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Importance of fungal decomposition of wood in the ecosystems of natural forests

Význam rozkladu dřeva houbami v ekosystémech přirozeného lesa

Diploma thesis

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Declaration:

I declare that all sources and literature are properly cited and that the content of this thesis or its major part was not previously used to obtain the same or other academic degree.

Prague, 15th August 2017

Bc. Lucie Štercová

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Abstract

The decomposition of organic substrates represents an important part of the global carbon cycle and affects its global change through CO_2 release. In temperate forests, deadwood represents a large carbon stock, its amount and decomposition is crucial for ecosystem biodiversity and functioning. The fungi are omnipresent powerful decayers in all terrestrial ecosystems. Their ability to decompose all deadwood compounds, mainly lignocellulose, is highly important. Without fungi, the wood decompositions and the release of withheld nutrients back to nutrient cycles couldn´t be performed. While many studies were concerned with the estimation of decomposition rates of deadwood, still deeper knowledge about microbial decomposition processes and the diversity of saproxylic species and their interaction is needed. The fungi are still underrepresented in dead wood studies. This study had two main objectives. First was to describe the fungal community on downed deadwood of *Fagus sylvatica* and *Abies alba* in natural forest of Salajka in the Czech Republic, to reflect the substrate changes during the different decay stages, and to link the enzyme activities to fungal community composition and their described ecophysiologies. Second aim was to describe the fungal communities on standing and downed dead logs of *Fagus sylvatica*, *Picea abies* and *Abies alba*, in natural forest of Žofín in the Czech Republic, to identify if there existe different pattern of decay, and which of the studied factors among tree species, time of decay and wood chemistry is the most important for fungal community composition. When examined the downed deadwood in Salajka, the fungal community was the most influenced by the tree species and its chemical composition, older the logs were, the more homogenous the community become. The difference between standing and downed logs weren´t confirmed statistically, nevertheless the results suggested a possible difference in fungal decay. More samples of standing logs would be necessary to confirm or disprove these suggestions. Although, this study already demonstrated the necessity to deepen the focus on various type of deadwood in forest ecosystems to implement their nutrient fluxes into ecological predictive models. The accurate prediction of ecosystem development, facing global climate change, is the next major challenge for environmental microbiology.

Keywords: deadwood dynamics, wood-decaying fungi, decomposition, microbial community, next-generation-sequencing

Abstrakt

Rozklad organických částí představuje důležitou součást globálního cyklu uhlíku a ovlivňuje jeho světové změny prostřednictvím uvolňování CO_2. V temperátních lesích představuje mrtvé dřevo velký zásobník uhlíku, jeho množství a rozklad je klíčovým pro biodiversitu afungování ekosystémů. Houby jsou všudypřítomní mocní rozkladači ve všech terestrických ekosystémech. Jejich schopnost rozkládat všechny součásti dřeva, halvně lignocelulózu, je vysoce důležitá. Bez hub by rozklad dřeva a uvolňování zadržovaných živin zpátky do nutričních cyklů nebyl možný. Zatímco se většina studií soustředila na odhad rychlosti tlení dřeva, stale hlubší poznání o mikrobiálních dekompozičních procesech a diverzitě saproxylických druhů a jejich interakcí je potřeba. Houby jsou stále nedostatečně zastoupeny ve studijích mrtvého dřeva. Tato studie měla dva hlavní cíle. Prvním bylo popsat houbovou komunitu na ležícím mrtvém dřevě *Fagus sylvatica* a *Abies alba* v přírodním lese Salajka v České republice, pro reflexi změn substrátu během různých tříd tlení a k propojení enzymových aktivit se složením houbového společenstva a jejich popsaných ecofyziologijí. A druhý cíl byl popsat houbové komunity na stojících a ležících mrtvých kmenech *Fagus sylvatica*, *Picea abies* a *Abies alba*, v přírodním lese Žofín, v České republice, k určení jestli existují různé vzorce tlení, a který ze studovaných faktorů mezi druhem stromu, časem tlení a chemickým složením dřeva ovlivňuje houbovou komunitu nejvíce. Při zkoumání ležícího mrtvého dřeva na Salajce byla houbová komunita ovlivněna nejvíce druhem dřeviny a jejím chemickým složením. Čím starší kmeny byli, tím podobnjší byla nalezená houbová komunita na nich. Rozdíly mezi stojícími a ležícími kmeny nebyli statisticky potvrzeny, nicméně výsledky indikovaly možný rozdíl ve způsobech tlení jinak položených kmenů. Pro potvrzení, či vyvrácení této hypotézy by bylo potřeba více vzorků stojících kmenů. Avšak již tato studie ukázala, že je potřeba prohloubit zájem o různé druhy mrtvého dřeva v lesních ekosystémech a implementovat tok jejich živin do ekologických prediktivních modelů. Správné modelování vývoje ekosystému, čelícímu globálním změnám klimatu, je další velkou vývou pro environmentální mikrobiologii.

Klíčová slova: dynamika tlejícího dřeva, dřevorozkladné houby, dekompozice, mikrobiální komunita, Ilumina MiSeq sekvenování

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List of abbreviations

5.8S - encoding gene of component of the large ribosomal subunit
ABTS - 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid
ANOVA - analysis of variance
BLASTn – nucleotide Basic Local Alignment Search Tool
CWD – coarse woody debris
DBH - diameter at breast height
DMAB - 3,3-dimethylaminobenzoic acid
DMSO - dimethylsulfoxid
EB – elution buffer
EcM – ectomycorrhizal
FWD- fine woody debris
G – guaiacyl subunit of lignin
H - *p*-hydroxyphenyl subunit of lignin
HSD - Tukey Honest Significant Difference test
ITS – internal transcribed spacer regions of the ribosome encoding
LSU- ribosomal large subunit encoding region
MBTH - 3-methyl-2-benzothiazolinone hydrazine
MnP - manganese peroxidase
MUF - 4-methylumbellyferol
MUFaG - 4-methylumbellyferyl-α-D-glucopyranoside
MUFC - 4-methylumbellyferyl-N-cellobiopyranoside
MUFG - 4-methylumbellyferyl-β-D-glucopyranoside
MUFN - 4-methylumbellyferyl-N-acetylglucosaminide
MUFP - 4-methylumbellyferyl-phosphate
MUFU - 4-methylumbellyferyl-β-D-galactopyranoside
MUFX - 4-methylumbellyferyl-β-D-xylopyranoside
MUFY - 4-methylumbellyferyl -heptanoate
NMDS - non-metric multidimensional scaling
OTU – operational taxonomic unit
S – syringyl subunit of lignin
SSU- ribosomal small subunit encoding region

1 Introduction

Deadwood is an important compound of forest ecosystems for both saproxylic and non-saproxylic species (Seibold *et al.*, 2015). It serves as a shelter, carbon pool, source of energy or platform for growth (Floudas *et al.*, 2012; Jonsson and Stokland, 2012). Deadwood is irreplaceable part of healthy, functioning forest biomes (Siitonen, 2001), nevertheless the decomposition processes aren´t fully understood. As wood grows, it locks carbon and other nutrients, essential for proper ecosystem functioning within its structure. The resulting lignocellulose is highly resistant to microbial attack (Pérez *et al.*, 2002; Watkinson, 2015) and without fungi, the wood decomposition would last hundreds years (Cornwell *et al.*, 2009). According to comparative phylogenomic and molecular clock data, fungi might have been using wood as a source of energy since the evolution of woody plants 400-500 million years ago (Watkinson, 2015). Since then, fungi adapted to all different kinds of lignocellulose material and nowadays play the main role in its decomposition. At the beginning of decay, the decomposition is slow due to dense wood structure and low moisture content. As the decomposition progress, the rate gradually increase and presumably peak in intermediated stages of decay (Mäkinen *et al.*, 2006). The density of wood decreases, becoming part of the soil during the last decay stages. It is essential for bio conservation purposes to establish the time necessary for wood decomposition, which factors influence the rate of wood decay (Přívětivý *et al.*, 2016).

The importance of fungi in forest ecosystems is mainly as primary decomposers of organic matter and its recycling for future use (Stokland, 2012; van der Wal *et al.*, 2013; Bässler *et al.*, 2016; Purahong *et al.*, 2016) hence, influencing the global carbon cycle (Watkinson, 2015). During the process of fungal decomposition, cellulose is freed from lignin and other organisms, without ability to fully decompose wood, like protozoa, nematodes, molluscs, crustacea, insects and arachnids (Watkinson, 2015; Kirchenbaur *et al.*, 2017), can feed on the delignified cellulose. The alternation of fungal biodiversity might modify the decomposition processes and consequently, the flux of nutrient ecosystem cycles (Kubartová, Ottosson and Stenlid, 2015; Peršoh, 2015). With progressive management of forest and deforestation, little by little, the natural habitats of fungi start to disappear and the fungal biodiversity progressively decreases. Most of the red listed fungi can be nowadays found only in old growth forests. Such decrease in fungal diversity might lead to deficiency in ecosystems functioning. Fungi use a specific set of powerful oxidative enzymes, hydrolases or other inventive techniques to decay the lignocellulose (Kubartová, Ottosson and Stenlid, 2015; Hoppe *et al.*,

2016), and they are the only organisms able to decay lignin under natural conditions (Peršoh, 2015). Despite the importance of these enzymes to deadwood decompositions, surprisingly their effect on decay rates has never been studied (Kahl *et al.*, 2017). The role of bacteria and their interaction with fungi in wood decay was widely discussed. Their function is estimated from commensal interaction, as fixators of nitrogen, to direct wood decayers (De Boer *et al.*, 2005; Hoppe *et al.*, 2014).

The importance, rate and mode of fungal wood decay are widely discussed topics in environmental ecology. The reasons why this topic stayed in the focus of scientific interest for so long, are numerous, mainly due to how difficult is to study microbial organisms in their native environment. The enlightenment came with the emergence of new technologies, mainly the next-generation sequencing. Processing of large data and the drop of sequencing costs, enabled many laboratories to use such technology in their studies, and access larger proportion of the microbial community. Numerous studies concerned with deadwood were published, still, there are some areas where the data are scarce. More information about fungal biomass and related enzymatic activities in decomposing logs (Noll *et al.*, 2016) are needed to further understand the wood decay and its dynamics. The composition of fungal community and climate are linked to the wood-decay rates and should be implemented into models of climate-carbon prediction models in forest ecosystems to accurately estimate the consequences of biodiversity changes on nutrient cycles (Kubartová, Ottosson and Stenlid, 2015; Venugopal *et al.*, 2016). Understanding the forces that structure the ecological communities is crucial in determination their contribution to ecosystem processes and the resilience to environmental change (Hiscox *et al.*, 2017). To fully understand the community development and therefore to predict the path which the community will take and how such development will most probably influence the decay rate, it is necessary to study the interaction between individuals inside the community, as a single organism might influence the community composition and development (Hiscox *et al.*, 2017).

This study aimed to describe the fungal communities in natural forest Salajka between different decay classes and the differences in fungal species composition of saproxylic communities on downed and standing dead trees in natural forest of Žofín.

2 Aims of the study

To describe the fungal community composition on decaying wood of beech and fir in natural forest of Salajka.

To compare the chemistry of two different tree species and found enzymatic activities and how they influence or are influenced by community composition in natural forest of Salajka.

To describe the fungal community on differently positioned decaying logs in natural forest of Žofín.

To determine if the position of logs has importance on wood decay and present fungal community, or what are the main factors influencing the decay of differently positioned deadwood.

3 Literature review

3.1 Function of deadwood in temperate forests

Forest biomes represent over 50 % of carbon (C) in terrestrial ecosystems, covering only 30 % of the Earth surface (Weedon *et al.*, 2009). The most important role of forest is the function of trees as primary producers, responsible for >90 % of the forest primary production (Baldrian, 2017). Forests act as carbon sinks, where C fixed by primary producers exceeds C loss by respiration by 7 %-25 % (Baldrian, 2017). Such large pool of carbon is stored in several ecosystem components, mainly dead wood (Cornwell *et al.*, 2009; Floudas *et al.*, 2012). Its amount and continuity is a crucial component of all forest ecosystems (Siitonen, 2001). Dead wood is an important habit for biodiversity of all species in forest ecosystems (Kirchenbaur et al., 2017). Globally, coarse dead wood represents 36-72 Pg of carbon and therefore is essential to understand the carbon cycling to accurately predict C cycle responses to global changes (Cornwell *et al.*, 2009). Despite the importance of coarse woody debris, the factors influencing flux rates and the size of dead wood pool sizes is still relatively poorly understood and their inclusion into climate models is usually in generalized form (Weedon *et al.*, 2009). It is well known that there is a connection between fungal ecology and carbon cycling, but the dynamics and interactions of fungi during decomposition processes needs further investigation (van der Wal *et al.*, 2013).

Deadwood can follow various path including microbial decomposition, combustion, extraction, consumption by insects or small animals and physical degradation (Cornwell *et al.*, 2009) and together with tree litter is an important source of recalcitrant organic matter (Baldrian, 2017). The decomposition of dead woody biomass is mostly affected by the activities of decomposer fungi (Venugopal *et al.*, 2016). The volume of coarse woody debris in natural forests can be up to 25–30 times more than in managed forests (Siitonen et al., 2000). Very few studies, look at the differences in decomposition processes between various forest ecosystems types (natural vs. managed) (Venugopal *et al.*, 2016).

Wood chemical composition is species specific, with high intra- and interspecies variability. The ration then changes but the key compounds remains. They are polysaccharides celluloses, hemicellulose, pectin, polymeric lignin, various extractives (free sugars, phenol compounds, terpenes, alkaloids, wax, lipids, ashes and others). They represent the essential reason why trees are extremely resistant to microbial attacks during their life, and sometime, even after them (Rytioja *et al.*, 2014).

Deciduous trees tend to have higher content of celluloses (38-47 %) and lower content of lignin (21-31 %), in the contrary to coniferous trees (celluloses 33-42 %; lignin 27-32 %). The main difference between softwood and hardwood is in their hemicelluloses composition. While softwood usually contains mostly galactoglucomannans, hardwood hemicelluloses are composed from glucuronoxylans (Rytioja *et al.*, 2014). Woody cells are composed from middle lamella, primary and secondary cell wall. Primary cell wall contains mainly cellulose, while secondary cell wall is strengthened by higher content of lignin, and its interconnection with other cell components. Carbon content in dead wood is significantly higher than nitrogen and phosphorus. The uneven ratio negatively influences the decomposition rate. Fungi decompose wood into CO_2, which is respired into atmosphere. With progressive decomposition, the carbon-nitrogen ration decreases and the decomposition rate increases. The formation of lignocellulose, high content of lignin and unfavourable C:N ration make microbial colonization of wood difficult (Floudas *et al.*, 2012) and influence decomposition. Decay of organic matter controls the balance between soil carbon storage and CO_2 release into the atmosphere, and releases mineral nutrients, which are again made available for plant growth.

When comparing softwood and hardwood, coniferous trees have lower concentration of phosphorus, calcium, nitrogen, potassium and higher ratio of C/N (Weedon *et al.*, 2009). Coniferous trees are characteristic by higher content of lignin, which is derived from conifer alcohol and it is more resistant to microbial degradation. It contains more alkaloids, phenols and other substances toxic for fungi. Therefore the decomposition of coniferous tress is slower in comparison to broadleaf trees (Cornwell *et al.*, 2009; Weedon *et al.*, 2009).

Certain fungi, mostly from genus Polyporales, restrict themselves to heartwood. Heartwood has, in general, a lower water content than sapwood of living trees, and a high water content is known to inhibit mycelial growth of many fungi and to limit decay because of low O_2 diffusion rates. Heartwood may also have high CO_2 content, and some of the specialist heart wood colonizers appear to be tolerant of high CO_2 levels while other less tolerant species are excluded. Heartwood is also rich in extractives, including alkaloids, resins, and phenolics, many of which colonize heartwood are clearly able to grow despite the presence of these extractives, and there is evidence that certain fungi that are selective for particular host trees are tolerant to extractives from those heartwoods (Dix, 2012).

Coarse woody debris (CWD; diameter > 10 cm), or fine woody debris (FWD; diameter < 10 cm) can be distinguished based on the diameter of dead wood in forests (Kruys and Jonsson, 1999; Allmér *et al.*, 2006; Bässler *et al.*, 2010; Venugopal *et al.*, 2016). Most of the studies of wood were done on CWD, and only few researches were focused on the contribution of FWD to ecosystems (Nordén *et al.*, 2004; Allmér *et al.*, 2006). But it seems that the contribution of all FWD, so to say, small fallen branches,

is not neglectable. Still it is very difficult to quantify the supply of the FWD to ecosystems flows. It is important to distinguished between wood above and below 10 cm diameter, as Harmon and Sexton (1996) identified different decomposition patterns in them. Below 10 cm the decomposition rate increased exponentially with decreasing diameter and above 10 cm it decreases slightly with increasing diameter (Stokland, 2001).

Decomposition of wood is defined as a process where the dry mass loss of wood per time is roughly proportional to the remaining wood mass. Wood decay was described in 1963 by Olson in single negative exponential model $D_t = D_0 e^{-kt}$, where D_0 is the initial dry mass, D_t is the dry mass left in t time, and k is the decay rate constant . Instead of wood mass is possible to use wood density, volume or cover of bark (Harmon *et al.*, 1986). This revealed that decomposition takes from few decades in tropical regions to several centuries in northern latitudes and high altitudes (Stokland, 2001). This has connection that majority of fungi grows and decompose in temperature ranges of 5-35°C, with optimum between 25 and 35°C (Stokland, 2012). The decay of deadwood can be expressed in terms of amount of carbon lost during the respiration. Following the thread of global climate warming, some studies have argued that as the temperature increases, the CWD temperature will increase, and so will the CO_2 respiration flux (Herrmann and Bauhus, 2013). This findings are in accordance with the ecology of wood-decaying fungi where a positive effect of rising temperature on the mycelia growth can be observed up to 30°C (Schmidt, 2006). Surprisingly, no apparent effect of differences in precipitation on wood decay rate wasn´t detected (Přívětivý *et al.*, 2016).

At the beginning of decay of small-sized logs, there is a lag-time period. In a case of warm-humid region it is 15 years, when the decompositions of beech logs is very slow (Přívětivý *et al.*, 2016). But same effects were observed also for *Picea abies* logs in the study of Næsset (1999), which lead to creation of lag-time model. This model work on principle of wood mass loss over time, taking into account that after the death of tree there is initial transition period until decomposers community establishes on wood (Harmon *et al.*, 1986). Přívětivý at al. (2016) confirmed that the greater the beech log diameter is, faster it decays. But beside trunk diameter, tree species and local climatic factors also influence the decomposition rate (Stokland, 2001). In Scandinavia, the average time for decomposition of spruce log close to sea was 70 years, and in northern Scandinavia around 200 years (Stokland, 2001). Næsset (1999) calculated, that 95% of spruce log in boreal forest would decompose 90 years. Mäkinen et al. (2006) estimated the decay time of log to 60-80 years. An average resident time for a beech log in European temperate forest was calculated in many studies. In Slovenia, total decomposition of beech log was calculated for 51 years, and 32 years to reach advanced decay stages (Kraigher *et al.*, 2002). In Denmark, the deadwood log should reach an advanced decay stage in 20-35 years (Christensen and Vesterdal, 2003). In Germany, Bavarian region, total residence time was estimated

to 24 years, by radiocarbon dating (Krüger *et al.*, 2014). And in other study was used density measuring to estimate the total residence time on 34 years and to reach the last decay class was 18 years (Müller-Using and Bartsch, 2009). When sampled more sites, the total residence time was measures to 54 years and the time to reach last decay stage to 33 years (Přívětivý *et al.*, 2016).

The high variability in time by the logs reach last decay stage might not be caused only by different climate, or other factors, but also because there is no uniform system for measuring the decay of logs. Mostly there aren´t information about the length of decay of every log (like in case of this study), and the decay classes are based on estimation of decay time. The estimation is done visually and physically, by touching the wood and evaluating its hardness and compactness. In almost every European country exist their own national system for evaluating wood decay class.

3.1.1 Composition of deadwood

Lignocellulose is the major component of the plant biomass and makes up about half of the organic matter produced by photosynthesis. It is formed by three types of polymers - cellulose, hemicellulose, and lignin (Fig. 1). They are all tightly combined and chemically bonded by non-covalent forces and by covalent cross-linkages (Pérez *et al.*, 2002).

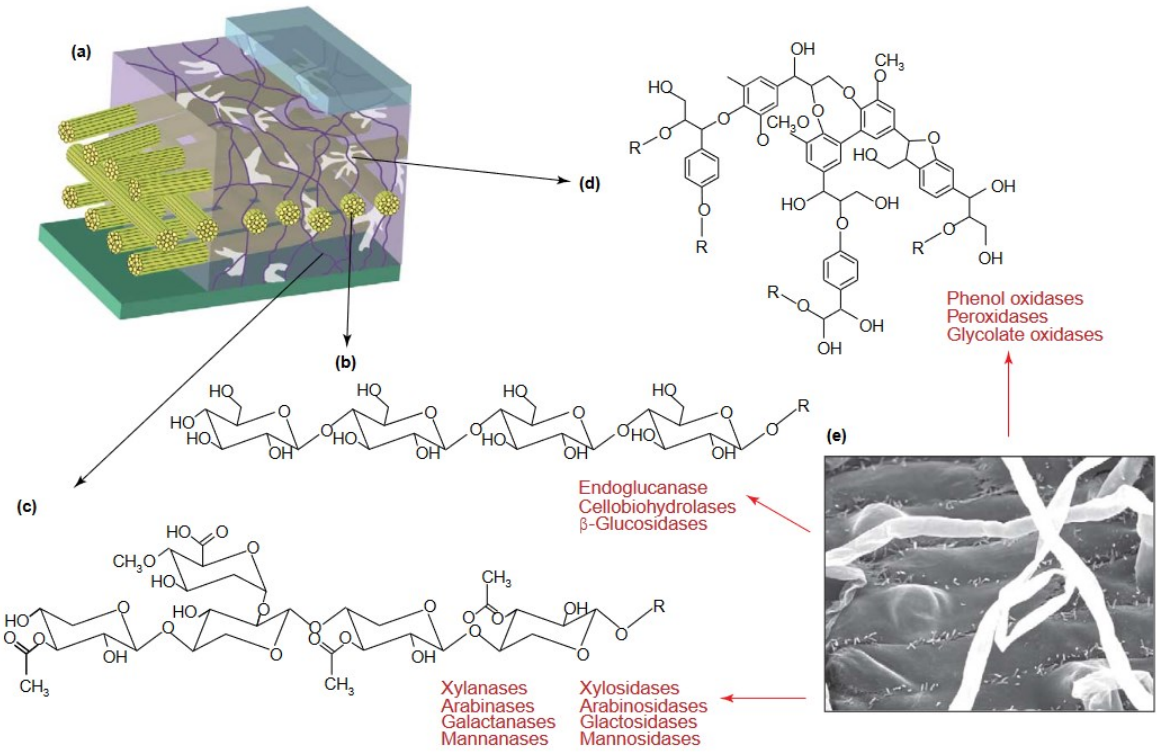


Fig. 1 The plant cell wall (a) and the distribution and chemical composition of cellulose (b), hemicellulose (c) and lignin (d). Hemicellulose refers to a variety of carbohydrates and shown is O-acetyl-4-O-methyl-dglucuronoxylan, which is common in angiosperms. Beneath each compound are listed the extracellular enzymes produced by soil microorganisms to depolymerize the plant cell wall compounds during decomposition processes, mediated by a community of microorganisms (e). *Source: Frank Dazzo. From Zak et al., 2006.*

Cellulose is linear and highly ordered heteropolymer, composed of D-glucose subunits linked by β-1,4-glycosidic bonds forming cellobiose molecules (Pérez *et al.*, 2002). The polymer represents over 50% of wood weight (Martínez *et al.*, 2005) and it is located predominantly in the secondary cell wall (Sjöström, 1993). The cellobiose molecules form long chains (over 10 000 glucose residues), called elemental fibrils, linked together by hydrogen bonds and van der Waal forces (Baldrian and Valášková, 2008). The elemental fibrils are clustered into microfibrils, that forms cellulose fibre, which is covered by hemicellulose and lignin. The orientation of microfibril is different in different wall levels. Cellulose can be seen in nature in a crystalline form or, a small percentage of cellulose is made by non-organised chains called amorphous cellulose. In this non-organised conformation, cellulose is more susceptible to enzymatic degradation (Pérez *et al.*, 2002).

Hemicellulose is a polysaccharide with a lower molecular weight than cellulose and it makes up 25–30% of total wood dry weight. Its role is to support the structure of the cellulose microfibrils in the primary and secondary walls of plant cells (Rytioja *et al.*, 2014). The complex carbohydrate polymer is made up of different pentose and hexose residues, which are often acetylated (Martínez *et al.*, 2005). The main difference with cellulose is that hemicellulose has branches with short lateral chains

consisting of different sugars. In contrast to cellulose, they are easily hydrolysable polymers. They do not form aggregates, even when they are co-crystallized with cellulose chains (Pérez *et al.*, 2002). Hemicelluloses are reported to be linked to lignin through cinnamate acid ester linkages, to cellulose through interchain hydrogen bonding, and to other hemicelluloses via covalent and hydrogen bonds (Decker, Siika-Aho and Viikari, 2008).

Structurally, lignin is an amorphous three-dimensional aromatic heteropolymer (Hatakka and Hammel, 2010). It is non-water soluble and optically inactive. It consists of three different phenylpropane units joined together by different types of linkages. The complex polymer is built up of dimethoxylated (syringyl, S), monomethoxylated (guaiacyl, G) and non-methoxylated (*p*-hydroxyphenyl, H) phenylpropanoid units, derived from the corresponding *p*-hydroxycinnamyl alcohols. Its basic units are linked together by different C-C and aryl-ether linkages, with aryl-glycerol β-arylether being the predominant structure (Pérez *et al.*, 2002). Some studies show that lignin can incorporate many more monolignols than the traditional three basic units (Vanholme *et al.*, 2008).The highest concentration of this recalcitrant polymer is found in the middle lamella, where it acts as a glue between wood fibres, but it is also present in the layers of the cell wall (especially the secondary cell-wall), forming, together with hemicelluloses, an amorphous matrix in which the cellulose fibrils are embedded and protected against biodegradation (Pérez *et al.*, 2002). Lignin composition varies between different plant species in terms of H, G, S subunits ratio. Softwoods have the highest lignin content, and their lignin is made up mostly of G units. On the other side, angiosperm´s lignin consists of S and G units. Lignin composition between the different wood tissues and cell-wall layers also varies. For example, middle-lamella typically has a lower S/G ratio than lignin from the secondary wall (Pérez *et al.*, 2002).

Other non-structural components of wood include compounds extractable with organic solvents (the so-called extractives) which can be either polar (e.g. phenols and tannins) or apolar (e.g. fats and sterols), water-soluble compounds (e.g. sugars and starch), as well as proteins and ashes. These components together generally represent less than 5% of the dry weight of wood but can reach 20% in some softwoods (e.g. in some Cupressaceae) (Martínez *et al.*, 2005).

3.2 Importance of wood-decaying fungi

Fungi interacting with dead wood represent a broad spectre of diverse fungal kingdom. A high diversity of various fungal lifestyles can be observed, from the most widespread and probably original one, saprotrophic, to specialised, like lichens, mycorrhizal fungi, endophytes, plant and animal pathogens and many other (McLaughlin *et al.*, 2009). Wood decaying fungi are the most important organisms participating in wood decomposition (Purahong *et al.*, 2016). In nature, only fungi possess an adequate range of secreted oxidoreductases and hydrolases to effectively decompose all compounds of wood (Kubartová, Ottosson and Stenlid, 2015; Purahong *et al.*, 2016). They are considered as the primary wood decomposer in forest biomes. The saprotrophic ability is spread across four fungal phyla, with ascomycetes and basidiomycetes being the most important one in degradation of lignocellulose complex (Kirk, Paul M. *et al.*, 2008). Ascomycetes contain 64 000 described species and their saprotrophic capabilities range from degradation of polysaccharides to lignocellulose (Xylariales). Basidiomycetes are known for high competence in degradation of lignin. The phylum contain 32 000 described species, with important saprotrophic decayers belonging to subphylum Agaricomycotina (Hatakka, 2001; Kirk, Paul M. *et al.*, 2008). Fungal mycelia represents an important pool of organic matter in forest litter and soil, containing mostly polysaccharides (80-90%), lipids and proteins (Baldrian *et al.*, 2013). Such large reservoirs of C and N, that typically contains, among polysaccharides, chitin, glucans and glucomannans, and phenolics like melanin, attract bacterial decomposers on readily decomposable substrate, comparing to lignocellulose (Baldrian *et al.*, 2013; Brabcová *et al.*, 2016). Bacteria are more important decayers of mycelia than fungi (Brabcová *et al.*, 2016).

3.2.1 Different paths of fungal deadwood decomposition

Activity of extracellular enzymes is one of the crucial part in estimating the rate of decay and usually reflects the fungal community structure, but also depends on many other chemical and environmental variables.

The structural integrity of cellulose is one of the main obstacles of enzymatic hydrolysis of cellulose. Cellulose can be crystalline, sub-crystalline and even amorphous (Hatakka and Hammel, 2010). The fungal decomposition of cellulose is catalysed by a set of extracellular hydrolytic enzymes typically composed of endoglucanase (EGs, EC 3.2.1.4), cellobiohydrolase (CBHs, EC 3.2.1.91) and β-

glucosidase (BGL, EC 3.2.1.21) (Baldrian and Valášková, 2008; Hatakka and Hammel, 2010). In some species, the absence of cellobiohydrolase is substituted by the production of processive endoglucanases combining the properties of both enzymes. In addition, systems producing hydroxyl radicals based on cellobiose dehydrogenase (CDH, EC 1.1.99.18), quinone redox cycling or glycopeptide-based Fenton reaction are involved in the degradation of several plant cell wall components, including cellulose. The complete cellulolytic complex used by a single fungal species is typically composed of more than one of the above mechanisms that contribute to the utilization of cellulose as a source of carbon or energy to ensure fast substrate colonization (Baldrian and Valášková, 2008). All the enzymes have pH optimum between 4 and 6, which follows observed pH in deadwood. Their temperature optima varies between 37 and 75 °C, meaning that under natural conditions the enzymes do not achieve their full potential activity (Baldrian and Valášková, 2008). The endo-cleaving EGs randomly cut along the cellulose chain to release glucooligosaccharides creating new terminal ends. They preferable cleave in cellulose amorphous regions (Pérez *et al.*, 2002). The exocleaving enzymes are cellobiohydrolases and exocellulases. CBHs cleave the polymeric cellulose releasing cellobiose from its reducing or non-reducing ends. β-glucosidase can be extra- or intracellular and their role in cellulolytic systems is to hydrolyse the resulting cellobiose or cello-oligosaccharides to glucose. But these can be subject to dehydrogenation by cellobiose dehydrogenase as well (Baldrian and Valášková, 2008; Hatakka and Hammel, 2010). Cellulose can be degraded under anaerobic conditions in large functional entities called cellulosomes by bacteria like *Clostridium thermocellum* (Pérez *et al.*, 2002). Products of cellulose hydrolysis are available as carbon and energy sources for cellulolytic microorganisms or other microbes living in the environment where cellulose is being degraded. In fact, this release of sugars from cellulose is the main basis of microbial interactions occurring in such environments (Martínez, 2002; Pérez *et al.*, 2002; Watkinson, 2015).

