the wood. This succession is often very strict, and in some cases the presence of one species depends on the preceding one (Renvall, 1995; Heilmann-Clausen, Boddy, 2005).

Contrary to the results in other studies of Europe (e.g. Renvall, 1995), in our study the majority of species were found on wood of early decomposition stages, and the lowest number of species was found on strongly decayed wood. However, this can be explained by the fact that the largest part of the sampled beechwood area is currently or recently managed, so most of the examined woody debris was in the earliest decay stage and fungal communities from the latest decay stages are not completely developed.

An overall change of fungal composition has been observed along the climatic gradient from acidophilous beechwoods with an oceanic character, to Pyrenean and basophilous beechwoods with a more continental character. This general pattern is repeated in the analysis of species composition among woody debris types, and consequently faithful species of each woody debris type have varied according to the climatic characteristics.

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**GRYBŲ ĮVAIROVĖS KAITA REMIANTIS MEDIENOS LIEKANIŲ TIPU (ŠIAURĖS ISPANIjos TYRIMŲ PAVYZDYS)**

**Santrauka**


**Raktažodžiai:** irimo stadija, diametras, *Fagus sylvatica*, lignofilinių grybų bendrija, makroskopiniai grybai, Navara