The structure of hemicellulose is very variable and therefore a specific set of enzymes is needed for its degradation to monomeric sugars and acetic acid (Pérez *et al.*, 2002). Depolymerizing and debranching enzymes act in concordance in order to decompose entirely the hemicellulose molecule structure up to free monosaccharides (Decker, Siika-Aho and Viikari, 2008). Depolymerizing enzymes works on the backbone sugar chain and can have endo- or exo-activity or the combination of both. Debranching enzymes are distinguished based on their activity on glyosidic linkage or ester-linkages. In this study were measured the activity of various enzymes acting on hemicellulose, some of them were not hemicellulose specific, but they were naturally acting on cellulose as well. The representing enzymes for hemicellulose degradation in this study were β-galactosidase (EC 3.2.1.23), β-xylosidase (EC 3.2.1.37) and endoxylanase (EC 3.2.1.8). The backbone of xylan is cleaved by endoxylanase into shorter oligomers. They are cleaved until xylobiose units, which are hydrolysed by β-xylosidase into

two xylose monomers. Xyloglucan backbone is structurally like cellulose, and it is cleaved by EGs, CBHs and BGLs. Where EGs cleaved β-glucans into oligosaccharides and BGLs works on galactoglucomannan backbone (Rytioja *et al.*, 2014). β-galactosidase acts as debranching enzyme. A recent study confirmed the enzymatic oxidative cleavage of hemicellulose (Agger *et al.*, 2014).

Wood decaying fungi can be classically referred to as white, brown and soft rot. Such division comes from different approaches they employ in the decay of lignocellulose. More than 90% of all wood decaying basidiomycetes are classified as white rot type, mostly from subphylum Agaricomycotina. In nature, it is more common to observe white rot type preferentially on angiosperm than on gymnosperm wood (Hatakka and Hammel, 2010). White rot fungi are the only organisms which are able to mineralise lignin completely to access cellulose and hemicellulose (Hatakka, 2001). Many of them colonize cell lumina and cause cell wall erosion, causing the typical white colour of decaying wood. The typical representative of white rot fungi is *Phanerochaete chrysosporium*, widely used as experimental model.

Brown rot fungi is a specific group of basidomycetes fungi belonging to the family Polyporales. It is estimated that only up to 10% of basidiomycetous wood decaying fungi exhibit this type of decay (Hatakka and Hammel, 2010). Brown rot fungi grow most frequently on gymnosperm wood and therefore are very important in boreal forests, for biomass recyclation in Scandinavien countries (Hatakka and Hammel, 2010). During brown rot decomposition, lignin in wood is selectively modified and left behind as a complex of aromatic ring-containing polymer. Only hemicellulose is removed rapidly, followed by degradation of cellulose. The wood affected by brown rot type is characteristic by brown color, with a pattern of cubical cracks and tends to become very fragile. Brown rot fungi are the most common cause of wood decay of timber structures and built environment, typically represented by *Serpula lacrymans* (Watkinson, 2015).

Soft rot fungi are considered pioneer species, usually followed by white and brown rot fungi (Rajala *et al.*, 2012). They differ from white and brown rot fungi mainly by their approach to lignin degradation, the way they enter the wood cell and that they forms decay cavities within secondary wall, but do not attack the middle lamella (Stokland, Siitonen and Jonsson, 2012). Soft rot fungi doesn´t decay the lignin entirely, rather use the free polysacharide compounds. Soft rot fungi are mostly from phylum Ascomycota, belonging to the family Xylariaceae (Liers *et al.*, 2006). The wood affected by soft rot is usually brown, soft, and when dry ressembles to brown rot decay type. They mainly degrade angiosperm wood but compare to basidiomycetes fungi still very little is known about the extent at which they are able to degrade lignin (Hatakka and Hammel, 2010). The most efficient fungus of soft rot type is *Daldinia concetrica* (Hatakka and Hammel, 2010). Soft rot fungi are very important under extreme conditions, where there is limitation by water potential, or gaseous regime. These are the one

which contribute to decomposition of submerged wood or wood in antartic conditions (Blanchette *et al.*, 2004).

Neverthless, such distinction among white, brown and soft rot fungi doesn´t comply with all wood decaying fungi, as some do not fit in either group, and show characteristics of both or neither groups, suggesting that not all of the fungi manifest only one type of decay and that its modified according to available substrate (Riley *et al.*, 2014). Therefore the decay of lignin is discussed in terms of the most commonly employed mechanism of decay.

Due to complex structure of lignin molecule, with no repeating pattern of bonds and hydrophobic nature, its degradation requires unspecific extracellular oxidative enzymes, small molecular weight mediators or factors like radicals (Hatakka and Hammel, 2010). Enzymes involved in biodegradation of lignin are high redox potential peroxidases (manganese-dependent peroxidase, lignin peroxidase and versatile peroxidase), oxidases and lacasses (Martínez *et al.*, 2005). To oxidative cleavage of lignin is sometimes referred as to oxidative burning, or enzymatic combustion, due to highly reactive and violent process necessary to performe extracellulary, near the substrate (Watkinson, 2015). Cooperation of both, peroxidase and oxidases, is necessary to break down lignin into smaller fragments. Manganese-dependent peroxidase (MnP) oxidise lignin phenols to phenoxy radicals, when coupled with organic acid produced by fungi, such as oxalate. MnP can be found in all white rot basidiomycetes, and therefore is the main enzyme acting in lignin breakdown in nature. Lignin peroxidase (LiP) is oxidase in the presence of hydrogen peroxide to an active state. It acts on non-phenolic part of lignin, generating a stable aryl cation radical which lead to ring cleavage and production of small lignin fragments. To support the function of these enzymes a set of accessory hydrogen peroxide-generating enzymes include aryl alcohol oxidase or glyoxal oxidase (Watkinson, 2015). Laccase can be found in most of the white rot fungi but cannot degrade all parts of lignin. The resulting lignin fragments doesn´t provide an energy source but can be taken up and degraded intracellularly through the cytochrome P450 system.

Presumable brown rot fungi, otherwise fungi which don´t rely on oxidative enzymes, use hydroxyl radicals (·OH) to overcome lignocellullose barrier. Their practice is to remove cellulose from within the lignocellulose complex, leaving behind modified residue of the lignin polymer. During brown rot decay most of the polysacharides are removed and the lignin is modified by side chain oxidation, demethylation of the aromatic rings and by partial depolymerisation (Eastwood *et al.*, 2011). Degradation of polysacharides probably doesn´t depend only on hydroxyl radicals, but use hydrolytic enzymes as well (Cohen, Suzuki and Hammel, 2005). Most of the brown rot fungi are not able to degrade crystalline cellulose (Baldrian and Valášková, 2008) but produce cellulases and hemicellulases,

which leads to suggestion that the lignin is transiently depolymerized, so it facilitate the access of these enzymes (Hatakka and Hammel, 2010). Presumable brown rot fungi seem to all lack the exoglucanases, used by white rot fungi to further degrade oligomeric carbohydrates. How this action is performed in brown rot fungi remains unclear (Watkinson, 2015). Still the enzymes needs ·OH to initiate the decay at a distance in order to increase the wood porosity for them to act on the wood (Yelle *et al.*, 2008). Under low pH conditions, Fenton´s reaction, in which organic acid and hydrogen peroxide are combined in redox reaction with ferrous iron, is used to generate highly reactive hydroxyl radical ·OH:

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + \cdot OH + H_2O$$

The hydroxyl radical must be produce in close proximity of the targeted site as it has a nanosecond half-life. Ascomycetes fungi doesn´t performe brown rot type of decay.

3.2.2 Factors influencing the decay and community composition

Fungi are known to respond to wide variety of environmental drivers, such as temperature (A´Bear *et al.*, 2013; Hiscox *et al.*, 2016), water content (Allison and Treseder, 2011), habitat quality (Lonsdale, Pautasso and Holdenrieder, 2008), substrate quality (Edman, Möller and Ericson, 2006) and wood physico-chemical factors like decay stage, log diameter, volume, density, C:N ration, lignin content and macro- and micro-nutrients correlate with the community strucutre of wood inhabiting fungi (Rajala *et al.*, 2010, 2011, 2012). Their modification can directly affect the decomposition process and C cycling (Venugopal *et al.*, 2016). Naturally, as the decay progress, the conditions inside wood changes and the change in community structure follows (Rajala *et al.*, 2012). It has been longtime known that the speed of wood decomposition differs depending on a wide variety of factors. For biodiversity conservation and determining the rate of carbon fixation in deadwood, it is very important to determined what are the factors and in which way they influence the decay rate (Přívětivý *et al.*, 2016). Nevertheless, it still remains unclear which factors and how they regulate the wood-inhabiting fungal community structure, their distribution and enzyme production (Purahong *et al.*, 2016).

Until the recent boom of next generation sequencing, most of the researches were done under laboratory conditions, the results not securely comparable with natural conditions, or in natur by fruting body surveys (Renvall, 1995). But even in the recent times, it is possible to see works based only on surveying sporocarps (Heilmann-Clausen *et al.*, 2014), nevertheless the ones done only by

next-generation sequencing, or combination of both, started to prevail (Kubartová, Ottosson and Stenlid, 2015; Ottosson *et al.*, 2015; Baldrian *et al.*, 2016). It has been proved that the molecular methods are able to distinguished and uncover more species than sporocarp surveys (Ovaskainen *et al.*, 2013), but there is unanswer question about the suitability of different molecular markers for PCR amplification and sequencing. Also, there is a call for improvement of databases and bioinformatic tools used in sequencing data processing.

Factors influencing the fungal community structure can be generely divided on abiotic and biotic agents, where abiotic represents all the different physical elements effecting fungi and biotic represent the interaction among fungi and various organisms. In studies carried out on monocultures, abiotic factors like moisture and temperature, followed by metabolic propreties of fungal species and substrate quality, played clearly the most important role in deciding the decay rate. In multiple decomposers community, such as find in nature, where the diversity is very high, the abiotic factors were still important for decomposition rates, but interactions between species seemed to be very important (van der Wal *et al.*, 2013).

Abiotic factors are mainly studied in laboratoris, where under pre-selected conditions, with known species, these factors can fundamentally change the outcomes. One of the very important factors is temperature. Considering the climat change threat in the following years, many scientist asked themselves a question how will the forest ecosystem react, if the temperature is about to grow by 0.3-4.8 °C by 2100, as predicted (IPCC, 2014). Temperature changes can alter fungal assembly history or reverse outcomes of interactions between cord-forming fungi and hence, possibly the ecosystem functioning (Toljander *et al.*, 2006; Hiscox *et al.*, 2016). Wood-inhabiting fungi differ in their microclimatic tolerances and preferences (Stokland, Siitonen and Jonsson, 2012; Heilmann-Clausen *et al.*, 2014). The temperature optima vary between species, most being mesothermic, but majority grows and decompose in the temperature range of 5-35°C, with optimum between 25-35°C (Stokland, Siitonen and Jonsson, 2012). Fungal decomposition rates increase with temperature until similar optima, and their enzymatic activity ceases when temperature falls below 0°C (Schmidt, 2006; Hiscox *et al.*, 2016). The positive effect of rising temperature on the growth of mycelia can be detected up to 30 °C (Schmidt, 2006). While the change of 4°C alters the fungal combative hierarchy and outcomes of competitive dominance between fungi (A´Bear *et al.*, 2013) in experimental settings in soil or on wood (Crowther, Boddy and Jones, 2011). This is in contrast to interactions in agar culture, where the outcomes becomes hidden behind intransitive dominance hierarchies in pair-wise interactions, when the combative skills of each species overpower other species, and itself is defeated by a different one A>B, B>C, C>A (Boddy, 2000). Temperature is therefore an important abiotic determinant of decomposer fungal growth and activity (A'Bear, Boddy and Hefin Jones, 2012).

Wood decaying fungal enzymes are extracellular and need at least basic water content to be carried to their substrate, otherwise the metabolic functioning, including fungal wood decay would stop (Boddy and Heilmann-Clausen, 2008). Wood decaying basidiomycetes cannot grow below -4,4MPa, unlike some xylarious ascomycetes which can grow at lower water potential (Boddy, 1983). Conversely, high level of moisture prevents the decomposition. Only certain type of specialised fungi can carry out the decomposition under low level (*Serpula lacrymans*) or high level of water (soft rot fungi). The water content is related to the amount of sun exposure of log and extent of canopy opening effect of forest on dead wood. The ability to keep the moisture level constant depends on the log diameter and is important for fungal community composition (Přívětivý *et al.*, 2016). As the decomposition process progress, the water content increases from the molecular water created during decomposition. An increase in the humidity inside the log is crucial for the higher decomposing rate of wood as well as the acces to air. Small invertebrae may increase the rate of aeration, due to their use of dead wood as their habitat. Tunnels and corridors created by animals serves as entry point for humidity and O_2, both necessary for activity of enzymes. The wood tends to become more acidic with progressive decay, due to fungal metabolic activity and secretion of organic acids. Therefore trees containing alkaline compounds, but also tannins and wax have tendency to be more resistant to microbial attacks. Many studies proved relationship between wood structure, density and quality of subtrate on fungal community. Heilmann-Clausen *et al.* (2014) showed that fungal communities were clearly structured by susbtrate quality, and that they responded to major gradient in climat and forest management conditions. It was found that fungal succession was specific to each tree species, reflecting physico-chemical wood quality. Such effects weren´t consistent, extending from shifting the fungal community structure to modifiying the decay rate, to neutral changes (Rajala *et al.*, 2010). But on decomposition of CWD and FWD participate different group of fungi, probably due to more serious fluctuation in temperature, moisture content and limited space to occupy in FWD (Bässler *et al.*, 2010). Diameter of logs is important for the biodiversity and composition of populations. Also bryophytes species richness increase with log diameter (Ódor *et al.*, 2006). Larger logs provide higher amount of various habitats, suitable for species requiring both large and small diameters (Stokland, Siitonen and Jonsson, 2012).

Fungal communities are strongly structured by local filters, like wood-decay stage of log, closely following decaying gradient shaping the fungal communities on beech logs (Heilmann-Clausen *et al.*, 2014; Seibold *et al.*, 2015). The highest fungal richness occurs in intermediate decay stage, as well for bryophytes (Heilmann-Clausen and Christensen, 2003; Táborská *et al.*, 2015), while the highest number of species of saproxylic beetles can be found on preferably large logs in advanced decay stage (Přívětivý *et al.*, 2016). Elevation, latitude and human influence have effects on diversity patterns and

differ among organismal groups (Fukami and Wardle, 2005; Sundqvist, Sanders and Wardle, 2013), but according to (Heilmann-Clausen *et al.*, 2014) it is still not adequatly explained why. It has been shown that latitude and mean annual temperature of distinct regions correlate with wood decomposition (Fukasawa, 2015). Many studies that deal with fungal distribution and enzymes secretion, suffer from a lack of geographic extent (Purahong *et al.*, 2016). Purahong´s study revealed that based on the physico-chemical propreties and secreted enzymes, the structure of fungal communities was similar across different regions, but variable in all regions. Neverthless, Heilmann-Clausen *et al.*, 2014 revealed that the geographical gradient was strongly related to longitude and temperature ranges. Also still little is known about large-scale biodiversity patterns of fungi (Ódor *et al.*, 2006; Heilmann-Clausen *et al.*, 2014). The amount of dead wood in forest seems to be important factor for maintaining fungal species diversity. In particular the presence of all decaying classes. Several studies linked lower dead wood amount and decreasing species richness, density and diversity of species in managed forests (Heilmann-Clausen *et al.*, 2014). In order to keep the continuity and deadwood effects on forest ecosystems, there should be a supply of dead stems at least once every 25-35 years, based on calculations of speed of wood decay (Přívětivý *et al.*, 2016). To maintain biodiversity, it is important to leave various type of deadwood (snaps, windthrows, snags and downed logs) in forest. Crucial role play thicker deadwood logs, which are communly not left in managed forest to decay (Přívětivý *et al.*, 2016). But as the input of FWD to forest ecosystems hasn´t been properly studied yet, sufficient amount of FWD might be also necessary for maintaining full functioning of forest ecosystems and promoting of saproxylic species (Allmér *et al.*, 2006). The amount of dead wood in forest seems to be quite important for other non-fungal forest taxa as well (Seibold *et al.*, 2015; Kirchenbaur *et al.*, 2017).

Climat and wood quality have decayer-specific effect on fungal wood decomposition (Venugopal *et al.*, 2016). But as was discussed at the beginning of the chapter, it is the combination of factors which creates the outcomes. There is no clear or distinctive hierarchy of factors, to distinguish the most powerful factor from the least strong. It is the combination of various forces of these factors which creates the results. For example, while Heilmann-Clausen *et al.* (2014) suggests that microclimatic conditions shape the fungal community structure, Bässler *et al.* (2010) find that resource availability was more important for the community than climat, on large-diameter dead wood. Supported by the hypothese, that temperature is less important on large-sized logs, as the thicker stems regulate more effectively the daily and seasonal fluctuations in tempertaure and humidity (Bässler *et al.*, 2010). Based on their enzymatic capacity, higher proportion of white and brown fungi lead to quicker decompostion in the warm-dry region, as these fungi decay wood more efficiently then other saprotrophs (Stokland, Siitonen and Jonsson, 2012). Climat itself fails to predict the speed of wood decomposition at regional scale. While local scale factors seems more suitable to explain most

of the variation (Bradford *et al.*, 2014; Přívětivý *et al.*, 2016). But most of the studies are done on regional scale, lacking extension of wider geographical scale.

The diversity of organism changes as mentioned in relation to decay stage, but also due to mortality agent of tree, its position during decomposition and the diameter at breast height (DBH) (Seibold *et al.*, 2015). Logs fully in contact with the ground buffer more successfuly against fluctuation in temperature regimes and water content in comparasion with suspended logs (Heilmann-Clausen and Christensen, 2003). Also downed logs are comparably moister than the topsoil during most of the vegetation period, which cause that CWD can be used as a refuge during hot and dry periods for litter- and soil-dweling organisms (Pichler *et al.*, 2012). Therefore, differently sized and positioned trees may provide a very large number of various substrate qualities and microhabitats for the wood-decaying fungi occupying advanced decay stages, as the tree is not subdue to an uniforme level of decay in all its size. Mortality agent are very important factors determining how the community will develop. Different causes for death of tree can open different entry points for decayers and expose the substrate to distinctive environmnetal conditions (Stokland, Siitonen and Jonsson, 2012). Logs broken at the stem are especially suitable for red-listed species of fungi on beech wood. In boreal forest the specificity of ´kelo´ tree, a Finnish term for very old Scots pine (*Pinus sylvestris* L.) trees, which grows slowly till end of their lives and then die slowly for decades or even centuries while standing. The specific physico-chemical composition of ´kelo´ trees seems to cause the high resistence of the wood and works as unique substrate for rare saproxylic species (Venugopal *et al.*, 2016).

Wood-chemical propreties such as decay class, remaining mass, density, extractives, total lignin, pH and different conditions of decay (forest, climate, soil and substrate), are the most important factors shaping the fungal community on regional scale (Heilmann-Clausen *et al.*, 2014; Purahong *et al.*, 2016). But together they fail to explain sufficiently variation in lignocellulolytic enzymes activities, which points to strong influence of biotic interaction, such as priority effect, on enzymes production and therefore the decomposition rate.

3.2.3 Biotic interactions inside microbial community

The biotic factors are believed to have large impact on species composition. However, after many years of research, they still haven´t been thoroughly examined. Studies have revealed that even under the same abiotic conditions, the output of biotic interactions is not the same. And that biotic interactions between various organisms, substantially modify the wood decay rate. Competition

between wood decaying fungi is manifested by interference, production of allopath, exploitation or occupying space and reducing the availability to another of resources (Boddy, 2000). The hierarchy of combative skill of fungi has been established, but the relationships is not straightforward and still local scale influences change the outputs. The complexity of biotic interactions is still viewed under the light of stochastic fungal assembly and it is believed that the outcomes of such interactions cannot be predicted yet (Baldrian *et al.*, 2016). Hence the it is not possible to accurately predict the possible decay rate and the speed and amount of nutrient flow back to the geochemical cycles. It is therefore still very complicated to evaluated the importance of fungi in nutrient cycling on global level and to implement such knowledge into predictive ecological models or forest management. Biotic factors can be distinguish as interactions between fungi and fungi (intra- or inter- species), fungi and bacteria, fungi and other organisms (invertebrate, small animals, humans, plants, lichens) (Stokland, Siitonen and Jonsson, 2012).

According to Boddy (2000) fungal competition can be divide into two categories depending on how they acquire resources. Fungi can capture uncolonized resource or to obtain resources already colonized by other fungi. The question remain in any first case scenario might take place for cord-forming fungi as most of the substrate is colonized already during the life of the tree by endophyte or arriving spores (Song *et al.*, 2017). During these interactions fungi employ variety of combative mechanisms, like antagonism at a distance, hyphal interference, mycoparasitism or gross mycelial contact. The results can be deadlock or replacement of one species by the other (Boddy, 2000). Biotic interactions can significantly alter mycelial physiological function, so they are potentially usable as biological control agents of fungal pathogens of trees and in service timber. Fungi arriving to uncolonized substrate are not common in nature because usually the log is already colonized and arriving fungi must deal with already established communities. But still, during the early decay stages, not all of the substrate is colonized and fungi may develop for a brief period of time, in absence of other organisms (Boddy, 2000). As the decay progress, established fungi expand their territories across wood and the chance of mycelial contact increase. The competition for substrate, space and nutrient resources is the most common interaction for wood decomposing heterotrophic mycelial fungi. When fungi approach each other, the success of one in combat depends on antagonistic mechanism employed. The recognition can be at distance or on a contact. Recognising presence of other fungi can be mediated or anticipated at a distance via volatile or diffusible antibiotics or waste products. If physical contact occurs two interspecific interaction may take place. Mycelial interaction is when a hypha contacts with another hypha or spore which is followed by death of contacted compartments – common amongst wood-decaying basidiomycetes, possible use in biocontrol. Other type of interaction is mycoparasitism, where shared nutrients are obtained biotrophically or necrotrophically (Boddy,

2000). The length of mutual fungal inhibition is unknown under natural conditions, but in agar culture was set on 15 mm. Gross mycelial contact is quite important in wood decaying fungi as it is accompanied by changes in metabolism. The outcomes of these interactions can be partial or complete replacement of one species by other(s), or deadlock, when none of the fungi gains the territory. The outcomes are still very unpredictable, even between the same combinations of fungi under apparently identical conditions. Like in study of (Rayner, Grifith,Ainsworth 1995) where 20 replicates of encounter of *Peniphora lycii* and *Coriolus versicolor* (synonym for *Trametes versicolor*) resolved into three different patterns. In 18 replicates *P. lycii* replaced *C. versicolor* and in only 2 others, *P. lycii* was replaced by *C. versicolor*. Other studies on agar malt showed how abiotic factors changes the outcomes of different fungal interactions, suggesting that their importance in natural conditions might have considerable effect on fungal community structure. *Daldinia concentrica* is strongly combative, and most of the tested fungi were subjected to deadlock with exception of *Coriolus versicolor*. These showed deadlock or partial replacement of *Daldinia* by *Coriolus*. But when the water potential was lowered to -1,3 MPa, *Coriolus* completely overgrew *Daldinia*, while when the concentration of CO2 was increased (20% O_2, 0% CO_2), *Daldinia* replaced *Coriolus* (Dix, 2012). The various abiotic factors affecting the outcome of interactions have been studied mostly on agar cultures, and should be interpreted with caution. *Hypholoma fasciculare* in agar culture is replaced *Steccherinum fimbriatum*, but their meeting resulted in deadlock in soil and *H. fasciculare* was replaced in wood by *S. fimbriatum* (Downson, Rayner and Boddy, 1988). Eventhough there still exists a big difference among species, some general patterns related to ecological and taxonomical groups are emerging. With elevated CO_2 or reduced O_2, the antagonistic ability of basidiomycetes decreases (Schoeman, Webber and Dickinson, 1996). As was mentioned, the size and quality of substrate is also important. As fungi occupying larger part of wood have higher success in combat then those occupying smaller parts, when met by the same species (Holmer and Stenlid, 1993). It is due to the fact, that fungi occupying bigger territory have access to more nutrients and therefore are more resistant on attacks. Fungi can fight not only through mycelial contact, but are known to use biochemical defence mechanisms against each other (Boddy, 2000), which modify their metabolism toward increase or decrease in secretion of lignin modifying enzymes, according to the type of interaction (Baldrian, 2004; Fukasawa, Osono and Takeda, 2009; Hiscox *et al.*, 2015). It was discovered that CO_2 evolution increases and decreases during deadlock and replacement interactions in wood (Owens, 1989). Interspecific interactions can vary with elevation. Among plants, the interactions are changed from positive (facilitating the interactions) at high elevation, to negative (competitive) at low elevation (Sundqvist, Sanders and Wardle, 2013). Most of the studies was studying the outcome of pariwise combination of fungi, to estimate hierarchy of fungal combative abilities or competence to decay. But it seems, that such approach simplify reality and omit the complexity of real multispecies communities (Hiscox *et al.*, 2017). Study of three-way

interactions between saprotrophic fungi in wood and across soil indicate that the pairwise interactions don´t predict the outcomes accuretly. Interaction of various species of fungi resulted into coexistence within resource over replacement (Hiscox *et al.*, 2017).

Bacteria in deadwood were less frequently studied when compared to fungal community (Lladó, López-Mondéjar and Baldrian, 2017). It has been long time known, that they inhabit decomposing wood, but still a lot remains to be found about their identity and ecology. The questions about involvement of wood-associated bacteria in direct degradation of lignocellulose remains unanswered. Lignin-degrading enzymes were found in genomes of certain bacteria (Lladó, López-Mondéjar and Baldrian, 2017). Therefore, it seems probable that bacteria are involved in decomposition of lignocellulose (Lladó, López-Mondéjar and Baldrian, 2017). Some bacterial genera are known to produce chitinolytic enzymes and considering that fungal biomass is more readily decomposable substrate than lignocellulose, bacteria seems to be more important mycelial degraders than fungi (Beier and Bertilsson, 2013; Brabcová *et al.*, 2016). Due to their probably limited ability to decompose polymeric lignin, bacteria were thought to be less important in ecology of deadwood then fungi (Cornelissen *et al.*, 2012). Many answers were delivered since then, but still a large part of the bacterial life inside deadwood deserves to be well-studied. Moreover, the attention was brought to the fungal-bacterial interactions. There are indications that both antagonistic and beneficial interactions are taking place, like modification of fungal behaviour, competition, production of volatile compounds, or production of secondary metabolism compounds (Kielak *et al.*, 2016). *Hypholoma fasciculare*, which cause a drop of pH after colonisation, creating a selective effect on wood inhabiting bacteria (de Boer *et al.*, 2010). They respond by adapting themselves to lower pH (Valášková *et al.*, 2009). Bacteria are able to compete with fungi because their metabolism is not necessarily dependent on oxygen level (Rinta-Kanto *et al.*, 2016). But it was found that bacteria-fungal consortia are able to decay wood rapidly than seldom community of fungi (Kielak *et al.*, 2016). Bacteria are becoming important during middle and late decay stages, thanks to their N-fixation ability and degradation of toxic wood compounds. The fixation ability has 25% of all bacteria in the stage and they belong to *Rhizobiales* (Hoppe *et al.*, 2015). This suggests mutualistic interactions with fungi. Them, providing C via wood decomposition and bacteria providing N. During the late stages of decomposition, bacterial abundance grow as they become specialized in the degradation of derivatives of lignin decomposition, mainly aromatic compounds (Kielak *et al.*, 2016). *Burkholderia*, *Phenylbacterium* and *Methylovirgula* genera, are known to degrade aromatic compounds and to use methanol as a sole carbon source, during middle and late stages of wood decomposition (Hoppe *et al.*, 2015; Kielak *et al.*, 2016). They are still voices echoing that direct influence of bacteria on decomposition of lignocellulose seems to be rather minor (van der Wal *et al.*, 2013).

Other organisms than bacteria can modify the fungal behaviour or be affected by it. Usually it comprise plants, invertebrates, small animals or human being (Stokland, Siitonen and Jonsson, 2012). The effect of invertebrates grazing or their ability to mechanically decompose the wood attracted more attention recently. Fungal mycelia are more readily decomposable substrate as they represent a large C and N pool that typically contains chitin and other polysaccharides (Brabcová *et al.*, 2016). Soil invertebrate grazer are known to limit the growth of individual mycelial systems, and having preferences for selective species (Tordoff, Boddy and Jones, 2008; A'Bear, Boddy and Hefin Jones, 2012). But only recently it was revealed that selective grazing can have an important impact on fungi, reversing fungal dominance and changing the outcome of fungal interactions in some cases (Crowther, Boddy and Jones, 2011; Crowther *et al.*, 2012). Blaser et al. (2013) showed in their study in microcosm that grazing by invertebrates is an important factor which has influence on the development and output of climatic change of saprotrophic fungal communities in woodland ecosystems. It stays unclear whether similar results would be achieved in more complex assemblages, like in nature, where the influence of fungal dominance hierarchy, and the roles of biotic and abiotic factors are more complexed and the outcomes might differ (A´Bear *et al.*, 2013). Saproxylic Diptera and Hymenoptera also contribute to biodiversity connected with deadwood, and are involved in processes like parasitoids or decomposers (Stokland, Siitonen and Jonsson, 2012; Seibold *et al.*, 2015). But only few experimental studies have focused on these taxa, considering that not many taxonomist are capable to distinguish the high number of very similar species (Seibold *et al.*, 2015). Nonetheless, even non saproxylic species benefit from the presence of dead wood in forest ecosystems. In study of Kirchenbaur *et al.* (2017) the addition of deadwood into the ecosystem had positive effect on gastropods feeding activity in proximity of woody debris in shady forest. Eventhough the activity was strongly mediated by canopy openness as effects of distance to dead wood. Small vertebrae use the deadwood as shelter (Stokland, Siitonen and Jonsson, 2012). Plants, bryophytes and lichens use deadwood as a platform for supported growth, and at the same time modify its structure or physico-chemical conditions inside the wood (Heilmann-Clausen *et al.*, 2014). Plants further disintegrate the wood composition by its roots. The deadwood is important for drought sensitive bryophytes due to conservationism reasons. On logs in intermediate stage of decay, are growing the most threatened species of drought sensitive bryophytes (Heilmann-Clausen *et al.*, 2014).

3.2.4 Fungal succession and community assembly

The community composition of organisms connected with deadwood is progressively changing along the decaying gradient (Fig. 2) (van der Wal *et al.*, 2013). Eventhough the exact community composition is in each log influenced by specific effect of abiotic factors and biotic interactions, some general patterns have merged in the past, providing deeper insight into the functioning of communities connected with wood decay.

The tree is colonized during life by various endophytic fungi, which initiate decay after the death of tree (Song *et al.*, 2017). The decay develops according to the way the tree died, its position and tree species. Most of the studies of deadwood was done on downed deadwood, partially or fully in contact with soil. These factors might influence the results when compared with standing dead trees, subjected to desiccation. Generally, the transformation of the fungal community progress from early ruderal species in initial decay stages, using mainly free polysaccharides, to secondary decomposers, that attack lignin. Amongst secondary decomposers belong competitive species and trunk rotters in intermediate decays stages, to cord former and specialist during late stages of decay (Heilmann-Clausen *et al.*, 2014). Representatives of each decay phase bear some characteristic attributes. The primary colonizers are generally good in dispersal, acquiring most of their territory by massive and rapid spore germination, or mycelial extension and ability to quickly use organic compounds available in previously uncolonized resources, like free sugars and easily available cellulose fractions. These fungi are, based on the general ecological theory, ruderal R-selected (Boddy, 2000, 2001). Primary colonizers comprise already presented endophyte and primary saprotrophs, mostly soft-rot ascomycetes or white rot fungi (Olsson *et al.*, 2011). Once the substrate is colonized, further community development is mediated through “priority effect” (Boddy, 2000; Boddy and Heilmann-Clausen, 2008; Fukami *et al.*, 2010; Dickie *et al.*, 2012; Hiscox *et al.*, 2015; Purahong *et al.*, 2016).. Reason why some fungi succeed and other won´t. Species inhabiting the wood, changes the substrate due to their actions by producing degradative enzymes, secondary metabolites, modifying overall decomposition of wood compounds. The modification of substrate might be crucial for following decayers, or in terms of easier, harder or neutral colonisation and establishment. The first arriving fungi may significantly influence the following fungal community assembly, and thus to affect the decay rate (Purahong *et al.*, 2016). The random variation in the primary decayers community and the differently positioned trees might provide a large number of various microhabitats, which may lead to non-uniform way of log decay, selecting different wood-inhabiting fungi in advanced decay stages (Halme *et al.*, 2013). It is known that some basidiomycetes needs a specific preceding fungi to prosper, and probably also to create fruiting bodies as suggested based on fruiting body survey in north-western European forests (Niemelä, Renvall and

Pentillä, 1995). This is probably due to changes in abiotic conditions, like lignocellulose compositions, inhibitory extractives, water content made by previous fungi (Boddy, 2001). The intermediate decayers decompose lignin and largely depend on their combative skill as they arrive on already colonize substrate (Olsson *et al.*, 2011). During the late decay stages, Ectomycorrhizal (EcM) fungi are become the most dominant (Rajala *et al.*, 2011). The presence of exudates, wood modifications, desiccation and invertebrate grazing, is not problematic for the very late stages colonizer as they become adapted for these conditions and often without them they are not prone to grow and prosper (Boddy, 2001). *Shizopora paradoxa* has very poor combative skills, but adapted on harsh conditions in late decay stages of wood by production of thick-walled chlamydospores. *Hyphoderma setigerum* can tolerate the invertebrates grazing.

Fungi can be divided into three groups according to how much stress, disturbances and competition they can bear. The groups are stress tolerant (S), combative (K) and ruderal (R), but fungi usually don´t belong only to one group, they tend to carry mixed characteristic (Boddy, 2001). Community structure development is from ruderal, and stress tolerant during early decay stages, through highly combative species at intermediate stage (Heilmann-Clausen, 2001; Boddy and Heilmann-Clausen, 2008), to again stress-tolerant during late decay stages. The identity within these groups can be changed through microclimatic conditions. Trunk rotters normally considered stress tolerant, might be preferred, under continental climate, as heart rot agents (Heilmann-Clausen *et al.*, 2014). The sequenation of first white rot wood-decaying fungi, *Phanerochaete chrysosporium* (Martinez *et al.*, 2004), revealed the rich set of different enzymes involved in decomposition of lignocellulose. After that, genome of brown rot fungi *Postia placenta* (Martinez *et al.*, 2009) was sequenced, showing completely different mechanisms used for wood degradation. The sequenation of other wood decaying fungi illuminated the great fungal decay diversity caused by very fine speciation and specialisations of fungi on wood decay. Each fungal specie adapted slightly differently on saproxylic lifestyle, but nevertheless their originality of solution, their activity leads to redundancy in features in wood (Ohm *et al.*, 2014). Generally, increased microbial diversity enhance nutrient cycling as greater intensity of resource exploitation and competition is taking place. Therefore, higher species richness might result in enhancement of wood decomposition rate via additive or synergistic activities. It was observed that combining two species on one substrate bring higher decomposition rate than monocultures on the same substrate separately. Combination of cellulolytic and sugar fungi on cellulose substrate, will have a positive effect, probably due to relief of catabolic repression of cellulase production by sugar fungi´s consumption of the released sugars (Tiunov and Scheu, 2005). Nevertheless, adding more species into cultures doesn´t show any consistent pattern and the resulting effect become less clear with increasing community complexity (Nielsen *et al.*, 2011). Hence, there is

a need for certain level of species diversity in ecosystems, which will turn into redundancy after saturating demand. This level seemed to be reached at 10 species per community, so comparing to natural conditions still relatively species-poor environment (Nielsen *et al.*, 2011). After further increasing the species number no increase of activity was observed. Possible explanations for such low level of saturation of diversity were the occurrence of redundancy in metabolic activities, limited resource dispersal, intensive competitions for space and interference with antagonistic interactions (van der Wal *et al.*, 2013). The diversity of fungal community, its size and connectivity depends on presence of various woody resources in forest ecosystems (Edman, Kruys and Jonsson, 2004; Heilmann-Clausen and Christensen, 2004). Even with all findings, it is still impossible to predict the community development in time.

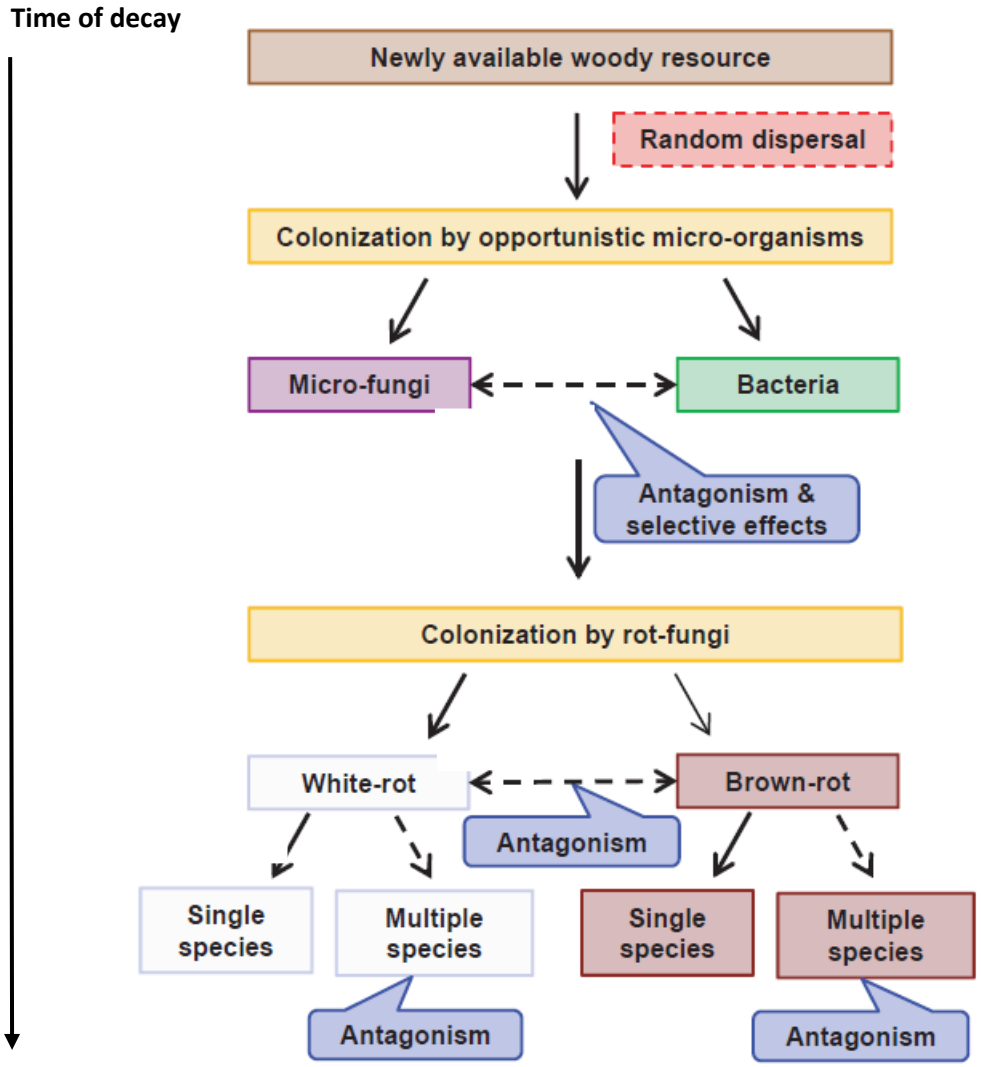


Fig. 2 Probable development of wood decaying communities and interactions during wood decay. From van der Wal et al., 2013.

4 Materials and Methods

4.1 Description of deadwood-associated fungal community in natural forest Salajka

The aim of this effort was to describe the development of fungal community during the decay on two different tree species, *Fagus sylvatica* L. and *Abies alba* Mill, along with deadwood properties and the activity of extracellular enzymes.

4.1.1 Study site

Deadwood sampling was conducted in the National Nature Reserve of Salajka (further reference as „Salajka“) situated on eastern border of the Czech Republic (49°24’ E; 18°25’ N) in the outer Western Carpathians (Král *et al.*, 2014). The total area is 21.9 ha, the altitude ranges from 715 to 815 m, mean annual total precipitation is about 1144 mm, and mean annual temperature is 5.4 ◦C (Tolasz et al., 2007). As a result of long-term repeated surveys and measurements of all living and dead trees (since the 1970s), a very detailed database is available with information about living and dead trees in this natural silver-fir beech forest. The dataset contains more than 3000 living and 1500 dead trees, with the latest update in 2014, before the sampling took place (Král *et al.*, 2014). Salajka has been strictly protected since 1937 (including no felling or removal of deadwood), and even before it was very little affected by management. Therefore, it is possible to assume that the natural processes taking place there are truly authentical and correspond to other similar natural forests (Šamonil and Vrška, 2007). The decay classes were decided based on the year of the first record when each tree was recorded as dead and fallen. Salajka is composed mainly of *Fagus sylvatica* L., and to a lesser extent by *Abies alba* Mill. and *Picea abies (*L.) Karsten (Král *et al.*, 2014).

4.1.2 Sampling design of deadwood

The selection of dead logs was done before site visit to avoided subjectivity of sampling. Only trees that were recorded as living before they fell, with diameters of 30-100 cm, were considered.

Selected 120 logs of beech and fir, were divided into four decay classes. The classification into decay classes was based on the year of the first record when each tree was recorded as dead and fallen; the surveys took place in the years 1974, 1994 and 2007. The oldest logs decaying at least 40 years (further reference as ">40, the fourth decaying class", recorded as fallen in 1974), the logs decaying between 20-40 years (further reference as "20-40, the third decaying class", recorded as fallen in 1994), the logs decaying between 7-19 years (further reference as "7-19, the second decaying class", recorded as fallen in 2007) and the logs decaying less than 7 years (further reference as "<7, the first decaying class", recorded as fallen in 2014), dated at the time of sampling, in autumn of 2014.

On the site, the length of tree was measured and drilled at 4 positions – in the 1/5, 2/5, 3/5, and 4/5 of length - using 40 cm drill with a 0.8 cm diameter (Makita, Japan), as described in (Baldrian *et al.*, 2016). The first and the last drilling was performed at least 30 cm from the end of the log. Samples from two adjacent drilling point were combined before processing and were jointly analysed. Before drilling, any plants, lichens and surface bark were removed. The drill was sterilised by washing in ethanol between drillings. Woody sawdust was collected into plastic bags and kept frozen untill processing in the laboratory in Prague. Material was then weighted, freeze-dried and weighted again to estimate the dry mass content of wood. Sawdust was milled in the Ultra Centrifugal Mill ZM 200 (Retsch, Haan, Germany) to a fine powder that was used for further analyses.

4.1.3 Extraction of DNA and preparation of samples for sequencing

Total genomic DNA was isolated from 150-200 mg of wood sawdust using the commercial kit NucleoSpin® Soil (Macherey-Nagel, Düren, Germany) according to the manufacturer instructions. Buffer SL1 with addition of Enhancer SX was used for lysis in the first step. The samples were homogenised using FastPrep®-24 (MP Biomedicals, Santa Ana, USA) at 5 m s^{-1} for 2× 30 sec in 4°C. The DNA was eluted from mini spin columns using 50 µl of elution buffer SE (5 mM Tris/HCl, pH 8.5). From each distinguished sample, the DNA was isolated twice independently (four isolations per log). The

concentration and purity of DNA was measured on spectrophotometer Nanodrop 1000 (Thermo Scientific, USA).

DNA extraction was followed by PCR amplification of Internal Transcribed Spacer 2 (ITS2) region (Fig. 3). DNA was amplified in triplicates by using tagged forward and reverse fungi-specific primers gITS7 (5´-GTGAATCATCGAATCTTTG-3´) and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) (White *et al.*, 1990).

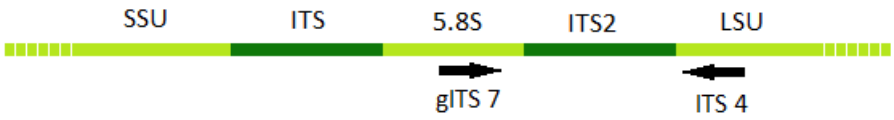


Fig. 3 Organization of ribosomal genes and target region of PCR primers for sequenation. ITS- internal transcribed spacer regions of the ribosome encoding genes, SSU- ribosomal small subunit encoding region, 5.8S- encoding gene of component of the large subunit, LSU- ribosomal large subunit encoding region. Modified from Ihrmark *et al.* (2012).

Tags were specific for each sample and were used for their identification after sequencing on platform Illumina MiSeq (Illumina, San Diego, California, USA). The reaction was performed in GenePro Thermal Cycler (Bioer, Hangzhou, China) using protocol as described in Table 1.

Table 1 PCR protocol used in ITS2 amplification

2,5 µl	10x buffer for DyNAzyme DNA Polymerase (Thermo Fischer Scientific)
1,5 µl	BSA (10 mg/ml, GeneON)
0,5 µl	PCR Nucleotide Mix (10 mM, Bioline)
1 µl	primer gITS7 (10 µM, Sigma Aldrich)
1 µl	primer ITS4 (10 µM, Sigma Aldrich)
0,75 µl	DyNAzyme II DNA polymerase (2 U/µl, Thermo Fischer Scientific)
1,0 µl	template DNA, concentration approx. 5-50 ng/µl
16,75	ddH_2O

Continuation of the table 1 from previous page

Amplification conditions	Temperature	Time
1. initial melting	94°C	5 minutes
2. melting	94°C	30s
3. annealing	56°C	30s
4. extending	72°C	30s
5. final extending	72°C	7 minutes
6. holding	4°C	Not specified

Repeated 35 times

Results of amplification were confirmed by gel electrophoresis on 1.5% agarose gel (agarose+ TAE buffer) with 4 µl EtBr (ethidium bromide). The triplicates of PCR amplicons were joined and cleaned by MinElute PCR Purification Kit (Qiagen, Hilden, Germany). DNA was concentrated by elution from the column by 20 µl of elution buffer (EB; 10 mM Tris/HCl, pH 8.5). dsDNA High Sensitivity Assay Kit on Qubit 2.0 Fluorometer (Life Technologies, Waltham, USA) was used for the measurement of the final DNA concentration. PCR products from individual samples were then mixed in an equimolar ratio to obtain equal DNA sequence counts from each sample. An aliquot containing 1 µg of DNA was diluted by EB buffer up to 50 µl, and the adaptors were ligated on the ends of amplicons according to the modified Illumina Pair-End Sample Preparation Protocol using the TruSeq DNA PCR-Free LT Kit (Illumina, San Diego, USA). Samples were sequenced on Illumina MiSeq, reading length 2x250 bp.

4.1.5 Bioinformatic data processing

Sequencing data were processed using the bioinformatic pipeline SEED 2.0.3 (Větrovský and Baldrian, 2013). Raw FASTQ files of pair-ends reads were joined together using Fastq-Join 1.1.2-621 with criteria of 40 bp minimum overlap and 15% of maximum difference (Aronesty, 2013). The sequences were filtered according to their quality and all sequences with quality mean under 30 were eliminated. The forward and reverse tags used for samples identification were removed and ITS2 sequence were extracted by the ITSx 1.0.11 tool (Bengtsson-Palme *et al.*, 2013). Sequences shorter than 40 bp were removed. USEARCH 9.2.64 package which contains UCHIME algorithm for detection and removal of chimeric sequences (Edgar *et al.*, 2011) and UPARSE clustering algorithm (Edgar, 2013)

were used to remove chimeric sequences and to cluster the resulting dataset at 97 % similarity level in order to create Operational Taxonomic Units (OTUs) which should represent fungal taxa at a species level. The distribution of individual OTUs in samples was subject to further data analysis. MAFFT64 7.222 algorithm (Katoh *et al.*, 2005) was applied for global alignment of sequences of all OTUs to create consensus sequences. These consensus sequences were identified by searching against downloaded NCBI nucleotide database for ITS2 spacer (database version from November 2016) employing BLASTn algorithm – MegaBLAST 2.5.0 - using a similarity threshold (E-value) of 0,00001. OTU consensus sequences with a similarity < 97 % or coverage 95 % identified to the closest genus, all other sequences to the closest species. The taxonomy for the best database hits was retrieved from GenBank. The fungal genera of the best hit, were classified into ecophysiological categories (e.g., White rot, Brown rot, Other saprotrophs, Ecomycorrhiza, Lichenized, Plant pathogen, Yeasts) based on the published data. The selected cathegories followed the work of Tedersoo *et al.* (2014). Fungal OTU non assigned to a known ecophysiology were labelled “Unknown”.

4.1.6 Diversity indices

To estimate the fungal community species richness and its diversity, various diversity indices (Shannon-Wiener diversity index, Evenness, Chao -1 species estimate and OTU richness) were calculated for 1000 randomly selected sequences from each sample in SEED 2.0.3 (Větrovský and Baldrian, 2013), to balance the uneven depth of sequencing. Spellerberg and Fedor (2003), call in their work for more rigorous use of the terms species richness and species diversity. In this study OTU richness is species richness, or the number of OTUs found in each sample. Species diversity is expressed as relation between number of species and number of individuals, in the form of species diversity indices (Spellerberg and Fedor, 2003). Shannon-Wiener diversity index (H) is commonly used to characterize species diversity in community, while considering both abundance and evenness of the species present. It is calculated by multiplying the proportion of species *i* relative to the total number of species (p_i), and the natural logarithm of this proportion ($\ln p_i$). The numbers are summed across species and multiplied by -1:

$$H = -\sum_{i=1}^{n} pi \ln pi$$

The value of the Shannon index increases as diversity increases (Shannon and Weaver, 1949).

Evenness, or Shannon's evenness (E_H) can be calculated by dividing H by H_{max} (H_{max} = ln$S$, S is total number of species). Evenness assumes a value between 0 and 1, where 1 means that all species are represented evenly in the sample:

$$E_H = \frac{H}{H_{\max}} = \frac{H}{\ln S}$$

Chao -1 species estimate uses the number of rare species found in a sample to calculate how many undiscovered species are left. The index works on the principle that if they are still singletons (F_1; rare species, occurring only once in the sample) discovered in the sample, it is very probable, that more species are about to be found. When all the found species occurs at least in the form of doubletons (F_2; species occurring in the sample exactly twice), there is higher probability that no more species will be found (Chao, 1984). The calculation of chao-1 species estimate is based on the following equation, where S_{obs}) is the number of specie observed in the sample:

$$S_1 = S_{obs} + \frac{F_1^2}{2F_2}$$

4.1.7 Enzymatic activities

The activity of extracellular enzymes was determined in all samples. Activity of exo-cleaving hydrolases was measured spectrophotometrically with fluorescently labelled substrates where substrates are covalently bound to 4-methylumbellyferol (MUF) which is fluorescent when released. The activity was measured for α-glucosidase (EC 3.2.1.20), β-glucosidase (EC 3.2.1.21), β-galactosidase (3.2.1.23), N-acetlyglucosaminidase (chitinase; EC 3.2.1.14), exocellulase (cellobiohydrolase; EC 3.2.1.91), β-xylosidase (EC 3.2.1.37), phosphomonoesterase (phosphatase; EC 3.1.3.1 – alkaline phosphatase) and lipase (EC 3.1.1.3) according to Baldrian, 2009.

For 2 h, 250 mg of lyophilised wood samples were left to be extracted, at 4°C on an orbital shaker (100 rpm) in 12 ml acetate buffer (50 Mm, pH=5) and the extract, diluted with 6 ml of acetate buffer, was used for the measurement of enzymatic activities.

40 µl of substrates or calibration solution were pipetted into wells of a microtiter plate and below described amount of calibration solutions in presented dilution (Table 2 and 3). Calibration range was prepared from 1 mM of MUF solution in dimethylsulfoxid (DMSO). Into wells containing β-glucosidase

(MUFG) and phosphomonoesterase (MUFP) was added 20 µl of DMSO extra, to ensure the solubilization.

Table 2 Enzymes and the substrate for their determination. Modified from Baldrian (2009).

Enzyme	**Substrate**
α-glucosidase	2,50 mM 4-methylumbellyferyl-α-D-glucopyranoside (MUFaG)
β-glucosidase	2,75 mM 4-methylumbellyferyl-β-D-glucopyranoside (MUFG)
β-galactosidase	2,50 mM 4-methylumbellyferyl-β-D-galactopyranoside (MUFU)
N-acetlyglucosaminidase	1,00 mM 4-methylumbellyferyl-N-acetylglucosaminide (MUFN)
Cellobiohydrolase	2,50 mM 4-methylumbellyferyl-N-cellobiopyranoside (MUFC)
β-xylosidase	2,50 mM 4-methylumbellyferyl-β-D-xylopyranoside (MUFX)
Phosphomonoesterase	2,75 mM 4-methylumbellyferyl-phosphate (MUFP)
Lipase	2,50 mM4-methylumbellyferyl -heptanoate (MUFY)
Calibration	4- methylumbellyferol (MUF)

At last, 200 µl of sample mixture with wood sawdust were pippeted into wells, and thus prepared microtiter plates were incubated in thermostat at 40 °C. Samples were also added into wells with MUF standards to correct the results for fluorescence quenching (Vepsäläinen *et al.*, 2001). The fluorescence was measured after 5 min incubation and 125 min incubation, on spectrophotometer Infinite 200 (TECAN, Austria) at an excitation wavelength 355 nm and an emission wave length 460 nm. The activity of enzymes was determined based on detected fluorescence, after subtraction of a blank, and compared with the calibration curves obtained with known MUF concentration. One unit of enzyme activity was defined as the amount of enzyme forming 1 µM of reaction product per min.

Table 3 Microtiter plate with distribution of substrates and calibration range. Numbers ("1","2","3") indicate different samples in triplicates.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 MUFG	1 MUFG	1 MUFG	2 MUFG	2 MUFG	2 MUFG	3 MUFG	3 MUFG	3 MUFG	1	2	3
B	1 MUFP	1 MUFP	1 MUFP	2 MUFP	2 MUFP	2 MUFP	3 MUFP	3 MUFP	3 MUFP	1+10 µl MUF 1/100	2+10 µl MUF 1/100	3+10 µl MUF 1/100
C	1 MUFC	1 MUFC	1 MUFC	2 MUFC	2 MUFC	2 MUFC	3 MUFC	3 MUFC	3 MUFC	1+20 µl MUF 1/100	2+20 µl MUF 1/100	3+20 µl MUF 1/100
D	1 MUFX	1 MUFX	1 MUFX	2 MUFX	2 MUFX	2 MUFX	3 MUFX	3 MUFX	3 MUFX	1+50 µl MUF 1/100	2+50 µl MUF 1/100	3+50 µl MUF 1/100
E	1 MUFN	1 MUFN	1 MUFN	2 MUFN	2 MUFN	2 MUFN	3 MUFN	3 MUFN	3 MUFN	1+20 µl MUF 1/10	2+20 µl MUF 1/10	3+20 µl MUF 1/10
F	1 MUFU	1 MUFU	1 MUFU	2 MUFU	2 MUFU	2 MUFU	3 MUFU	3 MUFU	3 MUFU	1+50 µl MUF 1/10	2+50 µl MUF 1/10	3+50 µl MUF 1/10
G	1 MUFY	1 MUFY	1 MUFY	2 MUFY	2 MUFY	2 MUFY	3 MUFY	3 MUFY	3 MUFY	1+10 µl MUF	2+10 µl MUF	3+10 µl MUF
H	1 MUFaG	1 MUFaG	1 MUFaG	2 MUFaG	2 MUFaG	2 MUFaG	3 MUFaG	3 MUFaG	3 MUFaG	1+20 µl MUF	2+20 µl MUF	3+20 µl MUF

In order to avoid possible interference of inhibitory small-molecular-mass compounds in determination of enzyme activities, the samples for the measurement of endo-cleaving hydrolases and oxidases were desalted on SephadexTM G-25 M columns (GE-Healthcare, United Kingdom) after acetate buffer extraction.

Endocleaving hydrolases, endo-1,4-β-glucanase (endocellulase; EC 3.2.1.21) and endo-1,4-β-xylanase (endoxylanase; EC 3.2.1.37) were measured with azo-dyed carbohydrate substrates, carboxymethyl cellulose for endocellulase and birch wood xylan for endoxylanase (Megazyme, Ireland). Following the manufacturer protocol, 150 µl of dyed substrate was mixed with 150 µl of desalted sample extract and the reaction mixture was incubated at 40 °C for 90-120 min (depending on the reactivity of samples). The reaction was stopped by adding 750 µl of ethanol and immediately followed by 10 s of vortexing. After centrifuging (10 000 × g) the samples for 10 minutes, the amount of released dye was measured spectrophotometrically at 595 nm (Valášková *et al.*, 2007). The enzyme activity was calculated according to standard curves correlating the dye release with the release of reducing sugars. One unit

of enzyme activity was determined as amount of enzyme which release 1 µmol of reducing sugars in one minute.

The activity of laccase (EC 1.10.3.2) was determined by measuring the oxidation of 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) in citrate-phosphate (100 mM citrate, 200 mM phosphate, pH 5.0) buffer (Bourbonnais and Paice, 1990). 150 µl of citrate-phosphate buffer, 50 µl of extract and 50 µl of ABTS substrate were pipetted on a microtiter plate. Absorbance at 420 nm was measured over 3 min of incubation.

Activity of manganese peroxidase (EC 1.11.1.13) was measured in succinate-lactate buffer (100 mM, pH 4.5; Hatakka and Hammel, 2010). The enzymes catalyzes oxidative condensation of 3-methyl-2-benzothiazolinone hydrazine (MBTH) and 3,3-dimethylaminobenzoic acid (DMAB) which creates purple indamine dye which is detected spectrophotometrically at 595 nm (Ngo and Lenhoff, 1980). The results were corrected based on the activities of samples without manganese ions (Mn^{2+}), by using equimolar amount of ethylenediaminetetraacetate (EDTA) or of H_2O, instead of $MnSO_4$. EDTA ensured that any remaining Mn^{2+} ions were chelated from the reaction solution. The analysis was conducted in quadruplicates. One unit of enzyme activity was defined as an amount of enzyme which creates 1 µmol of product per minute.

4.1.8 Measuring fungal biomass and chemical properties of deadwood

To determine the amount of fungal biomass in dead wood, ergosterol content was measured in samples. Ergosterol is the main sterol in the cell membranes of filamentous fungi and nevertheless the continuous discussion about its suitability for fungal biomass marker, it is still widely used for this purpose, with knowledge about limitation of this method (Pasanen *et al.*, 1999; Baldrian *et al.*, 2013). Another specific fungal marker of fungal biomass might be chitin as it is a major cell wall component of fungi, but ergosterol appears to be superior if metabolically active biomass is to be determined. The amount of ergosterol in fungal tissues is not constant which is a common argument of opponents of this method.

Total ergosterol was extracted and analysed as described in (Šnajdr *et al.*, 2008). Wood samples of 500 mg were sonicated in a solution of 3 ml of 10 % KOH and methanol at 70 ◦C for 90 min in a laboratory water bath. Then 1 ml of distilled water was added and the samples were extracted three times with 2 ml of cyclohexane. The samples were evaporated under nitrogen, redissolved in methanol and

analysed isocratically using a Waters Alliance HPLC system (Waters, USA). The flow rate of methanol as a mobile phase was 1 ml min^{-1}. Ergosterol was detected by UV detection at 282 nm and quantified by comparing with standards with known concentrations.
The determination of pH was done after mixing of 1 g of lyophilised wood sawdust with 10 ml of distilled water (1:10 w/v). Carbon and nitrogen content in samples was measured in the Research Institute for Soil and Water Conservation (Prague, Czech Republic) by combustion method.

To measure lignin content in plants, various methods are employed in its estimation. One of the most widely used and recommended for wood, is the determination of Klason lignin by using the hydrolysis of the wood with 72 % sulfuric acid and measuring the insoluble residue afterwards (Jung *et al.*, 1999). If wood is treated with strong acid, the polysaccharides are hydrolysed to water-soluble sugars, the lignin is recovered as an insoluble residue and can be measured gravimetrically (White, 1987).

Klason lignin was quantified in 200 mg of woodust, each sample was done in 3 parallels. The weight of beakers and frit filters (hot) used in analyses was recorded before the beginning. The samples were dried in 85 °C for 2 days in beakers till the constant weight. The total weight of beakers with samples was measured and sample dry mass was calculated. Then, 2 ml of 72 % H_2SO_4 were added and the beakers were covered by aluminium foil and carefully mixed. Samples were incubated for 1 h in 30 °C on an orbital shaker (100 rpm). Afterwards, 56 ml of ddH_2O were added and the liquid was transferred into Erlenmeyer flask, closed and covered by aluminium foil. The samples were left in autoclave for 1 h and after that, the samples were filtered, while still hot, through frit filters and washed by hot ddH_2O. The samples were then dried in 85 °C till constant weight and weighted while still hot. The content of lignin was calculated by dividing the mass of the residual with the mass of the original sample.

4.1.9 Statistical analysis

Programs PAST 3.14 (Hammer et al., 2001) and R platform 3.3.3 (R Development Core Team, 2016) were used for statistical analysis. Content of nitrogen, carbon and lignin and pH were tested by one-way ANOVA, followed by the Tukey Honest Significant Difference test (HSD) and the other environmental variables and enzymes were tested by Mann-Whitney U test, as it doesn´t assume the normality of the data, rather measure on a rank-order scale. Mantel test was performed with 99 999 permutations to study the correlations between dissimilarity matrices. Bray-Curtis distances were used as a measure of similarity in fungal community structures and Euclidean distances were used for the

other variables. Also non-metric multidimensional scaling (NMDS) ordination analysis with Bray-Curtis distances of fungal communities was calculated in vegan package (Oksanen *et al.*, 2013; R Development Core Team, 2016), which runs on platform R to estimate the influence of various factors on shape of fungal community. NMDS was based on dataset of relative abundances of 214 genera having at least in 3 samples abundance over 0.5 %, or at least 10 % abundance in one sample. The data were Hellinger-transformed and Bray-Curtis distances were used to create a matrix. For selected important variables, like pH, C, N, ergosterol, tree species and time of decay were calculated goodness-of-fit statistics (R_2) by *envfit* function of vegan package (Oksanen *et al.*, 2013) with P value based on 999 permutations. They were fitted into NMDS graphic of fungal communities as vectors. PERMANOVA test was calculated, which doesn´t assume the normality of data, with 9 999 permutations. One-way PERMANOVA was performed to determine the most influencing factor between tree species and time of decay on fungal community structure, and then two-way PERMANOVA was calculated for estimating interactions between fungal communities of different decay classes on beech and fir by Bray-Curtis distances. We also calculated one-way PERMANOVA for enzyme activities and environmental variables for different decay classes and tree species, using Euclidean distances. For PERMANOVA calculations was used PAST 3.14 (Hammer et al., 2001). The *indicspecies* package (De Cáceres and Legendre, 2009), running on R platform, was used for multilevel pattern analysis to identify indicator species associated with certain logs and decay classes. In all cases, differences at $p < 0.05$ were considered statistically significant.

4.2 Description of fungal community composition of standing and downed logs in the natural forest Žofín

The second part of this chapter is concerned with description of the fungal communities found on differently positioned logs in designated circular area in natural forest of Žofín. The focus of this study was to identify the main drivers of decay on downed and standing log, to determine enzymatic activities and wood chemistry between on these differently decaying logs.

4.2.1 Study site

The second study was realized in National Nature Reserve Žofínský prales (further reference as „Žofín"). Established already in 1838, Žofín is the oldest protected forest not only in the Czech Republic but in the whole Central Europe as well. It is situated in the Novohradské hory on south of the Czech Republic, near Austrian borders (48°40' N; 14°42' E). The total are is 25 ha, altitude ranges from 735 to 825 m, mean annual precipitation varies between 800 and 950 mm, and the mean annual temperature is 6,2 ◦C (Přívětivý *et al.*, 2016). *Fagus sylvatica* L. is predominant in tree species composition, followed by *Picea abies* L. Karsten and by *Abies alba* Mill., with additional occurrence of other tree species (*Acer platanoides* L., *Acer pseudoplatanus* L., *Betula pendula* Roth, *Populus tremula* L., *Salix caprea* L., S*ambucus racemosa* L., *Sorbus aucuparia* L., *Ulmus glabra* Huds) (Král *et al.*, 2014; Přívětivý *et al.*, 2016). The site was intensively studied since 1975 and was subjected to detailed survey of tree composition. Therefore, there is a very detailed information about the time of decay of every tree with trunk diameter larger than 10 cm, so it is possible to estimate quite precisely the time of decay elapsed. Censuses of tree composition were done during 1975, 1997, 2008, 2012, and with the latest in 2015, just before the sampling took place. In January 2007, the Kyrill windstorm caused a significant disturbance in the tree canopy layer of the protected forest.

4.2.2 Sampling design of deadwood

The sampling took place in selected circular area of 0.6 ha around the landmark point 151 (Fig. 4). All dead trees, downed (49 logs) and standing (14 logs), were sampled inside the depicted area. Due to small number of samples in the group of standing dead trees, the decay classes were reduced to two. Decaying >20 and <20 years, in autumn 2015 when the logs were sampled.

For downed logs, the same sampling pattern adapted for sampling deadwood in Salajka forest was followed. Standing dead trees had to be sampled differently due to accessibility and security reasons. They were drilled only at two positions, at 1 m height from the ground and at shoulder height. The two drillings were analysed separately like in a case for downed trees, where always the two samples from each half of the tree were put together and analysed separately. The drill was again sterilised between different logs by ethanol. The wood dust was collected in plastic bags and freezed until the analyses in laboratory in Prague. The material was weighted, lyophilised, weighted again and milled as was described in section 4.1.2.

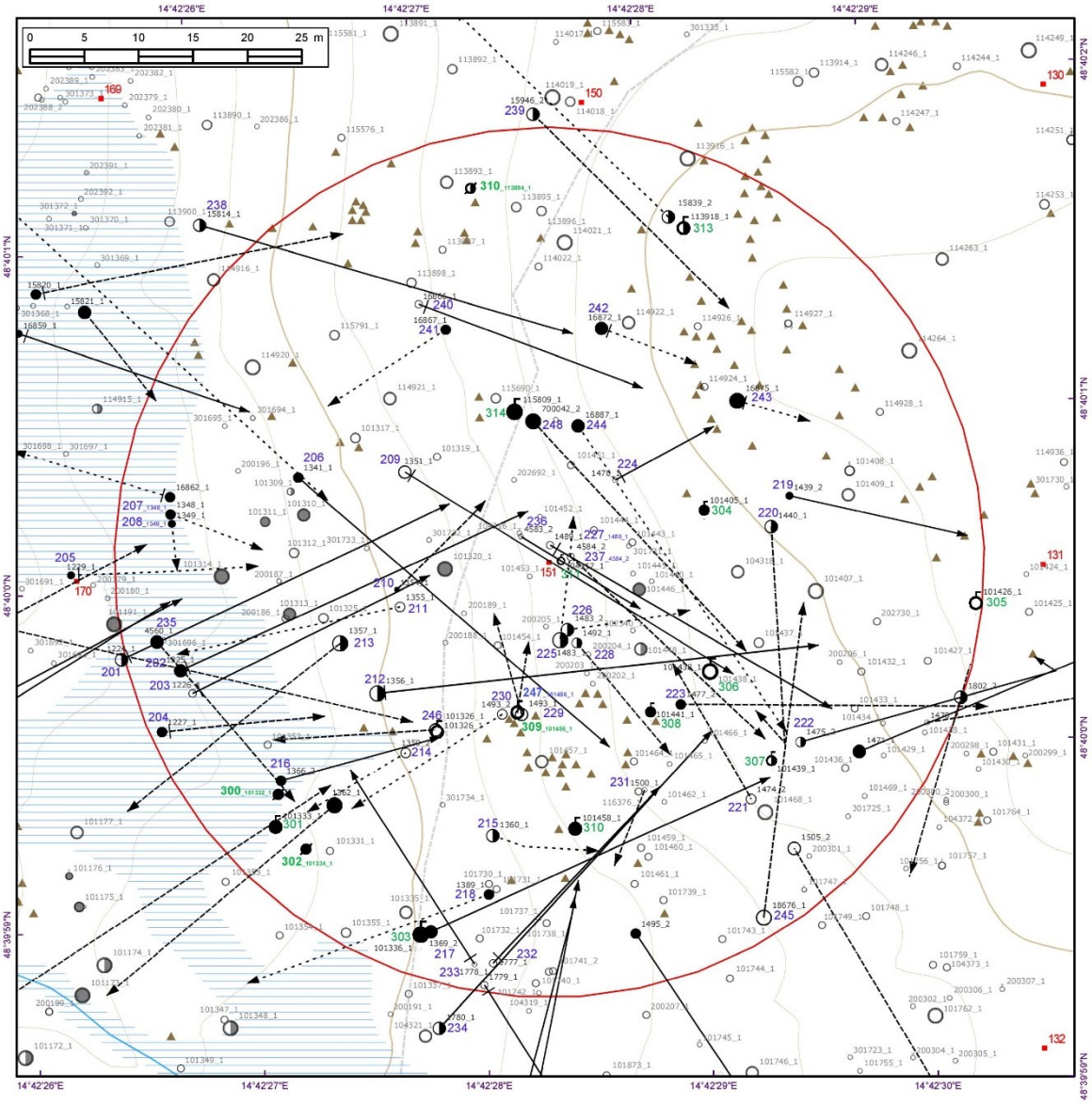


Fig. 4 The sampling map of circular area around landmark point 151. The circles symbolise living trees. Circles with little flag on top shows standing dead trees. Black arrows pointing from the circle symbolise dead downed tree, its position and state of decay. While filling of the circle represents tree species (e.g. empty – *Fagus sylvatica*, full- *Picea abies*, half filled- *Abies alba*). The triangles represent rocks and the blue lines shows the coverage by wetlands.

4.2.3 Processing of wood samples

DNA was extracted from milled wood sawdust, following the same methodology as in section 4.1.3, followed by ligation of adaptors and Miseq sequenation of samples, similarly as was described in section 4.1.4.

The data were bioinformatically processed in SEED 2.0.3, following the same pattern as in section 4.1.5 and the diversity indices were calculated for 1000 randomly selected sequences from each sample.

4.2.4. Determination of enzymatic activities, fungal biomass and chemical properties of deadwood

Enzymatic activities and ergosterol content were measured by the same methodology as employed in sections 4.1.7 and 4.1.8.

Chemical properties of deadwood were measured in samples of lyophilised sawdust in external analytical laboratory of the Institute of Botany of the CAS in Průhonice. Carbon and nitrogen content and pH were measured.

4.2.5 Statistical analysis

Statistical analysis of data was similar as in section 4.1.9, with minor differences in procedure. NMDS was based on 121 most abundant genera in dataset, having at least in 3 samples abundance over 0.5 %, or at least 10 % abundance in one sample. For selected variables like pH, nitrogen and ergosterol content, activity of MnP, tree species, time of decay and position of log were calculated goodness-of-fit statistics and they were fitted into NMDS graphic as vectors. One-way Permanova tests for tree species, time of decay, and position of logs and two-way Permanova test of tree species and position of logs were performed.

5 Results

5.1 Description of fungal community on *F. sylvatica* and *A. alba* deadwood in the natural forest Salajka

After MiSeq run, 907 080 suitable sequences were recorded in total, with a minimum length of 40 bp and a maximum of 319 bp. From these, 19 690 OTU were created and identified against NCBI local database. Nonfungal sequences that belonged mainly to the phylum Viridiplantae (widely represented by *F. sylvatica* sequences) were removed. After removal, 674 038 fungal sequences belonging to 14 917 OTUs were left for analysis.

Sampled logs were chosen to be maximally representative for all decay classes, while taking into consideration the natural character of the experiment (Table 4). As mentioned before in chapter 4.1.2, the selection of the logs was random and preceded the survey to avoid subjectivity.

Table 4 Distribution of sampled CWD among age classes and tree species. Numbers in parentheses indicate the total number of CWD across the whole study area.

	F. sylvatica	***A. alba***
Sampled CWD (all CWD)	57 (511)	63 (876)
CWD >40 years	8(8)	16 (73)
CWD 20-40 years	16(31)	19 (270)
CWD 7-19 years	18(201)	17 (428)
CWD <7 years	15(271)	11 (105)

5.1.1 Wood physico-chemical properties

Ergosterol content in decaying wood increased with the length of decay (Fig. 5). In logs of *F. sylvatica* most significant increase in fungal biomass were between <7 and 7-19 years of decay. In logs of *A. alba* there was also significant increase between <7 and 7-19 years of decay, but after that the development was rather toward significant decrease in class 20-40, only to be followed by increase up to level of class 7-19 content in the class >40. Increase of lignin content was proved to be significant

after <7 years of decay in *F. sylvatica*. In *A. alba,* the differences in increase of lignin content weren´t significant at desired confidence level. The content of carbon seemed to increase, but it wasn´t proved by Mann-Whitney U test. On the contrary, the nitrogen content significantly increased after first decay class for *F. sylvatica*, then the increase wasn´t proved to be significant. In *A. alba*, the increase was between <7 and 7-19 years, followed by decrease in 20-40 class. The >40 class registered increase again comparable to class 19-40. As expected, the values of pH progressively decreased with length of decay in both tree species.

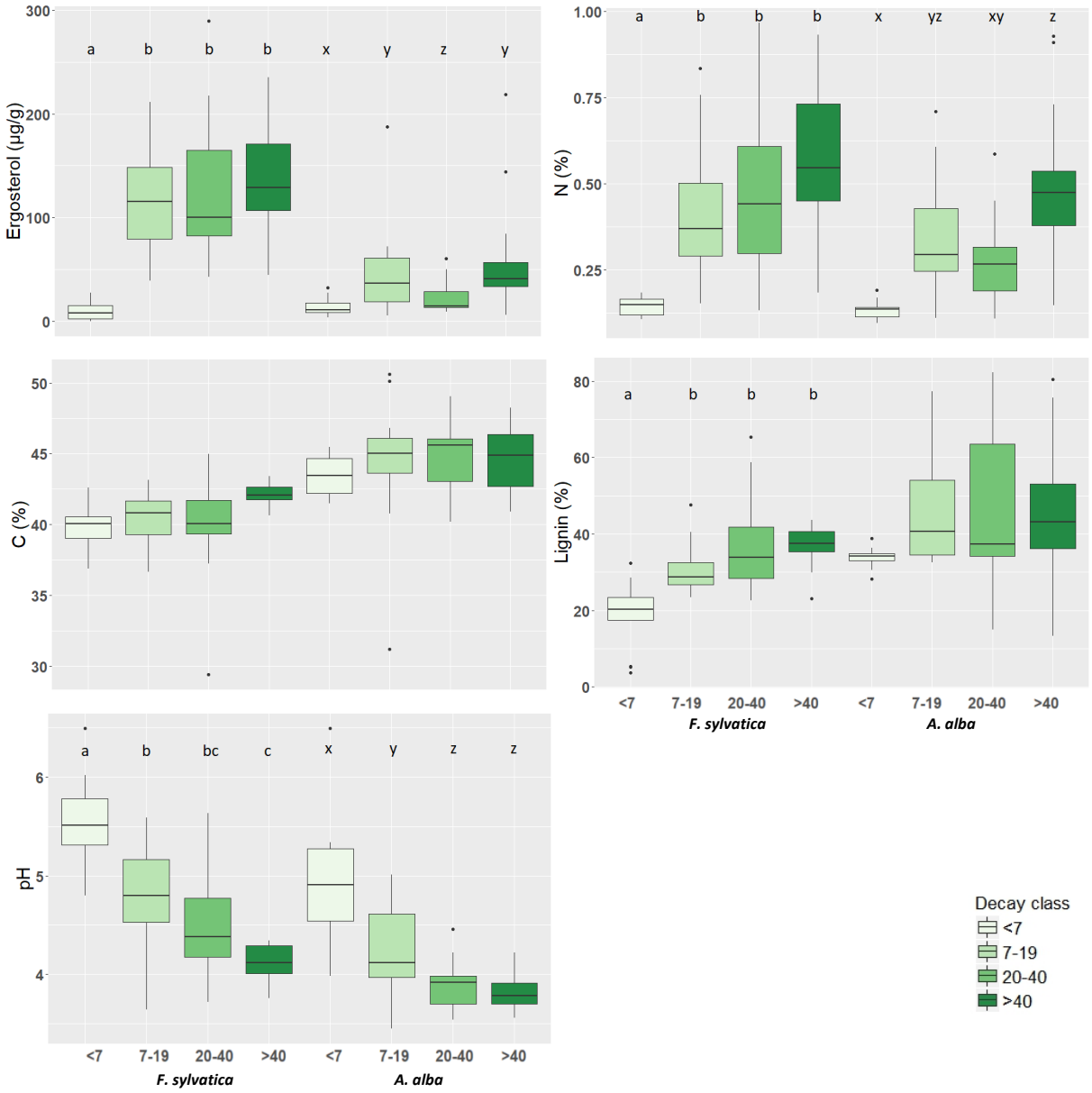


Fig. 5 Content of fungal biomass and chemistry of the coarse woody debris of *F. sylvatica* and *A. alba* in Salajka. Different letters indicate significant differences between the decay classes within each CWD tree species (Ergosterol was tested by Mann-Whitney U test, $p < 0.05$; C, N, pH and lignin content were tested by ANOVA, followed by Tukey Honest Significant Difference test, $p < 0.05$).

5.1.2 Diversity indices

None of the diversity indices showed significant differences between decay classes on either *F. sylvatica* or *A. alba* (Fig. 6).

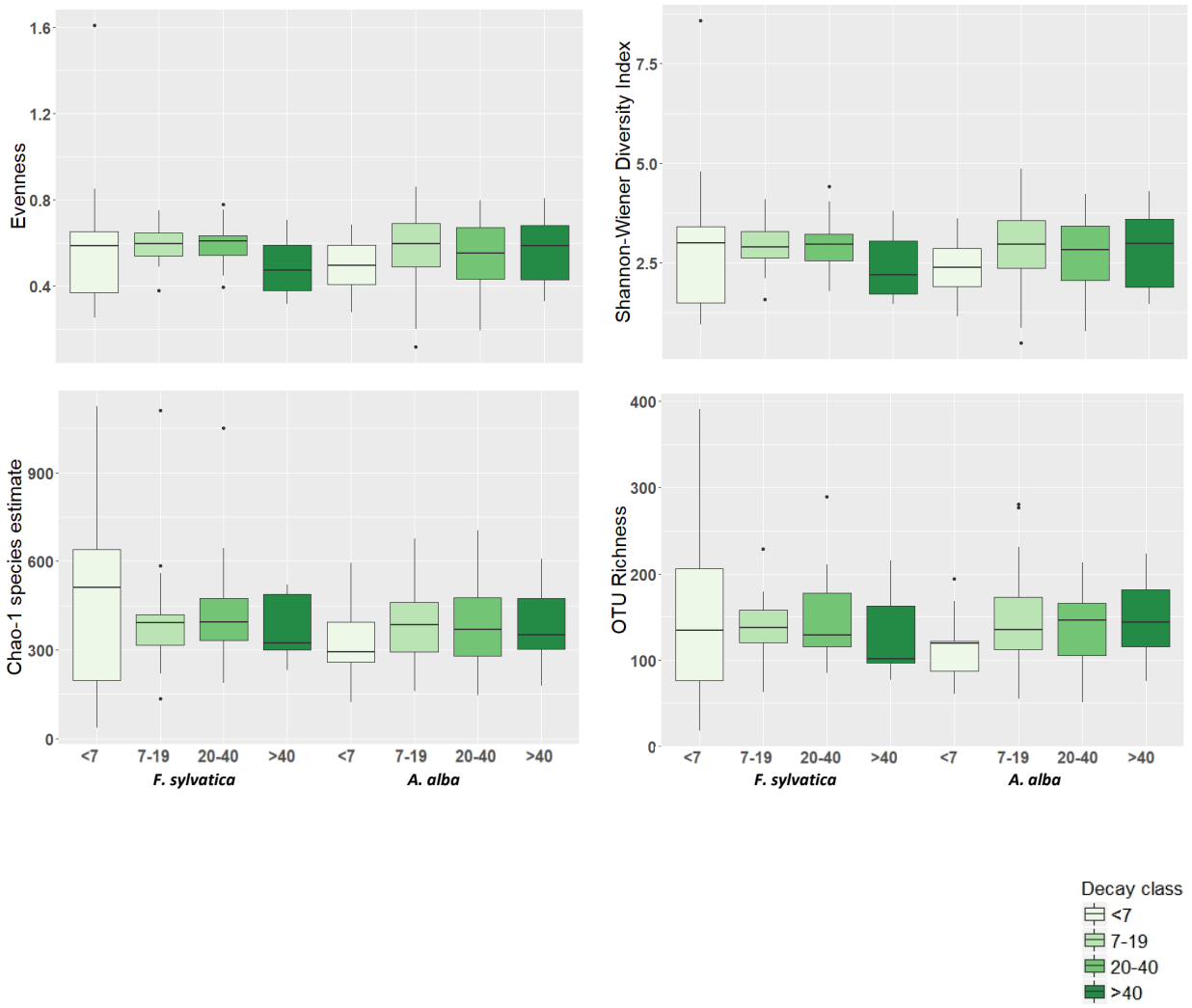


Fig.6 Diversity indices of fungal communities of different decay classes associated with *F. sylvatica* and *A. alba* CWD in Salajka (No significant differences were recorded using Mann-Whitney U test, $p < 0.05$).

5.1.3 Composition of fungal community on differently decayed logs

The ration between basidiomycetes and ascomycetes changed with developing decay, but also differed between *F. sylvatica* and *A. alba* (Fig. 7). In *F. sylvatica*, ascomycetes prevailed during the early stages of decay (<7 and 7-19 years); up to 60 %. These were progressively partially replaced by basidiomycetes, until in the logs >40, where the ratio reversed completely (basidiomycetes 60%, ascomycetes 30%). In the case of *A. alba* logs, the trend was not so clear, basidiomycetes were dominant (67 %), with an almost consistent relative abundance of ascomycetes (32%). Fungi from other phylum, like Mortierellomycotina, were in minority, becoming slightly more abundant on *A.alba* logs in advanced stages of decay (reaching 1.3 %).

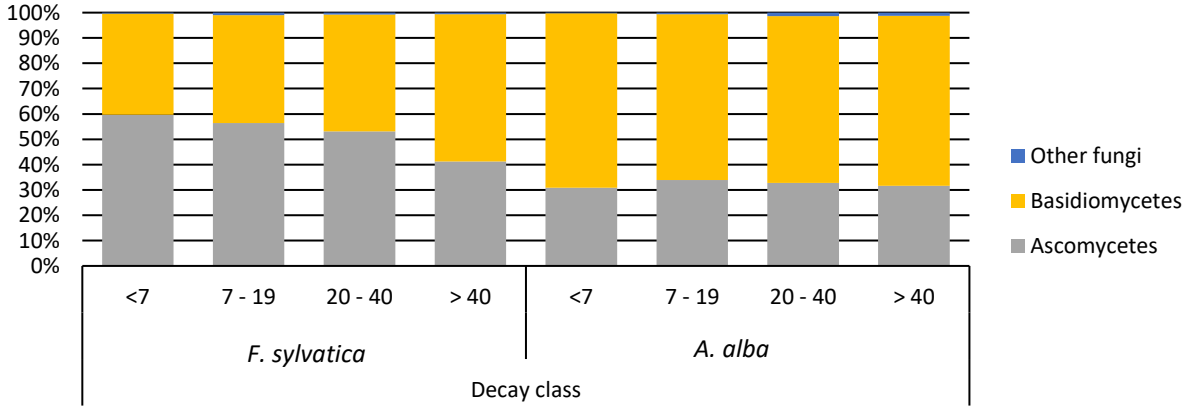


Fig. 7 Relative abundances of fungal phyla on decaying wood of *F. sylvatica* and *A. alba.* Displayed phyla are shown for orders with abundance >0.5% at least in 3 samples from the dataset. Phyla with lower abundances are labelled as „Other fungi “.

The most abundant orders on decaying beech in Salajka, reaching over 10 % of relative abundance on average in all sampled tree species, were Agaricales (21 %) and Polyporales (11 %) from the phylum basidiomycetes and Helotiales (17 %) and Xylariales (11 %) from phylum ascomycetes (Fig.8). On *A. alba*, the most abundant orders belonging to basidiomycetes were Agaricales (25 %) and Corticiales (15 %), from asomycetes it was the order Helotiales (14 %).

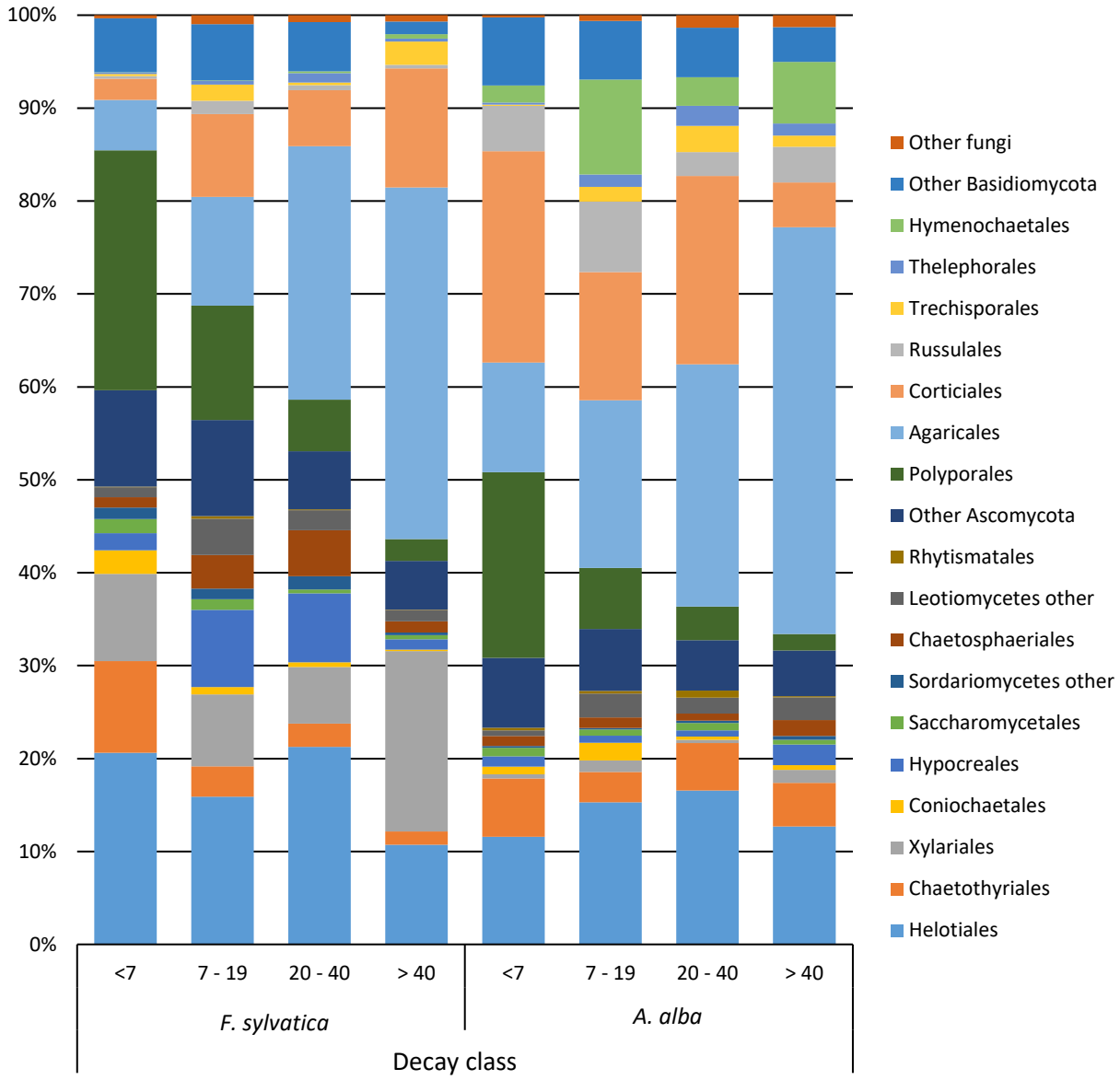


Fig.8 Relative abundances of fungal orders on decaying wood of *F. sylvatica* and *A. alba*. Displayed orders are shown for orders with abundance >0.5 % at least in 10 samples from the dataset, or >10 % in at least 3 samples. Orders with lower abundances are labelled as „Other Ascomycota", "Other Basidiomycota "and „Other fungi ".

On *F. sylvatica,* on average the most abundant genera were Mycena (8 %), Fomes (4.9 %), Ganoderma (3.8 %), Mycetinis (2.7 %), Pleurocybella (2.6 %), Hypholoma (1.4 %) and Megacollybia (1.2 %) from Basidiomycetes and Kretzschmaria (5.7 %), Trichoderma (4.0 %), Ascocoryne (3.8 %), Leptodontidium (3.8 %), Phialocephala (2.5 %), Sorocybe (2.4 %), Eutypa (2.1 %), Hypoxylon (1.5 %) and Zignoella (1.4 %) from Ascomycetes (Fig.3). While on average the most abundant genera found on *A. alba*, were Mycena (6.6 %), Pleurocybella (6.3 %), Hyphodontia (5.2 %), Resinicium (2.8 %), Ganoderma (2.3 %), Clitocybula (1.9 %), Ischnoderma (1.6 %), Sistotremastrum (1.4 %) and

Megacollybia (1.2 %) belonging to Basidiomycetes and Sorocybe (1.6 %), Arachnopeziza (1.2 %) and Phialophora (1.1 %) from Ascomycetes (Fig.9).

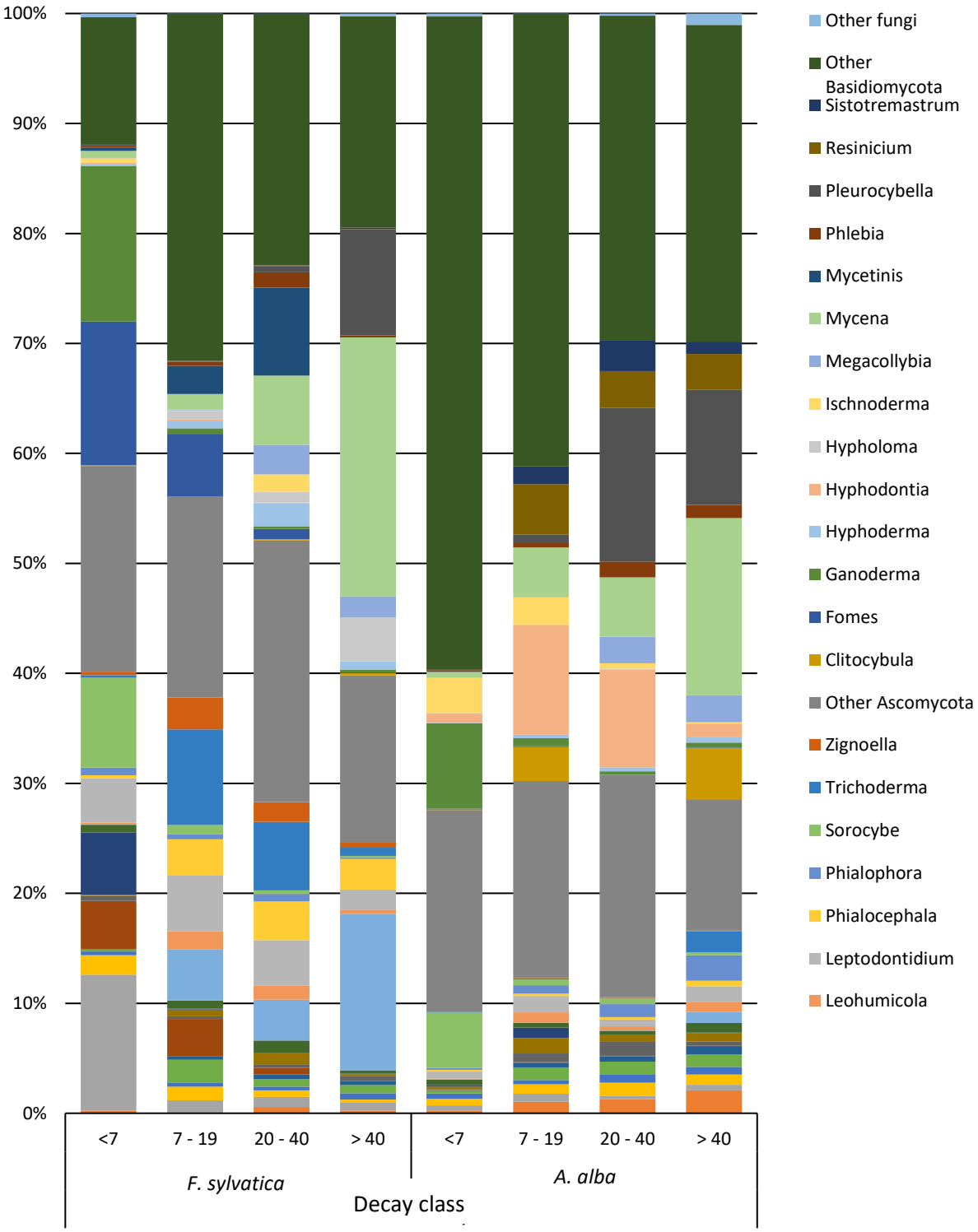


Fig.9 Relative abundances of fungal genera on decaying wood of *F. sylvatica* and *A. alba*. Displayed genera are shown for genera with abundance >0.5 % at least in 20 samples from the dataset, or >10 % in at least 3 samples. Genera with lower abundances are labelled as „Other Ascomycota “, “Other Basidiomycota “and „Other fungi “.

When considered various ecological groups of fungi present on dead wood, the most prevailing groups were other saprotrophs (50 % from all sequences) and white rot fungi (35 %) (Fig. 10). The relative abundance of other saprotrophs was growing with progressive decay, significantly among <7 and other decay classes on *F. sylvatica*. On *A. alba*, the relative abundance of other saprotroph was alternately increasing and decreasing with the highest values in 7-19 and >40 decay class (Fig. 4). White rot fungi seemed to be the most abundant on first decay stage logs (*F. sylvatica* = 42 %; *A. alba* = 57 %), and then their relative abundance followed individual development according to tree species. In *F. sylvatica*, the relative abundance of white rot fungi in <7 class showed high variability among logs. The relative abundances of white rot fungi on *A. alba* significantly decreased after 7 years of decay, reaching the lowest values (28 %) in >40, where the continuous higher importance of ectomycorrhizal (EcM) fungi since 20-40 class persisted. Brown rot fungi were almost non-existent on both trees, with one exception of 7-19 class on *A. alba*, where their relative abundance non-significantly increased due to dominance of *Wrightoporia* sp. and *Fomitopsis pinicola* on two sampled logs. Yeasts were presented in all decay classes on both trees, as well as the lichenized fungi. EcM fungi were more relatively abundant in the 20-40 class on *F. sylvatica*. Relative abundance of plant pathogenic fungi was progressively declining on both tree species.

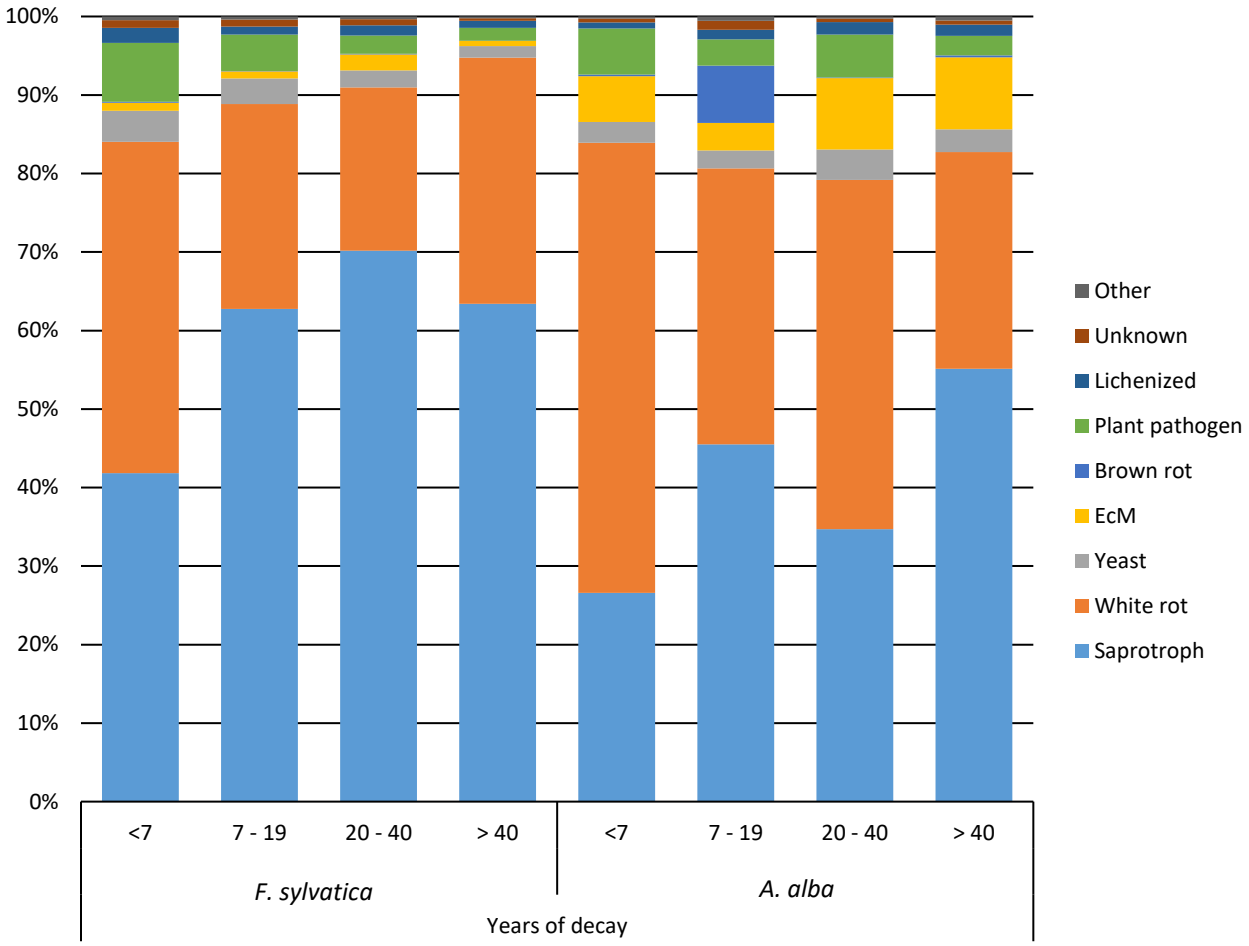


Fig.10 Relative abundances of various ecological groups on decaying wood of *F. sylvatica* and *A. alba.* Under label "Unknown" can be found fungi for which wasn´t possible to trace their ecology. Under "other" are fungi from minor various ecological groups, like arbuscular mycorrhizas, animal pathogens, coprophilous fungi, endophytic fungi, entomophagous fungi and mycoparasites.

Mann-Whitney U test showed only few significances for ecological groups on different decay classes inside one tree species (Fig.11). For *F. sylvatica*, other saprotrophs, ectomycorrhizal (EcM), brown rot, plant pathogenic fungi showed significant differences in relative abundance among age classes. Other saprotroph achieved the highest relative abundance in the 20-40 class (70.1 %) and the lowest in the <7 class (41.8 %). EcM fungi had the highest relative abundance in the 20-40 (2.1 %) and the lowest in the >40 class (0.7 %). Brown rot fungi and plant pathogen were the most abundant in the <7 class (0.1 %; 7.5 %), and less abundant in >40 class (0.04 %; 1.6 %). On *A. alba* logs the ecological groups which differed were white rot, plant pathogen and other saprotrophs. White rot fungi achieved the highest relative abundance in the <7 class (57.3 %), and the lowest in the >40 class (27.6 %). Other saprotrophs were the most abundant in the 20-40 class (55.1 %) and the less abundant in >40 class (34.7 %). Plant pathogen followed the same development pattern as in F. sylvatica logs and had the lowest abundance in <7 class (5.9 %) and the highest in >40 class (2.5 %).

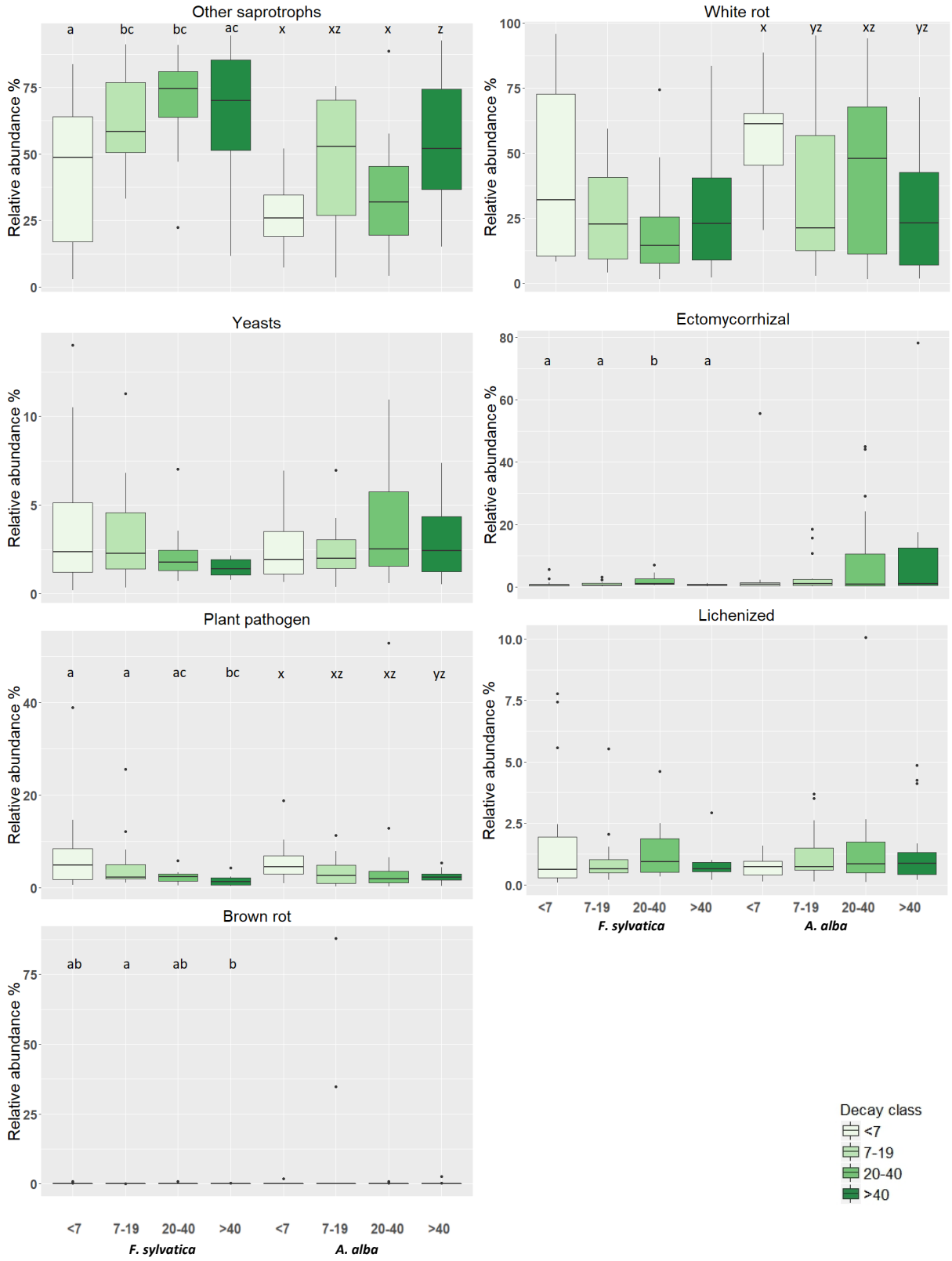


Fig.11 Relative abundance of ecological groups of fungi within individual coarse woody debris in Salajka. Different letters indicate significant differences between the decay classes within each CWD tree species (Mann-Whitney U test, $p < 0.05$).

Dominance of fungal species on logs was considered (Table 5). The highest number of dominated logs was recorded for *Pleurocybella porrigens*. It was dominant on 9 logs in total, 8 *A. alba* and 1 *F. sylvatica* and in total it appeared on 12 CWD with abundance over 10%. *Resinicium bicolor* was exceptionally added to the showed table, even without reaching the level of three dominated tree as it reached 93 % dominance on one *A. alba* log of second decay class, the highest recorded dominant abundance of one species on one log. Most of the fungi prefered one tree species over the other with the exception of a few some generalist like *Sorocybe* sp. and *Megacollybia platyphylla*.

Table 5 List of the most frequent fungal taxa dominating the sequence pools of *A. alba* and *F. sylvatica* logs in the Salajka forest. Shown only fungal species with dominance at least 10% on 3 CWD and more. Exceptionally showing one *R. bicolor* with dominance on only 2 CWD.

[1] number of CWD where the sequences of this taxon were most abundant; [2] number of CWD where this taxon represented >10% of all sequences; [3] maximal recorded relative abundance of sequences

Species	CWD with dominance[1]	*Fagus*[1]	*Abies*[1]	CWD >10%[2]	Max. abundance (%)[3]
Pleurocybella porrigens	9	1	8	12	70.35
Mycena niveipes	5	0	5	8	75.19
***Hypocrea* sp.**	6	6	0	8	45.37
Kretzschmaria deusta	2	2	0	7	54.62
Fomes fomentarius	5	5	0	5	71.48
Hyphodontia alutaria	1	0	1	5	56.45
***Sorocybe* sp.**	3	3	0	5	41.87
Mycena romagnesiana	4	3	1	5	39.88
Ganoderma applanatum	4	3	1	4	85.78
Mycena galericulata	3	2	1	4	75.38
Resinicium furfuraceum	4	0	4	4	68.30
Megacollybia platyphylla	2	1	1	4	40.98
Hypholoma sublateritium	0	0	0	4	14.66
Hyphodontia aspera	2	0	2	3	86.16
Ascocoryne sarcoides	1	1	0	3	68.82
Clitocybula lacerata	2	0	2	3	66.87
Marasmius alliaceus	3	3	0	3	59.62
Hypoxylon fragiforme	1	1	0	3	51.98
***Sistotremastrum* sp.**	1	0	1	3	39.96
Ischnoderma benzoinum	1	1	0	3	22.03
Eutypa spinosa	1	1	0	3	21.22
Zignoella pulviscula	0	0	0	3	18.22
Phialocephala lagerbergii	1	1	0	3	13.79
Resinicium bicolor	2	0	2	2	93.24

Mantel test revealed that tree species, time of decay, pH level, ergosterol content and β-glucosidase activity had significant important effect on fungal community structure (Table 6). N content alone wasn´t significant, while when combined with C content, or pH level, it showed

significance. The most important effect on fungal community structure had combination of factors tree species, pH level and N content (R=0.2771; p=1.00E-05).

Table 6 Mantel test results for tested fungal matrice with several factors of influence. Colors indicate the most important factors or combinations of factors. Deeper the color is, the more important the factors are in structuring the fungal community.

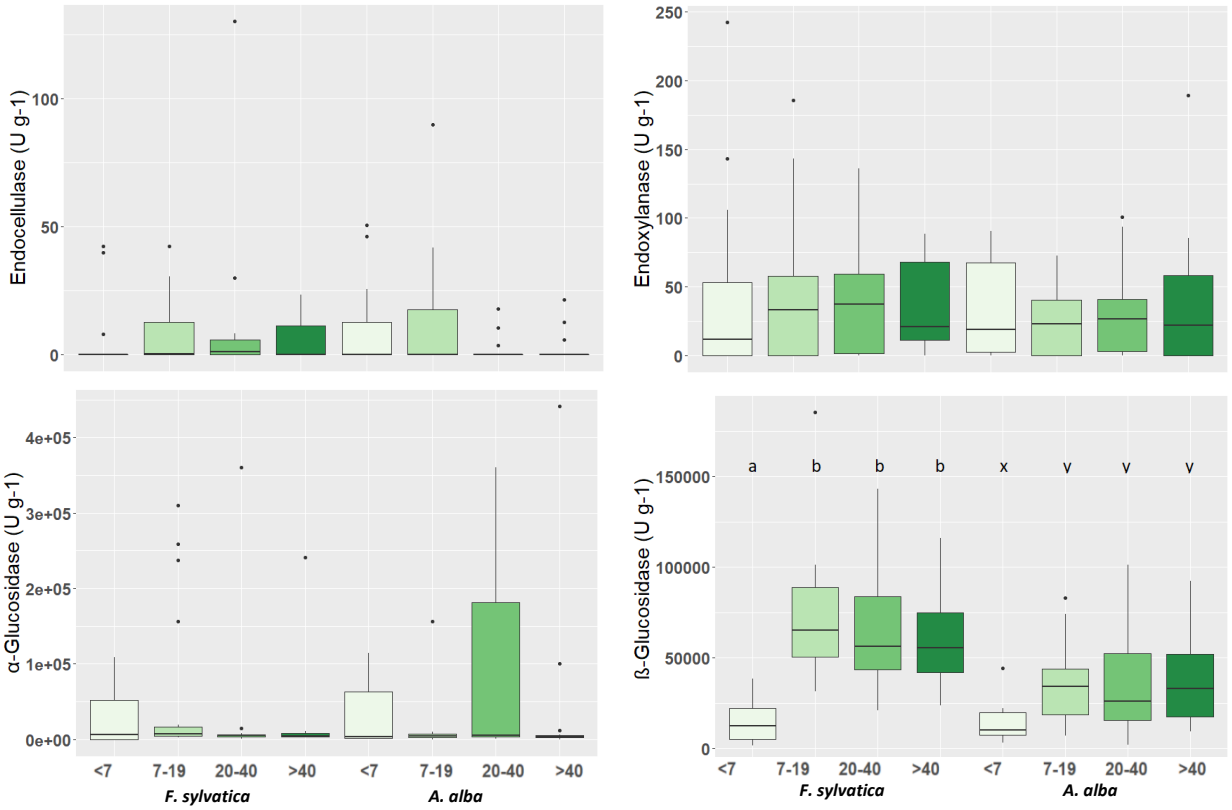
Matrices		R	p	
Fungi	Tree species	0.2199	1.00E-05	Good for fungi
Fungi	Time of decay	0.1319	2.00E-05	
Fungi	Tree species and time of decay	0.1360	1.00E-05	
Fungi	N	0.0332	0.16169	
Fungi	C and N	0.1228	0.00038	
Fungi	C, N and pH	0.1373	6.00E-05	
Fungi	pH	0.1968	1.00E-05	Good for fungi
Fungi	pH and N	0.1965	1.00E-05	Good for fungi
Fungi	Tree species, pH and N	0.2771	1.00E-05	Best for fungi
Fungi	Tree species, time of decay, pH and N	0.1359	1.00E-05	
Fungi	Enzymes	0.0636	0.04464	
Fungi	Ergosterol	0.0979	0.00555	
Fungi	MnP	0.0114	0.38226	
Fungi	β-glukosidase	0.0753	0.01916	
Fungi	Lignin	0.0605	0.06224	

One-way Permanova test of time of decay (F= 2.6; p=0.0001) showed less significance than the test of tree species (F=5.41; p=0.0001), two-way Permanova test was performed with tree species being the first grouping factors and decay class the second factor. Their interaction was also significant. The fungal community structure was influenced by the difference in tree species and the time of decay.

5.1.3 Activities of extracellular enzymes on downed decaying wood

In many cases, the activity of extracellular enzymes in <7 logs differed from all other decay classes (Fig. 12). Endo-cleaving enzymes were consistently present with stable level of activity (endoxylanase), or present with small level of activity (endocellulase), across all decay classes, without significant

change. Exo-cleaving cellulases and hemicellulases, showed some dependence on decay classes corresponding to the changes in their activity. With one exception of α-glucosidase, where wasn´t proved any significant change in neither of tree species within decay classes, enzymes β-glucosidase, β-galaktosidase, exocellulase and β-xylosidase followed the same development of activity pattern, where the <7 logs differed significantly from all other decay classes in both tree species. In these enzymes, the <7 logs showed little activity, in contrast with other classes where there was significant increase of activity, since when the level of activity followed individual patterns for each enzyme. In *F. sylvatica* activity of β-glucosidase culminated in 7-19 class and then started to decline. On the contrary in *A. alba* the activity was kept in more or less the same range after <7 class, which was the case for β-galaktosidase and exocellulase as well. Exocellulase activity in *F. sylvatica* stayed consistent after <7 class. N-acetlyglucosaminidase, phosphomonoesterase and lipase also followed the above mentioned pattern where their activity significantly increased after <7 class. Among lignin degrading enzymes, laccase exhibited significantly growing activity and after first decay class, in *F. sylvatica* the activity was fluctuating within decay classes. Manganese peroxidase on fir logs significantly increased in activity between <7 and 7-19 class, and then in classes 20-40 and >40 decreased to comparable activity level of <7 class.



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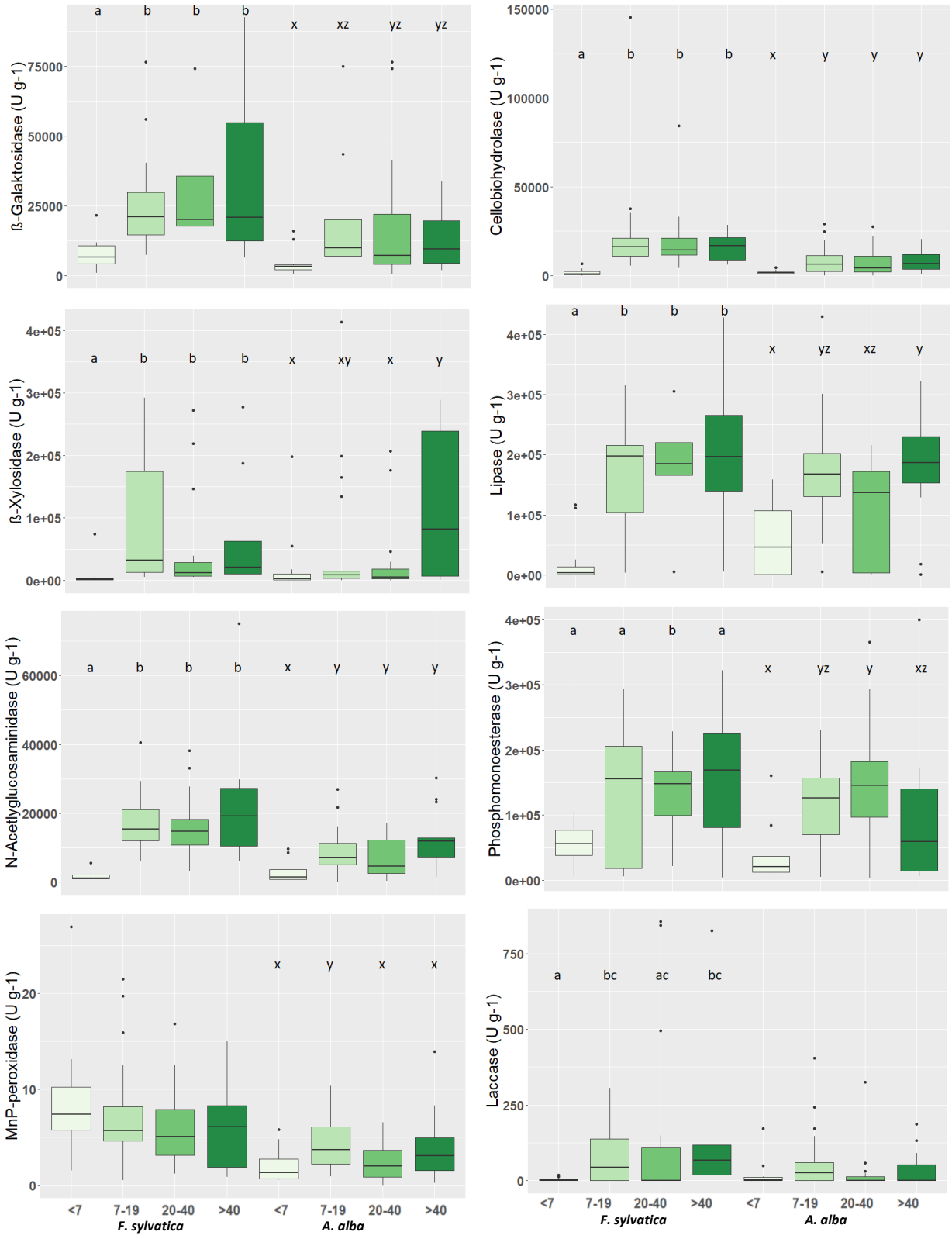


Fig. 12 Distribution of extracellular enzyme activities in coarse woody debris of *Fagus sylvatica*, *Abies alba* in the Salajka natural forest. Different letters indicate significant differences between the decay classes within each CWD tree species (Mann-Whitney U test, $p < 0.05$).

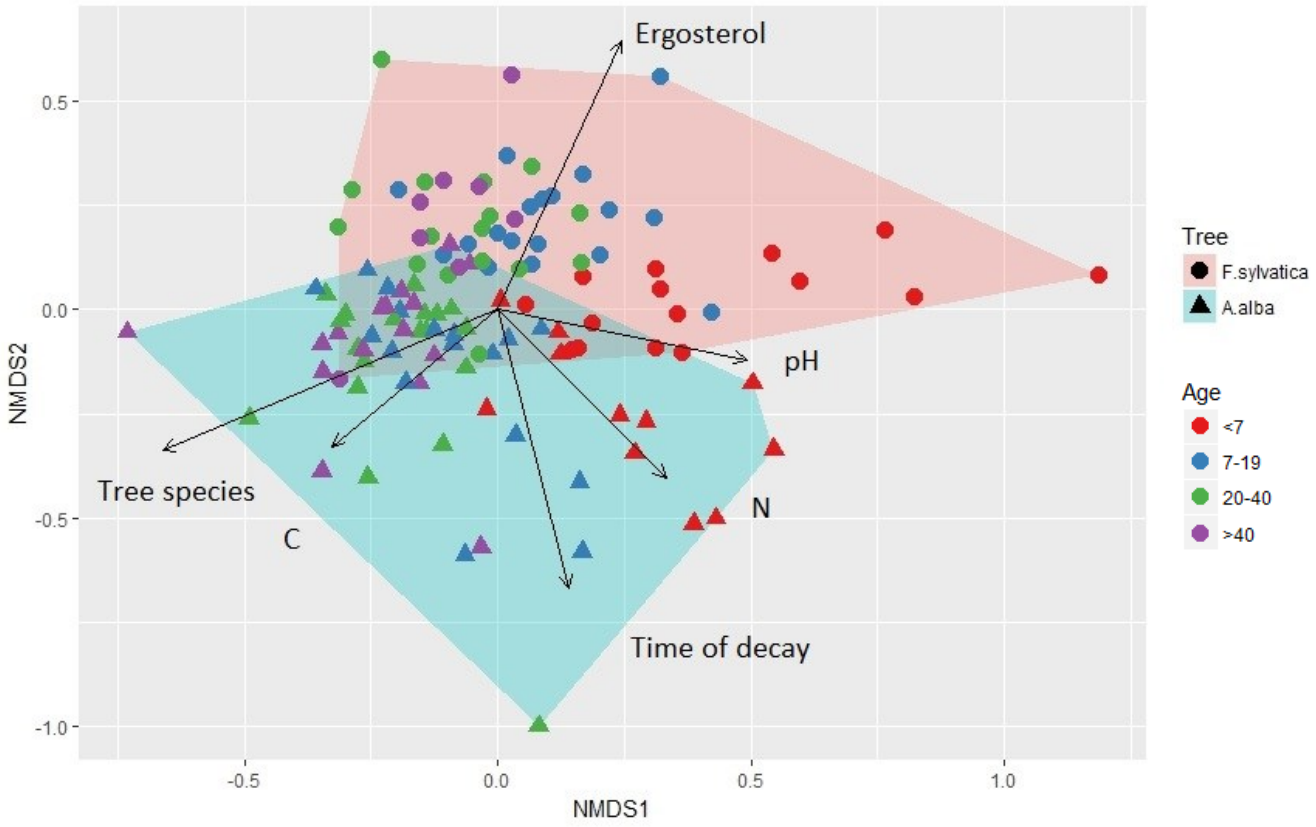
One-way Permanova tests showed significance for tested activity of enzymes and the time of decay (F = 7.105; p = 0.0001). Tested tree species weren´t significant. Mantel test showed the highest significance for tested enzyme activities with ergosterol content (F = 0.1729; p = 0.0026) and N content (F = 0.1406; p = 0.0055) (Table 7). Tree species alone wasn´t statistically significant, but combination of factors tree species and time of decay showed significance (F = 0.1220; p = 0.0020).

Table 7 Mantel test results for tested enzymatic matrices with several factors of influence. Colors indicate the most important factors or combinations of factors. Deeper the color is, the more important the factors are in structuring the fungal community.

Matrices		**R**	**p**	
Enzymes	N	0.1406	0.0055	Good for enzymes
Enzymes	pH	0.0189	0.3081	
Enzymes	pH and N	0.0356	0.2021	
Enzymes	C and N and pH	-0.0203	0.6140	
Enzymes	Time of decay	0.1219	0.0021	
Enzymes	Tree species	0.0022	0.3225	
Enzymes	Tree species and time of decay	0.1220	0.0020	
Enzymes	Tree species, time of decay, pH and N	0.0263	0.1891	
Enzymes	Time of decay and N	0.1225	0.0017	
Enzymes	Lignin	-0.0415	0.7654	
Enzymes	Ergosterol	0.1729	0.0026	Best for Enzymes
Enzymes	Ergosterol and N	0.1729	0.0031	

5.1.4 NMDS

Two-dimensional NMDS analysis of fungal communities on *F. sylvatica* and *A. alba* showed quite distinctive separation of the tested tree species (Fig.13). Also, it was possible to distinguish different decay classes clustering together due to higher similarity in hosted fungal species. The older the logs were, the more homogenous fungal community they hosted, as seen from the graphics (Fig.9). Projected vectors of environmental variables showed how the communities were shaped by their influence, and which of the factors were the most important predictor of fungal community composition.



NMDS stress: 0.2352

NMDS ordination

	r^2	p
pH	0.4758	0.0001
N	0.2745	0.0001
C	0.2145	0.0001
Time of decay	0.4674	0.0001
Tree species	0.5511	0.0001
Ergosterol	0.2571	0.0001

Fig. 13 Two-dimensional NMDS of fungal community composition on CWD in Salajka. Dataset was based on relative abundances of 214 fungal genera, which achieved at least 0.5 % in 3 and more samples, or more than 10 % in one and more samples. Coloured area represents defined territory covered by one of the tree specie (red = *F. sylvatica*; green = *A. alba*). Graph shows individual logs (CWD) and vectors of environmental variables.

5.1.5 Indicator species of beech and fir downed logs

Indicator species were the ones found repeatedly, on CWD certain decay class of one tree species and absent or rare in others (Table 8). The analysis of indicator species in Salajka revealed that, that only a few of species were shared among more decay classes and also that only some were typical

wood decayers, which would be expected on dead wood (with some exceptions like *Polyporus brumalis*, found on *F. sylvatica*, causing white rot decay). Only seven genera were shared among more decay classes, mostly associated to one of the two trees (*Arachnopeziza, Eutypa, Hypodontia, Mortierella, Xenochlora*). Others, like *Mycena* or *Hypholoma*, appeared on different tree species but also various decay classes.

Table 8 List of the indicator species on *A. alba* and *F. sylvatica* logs in natural forest Salajka based on the decay class and tree species.
* - p < 0.05; ** - p < 0.01; *** - p < 0.001.

***Fagus sylvatica* <7**		***Abies alba* <7**	
Ascocoryne sarcoides	***	*Pezicula* sp.	***
Hypoxylon fragiforme	**	*Phaeomoniella* sp.	***
Jobellisia sp.	***	*Helicodendron websteri*	***
Fomes fomentarius	***	*Hypholoma capnoides*	***
Absconditella lignicola	***	*Xenochalara* sp.	***
Cryptosporiopsis sp.	**	*Hyphodontia nespori*	**
Sorocybe sp.	**	*Acephala* sp.	*
Eutypa spinosa	**	*Hericium americanum*	***
Polyporus brumalis	*	*Exophiala* sp.	***
Thanatephorus cucumeris	*	*Helicoon* sp.	*
Phaeosphaeria sp.	**	*Alternaria tenuissima*	**
Neobulgaria pura	*		
Fusarium ciliatum	**	***Abies alba* 7-19**	
Annulohypoxylon cohaerens	*	*Mycena silvae-nigrae*	*
Botryobasidium subcoronatum	*	*Sphaerobasidium* sp.	*
		Hypochnicium wakefieldiae	**
***Fagus sylvatica* 7-19**			
Zignoella pulviscula	**	***Abies alba* 20-40**	
Microscypha ellisii	*	*Hyphodontia abieticola*	*
Polyscytalum sp.	*	*Mortierella parvispora*	*
Myxarium sp.	*	*Flagellospora* sp.	**
Pseudaegerita corticalis	*	*Lobaria* sp.	**
		Cladophialophora minutissima	**
***Fagus sylvatica* 20-40**		*Sistotrema* sp.	**
Cordana sp.	*	*Pleurocybella porrigens*	*
Hyaloscypha albohyalina	*	*Arachnopeziza aurata*	**
Hyphoderma sp.	*	*Thysanophora penicillioides*	**
Phlebia georgica	**	*Xenochalara juniperi*	*
		Scleropezicula sp.	**
***Fagus sylvatica* >40**		*Articulospora tetracladia*	*
Eutypa sp.	*	*Haplographium* sp.	*
Mycena galericulata	**		
Mycena romagnesiana	**	***Abies alba* >40**	
Xylaria sp.	**	*Mycena niveipes*	*
Kretzschmaria deusta	**	*Mortierella gemmifera*	**
Hypholoma sublateritium	*	*Ramularia pratensis*	*
Inonotus andersonii	**	*Arachnopeziza* sp.	*
		Russula ochroleuca	*
		Ceramothyrium sp.	*

5.2 Description of differences in fungal community on standing and downed deadwood in natural forest Žofín

The sequencing resulted in 400 166 sequences, with minimal length of 40 bp and maximal of 385 bp, for all 63 sequenced samples that were clustered into 8 563 operational taxonomic units. 359 704 sequences divided into 7 136 OTUs were left after exclusion of nonfungal sequences.

5.2.1 Wood physico-chemical properties

Measured C and ergosterol content and pH in differently positioned logs didn´t differ significantly (Fig. 14). Nitrogen content was significantly higher in downed over standing trees (F = 3.697; p = 0.05).

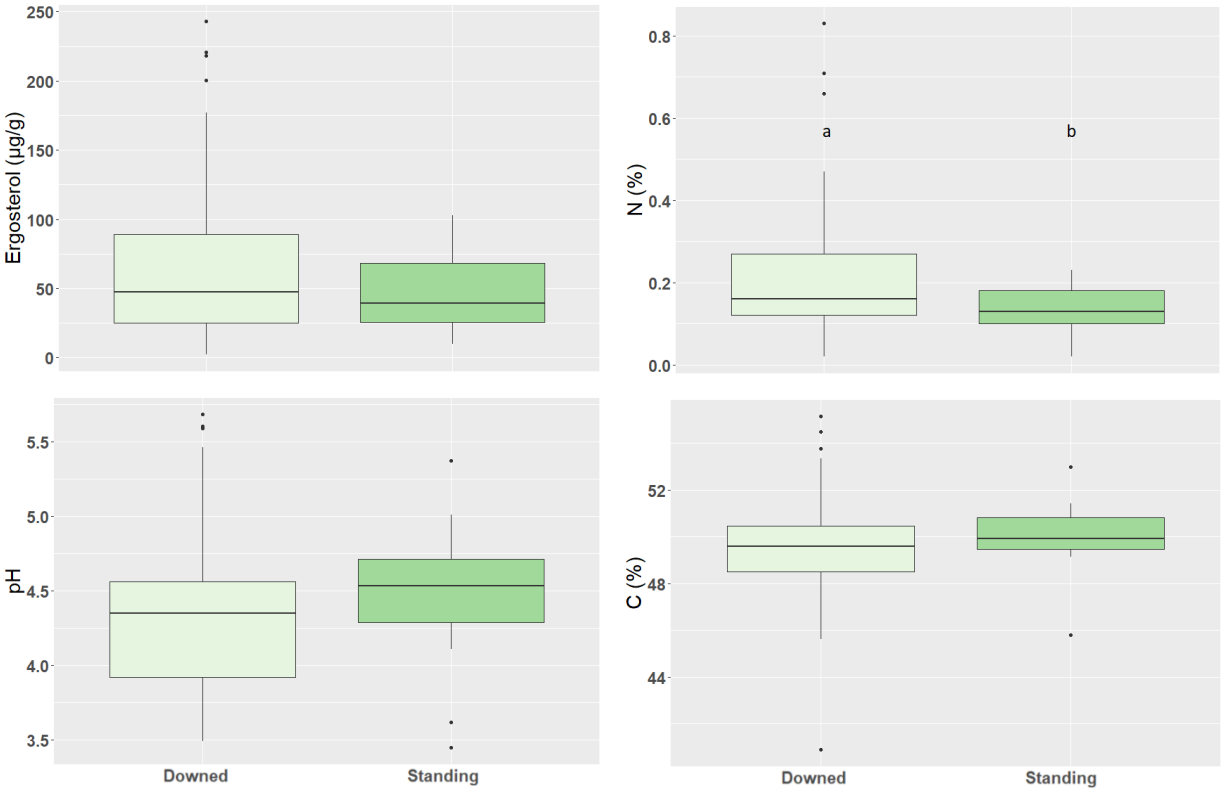


Fig. 14 Content of fungal biomass and chemistry of differently positioned logs in Žofín. Different letters indicate significant differences between differently positioned logs (Ergosterol was tested by Mann-Whitney U test, $p < 0.05$; C and N content and pH were tested by ANOVA and followed by Tukey Honest Significant Difference test, $p < 0.05$).

5.2.2 Diversity indices

None of the diversity indices was significantly different between standing and downed logs (Fig. 15).

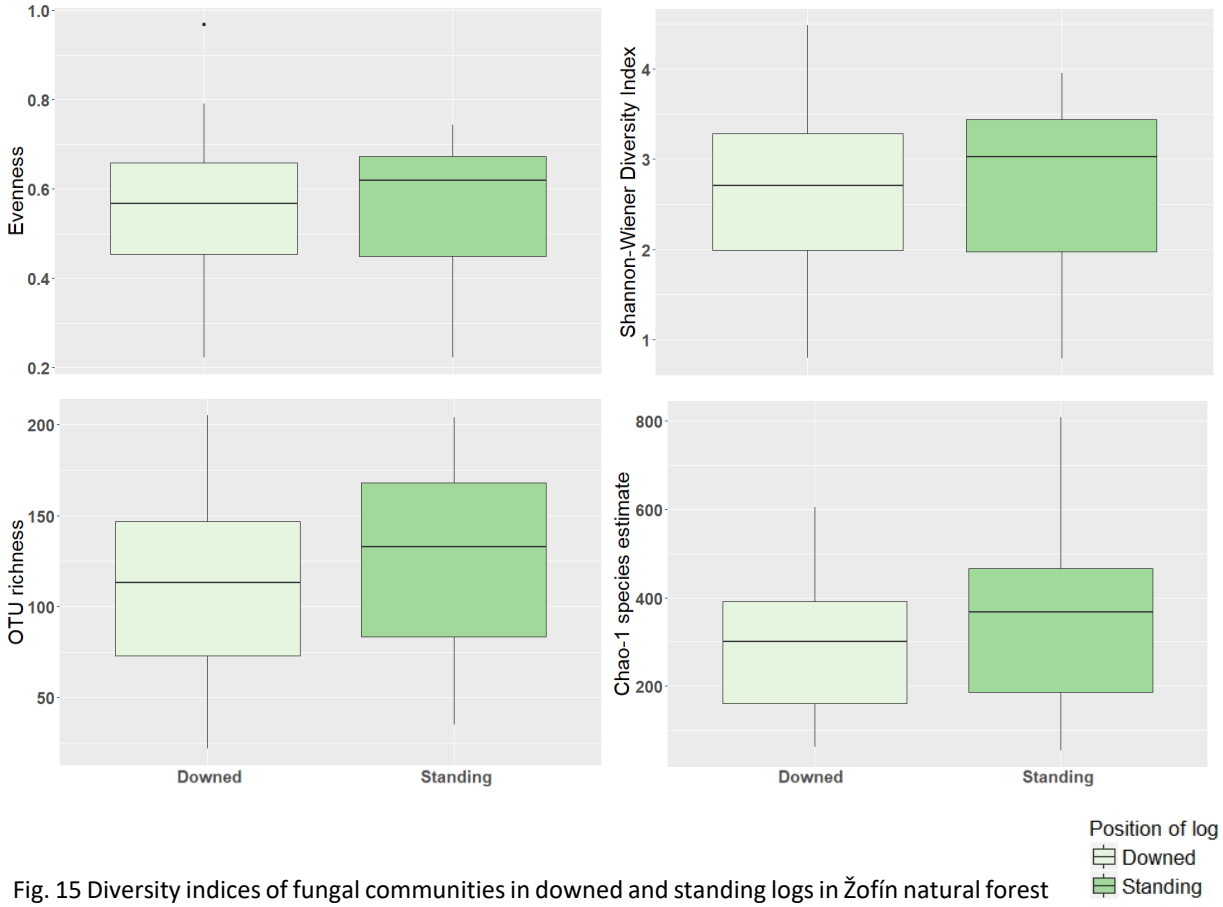


Fig. 15 Diversity indices of fungal communities in downed and standing logs in Žofín natural forest (No significant differences were recorded using Mann-Whitney U test, $p < 0.05$).

5.2.1 Composition of fungal communities on downed and standing dead trees

The relative abundance of ascomycetes in standing dead trees was slightly higher than of basidiomycetes (55 % and 45 %), the share of ascomycetes and basidiomycetes in downed trees was similar (Fig. 16).

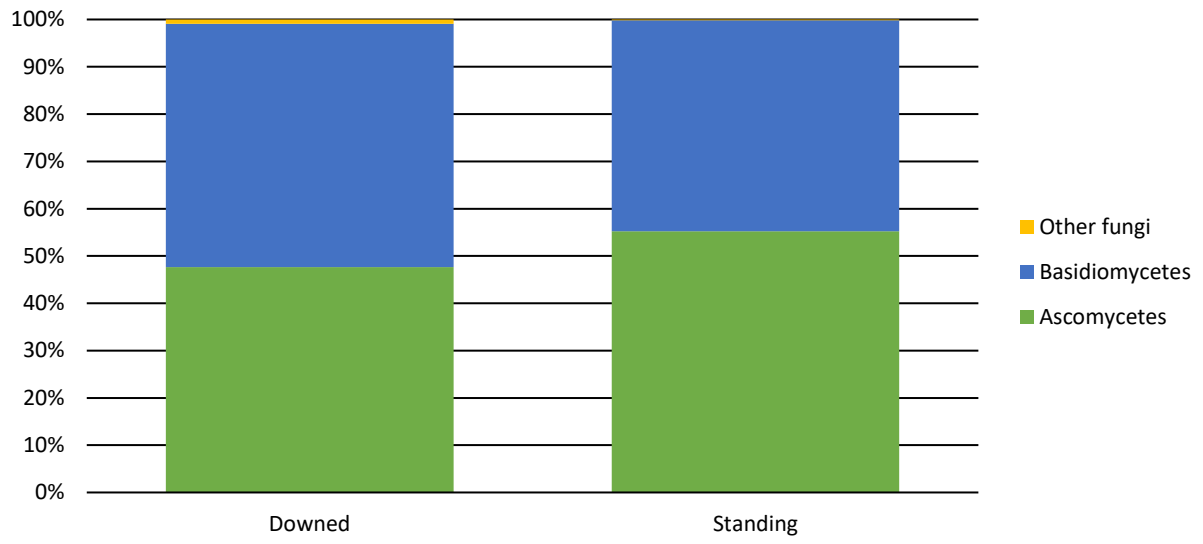


Fig.16 Relative abundances of fungal phyla on downed and standing decaying logs in Žofín natural forest.

When comparing tree species of downed and standing logs, the differences become more evident (Fig. 17). In downed logs of *A. alba* and *P. abies,* basidiomycetes have higher relative abundances then ascomycetes (*A. alba* 36 % Ascomycetes, 63 % basidiomycetes and *P. abies* 44 % ascomycetes, 56 % basidiomycetes), whereas in *F. sylvatica*, ascomycetes seemed to be more prevalent (60 % ascomycetes, 39 % basidiomycetes). In standing logs, relative abundance of fungal were different. In *A. alba* (60 % ascomycetes, 40 % basidiomycetes), *P. abies* (59 % ascomycetes, 40 % basidiomycetes) and *F. sylvatica* (39 % Ascomycetes, 61 % Basidiomycetes).

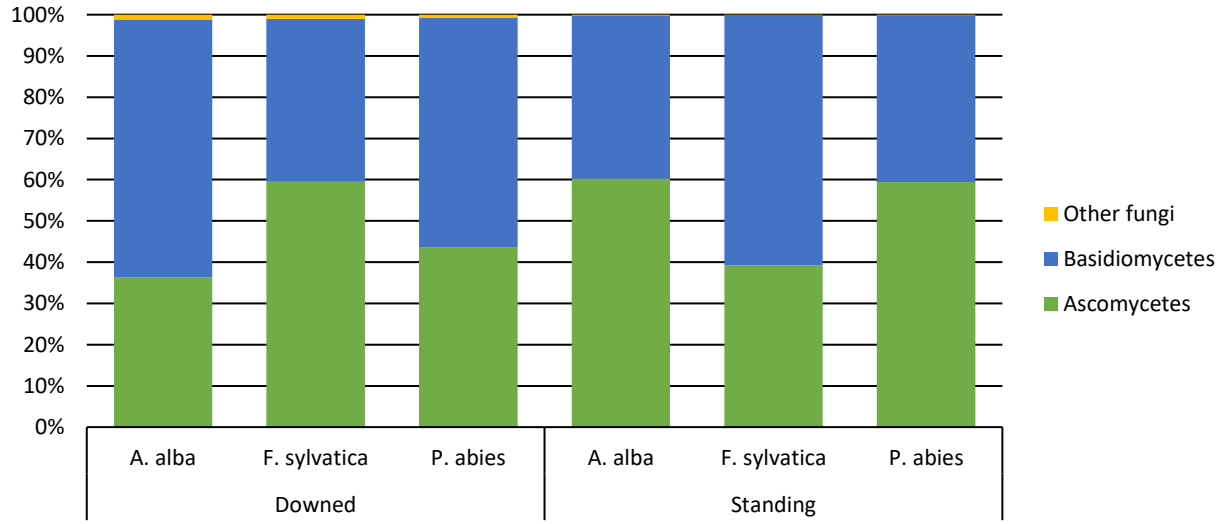


Fig.17 Relative abundances of fungal phyla on downed and standing decaying wood of *A. alba*, *F. sylvatica* and *P. abies.*

When comparing downed and standing dead trees based on their time of decay, it was possible to observe yet another difference (Fig. 18). While in downed dead wood the ratio changed only slightly between logs decaying for <20 years and >20 years being almost equally divided in basidiomycetes and ascomycetes, in standing dead trees, logs decaying for less than 20 years mostly caontained ascomycetes, in logs the relative abundances of ascomycetes and basidiomycetes was similar.

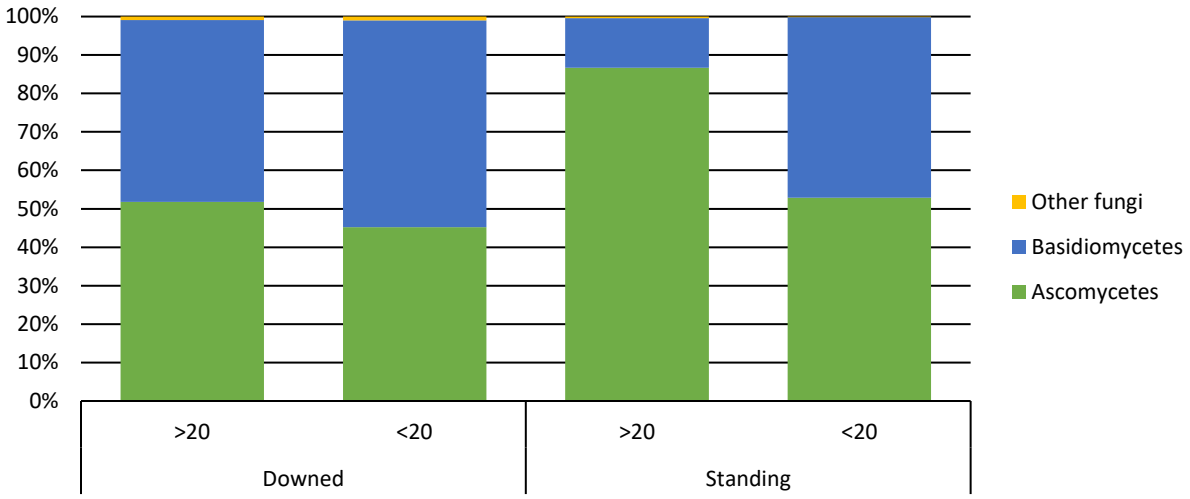


Fig.18 Relative abundances of fungal phyla on downed and standing decaying wood of decaying less than 20 years and more than 20 years.

The most abundant genera on downed logs were *Pseudogymnoascus, Ganoderma, Hyphodontia, Ischnoderma* and *Leiptodontiudium*. And on standing logs they were *Pseudogymnoascus, Ganoderma, Hyphodontia, Gorgomyces* and *Meliniomyces* (Fig. 19).

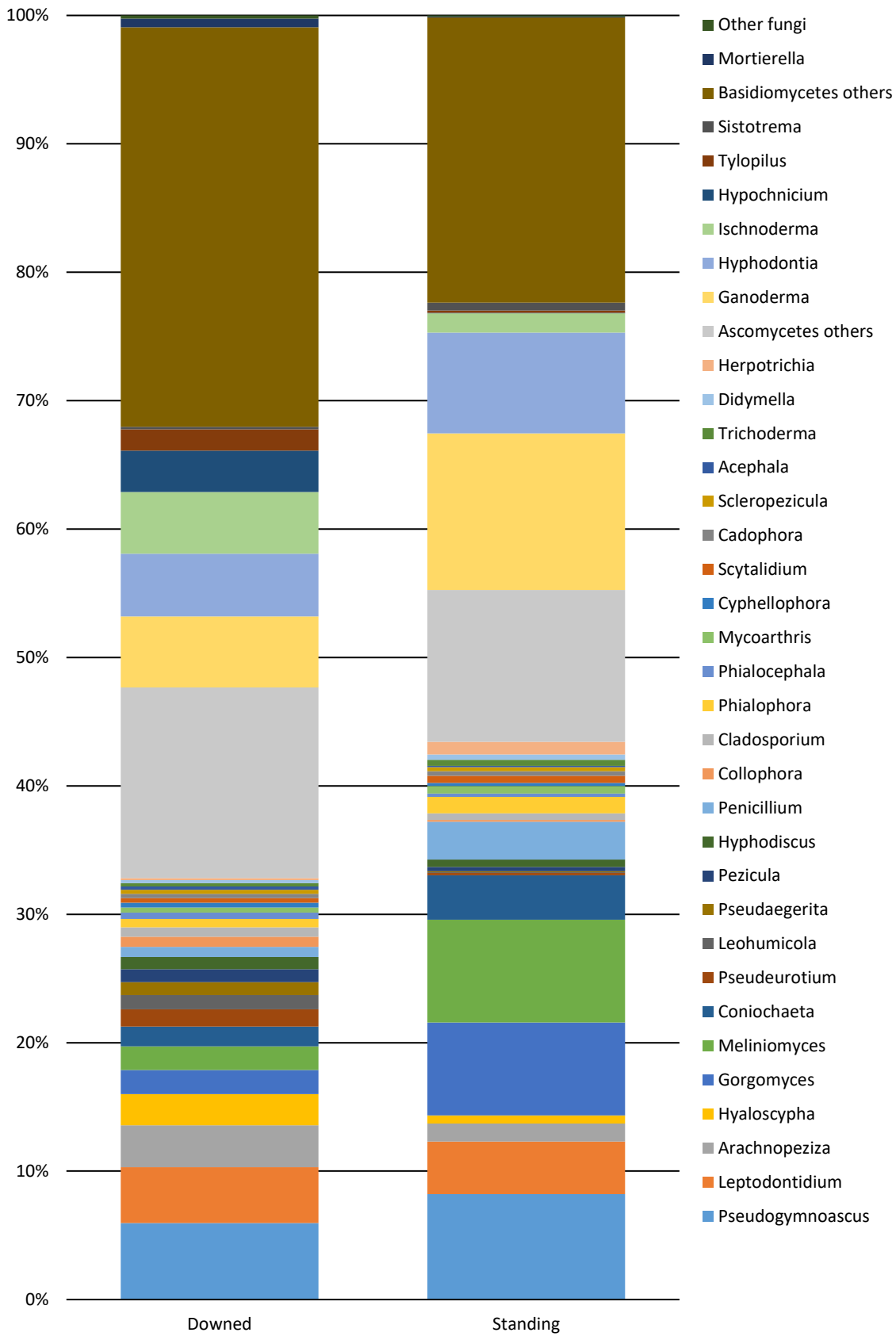


Fig. 19 Relative abundances of fungal genera on downed and standing decaying wood. Displayed genera are shown for genera with abundance > 0.5 % at least in 10 samples from the dataset. Genera with lower abundances are labelled as „Other Ascomycota “, “Other Basidiomycota “and „Other fungi “.

Relative abundances of various ecological groups found on differently positioned logs in Žofín varied only slightly (Fig. 20). On standing logs was possible to observe relative abundance 51 % of other saprotrophic fungi over downed with 43 %. The share of white rot fungi was 41 % on standing logs and 44 % on downed ones.

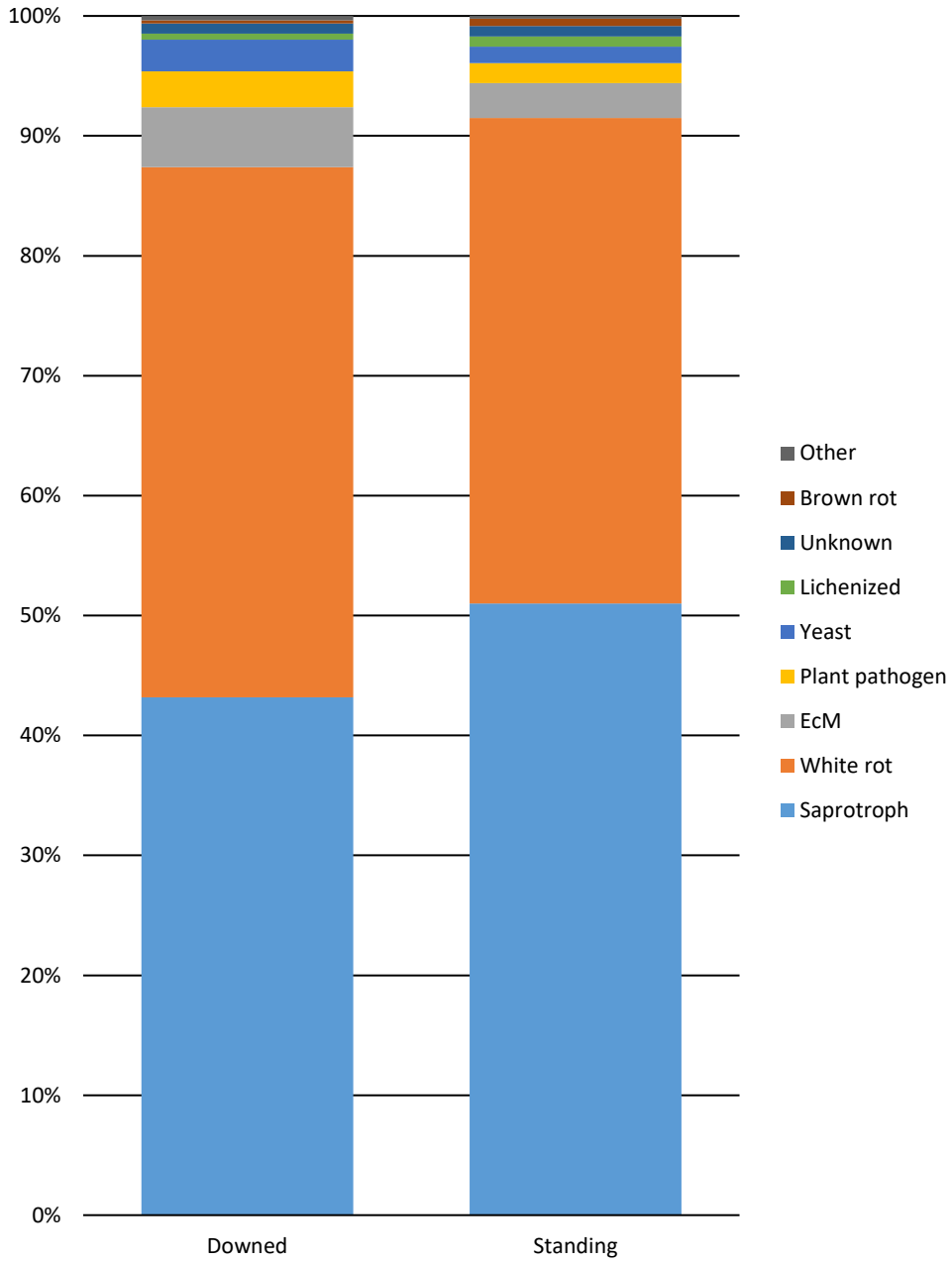


Fig. 20 Relative abundances of various ecological groups on differently positioned decaying wood in Žofín natural forest. Under label "Unknown" can be found fungi for which wasn´t possible to trace their ecology. Under "Other" are fungi from minor various ecological groups, like arbuscular mycorrhized, animal pathogens, coprophilous fungi, endophytic fungi, entomophagous fungi and mycoparasites. EcM signify ectomycorrhizal fungi.

The mean of relative abundance of various ecological groups in differently positioned logs didn´t vary significantly (Fig. 21).

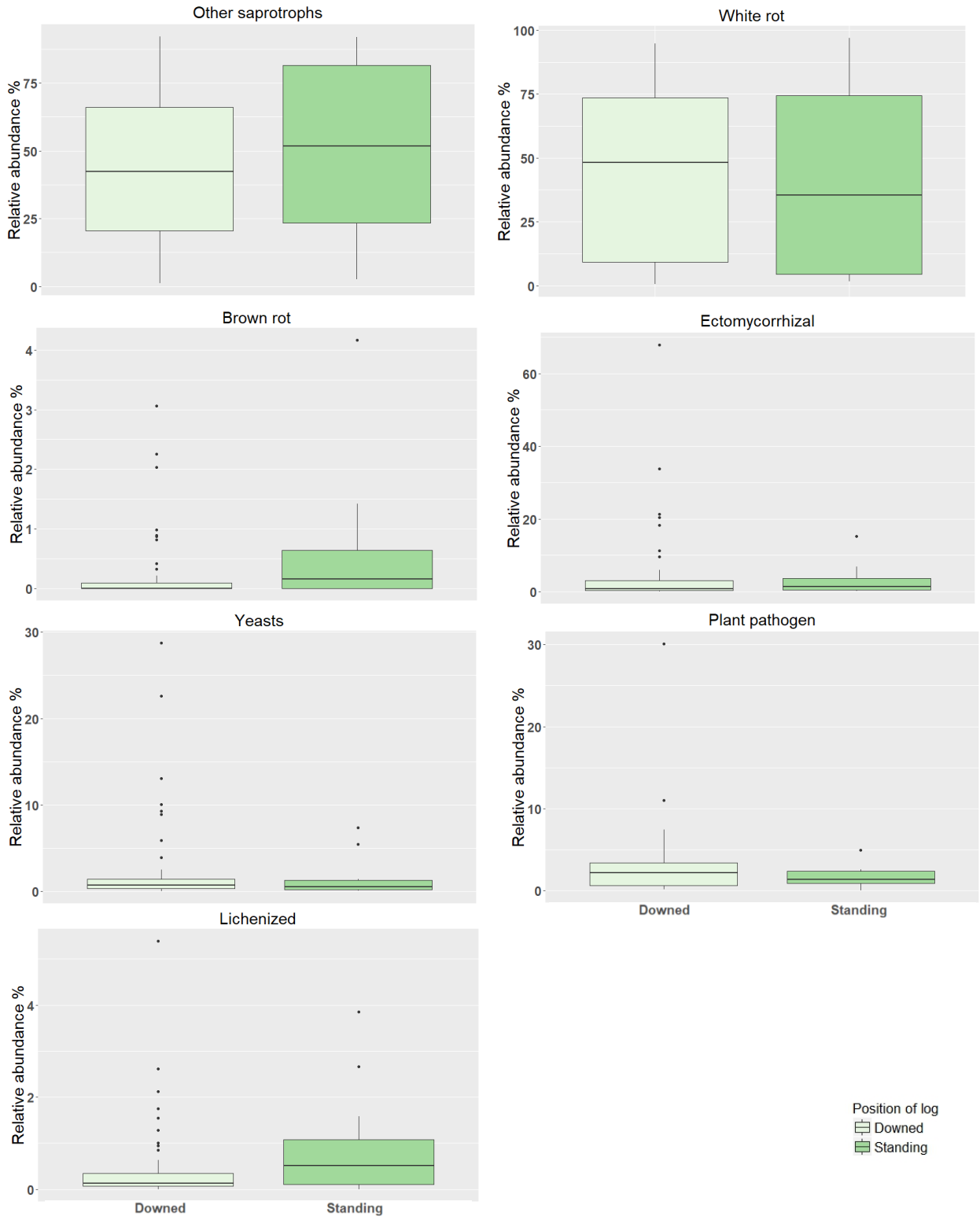


Fig. 21 Relative abundance of ecological groups of fungi in differently positioned logs in natural forest of Žofín. Different letters indicate significant differences between downed and standing logs (No significant differences were recorded using Mann-Whitney U test, $p < 0.05$).

Mantel test (Table 8) showed significance while tested separately tree species and time of decay. The most important effect on fungal communities had combination of factors tree species and time of decay (F = 0.118; p = 0.003). Factor of position of logs wasn´t significant. When combined with other factors, it didn´t add to significance.

Table 8 Mantel test results for fungal community composition and several factors of influence in Žofín natural forest. Colors indicate the most important factors or combinations of factors. Deeper the color is, the more important the factors are in structuring the fungal community.

Matrices		**R**	**p**
Fungi	Tree species	0.09504	0.00107
Fungi	Time of decay	0.1107	0.00448
Fungi	Tree species and time of decay	0.118	0.00246
Fungi	N	-0.008603	0.5603
Fungi	C and N	0.02046	0.32446
Fungi	C and N and pH	0.01846	0.33969
Fungi	pH	0.007559	0.40871
Fungi	pH and N	0.002714	0.45968
Fungi	Tree species, time of decay, pH, N	0.1162	0.00296
Fungi	Enzymes	0.0795	0.03191
Fungi	Ergosterol	0.005517	0.43204
Fungi	Position	0.005958	0.40344
Fungi	MnP	-0.009216	0.57711
Fungi	αG	0.04431	0.18712
Fungi	Lac	0.0007958	0.48355
Fungi	MnP,Lac,αG	0.04433	0.18428
Fungi	Position, tree species	0.1027	0.00052
Fungi	Position, tree species, time of decay	0.1176	0.003
Fungi	Position, time of decay	0.1114	0.00443

Three one-way Permanova tests of tree species (F = 2.152; p = 0.0001), time of decay (F = 1.424; p = 0.0144) and position of the logs during decay (F = 2.195; p = 0.0011) were performed. Two-way Permanova test didn´t show significance for interaction of tree species and position of dead trees.

With the exception of *Gorgomyces honrubiae, Ganoderma applanatum, Arachnopeziza variepilosa, Hyphodontia alutaria* and *Leptodontidium elatius,* fungi were dominant on either downed or standing decaying logs (Table 9). *Pseudogymnoascus pannorum* and *Meliniomyces* sp. achieved the highest number of dominated CWD, 8 logs, which is 12.7 % of all sampled trees, only with the highest recorded

relative abundance of 30 % in log. *Ganoderma applanatum, Fomes fomentarius, Resinicium furfuraceum, Hyphodontia alutaria* and *Climacocystis borealis* achieved the relative abundance over 80 % in one log. While most of these fungi attained such high relative abundance only in one log, *G. applanatum* and *R. furfuraceum* showed such high relative abundance in one log in 3 other logs.

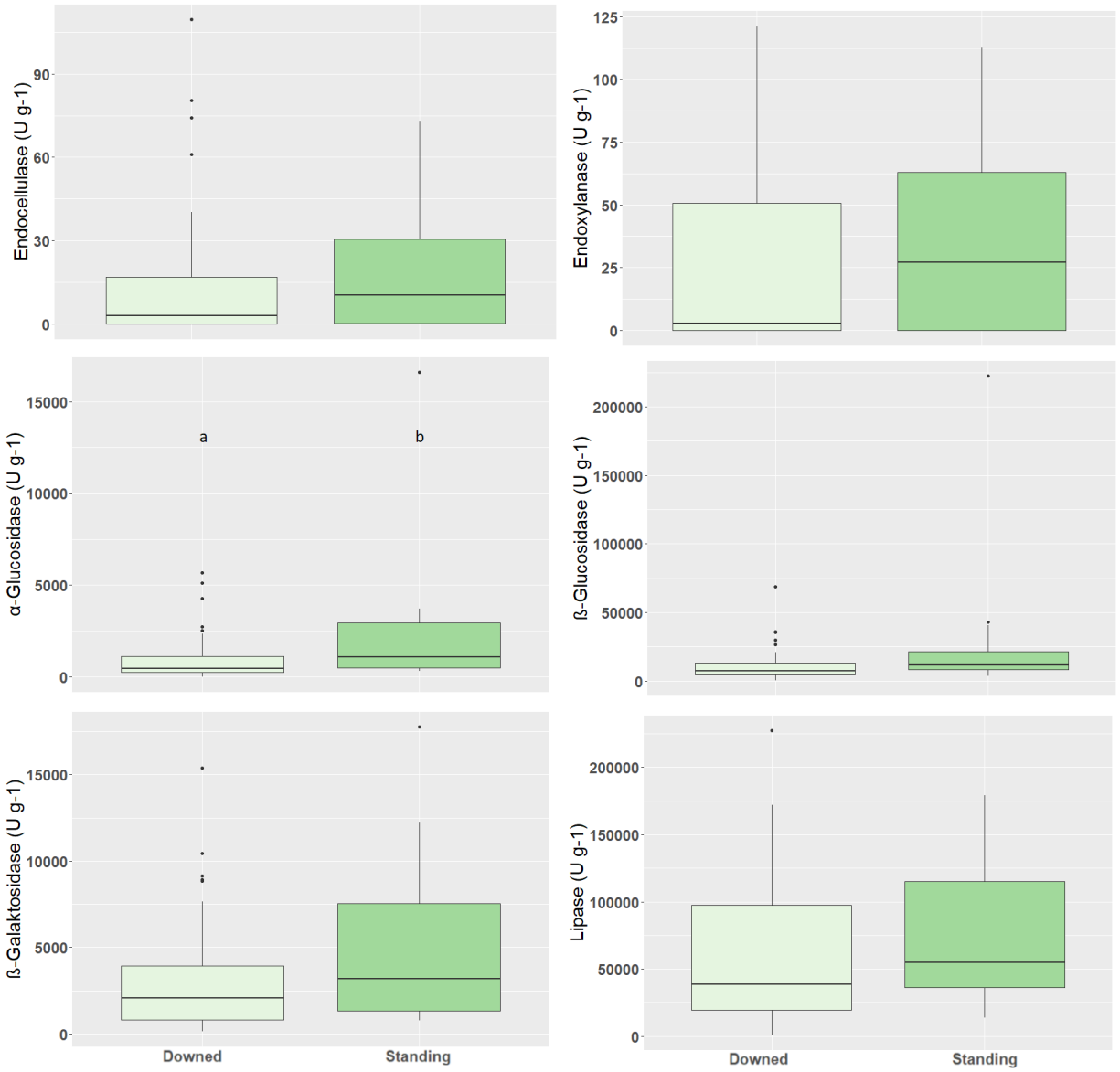
Table 9 List of the most frequent fungal taxa dominating the sequence pools of standing and downed dead trees in the Žofín natural forest. In the table are shown only fungal species with dominance at least 10 % on 2 CWD and more. Exceptionally showing one *Climacocystis borealis* with dominance on only 1 CWD.

[1] number of CWD where the sequences of this taxon were most abundant; [2] number of CWD where this taxon represented >10 % of all sequences; [3] maximal recorded relative abundance of sequences

Species	**CWD with dominance[1]**	***Downed*[1]**	***Standing*[1]**	**CWD >10%[2]**	**Max. abundance (%)[3]**
Gorgomyces honrubiae	6	3	3	6	32.1
Ganoderma applanatum	5	3	2	7	85.2
Fomes fomentarius	5	5	0	6	82.9
Resinicium furfuraceum	4	4	0	4	82.1
Pseudogymnoascus pannorum	3	3	0	8	29.8
Ischnoderma benzoinum	3	3	0	7	73.6
Arachnopeziza variepilosa	3	2	1	6	46.7
Hyphodontia alutaria	3	1	2	3	80.2
Phellopilus nigrolimitatus	3	3	0	3	66.6
Leptodontidium elatius	2	1	1	6	29.1
Tylospora fibrillosa	2	2	0	2	61.3
Hypochnicium wakefieldiae	2	2	0	2	53.7
Trametes versicolor	2	0	2	2	40.6
***Meliniomyces* sp.**	1	0	1	8	31.9
***Lecythophora* sp.**	1	1	0	5	19.1
Geomyces pannorum	1	0	1	3	30.0
Melanchlenus eumetabolus	1	1	0	3	25.6
Hyaloscypha albohyalina	1	1	0	2	47.3
Hyphodontia aspera	1	1	0	2	21.1
Tylopilus felleus	1	1	0	2	19.9
Ceriporiopsis gilvescens	0	0	0	3	20.0
Hyphodontia abieticola	0	0	0	3	16.7
Climacocystis borealis	1	0	1	1	82.4

5.2.4 Activities of extracellular enzymes on standing and downed decaying wood

Activity of lignin modifying enzymes was significantly higher in standing over downed for manganese peroxidases and laccase (Fig. 22). In celullase and hemicellulase degrading enzymes, statistically significant difference in favour to standing logs was possible to observe for enzymes β-xylosidase, cellobiohydrolase, α–glukosidase and the activity was higher in standing logs also for N-acetlyglucosaminidase.



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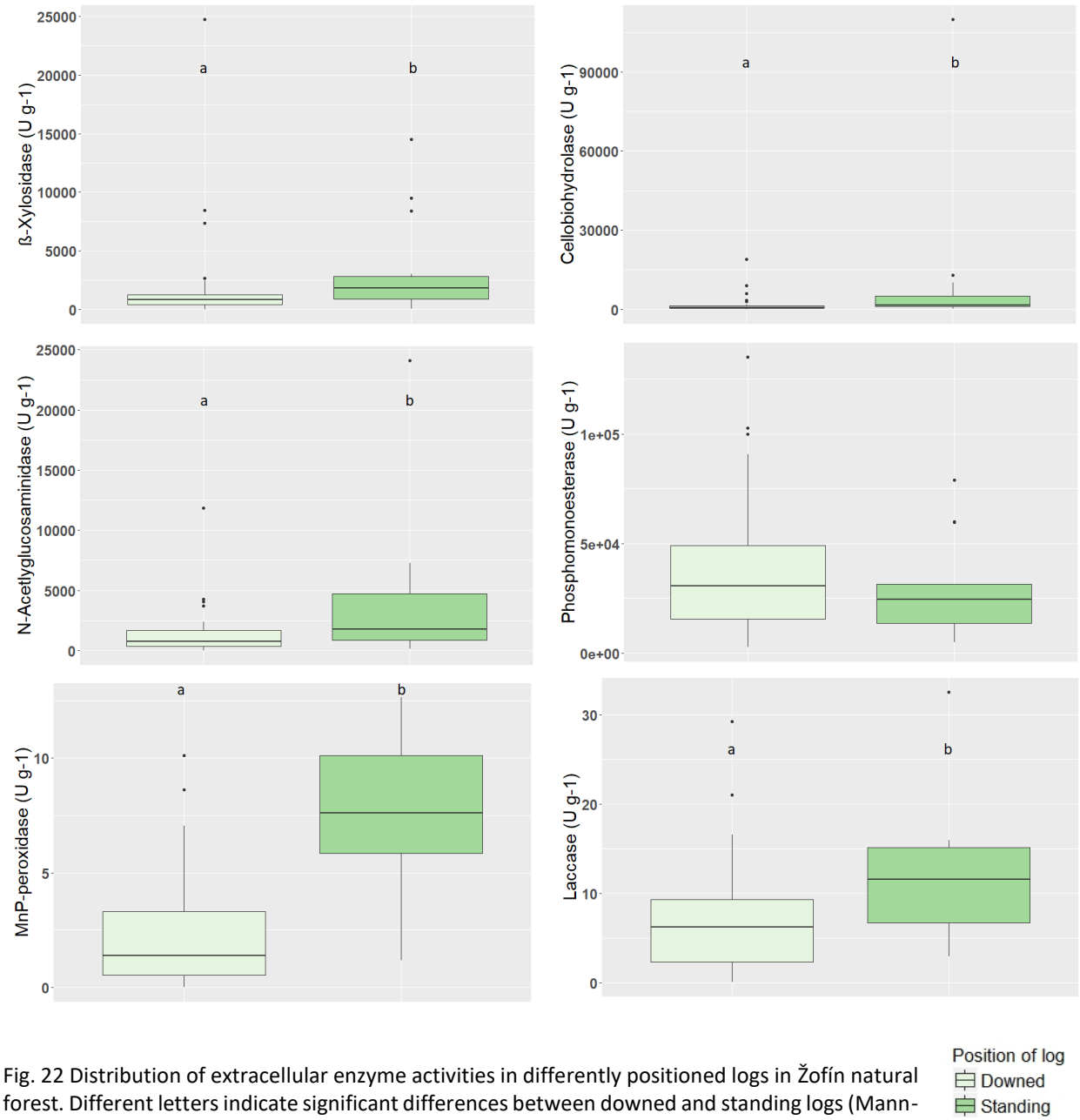


Fig. 22 Distribution of extracellular enzyme activities in differently positioned logs in Žofín natural forest. Different letters indicate significant differences between downed and standing logs (Mann-Whitney U test, $p < 0.05$).

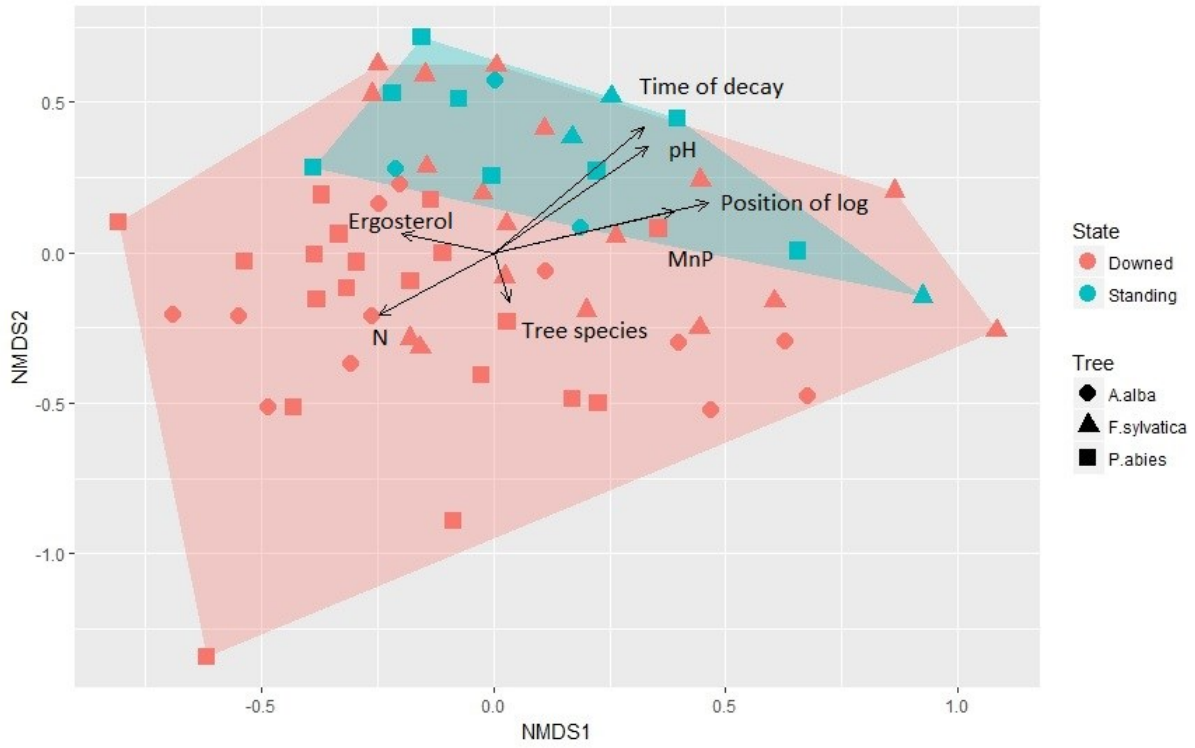
None of the performed one-way Permanova tests for enzyme activities of differently positioned logs (F= 2.153; p= 0.1138), tree species (F= 1.341; p= 0.2525) and time of decay (F= 0.1091; p= 0.1091) weren´t statistically significant at designated confidence α-level= 0.05. Therefore, the two-way Permanova test wasn´t performed. Mantel test of enzymatic activities (Table 10) revealed significance for the factor time of decay. The most important was the interaction of position, tree species and time of decay (F= 0.1865; p= 0.039).

Table 10 Mantel test results of enzyme activities and several factors of influence in Žofín natural forest. Colors indicate the most important factors or combinations of factors. Deeper the color is, the more important the factors are in structuring the fungal community.

Matrices		**R**	**p**
Enzymes	N	0.08129	0.14185
Enzymes	pH	-0.01719	0.53236
Enzymes	pH and N	-0.001251	0.44653
Enzymes	C and N and pH	-0.05844	0.76915
Enzymes	Time of decay	0.1834	0.04034
Enzymes	Tree species	0.0152	0.2672
Enzymes	Tree species and time of decay	0.1852	0.04063
Enzymes	Tree species, time of decay, pH, N	0.1856	0.04082
Enzymes	Time of decay and N	0.1849	0.04137
Enzymes	Ergosterol	0.02138	0.29863
Enzymes	Ergosterol and N	0.02138	0.3004
Enzymes	Position	0.08768	0.13807
Enzymes	Position, tree species	0.05805	0.07123
Enzymes	Position, tree species, time of decay	0.1865	0.03948

5.2.5 NMDS

Two-dimensional NMDS analysis of fungal communities on *F. sylvatica*, *A. alba* and *P. abies* in Žofín, revealed distinctive grouping of downed and standing trees (Fig. 23). The analysis didn´t show any separation of different tree species, and showed that the group of standing trees overlap with the group of downed decaying logs. The formation of such subgroup was due to mostly effects of time of decay, pH and activity of manganese peroxidases. The content of nitrogen had on the other hand, shaping effect on downed decaying logs.



NMDS stress 0.2362

NMDS ordination

	r2	p
pH	0.2358	0.0006
N	0.1039	0.0409
Time of decay	0.2771	0.0001
Tree species	0.0290	0.4181
Position of log	0.2418	0.0002
Ergosterol	0.0427	0.2769
MnP	0.1715	0.0038

Fig. 23 Two-dimensional NMDS of fungal community composition between differently positioned decaying tress in designated circle area of Žofín natural forest. Data set was based on relative abundances of 121 fungal genera, which achieved at least 0.5% in 3 and more samples, or more than 10% in one and more samples. Graph shows individual logs (CWD) and vectors of environmental variables

5.2.6 Indicator species of standing and downed deadwood

Analysis of indicator species showed more indicator species for standing over downed logs (Table 11). While for downed logs, all indicator species belonged to the ecological group of other saprotrophs, for standing trees the ecological variability was higher and included three white rot and twi ectomycorrhizal fungi.

Table 11 List of the indicator species on standing and downed logs in Žofín natural forest, with their ecological groups.

* - p < 0.05; ** - p < 0.01; *** - p < 0.001.

Downed logs	**Ecology**		**Standing logs**	**Ecology**	
Thysanophora penicillioides	Saprotroph	**	*Corynespora* sp.	Saprotroph	***
Collophora sp.	Saprotroph	*	*Scleroderma areolatum*	EcM	***
Meliniomyces variabilis	Saprotroph	*	*Helicosporium pallidum*	Saprotroph	***
Hyaloscypha hepaticola	Saprotroph	*	*Herpotrichia juniperi*	Saprotroph	***
Mortierella sp.	Saprotroph	*	*Calycina herbarum*	Saprotroph	***
Pseudaegerita corticalis	Saprotroph	*	*Penicillium citreonigrum*	Saprotroph	***
Acephala sp.	Saprotroph	*	*Heterobasidion irregulare*	White rot	***
Mortierella minutissima	Saprotroph	*	*Oliveonia pauxilla*	Saprotroph	***
			Geomyces pannorum	Saprotroph	**
			Helicodendron luteoalbum	Saprotroph	**
			Penicillium glabrum	Saprotroph	**
			Meliniomyces sp.	Saprotroph	*
			Gorgomyces honrubiae	Saprotroph	*
			Hyphodontia alutaria	White rot	*
			Cadophora sp.	Saprotroph	*
			Sistotrema sp.	White rot	*
			Cenococcum sp.	EcM	*
			Phialea strobilina	Saprotroph	*

6 Discussion

6.1 Community composition of downed decaying wood in natural forest of Salajka

The aim of the first part of study was to describe the fungal communities on different decay classes of beech and fir logs in natural forest Salajka. The fungal community was examined by sequencing the natural samples of deadwood and further bioinformatically processed to access the members of the most important fungal species participating on decay in different tree species and decay classes. The enzymatic activities and wood chemistry was put in context with present fungal species. Due to global warming and connected changes in temperature and climate, more studies are emerging concerned with ecosystems processes and the correlation with microbial diversity, as the loss of species might lead to deficiency in ecosystems functioning.

Ergosterol content in mycelia is variable and depends on the fungal species and environmental conditions, but is commonly used to estimate living fungal biomass in the substrate, as it shows less variation than internal transcribed spacer (ITS) numbers (Baldrian *et al.*, 2013). Ergosterol content in *F. sylvatica* significantly increased after <7 decay class from average 9.5 μg g^{-1} up till 139 μg g^{-1} in >40 class. In *A. alba,* the content varied alternatively significantly from 14.1 μg g^{-1} in <7 class to 57.6 μg g^{-1} in >40 class. The presumable fungal biomass was therefore higher in beech logs over fir, which is in accordance with literature (Noll *et al.*, 2016). Mantel test confirmed the influence of fungal biomass on community composition. Nitrogen content in logs increased with progressive decay due to microbial activity. Fungi are known to translocate in hyphae nutrients from nutrient-rich substrate to nutrient poor substrate (Frey, Six and Elliott, 2003), to recycle old mycelia or to store nitrogen as amino acids, specific storage proteins or chitin (Watkinson, 2015). Bacteria plays an important role in mycelia recycle and nitrogen fixation (Hoppe *et al.*, 2014). Overall the nitrogen content followed similar development pattern as ergosterol, rising significantly in beech after <7 class from 0.1 % to 0.6 % in >40 class. Logs of fir contained similar amount of nitrogen, rising from 0.1 % in <7 class to 0.5 % in >40 class. Such results are in line with literature, as nitrogen is limiting nutrient in decay process (Bebber *et al.*, 2011) and its higher content is necessary for successful decomposition of wood and influence the rate and dispersion of fungal colonisation of substrate. The reasons for higher fungal biomass in deciduous over coniferous trees are differences in wood anatomy, lignin composition and amount (higher in coniferous), microbial growth inhabiting substances like tannins, terpenoids and phenolics

substances (Schwarze, Engels and Claus, 2000; Cornwell *et al.*, 2009). Additionally, the higher content of N in deciduous trees may enhance the fungal growth in CWD (Weedon *et al.*, 2009). Both assumptions were confirmed in the study of Noll *et al.* (2016), and in this study as well, by correlation of ergosterol and nitrogen content with fungal community composition. Carbon content didn't vary significantly, but was relatively higher in beech over fir. In the logs of both tree species decaying <7 years, was very high the C:N ratio, supporting anti-microbial conditions in uncolonized substrate. As the decay proceeded, the ratio decreased because of nitrogen accumulation and microbial respiration of CO_2. Fungi accelerate their grow and the decay rate increases. This was in agreement with literature findings (Rajala *et al.*, 2011; Hoppe *et al.*, 2016). Lignin function as a barrier to microbial attack and therefore higher content of lignin, slow the decay (Freschet *et al.*, 2012). Content of lignin grew significantly in beech logs from 18.9 % to 36.4 %, while in fir logs the amount was higher and the intra-log variance was very important, ranging from 13 % to 80 % in the decay class 20-40. Mantel test showed independence of lignin content and fungal community composition. The pH level of both tree species significantly decreased as expected, due to acidification of substrate by fungal secondary metabolites as the decay progress (Weedon *et al.*, 2009). In beech logs, pH decreased from 5.5 to 4.1 and in fir logs from 5.0 to 3.8. The moisture content inside logs and therefore the decay rate can be influenced by presence of other organisms such lichens and bryophytes using dead wood as platforms for grow, and functioning as isolators , promoting more humid environment inside logs (Heilmann-Clausen *et al.*, 2014), hence it may be adequate for future studies to monitor the presence of surrounding species to implement such data into decay rate predictive models. Due to higher fungal biomass, overall lower C:N ratio and lower lignin content (30 % beech, 44 % fir) present in beech deadwood, over fir logs, the wood of broadleaf trees decompose faster than deadwood of coniferous trees (Weedon *et al.*, 2009; Noll *et al.*, 2016). In a study of decay rate of 13 different tree species, *F. sylvatica* and *Carpinus betulus* achieved the highest decay rates (Kahl *et al.*, 2017). According to Hoppe *et al.* (2015) all of the European high-throughput sequencing based studies were focusing on gymnosperm deadwood, *Picea abies*, in boreal forests (Ovaskainen *et al.*, 2010, 2013; Kubartová *et al.*, 2012), or angiosperm tree species, *Fagus sylvatica* and *Quercus robus*, in temperate ecosystems (Hiscox *et al.*, 2015; van der Wal, Ottosson and de Boer, 2015). Only recently, studies concerned with other tree species started to emerge (Noll *et al.*, 2016; Kahl *et al.*, 2017), which might potentially lead to unrevealing of other important factors with influence on community composition and decay rate. OTU richness was consistent across all decay classes and both tree species. This is in discrepancy with other studies, where the fungal species diversity increased with decay, achieving the highest diversity in the last decay stages. The most recent studies, conversely, confirmed observations of this study, suggesting that the diversity of fungi doesn't grow with decay. The average number of species was 143 in beech and 138 in fir. The assumption that wood of broadleaf trees, as easier to decay, inhabit larger

number of species (Kahl *et al.*, 2017) wasn't confirmed in this study. Such results are in contradiction with studies demonstrating a positive relationship between species richness and decay rate, supporting synergistic perception of fungal community function (Tiunov and Scheu, 2005). Also, the conclusion of numerous sequencing-based studies that the species richness increases with loss of mass and density of wood, (i.e., with progressive decay) wasn't confirmed (Kubartová *et al.*, 2012; Ovaskainen *et al.*, 2013; Rajala, Tuomivirta and Pennanen, 2015; Hoppe *et al.*, 2016). It was hypothesized that higher species richness might enhance decay rate because of the functional complementarity, variability in enzyme battery and more intense exploitation of resources (Kubartová, Ottosson and Stenlid, 2015). Neither the findings of fruiting bodies surveys, which showed the highest number of species during middle decay stages decay, didn´t convey with the result of this study (Renvall, 1995). On the contrary, Brown *et al.* (2001) suggest that observed pattern of stability in OTU richness might occur whenever limiting factors, as availability of energy for instance (deadwood), is relatively constant and when there are other species available to colonize with redundancy in features. It might then explain the relatively stable species richness in natural forests, where the deadwood is not scarcely distributed, and where it hosts known red-listed species. These conditions when met, the richness of ecosystem should maintain stable even while facing large changes in dominant vegetation, without influence of changes in species composition (Brown *et al.*, 2001; Sax, 2002). Therefore, such level of species richness should withhold global climate warming. A recent study showed that OTU richness was not correlated with wood density loss and further confirmed that it was mainly the composition of fungal community which influenced the decay rate, over OTU richness (Kubartová, Ottosson and Stenlid, 2015). Chao -1 species estimate predicted higher number of species than was found, on average 427 species in beech and 376 in fir, suggesting that sequencing depth wasn´t accurate. Shannon-Wiener index showed on average 2.8, of 5 maximal, therefore the population was quite rich in species, with uneven distribution. Evenness is on average 0.56 which shows rather unevenly distributed population with higher differences in species distribution. The evenness of dataset was 0.6 in beech and 0.5 in fir, meaning that the species were not evenly distributed but neither there was a clear dominance in most of the samples.

The relative abundances of basidiomycetes and ascomycetes varied between different tree species and among different decay classes. The composition of fungal communities changed along the decay classes, as the abiotic and biotic conditions of wood were modified by microbial activity (Hoppe *et al.*, 2016). In *F. sylvatica*, ascomycetes exceed basidiomycetes in first decay classes, and then progressively the ration changed till last decay class, when basidiomycetes exceeded ascomycetes. In *A. alba*, basidiomycetes had higher relative abundances through all decay classes (Hoppe *et al.*, 2016), which is not in line with studies made on coniferous trees, *Picea abies*, in boreal forest ecosystems (Rajala *et*

al., 2011, 2012; Kubartová *et al.*, 2012), where the ascomycetes were observed to be most dominant during early decay stages. Moreover, higher OTU diversity was found for taxonomically assigned species of ascomycetes then basidiomycetes (Ottosson *et al.*, 2015), which supports the hypothesis that most of the biological diversity can be access only via molecular based techniques (Hibbett *et al.*, 2011). On both tree species, the abundance of Agaricales in average progressively increased with time elapsed (from 8 % to 40 %), as in the contrary with Polyporales, which were more abundant on <7 (>20 %) than on >40 (2 %). Russuales were more important in *A. alba* (5 %) then on *F. sylvatica* (1 %). Vice versa for Hypocreales, which were more abundant on *F. sylvatica* (5 %) then on *A. alba* (1 %). Helotiales were present on both tree species (>10 %), while Xylariales were important only on *F. sylvatica*. It was possible to observe a pattern where one fungal order replaces other as the decay progress, and as the abiotic conditions changes to suit the latter. Polyporales little by little decreased, hypothetically giving up the territory to Agaricales. Such trend was possible to observe only for basidiomycetes, and the word hypothetically is employed here, as in this study wasn´t possible to confirm this assumption. Some of the most pronounced genera preferred certain decay classes, while other were present along the decay. In *A. alba*, were found more species of white rot fungi then on *F. sylvatica*, which was in line with found higher relative abundance of white rot fungi in *A. alba*. Genera *Fomes* and *Ganoderma* were important in <7 years decaying logs, while *Hyholoma* was the most abundant in the >40 class, in beech. *Ganoderma* and *Ischnoderma*, were quite abundant during <7 and 7-19 class, then were probably substituted by some more combative species of white rot fungi, like *Hyphodontia*, *Resinicium* or *Sistostrema* abundant during 7-40 years decaying logs, in fir. In fir classes 20-40 and >40, was the most abundant *Pleurocybella*. This was a highly probable scenario, as fungi belonging to ecological group of white rot were important decayers found on all decay classes. The replacement of one specie by other is in line with findings that the community composition is more important than OTU richness (Kubartová, Ottosson and Stenlid, 2015), and that the composition of fungal community reflect the changes of substrate during the decay, without a change in OTU richness. Additionally, fungi initializing the decay have less of the combative capacity, hence, they are gradually replaced by other species. Dominant fungi differed from other studies. In the study of Hoppe *et al.* (2016) was *Resinicium bicolor* most abundant in all decay classes on coniferous tree, while in this study *R. bicolor* was dominant on 2 fir logs in 7-19 and >40 class and wasn´t abundant in other classes. While *Hyphodontia alutaria*, *Ascocoryne cylichnium*, *Heterobasidion parviporum* and *Fomitopsis pinicola* were common in all decay classes (Hoppe *et al.*, 2016), or were classified as early colonizers (Kubartová *et al.*, 2012) on *Picea abies* logs. *Hyphodontia alutaria* was dominant on one fir log and was most abundant during 7-40 years of decay. *Ascocoryne cylichnium* was present on all decay classes in small abundance, *Fomitopsis pinicola* was abundant only in 7-19 decay class and *Heterobasidion parviporum* wasn´t abundant in this study. In beech logs, the dominant fungi differ among decay classes and were different from these

found in study of temperate forests (Hoppe *et al.*, 2016). The dominant taxa changes from *Ganoderma applanatum* in < 7 decay class to *Hypocrea* sp. and *Marasmius alliaceus* in 7-40 decay class and then *Kretzschmaria deusta* in > 40 class. Suggesting the development from white rot decayers to other saprotrophs. Basidiomycetes belonging among other saprotrophs (*Mycena*, *Clitocybula* and *Megacollybia*), were more important in logs decaying >20 years. *Mycena* in the decay class > 40 achieved relative abundance of 20 % on *F. sylvatica*. Ascomycetes were most important at the beginning or during the decay. *Ascocoryne*, *Eutypa*, *Hypoxylon* and *Sorocybe* were most important during < 7 class on beech logs. *Ascocoryne sarcoides* was simultaneously dominant on one beech log and was indicator species for < 7 decay class. *Eutypa* was also dominant on one log with 21 % of relative abundance, and was indicator species for beech < 7 and > 40 decay class. *Trichoderma* and *Zignoella* were abundant during 7-40 years of decay, *Kretzschmaria* and *Phialocephala* were abundant since < 7 class, while Leptodontium was present on all decay classes, on beech. Fir logs manifested lower diversity in abundant ascomycetes decayers, as the overall relative abundance of ascomycetes on fir was lower than on beech. Members of family Xilariaceae were found to cause low rates of decomposition as they are not able to produce MnP and impede secondary saprotrophs from colonisation of deadwood (Hoppe *et al.*, 2016). In this study were also found different members of Xylariaceae in beech logs from < 7 to >40 decay class, exhibiting lower rate of lignin degrading enzyme activity, which was in line with literature results (Hoppe *et al.*, 2016). Fungi were dominant always only on few logs (maximum 9 CWD of *Pleurocybella porrigens*) manifesting that the fungal community composition was highly variable among the logs, as observed in several studies (Kubartová, Ottosson and Stenlid, 2015; Ottosson *et al.*, 2015; van der Wal, Ottosson and de Boer, 2015; Baldrian *et al.*, 2016). This suggests that the species assembly is a stochastic process, powered by priority effect, facilitating or preventing some species from colonizing CWD, resulting into random community (Hiscox *et al.*, 2015; Baldrian *et al.*, 2016). Kubartová *et al.* (2015) reflect that the fungal communities in all samples were dominated by a few highly abundant key OTUs, and it was mainly their activity responsible for overall log density loss. The domination of fungal community by few species was possible to observe in this study as well. Hoppe *et al.* (2016) found that fungal communities on logs of the same tree species in different forest type are more similar than communities of deadwood of different species in the same forest. Therefore, he hypothesises that the substrate type has a greater impact on fungal community then forest type or geographical proximity. Such assumptions need to be confirmed with studies of more tree species with similar physico-chemical properties. Nevertheless, it was found that the physico-chemical composition of wood (decay class, C:N ratio, wood density, water and lignin content) has an impact on fungal community composition (Rajala *et al.*, 2012). Mantel test revealed, that the fungal community was influenced by factors tree species, time of decay, pH level and C and N content. But Mantel test showed the highest influence for the combination of factors tree

species, pH level and nitrogen content. Surprisingly, the correlation wasn´t higher when the factors of time of decay was added into the above mentioned combination. The possible explanation is, that the specificity of tree species itself and its chemical composition (pH level, nitrogen content) has more important effect on fungal community than the modification created during microbial decay. Or, as pH level and nitrogen content are themselves part of the microbial modifications during the decay, these two factors are in fact the most important variables in time of decay of tree and other aren´t as significant. But one-way Permanova test showed significance for the time of decay and tree species on the variation of community composition. And two-way Permanova test of interaction of these two factors was significant as well. Both factors are therefore important predictors of fungal community composition. Two-dimensional NMDS analysis manifested differences between the tree species and the time of decay. The less decayed trees were sparsely dispersed while when becoming more decayed logs tended to group together due to more similar fungal communities present on them. Logs of different tree species were visibly separated, though not entirely. It was possible to observe and overlap between fir and beech, probably due to stochastically occupied less decayed logs, which is in line with recent findings (Baldrian *et al.*, 2016). The main factors shaping the composition of community were pH level, tree species, time of decay, nitrogen, carbon and ergosterol content, all of them statistically significant. Therefore, again was shown that the chemical composition of tree and tree species itself was the most important community predictor.

Relative abundances of white rot fungi were alternately decreasing along the decay in fir logs, while the diversity inside decay classes was considerably high on beech logs, the differences weren´t significant. The highest relative abundance of white rot fungi was found significantly in < 7 and 20-40 decay class on fir and non-significantly on beech. Metabolically active white rot fungi were observed during initial phase of decay in study of Rajala *et al.* (2010). Brown rot fungi were abundant only in 7-19 decay class on fir, due to dominance of *Wrightoporia* sp. and *Fomitopsis pinicola* on two logs, while the presence of these species on other logs wasn´t that pronounced, as seen from the boxplot graphics. Ectomycorrhizal fungi (e.r., Inocybe, Tylospora, Russula, Tomentella) achieved the highest abundance in 20-40 and > 40 decayed fir logs, which was in line with the literature results from boreal forests (Rajala *et al.*, 2012; Rajala, Tuomivirta and Pennanen, 2015), but it wasn´t consistent with studies from temperate forests (Hoppe *et al.*, 2016). Hoppe *et al.* (2016) explain the relatively low abundance of EcM fungi in temperate forests in *Picea* logs by differences in availability of nitrogen between these two ecosystems, where temperate EcM fungi aim to nitrogen in soil, while boreal EcM fungi acquire N by decomposing wood, to avoid competition with forest floor vegetation (Rajala *et al.*, 2011). The higher relative abundance of EcM fungi in fir logs might be caused by niche opening due relatively low presence of brown rot fungi on fir. The decrease of white rot fungi on fir in > 40 class, might have been

caused by replacement of part of the community with ectomycorrhizal fungi, more adapted on harsh environmental conditions in logs in last decay stages, and as with progressive decay the wood structure and composition starts to resemble little by little to the soil, such results suggest the that primary habitat of EcM fungi is soil (Rajala, Tuomivirta and Pennanen, 2015). In boreal forests, EcM fungi compose an important part of the microbial biomass, where they symbiotically exchange carbon and nutrient with their host trees (Högberg and Högberg, 2002). Currently is discussed whether EcM fungi are obligate symbionts or facultative saprotrophs (Baldrian, 2009; Lindahl and Tunlid, 2015). Nevertheless, they colonize the woody substrate during their search of nitrogen from fungal mycelia. As the brown rot fungi were found mainly on coniferous logs and in intermediate stages of decay (Rajala, Tuomivirta and Pennanen, 2015), it support the results of fruiting body based surveys (Renvall, 1995) and it might indicate that heavily decayed logs are not suitable environment for brown rot fungi, selecting other, more adapted on stress condition of heavily decayed substrate (Rajala, Tuomivirta and Pennanen, 2015). Nonetheless, fungi with brown rot life strategy were found on heavily decayed logs as well, indicating that fungi can change their ecophysiology based on the environmental conditions (Riley *et al.*, 2014). As they, presumably, lack lignin decomposing enzymes, their appearance in intermediate decay stages isn´t surprising because they need disrupt structure of lignin to act on with their cellulases and hemicellulases. Yeasts were consistently present in all decay classes. Lichenized fungi were presented in all the samples, possibly indicating some level of contamination by epixylic organisms. In each decay class of each tree were found less than 1 % of sequences to which wasn´t assigned any ecophysiological role. The reason might be the cryptical lifestyle some fungi live and impossibility to examine their lifestyle, or to assign only one ecophysiology. The widely used designation (white, brown, soft rot) started to be insufficient to accurately describe fungal decay behaviour (Floudas *et al.*, 2012). Such partition seems uncorrected as fungi probably choose their life strategy based on the available resources (Song *et al.*, 2017), not only on their genomes. In this study was used the classical designation of fungi based on their known ecophysiology, as presumably the most common under standard conditions. The ecophysiology of most of the fungi were detected under laboratory conditions, imitating natural conditions and more field studies are needed to connect genetic identity and functional responses (McGuire *et al.*, 2010). Especially in terms of threatening climate change, more information to predict the development of fungal behaviour would be adequate.

The activity of most of the exo-cleaving enzymes significantly increased after the <7 decay class, suggesting that fungi start to decay wood efficiently after some period, once they establish properly on substrate. The lag time in decay was observed in other studies as well (Přívětivý *et al.*, 2016), probably necessary for an activation of enzyme production. Activity of enzyme cannot be linked to OTU diversity as it didn´t change with longer decay, but the activity of hydrolytic enzymes was

correlated with the increase or changes in fungal biomass in both tree species. Correlations between enzyme activities and the ergosterol content were found to be weak in the study of Noll *et al.* (2016), while in this study Mantel test showed that ergosterol content was one of the best predictors for overall activity of enzymes. Generally, the activity of exo-cleaving enzymes were higher in beech logs, which was in line with literature results (Noll *et al.*, 2016), suggesting higher decay rates of beech logs. Manganese peroxidase showed the highest activity in <7 class in *F. sylvatica*, while in *A. alba* the activity varied significantly. In *F. sylvatica* logs, the activity seemed to mirror the relative abundances of white rot fungi, while in *A. alba* the activity was more correlated with relative abundance of other saprotrophs. The tendency to relatively higher activity of MnP during initial phase of decay in beech, supports the hypothesis that lignin degrading enzymes are necessary to break the lignocellulose barriers for polysaccharide cleaving enzymes to act on the substrate. While in coniferous trees, the lignin degradation is usually carried by brown rot decayers, commonly found in early decay stages (*Fomitopsis pinicola*), using hydroxyl radicals to modify the lignin (Rajala, Tuomivirta and Pennanen, 2015), this wasn´t confirmed by results of this study. The input of laccase seemed rather uneven. Some logs didn´t show any activity, while some demonstrated outlining figures reaching the highest activity among lignin decaying enzymes. Such findings support the stochasticity of fungal community assembly, resulting into high inter-log variation within one decay class. Or the contribution of laccase to the detoxification of wood ingredients (e.g. aromatics) and released phenolics (Arnstadt *et al.*, 2016), or the role of laccase in enzymes production due to lignin modification, influence on fruiting body development, spore pigmentation and sexual differentiation (Boddy, 2000). Overall, lignin decaying enzymes on beech and fir, showed comparable activity with other studies of wood decaying enzymes (Noll *et al.*, 2016). Some of the detected higher activities of laccase and MnP might have been caused by two or more species interaction (Fukami *et al.*, 2010; Hiscox *et al.*, 2017). Surprisingly, the role of endocellulase, as a proxy for microbial activity from different organism (e.g., fungi, insects, bacteria), wasn´t confirmed (Kahl *et al.*, 2017), as the measured activity was quite low in both tree species and decay classes. It was expected that enzyme would reflect the composition of wood, constituted from up to 50 % of cellulose. The limiting nutrient for plants in boreal forest, is phosphorus, because essential part of the total phosphorus content is bound in organic compounds. Some EcM fungi can produce phosphomonoesterases that degrade organic phosphorus sources and free the nutrient into environment (Nygren and Rosling, 2009). Phosphomonoesterase activity significantly augmented in *F. sylvatica* in 20-40 decay class, but 7-19 and > 40 class had high variation in activity inside decay classes. In *A. alba*, the activity of phosphomonoesterase grew significantly till 20-40 class, and dropped in > 40 class. The decline in the last decay stage in *A. alba* might be caused by interspecific fungal interaction, preventing EcM fungi from expected higher phosphomonoesterase activity. As the phosphomonoesterase activity was in general higher in F. sylvatica, and the relative abundance of EcM

fungi was low (2.1 % highest relative abundance in 20-40 decay class), it would suggest that other organisms produce phosphomonoesterase, or that the activity of present EcM fungi was very high. The activity of lipase, enzyme which catalyse the hydrolysis of long-chain triglycerides, significantly increased after < 7 class, probably due to higher turnover of nutrients stored as lipids. The activity of N-acetylglucosaminidase also increased after < 7 class, reflecting the needs of microbial community. The enzyme is necessary in disposal of dead fungal cells, mycelia recycling, acquisition of nitrogen from chitin and in fungal interspecies combat (Boddy, 2000). With progressive decay, the community shifts to higher competitivness for territory, as the available resources become scarcely dispersed and fungi employ chitinase in interspecies combat. N-acetylglucosaminidase is also produced by mycophagous bacteria using chitin as their source of energy (Brabcová *et al.*, 2016; Baldrian, 2017). Unexpectedly, the Mantel test and one-way Permanova test didn´t showed significance for activity of enzymes and the factors tree species, as in study of Hoppe *et al.* (2016). They further hypothesise that such result might be due to functional redundancy of fungal communities, indicating that fungi in each community are adapted to their host tree species. Both test showed significance for activity of enzymes and the time of decay. And further Mantel test proved that the activity of enzymes was the most influenced by fungal biomass and nitrogen content. Both are indirectly linked to the time of decay, as they increased with the progressive decay. Such findings might indicate that time of decay would be a better predictor for the rate of enzyme activities. Enzymatic activities were generally higher in beech deadwood than in fir logs, with distinct differences between manganese peroxidases and hydrolytic enzymes (cellulolytic, hemicellulolytic and N-related). MnP were less active in fir logs, while hydrolytic enzymes achieved in both tree species considerable activity. The suggestion that activity of MnP might be lower in coniferous trees, as these enzymes are produced only by white rot fungi (Noll *et al.*, 2016) preferring deciduous trees, doesn´t seem to accord with results of this study. Because the highest relative abundance of brown rot was found in 7-19 decay class, where was also registered significantly the highest activity of MnP in fir logs, demonstrating the presence of white rot fungi, which was in consistence of various studies (Olsson *et al.*, 2011; Rajala *et al.*, 2012). The relatively high activity of hydrolytic enzymes was logical, as hydrolases are important in preservation of basic metabolic functions (energy metabolism and protein synthesis) through the acquisition of C and N sources (Noll *et al.*, 2016). In recent study of Hoppe *et al.* (2016) was suggested that the activities of lignin modifying enzymes are controlled by the succession of fungal communities and their competition rather than fungal OTU richness, which is in agreement with the results of one-way Permanova test and Mantel results, assuming that the time of decay is important predictor of enzyme activities. While the time of decay might be used in this context as another expression for the succession of fungal community. Strangely, pH level wasn´t correlated with enzyme activities. Such results are not in accord with published literature (Sinsabaugh *et al.*, 2008), as would be expected that as the enzyme activity is

positively linked to the time of decay, and since the pH level significantly decreases with the progressive decay, that the enzymes activity would be directly correlated with pH level. Sequenced ITS have variable copy number in fungal genome and might not directly correlate to biomass, which could influence the relationship between a fungi "abundance" in the dataset and the observed enzymatic activity (Hoppe *et al.*, 2016). A possible explanation of some of the low enzyme activities might be due to high level of interaction among fungal species inside one log, which put more energy into competing for resources than into production of wood-degrading enzymes under natural conditions (Fukami *et al.*, 2010; Dickie *et al.*, 2012). Therefore, as in consistence with results of this study, Hoppe *et al.* (2016) suggest that the high OTU richness of fungal communities on deadwood should not be associated with any increase in production of wood degrading enzymes or wood decay rate. Kubartová *et al.* (2015) further add that it is the composition of fungal community, not its richness, which influence the wood decay rate. The conclusion of Kahl *et al.* (2017), refine above written suggestions, by explaining that the fungal community composition and OTU richness were the best predictors for decay rate in the later stages of deadwood decay (van der Wal, Ottosson and de Boer, 2015).

Most of the dead wood studies were done in temperate forests, and in general, fungi are still underrepresented in experimental studies of dead wood, especially in the tropics and subtropics (Seibold *et al.*, 2015). Tropical forests rapidly degrade biomass and have high diversity of species, while boreal systems tend to have long residential times and some potential for buried dead wood stocks (Russell *et al.*, 2015). Temperate forests show both features, hence, by comparing all three ecosystems might lead to deeper understanding of the role of climate on decomposing processes. Most researches are funded for three to six years and therefore only few experimental studies follow the succession of dead wood for more than six years (Seibold *et al.*, 2015), which shows not to be sufficient when following the decay of deadwood decomposing on average 50-80 years. To put the results if this study into broader perspective of longer study would be beneficial. It was estimated that insects contribute for 10-20 % of wood decomposition (Ulyshen, 2016). In temperate ecosystems, where termites are absent, the presence of large wood-boring beetles is probably more influential and important than species richness itself, but knowledge about insect species richness on wood decomposition of different tree species is still lacking (Kahl *et al.*, 2017). In order to become closer the accurate quantify the carbon cycling, it is necessary to study more the interaction of deadwood and non-saproxylic species (Kirchenbaur *et al.*, 2017). Overall, in future more studies should be concerned with multitaxon interactions (fungi, bacteria, plants and invertebrate), to deepen the understanding of ecosystem processes. The combination of species in logs affect the rate of decomposition as different species have different ability to decay (Worrall, Anagnost and Zabel, 1997). Also the area occupied by fungi and the composition of logs is highly important in determining decomposition rate (Hiscox *et al.*, 2017).

The models of carbon sequestration would be more reliable if the composition of fungal communities could be implemented in them. Mainly, they are based on the chemical properties and environmental factors only. It is not possible to implement the whole fungal community in these models, but the reliability of models could be improved by implementing only the most abundant species that are responsible for the biggest mass loss. And identification of these key players in wood decomposition might help to find enzymes to facilitate production of lignocellulose - derived biofuels (Kubartová, Ottosson and Stenlid, 2015). Another practical use of theoretical knowledge would be use of "priority effect" in ecological restoration (Ottosson, 2013). Direct inoculation of pioneer fungi or rare specialist would promote composition of specific wood-inhabiting community and would also bring insight into functioning of "priority effect" on fungal community.

6.2 Difference in fungal community composition between downed and standing deadwood in natural forest of Žofín

Differently positioned decaying trees were studied in natural forest of Žofín, to distinguished which of the elements - tree species, time of decay and position of log - are more important for fungal composition and the level of enzymatic activities. The differences between the decay rate and fungal community composition found on downed and standing logs weren´t yet thoroughly studied. Presumably, the differences should be high as downed and standing logs are subjected to different microclimatic conditions and influenced by different factors.

With exception of nitrogen content, none of the measured chemical parameters of wood and ergosterol content didn´t statistically differ between standing and downed logs. The average content of nitrogen was significantly higher for downed (0.22 %) over standing (0.13 %), while the content of carbon was on average equal to 50 %. The higher nitrogen content in downed logs might suggest higher decay rate for downed wood over standing, as the C:N ratio would be lower (Rajala *et al.*, 2011). pH level was 4.4 in average the same in standing as in downed logs. The non-significantly higher ergosterol content and lower pH level in downed logs further indicate that there might be difference between the decay rate of differently positioned logs, as higher amount of fungal biomass would lead to higher enzyme activities, lowering pH in downed logs. Nonetheless, the enzyme activities were significantly found to be higher on standing dead trees, suggesting, together with presumable higher fungal biomass, more important competitive interactions taking place in downed logs, lowering enzyme production (Fukami *et al.*, 2010; Dickie *et al.*, 2012). The significantly higher content of nitrogen might be also explained by more important bacterial community on downed dead wood. The contact with

soil is essential in bacterial dead wood colonisation, and their activity in N-fixation and mycelia recycle would lead to increase in nitrogen content (Hoppe *et al.*, 2014; Brabcová *et al.*, 2016). None of the diversity indices were significantly different. OTU richness varied between 35 to 204 OTU for standing trees, and 22 to 205 in downed logs. The average of OTU found per logs was higher for standing dead trees (126 > 107). Chao -1 prediction estimate showed the the dispersion from 604 to 62 OTUs for downed logs and 808 and 54 OTUs for standing logs. According to Toljander *et al.* (2006), changing microclimatic conditions increase the OTU richness and the decay rate. It is considered that the microclimatic conditions in standing over downed logs would vary significantly, as the later are usually at least in some contact with forest floor, or are buffered from water content fluctuation by presence of epixylic species (Heilmann-Clausen *et al.*, 2014). Conversely, standing trees are subdue to desiccation and more various extreme environmental conditions (Přívětivý *et al.*, 2016). Therefore the results suggesting higher OTU richness in standing, over downed logs might be accurate. If the assumptions of Toljander *et al.* (2006) would be correct, the standing trees would decompose more quickly than downed, as they would host overall higher OTU richness, therefore supporting wider range niches for more diversified set of decayers (Kubartová, Ottosson and Stenlid, 2015). But as was found in Přívětivý at al. (2016), the lower availability of water, presumed to be in standing logs, extend the residence time of logs in forest, therefore suggesting a slower decay rate. The relative abundances of ascomycetes and basidiomycetes were very similar on differently positioned dead trees. When reflecting the different tree species and the time of decay, some differences emerged. On the same tree species, the composition of basidiomycetes and ascomycetes were conversely opposite to each other on differently positioned logs. The ratio of relative abundances of ascomycetes and basidiomycetes on downed logs in decay classes of <20 and >20 was similar, conversely to the ratio in standing dead trees. In >20 trees, ascomycetes dominated the community composition, while in >20 the ratio become closer the ratio in downed logs. The composition of fungal genera of downed and standing trees were mirroring each other in relative abundances present on logs. Most abundant genera were present on both groups, differently relatively abundant, but still present. That might indicate, that fungal colonisation was directed by other factors than position of decaying log. If it would be otherwise, some genera would be missing from standing or downed logs. The most abundant genera, each with presence in more than 7% of all sequences of standing logs , were *Pseudogymnoascus*, *Ganoderma*, *Hyphodontia*, *Gorgomyces* and *Meliniomyces*. Genera *Pseudogymnoascus*, *Ganoderma*, *Hyphodontia*, *Ischnoderma* and *Leptodontidium*, were the most abundant genera in downed logs with abundance of 4 % from all downed log sequences. These results are in coherence with index of evenness, which was lower for downed log than for standing ones, indicating more balanced and homogenous fungal community on downed logs. All of these genera are saprotrophs. *Ganoderma*, *Hyphodontia* and *Ischnoderma* are white rot fungi. None of the ecological

groups didn´t showed any statistical significance, therefore the ecological groups should be comparable in standing and downed logs. The relative abundance of EcM fungi was slightly higher in downed over standing trees, which was reflected by non-significantly higher activity of phosphomonoesterase in downed logs. Two-dimensional NMDS analysis revealed that standing dead trees groups together and creates with their fungal community structure a subgroup of downed logs. The most important factor in fungal community structure was the time of decay, position of logs and pH. pH understandably shapes the community and reciprocally is created by the shifts in community members. Indicator species revealed two EcM fungi associated with standing logs. It is because these two fungi were present in almost all sampled standing logs, and therefore due to small number of samples, they were selected as the indicator species. On downed logs, a lower diversity of the indicating species was found and they belonged among other saprotrophs. The lower number of indicator species on downed logs might have been caused by a variable fungal community among downed logs. As seen in study of Salajka forest, fungal community is mainly shape by the time of decay and tree species, sharing only few generalists across decay classes and tree species. This is in compliance with results of downed logs in Žofín, where the few indicator species represent the generalists adapted on the growth non-specifically on wider range of substrate. The indicator species of standing trees were more abundant, indicating that the found community was similar among logs. That may be cause by small number of samples, where few matching results gives significant results or due to the fact, that the communities on standing dead trees are preferentially similar. *Pseudogymnoascus pannorum* and *Meliniomyces* sp. achieved the highest number of dominated CWD, 8 logs, which is 12.7 % of all sampled trees, only with the highest recorded relative abundance of 30 % in log. Therefore the widely present fungi didn´t achieved total dominance, rather showed consistent higher relative abundance. *Ganoderma applanatum, Fomes fomentarius, Resinicium furfuraceum, Hyphodontia alutaria* and *Climacocystis borealis* achieved the relative abundance over 80% in one log. While most of these fungi attained such high relative abundance only in one log, *G.applanatum* and *R.furfuraceum* dominated in three other logs. That shows their ability to spread rapidly and successfully, without being limited ny substrate position. Conversely, the effect of log position and tree species on fungal community was confirmed by Mantel test. Adding other factors, like pH level and nitrogen content into combination of tested factors didn´t add to the significance. On downed logs, 44 % of all fungi belonged among white rot decayers, while on standing logs it was 41 %, that would support the possibility, that the genera on downed logs are more diversified, where more species with less relative abundance contribute to total abundance of white rot, while on standing trees there are few more abundant species joined by many slightly abundant. Mantel test was significant for fungal community structure and factors of the time of decay, tree species and activity of enzymes. The highest significance was for combination of tree species and time of decay. The addition of position into the

combination didn´t influence the significance. One-way Permanova was significant for tree species, time of decay and position of logs, but interaction of position of logs and tree species wasn´t significant. Suggesting that probably other non-measured factors influence the community composition. The activity of enzymes was in general higher in standing over downed logs, indicating higher decay rate in standing logs, which would be in contrast to assumptions of Přívětivý *et al.* (2016). Endocleaving enzymes demonstrated very high variance in activity range, not very active in none of the samples. Activity of exocleaving enzymes, β-xylosidase, cellobiohydrolase and α-glukosidase were statistically higher in standing over downed logs. The activity of N-acetylglucosaminidase was significantly higher in standing over downed logs, probably also due to higher specie richness in standing and a role of chitinase in the recycle of fungal cell walls, or in combative fungal interactions (Boddy, 2000). Lipase was quite active on both type of logs. Activity of all measured lignin modifying enzymes were statistically significantly higher in standing over downed logs. Despite the fact, that standing trees hosted lower relative abundance of white rot fungi. The possible explanation of lower enzyme activities in downed log, could be due to higher interspecies interactions. The downed log possibly provide lower number of microhabitats, causing competitive exclusion for substrate among close related species (Kubartová, Ottosson and Stenlid, 2015), decreasing enzymatic activities (Fukami *et al.*, 2010). Neither the position of logs, the time of decay or tree species didn't show significance for enzyme activities in one-way Permanova test. When performed Mantel test with combination of all three factors, the test showed the highest significance, indicating, that the factors alone cannot sufficiently explain the enzyme activities, it is their combination which reflect the enzyme activity.

To confirm all these suggestions and assumptions, a higher number of samples is required. Due to lower number of samples (50 downed and 13 standing), some patterns were only suggested. Higher number of sampled logs might cause a confirmation of these indications. The sampling of standing dead trees is difficult as they become eventually downed logs, therefore the number of suitable dead trees is limited with time. Plus, to sample the whole dead tree along its length is often not possible due to security reasons. The size of sampled area needs to be taken into consideration, otherwise there might be a bias in data because of dissimilarity between dead trees with growing distance. For forest management, it is necessary to decided how standing trees possibly influence the ecosystem processes. Whether they act as carbon storage, or oppositely they quickly release elevated amount of nutrient into environment. One of the positive effect is already established. Standing dead trees provide large number of different substrate and microhabitats for specific or endangered fungi (Halme *et al.*, 2013; Přívětivý *et al.*, 2016). The difference in decay among downed, standing and other differently positioned logs should be further studied. More precise knowledge about their decay will help to estimate carbon stock and to adjust ecological models of nutrient cycles (Harmon, Woodall and Sexton, 2011).

7 Conclusions

They were clear patterns of wood decay and separation of community composition between beech and fir logs.

The communities were becoming more homogenous with the time of decay elapsed and with progressive decay could have been distinguished successional patterns of different orders, genera and ecophysiological groups on both tree species.

Enzyme activities reflected the relative abundances of presumable ecophysiological groups and were correlated with the time of decay of logs.

OTU richness and none of the diversity indices didn´t vary between decay classes, suggesting that a consistent level of diversity was present in all decay classes, and that it was the community members changing and reflecting the substrate conditions.

The results suggest that they are no significant differences among standing and downed dead trees. Standing dead trees acting as a specific subgroup of all deadwood, nevertheless, the differences wasn´t proved to important enough to clearly detach standing trees from downed logs.

NMDS analysis showed that standing trees have a specific homogenous fungal community, mainly caused by high number of only slightly abundant species, inhabiting standing logs thanks to presumably higher number of niches.

To adequately study the fungal communities on decaying logs in time and on various substrate, longer studies with high number of samples are required.

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